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VOLUME 3

DETAILED OPERATIONAL PLANS FOR STUDIES

IN THE

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

CONFIDENTIAL

Fisheries Studies 18 - 26

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STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DETAILED STUDY PLAN

Project Title:	Prince William Sound Trawl Assessment
Study ID Number:	Fish/Shellfish Study Number 18
Lead Agencies:	State of Alaska, ADF&G; Commercial Fish Division National Marine Fisheries Service
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Date Submitted:	October 12, 1989

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Supervisor ADFG:	[signature]	10/5/89
Supervisor NMFS:	George Snyder	10/12/89
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INTRODUCTION

The goal of this project is to determine the short and long term impacts of the Exxon Valdez oil spill on commercially important bottom dwelling species of fish and shellfish in Prince William Sound and adjacent waters of the Gulf of Alaska. This area supports major stocks of bottomfish and shellfish which in turn support commercial fisheries. These fisheries include pot fisheries for king, Tanner, and Dungeness crab; trawl and pot fisheries for shrimp; and longline, trawl, and jig fisheries for halibut, pollock, sablefish, Pacific cod, flatfish, rockfish and others. These stocks are an important part of the economy of Alaska.

It is hypothesized that the oil spill could adversely affect bottom dwelling fish and shellfish populations in two ways. First, most benthic species go through a planktonic stage which ranges widely through the water column. It is well documented that these plankton are extremely sensitive to the water soluble fractions of crude oil (Mecklenburg et al., 1977, Wells and Sprague, 1976). A multispecies stock assessment permits appraisal of shellfish and bottomfish abundance and distribution. Data from this study will allow detection of reduced year-class strength consistent with the timing of the oil spill. Otoliths (ear bones) of fish and size frequency data of shellfish will be used for determining age composition of various species. Secondly, adult populations could be impacted through movement of oil to the benthic environment causing direct mortality on bottomfish and shellfish through immediate toxic effects, contamination of prey, direct ingestion of tar balls, and detrimental effects on functions of tissues and organs. Trawl surveys are the best method for determining changes in abundance of marine populations living in trawlable habitats and for providing specimens for analysis of oil contamination. The number of organisms caught and the area swept by trawl tows provide the data necessary to calculate abundance.

Establishing actual degree of damage to these stocks by oil is required to advise the public on the full scope of impact on the commercial fishing economy due to the spill. Determining the level of damage to these stocks is also necessary so that it can be factored into future management decisions on harvest levels.

OBJECTIVES

1. Determine abundance of Tanner crab, sidestripe shrimp, halibut, sablefish and other commercially important species.

2. Determine the age composition for primary species (Tanner crab, king crab, Dungeness crab, Dover sole, flathead sole, arrowtooth flounder and pollock) such that the estimated

proportion for each age is within ±5% of the true value 90% of the time. This objective is to be met by a trawl survey in August/September, 1989.

3. Determine the incidence of abnormalities in tissues and organs in fish and shellfish captured in oiled and non-oiled areas and whether such abnormalities result in adverse changes in viability for the resource. This objective is to be met by a trawl survey in May/June, 1989 and augmented by information from a trawl survey in August/September, 1989.

4. Determine the incidence of tar balls in the demersal environment and in stomachs of groundfish captured in oiled and non-oiled areas. This objective is to be met by a trawl survey in May/June, 1989 and augmented by information from a trawl survey in August/September, 1989.

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

METHODOLOGY/DATA ANALYSIS

Two trawl surveys will occur in 1989 to meet the objectives of this study. The May/June trawl survey will duplicate the 1978 NMFS trawl survey in Prince William Sound (Parks and Zenger, 1979) to the extent that time permits. This survey will focus on obtaining hydrocarbon and necropsy samples from selected fish and shellfish in oiled and non-oiled areas.

Abundance, species composition, and other biological attributes of the catch will be estimated for the entire area covered by the August/September trawl survey. Sampling in future years and/or post stratification of the sample area into oiled and non-oiled areas will allow comparison of changes in abundance, species composition, age composition, or other biological parameters that may be related to the oil spill.

Study Design

The design for the May/June survey is to duplicate the 1978 NMFS trawl survey in Prince William Sound (Appendix A.1, Figure A.1, Figure A.2). As part of this survey, hydrocarbon samples are to be taken at eight different sites representing two levels of oil contamination based on the surface distribution of oil as follows:

Oiled	Non-oiled
Naked/Glacier	Orca Bay
North Knight Is. Passage (or Green Island if not trawlable)	Port Fidalgo
Upper Montague Passage	Montague Trench
Lower Montague Passage	Port Wells

Number of tows by area are given in Appendix A.2. Results from the May/June (spring) survey, groundfish and shellfish commercial catch distributions, and Tanner crab pot survey results will be used to divide the study zone into geographic sampling areas for the fall stratified random survey, which will occur in August/September (Brannian 1989) (Appendix B.1). Depth strata for geographic sampling areas are based on the 1978 PWS trawl survey. These area strata were outlined on a nautical chart and bottom surface was calculated in square nautical miles using a Sumigraphics digitizing pad (Appendix B.2). Each area was then divided by stratum into a grid of stations, each representing 1.56 square nautical miles.

In the fall survey, a total of 75 tows were optimally allocated to area-strata based on the standard deviation of abundance weighted by bottom surface area for each of the 13 depth-area stratum (Appendix B.3). A compromise survey design was established after evaluating the optimal allocation of effort using Tanner crab, flathead sole, Pacific cod, sablefish, and sidestripe shrimp CPUE from the May/June survey (Appendix B.6). It was assumed that cost of sampling among strata was equal.

Fiscal restraints limit the August/September survey to 75 trawls. Appendix B.3 presents the number of trawl tows to be conducted in each depth-area stratum. These will be randomly chosen from the total available stations in each depth-area stratum. Tows which could be dropped or added have also been prioritized (Appendix B.4). Locations of the proposed stations are given in Appendix B.5.

The following procedure will be followed in conducting a tow in a chosen station. For each station, the vessel first would go to the station center and search for 20 to 30 minutes for trawlable bottom. If no trawlable bottom was found, the vessel would proceed to the location of the nearest successful historical tow. At the location of the nearest successful tow, the vessel would again search 20 to 30 minutes for trawlable bottom and presumably trawlable bottom would be found. If trawlable bottom is found while travelling from the station location to the location of the nearest successful tow, a haul would be made. If no trawlable bottom was found at the station location, enroute to the location of the nearest successful tow, or at the location of the nearest successful haul, the vessel would move to the next station.

Standard 400 mesh eastern otter trawls without roller gear (Appendix C) will be fished for 15 minutes at a speed of 2-3 knots. At the completion of each haul a CTD cast will be made to profile

water characteristics.

Data Collection - General

Station information including location (latitude and longitude), depth, time and duration of tow, direction of tow, weather, surface temperature, and sea conditions, etc. will be recorded by the vessel skipper on a paper form (Appendix D).

The catch will be processed following procedures used by the Resource and Conservation Engineering Division (RACE) of the Alaska Fisheries Center (N.M.F.S.) in assessing population abundance during fisheries surveys. These procedures are described in the Catch Sampling and Data Recording Manual of the RACE Division. (Appendix H.1). This manual includes all aspects of sample collection and processing, such as sorting, counting, weighing, sex determination, length measurements, recording, etc.

Shellfish Samples:

Sample size for Tanner, king, and Dungeness crabs will be 100% of the catch because the numbers are expected to be fairly low. Weight and number of all crabs by species will be recorded. For king, Tanner, and Dungeness crabs sex, size (carapace length or width), shell age and shell condition will be recorded. For female crabs of these species, stage of maturity (juvenile or mature) and relative egg clutch size and stage of egg development will be recorded. Degree of black mat infestation will be recorded for all crabs (Appendix H.2).

Weight and number of shrimp by species will be recorded. Carapace length, sex (Appendix I), and egg development stage will be recorded for a representative sample of shrimp from tows with 250 shrimp or more (Appendix H.3 and H.4).

Data Collection - May/June

Hydrocarbon and Necropsy Samples:

Hydrocarbon and histopathology tissue samples are to be taken during the May/June survey using standard procedures outlined by the Damage Assessment Program.[1] Three replicates for hydrocarbon analysis will be taken at each of eight sites (four oiled and four

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[1] Procedures are outlined in the Standard Operating Procedure (Appendix E); forms are found in Figures E.1 and E.2; hydrocarbon and necropsy procedures are also described in Appendices F.1 and F.2; and Chain of Custody procedures are described in Appendix G.

non-oiled, see above) of the following tissues:

a. Pollock muscle, viscera, bile
b. Tanner crab muscle, hepatopancreas, eggs
c. halibut muscle, viscera, bile
d. Pacific cod muscle, viscera, bile
e. Flathead sole muscle, viscera, bile
f. Sablefish muscle, viscera, bile
g. Sidestripe shrimp (or pink shrimp) muscle

In total, 57 tissue samples are to be taken at each site (3 replicates X 19 tissues), or 456 samples are to be collected from all eight sites (57 samples X 8 sites). Each sample is to be a composite of at least three individuals. Sample sizes and replicates are as recommended by S. Rice, NMFS, Auke Bay Lab.

Histopathology samples will be taken from pollock and sidestripe shrimp with a sample size of 20 specimens per site (three oiled sites, one non-oiled site). Sample sizes and number of sites were recommended by T. Meyer, ADF&G, FRED, Pathology.

Data Collection - August/September

Age Composition (otolith) Samples:

A random sample of 500 otoliths per species will be taken during the August/September survey. Dover sole, flathead sole, arrowtooth flounder and pollock were chosen as the target species for their economic importance, ease of otolith aging, and foraging strategies. (Dick Haight, NMFS Auke Bay Lab., personal communication). The sample size per species was set to simultaneously estimate proportions by age when sampling from a multinomial population such that the probability will be at least 1 - a (precision) that all of the estimated populations will be simultaneously within 5 percentage points (accuracy = 0.05) of the true population age proportions. The largest sample size for a = 0.1 occurs when there are only 3 age classes present in equal proportions and guarantees at least this level of precision and accuracy for any number of age classes and proportions. This "worst case" sample size (Thompson, 1987) of 403 ageable otoliths will be used to estimate the age composition with the desired level of precision and accuracy in the absence of a better estimate of population proportions. An unreadable rate of 25% was estimated resulting in a sample size of 500. Otoliths will be read by the Aging Unit, RACE Division.

Sampling should be spread over a number of tows and represent the entire cruise. For widely distributed species such as arrowtooth flounder or rex sole, 20 otoliths per tow should result in the sample size objective being met. For pollock whose distribution

is more patchy, 50-75 otoliths should be collected when major catches are made.

Flatfish otoliths will be stored in normal glycerin solution. Pollock otoliths will be stored in 50% ethyl alcohol. All will be labled in a manner consistent with the NMFS triannual Gulf of Alaska survey. The form for otilith collection is Appendix H, Figure 6.

All individuals selected for otolith samples will be examined for tarballs, during sex determination. If tarballs are discovered, if the gear if fouled with oil, or if other projects report the presence of oil, hydrocarbon and histopathology samples will also be taken during the August/September survey. The occurance of tarballs is recorded on the form given in Appendix E.2. The procedure for taking hydrocarbon and necropsy samples, as well as the tissues to be sampled are identical to those stated above for the May/June trawl, and recorded on the form given in Appendix E.1.

DATA ANALYSIS

In order to meet objective 1, trawl catch data[2] will be analyzed to generate abundance estimates with operational computer statistical programs utitlized for population estimates from annual Gulf trawl surveys (Blackburn et. al., 1989). Absolute abundance will be estimated in numbers for each target crab species by size, relative age, and sex. Absolute abundance in weight will be estimated for each target fish species by size. Absolute abundance in weight will be estimated by species for miscellaneous shellfish and finfish. Absolute abundance in weight will be estimated for shrimp by species, size, and sex for areas not included in the semi-annual shrimp trawl survey. Shrimp abundance for areas covered by the shrimp trawl survey will be determined from that survey (Hammarstrom, 1988).

Analysis of variance will be used to test for differences in abundance and density between depths and geographic strata. Confidence intervals for abundance estimates will be used to document changes in abundance. Chi-square tests may be used to test for differences in sex composition.

Objective 2 will be met by determining the age composition of primary commercial species available to the trawl net through age

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[2] Logistic contraints dictate that only one haul is made at each station and sample size will rarely be less than 100% of the catch. Catchability of the trawl is assumed to be 1.0. Scan-Mar mensuration equipment will be used to determine the net opening at various depths.

frequency diagrams. Size frequency distribution of crabs by species, sex and relative age and size frequency distribution for target species of groundfish will also be examined.

To meet objective 3, statistics for analysis of variance will be computed to detect any differences in hydrocarbon content and incidence of tissue abnormalities between oiled and non-oiled areas.

Negative impacts of oil contamination on abundance of shellfish and groundfish stocks can be determined by testing for differences in species composition and abundance with categorial data analysis techniques. Catch-age analyses for principal species will be conducted to detect potential recruitment failures. Multivariate statistics could be used to identify more complex associations among the biological and physical parameters between oiled and non-oiled areas.

Data can be post-stratified to determine impact levels based on presence of oil contaminants and mapping information from the coastal habitat project. Analysis of variance (parametric) or Kruskal-Wallis test(non-parametric) will be used to test for differences between years and impact levels for: 1) average number of mature female crab; 2) average relative clutch size of female crab; 3) average stage of egg development; and 4) average number of abnormal egg clutches. Egg development stage will be determined by color and presence or absence of eyes.

To meet objective 4, the number of tarballs encountered in the environment or in groundfish stomachs will be enumerated. Differences between oiled and non-oiled sites will be determined by chi-square tests.

To meet objective 5, and to identify potential methods or strategies for restoring populations if damaged by oil, it will be necessary to test changes in catch per unit effort, age class strength, and reproductive viability. All data will be analyzed for evidence of damage. Depending on the degree of damage to the stock, fishery management actions may be necessary to reduce additional mortality to an oil damaged stock. This may include fishery restrictions, total closure or other measures. Additionally, the need for continual study and monitoring of stock conditions will be assessed.

Analyses will be conducted in the area offices of ADF&G following each survey using microcomputers running R:base applications. Statistical analyses will be conducted on microcomputers using Stats Plus or other appropriate software by ADF&G. Groundfish and shellfish data will be analyzed by ADF&G.

SCHEDULES AND REPORTS

DATE	ACTIVITY
May-June 1989	Repeat 1978 PWS trawl survey
June 28-29, 1989	Joint planning session with ADF&G and NMFS, Auke Bay Lab, in Juneau
August-September, 1989	Fall Survey completed
September-December, 1989	Data entry and analysis
December 21, 1989	Preliminary report and data analysis due
February 28, 1989	Final Report

PROJECT BUDGET

Line item	category	budget ADF&G	budget NMFS	Totals
100	Personnel	127.3	125.0	252.3
200	Travel	13.0	5.0	15.0
300	Contracts	0.0	282.0	282.0
400	Commodities	7.0	37.5	44.5
500	Equipment	52.0	90.0	142.0
		199.3	539.5	738.8

PERSONNEL

Alaska Department of Fish and Game

Name	Class	PCN	PFT_mm	SFT_mm	Cost
Dan Urban	FB II	11-	12		58.0
Dan Coyer	FT II	N-197		6	16.0

National Marine Fisheries Service

Name	Grade	months	Total cost
Haynes	13	8.0	66.4
Haight	11	2.0	14.0
Rutecki	9	2.0	11.4
Wing	12	2.0	15.4
Karinen	13	0.2	1.7
Sigler	9	0.2	1.1
Heifetz	11	0.2	1.4
Kamikawa	5	2.0	6.8
Derrah	5	2.0	6.8

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APPENDIX A.1

MAY/JUNE, 1989

MULTISPECIES TRAWL DESIGN

APPENDIX A.1
SURVEY DESIGN AND STATION DEFINITION
FOR THE MAY-JUNE 1989 MULTISPECIES TRAWL SURVEY

The May-June leg of the 1989 multispecies trawl survey would repeat the survey conducted in Prince William Sound in 1978. The survey design for the 1978 multispecies trawl survey (Parks and Zenger 1979) was a stratified random sample. Prince William Sound was divided into four quadrants (Figure B.2). The area within each quadrant was divided into depth intervals (10-50, 51-100, 101-150, 151-200, and 201-600 fm) called strata. Each depth stratum was then divided into 25 square mile areas designated as stations and randomly chosen for sampling.

For the May-June cruise in 1989, the 63 of the 70 stations fished in 1978 will again be sampled (Figure B.1). These consist of 54 stations located within Prince William Sound (Figure B.2) and 9 located outside Montague Island (Montague Trench area) and lower Montague passage (south of 60° N). Additional stations (about 30) will also be defined to include the northern Knight Island Passage, Port Wells, the southwestern passages and Bays, Port Bainbridge, and the Montague Trench area. New stations are chosen from a list of existing stations previously trawled in Prince William Sound to insure broad area and depth coverage. Poststratification of stations by depth within larger geographic strata may be attempted.

For each station, the vessel first would go to the station center and search for 20 to 30 minutes for trawlable bottom. If no trawlable bottom was found, the vessel would proceed to the location of the nearest successful haul. At the location of the nearest successful haul, the vessel would again search 20 to 30 minutes for trawlable bottom and presumably trawlable bottom would be found. If trawlable bottom is found while travelling from the station location to the location of the nearest successful haul, a haul would be made. If no trawlable bottom was found at the station location, enroute to the location of the nearest successful haul, or at the location of the nearest successful haul, the vessel would move to the next station.

Data on distribution and abundance of species collected on the May-June survey will be used to select strata and sampling intensity for the August survey, which will be a stratified random sample.

FIGURE A.1

1978 NMFS TRAWL SURVEY

STN. NO.	GEAR	DATE	LATITUDE	LONGITUDE	LORAN		DEPTH RANGE FATHOMS	HAUL TIME HRS.	REMARKS	CATCH IN POUNDS TOTAL	SPECIES 1	SPECIES 2	SPECIES 3	SPECIES 4
1	OTE	5/13/78	S 60° 38.1' E 60° 37.6'	145° 50.7' 145° 52.7'	-- --	-- --	16-39	0.5	Clear	2,237	Yellowfin sole 663	Pollock 499	Starry flounder 373	Tanner crab 186
2	"	5/14/78	S 60° 36.7' E 60° 36.7'	146° 37.1' 146° 35.0'	-- --	-- --	65	"	"	1,050	Pollock 511	Tanner crab 306	Flathead sole 115	Arrowtooth flounder 25
3	"	"	S 60° 35.6' E 60° 35.5'	146° 31.0' 146° 31.4'	-- --	-- --	237-243	"	"	1,200	Eulachon 446	Tanner crab 410	Pollock 107	Arrowtooth flounder 83
4	"	"	S 60° 28.8' E 60° 29.8'	146° 33.3' 146° 32.0'	-- --	-- --	223-225	"	"	1,250	Halibut 458	Arrowtooth flounder 260	Heart urchins 208	Pollock 109
5	"	"	S 60° 23.6' E 60° 24.6'	146° 50.2' 146° 48.8'	-- --	-- --	131-137	"	"	922	Skates 310	Arrowtooth flounder 263	Tanner crab 172	Halibut 58
6	"	5/15/78	S 60° 17.0' E 60° 18.2'	146° 45.0' 146° 45.8'	-- --	-- --	104-120	"	"	1,285	Tanner crab 806	Skates 126	Arrowtooth flounder 71	Whelks 45
7	"	"	S 60° 21.4' E 60° 22.5'	146° 47.8' 146° 48.1'	-- --	-- --	133-136	"	"	1,977	Skates 1,309	Tanner crab 363	Arrowtooth flounder 124	Pollock 78
8	"	"	S 60° 25.0' E 60° 25.2'	146° 45.3' 146° 47.8'	-- --	-- --	128-153	"	"	1,022	Skates 306	Tanner crab 270	Arrowtooth flounder 233	Flathead sole 51
9	"	5/16/78	S 60° 28.5' E 60° 27.3'	146° 41.6' 146° 42.2'	-- --	-- --	93-99	"	"	458	Tanner crab 92	Pollock 73	Flathead sole 58	Halibut 52
10	"	"	S 60° 30.5' E 60° 29.6'	146° 45.9' 146° 47.4'	-- --	-- --	193-198	"	"	552	Arrowtooth flounder 126	Halibut 95	Pollock 91	Skates 82
11	"	"	S 60° 31.2' E 60° 30.3'	146° 36.6' 146° 38.1'	-- --	-- --	70-84	"	"	821	Tanner crab 562	Flathead sole 115	Pollock 76	Starfish 55
12	"	"	S 60° 35.7' E 60° 35.6'	146° 37.4' 146° 36.9'	-- --	-- --	61-66	"	"	283	Tanner crab 108	Pollock 77	Flathead sole 63	Arrowtooth flounder 12
13	"	"	S 60° 35.4' E 60° 35.6'	146° 16.8' 146° 19.1'	-- --	-- --	65-66	"	"	272	Tanner crab 92	Pollock 59	Flathead sole 50	Rex sole 23
14	"	5/17/78	S 60° 30.6' E 60° 31.9'	146° 26.2' 146° 24.6'	-- --	-- --	43-50	"	"	2,010	Pollock 1,516	Sculpins 95	Dungeness crab 83	Flathead sole 82
15	"	"	S 60° 33.9' E 60° 34.0'	146° 13.4' 146° 15.5'	-- --	-- --	71-73	"	"	228	Flathead sole 55	Tanner crab 54	Octopus 37	Pollock 24
16	"	"	S 60° 25.7' E 60° 26.7'	147° 01.0' 147° 01.8'	-- --	-- --	111-118	"	"	197	Tanner crab 56	Pollock 45	Flathead sole 27	Arrowtooth flounder 21
17	"	"	S 60° 25.1' E 60° 25.1'	147° 06.3' 147° 03.8'	-- --	-- --	87-92	"	"	175	Pollock 45	Tanner crab 44	Flathead sole 30	Arrowtooth flounder 27
18	"	5/18/78	S 60° 37.2' E 60° 37.8'	147° 15.7' 147° 13.8'	-- --	-- --	122-124	"	"	206	Tanner crab 89	Starfish 31	Flathead sole 20	Pollock 17
19	"	"	S 60° 36.9' E 60° 36.5'	147° 10.8' 147° 08.4'	-- --	-- --	121-134	"	"	226	Tanner crab 92	Skates 24	Pollock 23	Halibut 19
20	"	"	S 60° 37.5' E 60° 36.8'	147° 08.4' 147° 06.5'	-- --	-- --	150-160	"	"	231	Tanner crab 105	Pollock 46	Halibut 20	Sculpins 17
21	"	5/19/78	S 60° 47.5' E 60° 47.3'	147° 16.0' 147° 13.5'	-- --	-- --	193-196	"	"	445	Tanner crab 175	Pollock 104	Dogfish 38	Skates 37
22	"	"	S 60° 47.5' E 60° 46.4'	147° 02.2' 147° 02.7'	-- --	-- --	230-238	"	"	1,264	Tanner crab 405	Dogfish 315	Eulachon 106	Pollock 88
23	"	"	S 60° 43.5' E 60° 44.7'	146° 59.0' 146° 59.0'	-- --	-- --	241	"	"	1,194	Tanner crab 418	Eulachon 266	Pollock 119	Arrowtooth flounder 103
24	"	5/20/78	S 60° 43.4' E 60° 42.3'	146° 51.4' 146° 51.7'	-- --	-- --	228-235	"	"	1,077	Tanner crab 373	Arrowtooth flounder 139	Dogfish 103	Halibut 98
25	"	"	S 60° 38.2' E 60° 39.3'	146° 52.0' 146° 51.7'	-- --	-- --	241-245	"	"	365	Dogfish 109	Halibut 69	Arrowtooth flounder 65	Tanner crab 40
26	"	"	S 60° 44.2' E 60° 45.2'	146° 47.8' 146° 48.7'	-- --	-- --	116-117	"	"	383	Tanner crab 141	Arrowtooth flounder 54	Pollock 36	Halibut 24
27	"	"	S 60° 46.4' E 60° 45.2'	146° 44.5' 146° 45.7'	-- --	-- --	89-96	"	"	359	Tanner crab 151	Arrowtooth flounder 63	Sculpins 63	Pollock 62
28	"	5/21/78	S 60° 37.3' E 60° 47.4'	146° 38.1' 146° 40.6'	-- --	-- --	84-86	"	"	892	King crab 487	Tanner crab 94	Arrowtooth flounder 68	Pollock 55

FIGURE A.1 (pg 1 of 3)

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STN. NO.	GEAR	DATE	LATITUDE	LONGITUDE	LORAN		DEPTH RANGE FATHOMS	HAUL TIME HRS.	REMARKS	CATCH IN POUNDS TOTAL	SPECIES 1	SPECIES 2	SPECIES 3	SPECIES 4
29	OTR	6/21/78	S 60° 48.5' E 60° 48.2'	166° 22.3' 166° 25.6'	-- --	-- --	108-110	0.5	Clear	323	Tanner crab 151	Flathead sole 38	Pollock 33	Arrowtooth flounder 29
30	"	"	S 60° 51.6' E 60° 51.4'	166° 13.1' 166° 13.5'	-- --	-- --	69	0.1	Mudded down	-0-	--	--	--	--
31	"	"	S 60° 51.1' E 60° 50.6'	166° 13.7' 166° 16.0'	-- --	-- --	76-90	0.5	Clear	791	Pollock 483	Tanner crab 101	Halibut 47	King crab 46
32	"	6/22/78	S 60° 45.3' E 60° 45.1'	166° 19.2' 166° 19.2'	-- --	-- --	17-19	0.1	Snag tore net	283	Starry flounder 112	Sculpins 61	Yellowfin sole 31	Rock sole 16
33	"	6/23/78	S 60° 40.9' E 60° 41.5'	166° 18.0' 166° 15.8'	-- --	-- --	66-73	0.5	Clear	540	Pollock 288	Flathead sole 60	Tanner crab 39	True cod 24
34	"	"	S 60° 42.5' E 60° 42.5'	166° 19.5' 166° 19.3'	-- --	-- --	30	"	Hung up	38	Tanner crab 16	Sculpins trace	Flathead sole trace	Pollock trace
35	"	"	S 60° 38.6' E 60° 38.9'	166° 23.7' 166° 21.5'	-- --	-- --	67-69	"	Clear	361	Pollock 81	Skates 75	Tanner crab 49	Flathead sole 39
36	"	"	S 60° 37.7' E 60° 37.6'	166° 31.3' 166° 28.8'	-- --	-- --	52-58	"	"	1,550	Pollock 1,261	Flathead sole 117	Tanner crab 59	Arrowtooth flounder 43
37	"	"	S 60° 38.6' E 60° 37.9'	166° 25.3' 166° 27.6'	-- --	-- --	66-67	"	"	1,450	Pollock 1,183	Flathead sole 109	Tanner crab 48	Sculpins 27
38	"	6/24/78	S 60° 17.3' E 60° 18.2'	167° 18.0' 167° 16.8'	-- --	-- --	65-73	"	"	593	Flathead sole 303	Tanner crab 95	Halibut 79	Pollock 31
39	"	"	S 60° 23.1' E 60° 22.1'	167° 12.7' 167° 13.8'	-- --	-- --	58-60	"	"	1,450	Pollock 1,215	Tanner crab 102	Flathead sole 56	King crab 42
40	"	"	S 60° 23.8' E 60° 22.6'	167° 31.9' 167° 32.6'	-- --	-- --	91-93	"	"	289	Pollock 226	Tanner crab 25	Pink shrimp 16	Flathead sole 13
41	"	6/25/78	S 60° 12.9' E 60° 13.9'	167° 40.0' 167° 39.3'	-- --	-- --	139	"	"	200	Flathead sole 73	Tanner crab 58	Pollock 30	Starfish 16
42	"	"	S 60° 10.0' E 60° 08.8'	167° 37.7' 167° 38.5'	-- --	-- --	73-75	"	Clear	367	Flathead sole 122	Tanner crab 93	Pollock 53	Starfish 46
43	"	"	S 60° 06.1' E 60° 05.2'	167° 34.7' 167° 36.3'	-- --	-- --	66-71	"	"	400	Skates 296	Flathead sole 32	Tanner crab 25	True cod 12
44	"	"	S 60° 07.9' E 60° 06.7'	167° 43.1' 167° 43.9'	-- --	-- --	135-139	"	"	253	Tanner crab 96	Flathead sole 53	Pollock 45	Arrowtooth flounder 23
45	"	6/26/78	S 60° 11.5' E 60° 12.5'	167° 56.6' 167° 55.8'	-- --	-- --	226-231	"	"	927	Eulachon 503	Tanner crab 175	Pollock 130	Flathead sole 46
46	"	"	S 60° 08.9' E 60° 09.9'	167° 53.7' 167° 56.7'	-- --	-- --	149-157	"	Hung up	62	Tanner crab 26	Flathead sole 11	Pollock trace	Eulachon trace
47	"	"	S 60° 01.9' E 60° 03.0'	167° 46.5' 167° 45.3'	-- --	-- --	151-158	"	Clear	208	Arrowtooth flounder 50	Skates 36	Tanner crab 35	Halibut 34
48	"	"	S 60° 01.0' E 59° 59.9'	167° 49.6' 167° 50.3'	-- --	-- --	165-166	"	"	436	Skates 126	(golden) King crab 80	Tanner crab 51	Arrowtooth flounder 48
49	"	6/27/78	S 59° 57.0' E 59° 56.5'	167° 42.2' 167° 49.7'	-- --	-- --	89-96	"	"	178	Halibut 41	True cod 36	Tanner crab 20	Arrowtooth flounder 19
50	"	"	S 60° 13.7' E 60° 16.5'	168° 06.6' 168° 06.1'	-- --	-- --	256	"	"	218	Tanner crab 60	Pollock 48	Sculpins 32	Halibut 16
51	"	6/28/78	S 60° 37.3' E 60° 37.0'	167° 58.6' 167° 58.3'	-- --	-- --	256-264	0.3	Mudded down	43	Tanner crab 30	Skates trace	Squid trace	Dungeness crab trace
52	"	"	S 60° 28.0' E 60° 29.3'	168° 15.1' 168° 15.2'	-- --	-- --	96-103	0.5	Clear	232	Dogfish 137	Pollock 20	Tanner crab 11	Flathead sole trace
53	"	6/29/78	S 60° 43.8' E 60° 42.6'	168° 04.0' 168° 04.0'	-- --	-- --	232-235	"	"	393	Tanner crab 212	Skates 32	Sablefish 21	Arrowtooth flounder 18
54	"	"	S 60° 44.1' E 60° 43.0'	167° 51.8' 167° 50.7'	-- --	-- --	251-265	"	Mud and rocks	186	Tanner crab 103	Sculpins 55	Skates trace	Pollock trace
55	"	"	S 60° 47.6' E 60° 46.9'	167° 24.6' 167° 26.8'	-- --	-- --	240-249	"	Clear	560	Tanner crab 263	Heart urchins 70	Skates 59	Pollock 31
56	"	6/30/78	S 60° 46.9' E 60° 47.0'	167° 20.2' 167° 22.6'	-- --	-- --	229-238	"	"	420	Tanner crab 256	Arrowtooth flounder 41	Pollock 35	Skates 34

URE A.1 (pg 2 of 3)

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STN. NO.	GEAR	DATE		LATITUDE	LONGITUDE	LORAN		DEPTH RANGE FATHOMS	HAUL TIME HRS.	REMARKS	CATCH IN POUNDS TOTAL	SPECIES 1		SPECIES 2		SPECIES 3		SPECIES 4	
57	OTE	4/30/78	S E	60° 50.9' 60° 49.8'	167° 30.9' 167° 31.5'	-- --	-- --	227-229	0.5	Clear	679	Tanner crab	418	Pollock	81	Skates	52	Halibut	46
58	"	"	S E	60° 48.2' 60° 48.2'	167° 16.4' 167° 18.6'	-- --	-- --	208-209	"	"	651	Tanner crab	197	Skates	66	Arrowtooth flounder	39	Dogfish	38
59	"	5/2/78	S E	60° 08.9' 60° 08.5'	166° 46.5' 166° 48.7'	-- --	-- --	66	"	"	2,340	Pollock	2,090	Tanner crab	80	Flathead sole	70	Arrowtooth flounder	65
60	"	"	S E	60° 12.6' 60° 12.1'	166° 29.6' 166° 31.8'	-- --	-- --	73	"	"	262	Flathead sole	104	Tanner crab	54	Pollock	29	Arrowtooth flounder	21
61	"	5/3/78	S E	60° 11.6' 60° 11.7'	166° 22.7' 166° 25.2'	-- --	-- --	60	"	"	899	Flathead sole	158	Tanner crab	86	Arrowtooth flounder	55	Halibut	50
62	"	"	S E	59° 58.6' 59° 58.3'	166° 59.2' 167° 01.5'	-- --	-- --	86	"	"	824	Pollock	355	Flathead sole	224	Tanner crab	127	Arrowtooth flounder	102
63	"	"	S E	60° 00.3' 59° 59.3'	166° 52.0' 166° 52.6'	-- --	-- --	76	"	"	1,483	Pollock	916	Arrowtooth flounder	147	Flathead sole	138	Halibut	135
64	"	"	S E	59° 50.7' 59° 52.1'	167° 13.6' 167° 12.1'	-- --	-- --	76	"	"	1,002	Pollock	637	Tanner crab	190	Flathead sole	70	Arrowtooth flounder	43
65	"	5/4/78	S E	59° 39.8' 59° 40.8'	168° 16.2' 168° 17.8'	-- --	-- --	100	"	"	755	Pollock	296	Arrowtooth flounder	183	Flathead sole	109	Pacific cod	91
66	"	"	S E	59° 49.8' 59° 50.1'	168° 22.9' 168° 23.5'	-- --	-- --	73	0.2	Obstruction	1,170	Pollock	969	Tanner crab	77	Pacific cod	55	Flathead sole	38
67	"	"	S E	59° 53.2' 59° 52.5'	168° 26.8' 168° 25.3'	-- --	-- --	88	0.5	Clear	256	Pollock	56	Halibut	50	Tanner crab	50	Arrowtooth flounder	42
68	"	"	S E	59° 53.0' 59° 56.8'	168° 25.3' 168° 25.2'	-- --	-- --	86	0.8	"	666	Tanner crab	136	Halibut	102	Pollock	77	Flathead sole	31
69	"	5/5/78	S E	60° 40.2' 60° 39.4'	166° 18.3' 166° 20.1'	-- --	-- --	69	0.5	"	539	Pollock	282	Tanner crab	156	Flathead sole	42	Pacific cod	20
70	"	"	S E	60° 41.6' 60° 40.9'	166° 15.2' 166° 17.8'	-- --	-- --	72	"	"	306	Pollock	129	Tanner crab	53	Flathead sole	48	Pacific cod	32
		END																	
3	PGS	ONLY																	

OREGON 78-1

APPENDIX A.2

FIGURE A.2

OILED AND NON-OILED SITES

APPENDIX A.2

Tow Number and location for the 1978 survey by oil contamination area. The following 8 sites are defined by Figure A.2 and include the following hauls conducted in 1978.

I. Oiled areas:

1. Naked/Glacier - Tow # 21, 22, 55, 56, 58
2. North Knight Is. - Tow # (not surveyed in 1978)
3. Upper Montague - Tow # 16-18, 39, 40
4. Lower Montague - Tow 47-49 and areas not surveyed
5. If no trawlable stations are found in North Knight Is. Passage, Green Island will be substituted - Tow # 38, 41, 42, 44

II. Unoiled areas:

1. Orca Bay - Tows # 2, 12, 13, 15, 35-37
2. Port Fidalgo - Tows # 26-28
3. Montague Trench - Tow # (not surveyed in 1978)
4. Port Wells - Tow # 53

A new station pattern has been defined for PWS and will include stations for the North Knight Is, Lower Montague, Montague Trench, and Port Wells area.

Figure 1.--Prince William Sound showing all drags made during the groundfish survey by NOAA research vessel Oregon during April 1978, and the quadrant division of the Sound (dotted lines).

FIGURE A.2

APPENDIX B

AUGUST/SEPTEMBER, 1989

TRAWL SITE SELECTION

APPENDIX B.1
SURVEY DESIGN AND STATION DEFINITION
FOR THE AUGUST 1989 MULTISPECIES TRAWL SURVEY

Prince William Sound was divided into geographic areas based on the May-June 1989 trawl survey results, groundfish and shellfish commercial catch distributions, and Tanner crab pot survey results (Brannian 1989). Depth strata were defined based on the 1978 PWS trawl survey (Parks and Zenger 1979). Bottom surface area measurements for the depth-area strata were made in square nautical miles (Appendix B.2). Each depth-area stratum was then divided into a grid of stations each representing 1.56 square nautical miles. Potentially, a trawl haul could be located at the center of each station. A total of 75 tows were optimally allocated to depth-area strata based on the standard deviation of each of the 13 stratum (Appendix B.3). A compromise survey design was established after evaluating the optimal allocation of effort using Tanner crab, flathead sole, Pacific cod, sablefish, and sidestripe shrimp CPUE. It was assumed that cost of sampling among strata was equal.

Appendix B.3 presents the number of trawl tows to be conducted in each depth-area stratum. These will be randomly chosen from the total available stations in each depth-area stratum. Tows which could be dropped or added have also been prioritized (Appendix B.4). Appendix B.5 presents the location of the 75 priority tows by depth-area stratum and 8 additional tows.

The following procedure will be followed in conducting a tow in a chosen station area. For each station area, the vessel first goes to the station area center and searches for 20 to 30 minutes for trawlable bottom. If no trawlable bottom is found, the vessel proceeds to the location of the nearest successful haul (a haul without a tear-up during a prior survey). At the location of the nearest successful haul, the vessel again searches 20 to 30 minutes for trawlable bottom and presumably trawlable bottom is found. If trawlable bottom is found while travelling from the station area center to the location of the nearest successful haul, a haul is made. If no trawlable bottom is found at the station area, enroute to the nearest successful haul, or at the location of the nearest successful haul, the vessel transits to the next station area and repeats the searching procedure.

Appendix B.2. Bottom surface area measurements for depth-area strata developed for the multispecies trawl survey in Prince William Sound August, 1989.

Depth Strata (fm)	Hinchin-brook	Orca-Fidalgo	Central Basin	Montague-Knight Is	Port Wells	Outside	Total
	Geographic Areas (Square Nautical Miles)						
10 - 50	0.0	111.5	0.0	0.0	0.0	0.0	111.5
51 - 100	89.6	175.0	0.0	359.3	0.0	117.4	741.2
101 - 200	128.5	0.0	161.0	155.4	57.4	191.3	693.6
201 - 400	0.0	0.0	309.0	21.6	48.5	0.0	379.1
Total	218.1	286.5	470.0	536.2	105.9	308.7	1925.4

Depth Strata (fm)	Hinchin-brook	Orca-Fidalgo	Central Basin	Montague-Knight Is	Port Wells	Outside	Total
	Geographic Areas (Percent of Total Area)						
10 - 50	0.0	5.8	0.0	0.0	0.0	0.0	5.8
51 - 100	4.7	9.1	0.0	18.7	0.0	6.1	38.5
101 - 200	6.7	0.0	8.4	8.1	3.0	9.9	36.0
201 - 400	0.0	0.0	16.0	1.1	2.5	0.0	
Total	11.3	14.9	24.4	27.9	5.5	16.0	100.0

APPENDIX B.3

Proposed number of trawl tows by depth-area stratum for the multispecies trawl survey in Prince William Sound August, 1989.

depth/strata (fm)	Geographic Areas						
	Hinchin-brook	Orca-Fidalgo	Central Basin	Montague-Knight Is.	Port Wells	Outside	Total
10 - 50		3					3
51 - 100	7	8		11		3	29
101 - 200	6		4	10	3	3	26
201 - 400			11	3	3		17
Total	13	11	15	24	6	6	75

APPENDIX B.4

A list of tows that can be added or dropped by Depth-Area strata for the multispecies trawl survey in Prince William Sound, August 1989.

Tows that could be dropped in case of inclement weather:

1. Drop 3 tows at >200 fm in the Montague Strait & Knight Island Area
2. Drop 1 tow at >200 fm in the Central Basin Area
3. Drop up to 2 tows at 51-100 fm in the Montague Strait & Knight Island Area

Tows that can be added if additional time becomes available

1. Add 1 tow at 51-100 fm in the Outside Area
2. Add up to 2 tows at 101-200 fm in the Outside Area (Montague Trench)
3. Add 1 tow at 51-100 fm in the Hinchinbrook Area
4. Add up to 2 tows at 51-100 fm in the Orca Bay and Port Fidalgo Area
5. Add up to 2 tows at 101-200 fm in the Central Basin Area

Appendix B.5. Location of proposed trawl tows for the August 1989 Prince William Sound multispecies trawl survey.

Depth	Area	Latitude	Longitude	Station Number
51-100	Outside	1. 59.44.00	147.38.30	62024
		2. 59.40.38	147.43.45	62058
		3. 59.44.15	147.11.45	62044
101-200	Outside	4. 59.49.22	147.03.45	63089
		5. 59.43.07	147.01.15	63110
		6. 60.08.07	147.03.45	63026
51-100	Montague/ Knight I.	7. 59.45.37	148.21.15	42190
		8. 59.54.30	148.25.30	42104
		9. 59.50.38	148.21.30	42138
		10. 59.46.52	148.26.15	42176
		11. 60.08.07	147.45.10	42055
		12. 59.52.45	148.16.20	42117
		13. 59.54.30	147.51.15	42110
		14. 60.00.40	148.10.35	42079
		15. 59.51.55	148.13.40	42130
		16. 60.25.37	147.26.45	42020
		17. 59.44.22	148.26.15	42199
101-200	Montague/ Knight I.	18. 60.05.37	147.48.45	43069
		19. 60.34.25	147.30.25	43004
		20. 60.21.25	147.48.20	43019
		21. 59.59.30	147.44.30	43094
		22. 60.36.30	147.33.45	43001
		23. 60.08.07	147.51.15	43059
		24. 59.59.40	147.50.30	43091
		25. 60.12.45	148.00.00	43038
		26. 60.09.22	147.53.45	43052
		27. 60.17.45	147.33.45	43026
201-400	Montague/ Knight I.	28. 60.18.07	147.58.45	44003
		29. 60.13.10	148.03.00	44009
		30. 60.13.45	147.58.35	44008
51-100	Hinchen-brook	31. 60.28.25	147.16.40	12016
		32. 60.18.07	147.17.30	12053
		33. 60.24.22	147.13.45	12033
		34. 60.23.07	147.16.15	12040
		35. 60.24.22	147.18.45	12031
		36. 60.18.07	146.52.50	12051
		37. 60.25.37	147.16.15	12025
101-200	Hinchen-brook	38. 60.12.15	146.46.30	13081
		39. 60.29.22	147.03.45	13004

Appendix B.5 (continued)

Depth	Area	Latitude	Longitude	Station number
101-200	Hinchen-brook	40. 60.25.30	147.00.45	13027
		41. 60.27.05	146.50.50	13023
		42. 60.24.22	146.48.45	13039
		43. 60.29.15	147.16.10	13001
51-100	Orca/ Fidalgo	44. 60.39.22	146.38.45	21020
		45. 60.29.25	146.34.25	21066
		46. 60.36.52	146.18.45	21042
51-100	Orca/ Fidalgo	47. 60.30.30	146.33.45	22096
		48. 60.34.30	146.33.45	22059
		49. 60.28.07	146.40.30	22099
		50. 60.41.52	146.11.15	22022
		51. 60.33.07	146.41.05	22072
		52. 60.29.35	146.38.00	22098
		53. 60.46.52	146.41.15	22015
		54. 60.33.07	146.35.15	22074
101-200	Central Basin	55. 60.39.22	147.08.45	33044
		56. 60.43.07	147.11.15	33029
		57. 60.34.22	147.08.45	33080
		58. 60.49.22	147.01.15	33009
201-400	Central Basin	59. 60.39.22	146.53.45	34098
		60. 60.44.22	147.06.15	34063
		61. 60.36.52	147.58.45	34114
		62. 60.41.52	146.58.45	34083
		63. 60.28.00	146.51.45	34183
		64. 60.35.37	147.56.15	34127
		65. 60.45.37	147.41.50	34042
		66. 60.44.22	147.01.15	34065
		67. 60.43.07	147.33.45	34070
		68. 60.50.30	147.31.55	34001
		69. 60.46.52	147.01.15	34040
101-200	Pt.Wells	70. 60.50.00	148.15.45	53017
		71. 60.58.07	148.03.45	53005
		72. 60.46.52	148.00.25	53024
201-400	Pt.Wells	73. 60.46.30	148.08.30	54018
		74. 60.49.22	148.13.50	54011
		75. 60.45.37	148.02.30	54019

APPENDIX B.6

Table. Allocation of effort among individual depth-area strata to minimize the variance an estimate of Tanner Crab abundance with 1989 area measurements. a
15-Jul-89 12:57 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	12.3	111.5	0	1	0	0	0	0
51 - 100	190.2	741.2	15	11	0	6	0	5
101 - 200	124.8	693.6	7	0	7	15	1	0
201 - 400	37.5	379.1	0	0	7	0	1	0
Total		1,925.4	22	12	14	21	1	6

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance an estimate of Tanner Crab abundance with 1989 area measurements. a
15-Jul-89 12:58 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	12.3	111.5	0	0	0	0	0	0
51 - 100	190.2	741.2	7	5	0	3	0	3
101 - 200	124.8	693.6	3	0	3	7	0	0
201 - 400	37.5	379.1	0	0	3	0	0	0
Total		1,925.4	11	6	7	10	1	3

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance an estimate of Flathead Sole abundance with 1989 area measurements. a
15-Jul-89 12:07 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	116.0	111.5	0	4	0	0	0	0
51 - 100	293.6	741.2	5	6	0	31	0	1
101 - 200	260.0	693.6	4	0	1	14	0	5
201 - 400	46.7	379.1	0	0	4	0	0	0
Total		1,925.4	10	10	5	45	0	6

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance an estimate of Flathead Sole abundance with 1989 area measurements. a
15-Jul-89 12:08 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	116.0	111.5	0	2	0	0	0	0
51 - 100	293.6	741.2	3	3	0	15	0	0
101 - 200	260.0	693.6	2	0	0	6	0	3
201 - 400	46.7	379.1	0	0	2	0	0	0
Total		1,925.4	5	5	2	22	0	3

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance f
an estimate of Pacific Cod abundance with 1989 area measurements. a
15-Jul-89 12:17 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	65.6	111.5	0	0	0	0	0	0
51 - 100	89.8	741.2	7	11	0	15	0	3
101 - 200	135.7	693.6	6	0	2	4	0	18
201 - 400	42.5	379.1	0	0	10	0	0	0
Total		1,925.4	12	11	12	19	0	21

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance
an estimate of Pacific Cod abundance with 1989 area measurements. a
15-Jul-89 12:17 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	65.6	111.5	0	0	0	0	0	0
51 - 100	89.8	741.2	3	5	0	7	0	1
101 - 200	135.7	693.6	3	0	1	2	0	9
201 - 400	42.5	379.1	0	0	5	0	0	0
Total		1,925.4	6	5	6	9	0	10

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance an estimate of Sablefish abundance with 1989 area measurements. a
15-Jul-89 01:03 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	28.6	111.5	0	0	0	0	0	0
51 - 100	26.9	741.2	2	2	0	3	0	2
101 - 200	223.2	693.6	11	0	1	14	2	22
201 - 400	123.1	379.1	0	0	16	0	2	0
Total		1,925.4	12	2	16	17	4	24

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance an estimate of Sablefish abundance with 1989 area measurements. a
15-Jul-89 01:03 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	28.6	111.5	0	0	0	0	0	0
51 - 100	26.9	741.2	1	1	0	1	0	1
101 - 200	223.2	693.6	5	0	0	7	1	10
201 - 400	123.1	379.1	0	0	8	0	1	0
Total		1,925.4	6	1	8	8	2	11

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance an estimate of Sidestripe Shrimp abundance with 1989 area measurements. a
15-Jul-89 01:06 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	0.2	111.5	0	0	0	0	0	0
51 - 100	13.3	741.2	4	3	0	5	0	0
101 - 200	36.7	693.6	1	0	5	12	3	1
201 - 400	78.2	379.1	0	0	39	0	3	0
Total		1,925.4	5	3	43	18	6	1

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance an estimate of Sidestripe Shrimp abundance with 1989 area measurements. a
15-Jul-89 01:07 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	0.2	111.5	0	0	0	0	0	0
51 - 100	13.3	741.2	2	1	0	3	0	0
101 - 200	36.7	693.6	0	0	2	6	2	0
201 - 400	78.2	379.1	0	0	19	0	1	0
Total		1,925.4	2	1	21	8	3	0

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance an estimate of Arrowtooth Floundeabundance with 1989 area measurements. a
15-Jul-89 12:19 PM

Depth Strata (fm)	Depth SD	Depth Area	Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
			Sample Size by Depth-Area Strata					
10 - 50	164.5	111.5	0	1	0	0	0	0
51 - 100	2078.5	741.2	1	2	0	47	0	5
101 - 200	810.2	693.6	1	0	1	7	0	1
201 - 400	505.8	379.1	0	0	8	0	0	0
Total		1,925.4	2	3	9	55	0	6

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance an estimate of Arrowtooth Floundeabundance with 1989 area measurements. a
15-Jul-89 12:21 PM

Depth Strata (fm)	Depth SD	Depth Area	Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
			Sample Size by Depth-Area Strata					
10 - 50	164.5	111.5	0	0	0	0	0	0
51 - 100	2078.5	741.2	1	1	0	23	0	2
101 - 200	810.2	693.6	1	0	0	4	0	1
201 - 400	505.8	379.1	0	0	4	0	0	0
Total		1,925.4	1	2	4	26	0	3

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance f
an estimate of Halibut abundance with 1989 area measurements. a
15-Jul-89 01:00 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	128.9	111.5	0	4	0	0	0	0
51 - 100	192.8	741.2	3	5	0	16	0	12
101 - 200	234.9	693.6	3	0	3	14	2	1
201 - 400	153.7	379.1	0	0	11	0	1	0
Total		1,925.4	6	9	14	30	3	14

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance
an estimate of Halibut abundance with 1989 area measurements. a
15-Jul-89 01:01 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	128.9	111.5	0	2	0	0	0	0
51 - 100	192.8	741.2	1	2	0	8	0	6
101 - 200	234.9	693.6	2	0	1	7	1	1
201 - 400	153.7	379.1	0	0	5	0	1	0
Total		1,925.4	3	4	7	14	2	7

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance an estimate of Rougheye Rockfish abundance with 1989 area measurements. a
15-Jul-89 01:04 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	6.4	111.5	0	1	0	0	0	0
51 - 100	70.0	741.2	2	13	0	20	0	0
101 - 200	106.4	693.6	5	0	28	2	1	1
201 - 400	13.7	379.1	0	0	1	0	1	0
Total		1,925.4	7	13	28	23	3	1

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance an estimate of Rougheye Rockfish abundance with 1989 area measurements. a
15-Jul-89 01:05 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	6.4	111.5	0	0	0	0	0	0
51 - 100	70.0	741.2	1	6	0	10	0	0
101 - 200	106.4	693.6	2	0	13	1	1	0
201 - 400	13.7	379.1	0	0	0	0	1	0
Total		1,925.4	3	6	13	11	1	1

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance f
an estimate of Shortracker Rockfiabundance with 1989 area measurements. a
15-Jul-89 01:05 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	0.0	111.5	0	0	0	0	0	0
51 - 100	0.0	741.2	0	0	0	0	0	0
101 - 200	5.5	693.6	0	0	4	3	4	0
201 - 400	73.7	379.1	0	0	60	0	4	0
Total		1,925.4	0	0	64	3	8	0

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance
an estimate of Shortracker Rockfiabundance with 1989 area measurements. a
15-Jul-89 01:06 PM

Depth Strata (fm)	Depth SD	Depth Area	Sample Size by Depth-Area Strata: Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
10 - 50	0.0	111.5	0	0	0	0	0	0
51 - 100	0.0	741.2	0	0	0	0	0	0
101 - 200	5.5	693.6	0	0	2	1	2	0
201 - 400	73.7	379.1	0	0	29	0	2	0
Total		1,925.4	0	0	31	1	4	0

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance an estimate of Pollock abundance with 1989 area measurements. a
15-Jul-89 12:24 PM

Depth Strata (fm)	Depth SD	Depth Area	Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
			Sample Size by Depth-Area Strata					
10 - 50	70.7	111.5	0	1	0	0	0	0
51 - 100	1009.9	741.2	2	2	0	34	0	32
101 - 200	56.1	693.6	1	0	1	1	0	1
201 - 400	59.2	379.1	0	0	2	0	0	0
Total		1,925.4	2	3	3	34	0	33

a Rounding errors may result in column and row totals not being correct.

Table. Allocation of effort among individual depth-area strata to minimize the variance an estimate of Pollock abundance with 1989 area measurements. a
15-Jul-89 12:28 PM

Depth Strata (fm)	Depth SD	Depth Area	Hichen-Brook	Orca-Fidalgo	Central Basin	Montague Knight	Port Wells	Outside
			Sample Size by Depth-Area Strata					
10 - 50	70.7	111.5	0	0	0	0	0	0
51 - 100	1009.9	741.2	1	1	0	16	0	15
101 - 200	56.1	693.6	0	0	0	0	0	1
201 - 400	59.2	379.1	0	0	1	0	0	0
Total		1,925.4	1	1	1	16	0	16

a Rounding errors may result in column and row totals not being correct.

le . A worksheet for estimating mean catch and its variance for the May-June trawl survey in PWS, 1989. 15-Jul-89 11:58 AM
*This table is for Tanner Crab — With 1989 area measurements

Tanner Crab Depth	Quadrants - Mean Catch 1	2	3	4	5	Total-Pooled
1		22.2				12.1
2	126.7	231.2	33.9	133.5	63.0	159.5
3	122.4	120.5	39.6	130.8	2.4	101.6
4	57.8		58.7			55.5
Total	100.9	156.7	51.6	-132.4	26.6	104.5

Tanner Crab Depth	Quadrants - Variance Catch 1	2	3	4	5	Total-Pooled
1		35.3				158.4
2	8,141.0	16,293.9	1,280.2	81,938.0	6,962.0	36,166.1
3	24,200.0	7,190.4	1,214.6	34,177.0	6.2	15,563.8
4	1,946.0		1,465.3			1,409.2
Total	7,431.1	15,011.3	1,318.8	57,267.3	2,845.3	28,010.9

Tanner Crab Depth	Area - Mean Catch 1	2	3	4	5	6	Total-Pooled
1		14.8					12.1
2	453.3	162.3		36.2		63.0	159.5
3	155.1		103.2	112.1		2.4	101.6
4			58.8		47.6		55.5
Total	300.1	128.2	72.7	71.6	47.6	26.6	104.5

Tanner Crab Depth	Area - Variance for Catch 1	2	3	4	5	6	Total-Pc
1.0		181.9					1
2.0	90,617.3	12,514.8		992.9		6,962.0	36.1
3.0	10,352.1		5,502.4	30,926.4		6.2	15.5
4.0			1,789.7		375.0		1.
Total	68,060.1	13,598.1	3,111.5	15,290.0	756.6	2,345.3	28.

Tanner Crab Depth	Quadrant- Statified mean catch- Weighted by stratum 1	2	3	4	5	Total
1		1.0				1.0
2	12.7	18.3	1.0	12.4	1.8	46.2
3	12.2	14.8	3.9	10.7	0.1	41.8
4	3.6		5.9			9.5
Stratified Mean Catch for PWS					97.6	97.6

Tanner Crab Depth	Quadrants - Variance weighed by stratum area 1	2	3	4	5	Total
1		0.0				0.0
2	16.4	14.6	0.6	88.5	2.8	122.9
3	120.4	15.6	3.9	38.4	0.0	178.4
4	1.9		1.5			3.4
Variance for the stratified mean catch					304.6	304.6
Relative Error						17.9%

Tanner Crab Depth	Area - Statified mean catch - Weighted by stratum 1	2	3	4	5	6	Total
1		0.9					0.9
2	23.0	14.7		6.7		3.8	48.3
3	10.3		8.6	10.3		0.2	29.5
4			5.4		1.2		10.6
Stratified Mean Catch for PWS					89.3		89.3

Tanner Crab Depth	Area - Variance Weighted by stratum area 1	2	3	4	5	6
1		0.2				
2	65.4	10.3		4.3		12.9
3	11.5		7.7	37.3		0.0
4			4.2		0.0	
Variance for the stratified mean catch					154.0	
Relative Error						

random sampling

. 6 worksheet for estimating mean catch and its variance for the May-June trawl survey in PWS, 1989. 15-Jul-89 12:06 PM

This table is for Flathead Sole — With 1989 area measurements

Flathead Sole Depth	Quadrants - Mean Catch 1	2	3	4	5	Total-Pooled
1		130.4				90.4
2	295.1	217.0	158.0	532.8	76.0	346.6
3	117.2	80.8	54.9	337.2	130.3	175.6
4	72.4		6.4			24.8
Total	181.8	146.6	36.3	449.0	108.6	192.1

Flathead Sole Depth	Quadrants - Variance Catch 1	2	3	4	5	Total-Pooled
1		30,159.7				13,453.9
2	52,726.7	20,082.2	42,632.0	141,342.7	648.0	86,218.3
3	27,471.7	16,316.5	1,663.3	166,047.7	14,899.4	67,580.3
4	6,367.5		35.2			2,184.9
Total	37,795.1	20,938.5	6,132.8	150,067.1	5,495.2	68,660.0

Flathead Sole Depth	Area - Mean Catch 1	2	3	4	5	6	Total-Pooled
1		55.2					90.4
2	292.1	287.8		504.2		76.0	346.6
3	129.6		14.1	303.5		130.3	175.6
4			29.2		0.5		24.8
Total	205.0	242.0	24.5	410.6	8.9	108.6	192.1

Flathead Sole Depth	Area - Variance for Catch 1	2	3	4	5	6	Total-Pooled
1.0		20,172.2					13,453.9
2.0	72,380.1	22,740.0		147,889.6		648.0	86,218.3
3.0	21,868.5		282.4	146,315.6		14,899.4	67,580.3
4.0			3,099.4		47.6		2,184.9
Total	41,875.1	28,003.5	2,193.8	147,401.4	38.6	5,495.2	68,660.0

Flathead Sole Quadrant- Statified mean catch- Weighted by stratum Depth	1	2	3	4	5	Total
1		6.0				6.0
2	25.6	17.2	4.7	49.5	2.2	103.2
3	11.7	10.0	5.4	27.7	7.4	62.2
4	4.5		0.6			5.1
Stratified Mean Catch for PWS					170.5	170.5

Flathead Sole Depth	Quadrants - Variance weighed by stratum area 1	2	3	4	5	Total
1		32.2				32.2
2	106.2	18.0	18.7	152.6	0.3	295.8
3	136.7	35.3	5.4	186.5	9.7	373.7
4	6.1		0.0			6.2
Variance for the stratified mean catch					707.8	707.8
Relative Error						15.5%

Flathead Sole Area - Statified mean catch - Weighted by stratum Depth	1	2	3	4	5	6	Total
1		5.2					5.2
2	13.5	26.2		94.1		4.6	138.5
3	9.3		1.2	27.9		12.9	51.3
4			4.7		0.2		4.9
Stratified Mean Catch for PWS					199.9		199.9
					192.1		

Flathead Sole Depth	Area - Variance Weighted by stratum area 1	2	3	4	5	6	To[tal]
1		22.5					[illegible]
2	52.6	18.8		643.6		1.2	[illegible]
3	24.3		0.4	176.6		49.0	[illegible]
4			7.3		0.0		
Variance for the stratified mean catch					996.4		
Relative Error							

Variance for mean catch from simple random sampling [illegible]

Relative Error

le . A worksheet for estimating mean-catch and its variance for the May-June trawl survey in PWS, 1989.
This table is for Pacific Cod

15-Jul-89 12:15 PM
With 1989 area measurements

Pacific Cod Depth	Quadrants - Mean Catch 1	2	3	4	5	Total-Pooled
1		4.6				35.8
2	112.7	111.8	51.0	71.1	106.0	95.0
3	41.4	68.3	51.1	61.6	379.5	112.6
4	42.3		5.4			15.7
Total	74.1	75.6	28.6	67.0	270.1	75.8

Pacific Cod Depth	Quadrants - Variance Catch 1	2	3	4	5	Total-Pooled
1		42.3				4,384.7
2	13,852.9	9,299.5	2,738.0	7,580.0	1,568.0	8,872.4
3	3.9	3,689.3	2,006.6	1,911.6	23,155.3	18,419.9
4	7,157.2		155.6			1,310.1
Total	9,852.8	6,532.8	1,877.2	4,797.3	34,404.8	10,650.9

Pacific Cod Depth	Area - Mean Catch 1	2	3	4	5	6	Total-Pooled
1		3.1					35.8
2	139.1	118.0		55.1		106.0	95.0
3	83.8		38.0	66.1		379.5	112.6
4			28.3		2.8		15.7
Total	107.5	91.4	25.8	60.3	2.8	270.1	75.8

Pacific Cod Depth	Area - Variance for Catch 1	2	3	4	5	6	Total-Pool
1.0		28.2					4,384.
2.0	13,770.6	9,397.7		4,248.8		1,568.0	8,872.
3.0	4,838.6		575.8	1,735.2		23,155.3	18,419.
4.0			2,577.9		0.0		1,310.
Total	7,882.2	9,591.5	1,943.7	2,980.5	39.2	34,404.8	10,650

Pacific Cod Depth	Quadrant- Statified mean catch- Weighted by stratum 1	2	3	4	5	Total
1		0.2				0.2
2	11.3	8.8	1.5	6.6	3.0	31.3
3	4.1	7.4	5.0	5.1	21.6	43.3
4	2.6		0.5			3.2
Stratified Mean Catch for PWS					77.7	77.7

Pacific Cod Depth	Quadrants - Variance weighed by stratum area 1	2	3	4	5	Total
1		0.0				0.0
2	27.9	8.3	1.2	8.1	0.6	46.2
3	0.0	7.8	6.5	2.1	15.1	31.5
4	6.9		0.2			7.1
Variance for the stratified mean catch					84.8	84.8
Relative Error.						11.9%

Pacific Cod Depth	Area - Statified mean catch - Weighted by stratum 1	2	3	4	5	6	Total
1		0.2					0.2
2	6.5	10.7		10.3		6.5	33.9
3	5.6		3.2	6.1		37.7	52.5
4			3.3		0.1		3.3
Stratified Mean Catch for PWS					98.0		98.0

Pacific Cod Depth	Area - Variance Weighted by stratum area 1	2	3	4	5	6	Tc
1		0.0					
2	9.9	7.8		18.5		2.9	
3	5.4		0.8	2.1		76.2	
4			6.0		0.0		
Variance for the stratified mean catch					129.6		
Relative Error							

75.8

Variance for mean catch from simple random sampling
Relative Error

ble . A worksheet for estimating mean catch and its variance for the May-June trawl survey in PWS, 1989.

This table is for _ Arrowtooth Flounder

15-Jul-89 12:10 PM
With 1989 area measurements

Arrowtooth Flounder Depth	Quadrants - Mean Catch 1	2	3	4	5	Total-Pooled
1		435.4				382.2
2	401.1	679.2	74.0	2759.2	938.4	1405.4
3	713.6	238.4	143.6	1325.4	254.9	640.7
4	741.4		91.6			247.1
Total	616.0	455.9	99.7	2144.7	528.3	791.1

Arrowtooth Flounder Depth	Quadrants - Variance Catch 1	2	3	4	5	Total-Pooled
1		224.7				27,054.1
2	49,446.1	108,142.9	10,952.0	9,335,663.1	976,642.9	4,320,114.5
3	348,779.5	59,569.4	19,303.8	1,325,497.8	17,647.4	656,489.7
4	829,492.7		4,267.6			255,364.3
Total	320,791.2	112,495.6	6,837.0	6,078,843.3	393,122.4	2,014,719.7

Arrowtooth Flounder Depth	Area - Mean Catch 1	2	3	4	5	6	Total-Pooled
1		323.1					382.2
2	776.0	699.3		2,636.6		938.4	1405.4
3	375.6		172.0	1,140.8		254.9	640.7
4			310.9		92.5		247.1
Total	547.2	612.5	267.5	1938.5	92.5	528.3	791.1

Arrowtooth Flounder Depth	Area - Variance for Catch 1	2	3	4	5	6	Total-Pooled
1.0		37,968.7					27,054.1
2.0	94,227.0	99,035.0		9,912,779.8		976,642.9	4,320,114.5
3.0	59,019.4		13,239.7	1,343,131.1		17,647.4	656,489.
4.0			367,264.6		6,526.3	-	255,364.
Total	106,724.5	107,031.5	252,792.1	6,128,619.3	7,794.7	393,122.4	2,014,719.

Arrowtooth FloQuadrant- Statified mean catch- Weighted by stratum Depth	1	2	3	4	5	Total
1		28.1				28.1
2	48.3	53.8	2.2	256.4	26.8	387.5
3	71.2	29.4	14.2	108.8	14.5	238.1
4	46.1		9.2			55.3
Stratified Mean Catch for PWS					680.8	680.8

Arrowtooth Flounder Depth	Quadrants - Variance weighed by stratum area 1	2	3	4	5	Total
1		0.2				0.2
2	99.6	97.0	4.8	10,077.9	396.8	10,676.1
3	1,736.0	129.1	62.6	1,489.0	11.5	3,428.1
4	800.8		4.3			805.1
Variance for the stratified mean catch					14,909.6	14,909.6
Relative Error						17.9%

Arrowtooth FloArea - Statified mean catch - Weighted by stratum Depth	1	2	3	4	5	6	Total
1		18.7					18.7
2	36.1	63.5		492.0		57.2	648.8
3	25.1		14.4	184.9		25.3	169.6
4			49.9		2.3		52.2
Stratified Mean Catch for PWS					889.4		889.4
[illegible]					791.1		

Arrowtooth Flounder Depth	Area - Variance Weighted by stratum area 1	2	3	4	5	6	To
1		42.4					
2	68.0	31.8		43,140.3		1,315.2	45.1
3	65.7		18.5	1,521.3		58.1	1.
4			860.0		4.9		
Variance for the stratified mean catch					47,772.1		47.
Relative Error							
Variance for mean catch from simple random sampling							35
Relative Error							

.le . A worksheet for estimating mean catch and its variance for the May-June trawl survey in PWS, 1989.
This table is for Halibut

15-Jul-89 12:22 PM
With 1989 area measurements

Halibut Depth	Quadrants - Mean Catch 1	2	3	4	5	Total-Pooled
1		120.4				110.6
2	67.7	76.7	29.2	124.3	497.4	120.2
3	231.6	107.6	46.6	285.5	35.5	140.6
4	275.2		153.9			196.8
Total	182.4	95.7	115.8	193.4	220.2	148.9

Halibut Depth	Quadrants - Variance Catch 1	2	3	4	5	Total-Pooled
1		20,992.3				16,514.6
2	6,435.5	9,628.4	1,705.3	31,996.7	174,050.0	37,161.4
3	19,681.3	27,766.7	2,129.3	137,724.2	944.9	55,171.7
4	30,424.0		15,601.2			23,622.4
Total	22,155.2	17,206.0	13,587.9	77,056.3	107,999.6	37,100.3

Halibut Depth	Area - Mean Catch 1	2	3	4	5	6	Total-Pooled
1		80.3					110.8
2	164.5	101.7		106.1		497.4	120.2
3	40.7		59.0	257.9		35.5	140.6
4			249.7		68.0		196.8
Total	98.3	96.7	190.1	177.0	68.0	220.2	148.9

Halibut Depth	Area - Variance for Catch 1	2	3	4	5	6	Total-Poo
1.0		19,328.2					16,514
2.0	15,281.7	11,006.4		31,331.1		174,050.0	37,161
3.0	9,486.8		3,909.5	120,106.0		944.9	55,171
4.0			21,044.4		14,951.9		23,622
Total	13,670.8	11,554.3	23,400.2	73,286.7	12,560.6	107,999.6	37,18

Halibut Depth	Quadrant- Statified mean catch- Weighted by stratum 1	2	3	4	5	Total
1		5.6				5.6
2	8.8	6.1	0.9	11.6	14.2	41.5
3	23.1	13.3	4.6	23.4	2.0	66.4
4	17.2		15.4			32.6
Stratified Mean Catch for PWS					140.5	140.5

Halibut Depth	Quadrants - Variance weighed by stratum area 1	2	3	4	5	Total
1		30.9				30.9
2	13.0	8.6	0.7	34.5	70.7	127.6
3	98.0	60.2	6.9	154.7	0.6	320.3
4	29.4		15.7			45.1
Variance for the stratified mean catch					523.9	523.9
Relative Error						16.3%

Halibut Depth	Area - Statified mean catch - Weighted by stratum 1	2	3	4	5	6	Total
1		4.6					4.6
2	7.7	9.2		19.8		30.3	67.0
3	3.2		4.9	23.7		3.5	35.4
4			40.1		1.7		41.8
Stratified Mean Catch for PWS					148.9		148.9

Halibut Depth	Area - Variance Weighted by stratum area 1	2	3	4	5	6	T
1		21.6					
2	11.0	9.1		136.4		323.5	
3	10.6		5.5	145.0		3.1	
4			49.3		1.9		
Variance for the stratified mean catch					716.9		
Relative Error							

[illegible]le mean 148.9

Variance for mean catch from simple random sampling
Relative Error

. A worksheet for estimating mean catch and its variance for the May-June trawl survey in PWS, 1989.
This table is for Pollock

15-Jul-89 12:24 PM
With 1989 area measurements

llock epth	Quadrants - Mean Catch 1	2	3	4	5	Total-Pooled
1		30.4				54.1
2	165.3	225.5	116.1	569.7	2049.6	511.9
3	62.6	72.6	109.1	35.3	87.1	75.5
4	26.7		53.1			39.9
tal	95.2	141.7	73.0	340.6	872.1	222.1

Pollock Depth	Quadrants - Variance Catch 1	2	3	4	5	Total-Pooled
1		9,688.3				5,000.5
2	15,860.3	16,404.6	24,156.0	1,026,733.1	8,293,849.9	1,019,983.2
3	752.7	3,303.2	6,176.7	2,506.4	4,348.2	3,147.0
4	1,063.2		4,862.3			3,585.6
Total	11,285.0	14,380.1	6,585.6	629,136.4	3,231,097.7	407,404.5

llock epth	Area - Mean Catch 1	2	3	4	5	6	Total-Pooled
1		67.7					54.1
2	235.7	179.4		592.0		2,049.6	511.9
3	75.3		106.9	34.1		87.1	75.5
4			51.1		16.7		39.9
tal	146.1	153.6	68.6	331.7	16.7	872.1	222.1

Pollock Depth	Area - Variance for Catch 1	2	3	4	5	6	Total-Pooled
1.0		6,385.5					5,000.5
2.0	33,788.2	11,223.1		1,004,141.7		8,293,849.9	1,019,983.2
3.0	3,327.3		3,417.4	2,097.8		4,348.2	3,147.0
4.0			4,729.0		181.3		3,585.6
Total	20,280.3	11,386.4	4,776.3	585,965.1	158.6	3,231,097.7	407,404.5

llock epth	Quadrant- Statified mean catch- Weighted by stratum 1	2	3	4	5	Total
1		4.2				4.2
2	16.6	17.9	3.5	52.9	58.4	149.3
3	6.2	8.9	10.8	2.9	5.0	33.0
4	1.7		5.3			7.0
Stratified Mean Catch for PWS					190.1	190.1

Pollock Depth	Quadrants - Variance weighed by stratum area 1	2	3	4	5	Total
1		10.3				10.3
2	31.9	14.7	10.6	1,100.4	3,359.0	4,535.5
3	3.7	7.2	20.0	2.0	2.8	36.6
4	1.0		4.9			5.9
Variance for the stratified mean catch					4,500.3	4,500.3
Relative Error						35.6%

llock epth	Area - Statified mean catch - Weighted by stratum 1	2	3	4	5	6	Total
1		3.9					3.9
2	11.2	16.3		110.5		125.0	262.9
3	5.3		8.9	3.1		8.6	26.0
4			6.2		0.4		8.6
Stratified Mean Catch for PWS				301.4			301.4

Pollock Depth	Area - Variance Weighted by stratum area 1	2	3	4	5	6	Total
1		7.1					7.1
2	24.4	9.3		4,370.0		15,415.0	19,515.6
3	3.7		4.8	2.5		14.3	25.[illegible]
4			11.1		0.0		11.[illegible]
Variance for the stratified mean catch					19,562.2		19,56[illegible]
Relative Error							46.[illegible]

Simple random sample mean 222.1

Variance for mean catch from simple random sampling 7,27[illegible]
Relative Error 3[illegible]

le . A worksheet for estimating mean catch and its variance for the May-June trawl survey in PWS, 1989.
This table is for Sablefish

15-Jul-89 12:29 PM
With 1989 area measurements

Sablefish Depth	Quadrants - Mean Catch 1	2	3	4	5	Total-Pooled
1		0.0				14.3
2	0.0	11.7	0.2	24.7	28.6	12.7
3	1.6	132.8	0.0	169.8	345.1	156.5
4	265.8		95.1			126.8
Total	96.9	63.2	63.4	86.9	218.5	87.5

Sablefish Depth	Quadrants - Variance Catch 1	2	3	4	5	Total-Pooled
1		0.0				815.0
2	0.0	907.2	0.1	1,072.6	1,635.9	725.2
3	5.1	36,307.0	0.0	63,463.4	86,420.2	49,796.3
4	22,324.7		4,919.7			15,157.9
Total	24,620.0	18,915.9	5,313.7	30,541.8	73,664.4	23,348.4

Sablefish Depth	Area - Mean Catch 1	2	3	4	5	6	Total-Pooled
1		0.0					14.3
2	30.7	8.3		13.5		28.5	12.7
3	225.1		5.8	145.6		345.1	156.5
4			155.3		61.3		126.8
Total	141.8	6.4	108.6	75.1	61.3	218.5	87.5

Sablefish Depth	Area - Variance for Catch 1	2	3	4	5	6	Total-Poo
1.0		0.0					315
2.0	2,641.3	635.3		405.9		1,635.9	725
3.0	45,920.1		170.5	57,006.6		86,420.2	49,796
4.0			17,142.7		8,051.0		15,157
Total	34,641.0	489.8	16,594.2	29,289.3	7,196.5	73,664.4	23,348

Sablefish Depth	Quadrant- Statified mean catch- Weighted by stratum 1	2	3	4	5	Total
1		0.0				0.0
2	0.0	0.9	0.0	2.3	0.8	4.0
3	0.2	16.4	0.0	13.9	19.7	50.1
4	16.5		9.5			26.1
Stratified Mean Catch for PWS					80.2	80.2

Sablefish Depth	Quadrants - Variance weighed by stratum area 1	2	3	4	5	Total
1		0.0				0.0
2	0.0	0.0	0.0	1.2	0.7	2.5
3	0.0	78.7	0.0	71.3	56.2	206.2
4	21.6		5.0			26.5
Variance for the stratified mean catch					235.3	235.3
Relative Error						19.1%

Sablefish Depth	Area - Statified mean catch - Weighted by stratum 1	2	3	4	5	6	Total
1		0.0					0.0
2	1.4	0.8		2.5		1.7	6.4
3	15.0		0.5	13.4		34.3	53.2
4			24.9		1.5		26.5
Stratified Mean Catch for PWS					96.1		36.1

Sablefish Depth	Area - Variance Weighted by stratum area 1	2	3	4	5	6	T
1		0.0					
2	1.9	0.5		1.3		3.0	
3	51.1		0.2	58.8		284.3	
4			40.1		1.0		
Variance for the stratified mean catch					452.9		
Relative Error							

Simple random sample mean 87.5

Variance for mean catch from simple random sampling
Relative Error

1[illegible] . A worksheet for estimating mean catch and its variance for the May-June trawl survey in PWS, 1989. 15-Jul-89 12:34 PM
This table is for Rougheye Rockfish — With 1989 area measurements

Rougheye Rockfish Quadrants - Mean Catch						
Depth	1	2	3	4	5	Total-Pooled
1		5.8				4.3
2	60.9	89.9	24.0	47.8	2.0	62.0
3	53.4	22.3	169.3	23.8	3.3	48.6
4	1.6		5.5			3.8
Total	37.9	49.9	40.7	37.5	2.8	38.8

Rougheye Rockfish Quadrants - Variance Catch						
Depth	1	2	3	4	5	Total-Pooled
1		92.5				41.3
2	3,196.0	7,970.4	1,152.0	4,448.5	8.0	4,894.8
3	1,824.1	1,456.3	69,029.3	279.8	33.3	11,322.7
4	9.6		382.5			198.6
Total	2,304.3	5,122.9	14,608.6	2,654.5	19.2	5,792.4

Rougheye Rockfish Area - Mean Catch							
Depth	1	2	3	4	5	6	Total-Pooled
1		5.7					4.3
2	22.4	85.6		53.8		2.6	62.0
3	19.8		133.8	20.4		3.3	48.6
4			0.6		11.0		3.8
Total	25.2	67.1	42.2	38.2	11.0	2.8	38.8

Rougheye Rockfish Area - Variance for Catch							
Depth	1	2	3	4	5	6	Total-Pooled
1.0		49.7					41.3
2.0	842.1	6,456.5		4,081.6		8.0	4,894.8
3.0	1,568.2		36,892.5	313.9		33.3	11,322.7
4.0			3.5		756.3		198.[illegible]
Total	1,110.1	6,076.[illegible]	13,906.0	2,472.3	605.6	19.2	5,792.[illegible]

Rougheye RockfQuadrant- Statified mean catch- Weighted by stratum						
Depth	1	2	3	4	5	Total
1		0.3				0.3
2	6.1	7.1	0.7	4.4	0.1	18.4
3	5.3	2.8	16.7	2.0	0.2	26.9
4	0.1		0.6			0.6
Stratified Mean Catch for PWS					46.0	46.0

Rougheye Rockfish Quadrants - Variance weighed by stratum area						
Depth	1	2	3	4	5	Total
1		0.1				0.1
2	6.4	7.2	0.5	4.8	0.0	18.3
3	9.1	3.2	223.8	0.3	0.0	236.4
4	0.0		0.3			0.3
Variance for the stratified mean catch					255.7	255.7
Relative Error						34.8%

Rougheye RockfArea - Statified mean catch - Weighted by stratum							
Depth	1	2	3	4	5	6	Total
1		0.3					0.3
2	1.5	7.0		10.0		0.1	19.4
3	1.3		11.2	1.9		0.3	14.7
4			0.1		0.3		0.4
Stratified Mean Catch for PWS					34.8		34.8

Rougheye Rockfish Area - Variance Weighted by stratum area							
Depth	1	2	3	4	5	6	To[illegible]
1		0.1					
2	0.6	5.3		17.8		0.0	
3	1.7		51.6	0.4		0.1	
4			0.0		0.1		
Variance for the stratified mean catch					77.7		
Relative Error							

Simple random sample mean 38.8

Variance for mean catch from simple random sampling
Relative Error

Te . A worksheet for estimating mean catch and its variance for the May-June trawl survey in PWS, 1989. 15-Jul-89 12:44 PM

This table is for Shortracker Rockfish — With 1989 area measurements

Shortracker Rockfish Depth	Quadrants - Mean Catch 1	2	3	4	5	Total-Pooled
1		0.0				0.0
2	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	3.4	0.0	3.4	0.0	1.3
4	16.9		72.6			50.9
Total	5.1	1.5	48.4	1.5	0.0	13.7

Shortracker Rockfish Depth	Quadrants - Variance Catch 1	2	3	4	5	Total-Pooled
1		0.0				0.0
2	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	82.3	0.0	78.7	0.0	38.3
4	897.5		7,327.4			5,434.2
Total	341.5	36.0	5,966.2	38.3	0.0	1,867.0

Shortracker Rockfish Depth	Area - Mean Catch 1	2	3	4	5	6	Total-Pooled
1		0.0					0.0
2	0.0	0.0		0.0		0.0	0.0
3	0.0		4.8	2.9		0.0	1.3
4			66.3		12.8		50.9
Total	0.0	0.0	47.1	1.4	12.8	0.0	13.7

Shortracker Rockfish Depth	Area - Variance for Catch 1	2	3	4	5	5	Total-Pool
1.0		0.0					0
2.0	0.0	0.0		0.0		0.0	0
3.0	0.0		115.2	68.6		0.0	38
4.0			7,002.6		1,024.0		5,434
Total	0.0	0.0	5,567.2	28.3	319.2	0.0	1,367

Shortracker RoQuadrant- Statified mean catch- Weighted by stratum Depth	1	2	3	4	5	Total
1		0.0				0.0
2	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.4	0.0	0.3	0.0	0.7
4	1.0		7.3			8.3
Stratified Mean Catch for PWS					9.0	9.0

Shortracker Rockfish Depth	Quadrants - Variance weighed by stratum area 1	2	3	4	5	Total
1		0.0				0.0
2	0.0	0.0	0.0	0.0	0.0	0.0
3	0.0	0.2	0.0	0.1	0.0	0.3
4	0.9		7.4			8.2
Variance for the stratified mean catch					8.5	6.5
Relative Error						32.3%

Shortracker RoArea - Statified mean catch - Weighted by stratum Depth	1	2	3	4	5	6	Total
1		0.0					0.0
2	0.0	0.0		0.0		0.0	0.0
3	0.0		0.4	0.3		0.0	0.7
4			10.6		0.3		11.0
Stratified Mean Catch for PWS					11.6		11.5

Shortracker Rockfish Depth	Area - Variance Weighted by stratum area 1	2	3	4	5	6	T:
1		0.0					
2	0.0	0.0		0.0		0.0	
3	0.0		0.2	0.1		0.0	
4			15.4		0.1		
Variance for the stratified mean catch					15.2		
Relative Error							

Simple random sample mean 13.7

Variance for mean catch from simple random sampling

Relative Error

Tie . A worksheet for estimating mean catch and its variance for the May-June trawl survey in PWS, 1989.
This table is for Sidestripe Shrimp

15-Jul-89 12:47 PM
With 1989 area measurements

Sidestripe Shrimp — Quadrants - Mean Catch

Depth	1	2	3	4	5	Total-Pooled
1		0.2				0.1
2	12.2	8.9	0.1	13.6	0.0	10.5
3	21.6	25.1	49.4	36.0	4.0	30.0
4	113.4		104.3			96.8
Total	50.7	16.7	79.4	23.2	2.4	38.6

Sidestripe Shrimp — Quadrants - Variance Catch

Depth	1	2	3	4	5	Total-Pooled
1		0.1				0.0
2	163.6	91.0	0.0	312.6	0.0	176.9
3	32.0	330.8	1,092.7	3,542.1	4.0	1,350.2
4	14,987.8		1,787.7			5,111.5
Total	7,049.5	385.7	4,123.3	1,663.6	6.8	3,310.5

Sidestripe Shrimp — Area - Mean Catch

Depth	1	2	3	4	5	6	Total-Pooled
1		0.1					0.1
2	17.9	13.7		7.4		0.0	10.5
3	15.2		50.2	22.1		4.0	30.0
4			100.2		71.2		96.8
Total	16.3	10.6	90.1	19.4	71.2	2.4	38.6

Sidestripe Shrimp — Area - Variance for Catch

Depth	1	2	3	4	5	6	Total-Poole
1.0		0.1					0.0
2.0	759.3	102.6		109.2		0.0	176.
3.0	27.6		431.8	3,009.4		4.0	1,350.
4.0			7,604.6		1,716.7		5,111.
Total	268.9	112.2	5,957.6	1,521.6	2,364.6	6.8	3,310.

Sidestripe ShrQuadrant- Statified mean catch- Weighted by stratum

Depth	1	2	3	4	5	Total
1		0.0				0.0
2	1.2	0.7	0.0	1.3	0.0	3.2
3	2.2	3.6	4.9	3.0	0.2	13.8
4	7.0		10.5			17.5
Stratified Mean Catch for PWS					34.5	34.5

Sidestripe Shrimp — Quadrants - Variance weighed by stratum area

Depth	1	2	3	4	5	Total
1		0.0				0.0
2	0.3	0.1	0.0	0.3	0.0	0.7
3	0.2	0.7	3.5	4.0	0.0	5.4
4	14.5		3.8			18.3
Variance for the stratified mean catch					27.4	27.4
Relative Error						15.2%

Sidestripe ShrArea - Statified mean catch - Weighted by stratum

Depth	1	2	3	4	5	6	Total
1		0.0					0.0
2	0.8	1.2		1.4		0.0	3.4
3	1.0		4.2	3.0		0.4	8.7
4			17.4		1.8		19.2
Stratified Mean Catch for PWS					31.3		31.3

Sidestripe Shrimp — Area - Variance Weighted by stratum area

Depth	1	2	3	4	5	6	T:
1		0.0					
2	0.5	0.1		0.5		0.0	
3	0.0		0.6	3.6		0.0	
4			17.8		0.2		
Variance for the stratified mean catch					23.4		
Relative Error							

38.6

Variance for mean catch from simple random sampling
Relative Error

APPENDIX C

TRAWL SPECIFICATIONS

APPENDIX C. TRAWL GEAR SPECIFICATIONS

NET: 400 MESH EASTERN OTTER TRAWL

Footline:
1. 95' footline of 1/2" cable wrapped with 7/16" corsair line and 3/8" chain attached snug to footline every 10".

Headline:
1. 70' headline of 3/8" cable wrapped with 3/8" poly with 18 @ 8" aluminum floats with ears, attached about every 4 feet.

Web:
1. 42 thread 4" mesh in the wings and body.
2. 60 thread 3-1/2" mesh in the intermediate.
3. 18 thread 1-1/4" mesh cod end liner extending 3 feet beyond cod end.
4. 120 thread 3-1/2" mesh cod end
5. 3-1/2" 6.00 mm P.E. web chaffing gear around cod end.

Other:
1. Flymesh on back end of body of net, both ends of intermediate, and front end of cod end. This is for attachment of the body of the net to the intermediate with a zipperline and for attachment of the intermediate to the cod end with a similar zipperline arrangement.

2. The intermediate is straight 110 open meshes around and 120 meshes deep attached to the body of the net with a flymesh zipperline configuration. The other end of the intermediate with 1 flymesh every two meshes, attached to cod end with a zipperline with the same flymesh configuration. The end of the cod end will have a 2" x 3/8" galvanized ring for every 4 meshes.

3. 3/4" poly-dac rib lines with eyes at intermediate and cod end seams.

4. 1/2' poly-dac spider lines from corners of bosom across belly of net.

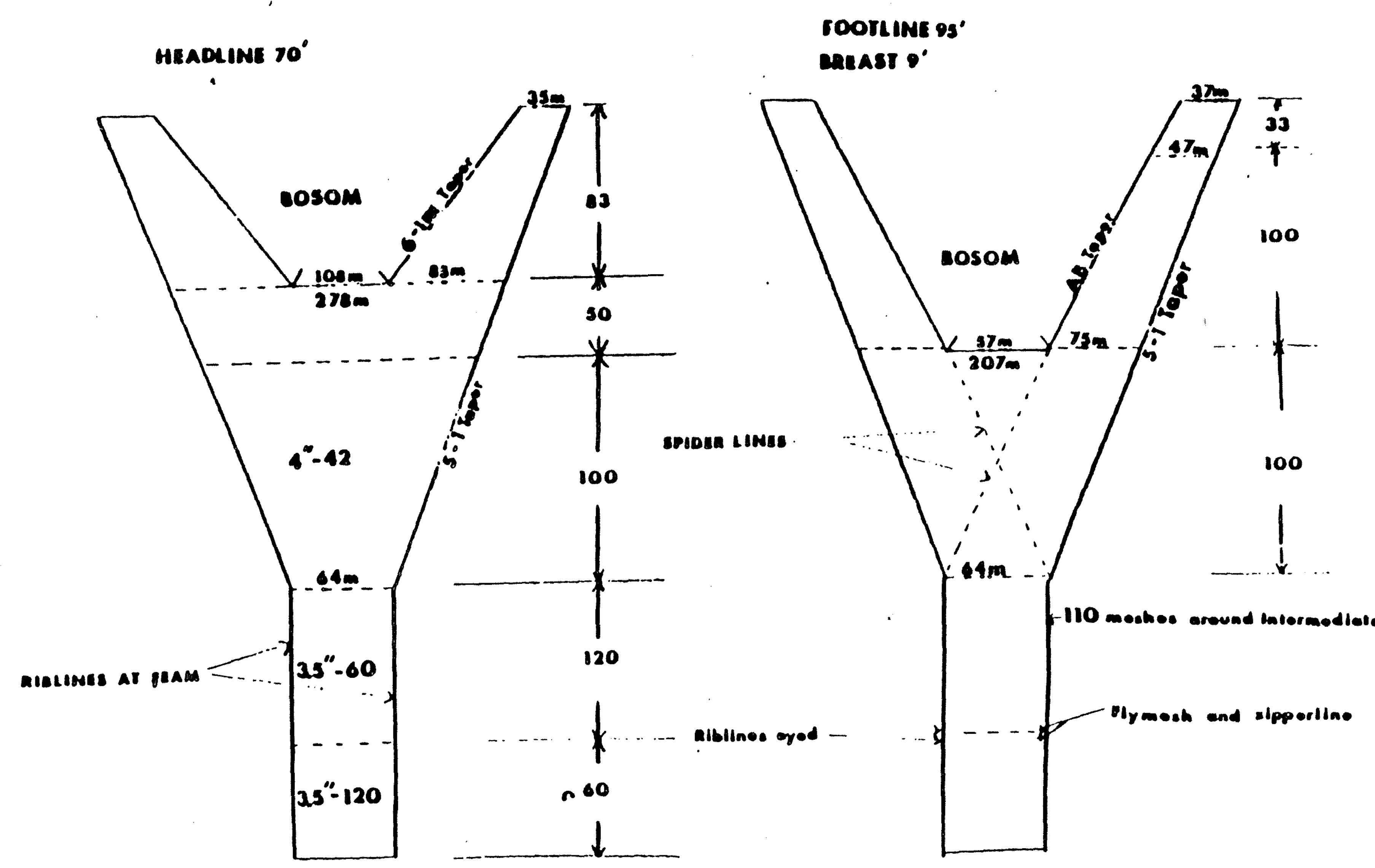
5. 5 @ 4" x 1/2" splitting strap rings sewn to cod end 21 meshes from end of cod end. These rings should be evenly spaced around the net.

6. A 1/2" poly bolsh line will be attached to the foot line and web should be drop hung from the bolsh line. The bolsh line should be attached to the footline every 8".

DOORS: NOR'EASTERN TRAWL SYSTEMS INC. NOR'EASTERN ASTORIA VEE DOOR

1. 5'x 7'; 800 lbs. each

Appendix C, Figure 1



APPENDIX D

HAUL POSITION FORM

APPENDIX D

Figure 3.

HAUL-POSITION FORM

VESSEL [2 3 4] ________ CRUISE [6 7 8] HAUL [10 11 12]

YEAR [14 15] MONTH [16 17] DAY [18 19] YOUR NAME ________

POSITION CARD

LATITUDE / LONGITUDE

POSITION START... [20 21 22: + DEGREE] [23 24 26 27: MIN.] [28 29 30 31: DEGREE] [32 33 35 36: MIN.]

"+" = WEST
"-" = EAST
COL. 28 & 45

END..... [37 38 39: +] [40 41 43 44] [45 46 47 48] [49 50 52 53]

RATE / FIRST READING / RATE / SECOND READING

LORAN START [55 56] [58 59 60 61 62 64 65] [67 68] [70 71 72 73 74 76 77]

LORAN END... [79 80] [82 83 84 85 86 88 89] [91 92] [94 95 96 97 98 100 101]

---------- DUP. COL. 1-36 FROM ABOVE

HAUL CARD

GEAR DEPTH WTD. AVG. (FM) [37 38 39 40] MIN ________ MAX ________

BOTTOM DEPTH WTD. AVG. (FM) [41 42 43 44] MIN ________ MAX ________

TIME STANDARD USED ________

EQUIL. HOUR [46 47] DURATION (HRS.) [48 49 51 52]

TIME START ________ OUT ________

EQUILIBRIUM ________

DISTANCE FISHED (N.M.) [54 55 57 58] HAUL TYPE [60 61]

HAUL ________

IN ________

STRATUM [62 63 64] WTD. AVG. TRACE DEPTH (FM) [65 66 67 68] MIN ________ MAX ________

DESCR. ________

WATER TEMPERATURES (DEGREES C.) SURFACE [70 71 73] GEAR [75 76 78] METHOD ________

BOTTOM TYPE [83 84] DESCR. ________

WIRE OUT (FM) [85 86 87 88] GEAR TYPE [94 95 96] DOOR + ACCES. [97 98 99]

PERFORMANCE [103] ________ WEATHER, SEA CONDITIONS ________

REMARKS

DP-001 83-01-15

APPENDIX E

HYDROCARBON AND NECROPSY SAMPLES

Appendix E

PROCEDURES FOR TAKING HYDROCARBON AND HISTOLOGY SAMPLES MULTI-SPECIES TRAWL SURVEY, PRINCE WILLIAM SOUND

The following procedures should be used in taking hydrocarbon and histology samples during trawl surveys in Prince William Sound. The hydrocarbon and histology procedures were developed during the May/June 1989 trawl survey. The procedures developed at that time have been modified slightly based on subsequent information on taking samples and proper custody of the samples. Two handouts should be consulted prior to taking the samples, 1) Histopathology Technical Group for Oil Spill Assessment Studies in Prince William Sound, Alaska, and 2) Chain-of-Custody Procedures. The handouts are appended to these Procedures.

Equipment

1. Stainless steel pans, with lids: three assorted sizes for holding and storing knives, scalpels, and scissors.
2. Paper towels.
3. Scalpels: four styles of Bard Parker blades: #10 deep belly, larger deep belly, #11 straight point, and #12 hooked. For cutting tissue and puncturing bile gland.
4. Short blade boning knives: two. For incising and cutting tissue.
5. Long, thin blade fish fillet knives: two. For incising and cutting tissue.
6. Scissors: surgical type with thin, pointed blade and larger, heavy duty type. For cutting tissue and exoskeleton.
7. Forceps: three sizes, small to large. For holding delicate tissue up to large visceral mass.
8. Methylene chloride (MC) for rinsing equipment.
9. Teflon squeeze bottles for using the MC.
10. Steel for knife sharpening.
11. Surgical gloves.
12. Liquid dish washing detergent.
13. Aluminum foil. To cover surfaces to prevent contamination of tools and specimens.
14. Unpainted plywood cutting boards.
15. Pre-baked 4 g amber vials, with caps, for bile samples.
16. IChem prebaked, custody sealed 4 oz jars with Teflon lined caps.

Work Area

The samples should not be taken on a surface painted with oil-based paint. This problem can be solved by laying a sheet of unpainted plywood on top of the painted surface and using aluminum foil to cover work surfaces.

Water dripping from vessel must not be allowed to splash work area.

Preparation of Equipment

At the end of sampling and prior to use, wash all cutting tools, forceps, pans, cutting surfaces, etc. with hot water and detergent. Hot water is available on deck. Rinse thoroughly with hot water and then MC. On cold days the MC may freeze the water. If so, wipe equipment with paper towels prior to rinsing with MC. Flush all tools, pans, etc. with MC and arrange tools for use. Knife blades are positioned so that they do not touch any surface. Scalpels, etc. are kept in the MC rinsed stainless steel pans. Air dry all utensils after rinsing with MC. Use indelible pen to mark labels, not pencil. NO plastics touch the sample.

Capturing the Specimens

All samples are from internal tissue and thus capturing specimens with otter trawls is valid. Because of large catches, tows are usually limited to 15 minutes. The catch is dumped onto the sorting table and sample specimens immediately put aside for processing.

Selecting the Specimens

Hydrocarbon samples:

Decide beforehand which species and sizes will be sorted from a given catch. Avoid large individuals - their viscera are too large to manipulate effectively. At least four specimens of a given species should be retained from the catch, three for hydrocarbon sampling and one for backup. The selected fish are put in fish baskets and kept cold by covering them with ice.

Histology samples:

Only live or moribund specimens are suitable for processing. Do not process dead fish. Tissues in dead fish autolyze rapidly and mask the subtle changes caused by toxic chemicals. Do not over-ice fish to the extent that tissues freeze. Frozen tissues are worthless for histological examination.

Preparing the Specimen

The specimen is wiped repeatedly with paper towels to remove all slime, mud, scales, etc. The table where the specimen will be placed is also wiped clean.

Taking Samples

Always wear surgical gloves to avoid contamination of samples by

skin oils. Use new gloves rather than trying to reuse gloves. Used gloves are difficult to put on and trying to put them on runs the risk of contamination.

Hydrocarbons:
Removing the pectoral fin creates a hole that allows the knife blade to enter the abdominal cavity without contaminating the internal tissues. Use a short blade boning knife to remove the pectoral fin by slicing the fin away with short strokes in the direction of the head. Wipe knife and put it aside. Remember that each tool must be clean (rinsed with MC) before it touches the sample.

The abdominal wall must be removed to expose the internal organs. Insert the long thin blade of the fillet knife into the pectoral fin hole with the cutting edge of the blade toward the ventral side of the fish, and run the blade very carefully under the surface of the abdominal wall to a point above and behind the anus. The cut is made high enough along the side of the fish that the belly portion prevents the viscera from spilling out onto the work table. The knife point is then pushed through the body wall and the knife pulled downwards and backwards to cut through the flesh. The cut is thus made from the inside out and prevents contamination of the abdominal cavity. The knife is then wiped cleaned and put aside in a clean area.

Subsequent cuts are made holding the body wall away from the internal organs. To lift the body wall, carefully insert the knife point under the cut body wall and lift the body wall up. The body wall is then cut away, the cut being made from the inside out. and the knife wiped and then rinsed with MC before each cut. After the cut, the body flap is discarded, the knife wiped cleaned and put in the stainless steel pan.

Collecting bile

The bile duct opens into the small intestine. The duct is grasped with forceps, gently pulled upwards, and connective tissue around the gall bladder is gently cut away (a deep belly scalpel seems best for this cutting). When free from connective tissue, the gall bladder is set on top of, and slightly to one side of the 4 g amber vial. The #11 straight point scalpel is used to pierce the bladder so that the point of the blade projects inside the vial and acts as a guide for the bile to flow into the vial. Care is needed to prevent the gall bladder or bile from creating a seal or bubble over the top of the vial. Such a seal prevents the bile from flowing into the vial. One technique to aid the puncturing is to hold clean forceps against the bladder on the opposite side from the puncture by the #11 scalpel. Fortunately, only a few drops of bile are needed for analysis. The vial is then capped tightly.

Collecting intestinal tissue

Intestinal tissue includes stomach contents. Indeed, stomach

contents is more valuable for detecting intestinal oil than intestinal tissues themselves. Use forceps (large or small, depending on size of fish) to grasp firmly the esophagus just behind the pharyngeal area. The esophagus is severed anterior to the forceps, and pulled upward as connective tissue is cut away with the deep bellied scalpel. The liver is also cut away. The entire intestine is pulled free of all connective tissue and the lower intestine severed at the anus. Small digestive systems are put directly into the 4 oz IChem jars. Large digestive systems are laid onto a clean and rinsed stainless steel pan and small sections cut from the esophagus, stomach wall, and intestine. These sections and as much of the stomach contents as possible are put into the jar.

Muscle tissue

Muscle tissue is removed from the tail region just posterior to the gut. Using a fillet knife, two longitudinal and parallel cuts are made, one above and the other below the lateral line. Using the same knife, the flesh above and below the two cuts is filletted away. This cutting exposes the sides of the muscle sample such that the knife can be inserted under the sample without contacting the skin and scales. A clean and rinsed knife is used to fillet the sample from the backbone the distance of the two parallel cuts. Care should be taken to avoid contaminating the center area of the sample with the portion of the knife blade that touches the edge of the sample. This care is needed because the edges of the sample could have become contaminated from the fillet knife cutting vertically through the skin.

The rectangular sample is laid skin down on a clean surface. The flesh is shaved away from each side with a scalpel in such a way that the scalpel blade enters the flesh from the clean side and the cuts made so that the blade exits in the unclean areas. The flesh is then filletted from the skin and put into a clean 4 oz IChem jar.

Sampling Crab

Three kinds of tissue are removed from tanner crab (*Chionoecetes bairdi*), hepatopancreas, muscle, and eggs. A single crab does not provide enough material for a sample and several crab, usually three, are needed to obtain sufficient tissue.

Eggs

The crab are wiped thoroughly with tissue paper. The eggs are removed first. The abdomen is forced apart from the thorax and the outermost (posterior) pair of pleopods excised and discarded. The next two pairs of pleopods with attached eggs are excised and placed into a 4 oz IChem jar. This procedure is repeated on as many crab as needed to provide the sample.

Hepatopancreas

Hepatopancreas is taken from crab of either sex. The carapace is pried off the body of the crab using gloved hands. Part of the hepatopancreas may be lying on the inner surface of the carapace, but most will be in the main body area between the gills. It is a brownish material and is removed with clean forceps directly into a 4 oz IChem jar. As many crab as necessary are use to provide the composite sample.

Muscle

The muscle is removed from the merus segment of each walking leg after the hepatopancreas is removed from the body. First, the merus is severed at its proximal end either by breaking or severing with scissors. To remove the muscle, the joint between the merus and carpus is broken by bending the carpus in the opposite direction that it normally bends. With the joint broken, the carpus is used to pull the muscle out of the merus. The muscle, still attached to the carpus, is held inside the 4 oz jar and the distal end attached to the carpus severed with clean scissors.

Sampling Shrimp

Muscle is taken from the sidestripe shrimp (Pandalopsis dispar). The shrimp is wiped clean, although the interstices between segments is difficult to clean properly. These sections, however, are not likely to come into contact with the muscle sample. Removing shrimp muscle is frustrating, because the muscle tears easily. One procedure is to cut the abdominal exoskeleton laterally along its ventral surface. The abdomen is the spread apart along the cut and held in this position while a second person removes the muscle with clean forceps.

Histology Sampling

Histological sample preparation for fish and shellfish should follow the instructions written by the Histopathology Technical Group.

Chain-of-Custody Procedures

Chain-of-Custody procedures should follow the general instructions "EQM&LO Chain-of-Custody Procedures" (Appendix F). When sample taking must terminate for a short period, such as for meals, a workable procedure to retain custody is to place the on-deck samples in a closed container (such as a Coleman cooler) and seal the container with evidence tape. The tape is broken when sapling is resumed. All sealing and breaking of evidence tape should be noted for record.

FIGURE E.1

MULTISPECIES TRAWL SURVEY
PWS OIL IMPACT ASSESSMENT PROJECT
HYDROCARBON AND NECROPSY SAMPLE FORM

Vessel ________ Cruise ________

Date ________ Tow Number ________ Gear ________

Latitude ________ Longitude ________ Agency ________

SAMPLE COLLECTION - CHECK BOX IF SAMPLE WAS COLLECTED AND INDICATE SAMPLE

Species/Tissue	Hydrocarbon Yes	Hydrocarbon Sample #			Necropsy Yes	Necropsy Sample #
Pollock/muscle	☐	____	____	____	☐	____
Pollock/viscera	☐	____	____	____		
Pollock/bile	☐	____	____	____		
S.Shrimp/muscle	☐	____	____	____	☐	____
P.Cod/muscle	☐	____	____	____		
P.Cod/viscera	☐	____	____	____		
P.Cod/bile	☐	____	____	____		
Flthed sole/mscle	☐	____	____	____		
Flthed sole/vscra	☐	____	____	____		
Flthed sole/bile	☐	____	____	____		
Tanner Crab/mscle	☐	____	____	____		
Tanner C/hepat.	☐	____	____	____		
Tanner Crab/egg	☐	____	____	____		
Sablefish/muscle	☐	____	____	____		
Sablefish/viscera	☐	____	____	____		
Sablefish/bile	☐	____	____	____		
(other)____	☐	____	____	____		
________	☐	____	____	____		
________	☐	____	____	____		
	☐	____	____	____		

FIGURE E.2

MULTISPECIES TRAWL SURVEY
PWS OIL IMPACT ASSESSMENT PROJECT
TARBALL SAMPLING FORM

Vessel ____________________ Cruise ______________
Date __________ Tow Number __________
Area ______________ Sampler __________

SAMPLE COLLECTION - CHECK BOX IF OBSERVATION WAS MADE AND INDICATE # OBSERVED

Species		Tarballs # sampled/# with tarballs		Stomach contents (optional) # crab/ species	other contents/ comments
Arrowtooth flounder	☐	______ ______	☐	______	______
Dover Sole	☐	______ ______	☐	______	______
Rex Sole	☐	______ ______	☐	______	______
Pollock	☐	______ ______	☐	______	______
(other) ______	☐	______ ______	☐	______	______
______	☐	______ ______	☐	______	______
______	☐	______ ______	☐	______	______

APPENDIX F.1

SAMPLE HANDLING AND COLLECTION

September 15, 1989

STATE/FEDERAL DAMAGE ASSESSMENT PLAN

ANALYTICAL CHEMISTRY

COLLECTION AND HANDLING OF SAMPLES

FOR AGENCY USE ONLY
NOT FOR RELEASE
ATTORNEY WORK PRODUCT

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6. SAMPLE PRESERVATION AND HOLDING TIME
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7. SAMPLE SHIPPING

8. CHAIN-OF-CUSTODY PROCEDURE

1. Introduction

In response to the release of more than 10 million gallons of crude oil into Prince William Sound, the State of Alaska and four Federal Agencies, the Departments of Agriculture, Commerce and Interior and the Environmental Protection Agency are acting together to assess the damages to the natural resources. Authority for this action is provided by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and the Clean Water Act (CWA).

A damage assessment requires documentation of the exposure of the resources to oil released from the EXXON VALDEZ, identifying which resources were injured by that exposure, measuring the magnitude of the adverse affects on each resource over time and assigning economic values for that injury. Once this is done, monetary compensation can be sought from the potentially responsible parties to restore and/or replace the injured resources.

Recovery of monetary damages may involve civil court actions. It will then be necessary to prove that the samples were collected in a scientifically approved manner and that the samples were protected from outside contamination (non-incident related) and accidental mix-ups during handling and analyses. It is, therefore, extremely important that every sample be readily identified and their location and analytical status known and documented at all times.

This document and the associated training sessions, were prepared to assist field personnel in collecting samples that will provide scientifically sound and legally defensible data to support the State/Federal Natural Resource Damage Assessment for the EXXON VALDEZ oil spill.

2. Record Keeping and Documentation

Standard operating procedures (SOPs) for all sampling procedures, including chain of custody procedures; sampling protocols; cleaning and preparation of sample collection and storage devices; and labeling, handling, and sample

preservation and holding time must be written in detailed, clear, simple and easy to follow language.

Personnel must be knowledgeable and experienced in the described sampling techniques and must adhere to the SOPs.

Any changes in procedures must be recorded in detail in the field logbook. The log entry must include reasons that the change in procedure was unavoidable.

Field logbooks are issued by the Team Leader or their representative. The logbooks should be serially numbered, sturdy, bound books with sequentially numbered pages. Waterproof logbooks should be used if available.

Field data sheets, if used, must be consecutively numbered by project. The field data sheets must be referred to in entries in logbooks which reference, the precise data sheet involved and the relationship to specific data in the logbook noted.

All information pertinent to field activities, including descriptive notes on each situation, must be recorded in indelible marker in the field logbook. The information must be accurate, objective, up-to-date and legible. It should be detailed enough to allow anyone reading the entries to reconstruct the sampling situation. Additional information may be provided by field data sheets, sample tags or photographs.

Entries should be made in the logbook or on field data sheets with indelible marker at the earliest possible time. Notes should never be written on scrap paper and then transferred to the logbook.

Entries into field logbooks or field data sheets are signed or initialed, and dated by the person making the entry at the time of entry.

Each day's entries are closed out with a horizontal line, date and initial.

Errors in field logbooks or other records are corrected by drawing a single line though the error, entering the correct information and signing and dating the correction. Never erase an entry or any part of an entry.

Do not remove pages from the logbook.

Completed logbooks and field data sheets are returned to the Team Leader or their representative to be archived in a central location under chain-of-custody procedures until the Trustees indicate that they may be released.

3. Sample Identification and Labelling

A tag or label identifying the sample must be completed and attached to each sample. Waterproof (indelible) marker must be used on the tag or label. The minimum information to be included on the tag are the sample identification number, the location of the collection site, the date of collection and signature of the collector (who, what, where & when). This information and any other pertinent data such as the common and scientific names of the organism collected, the tissue collected and any remarks are recorded in the logbook. Field sample data sheets, photographs, any pertinent in-situ measurements (such as temperature, salinity, depth) and field observations are recorded in the logbook.

The location of the sampling site is determined with the aid of USGS grid maps, NOAA charts or navigational systems such as LORAN C. The site locations should be plotted on a chart of appropriate scale and photocopies incorporated into the logbook. In addition, a clear, detailed descriptive location as well

as the latitude and longitude, in degrees, minutes and seconds, of the collection site must be recorded in the logbook.

4. Sampling Equipment and Sample Containers

All sample containers must be either organic-free (solvent-rinsed) glass or organic-free (solvent-rinsed) aluminum foil. Lids for the glass containers must be lined with either teflon or solvent-rinsed aluminum foil.

Certified-clean glass jars are available from various vendors and if obtainable, may be used without cleaning.

Sample collection and storage devices are cleaned by washing with soap and hot water, rinsed extensively with clean water and then rinsed with either methylene chloride or acetone followed by pentane or hexane and allowed to dry before use.

First rinse: tap water, then re-rinse in distilled water.

Second rinse: methylene chloride or acetone

Third rinse (if acetone is used): pentane or hexane

The solvents (methylene chloride, acetone, pentane and hexane) used for cleaning sample collection and storage devices must be of appropriate quality for trace organic residue analysis and be stored in glass or Teflon containers, not plastic.

New glass jars or unused aluminum foil do no need to be washed with soap and

water. They must however, be solvent-rinsed as described above before use.

Glass jars may be cleaned by heating to 440°C for a minimum of 1 hour.

Clean glassware should be stored inverted or tightly capped with either solvent-rinsed aluminum foil or teflon-lined caps.

The dull side of the aluminum foil should be the side that is solvent-rinsed. Pre-cleaned squares may be stored with the clean sides folded together.

All equipment that comes in contact with the sample such as dredges or dissecting equipment must be solvent-rinsed before contacting each sample. Equipment should be steam-cleaned or washed with soap and hot water at the end of each day or between sampling locations.

5. Sampling Procedures

The method of collection must not contaminate the samples. Do not collect any subsurface samples through surface slicks. Do not collect any samples with oil-fouled equipment, such as nets or dredges. Do not touch or collect any sample with your bare hands.

Sample container volume must be appropriate to sample size; fill the jar to just below the shoulder. Overfilled jars will break when they freeze; underfilled jars will allow the sample to dry out.

At least one field blank and replicate sample should be taken for each collection site, batch of samples or 20 samples taken. (A field blank is a sample container opened in the field, closed and stored as if it contained a sample. A replicate sample is a second sample from the same site.) Rinsate blanks should be taken if appropriate.

5.1 <u>Water</u> - The method must be described or adequately referenced in sampling SOPs. Recommended sample size is 1-4 liters depending on the analytical methodology.

Water samples for volatiles analyses should be taken in 40 ml amber vials with no head space or bubbles.

5.2 <u>Sediment</u> - Any accepted methods of collecting undisturbed surface sediment samples such as box cores, hand corers, or grabs may be used. The method must be described or adequately referenced in sampling SOPs. Recommended sample size is 10-100 grams (a 4 oz. jar).

5.3 <u>Tissue</u> - Organisms to be analyzed for petroleum hydrocarbons should be freshly killed or recently dead. Decomposed organisms are rarely of any value for analysis.

Whole organisms may be stored in solvent-rinsed glass jars or wrapped in solvent-rinsed aluminum foil.

Tissue sections may be taken either on site from freshly killed organisms or in the laboratory from carefully collected and preserved - cold or frozen - whole organisms. Tissue should include flesh and internal organs, especially liver. Recommended sample size is 10-15 grams.

Tissue samples need to be protected from external contamination at time of collection. Contents of the intestinal tract, external slime coating, contaminated collecting utensils, etc. are all potential sources of contamination when collecting internal tissue samples.

All instruments used in handling samples must be made of a non-contaminating material (e.g. stainless steel, glass, teflon, aluminum) and solvent-rinsed between each sample collection.

Instruments used for exterior dissection must not be used for internal dissection.

Avoid hand contact with tissue sample.

Collect stomach and intestinal tract last.

Bird eggs are wrapped in solvent-rinsed aluminum foil and transported by any convenient means that will prevent breakage. They should be opened or refrigerated as soon as possible. Eggs are opened by cutting them with a solvent-rinsed scalpel or by piercing the air cell end and pouring/pulling the contents out. Avoid including pieces of egg shell with the contents or touch-

ing the contents with your hands. Total weight, volume (measured or calculated), length, width and contents weight must be recorded for each egg. Bile is collected by removing the gall bladder, puncturing it with a scalpel fitted with a new #11 blade, and collecting the contents in a 4 mL amber glass vial.

6. Sample Preservation and Holding Time

Samples must be kept cool, i.e. on ice.

Samples that are to be frozen, sediment and tissue, should be frozen quickly and rapidly. That is, these samples should be frozen as soon after collection as possible and the freezing process should be rapid.

Frozen samples must be kept frozen, at -20°C or less, until extracted or prepared for analysis. Repeated freezing and thawing of samples can destroy the integrity of the samples resulting in questionable data or the loss of data.

6.1 Water - All water samples must be immediately extracted with methylene chloride or preserved with HCl to pH<2. If preserved, water samples are stored in the dark at 4°C and extracted within 7 days. All extracts must be stored in the dark in air tight chemically clean containers until analysis.

6.2 Sediment and Tissue - Samples should not be extracted until immediately before analysis; if there is a lag between sample extraction and sample analysis, extracts must be stored in air tight containers kept in the dark at 4°C.

7. Sample Shipping

All samples, except water samples, must be kept frozen throughout the shipping process.

Samples must be packaged to prevent breakage. Glass jars should be individually wrapped so that they will not contact each other if padding shifts in transit (which styrofoam chips do). Bubble wrap or the divided boxes that new jars are shipped in work well. Pack samples in insulated containers (e.g. ice chests) with enough frozen mass to remain frozen in transit.

It is the responsibility of the sample shipper to arrange for sample receipt. Do not send samples off without arranging for pickup and storage.

To insure that samples are not compromised, shipment should not be initiated later in the week than Wednesday nor should samples be shipped in any week in which there is a holiday.

Shipments must comply with Department of Transportation regulations.

8. Chain-of-Custody Procedure

Samples must be kept in such a manner that they cannot be altered either deliberately or accidentally. Any indication that a sample has been subjected to tampering or physical alteration could disqualify it as evidence for possible

legal action.

The field sampler is personally responsible for the care and custody of the samples collected until they are transferred under chain-of-custody procedures.

A sample is considered in "custody" if:

it is in your actual physical possession or view;

it is retained in a secured place (under lock) with restricted access

or it is placed in a container and secured with an official seal(s) such that the sample cannot be reached without breaking the seal(s)

Evidence tape or sample seals are used to detect unauthorized tampering of samples following sample collection. The seal must be attached in such a way that it is necessary to break it in order to open the container. Seals must be affixed to the container before the samples leave the custody of sampling personnel.

All samples must be accompanied by a chain-of-custody record or field sample data record (Figure 1). When samples are transferred from one individual's custody to another's, the individuals relinquishing and receiving the samples will sign and date the chain of custody record. This record documents the transfer of custody of samples from the sampler to another person or to a specified analytical laboratory.

Shipping containers must be custody-sealed for shipment. The seal must be signed before the container is shipped. The chain-of-custody record must be dated and

signed to indicate any transfer of the samples. The original chain-of-custody record accompanies the shipment; a copy is retained by the sample shipper. If samples are sent by common carrier, copies of all bills of lading or air bills must be retained as part of the permanent documentation.

Whenever samples are split, a separate chain-of-custody record is prepared for those samples and marked to indicate with whom the samples are being split.

APPENDIX F.2

NECROPSY HANDLING AND COLLECTION

HISTOLOGICAL SAMPLE PREPARATION FOR FISH

Histopathology Technical Group

NOTE: Only live or moribund fish will be suitable for processing. Histopathological changes caused by toxic chemicals are often very subtle at best. Tissues in dead fish autolyze very quickly and will mask these changes. Do not collect and process dead fish. Keep fish alive as long as possible during transport to the site of necropsy. Do not over-ice fish such that tissues freeze while in transit. Frozen tissues are worthless for histological examination.

1. The fixative to be used is 10% neutral buffered formalin (formula attached). Formalin should be handled wearing rubber or latex gloves.

2. The volume of fixative should be ten times the volume of the tissue. This is important since any less fixative may result in tissue autolysis and worthless samples. After 77 hours, the formalin fixative may be poured off and replaced with 70% ethyl alcohol for storage and transport. This accomplishes an important objective; i.e., it prevents tissues from becoming too hard and brittle when stored in fixatives for long periods. Also, the fixative poured off may be saved and strained of tissue fragments and used one more time for other samples.

3. The sample size per site or species will be 20 fish, live or moribund.

4. Fish less than 3 cm may be fixed whole by dropping into preservative.

5. Fish 4 cm-10 cm should have the belly slit with a scalpel or scissors, the intestine detached at the vent, and the internal organs pulled out slightly for proper fixative penetration.

6. Larger fish (11 cm-20 cm) will require on site excision of 0.5-cm sections of major tissues and internal organs (attached diagram) as listed. Do not send whole fish.

   Excise: Whole head detached from just behind the opercular opening, liver, spleen, GI tract (anterior intestine, stomach, pyloric cecae, posterior intestine, and rectum), air bladder, kidney (anterior and posterior), heart, gonads. Also, take a 0.5-cm square of musculature and attached skin intersected by the lateral line midway between the head and tail on the right side of the fish. Take a second 0.5-cm section of muscle and skin from the body wall covering the viscera from the right side of the fish.

   Organs and tissue samples from a single fish should be placed in tissue processing cassettes, 4 to 5 tissue samples to one cassette. Each cassette must be labelled with the animal number from which it was taken. Place cassettes in a jar of fixative.

   Fish larger than 20 cm will also require that 0.5-cm portions of each major organ be utilized (if larger than 0.5 cm) and the whole head will be eliminated from the sample. In this case the first right gill arch must be excised and fixed before

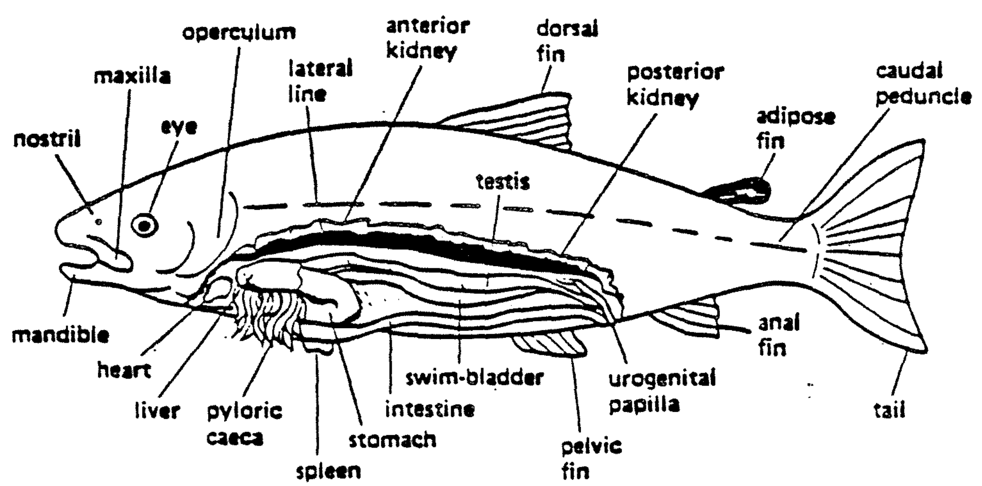


Fig. 2.6. Diagram of the basic anatomy of a salmonid fish.

NECROPSY FIELD DATA SHEET FOR HISTOLOGICAL SAMPLES
ADF&G, FRED Division Fish Pathology Lab

Collector/Address/Telephone #

Species

Number Specimens in Sample

Size Range

Life Stage

Date of Collection

Location of Collection (Site Name or Number)

Abnormalities Observed Per Specimen Number

PHOSPHATE BUFFERED FORMALIN

Fixative for Histological Samples of Fish, Bivalves, and Crabs
Histopathology Technical Group

1.	37%-40% Formalin	100.0 ml
2.	Tap water	900.0 ml
3.	$NaH_2PO_4 \cdot H_2O$	4.0 g
4.	Na_2HPO_4	6.0 g

1. Protozoa

Blood Smears

It is essential that slides used in making smear preparations be unscratched, noncorroded, and meticulously clean, free from grease, dust, acid, or alkali; that slides be handled by their edges; that the blood be taken as it exudes; that the process be done rapidly so as to prevent coagulation; and that smears be left to dry in a horizontal position away from flies and dust. The finger tip or structure to be pricked is cleaned with 70% alcohol, after which a prick is made with a blood lancet or a sterilized needle. The first drop is wiped off with absorbent cotton or gauze. Mark necessary data with wax pencil on the end of each slide. Blood films should be stained as soon as possible after drying to insure proper staining.

1. *Thin film*: On slide "A" place a drop of blood about one-half inch from the end. Take a second slide "B" and place it on the surface of the first slide at about a 45° angle, as indicated in Figure 259, and move it to the right until contact is made with the drop of blood. The free end of slide "B" may be supported by the third finger. As soon as it touches the blood, the latter will spread. Now push slide "B" toward the left, being careful to keep the edge pressed uniformly against the surface of slide "A."

In this way a thin smear with uninjured host cells and protozoans and/or microfilariae will be obtained. The size of the drop of blood and acuteness of the angle formed between the slides, will determine the thickness of the film, a more acute angle resulting in a thicker film. Allow film to dry thoroughly.

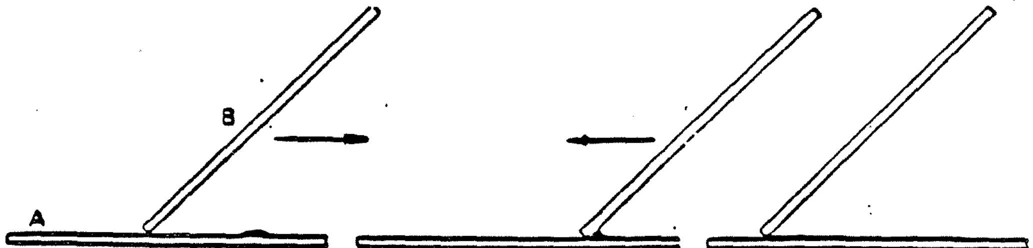


Figure 259. Preparation of thin blood smear.

epidermis overlying the heart and save for fixation, then proceed to uncover the visceral cavity. Also, fix a 1-cm square piece of the carapace.

c. Once the cavity is exposed, the heart, cardiac stomach, hepatopancreas, gonads (posterior to heart in Alaskan crabs), and gills become obvious (Figure 4).

d. Remove the right rear gill arch and take a 0.5-cm portion thereof.

e. Remove a 0.5-cm portion of the heart which will be beating if the animal has been freshly killed.

f. Remove a 0.5-cm square of hepatopancreas to the left of the heart.

g. Remove both antennal glands (green glands). Each lies on either side against the frontal carapace of the crab and is surrounded by urinary bladder and hepatopancreas (Figure 7). This can be a difficult organ to find and should be retrieved early on before other tissues are disturbed and landmarks are lost.

h. Remove the entire GI tract starting with the esophagus, which is ventral and anterior to the cardiac stomach (Figure 4) continuing with the entire stomach and intestine ending with the rectum that terminates at the vent on the ventral surface of the abdominal apron or flap underneath the crab. The intestine is long, curling down posterior to the heart (Figure 4) and extending anteriorly into the abdominal flap. It is fragile and requires some digging with forceps and cutting away from hepatopancreas with scissors to free the specimen. Remove 0.5-cm portions of the esophagus, cardiac stomach, pyloric stomach, midgut, hindgut, and rectum.

i. Remove a 0.5-cm section of the gonads also located posterior to the heart on either side. Gonads are part of the tissue in the way of extracting the intestine.

> Ovaries are large diameter, tubular organs that can be white, yellow, blue, or dark brown in color, depending upon the crab species.
>
> Testes are thin, very white, twisted threads containing viscous gametogenic material. Remove anterior, mid, and posterior lengths of the testes.

j. Expose the thoracic ganglion (Figure 7), which lies beneath the heart on the floor of the body cavity, by removing the residual hepatopancreas. Remove a 0.5-cm portion of the thoracic ganglion. The correct organ has been obtained if severance of the radiating peripheral nerves causes violent twitching of the respective walking leg of the crab, if the animal has been freshly killed.

NECROPSY FIELD DATA SHEET FOR HISTOLOGICAL SAMPLES

ADF&G, FRED Division Fish Pathology Lab

Collector/Address/Telephone #

Species

Number Specimens in Sample

Size Range

Life Stage

Date of Collection

Location of Collection (Site Name or Number)

Abnormalities Observed Per Specimen Number

HISTOLOGICAL SAMPLE PREPARATION FOR SHRIMP

Histopathology Technical Group
(Taken from Bell and Lightner 1988)

NOTE: Only live or moribund shrimp will be suitable for processing. Histopathological changes caused by toxic chemicals are often very subtle at best. Tissues in dead shrimp autolyze very quickly and will mask these changes. Do not collect and process dead shrimp. Keep shrimp alive in containers of seawater if they must be transported to the processing site. Also, minimize the handling stress on the live shrimp to be preserved so that stress-mediated histological artifacts do not occur. Do not over-ice animals such that tissues freeze while in transit. Frozen tissues are worthless for histological examination.

1. The fixative to be used is 10% neutral buffered formalin (formula attached). Formalin should be handled wearing rubber or latex gloves.

2. The volume of fixative should be ten times the volume of the tissue. This is important since any less fixative may result in tissue autolysis and worthless samples. After 72 hours, shrimp specimens should be transferred to 70% ethyl alcohol for shipment and storage. This prevents tissues from becoming too hard and brittle when stored in fixative for long periods. Also, the fixative poured off may be saved and strained of tissue fragments and used one more time for other samples.

3. The sample size per site or species will be 20 shrimp, live or moribund.

4. The chitinous exoskeleton of shrimp prevents adequate penetration of any fixative by simple immersion. Consequently, the fixative must be injected into strategic internal areas of each animal prior to dropping the whole shrimp into the fixative. Inject fixative into the living shrimp using a 10-ml syringe and appropriately sized needle, depending upon the size of the animal (small shrimp; i.e., small-gauge needle). This procedure is described by the following:

   a. First inject laterally into the hepatopancreas; i.e., cephalothorax region (Figure 1a).
   b. Then inject dorsally into the region anterior to the hepatopancreas; i.e., between the thorax and the eyestalks (Figure 1b).
   c. Inject the posterior abdominal region (Figure 1c).
   d. Inject the anterior abdominal region (Figure 1d).

   Inject more of the fixative into the hepatopancreas than the other sites but overall use about 5%-10% of the shrimp's body weight. All signs of life should disappear.

   e. Immediately after injection, slit the cuticle of the animal from the last (6th) abdominal segment to the base of the rostrum. The incision in the cephalothoracic region should be just lateral to the dorsal midline and that in the abdominal region should be mid-lateral (Figure 2). Do not cut too

Table 1.--Definition of times used on the Haul-Position form.

Time Start	--	Time when the trawl begins to be pulled off the deck. Always use local time indicating time zone and daylight or standard time.
Time Out	--	Time when the amount of cable specified for the haul is out and the brakes on the trawl winch have been set.
Equilibrium Time	--	Time when the gear reaches bottom. Would be same as Time out without on-bottom indicator. Duration is computed as the difference between the Equilibrium time and the Haul time.
Haul Time	--	Time when trawl winches begin retrieving the gear. Wit on-bottom indicator would be the time when gear leaves bottom.
Time In	--	Time catch is on deck.

C. Length-Frequency form

Data from the length-frequency strips will be transcribed to these forms in addition to some haul identification data, the weight of the length-freque subsample, and the total catch weight for the species (Figure 5).

D. Specimen data form

All length-weight, length-fecundity, and length-maturity data collected from individual fish will be transcribed on this form. Entries will be made using the ADP and species code book. At the top of the form enter columns 1 vessel, cruise, and haul. Columns which are not applicable will be left bla See Figure 6 for an example of a completed form.

The field party chief will return all original data to Seattle persona. It is important that data forms be completely filled out, the information

coded, and entries checked prior to the completion of each leg. It is suggested that this work be kept up on a daily basis if possible. When data form have been double-checked, initial upper right-hand corner of page and date.

Immediately upon return to the laboratory, data will be punched onto cards for further processing and analysis.

LITERATURE CITED

Hughes, S.E.

1976. System for sampling large trawl catches of research vessels. J. Fish. Res. Board Can. 33(4): 833-839.

APPENDIX I

MATURITY INDICES FOR POLLOCK AND ROCKFISH

WALLEYE POLLOCK--FIVE POINT MATURITY SCALE

Code	Gonad Condition	Description
1	Immature	Testes thread-like. Testes contained within a transparent membrane. Ovaries tapered and paired (in fish as small as 10 cm), transparent also. (Will not spawn this year.)
2	Developing	Testes uniformly ribbon-like. Ovaries tapered, forming two distinct lobes having well-developed red blood vessels. Surface of testes appear smooth and uniformly textured; ovaries can be somewhat granular (some ova are distinct). (Time of spawning --whether later this year or next year--not apparent.)
3	Mature	Ova are distinctly visible but cannot be extruded when ovaries are compressed. Ovaries form two large distinct lobes. Testes large and highly convoluted, sperm cannot be extruded. Body wall incision causes gonads to be expelled from opening (both sexes). (Will spawn this year.)
4	Spawning	Sperm and ova extruded when gonads are compressed or ova are loose in ovaries and testes milk freely. (Spawning.)
5	Spent	Gonads large but flaccid and watery. Ovaries may contain remnants of disintegrating ova and associated structures. Testes bloodshot.

ROCKFISH MATURITY CODES

MALES

Code	Maturity Stage	Description
1	Immature	Testes string-like, translucent or translucent white.
7	Maturing	Testes large and swollen, somewhat rounded in cross-section, white. Sections of fresh testes produce free-flowing sperm.
8	Copulation	Milt can be expressed by applying pressure on the body. Testes divided into an inner layer of lighter color with sperm in ampullal, and a more transparent outer layer with voided ampullae.
9	Sexually Inactive	Testes ribbon-like, triangular in cross-section, brown. Sections of fresh testes do not have free-flowing sperm.

FEMALES

Code	Maturity Stage	Description
1	Immature	Ovary small and translucent or small and yellow.1/
2	Maturing	Ovary firm, eggs yellowish and opaque.
3	Yolk Cleared (Eggs Fertilized)	Ovary not firm, eggs yellowish and translucent.
4	Ripe (With Embryos or Larvae)	Ovary not firm, eggs translucent with black dots or visible larvae.
5	Spent	Ovary large and flaccid with a reddish-purple or dark grey color.
6	Sexually Inactive	Ovary firm, grey or pink, some with black blotches.

1/ Several species including *S. aleutianus*, *S. borealis*, *S. brevispinis*, *S. crameri*, *S. entomelas*, and *S. paucispinis* have been found to have cream-colored ovaries.

GONAD CONDITION INDICES FOR HALIBUT, POLLOCK, ROCKFISH, AND DOVER SOLE

POLLOCK

Immature ovary -- ovary small, color varies from opaque to reddish, eggs small, non-yolked, diameter less than 0.4-0.5 mm (Summer-Fall).

Immature ovary -- eggs still opaque but diameter 0.8-0.9 mm, eggs clearly noticeable through ovarian membrane (Nov.-Jan.).

Mature --

(a) mixture of opaque and mature transparent eggs, diameter 1.3-1.5 mm.

(b) transparent eggs in aggregate form are seen in the center or the periphery of the ovary.

(c) mostly transparent eggs, percent of opaque eggs is small.

Spent ovary -- the ovary shrinks and the ovarian membrane becomes very thick.

HALIBUT

Immature testes usually small, fiber-textured and pink colored. Mature testes especially soft and plump, pink to whitish in color and enlarged.

DOVER SOLE

Immature testes -- small and yellowish.

Ripening testes -- large sperm, evident, whitish.

Spent testes -- shrunken, yellow, brown and green in color.

Immature ovary -- small, pink, somewhat gelatinous.

Gravid ovary -- full, yellowish with granular eggs becoming transluce[nt] at spawning.

Spent ovary -- flaccid, few translucent eggs, membrane bloodshot and sac-like.

Resting ovary -- becoming firm, no eggs discernible to naked eye, pinkish, gelatinous.

ROCKFISH

Maturing ovary -- firm and yellow.

Mature ovary -- eggs fertilized.

(a) Eggs translucent

(b) With visible larvae

Spent ovary -- not firmn, flabby, red or gray.

Transitional ovary -- firm and small, dark gray.

HAKE

Immature ovary

(a) Small, eggs not yolked, pinkish translucent

(b) Small, eggs yolked

(c) Large, eggs yolked

(d) Large, some eggs translucent.

Mature ovary -- large, all eggs translucent.

APPENDIX II

COLLECTION AND STORAGE OF OTOLITHS AND SCALES

The clearness of the age marks on otoliths and scales depends greatly on the collection and storage procedures used. The following notes are intended to be of assistance to those who have had little or no practical experience i the collection of age structures. Also, current information is summarized on the age structures and storage media preferred by the Age Determination Unit for the various species in the North Pacific Region (Table 1).

Removal of the Age Structure From the Fish

OTOLITHS

The method to remove the otoliths is to cut open the head (Fig. 1), exposing the cavities in which the otoliths are located (Figs. 2-3). A knif is usually sufficient to make the cut, although a hacksaw may be useful for large specimens. The otoliths are in the otic capsule, a cavity at the base of the skull. A few exploratory cuts and probings in the skull cavities wil usually be necessary to get the "feel" for the location of the cut and to fi the otoliths. Frequently, the otoliths can be quite difficult to locate.

There are six otoliths in the otic capsule, three on each side. The sagitta (Fig. 4), by far the largest and usually the only one readily visib is the one that is collected. Its size varies with the species and the siz of the fish and will range from about as small as a grain of rice to as lar as 4 cm.

The otolith is easily removed with tweezers or the fingers. Rinse the otolith in running water or in a bucket to remove slime and tissue. Then, store it in the appropriate media (Table 1) and container. Small paper

envelopes are usually used when storage is dry, and glass or plastic vials, leak-proof compartmented boxes, or plastic envelopes are used when storage is wet. The otoliths usually must be identified so that date of collection, area and other information can be related to them. It is very important to have a clear understanding of the scheme used to identify the otoliths being collected. A mistake in the numbering sequence or procedure used to relate the otolith to associated biological and time-area data can make a collection useless. If at all possible, practice the entire procedure on a few fish of each type (flatfish and roundfish) before attempting to process large samples.

SCALES

At present scales are collected from Pacific cod and lingcod. The procedure is to take a scrape sample (see below) of about 50 scales from A (the preferred zone), or B (next preferred zone), on either the right or left side of the fish (Fig. 5). If scales are missing from these zones take them from any location (zone C). The scales are usually stored in a coin envelope.

SAMPLING PROCEDURE

1. Examine fish and select zone A, B, or C. RECORD ZONE on envelope or data sheet.
2. Wipe the area to be sampled with a sponge, paper towel, or cloth. This is to minimize contamination of the sample with scales of other fish.
3. Using any thin edged instrument (knife, scalpel), scrape within the zone in an anterior direction (toward the head).
4. Wipe off inside the coin envelope the scales that adhere to the instrument. Be certain the envelope is properly labeled.
5. Remove excess scales from instrument before sampling the next fish.

REMARKS

These instructions may be modified from time to time. The best age structure and storage media has not been determined for some species. On occasion, determined by the biologist in charge, two different age structures or both otoliths may be collected.

These instructions are based on ideal sampling conditions. It is recognized that strict adherence to the methods will sometimes be impossible or impractical. Keep a record of the deviations from instructions so that the effect can be evaluated.

Table 1.--Age structure and storage media for flatfish and roundfish.

Type of Fish	Age Structure	Storage Media
Roundfish		
Pacific cod	Scale	Dry in envelope
Lingcod	Scale	Dry in envelope
Rockfish, pollock, sablefish, and other roundfish	Otolith from right side[1]	50% Ethyl Alcohol
Flatfish	Otolith from eyed side[1,2]	Dry and clean

1 - If preferred otolith is damaged, take from other side.

2 - Take both otoliths from Greenland halibut (turbot) and take both otoli· from all flatfish when possible.

APPENDIX III

Predator Information

(1) Specimen number (0001 to 9999)

Each individual examined must have a number assigned to it that is unique within each haul. For example, haul 1 might have specimen numbers 1-20, and haul 2 might have specimen numbers 1-52. The unique identifiers for each individual are the vessel code, haul number, and specimen number (VVHHH, NNNN). These should be recorded on specimen labels if stomachs are preserved for later analyses in the laboratory.

(2) Stomach fullness index (0 to 10)

0 = empty stomach

1 = 10% full

10 = 100% full (fully distended)

(3) Food condition code (1 to 5)

1 = fresh, no digestion

5 = extremely digested (mush)

Prey Information

If the stomach was empty, this information is left blank. If a stomach did contain identifiable prey or other materials, then an attempt should be made to identify the contents as best possible, record the amount of each food item, and if possible, record each item's mean size.

(1) Contents should be identified by name and 5-digit species code. If an appropriate code is not available, leave the code area blank until your return to Seattle.

(2) The amount of each food item should be accurately and consistently evaluated within a cruise. Data should be entered into at least one of the three columns, "Percent," "Weight," or "Number," for each food item.

"Percent" = Estimated percentage of total stomach contents (by volume). For most field programs, this is the observation that should be consistently made for all predator samples and food items.

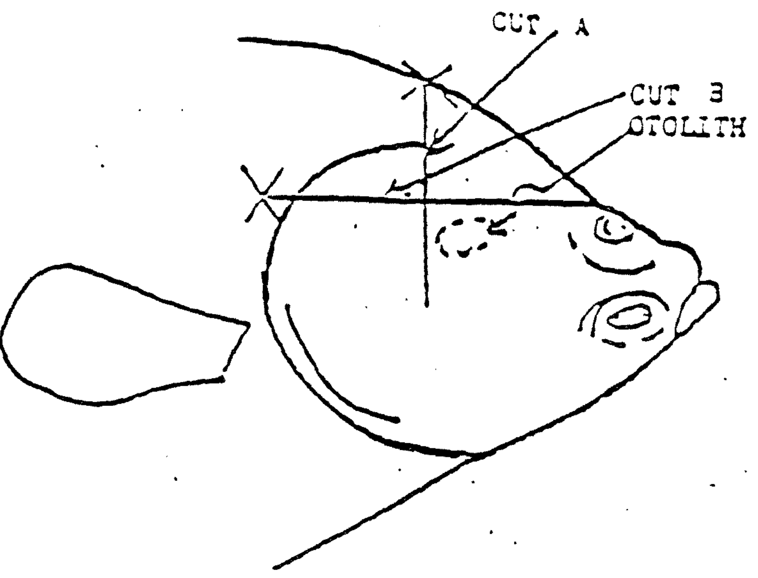
"Weight" = Wet weight. A weight should only be entered if it has been accurately determined using a laboratory balance. Otherwise, leave this field blank.

"Number" = Number of prey individuals. For some food items, it may be possible to accurately count or make a good estimate of numbers Only good estimates should be recorded. Otherwise, leave this field blank.

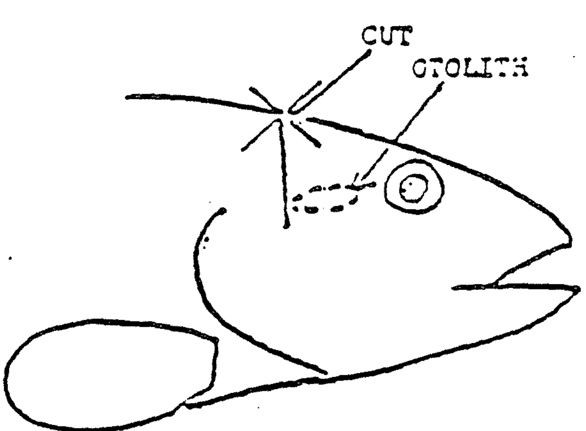
(3) The approximate mean size (mm) of prey items should be recorded under th column headed "Size." Leave this field blank if difficult to describe prey size.

COMMONLY-USED PREY CODES

00003	Fish unident
50000	Annelid worm unident
60100	Amphipod unident
63500	Euphausid unident
64500	Copepoda unident
79000	Squid unident
99990	Invertebrate unident
99992	Unidentifiable stomach contents
66000	Shrimp unident



FLATFISH



ROUNDFISH AND ROCKFISH

Fig. 1 Approximate location of the otoliths (sagitta) and the cu
the removal of otoliths from flatfish, roundfish and rock
(Use either cut A or Cut B for flatfish)

FLATFISH ROUNDFISH ROCK

Top view

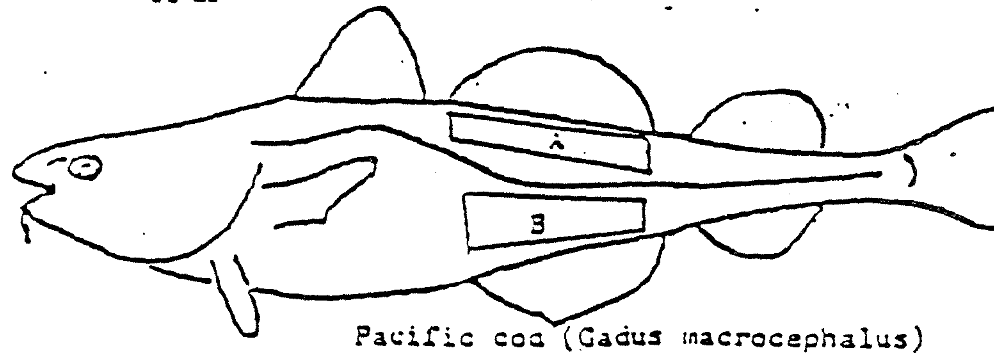
Side view

Yellowfin sole (Limanda aspera)

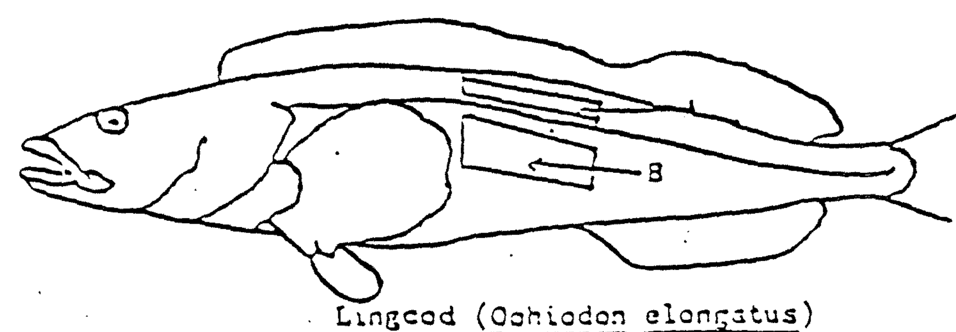
Walleye pollack (Theragra chalcogramma)

Pacific Ocea (Sebastodes

Fig. 4. Diagrammatic sketches giving top and side view of represent of flatfish, roundfish, and rockfish.



Pacific cod (Gadus macrocephalus)

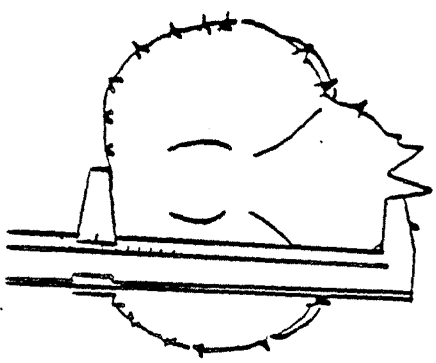


Lingcod (Ophiodon elongatus)

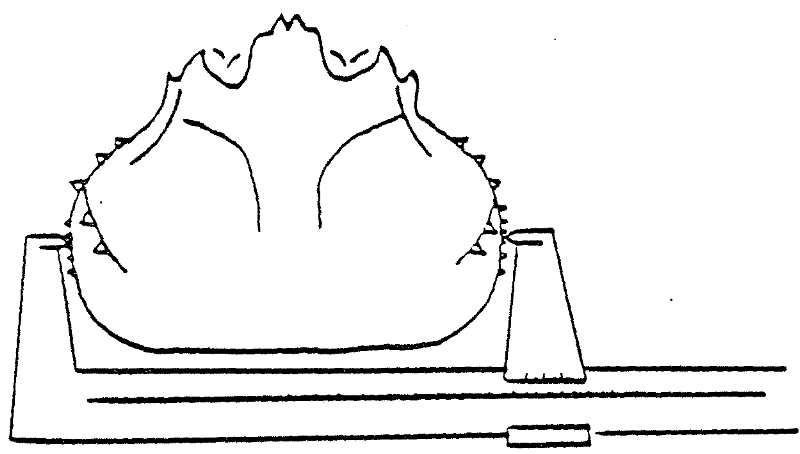
Fig. 5. Body location for collecting scales from Pacific cod and Lingcod. A is preferred zone and B is next preferr All other zones are zone C.

APPENDIX IV

Item	Column	Code Instructions
Carapace Length (for king crabs)	30-32	Enter measurement to nearest mm.



Carapace Width (for Tanner crabs)	33-35	Enter measurement to nearest mm.



Shell Condition	36	0-Molting
		1-Carapace--soft and pliable, brick r
		2-Carapace--firm to hard, clean, bric red to yellow brown on topside; (gr colored crabs were sometimes encoun in certain areas of Bristol Bay) ep limited except that leech cases may sometimes be present; dactyli relat sharp; pterygostomial and branchial

Item	Column	Code Instructions
	36	sharp; thoracic sternum and underside of legs white to yellowish, white, with none or only a few scratches; spines on meri and metabranchial region sharp, we defined not rounded.
		3-Carapace hard; topside usually yellow to yellowish brown; thoracic sternum and underside of legs yellow with numerous scratches; pterygostomial and bronchial spines worn and polished; dactyli on meri and metabranchial regio rounded; epifauna (barnicales and leech cases) usually present but not always.
		4-Carapace hard, topside yellowish-brown to dark brown; thoracic sternum and undersides of legs dark yellow with many scratches and dark stains; pterygolstomial and branchial spines rounded with tips sometimes worn off; dactyli very worn sometimes flattened on tips; spines on meri and metabranchi region worn smooth, sometimes completel gone; epifauna most always present (large barnacles and bryozoans).
		5-Conditions observed in shell condition #4 much advanced; large epifauna almost completely covers crab; carapace is worn through in temabranchial region, along pteygostomial branchial spines, or on meri; dactyli flattened sometimes worn through, mouth parts and eyes some times nearely immoblized by barnicles.
Egg color	37	2-purple 3-brown 4-orange 5-purple-brown
Egg condition	38	2-eyed 3-dead

Item	Column	Code Instructions
Clutch size	39	0-immature 1-no eggs 2-trace-1/8 full 3-1/4 full 4-1/2 full 5-3/4 full 6-full 7-eggs bulging outside tail flap
Species code	40	1-Lithodes aequispina (Golden king crab) 2-Paralithodes camtschatica (Red king crab 3-P. platypus (Blue king crab) 4-P. brevipes (Brown king crab) 5-P. sp. (king crab?sp.) 6-Chionoecetes bairdi 7-C. opilio 8-C. sp. 9-C. bairdi/c.opilio hybrid
Weight	41-45	Leave blank
Primary sampling factor	49-55	Enter primary sampling factor denominator "N" in Columns 52-55.[1] Leave Comumns 45-51 blank. Note: If small crab sample, enter weight of baskets sampled in Columns 49-51. Enter total weight of crabs in Columns 52-55. See Requirements for small crab samples.
Secondary sampling factor	56-57	Enter secondary sampling factor denominatc "N" in Columns 56 & 57.[2]
Station type	59	2-Vessel comparison tow. 3-Gear comparison tow. 4-Intra block variance tow. 5-Tag release tow.
	60	0-Extra tow (not used for a monitor tow). 1-Monitor tow in center of station square 2-Monitor tow on verticle edge of statior 3-Monitor tow on horizontal edge of stati 7-Monitor tow on station corner. 8-Monitor tow inside boundries of statior square-but not in center.

1. See step 3a to determine Primary Sampling Fraction
2. See 3c to determine Secondary Sampling Fraction.

Item	Column	Code Instructions
Area Tow	62-64	Leave blank
Tow	78-80	Enter haul number, consecutive throu all legs of curise, for all station except vessel comparative hauls. For vessel comparative tows enter 9 Column 78 and number in sequence sta from 1st comp. tow. (i.e., 901, 902,

9. The Crab Data Summary Form (Front Page).

The Crab Data Summary Form should be filled out accurately and neatly to avoid some of the errors found during the last BLM/OCS cruise. Fill out this form as soon as possible while the information is still fresh in your mind. This form should be double checked to make sure all data agrees with the Crab Data Forms and wheel house log books.

Item	Column	Code Instructions
Vessel	1-2	Same as Crab Data Form Columns 1 and 2.
Cruise	3-5	Enter 761.
Date	6-11	Same as Crab Data Form Columns 7-12.
Station	12-14	Same as Crab Data Form Columns 4-6.
Tow	15-17	Same as Crab Data Form Columns 78-80.
Position	18-65	Enter beginning and ending LORAN C and Latitude-Longitude co-ordinates from wheel house log book. Omit 1 for all degrees of longitude. DG=Degrees. MN=Minutes. D=Direction E (east) W (west)
Distance towed	66-68	Enter estimate of distance towed, to nearest tenth of a mile, from wheel house log book.
Average Depth	69-71	Enter average depth of tow. Calculate by adding start depth to end depth and dividing by two. (Use start and end depths recorded in wheel house log book.
Bottom Temperature	72-75	Enter bottom temperature in degrees centigrade to nearest tenth degree. Record any minus signs (-) in Column 72. Leave blank if unknown.
Station Type	76-77	Same as Crab Data Form Columns 59-60.

(Back Page)

Item	Column	Code Instructions
Station	1-3	Same as Crab Data Form Columns 1-2 and Front Page Columns 12-14.
Tow	4-6	Same as Crab Data Form Columns 78-80 and Front Page Columns 15-17.
Numbers Measured & Caught	7-76	Enter total numbers of crabs measured and caught, by sex and species. If the catch for this haul was subsampled the numbers caught is determined by multiplying the numbers measured by the primary sampling factor ('N", or weight factor for small crabs) or the product of the primary and secondary sampling factor (if a secondary subsample was take If the catch was not subsampled, the numbers measured and the numbers caught should be the same.
Small Crab Species Codes	77-80	Leave blank.

FIG 1 (APPENDIX A.2

NMFS/ADFG OSIA PRINCE WILLIAM SOUND
MULTI-SPECIES TRAWL SURVEY
RESPONSIBILITY ASSIGNMENT SHEET

VESSEL ____________ SURVEY NUMBER ____________
SURVEY LEG ________ DATES ___/___/___ TO ___/___/___
CHIEF SCIENTIST ____________

CHAIN OF CUSTODY FORMS ____________
TRIP REPORT FORM ____________
PMC REPORT ____________

NECROPSY:
1. PROCESSING SAMPLES ____________
2. SAMPLE RECORDING FORMS ____________
3. ONBOARD SAMPLE SECURITY AND CUSTODY ____________
4. CHAIN OF CUSTODY FORMS ____________
5. SHORESIDE SAMPLE SECURITY AND CUSTODY ____________
6. FINAL LOCATION AND SHIPPING ____________

HYDROCARBON:
1. PROCESSING SAMPLES ____________
2. SAMPLE RECORDING FORMS ____________
3. ONBOARD SAMPLE SECURITY AND CUSTODY ____________
4. CHAIN OF CUSTODY FORMS ____________
5. SHORESIDE SAMPLE SECURITY AND CUSTODY ____________
6. FINAL LOCATION AND SHIPPING ____________

FINFISH DATA:
1. **DATA COLLECTION** ____________
2. **DATA ENTRY** ____________
3. **DATA FORM CUSTODY** ____________
4. **DATA DISK CUSTODY** ____________
5. **DATA ERROR CORRECTION** ____________

SHELLFISH DATA:
1. DATA COLLECTION ____________
2. DATA ENTRY ____________
3. DATA FORM CUSTODY ____________
4. DATA DISK CUSTODY ____________
5. DATA ERROR CORRECTION ____________

CONTINUED ON BACK

Figure 6.

SPECIMEN FORM

VESSEL [1 2 3] ______________________ CRUISE [5 6 7] HAUL [9 10 11]

STRATUM [13 14 15] SPECIES CODE [17 18 19 20 21] SPECIES NAME ______________________

FREQUENCY [36: 1] SUBSAMPLE TYPE [48] WEIGHT DETERMIN. [49] AGE STRUCTURE [50] AGE DETERMIN. [51]

MATURITY TABLE [59 60] YOUR NAME ______________________ DATE ______________

23	25	28 29 30 31	37 38 39 40 41	45 46	53 54 55 56 57	62 63 64 65 66	68 69 70 71 72
SEX	MATUR	LENGTH IN MM.	WEIGHT (GRAMS)	AGE	SPECIMEN NUMBER		

LENGTH-FREQUENCY FORM

VESSEL [1 2 3] ________ CRUISE [5 6 7] HAUL [9 10 11]

DATE ________ TOTAL WEIGHT (LB.) ________ SUBSAMPLE WEIGHT (LB.) ________

SPECIES CODE [13 14 15 16 17] SPECIES NAME ________

YOUR NAME ________ SUBSAMPLE TYPE [32] LENGTH TYPE (IF NOT FORK LEN.) [33 34]

19 20 21 22 23 LENGTH IN MM.	26 27 28 FREQUENCY	30 SEX
0		
0		
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0		
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19 20 21 22 23 LENGTH IN MM.	26 27 28 FREQUENCY	30 SEX
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APPENDIX H.2

(pg 1 of 3)

ADF&G / NMFS MULTISPECIES TRAWL SURVEY
CRAB DATA FORM

SAMPLERS NAME ______________ SPECIES NAME ______________

VESSEL [ ] CRUISE [ ] HAUL [ ] STATION [C01] TOTAL CATCH WEIGHT [ ]

TOTAL CRAB WEIGHT [ ] TOTAL WEIGHT OF CRAB SPECIES X [ ] SUBSAMPLE WEIGHT [ ]

TOTAL NUMBER OF CRAB [ ] TOTAL NUMBER OF CRAB SPECIES X [ ] TOTAL NUMBER IN SUBSAMPLE [ ]

COMMENTS ______________

	SPECIES	SEX	CARAPACE LENGTH	CARAPACE WIDTH	SHELL COND.	EGG	CLUTCH SIZE	BLACK MAT D	V	L	WEIGHT	SPECIMEN NUMBER
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												
26												
27												
28												
29												
30												

Crab Species Codes: 1=golden king crab; 2=red king crab; 3=blue king crab; 4= hair crab; 5=Telmessus crab; 6=C. bairdi; 7=Dungeness crab; 8=Cancer angulatus; 9=Cancer (hybrid)

Sex Code: 1=male; 2=female; 3=unidentifiable

Shell Age Code: 0=molting; 1=soft & pliable; 2=firm to hard, clean; 3=hard, scratches; 4=hard, worn; 5=hard, wishes it were 2

Egg Color Code: 0=no eggs; 2=purple; 3=brown; 4=orange; 5=purple-brown; 6=pink

Egg Condition Code: 0=no eggs; 1=uneyed eggs; 2=eyed eggs; 3=dead eggs; 4=empty egg cases-filamentous material attached to pleopods

Clutch Size Code: 0=immature; 1=mature female/no eggs; 2=trace 1/8 full; 3=1/4 full; 4=1/2 full; 5=full; 6=eggs bulging tail flap

APPENDIX H.3

ADF&G / NMFS MULTISPECIES TRAWL SURVEY
SHRIMP SUBSAMPLE DATA ENTRY FORM

SAMPLERS ______________________ VESSEL ☐☐ LANDING DATE (mm dd yy) ☐☐ ☐☐ ☐☐ SPECIES NAME ________

CRUISE ☐☐☐ HAUL ☐☐☐ TOTAL SHRIMP CATCH ☐☐☐☐☐ SUBSAMPLE WEIGHT ☐☐☐☐ NUMBER IN SUBSAMPLE ☐☐☐ SPECIES ☐☐☐

0.55 mm RANGE MIDPOINT	# MALE	# TRANS.	# NON OVIGER. FEMALES	# OVIGER. FEMALES	TOTAL
55					
60					
65					
70					
75					
80					
85					
90					
95					
100					
105					
110					
115					
120					
125					
130					
135					
140					
145					
150					
155					
160					
165					
170					
175					

0.55 mm RANGE MIDPOINT	# MALE	# TRANS.	# NON OVIGER. FEMALES	# OVIGER. FEMALES	TOTAL
180					
185					
190					
195					
200					
205					
210					
215					
220					
225					
230					
235					
240					
245					
250					
255					
260					
265					
270					
275					
280					
285					
290					
295					
300					

APPENDIX H.4

ADF&G / NMFS MULTISPECIES TRAWL SURVEY
SHRIMP SUBSAMPLE TALLY FORM

MPLERS ______ VESSEL ☐☐ LANDING DATE ☐☐ (mm) ☐☐ (dd) ☐☐ (yy) SPECIES NAME ______

UISE ☐☐☐ HAUL ☐☐☐ TOTAL SHRIMP CATCH ☐☐☐☐☐ SUBSAMPLE WEIGHT ☐☐☐☐ NUMBER IN SUBSAMPLE ☐☐☐ SPECIES ☐☐☐

.55 mm RANGE IIDPOINT	# MALE	# TRANS.	# NON OVIGER. FEMALES	# OVIGER. FEMALES	TOTAL
7 5					
8 0					
8 5					
9 0					
9 5					
1 0 0					
1 0 5					
1 1 0					
1 1 5					
1 2 0					
1 2 5					
1 3 0					
1 3 5					
1 4 0					
1 4 5					
1 5 0					
1 5 5					
1 6 0					
1 6 5					
1 7 0					

0.55 mm RANGE MIDPOINT	# MALE	# TRANS.	# NON OVIGER. FEMALES	# OVIGER. FEMALES	TOTAL
1 7 5					
1 8 0					
1 8 5					
1 9 0					
1 9 5					
2 0 0					
2 0 5					
2 1 0					
2 1 5					
2 2 0					
2 2 5					
2 3 0					
2 3 5					
2 4 0					
2 4 5					
2 5 0					
2 5 5					
2 6 0					

APPENDIX I

SHRIMP SEX DETERMINATION

APPENDIX I

STANDARD OPERATING PROCEDURE
FOR SEX DETERMINATION OF PANDALID
SHRIMPS IN PRINCE WILLIAM SOUND

by

Charlie Trowbridge

October 10, 1989

Determining the sex of a Pandalid shrimp is best accomplished using secondary sexual characteristics. Sexing based upon primary sex organs is difficult and time consuming. Using a secondary sex characteristic such as endopod development, which closely tracks the gonad development, allows sex to be determined with relative ease. The sex of large Pandalids such as P. platyceros can be found by visual inspection, without aid of magnification.

Materials used in sexing are a sharp needle probe, a good light preferably with a dark background, and a source of magnification, two to seven power (2x-7x) in strength in order to clearly view endopod characteristics. A visor with two-power optics in place has worked well however a stronger magnification would be helpful for small specimens.

Pandalid shrimp in Alaska are typically protandric hermaphrodites. Therefore three sexual stages can be identified, male, female, and transitional. Sexing of Pandalid shrimp in Prince William Sound is performed according to Butler's description in his work Shrimps of the Pacific Coast of Canada (1980).

The size of a shrimp may give some indication of its sex eg. a small specimen would probably be a male and a large specimen a female. The endopod of the first pleopod is first inspected. If the endopod terminates in two rounded lobes approximately equal in length it is a male. Depending upon specimen size and magnification a small clump of "hooklike setae" may be visible on the inner lobe. A female will be indicated by the endopod of the first pleopod terminating in a single firmly-pointed lobe. A transitional could be described as being intermediate between the characteristics of the male and the female in that the two lobes are still present however the inner lobe is shrunken to form a small stiff appendage which may be hidden along the inner margin of the endopod. The outer lobe of the endopod in the transitional is usually somewhat larger, firmer, and more pointed than the male stage.

The second pleopod may also be inspected to determine sex and Butler cautions that for a neophyte this is the characteristic to use first. A male can be identified as having two small processes

nearly the same length branching from the medial end of the endopod. The inner process is the appendix masculina and will have spines along its tip. The outer process is the appendix interna which will be tipped with "hooklike setae". In the female only the appendix interna will be present. A transitional may be identified as having both processes with the appendix masculina being approximately one-half (or less) the length of the appendix interna.

Allen (1959) gives a detailed account of these same morphological changes which aid in determination of sex in Pandalus borealis. Drawings in his paper are more extensive than those in Butler but both authors appear to agree on the use of endopods in sexing Pandalid shrimp.

BIBLIOGRAPHY

Allen, J. A. 1959. On the Biology of Pandalus borealis Kroyer, with reference to a population off the Northumberland Coast. Journal of Marine Biology Association U.K. 38:189-220 Great Britain

Butler, T. H. 1980. Shrimps of the Pacific Coast of Canada. Canadian Bulletin of Fisheries and Aquatic Sciences 202, Department of Fisheries and Oceans, Ottowa.

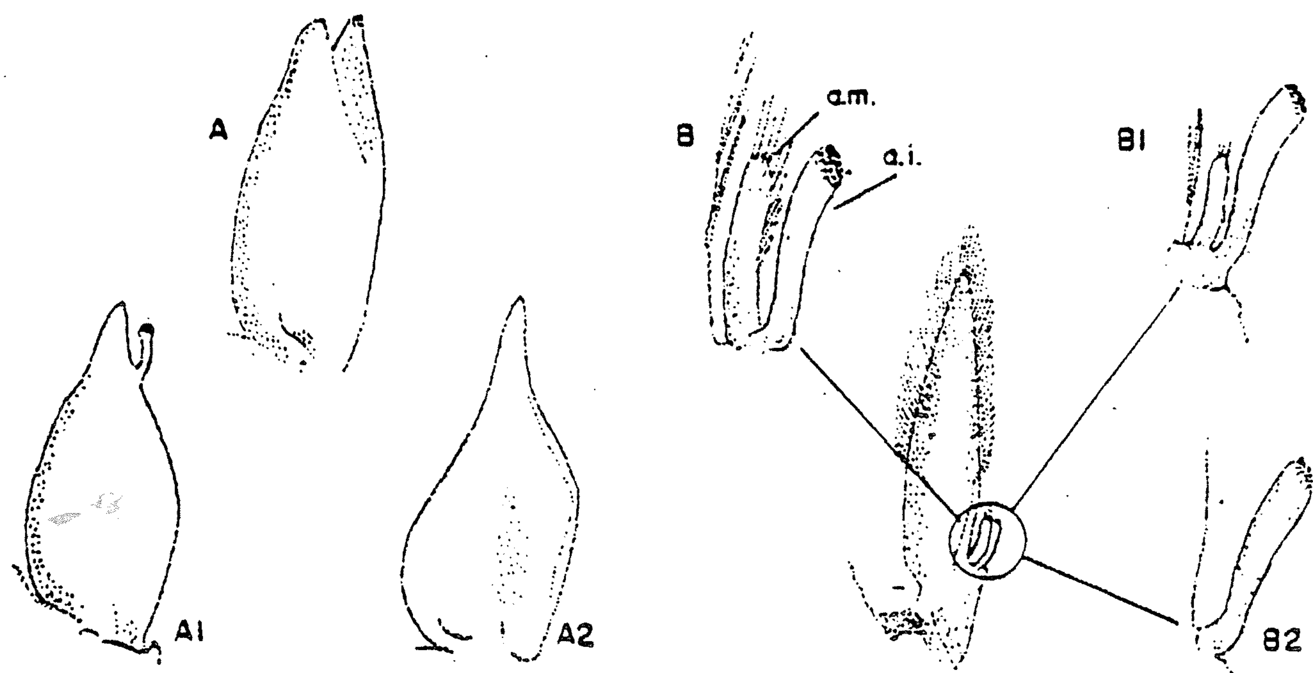


FIG. 1. *Pandalopsis dispar.* Endopod of first pleopod: (A) active male phase. (A_1) transitional phase. (A_2) female phase. Endopod of second pleopod: (B) active male phase. (B_1) transitional phase. (B_2) female phase. a.i., appendix interna; a.m., appendix masculina. (from Butler, 1980)

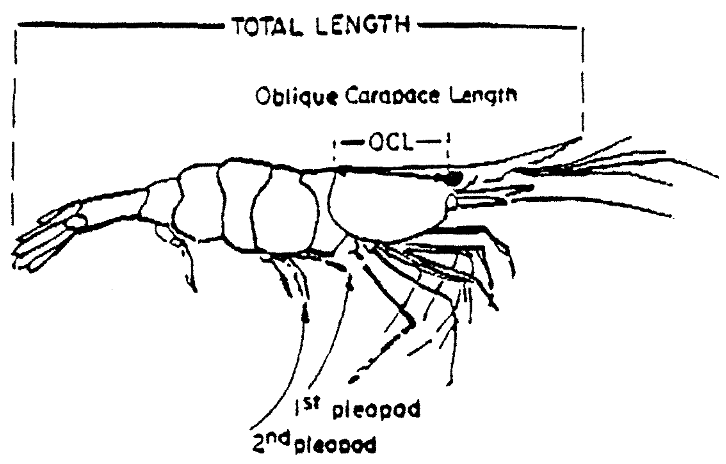


Figure 9.—Method of measuring the carapace of *Pandalus borealis* and location of the first and second pleopods.

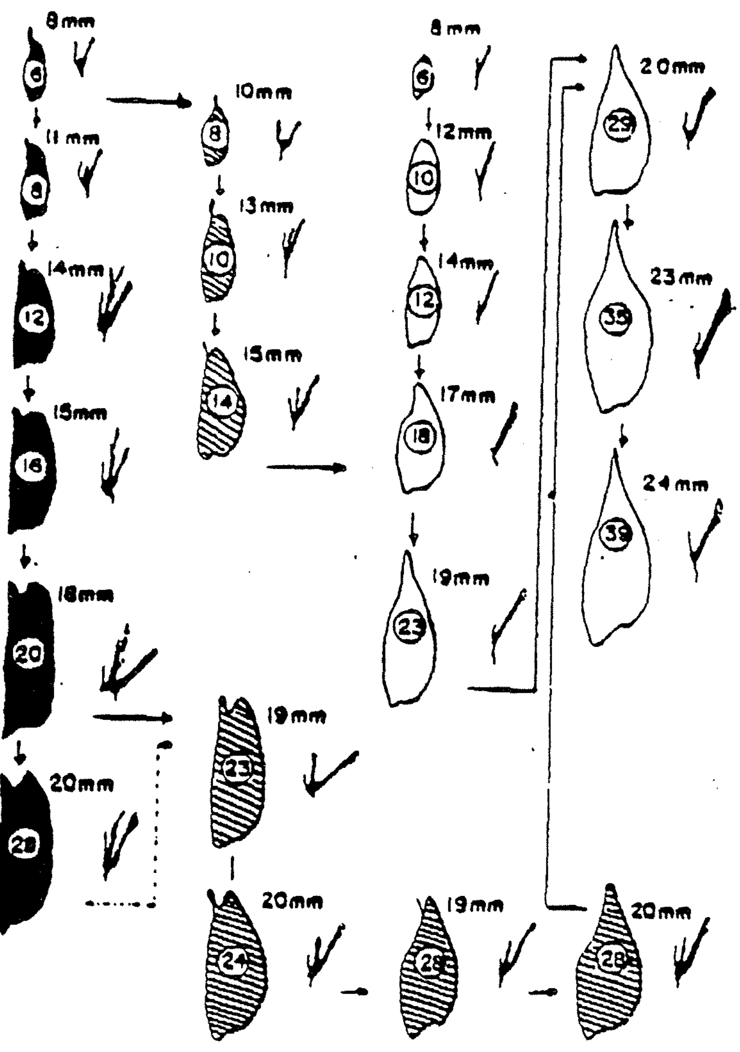
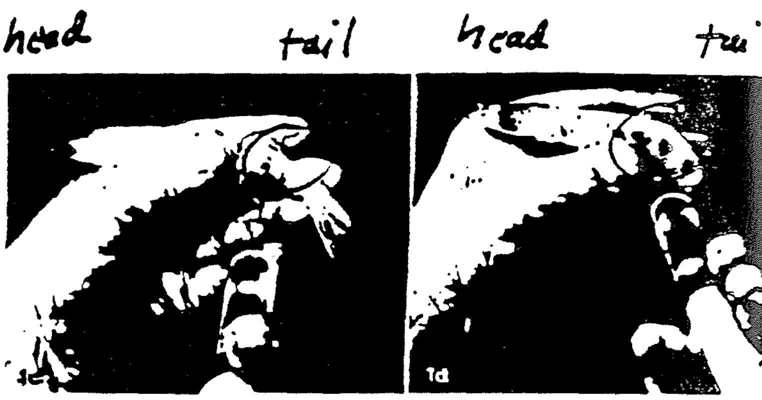


Figure 10.—Changes in form with increasing age of the endopodite of the first pleopod and the corresponding appendix interna and appendix masculina of the second pleopod of *Pandalus borealis* from the Northumberland population. Age in months is given in the ring in each endopodite and the carapace length (mm) above each figure. Male endopodite, black; transitional, cross-hatched; female, outlined. Arrows indicate sequence (from Allen 1959).

1b

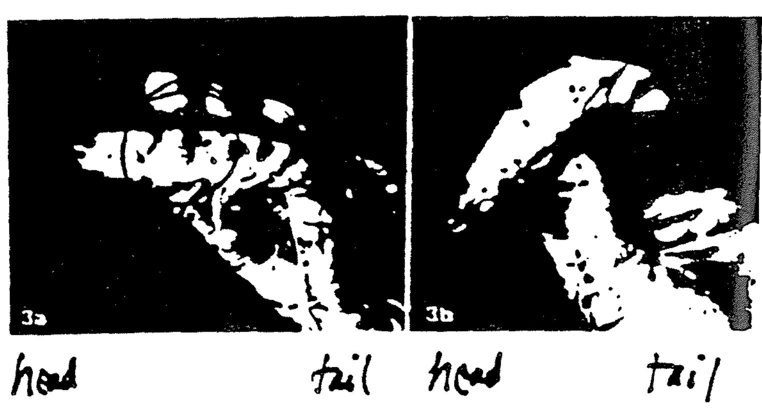


1d

4) Immediately following injection, slit the cuticle, with dissec scissors, from the sixth abdominal segment to the base of rostrum, paying particular attention not to cut deeply into underlying tissue. The incision in the cephalothoracic re should be just lateral to the dorsal midline, while that in abdominal region should be approximately mid-lateral (Figur

2

5) Shrimp larger than 12 grams, should then be transversely slit at the abdomen/cephalothorax junction (Figure 3a) or again abdominally (Figure 3b).
6) Following injection, incisions and bisection/trisection, imm the specimen in the remainder of the fixative.



3a 3b

NECROPSY FIELD DATA SHEET FOR HISTOLOGICAL SAMPLES

ADF&G, FRED Division Fish Pathology Lab

Collector/Address/Telephone #

Species

Number Specimens in Sample

Size Range

Life Stage

Date of Collection

Location of Collection (Site Name or Number)

Abnormalities Observed Per Specimen Number

APPENDIX G

CHAIN OF CUSTODY

EQM&LO CHAIN-OF-CUSTODY PROCEDURES

Chain-of-Custody is necessary if there is a possibility that the conclusions based upon analytical data will be used in litigation. The components of chain-of-custody are : sample seals, a field log book, chain-of-custody record, and the Request for Laboratory Services (RLS); the procedures for their use are described in the following sections.

Due to the evidentiary nature of samples collected during enforcement investigations, possession must be traceable from the time samples are collected until they or their derived data are introduced as evidence in legal proceedings. To maintain and document sample possession, chain-of-custody procedures are followed.

Admissibility of Analyses as Evidence. To be admissible as evidence, samples must be proved conclusively to be in an appropriate person's possession until the analyses resulting therefrom have been introduced as evidence. Rigid controls must be maintained to establish a chain-of-custody for the samples from the time of sampling until ultimate disposition of the particular case.

CUSTODY DEFINITION

A sample is under custody if:

If it is in your possession, or
It is in your view, after being in your possession, or
It was in your possession and you locked it up, or
It is in a designated secure area.

1. Evidence tape or sample seals are used to detect unauthorized tampering of samples following sample collection up to the time of analysis. The seal must be attached in such a way that it is necessary to break it ~~in order~~ to open the container. Seals must be affixed to containers before the samples leave the custody of sampling personnel.

2. Samples must be kept in such a manner that they cannot be altered wether deliberately or accidentally. Until the samples can be sent to the laboratory they should be kept in a cool, dark, dry place. Refrigeration, freezing or other chemical method of preservation are usually required. Chemical preservatives are added at the laboratory.

   Any indication that a sample has been subjected to tampering or physical alteration could disqualify it a evidence for possible legal action. Therefore, the instructions given herein must be followed strictly.

opening. A evidence tape is placed on the openings of the shipping container, signed and dated.

Sample tags and custody forms must be legible and filled out using waterproof, non-fading ink. Secure individual sample containers or group of sample containers using tamperproof evidence tape or seals.

4. Maintain an up-to-date Field Data Record Logbook. Record field measurements and other pertinent information necessary to refresh the sampler's memory if, later on, he/she takes the stand to testify regarding his/her actions during the evidence gathering activity. Maintain a separate set of field notebooks for each survey; store them in a safe place where they can be protected and accounted for at all times.

5. The field sampler is responsible for the care and custody of the collected samples until they are properly dispatched to the receiving laboratory, or turned over to an assigned custodian. The field sampler should verify that each container is in his/her physical possession or in his/her sight at all times, or is locked so that no one can tamper with it.

6. Colored slides or photographs are often taken to show the outfall sample location and any visible water pollution. Written documentation on the back of the photo should include the photographer's signature, and the time, date and site location. These photographs can be used as evidence, and are handled by chain-of-custody procedures to prevent alteration.

TRANSFER OF CUSTODY AND SHIPMENT

1. Samples are accompanied by a Request for Laboratory Services which has a chain of custody section. When transferring the possession of samples, the individual relinquishing and receiving the samples will sign, date and note the time. This record documents sample custody transfer from the sampler, often through another person, to the laboratory Sample Custodian.

2. Ensu[illegible] that samples are properly packed in shipping cont[illegible]ners (for example, ice chests) to avoid breakage. Ensu[illegible] that shipping containers are sealed for shipment to t[illegible] laboratory.

3. If th[illegible] package is sent by the US mail, ensure that it is se[illegible]t with a return receipt. If the package is hand-delivered, note that it was hand carried in the method of shipment block in the chain of custody record. Send field receipts from the post office and bills of lading to the laboratory custodian for retention as part of [illegible]

APPENDIX H

R.A.C.E.

SAMPLING AND RECORDING MANUAL

APPENDIX H.1

CATCH SAMPLING
AND
DATA RECORDING MANUAL

GULF OF ALASKA GROUNDFISH SUBTASK
RACE DIVISION
NORTHWEST AND ALASKA FISHERIES CENTER

JANUARY 1982

PROCESSING THE CATCH

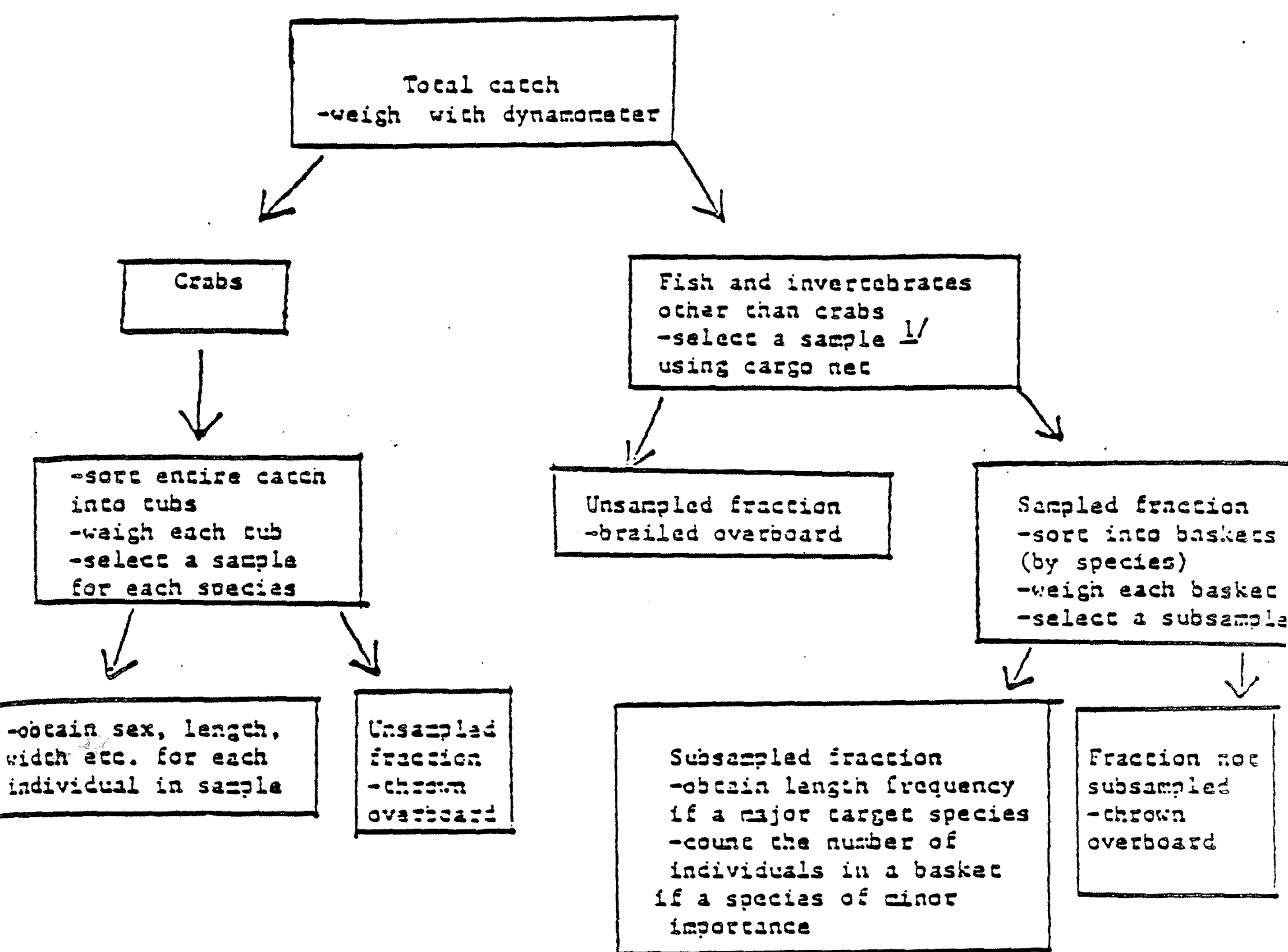
Initial Handling

Methods employed to handle trawl catches can vary, depending upon the size of the catch. The methods presented are tried and proved but are not the only methods. The primary concern is to obtain data on the total catch or a random sample of the total catch.

A. Catches of 8,000 pounds

1. If there is a load cell on board, the easy solution is to weigh all splits subtracting each time the weight of the trawl to obtain the total catch weight. Two or three splits, such as the first and last, or first, middle and last, are selected for biological sampling, depending upon the size of the catch and the size of the deck bin on the vessel being used. When the selected splits are in the deck bin, the sample will be further subsampled using the methods described in the section for catches from 2,000-8,000 pounds. In the Gulf of Alaska catches of this magnitude will usually consist of pollock or a mixture of pollock and other roundfish. When the catch is principally of one species with the same size composition throughout the cod end or a mixture of roundfish, the above selection of splits will usually provide an adequate sample. If, however, there are substantial numbers of "heavy" fish such as flatfish, sablefish, or Atka mackerel in the catch, the above system will probably not prove adequate as a disproportionate number of these fish will end up in the last split. If these circumstances occur, the splits selected for biological sampling from the forward portion of the net must be handled and sorted separately from the last split and then finally combined to obtain the total catch.

Figure 1.--Typical Processing of a Catch Greater Than 2500 lbs.



1/ This step is omitted if the catch is less than about 2500 lbs.

2. When a load cell is not available, acceptable results can usually be obtained by counting the number of splits and weighing the components of one or two splits which were selected for sampling. As in Section One above, you may have to treat the last split separately if there is a good showing of flatfish in the catch. The total catch weight is then obtained by assuming that each split is identical and use the weights of the sample splits.

3. When you are working aboard a large vessel, a third alternative is available. First estimate the total catch from the size of the cod end which you can see completely when it is hauled up on deck. Then dump the entire catch on deck. This will usually provide an opportunity to observe the species composition and possible clumping. The subsample can then be selected by filling the desired number of baskets directly from the deck. Be sure to select baskets from all portions of the catch.

B. Catches from 2,000-8,000 pounds

The catches in this size range are generally much easier to handle with the maximum size depending upon the capacity of the checker or deck bin aboard the vessel. The typical processing system of a catch greater than 2,000 pounds is diagrammatically shown in Figure 1. First you must estimate the total amount of the catch or weigh the splits with a load cell, so you will know what percentage to select for a subsample. Then the deck bin is lined with the cargo net and adjusted to line that portion of bin necessary to give a resulting subsample of approximately 2,000 pounds. The cargo net must be positioned so that it divides the deck bin athwartship. If the total catch is 6,000 pounds, you would adjust the cargo net to line 1/3 of the deck bin while for a 4,000 pound catch you would adjust the cargo net to line 1/2 of the deck bin, etc. Following the technique developed by Hughes (1976), it is vitally

important that the cod end be positioned so that the top and bottom of the trawl are facing athwartship prior to dumping the catch. Proper orientation of the cod end can be facilitated by tying a short marker line to the top and bottom of the cod end.

1. If a load cell is available the splits can be individually weighed and net weight subtracted to obtain the total catch weight. When the catch is in the deck bin, the cargo net is lifted by the boom removing the subsample from the deck bin and dumping it on the sorting tables.

2. If a load cell is not available the total catch weight can be obtained by leveling off the catch after it has been dumped into the deck bin. Measure the total volume of the catch by bin boards; that is, does the catch fill 1-1/2 or 2-1/2 or 2-3/8 bin boards. After removing the subsample and placing it in the sorting table, again level the remaining catch in the deck bin and record how many boards the catch now fills. After the weight of the subsample is obtained the total catch weight is obtained by extrapolation from the weight of the subsample.

C. Catches of 2,000 pounds or less

Catches in this weight range are dumped directly onto the sorting table.

Sorting and Weighing the Catch or Subsample

The first step in processing the catch or the subsample thereof on the table is to sort the dominant fish species in a manner to provide a random sample. Place three or more baskets on the sorting table, depending on the total species weight, and fill them simultaneously with the most dominant species. In filling the baskets, each person rotates from basket to basket, putting one fish in each basket. When the baskets are filled, repeat this procedure with another set of three baskets until the dominant species is

completely sorted. Other species can be sorted into separate basket sets at the same time the dominant species is being sorted. If no single species is highly dominant in the catch, sets of two or single baskets can be used for any species. Place the filled basket sets or single basket aside in the orde they are removed from the table. Do not combine partially filled baskets. I most situations, it is usually convenient to weigh the baskets as they are removed from the table and before they are set aside for further processing.

Sort all species, including invertebrates, and determine the weight and number of each species for the catch or that portion of the catch processed and record on the on-deck sampling form. For species with only a few specimens, this can be done by direct count. For those species where a length-frequency sample is taken, the sample weight and number will be used to estimate the total number in the catch. It is not necessary to do this in the field--the expansion will be done by computer at the Center from the sample data supplied on the length-frequency form. For other species where length-frequencies are not taken, but the number of individuals is large, a subsample should be weighed and counted and recorded on the on-deck sampling form. These sample numbers will also be expanded by computer. It is not necessary to get sample or total counts on miscellaneous species for all hauls. When time is not available, don't bother with these counts. Numbers will be expanded by strata and all that is required for this computation is good estimate of mean individual weight within each stratum. A further expansion of the weights and numbers to adjust them to the total catch will necessary in those cases where only a portion of the catch was processed. This will be done in the field. For those invertebrates that are difficult identify to species, they may be grouped into one of the following categor:

Octopus unidentified	Starfish unidentified
Jellyfish "	Clams "
Squid "	Hermit crabs "
Snails "	Other invertebrates

Hermit crabs should be weighed in the shell. It is not necessary to weigh empty shells. See the section on Data Records for an example of how to record catch data.

Biological Sampling of Fish and Shellfish

A. Species

Species of fish from which biological data is desirable are listed below:

Pacific cod (*Gadus macrocephalus*)

Pollock (*Theragra chalcogramma*)

Rex sole (*Glyptocephalus zachirus*)

Dover sole (*Microstomus pacificus*)

Rock sole (*Lepidopsetta bilineata*)

Flathead sole (*Hippoglossoides elassodon*)

Halibut (*Hippoglossus stenolepis*)

Sablefish (*Anoplopoma fimbria*)

Pacific ocean perch (*Sebastes alutus*)

Atka mackerel (*Atheresthes stomias*)

Snow crab (*Chionoecetes bairdi*)

King crab (*Paralithodes camtschatica*)

It is anticipated that only three or four species will be available in any one haul in sufficient numbers to take biological data. The field party chief wil decide the species and number of specimens from which to take data based on need and the time available.

B. Selecting a random sample

The next step in processing the catch is to reduce the number of baskets (set aside during the sorting procedures) to a random subsample of from 200-3 fish of other species (300 fish for Pacific cod) which will be processed for biological data. Handle juvenile pollock (<20 cm) as a separate sample from the adults. With a little experience, the number of baskets to be filled at one time to acquire these sample sizes will be apparent. Randomly select one row from the 3-5 basket sets of fish. If the subsample is too large because too few baskets were filled at one time, the subsample can be further reduced by selecting baskets from the front, middle, and end of the row to obtain the subsample for processing. Another procedure is to dump the subsample on the table and resort into baskets a second time, using the number of baskets nee and randomly selecting one of the two sets a second time.

After the subsample is selected, the unused baskets of fish can be discarded overboard after their weights have been recorded. Weigh and count al species not requiring further processing.

C. Length-frequency samples for fish species

Take length-frequencies from as many of the 13 commercially-important species as time permits from each tow. Normally only four or five of these species will be present in any given haul. If time is not available to take frequencies from all commercially-important species of fish present, take frequencies from species having the highest priority. If necessary, separa length-frequency samples can be taken for adult and juvenile pollock and th length for each group recorded on separate forms--only about 30 juvenile pollock need to be measured. If possible, determine the sex of juveniles. Record the weight of the length-frequency sample and total weight of the ad and juveniles in the catch on the length-frequency forms.

Using plastic strips, length-frequencies will be taken at all stations where target species are captured using the random subsample of 200-250 fish previously selected. In the case of halibut, all specimens will be measured using a metric tape for large specimens. Frequencies will be reported by sex, recording lengths for one sex on the upper part of the plastic strip and the other sex on the lower half. Lengths will be recorded by centimeter interval and measured from the tip of the snout to the end of the middle rays of the caudal fin.

The plastic length-frequency strip is attached to the measuring board using thumb tacks. The first line on the measuring board is 9.5 centimeters from the front of the board so that when the first "0" interval on the length-frequency strip is properly aligned on this mark, the lengths tallied in this interval will represent those fish that are 10 cm long. Length-frequency measurements should be transferred to length-frequency forms daily.

Sex determination will be made by opening the abdominal cavity. Flatfish species can best be sexed by observing the shape of the reproductive gland. The ovary is generally triangular with a long tail lobe which extends posteriorly. If mature, eggs are generally visible within the ovary. Male testes do not have the tail lobe and are white in mature specimens. Some species of flatfish can be sexed without making an incision by holding them up against a light and observing the presence or absence of the long tailed lobe of the ovary (see data code book).

In Pacific ocean perch, which are oviparous, the ovaries of immature females appear yellow in color and are firm in texture. Mature females will have embryos at various stages of development and will appear red or gray in color. Mature males' testes should be whitish-colored (see Appendix I).

The gonads of pollock and Pacific cod should appear quite similar to on another. Mature females will have large grayish ovaries full of eggs while the male testes will be white and composed of many leafy lobes. The smaller immature specimens can be separated by the shape of the gonad. In the case pollock, rather small fish of less than 20 cm can be sexed by the presence o absence of ovaries. The ovaries are oblong and clear to reddish, and appear toward the back of the body cavity. The absence of ovaries identifies a mal (see data code book).

If sexes cannot be separated in the case of small specimens, record the length measurements as unsexed.

See the section on Data Records below for the method of recording leng frequencies.

D. Length-frequency samples for crabs

Length-frequency samples will be selected in the same manner as for th fish species. King crab are measured by length, from the posterior edge of the carapace. Tanner crabs are measured by width, measured at the widest point on the lateral-posterior lobes of the carapace. Both king and Tanner crabs can be sexed by the shape of the abdominal flap. Females of both spe have wide abdominal flaps, while the males are narrow. Shell condition wil recorded for all Tanner crabs. Incidence of black mat disease and infectic due to chinoclastic bacteria will be noted.

E. Age structure samples

Otolith or scale samples will be collected from the principal demersa fish species captured during the cruise. A random sample will be taken fr each station where a species is a dominant form. If time does not allow aging samples for all species captured, the field party chief will select species in order of priority.

Vials held in polystyrene boxes will be used to store the otoliths and paper envelopes for scale samples. Otoliths for a length-sex category will be stored in a single vial. Each container vial will have a label giving the species, sex, length groups, and vessel. Keep a permanent record of the number of otoliths by sex-centimeter group for each completed sample and return this record to the Center.

Scales rather than otoliths will be taken from Pacific cod. See Appendix III for methods of collecting scales and otoliths.

Use Specimen Data forms for recording otolith data. See the section on Data Records below for example of data required on the form.

F. Length-weight

To establish length-weight relationships for the various species, weights and lengths for individual fish will be taken. This can be a sample independent of the otolith and length-frequency or the same sample. The sample will be random with lengths and weights taken from all specimens in the sample. Stomach contents should be removed prior to taking the weight measurement. Weights will be determined on a triple-beam balance or during periods of rough weather by hand-held spring scales. When possible, a K-TRON electronic balanc will be used.

G. Maturity

An effort will be made to determine the maturity status of all species i order to define spawning areas and times. The timing of the survey should be during the spawning period for some species. Use Specimen Data forms for thi purpose (see the section on Data Records below for the method of filling out forms).

Figure 2. pg 1 of 2

ON-DECK SAMPLING FORM — SPECIES COMPOSITION

Page ______ Of ____

Vessel ______________ Cruise ______________ Haul No. ______________

Date __________ Haul Type --- Regular Other (circle) Surface (bucket) Temperature __________ C°

1. If the trawl sample was subsampled, check box here ☐ and then enter data and computations on back side of page.

2. If the same portion of all species was processed, enter the percent here ☐☐☐☐ %

Otherwise enter percents for each species on next lines

Species name and code	Non-sub-sample basket weights	Sub-sample basket weights	Sub-sample numbers	Perc

79-3-10

When data have been recorded on terminal, mark an 'X' here ◯

Figure 2. pg 2 of 2

ON-DECK SAMPLING FORM – SUBSAMPLE CALCULATIONS

Vessel ____________ Cruise ____________ Haul No. ____________

Split Weights	1.	2.	3.	4.	5.	Total
Dynamometer						
– Bag weight						
– Debris removed before subsampling						
= Animal Weight						

Calculation Of Percent Processed

1. In cases where the proportions of fish and crab processed are the same:

$$\text{Percent of fish and crab processed} = \frac{\text{weight of fish and crab processed}}{\text{total weight of catch}} \times 100\%$$

$$= \frac{\qquad}{\qquad} \times 100\%$$

= [%]

2. In cases where some items in the catch are completely processed, but only a fraction of the fish are processed:

(a) Total weight of catch (A) = ____________

(b) Weights of items completely processed: Crab = ____________

Halibut = ____________

Other = ____________

Total (B) = ____________

(c) Total weight of all items (including debris) processed in groundfish subsample (C) = ____________

(d) Percent of fish processed $= \frac{C}{A-B} \times 100\% =$ [%]

Maturity determinations will be estimated by visual observation and w
be somewhat subjective because of the lack of precise guidelines for the
classification of gonads. Five classifications (except for rockfish) will
used--immature, maturing, spawning, spent, and sexually inactive (see ADP c
book for criteria or Appendix II for rockfish criteria). Attempt to photoc
each classification in color for as many species as possible.

Data Records

A. On-deck sampling form

This form will be used for initial recording of catch and sample data
It will be kept as a permanent record, although most of the data it contai
will be transcribed onto the Trawl Catch Form (Figure 4) after the haul or
the end of the day. An example of how data should be recorded on this for
shown in Figure 2.

For each species, the individual basket weights will be recorded for
total catch or that proportion of the catch processed. Individual basket
weights will be summed and the total weight for each species recorded. Al
record the total number in the sample, or a subsample weight and number, i
the total number for any species is large. It is not necessary to count c
record the number of fish in a length-frequency subsample, since this wil
done by computer.

B. Haul-position and species catch form

Entries on the trawl catch form are described in the ADP codebook (F
3). On the front side of this form, information for each haul will be
recorded pertaining to the location, depth, duration and distance of the
and weather and sea conditions (Figure 3). Much of this information wil

from the bridge, and the field party chief should make arrangements with the Captain for the most convenient time and method of acquiring this information.

When recording loran readings for the start and end positions, be sure to use the correct loran rates as shown in the ADP Code Book (Table 2. Pg. 32). Geographic start and end positions will be plotted on the appropriate hydrographic chart or mylar overlay and by loran plotter if available.

The distance fished will be determined by the best available method. If a tow deviates from a straight line, indicate in the "remarks" section of the Haul-Position form and calculate distance fished from the loran plotter. The duration of tow which is computed as the difference between the Equilibrium and Haul time will also be measured by the best available method. A net sonde unit or other electronic on-bottom indicator is required to determine the moment the gear reaches and leaves the bottom. This corresponds to the Equilibrium and Haul times on the Haul-Position form (Table 1). If these devices are not available, the duration of the tow will be measured as the difference between when the winch brakes have been set (Time Out = Equilibrium Time) and when the winches begin retrieving the trawl gear (Haul Time = Time gear leaves bottom). All times will be local to the survey area and noted on each haul form.

Figure 4.

SPECIES CATCH FORM

DP-002 83-01-15

VESSEL [1 2 3] CRUISE [5 6 7] HAUL [9 10 11]

YOUR NAME ______________________

SPECIES NAME	CODE (13 14 15 16 17)	WEIGHT TO A TENTH LB (19 20 21 22 23 . 25)	NUMBER (27 28 29 30 31 32)	COMMENTS
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CONFIDENTIAL

TITLE: Injury to Larval Fish in Prince William Sound

STUDY ID NUMBER: 19

PROJECT LEADER: Brenda L. Norcross

LEAD AGENCY: Alaska Department of Fish and Game

COOPERATING AGENCY: University of Alaska Fairbanks, Institute of Marine Science

COST OF PROPOSAL: $413,400

DATES OF STUDY PLAN: March 1989 - February 1990

Brenda L. Norcross 19 October 1989

Brenda L. Norcross (907)474-7990 Date
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Vera Alexander 19 October 1989

Vera Alexander (907)474-7531 Date
Dean, School of Fisheries and Ocean Sciences
Director, Institute of Marine Science
University of Alaska Fairbanks
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Joan Osterkamp 10-20-89

Joan Osterkamp (907)474-6734 Date
Executive Officer, School of Fisheries and Ocean Sciences
University of Alaska Fairbanks
Fairbanks, Alaska 99775-1080

OSIAR Senior Biometrician: ____________________ Date

OSIAR Program Manager: Ralph Holmes 10/24/89 Date

OSIAR Director: ____________________ Date

INTRODUCTION

The overall goal of this project is to determine whether the oil spilled from the T/V Exxon Valdez had a measurable negative impact on the survival of finfish and shellfish larvae in Prince William Sound. There are few quantitative studies on the effects of an oil spill in arctic and subarctic waters which included measurements of oil in the water column. Not knowing concentrations of oil which might be encountered in an actual field spill situation inhibits educated choice of exposure levels to use in laboratory experiments (Rice et al. 1976). Therefore a priori knowledge of the effects of this oil spill on larval finfish and shellfish survival is negligible.

The larval period is often designated the "critical period" in survivorship of young fish (Hjort 1926). Several species of finfish, especially the gadids, have been shown to be sensitive to the toxicity of hydrocarbons (Kuhnhold 1974, 1977; Falk-Petersen and Kjorsvik 1987). Because of the frequency of molt, the larval period of shellfish has been shown to be more susceptible to oil toxicity than adult stages (Chia 1973; Renzoni 1973; Rice, et al. 1976). Toxic effects of hydrocarbons on finfish and shellfish eggs and larvae can be both immediate and acute (Rice et. al 1976; Smith and Cameron 1979) and/or prolonged and sublethal (Kuhnhold 1977; Rice et al. 1978; Solbakken et al. 1984). The effects of oil pollutants on larvae are dependent on species age/weight/length (Foyn and Serigstad 1987), environmental factors such as temperature and salinity (Rice et al. 1978) and timing and distribution of the sensitive stages (Foyn and Serigstad 1987). Emulsifiers and dispersants used to clean up spilled oil may be even more toxic to egg and larval survival than the oil itself (Wilson 1976, 1977; Lonning 1977; Lonning and Falk-Petersen 1978).

Since there are no pre-oil spill larval data to provide a baseline, results of post-oil spill collections cannot be directly compared to "normal" conditions to estimate loss. Therefore, the major product of this study will be charts mapping the distribution of larvae in Prince William Sound over space and time. These will be compared to distribution charts of the hydrocarbons over a similar time frame. Potential for impact will be inferred from literature documenting effects of oil toxicity on similar species. The exposure of the larvae to hydrocarbons is hypothesized to have continued after the initial spill of oil due to oil and cleaning agents which continued to wash off beaches and enter the water column.

This plan includes accomplishments completed since March 1989, anticipated through February 1990, and required after February 1990.

OBJECTIVES

1. For each species within each community, test the hypothesis that the oil spill did not change the abundance ($\alpha = 0.05$).

2. While controlling for environmental variables if necessary, test the hypothesis that the oil spill did not change species composition of the communities within Prince William Sound ($\alpha = 0.05$).

The following tasks will be accomplished to provide estimates of key parameters:

a. Estimate temporal and spatial presence of larval finfish and shellfish in Prince William Sound.

b. Estimate temporal and spatial distribution of larval finfish and shellfish in relation to the distribution of hydrocarbons in Prince William Sound.

c. Estimate the potential for loss of larval finfish and shellfish as a result of the March 1989 oil spill based on published literature documenting toxicity of oil to the same or related species.

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

METHODS

There are basic constraints in designing any oil spill survey: 1) non-repeatability, i.e. there is only one experimental unit, and therefore effects must be judged as differences between affected and unaffected areas; 2) the total cost of the effort is unknown at the start of the survey which makes planning sampling difficult; and 3) baseline data are not available (Smith 1979).

When no before-impact data can be collected, impact effects must be demonstrated and described from spatial pattern (Green 1979). Because there were no previous data with which to design a statistical study for population analysis, this study was designed to determine what species of finfish and shellfish have larval stages present in Prince William Sound. The first step in such a survey is to divide the area of the spill into strata that are relatively homogeneous with respect to biological, chemical, and physical properties (Smith 1979). Strata which were representative of the characteristic hydrological, geological and ecological areas within the sound were chosen to cover as much area of Prince William Sound as possible. Both oiled and non-oiled areas were chosen. The specific locations of sampling stations were randomly chosen within these representative strata. These sampling stations were coordinated with the sites initially sampled by investigators from UAF aboard the R/V Alpha Helix in April 1989. The final

combination of number and location of stations was also dependent upon what could reasonably be accomplished on a 7-day cruise when sampling 24 hours per day. The same stations that were sampled on the first cruise were sampled on following cruises (weather permitting). Additional sites were added over the progression of the summer sampling, and they were also resampled on successive cruises. Returning to the same station on successive cruises increases the accuracy of the sampling program (Smith 1979).

At each station, CTD (conductivity, temperature and depth) profiles and ADCP (Acoustic Doppler Current Profiler) data were collected and will be used to characterize water movement throughout the sound. Zooplankton (net) samples were collected at a subset of those stations at which physical parameters were collected (Smith 1979). A list of all physical stations sampled is presented which notes at which stations zooplankton were sampled on specific cruises (Appendix A). A charlet depicting locations (Appendix B) is also attached.

To assess the temporal presence and distribution of the larvae in 1989, sampling was planned to take place once a month for seven months, April-October. Because of logistic problems securing a vessel, only one cruise was conducted in August and September. Additionally, the October cruise will be at the end of the month and overlap into November. The actual cruise sampling dates for the 1989 field season were as follows:

	Vessel	Cruise	Dates
	R/V Alpha Helix	HX121	6 - 12 April 1989
	R/V Alpha Helix	HX123	5 - 11 May 1989
	R/V Alpha Helix	HX125	1 - 7 June 1989
	NOAA Ship John Cobb	CO8902	8 - 14 July 1989
	F/V Jennie Girl	JG001	30 August - 9 September 1989
*	R/V Alpha Helix	HX134	27 October - 3 November 1989 *(planned)

The gear used to sample larvae on the first five cruises was a $1 \cdot m^2$ NIO (Tucker Trawl) with 505u or 1,000u mesh net. During the first three cruises, discrete depths to 100 m were sampled horizontally. Starting with the July cruise, discrete depth increments to 600 m were sampled obliquely. Thus additional depth samples have been collected as the sampling season progressed. Replicate samples were not collected for most of these samples, though there are some replicates of the latter oblique tows. More information about species presence/absence will be gained by increasing the number of sites sampled than increasing the number of samples per site (S. Thompson, UAF, pers. comm.).

During the October/November cruise, a 1 m^2 MOCNESS (multiple opening/closing net) will be employed. This net can collect up to nine samples per deployment, as compared to three samples possible with the Tucker Trawl. Therefore, we will attempt to sample all stations previously occupied, but will collect oblique samples from additional depth ranges covering the water column. Replicate samples will be possible with this new net. Numbers of replicates depend on time available and depth increments. We will also deploy

the Tucker Trawl at several sites for comparison tows.

Standard Operating Procedures for deploying and maintaining all gear, preserving and sorting samples, and recording data are included in Appendix C. An SOP will be developed for the MOCNESS after the October cruise when we have become familiar with that specific gear. Finfish and shellfish larvae will be sorted and identified from all zooplankton samples. A technician will soon be hired whose primary responsibility will be to identify the larval fish. We are in the process of purchasing a video system which will allow the fish larvae to be measured and the data to be entered into a computer data base. In November or December we will host Art Kendall (NOAA/NMFS/NWAFC), the recognized expert in identification of north Pacific fish, who will conduct a workshop to instruct us in the identification of Alaskan larvae. SOPs will be developed for identifying larvae and use of the video system once we are familiar with these procedures.

No hydrocarbon analysis can be directly performed on the larvae because we cannot collect enough larval weight of a specific species to separate and preserve the sample properly at sea. Species-specific laboratory toxicity experiments, though lacking and definitely needed (Fyhn et al., 1987) were deemed cost prohibitive. To accomplish the objectives, Task 2 requires input from another component, i.e. the analysis of distribution of hydrocarbons within Prince William Sound over time. Geographic distribution plots of the oil over time, preferably with isopleths of concentration, are needed. The hydrocarbon analysis needed to complete this task should include distribution and concentration of oil in Prince William Sound throughout the summer of 1989. Information depicting location, amount and concentration of dispersants used on the beaches and their effect on surrounding water is also needed to compare to larval fish data.

DATA ANALYSIS

This survey is designed to document what species are present and what their horizontal and vertical distribution is over time. Since no previous information is available with which to compare 1989 species presence/absence or abundance information, sampling over one season cannot provide assessment regarding usual occurrence/abundance or lack of occurrence/abundance in relation to the effect of the oil spill.

Sites sampled were chosen to be hydrologically, geologically and ecologically representative of Prince William Sound. This sampling design enables Task #1 to spatially and temporally establish the presence of larval fish and shellfish. Results will be depicted as distribution maps of areas occupied by specific species at specific times. Task #2 will overlay distribution maps of hydrocarbon concentrations (provided by the GIS and hydrocarbon component) on the maps generated for Task #1.

As part of Task #3, the maps resulting from Tasks #1 and #2 will

be interpreted using available literature to estimate the temporal and spatial potential for injury to the larval finfish and shellfish populations by the oil spill. Since not all species collected in PWS will have had laboratory hydrocarbon analyses conducted and reported, closely related species in the same family will be used to interpret the impact.

To meet Objective #1, after grouping by habitat type, the hypothesis that there was no change in abundance in relation to concentration of hydrocarbon will be evaluated by a chi-square test. If there is no effect due to oil an equal amount of larvae at all stations would be expected ($\alpha = 0.05$). Absolute abundance changes may be difficult to detect since "the wide annual variability in egg and larval distribution and survival combined with sampling problems results in such large confidence limits on field survey estimates that mortality below an order of magnitude greater than normal would be virtually impossible to detect" (Reed 1981). Therefore, we will also investigate relative abundances and species composition. The relationship (correlation/regression) between hydrocarbon concentration and species abundance will be tested ($\alpha = 0.05$).

To meet Objective #2, habitats will be grouped by physical parameters, i.e. temperature, salinity, depth, flow patterns. Species composition will be compared between sites in habitat groups. Species will be ranked by abundance for each station within the group for oiled and non-oiled areas. The hypothesis that there is no difference in species composition will be tested using Kendall's coefficient of concordance ($\alpha = 0.05$). This test is a measure of agreement in rankings and does not require matched pairs of data (Conover 1971). This may detect potential competitive replacement of a resilient species between oiled and non-oiled areas.

To further interpret the effect of the oil spill, trajectories of larval patches through oiled areas will be estimated based on the physical transport of the water masses within and through Prince William Sound. CTD and ADCP data are being collected on the cruises in PWS and UAF/IMS deployed four current meters in April which will be retrieved in November. However, there is no physical circulation component of the CERCLA program to fund analysis of these important data. Dr. Thomas Royer, UAF/IMS, has agreed to analyze these 1989 physical date as part of his contract to analyze historic Prince William Sound physical data for Exxon. This cooperative agreement will provide the 1989 PWS data to Exxon through Royer and the analysis of these data to CERCLA through Norcross. This analysis of physical transport of water is applicable to larvae and hydrocarbons. Transport of ichthyoplankton in relation to hydrocarbons is an important component when calculating the potential loss of larvae due to an oil spill (Reed 1981).

Length/frequency histograms of specific species will be analyzed for "missing" cohorts, i.e. a gap in lengths indicating a time when larvae appear to be "lost". Regression of abundance of larvae on

length increments should reveal deviations from a linear relationship indicating underrepresentation of a specific age class. Published literature on the growth rates of these species will be used to back calculate to the time these fish should have encountered the oiled areas. Individual larvae will also be screened for any obvious abnormalities which may have been caused by association with the oil.

Task #4 will be considered based on the results of the data and may include some form of recommendation for a long range study of natural variability which will allow anthropogenic effects to be readily identified and quantified.

SCHEDULE & PLANNING

1989 Field collection schedule:

SHIP	CRUISE	DATES	# SAMPLES
R/V Alpha Helix	HX121	6 - 12 April	19
R/V Alpha Helix	HX123	5 - 11 May	44
R/V Alpha Helix	HX125	1 - 7 June	39
NOAA Ship John Cobb	CO8902	8 - 14 July	107
F/V Jennie Girl	JG001	30 August - 9 September	84
R/V Alpha Helix	HX134	27 October - 3 November	~200

Sample analysis:

literature search	July - December 1989
ichthyoplankton sorting	May 1989 - February 1990
shellfish plankton sorting	January - May 1990
ichthyoplankton identification	November 1989 - May 1990
shellfish identification & staging	June - July 1990
ichthyoplankton length measurements	March - July 1990
fish length/frequency plots	August 1990
distribution maps (by GIS)	March - August 1990
analysis and interpretation	August - October 1990
interim/status reports	quarterly as required
cruise reports	30 days after sample period
final 1989 report	December 1990

Sample and data archival:

All samples will be shipped to UAF/IMS for sorting. They will later be shipped to UAF/IMS/Seward Marine Center for decapod sorting. Ultimately, samples will all be stored at UAF. All original data, logs, etc. will be stored at UAF/IMS in the care of Brenda Norcross or Brenda Holladay.

Depending on the number of species on the number of depth strata plotted, there will be 100 - 250 distribution maps of species and hydrocarbons.

Management:

Brenda L. Norcross	Principle Investigator/Project Leader
Brenda A. Holladay	Technician - field collections, forming data archives, reports, data analysis

(TBN)	Lab Assistant II - identify and measure finfish larvae, length/frequency analysis
Undergraduates (10)	Student Assistants - sort finfish larvae
A. J. Paul	Scientist - identify and stage shellfish larvae
J. McDonald	Technician - sort shellfish larvae
J. Smithhisler	Physical Oceanography technician
G. Mimkin	Electronics technician
D. Nebert	Computer specialist
C. Chu	Data base manager
F. Mueter	Graduate student

Logistics: See attached list and chart of station locations.

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Appendix A

OIL SPILL STUDY

	Approximate Station Locations			Zooplankton Sampled					
				HX121	HX123	HX125	CO8902	JG001	HX134* *(planned)
Resurrection Bay	RES 2.5	60 01.5'	149 21.5'			X		X	X
	GAK 1	59 50.7'	149 28.0'			X			X
Cape Fairfield Line	CF 13	59 31.0'	148 50.0'						
	CF 12	59 33.0'	148 50.0'	X					
	CF 11	59 35.0'	148 50.0'						
	CF 10	59 37.0'	148 50.0'						
	CF 9	59 39.0'	148 50.0'						
	CF 8	59 41.0'	148 50.0'						X
	CF 7	59 43.0'	148 50.0'						
	CF 6	59 45.0'	148 50.0'	X	X	X			X
	CF 5	59 47.0'	148 50.0'						
	CF 4	59 49.0'	148 50.0'						X
	CF 3	59 51.0'	148 50.0'						
	CF 2	59 53.0'	148 50.0'						X
	CF 1	59 54.7'	148 50.0'						
Montague Buoy	MS/3	59 58.47'	147 48.7'						
	MSX	59 58.5'	147 48.7'		X	X	X	X	X
Rocky Bay	RB 1	60 26.0'	147 10.0'				X	X	X
	RB 2	60 21.8'	147 01.7'	X					
Hinchinbrook Entrance	HE/6	60 19.09'	146 50.07'						
	HE-A	60 13.5'	146 55.9'		X	X	X	X	X
	HE 1	60 18.1'	146 52.8'		X	X	X	X	X
	HE 2	60 17.8'	146 50.0'						
	HE 3	60 17.3'	146 46.1'	X	X	X	X	X	X
	HE 4	60 17.0'	146 43.2'						
	HE-B	60 13.0'	146 40.0'				X	X	X
Orca Bay Transect	OB-1	60 35.0'	146 01.9'		X		X	X	X
	OB-2	60 34.9'	146 11.0'						
	OB-3	60 35.0'	146 22.0'						
	OB-4	60 35.0'	146 31.1'						
	OB-5	60 34.9'	146 43.0'	X					
	OB-6	60 34.8'	146 54.1'	X	X	X	X	X	X
Central Basin	CB	60 43.2'	147 02.7'						
Port Fidalgo	PF	60 45.0'	146 54.4'						
Bligh Island	BI	60 50.2'	146 55.0'		X	X	X		X
Valdez Arm	VA	60 58.2'	146 48.0'						

Appendix A (cont.)

	Approximate Station Locations			Zooplankton Sampled					
				HX121	HX123	HX125	CO8902	JG001	HX134* *(planned)
Valdez	PV-40	61 06.2'	146 28.6'	X					
	PV-50	61 06.4'	146 35.7'	X	X	X	X	X	X
Columbia Glacier	CG	60 55.7'	147 06.2'						
Naked Island Transect	NI-1	60 49.9'	147 26.1'						
	NI-2	60 48.5'	147 26.1'						
	NI-3	60 46.9'	147 26.1'						
	NI-4	60 44.9'	147 26.1'	X	X	X	X		X
W of Naked Is	NI-5	60 43.9'	147 39.0'						
Esther Island	EI	60 46.3'	148 03.3'	X	X	X	X		X
SW of Naked Is	NI-6	60 35.4'	147 46.7'			X	X	X	X
Main Bay	MB	60 34.0'	147 57.0'		X	X	X		X
Herring Bay	HB	60 28.2'	147 44.3'						
Knight Island Passage	KI-1	60 20.5'	147 57.0'		X		X	X	X
	KI-2	60 10.8'	147 53.4'			X			
Snug Harbor	SH-2	60 15.5'	147 43.3'						
	SHX/4	60 15.73'	147 41.72'						
Green Island Transect	GI-1	60 15.0'	147 40.0'						
	GI-2	60 14.5'	147 37.0'	X	X	X	X	X	X
	GI2/5	60 14.55'	147 37.03'						
	GI-3	60 14.0'	147 34.0'						
	GI-4	60 13.5'	147 31.2'						
	GI-5	60 12.8'	147 27.7'						
	GI-6	60 12.5'	147 24.6'	X	X	X	X	X	X
	GI-7	60 12.0'	147 21.9'						
Sawmill Bay	SB	60 03.5'	147 58.0'		X	X	X	X	X

Appendix A

OIL SPILL STUDY

Location	Approximate Station Locations			Zooplankton Sampled HX121	HX123	HX125	CO8902	JG001	HX134*
Resurrection Bay	RES 2.5	60 01.5'	149 21.5'			X		X	X
	GAK 1	59 50.7'	149 28.0'			X			X
Cape Fairfield Line	CF 13	59 31.0'	148 50.0'						
	CF 12	59 33.0'	148 50.0'	X					
	CF 11	59 35.0'	148 50.0'						
	CF 10	59 37.0'	148 50.0'						
	CF 9	59 39.0'	148 50.0'						
	CF 8	59 41.0'	148 50.0'						X
	CF 7	59 43.0'	148 50.0'						
	CF 6	59 45.0'	148 50.0'	X	X	X			X
	CF 5	59 47.0'	148 50.0'						
	CF 4	59 49.0'	148 50.0'						X
	CF 3	59 51.0'	148 50.0'						
	CF 2	59 53.0'	148 50.0'						X
	CF 1	59 54.7'	148 50.0'						
Montague Buoy	MS/3	59 58.47'	147 48.7'						
	MSX	59 58.5'	147 48.7'		X	X	X	X	X
Rocky Bay	RB 1	60 26.0'	147 10.0'				X	X	X
	RB 2	60 21.8'	147 01.7'	X					
Hinchinbrook Entrance	HE/6	60 19.09'	146 50.07'						
	HE-A	60 13.5'	146 55.9'		X	X	X	X	X
	HE 1	60 18.1'	146 52.8'		X	X	X	X	X
	HE 2	60 17.8'	146 50.0'						
	HE 3	60 17.3'	146 46.1'	X	X	X	X	X	X
	HE 4	60 17.0'	146 43.2'						
	HE-B	60 13.0'	146 40.0'				X	X	X
Orca Bay	OB-1	60 35.0'	146 01.9'		X		X	X	X

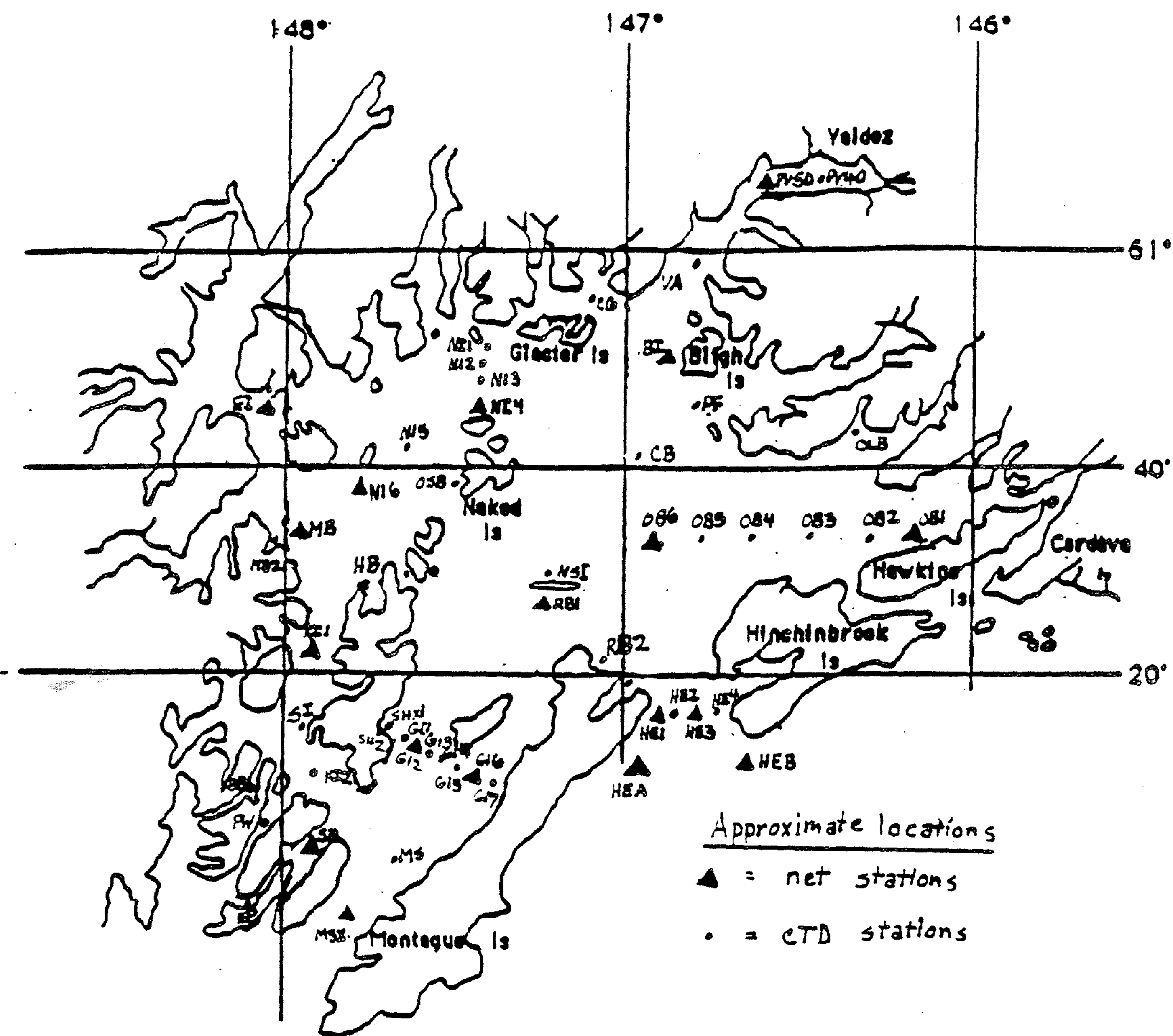
*(planned)

Transect	OB-2	60 34.9'	146 11.0'						
	OB-3	60 35.0'	146 22.0'						
	OB-4	60 35.0'	146 31.1'						
	OB-5	60 34.9'	146 43.0'	X					
	OB-6	60 34.8'	146 54.1'	X	X	X	X	X	X
Central Basin	CB	60 43.2'	147 02.7'						
Port Fidalgo	PF	60 45.0'	146 54.4'						
Bligh Island	BI	60 50.2'	146 55.0'		X	X	X		X
Valdez Arm	VA	60 58.2'	146 48.0'						

Appendix A (cont)

	Approximate Station Locations			Zooplankton Sampled					
				HX121	HX123	HX125	CO8902	JG001	HX134*
Valdez	PV-40	61 06.2'	146 28.6'	X					
	PV-50	61 06.4'	146 35.7'	X	X	X	X	X	X
Columbia Glacier	CG	60 55.7'	147 06.2'						
Naked Island	NI-1	60 49.9'	147 26.1'						
Transect	NI-2	60 48.5'	147 26.1'						
	NI-3	60 46.9'	147 26.1'						
	NI-4	60 44.9'	147 26.1'	X	X	X	X		X
W of Naked Is	NI-5	60 43.9'	147 39.0'						
Esther Island	EI	60 46.3'	148 03.3'	X	X	X	X		X
SW of Naked Is	NI-6	60 35.4'	147 46.7'			X	X	X	X
Main Bay	MB	60 34.0'	147 57.0'		X	X	X		X
Herring Bay	HB	60 28.2'	147 44.3'						
Knight Island	KI-1	60 20.5'	147 57.0'		X		X	X	X
Passage	KI-2	60 10.8'	147 53.4'			X			
Snug Harbor	SH-2	60 15.5'	147 43.3'						
	SHX/4	60 15.73'	147 41.72'						
Green Island	GI-1	60 15.0'	147 40.0'						
Transect	GI-2	60 14.5'	147 37.0'	X	X	X	X	X	X
	GI2/5	60 14.55'	147 37.03'						
	GI-3	60 14.0'	147 34.0'						
	GI-4	60 13.5'	147 31.2'						
	GI-5	60 12.8'	147 27.7'						
	GI-6	60 12.5'	147 24.6'	X	X	X	X	X	X
	GI-7	60 12.0'	147 21.9'						
Sawmill Bay	SB	60 03.5'	147 58.0'		X	X	X	X	X

*(planned)


148°
147°
146°
61°
40°
20°
Valdez
Glacier Is
Bligh Is
Naked Is
Hawkins Is
Hinchinbrook Is
Cordova
Montague Is
Approximate locations
= net stations
= CTD stations

STANDARD OPERATING PROCEDURES
Deployment of Portable SeaBird CTD

Date: 13 October 1989

Author: Brenda A. Holladay
Laboratory Technician
Institute of Marine Science
University of Alaska Fairbanks
Fairbanks, AK 99775
(907) 474-7990

Purpose: to attain temperature and salinity profiles at specific station locations; to record depth, temperature and salinity data during each net tow

Procedures

Equipment:

SeaBird SBE 19 Profiler S/N 192411-261, enclosed in plastic housing effective to 600m depth; pressure sensor good to 680m; 256K (6 hours) memory; 600 baud output for real time
cannonball weight (25-40#) or NIO net frame
length of 3/16-5/16" wire or line equal to depth to bottom
Triton X-100 non-ionic detergent
fresh water and/or distilled water

Methods:

Rigging

1. For deployment alone or with Nansen bottle: with shackles, attach cannonball weight to end of CTD on which profiler switch is, attach wire thimble to other end of CTD
2. For deployment with NIO net: with shackles, attach end of CTD on which profiler switch is to top corner of NIO net frame, leave other end of CTD hanging free

Deployment

1. Turn CTD on using profiler switch; record cruise number, station name, consecutive station number, fathometer depth reading, latitute and longitude, date, time and CTD cast number *
2. Allow CTD to equilibrate to surface water temperature for 2 minutes; record time

3. Spool wire out at a constant speed (60m/min is optimal for flushing of CTD conductivity cell) until CTD is at desired depth (estimate by wire angle and length); desired depth is variable when CTD is deployed in conjunction with NIO net; when deployed alone or with Nansen bottle, CTD is deployed within 5m of the bottom in calm seas, 10m if ship is rolling; record time, length of wire out, wire angle, estimated CTD depth
5. Pull wire in at constant speed until CTD reaches surface
6. Turn off profiler switch; record time
7. For CTD casts without NIO net: record required data on hydrographic data sheets: cruise number, consecutive station number, date, time CTD was at depth, station location, time zone, and ship code

Cleaning
1. After each cast, flush conductivity cell with distilled (preferred) or fresh water, fill protective tube with distilled (preferred) or fresh water and secure tube at both ends of conductivity cell
2. At end of each sampling day, flush conductivity cell with 1/4 cup 1% Triton solution, flush thoroughly with fresh water, then store with protective tube filled with distilled (preferred) or fresh water and connected to both ends of conductivity cell; rinse exterior of CTD with fresh water

Retrieving and storing data
1. After recording approximately 3 hours of data on CTD, transfer memory to computer disk and initialize CTD memory according to instructions in the <u>SeaCat SBE 19 Conductivity, Temperature, Depth Recorder Operating Manual</u>
2. Make a backup copy of each data disk

* record data in field notebook unless otherwise indicated

STANDARD OPERATING PROCEDURES
Deployment of Nansen bottle

Date: 13 October 1989

Author: Brenda A. Holladay
Laboratory Technician
Institute of Marine Science
University of Alaska Fairbanks
Fairbanks, AK 99775
(907) 474-7990

Purpose: collection of temperature and salinity information to calibrate the SeaBird CTD

Procedures

Equipment:

SeaBird SBE 19 Profiler S/N 192411-261, enclosed in plastic housing effective to 600m depth, pressure sensor good to 680m. 256K (6 hours) memory, 600 baud output for real time
cannonball weight (25-40#)
length of 3/16-5/16" wire or line equal to water depth
2 liter Nansen bottle
reversing thermometer holder
2 "protected" reversing thermometers (approx. range -2 - 10 deg C, auxillary therm -15 - 50 deg C)
1 "unprotected" reversing thermometer (approx. range -2 - 30 deg C, auxillary therm -20 - 60 deg C)
magnifying ocular for reversing thermometers
8oz salinity bottles

Methods: **Rigging**

1. Place protected thermometers in left and center positions of reversing thermometer holder, unprotected thermometer in right position; record thermometer serial numbers and positions in hydrographic data book and field notebook *
2. With shackles, attach cannonball weight to end of CTD on which profiler switch is, attach wire thimble to other end of CTD
3. Attach Nansen bottle on wire 1m above CTD in tripping position
4. Attach reversing thermometer holder to Nansen bottle

Deployment

1. Turn CTD on using profiler switch; record cruise number, station name, consecutive station number, date, fathometer depth reading, latitude and longitude, CTD cast number and time
2. Allow CTD to equilibrate to surface water temperature for 2 minutes; record time
3. Spool wire out at a constant rate of approximately 60m/min until CTD is within 5m of bottom in calm seas or 10m if ship is rolling (estimate depth by wire angle and length); record time, length of wire out, wire angle, estimated CTD depth
4. Allow reversing thermometers to equilibrate to water temperature at depth for 5 min
5. Release messenger to trip Nansen and collect water sample; record time messenger sent
6. Allow sufficient time for Nansen bottle to trip, then pull wire in at constant speed of approximately 60m/min to surface
7. Turn off CTD profiler switch; record time

Data collection

1. Using ocular, read thermometers from left to right. Read reversing thermometers to 3 decimal places and auxillary thermometers to 2 decimal places; record readings on hydrographic data sheets
2. Record salinity bottle number; rinse bottle twice in water collected at depth, drain; fill bottle to shoulder; repeat with a second salinity bottle
6. Record remaining data on hydrographic data sheets: cruise number, consecutive station number, date and time messenger was sent, station location, time zone, ship code and salinity bottle numbers

Cleaning

1. After collecting water sample, drain Nansen bottle

* record data in field notebook unless otherwise indicated

STANDARD OPERATING PROCEDURES
Deployment of National Institute of Oceanography (NIO) net

Date: 13 October 1989

Author: Brenda A. Holladay
Laboratory Technician
Institute of Marine Science
University of Alaska Fairbanks
Fairbanks, AK 99775
(907) 474-7990

Purpose: to sample ichthyoplankton from discrete depth increments of the water column

Procedures

Equipment:
- $1m^2$ NIO net frame
- 505u or 1000u nets
- 505u or 1000u codends
- double tripper mechanism
- flowmeter(s)
- SeaBird SBE 19 Profiler S/N 192411-261, enclosed in plastic housing effective to 600m depth, pressure sensor good to 680m. 256K (6 hours) memory, 600 baud output for real time
- 1-kg messengers
- 505u mesh sieve
- seawater hose
- sample jars

Methods: Rigging

1. Prior to first net tow, attach 2-3 nets to NIO frame: currently using 1000u in bottom net for oblique tow to depth, 505u in middle and top nets to increment the water column; can use drogue net for bottom net, fish with middle net or middle and top net
2. Attach double tripper to NIO frame; attach wire thimble to double tripper
3. With twine or string, attach flowmeter 18" to the rear of net opening; balance flowmeter in center of net opening
4. With shackles, fasten SeaBird portable CTD to upper corner of NIO frame

Fishing (each net tow)

1. Depths to be fished will be determined for each station based on depth of station, CTD profiles and previous plankton distributions
2. Rig double tripper with chains from net frame
3. Attach codend to each fishing net

4. Record flowmeter serial number(s) and take reading from each flowmeter before tow *
5. With bottom net open, lower net into water until CTD is approximately 0.5m below surface; record time and depth
6. Fish at surface for 2 minutes while temperature sensor of CTD equilibrates to surface temperature; record time
7. Spool wire out at a constant rate (30-60m/min) OR do a stepped oblique tow by stopping wire motion at equal depth intervals for equal lengths of time until net reaches desired depth (calculated from wire angle and length of wire out); record depth and time
8. Release 1st messenger to trip the bottom net closed, middle net open; record time messenger sent and time net tripped
9. Fish middle net at discrete depth, releasing 2nd messenger exactly 5 min after first OR spool wire in at constant speed (20-40m/min) OR do a stepped oblique tow to desired depth before releasing 2nd messenger; 2nd messenger closes middle net and will open top net if 3 nets are rigged in frame; record depth, time messenger sent, and time net tripped
10. Spool wire in to surface at a constant speed of 60m/min if not fishing; if fishing, spool wire in to surface at 20-40m/min OR perform a stepped oblique tow; record surface time
11. Turn off CTD; record time
12. Wash zooplankton down nets with seawater hose, concentrate plankton by draining in sieve
13. Contain in sample jars (label format below) with preservative in following proportions: 50% plankton:50% isopropanol, 30% plankton:70% ethanol, or 90% plankton:10% formalin
14. Label with yellow tape and display information about tow in this format:

CRUISE #	[STATION NAME]		DATE
CONSECUTIVE STATION #			PLANKTON #
DEPTH SAMPLED	NET	MESH	DURATION OF TOW

14. Identify the preservative with an additional color-coded label:
    green = isopropanol
    red = ethanol
    blue = formalin
15. Copy information about net tow onto rite-in-rain form; detach perforated square from rite-in-rain form and insert in sample jar

* record data in field notebook unless otherwise indicated

STANDARD OPERATING PROCEDURES
Laboratory Sorting of Zooplankton

Date 13 October 1989

Author: Brenda A. Holladay
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Fairbanks, AK 99775
(907) 474-7990

Purpose: to isolate fish and fish eggs from the remainder of sampled zooplankton

Procedures

Equipment and supplies:

jar containing zooplankton sample in preservative
505u mesh sieve
funnel
assorted graduated cylinders
additional jar
forceps
sorting tray
dissecting microscope
two vials

Method: Choosing a plankton sample

1. Laboratory supervisor determines order in which to sort samples and designates a case of highest priority samples
2. Plankton sorter obtains unsorted sample from the designated case

Determining volume of zooplankton

1. Copy information from lid of jar to the sample checkout form; label lid with sorter's name
2. Transfer sample and preservative to large graduated cylinder; measure total volume in ml of zooplankton and preservative to get **Total Volume**; record on sample checkout form
3. Decant preservative off zooplankton into another graduated cylinder by sieving entire sample through funnel and 505u mesh sieve; measure **Preservative Volume** in ml; record on sample checkout form
4. Subtract **Preservative Volume** from **Total Volume** to calculate **Zooplankton Volume** in ml; record **Zooplankton Volume**, sorting date and sorter's name on sample checkout form
5. Replace sample in jar and add an equal amount of water

Preparing jar labels and preservative

1. Copy information from jar lid and rite-in-rain label inside jar onto the sample sort form; label sample jar **unsorted**
2. Label the additional jar identically to the sample jar but identify it as **sorted**; fill jar to approximate **Zooplankton Volume** with fresh isopropanol
3. If sample was originally preserved in formalin or ethanol, record preservative change to isopropanol on jar lid, rite-in-rain label inside jar and sample sort form

Isolating fish and fish eggs

1. Transfer a small amount of zooplankton into a sorting tray
2. Under dissecting microscope, methodically sort through plankton, picking out all fish and fish eggs
3. Transfer fish to one vial (labeled as below and filled with isopropanol) and eggs to a separate vial
4. After sorting this portion of the sample, transfer it into the additional jar
5. Sort through entire sample jar in small increments

VIAL LABELS: use yellow labeling tape and circular stickers

CRUISE
STATION NAME
CONSECUTIVE STATION NUMBER
DATE OF NET TOW
TYPE OF GEAR
MESH SIZE
PLANKTON #
DEPTH OF TOW
DURATION OF TOW
PRESERVATIVE
VIAL # (CONSECUTIVE JAR # AND "A" FOR FISH OR "B" FOR EGGS)

Label 1/4" diameter yellow circular sticker with vial #, and attach to vial lid

6. If sample is not completely sorted, store the unsorted portion in water for up to 5 days; after 5 days, store both sorted and unsorted portions in isopropanol
7. When jar is completely sorted, label lid with 3/4" diameter yellow sticker stating sorter's name, date sorted and consecutive jar #

STANDARD OPERATING PROCEDURES
Counting, Measuring Length and Identifying Fish Larvae and Eggs

Date: 13 October 1989

Author: Brenda A. Holladay
Laboratory Technician
Institute of Marine Science
University of Alaska Fairbanks
Fairbanks, AK 99775
(907) 474-7990

Purpose: To record information for each fish species about number, size and condition of fishes and eggs collected by individual plankton tows

Procedures

Equipment and supplies:

dissecting microscope
forceps
vials of collected fish and eggs
Clay Adams laboratory counter with depressible keys
computerized video system with light pen

Methods:

Counting

1. For each vial, record cruise name, station name and consecutive station number, plankton number, plankton depth
2. Place all fish or eggs from one vial in a sorting tray; view under dissecting microscope in a methodical manner
3. Depress counter once for each fish head or egg until vial is completely counted
4. Record total number of fish or eggs

Identification, length measurement and condition factor

1. Procedures are currently uncertain, but will be detailed at a later time

Biographical Sketch of Appendix D

Brenda L. Norcross

SS# 355-42-8879

Education: Ph.D., Marine Science, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, Virginia, 1983

M.S., Biology, St. Louis University, St. Louis, Missouri, 1976

A.B., Biology, MacMurray College, Jacksonville, Illinois, 1971

Professional Experience: Assistant Professor, Institute of Marine Science, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 1989-present

Assistant Professor, Division of Biological Oceanography and Fisheries Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, Virginia, 1986-1988

Assistant Professor, Computer Center, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, Virginia, 1984-1986

Research Biologist/Oceanographer, Ocean Research and Education Society, Inc., Gloucester, Massachusetts, 1984

Publications: International Symposium Proceedings

Austin, H. M., D. A. Evans and B. L. Norcross. 1986. Time series analysis as a means of examining long term biological data sets. IEEE Oceans 1986 Conference Proceedings. 3:946-952.

Journal Publications

Norcross, B. L. and H. M. Austin. 1988. Middle Atlantic Bight meridional wind component effect on bottom water temperatures and spawning distribution of Atlantic croaker. *Cont. Shelf Res.* 8(1):69-88.

Norcross, B. L. and R. F. Shaw. 1984. Oceanic and estuarine transport of fish eggs and larvae: A review. *Trans. Am. Fish Soc.* 113(2):153-165.

Selected Unpublished Reports

Norcross, B. L. 1988. Transport and recruitment of spot (*Leiostomus xanthurus*) into the Chesapeake Bay, USA. ICES Early Life History Symposium, 1988, Paper No. 82, 16 pp.

April 1989

DETAILED BUDGET

Note - $413.000 is the amount allocated for Study #19 in the State/Federal Plan through February 1990. Expenses projected here are for collection of samples in 1989 and for workup of samples and analysis of data. This budget is project to complete one year only of the project and does not include additional sampling which would be covered under a separate budget.

	April 1989 -June 1990		July-December 1990	
100 - SALARIES				
Name	Months	Cost	Months	Cost
B.L. Norcross, PI	8	35016	4	18384
B.A. Holloday, Tech	14	39769	3	8948
Lab Ass't II (TBN)	8	16631	6	13105
A.J. Paul, Scientist	2	9527	1	5807
J. McDonald, Tech	5	14575	0	
Student Ass't III	10	11943	4	4777
Student Ass't II	100	98107	10	9811
G. Mimkin, Elect. Tech	2	9906	0	
D. Nebert, Computers	2	11602	0	
J. Smithhisler, Mar. Tech	3	11105	0	
F. Mueter, MS Student	6	5200	3	2691
Lab Ass't I (Misc.)	3	5667	0	
Sub-Total		269048		63523
Staff Benefits		37971		12270
TOTAL SERVICES		307019		75793
200 - TRAVEL				
Field collections		15,000		0
Meetings		15,000		2500
TOTAL TRAVEL		30,000		2500
300 - SERVICES				
Boat charter (F/V Jennie Girl)		31600		
Phone, Fax, Copying		5000		1000
Publications & Drafting		3000		5000
Data Management @ $36/hr		15000		5000
Shipping		7000		
Gear repair/replacement/calibration		6000		
MOCNESS consultant		4000		
GIS Consultant		5000		15000
Biometrician Consultant		5000		5000
Sub-Total		81600		31000

Equipment Use	Months	Cost	Months	Cost
Wild Microscope @ $350	1	350		
@ $175	13	2275	3	525
Wild Microscope @ $250x4	1	1000		
@ $125 x 4	7	3500		
Video system @ $2000	1	2000		
@ $1000	7	7000	3	3000
MOCNESS @ $6500	1	6500		
@ $3250	1	3250		
Sub-Total		25875		3525
TOTAL SERVICES		107475		34525

400 - SUPPLIES

Software, disks, office supplies, etc.	5000	2000
Field & Lab - Nets, jars, flowmeters, preservatives, labels, etc.	20000	
TOTAL SUPPLIES	25000	2000

500 - EQUIPMENT

PC Office computer	3000	
Computer printer x 3	3300	
Portable computer	1600	
Portable computer printer	600	
AT Lab computer	4000	
TOTAL EQUIPMENT	12500	

TOTAL	456119	114818

CONFIDENTIAL

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT

DETAILED STUDY PLAN

DRAFT

Project Title:	Undersea Observations of Submerged Oil
Study ID Number:	Fish/Shellfish Study Number 20
Lead Agency:	State of Alaska, ADF&G; OSIAR Division
Cooperating Agencies:	Federal: NOAA State: DEC
Principal Investigator:	Dan Huttunen, Fishery Biologist
Assisting Personnel:	Paul Skvorc, Biometrician Alan Kimker, Fishery Biologist
Date Submitted:	September 15, 1989

	Signature	Date
Principal Investigator:	[signature]	10-13-89
Supervisor:	[signature] Paul A. Skvorc	Oct 13-89
OSIAR Sr. Biometrician:		
Osiar Program Manager:	[signature]	10-18-89
OSIAR Director:		

I. TITLE: UNDERSEA OBSERVATIONS OF SUBMERGED OIL

II. INTRODUCTION:

Information exists about the surface distribution of crude oil spilled from the Exxon Valdez. Ancillary information also exists suggesting that some of the spilled oil has entered the benthos of Prince William Sound and Katchemak Bay which are home to a variety of highly valuable organisms including dungeness and brown king crab, spot shrimp, halibut, ling cod, and various rockfish species. However, the extent and distribution of submerged oil is largely unknown due to a paucity of viable techniques capable of sampling large areas of the ocean bottom.

This project will ground-truth visual observations for oil by remotely-operated vehicle (ROV) with scoop samples and bottom trawls at depth. The presence of petroleum hydrocarbons in scoop samples sealed at depth or on bottom trawls will solely verify the presence or absence of submerged oil. This project will support the overall resource damage assessment by determining whether visual observation from an ROV is a cost-effective method of sampling large areas of the ocean bottom for submerged crude oil residues. It will also provide direct observations of semi-pelagic and benthic species in waters near heavily oiled beaches.

The project will be determined to be successful if at least 50 matched physical and optical samples can be collected at depth during one five-day sampling session in Prince William Sound and 40 matched physical and optical samples collected during one four-day sampling session in Katchemak Bay.

Background Data

Institutional investigators from various state (ADF&G, ADEC) and federal (NOAA) agencies have collected intertidal and subtidal sediment samples within heavily oiled embayments within Prince William Sound. Results are still unpublished, but no large concentrations of benthic oil have yet been reported.

Experimental Design

It is hypothesized that a significant amount of crude oil has entered the benthos. Because the heaviest distillate fractions of North Slope Crude oil are characterized by a specific gravity of 0.9, this project is operating under the assumption that the mechanism causing oil to sink is one where oil becomes mixed with gravel or rocky sediment along oiled shorelines. Therefore all sampling in Prince William Sound will be conducted in embayments documented as having been heavily oiled, and in deep areas of Katchemak Bay where

submerged oil may have settled (Table 1). Additionally, attempts will be made to sample in areas previously sampled for benthic oil during studies conducted shortly after the oil spill.

The original intent of this study (Fish/Shellfish Study No. 20) was to identify, through direct observation, oiled and non-oiled benthic sample areas for incorporation into ongoing Fish/Shellfish studies 14, 17, and 23 as experimental and control areas. However, preliminary information from other related oil spill studies indicates that any submerged oil exists primarily within sediment and may not be visible. Therefore, this portion of the undersea observation project is tasked to ground truth the ROV as a technique of observing oil at depth since grab samples are limited in scope and extremely costly to analyze. Therefore, during each dive, the ROV will be used to first visually look for oil on or near the bottom. If a suspicious substance is observed, the ROV will collect a sample. If no suspicious substances are observed, the ROV will collect a sediment to verify the absolute presence or absence of any oil in the sediment.

III. OBJECTIVES:

A. Collect at least 50 coincident visual (video-taped) and physical (sealed scoop) samples of the ocean bottom in selected (heavily oiled) areas of Prince William Sound, Alaska.

B. Collect at least 20 coincident visual (video-taped), physical (bottom trawl), and acoustic samples of the ocean bottom in selected (heavily oiled) areas of Katchemak Bay, Alaska.

C. Determine if a Remotely-Operated Vehicle can be used to detect the presence of submerged oil located by physical methods.

IV. METHODOLOGY/DATA ANALYSIS:

This study plan addresses only the initial ground-truth phase of this project. Because of the urgency of this phase of the project, the scope of this study plan will be limited specifically to that task.

A. Location:

Western Prince William Sound waters near beaches documented as having been heavily oiled including the following bays: East Twin Bay of Perry Island; Northwest Bay of Elenor Island; Herring Bay, Bay of Isles, and Snug Harbor of Knight Island; Shelter Bay of Evans Island; Sleepy Bay of Latouche Island; and the northern shore of Greene Island.

Central Katchemak Bay waters near Homer Alaska.

B. Data to be Collected:

1. Date, dive number (sequential), depth, physical sample number, time, location by latitude, longitude, and name, remarks (visual presence or absence of oil), collectors initials, and date and time of recording for each dive and/or sample taken. Recorded by principal investigator in log book.

2. Exact location plotted on high resolution nautical chart of sampled waters.

3. Presence or absence of petroleum odor in samples when transferred to clean sample bottles.

4. Presence or absence of petroleum odor or residue on bottom trawl nets after tows.

5. Digital or converted analog tapes of acoustic output associated with trawl and visual tapes from the ROV.

6. Number, type, and condition of animals observed while looking for oil.

C. Sample Collection Methods:

1. Scoop samples:

a. Upon arrival on station near shore in previously identified areas of heavy onshore oiling, the vessel will anchor and the exact latitude and longitude precisely calculated from published maritime charts and recorded.

b. The ROV will be deployed by hand, and the tether/umbilical monitored by the deck hand and one Department representative.

c. Video-taping will begin and the sample collecting containers will be opened during the descent toward the bottom.

d. Visual sampling will begin immediately upon reaching the bottom in a 100 ft radius of the station for presence of oil.

e. A scoop sample of any suspected oil will be collected and sealed, and if no suspicious substance is observed, a sediment sample will be collected and sealed for subsequent chemical

analysis.

f. The ROV will be retrieved as quickly as possible after the sealed samples are secured aboard the unit.

g. At the surface, the samples will be unsealed and transfered to certified clean teflon lid sample jars, labeled as to date, time, location, and investigator and frozen immediately. The presence or absence of any petroleum odors in samples will be noted during the transfer process.

2. Bottom trawl samples:

a. Upon arrival on station the exact latitude and longitude will be calculated from published maritime charts, and the bottom trawl net and doors will be deployed.

b. A 30-minute tow will be conducted while video-taping color-coded acoustic samples on standard (1/2 inch) VHS format and video-tapes.

c. The net will be retrieved and inspected for visual and/or olfactory evidence of petroleum residues on webbing and captured fish.

d. The vessel will return to the exact starting point of the transect and will either "live-boat" (free swim) or anchor and deploy the ROV.

f. Video-taping will begin during the descent and will continue uninterrupted throughout the duration of the dive.

g. Visual sampling of a 100 radius will begin upon reaching the bottom and the presence of any suspicious substance will be recorded.

h. At the surface, any suspicious substances or the tissues from any captured animals suspected to have been contaminated will be transferred to certified clean teflon lid sample jars, labelled as to date, time, location, and investigator, and frozen immediately.

V. SCHEDULES:

A schedule of tasks to be completed includes:

Task	Date
Prince William Sound Embayment Sampling	Late September
Katchemak Bay Bottom Trawl Sampling	Early October
Data Analysis and Report Preparation	10/9-2/15

VI. REPORTS:

All results of this investigation will be reported to ADF&G, Division of Oil Spill Impact Assessment and Restoration. These data will become available in report form as litigation is resolved.

VII. BUDGET SUMMARY:

Line Item	FY 89	OY 90*
Personnel	60.0	60.0
Travel	22.5	22.5
Services	218.8	178.8**
Commodities	18.8	18.8
Equipment	230.0	230.0
Total	550.1	510.1

* Oil Year, March 1989 through February 1990.

** Reduced by 40.0 due to reallocation to fish/shellfish study no. 26.

Table 1. Prospective locations for Prince William Sound and Kachemak Bay ROV sampling, 1989.

Location	Latitude	Longitude	Depth (fath)
Prince William Sound:			
East Twin Bay	60° 43.50'N	147° 55.88'W	5 - 40
Crafton Island	60° 30.06'N	147° 57.02'W	5 - 40
Smith Island	60° 32.00'N	147° 21.70'W	5 - 30
Upper Passage	60° 31.75'N	147° 36.95'W	5 - 50
Greene Island	60° 16.63'N	147° 27.80'W	5 - 25
Bay of Isles	60° 23.25'N	147° 41.80'W	5 - 45
Snug Harbor	60° 15.00'N	147° 43.35'W	5 - 55
Shelter Bay	60° 07.80'N	147° 55.28'W	5 - 40
Sleepy Bay	60° 04.10'N	147° 50.05'W	5 - 60
Evans Island	60° 09.73'N	147° 53.35'W	5 - 30
Herring Bay	60° 25.69'N	147° 46.92'W	5 - 50
Northwest Bay	60° 33.47'N	147° 34.82'W	5 - 60
Louis Bay	60° 28.38'N	147° 40.71'W	5 - 50
Knight Island	60° 30.58'N	147° 42.68'W	5 - 20
Kachemak Bay:			
Upper Kachemak Bay	59° 40.38'N	151° 12.49'W	15 - 25
Middle Kachemak Bay	59° 35.92'N	151° 28.11'W	25 - 50
Outer Kachemak Bay	59° 31.48'N 59° 33.70'N	151° 40.13'W 151° 29.79'W	60 - 95 60 - 95
Tutka Bay	59° 26.20'N 59° 27.70'N	151° 22.02'W 151° 24.30'W	40 - 90 40 - 90

CONFIDENTIAL DRAFT

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DETAILED STUDY PLAN

Project Title:	INJURY TO CLAMS OUTSIDE PRINCE WILLIAM SOUND
Study ID Number:	Fish/Shellfish Study Number 21
Lead Agency:	State of Alaska, ADF&G; Commercial Fish Division
Cooperating Agency(ies):	Federal: USDI (NPS), USFS State: DNR
Principal Investigator:	Alan S. Davis, Fishery Biologist
Assisting Personnel:	Ron Regnart, Fishery Biologist Charlie Trowbridge, Fishery Biologist Peggy Murphy, Biometrician
Date Submitted:	October 12, 1989

	Signature	Date
Principal Investigator:	[signature]	[illegible]
Supervisor:	[signature]	10/13/89
OSIAR Senior Biometrician:		
OSIAR Program Manager:	[signature]	10/17/89
OSIAR Director:		

INTRODUCTION

This project is to determine impacts of the Exxon-Valdez oil spill on bivalve resources outside Prince William Sound. Bivalve mollusks are an important component of the food chain, and they support subsistence and sport fisheries in lower Cook Inlet and Kodiak Island. Because they are relatively sedentary and occupy nearshore areas, bivalves may be particularly susceptible to contamination by oil. It is hypothesized that increased hydrocarbons in nearshore sediments could affect bivalves by increasing mortality, decreasing growth, or causing sublethal injuries. This study seeks to evaluate these potential effects by comparing data obtained from oiled and non-oiled beaches. Data on growth obtained in 1989 will provide a baseline for comparison with growth data to be taken at a future date. Documenting effects on littleneck clams is required to advise the public of the full scope of impact by the oil spill on current and future employment, recreation, and lifestyles of coastal communities on Prince William Sound.

OBJECTIVES

A1. Test the hypothesis that the level of hydrocarbons in bivalves is not related to the level of oil contamination of a beach. The experiment is designed to detect a difference of 1.4 standard deviations in hydrocarbon content with the probability of making a type I and type II error of 0.05 and 0.1, respectively.

A2. Test the hypothesis that the level of hydrocarbons in sediments is not related to the level of oil contamination of a beach. The experiment is designed to detect a difference of 1.4 standard deviations in hydrocarbon content with the probability of making a type I and type II error of 0.05 and 0.1, respectively.

B. Document the presence and type of damage to tissues and vital organs of bivalves sampled from beaches with no and high levels of oil contamination such that differences of ±5% can be determined between impact levels 95% of the time.

C. Test the hypothesis that the proportion of dead bivalves sampled on beaches is not related to the level of oil contamination of a beach. The experiment is designed to detect a difference of 5% in the proportion of live to dead bivalves from beaches with no and high levels of oil contamination with the probability of making a type I and type II error of 0.05 and 0.1, respectively.

D. Test the hypothesis that the growth rate of littleneck clams is the same at beaches of no and high levels of oil impact. This experiment was designed to detect a difference in mean shell height equal to the difference between the mean shell height at age i and age i+1 clams with the probability of making a type I error equal to 0.01 and probability of making a type II error equal to 0.05.

E. Document any changes in numbers of young-of-the-year clams.

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

METHODS/DATA ANALYSIS

This project will be conducted in two phases. During phase I, (August-September 1989) littleneck clams will be sampled from oiled and non-oiled beaches in lower Cook Inlet and Kodiak for hydrocarbon samples, necropsy samples and growth information. Phase II of the project is to begin in spring 1990. Hydrocarbon, necropsy and growth samples will be collected for littleneck clams from oiled and non-oiled beaches in Resurrection Bay and along the Alaska Peninsula during phase II. All sites in Cook Inlet, Kodiak, Resurrection Bay and Alaska Peninsula will also be sampled during phase II to document any changes in numbers of young-of-the-year clams. A change in the numbers of young-of-the-year clams can be detected by comparing counts of the small clams over multiple years.

The study plan for clams outside Prince William Sound in the State/Federal natural resource damage assessment plan for the Exxon Valdez oil spill states species to be included are cockle, littleneck clam, butter clam, and razor clam. The Fisheries Management Team subsequently determined the best use of resources was to sample one species, littleneck clams, therefore cockle, butter clam, and razor clam have not been sampled[1]. The study plan also indicated samples would be collected from Resurrection Bay, lower Cook Inlet, Kodiak, and the Alaska Peninsula. The limited number of extreme low tides during daylight hours during the sampling period for this study precluded complete sampling of all areas. Resurrection Bay and the Alaska Peninsula are scheduled to be sampled spring of 1990 during Phase II.

[1] Alaska Department of Fish and Game, Sport Fish Division personnel sampled razor clams for hydrocarbon analysis at Clam Gulch and Ninilchik Beaches prior to any oil reaching the area. Susequent to oiling of Clam Gulch beach, another hydrocarbon sample was obtained.

Study Sites

Beaches known to contain clams were surveyed to determine the level of oil contamination. Eight study sites representing two levels of oil contamination (subjectively rated as no or high contamination) were chosen to be sampled (Table 1, Figure 1-2). Beaches with no oil contamination are Jakalof Bay and Seldovia Bay in lower Cook Inlet and Port Bailey and McDonald Lagoon on Kodiak Island. Beaches with heavy oil contamination are Windy Bay and Port Dick in lower Cook Inlet and Ruth Bay and Kupreanof Strait on Kodiak Island.

Table 1. Study beaches representing two levels of oil impact.

IMPACT LEVEL	
NONE	HIGH
Jakalof Bay (Cook Inlet)	Windy Bay (Cook Inlet)
Seldovia Bay (Cook Inlet)	Port Dick (Cook Inlet)
Port Bailey (Kodiak)	Ruth Bay (Kodiak)
McDonald Lagoon (Kodiak)	Kupreanof Strait (Kodiak)

For each sample site the following site description information will be recorded (Appendix A, Form 1 and Form 2): site orientation (N-NW etc.), latitude, longitude, beach slope, low tide height, percent dominant substrate composition, temperature and salinity of the water, weather and wave action. Temperature and salinity of the water will be measured at a distance of approximately 5 meters offshore from the sampled beach at the daily low slack tide.

Sample Design

Beaches will be sampled at maximum low tides for a monthly tidal cycle beginning in September. At each beach, three sampling transects will be run to insure complete coverage of the beaches as distribution of oil on the beaches is unknown. Transects will be perpendicular to the water's edge and parallel to each other with a total distance between each transect of 15 meters (Appendix B). Transects were perpendicular to the water to insure complete sampling of clam habitat. The top of each transect is placed at the +1.6 meter tide level and the bottom of the transect at the lowest tide level. Tidal height will be determined using reference points from a standard tide table, a hand level, and a stadia rod. Transect number, bottom of the transect tidal height, top of the transect tidal height, middle of the transect tidal height, and distance from the top of the transect to each remaining sampling quadrate will be recorded.

Prior to sampling, the upper distribution of clams will be determined by removing sediment to a depth of 30 cm (12 in) along a trench adjacent to the proposed transect. The trench is dug starting from

the top of the transect and continuing until clams are encountered. The distribution of clams will extend below the low tide levels occurring during this project. The bottom of each transect and the bottom sampling quadrate will occur at the daily low tide level.

A total of seven quadrates will be sampled from each transect to obtain hydrocarbon and necropsy specimens. Sample quadrates are each 0.5 m^2. Additional sampling or complete sampling of each transect (all possible sampling quadrates) may be necessary if an insufficient number of clams is recovered within the seven sampling quadrates to meet project objectives. Quadrates will be sampled from the top to the bottom of each transect as the tide recedes.

The first sample quadrate will be located where the first clam was encountered in the preliminary trench. The second quadrate will be located equal distance from the top and bottom sampling quadrates. The tidal height of the second quadrate is calculated as half the difference between the tidal height of the first quadrate and the low tide height subtracted from the tidal height of the first quadrate. The third quadrate is taken at an equal distance from the first and second quadrates (a tidal height approximately one quarter the difference between the tidal height of the first quadrate and the low tide height subtracted from the tidal height of the first quadrate). The fourth sample is taken at a point equal distance between the second and third sample quadrates. The fifth sample is taken at a point equal distance between the low tide level and the second quadrate, a tidal height equal to three quarters the difference between the tidal height of the first quadrate and the low tide height subtracted from the tidal height of the first quadrate. The sixth sample is taken in the area equal distance between the fifth and second sample quadrates. The last sampling quadrate is placed at the low tide level (Appendix B).

Sediment Sample Collection for Hydrocarbon Analysis

A total of three sediment samples will be collected from each beach site (one from each transect). All sediment samples will be collected before bivalve sampling is performed. The hydrocarbon sample from each transect will be a composite sediment sample which will be collected by scooping one tablespoon of sediment to a depth of 2 to 3 cm from each of the seven sample quadrates on a transect.

All samples from each transect will be placed in an 8 oz glass jar rinsed with methylene chloride. Each jar will be labelled with the site name, latitude, longitude, date, "SEDIMENT", transect number, sample number, names of the sampling team members, "BIVALVE", and "ADF&G".

Three composite sediment samples (one per transect) will be obtained from each beach sampled. This will provide a total of 12 samples for each impact level (1 hydrocarbon sample/transect * 3 transects/site = 3 hydrocarbon samples/site; 3 hydrocarbon samples/site * 4

sites/impact level = 12 hydrocarbon samples/impact level).

The small sub-samples of sediment taken from each sampling quadrate will provide a representative mixture of sediment composition and contamination throughout the transect. One composite sediment sample for each transect at each site provides 12 composite samples for each impact level (none and high) exceeding the industry standard of 8 samples for each treatment level. A sample size of 12 composite samples per impact level is considered an adequate number of samples to detect a difference in sediment contamination between impact levels at the desired α and β levels.

Bivalve Sample Collection

One species, littleneck clam, will be sampled for hydrocarbon analysis, necropsy, and to estimate percent of live and dead clams. Littleneck clams will also be collected to estimate numbers of young-of-the-year clams and age and growth statistics.

Hydrocarbon Samples:

Specimens for hydrocarbon analysis will be taken from all sampling quadrates before any other specimen sampling is conducted. Clams will be randomly selected for hydrocarbon analysis from sampling quadrates at each site. Care will be taken to avoid contamination of a specimen by sediments not immediately surrounding the specimen. Each sample will be placed immediately in a sample container before another bivalve is obtained.

One composite hydrocarbon sample will be obtained from each transect. The desired size of each composite tissue sample is 15 gm. The number of bivalves to provide this sample from each transect was estimated based on the average size of littleneck clams. Each hydrocarbon sample will be composed of 14 specimens. The 14 samples from each transect (1 hydrocarbon sample) will be selected by randomly picking two clams from each of the seven sampling quadrates. Each clam must have a shell length of 2-5 cm.

Bivalve samples are being limited to a particular size range because rates of uptake, metabolism, and depuration by clams probably change with size. If specimens of the desired size are not found in each of the sampling quadrates, then the desired number of additional specimens will be collected from the other sample quadrates.

Specimens of each species from each transect will be placed together in a non-plastic container and the air will be evacuated from the container. A 16 oz squat jar should be large enough for 14 littleneck clams. Alternatively, clams can be wrapped in paper towels then wrapped in aluminum foil. The foil packages can be stored in metal coffee cans covered with tin foil prior to sealing with the plastic lid. All containers and pieces of aluminum foil must be rinsed with methylene chloride before they are used. Each

container will be labelled with the site name, latitude, longitude, date, species, transect number, names of the sampling team members, "BIVALVE" and "ADF&G". All hydrocarbon samples will be stored at -40° F until hydrocarbon analysis is initiated.

Combined tissue samples from each sampling quadrate will provide a representative mixture of bivalve tissue composition and contamination throughout the transect. An estimate of 3 hydrocarbon samples from each site is needed for detecting contamination between impact levels (Dr. B. Clark, personal communication). A sample of 12 composite samples per impact level will allow the detection of differences in hydrocarbon content of 1.4 standard deviations with α and β levels of 0.05 and 0.1, respectively.

Necropsy Samples:

Collection of specimens for necropsy will begin only after all hydrocarbon samples have been taken. Total sample size is 20 live or moribund littleneck clams from each beach site. With 20 bivalves sampled from each beach, the total sample for each treatment (no and high oil contamination) will be 80. This sample size will allow detection of differences in presence of tissue damage of ±5% with 95% confidence between sample obtained from beaches with different levels of oil impact (Dr. Ted Meyers, Alaska Department of fish and game, personal communication). This sample size will allow detection of gross differences between beaches with no and high oil impact.

One specimen will be randomly selected from each sampling quadrate. This will yield a total of 21 specimens. One specimen from the 21 collected will be randomly selected and discarded from the sample to achieve a sample size of 20 specimens. Sampling procedures and quality assurance will be conducted as outlined in the histopathology guidelines, and sample preparation will be followed as outlined in Appendix C. Histopathological analysis of bivalve tissues will include all criteria listed in the histopathology guidelines. Necropsies will be performed by a qualified contractor approved by the Histology Technical Group.

Bivalve Mortality Samples:

All live and recently dead specimens of littleneck clams from each sampling quadrate will be separated from the sediment and placed in containers. Recently dead bivalves are defined as those bivalves with tissue remaining attached to the shell. Each container will be identified with the species name, transect number, and sampling quadrate number. Each container will be set aside until quadrate sampling is completed. After all quadrates in each transect have been sampled, live specimens and recently dead specimens will be counted and placed in separate containers. Numbers of live and dead bivalves will be recorded by sampling quadrate and transect on Form 4 (Appendix A).

An additional 30 meter transect located along the high tide line above the sampling stations will be sampled. All recently dead bivalves along this transect will be counted.

Growth and Age Samples:

Littleneck clams will be collected for growth and age estimation. A total of 100 specimens will be collected from each transect at each site. From each transect five sampling quadrates will be selected at random. From each of these, 14 specimens will be randomly sampled from the quadrate containers. Fifteen specimens will be randomly sampled from the remaining two quadrate containers. The specimens from each sampling quadrate container will be placed in separate bags. Each bag will be labelled with the site name, latitude, longitude, date, transect number, sampling quadrate, names of the sampling team, "BIVALVE" and "ADF&G". Specimens from each sampling quadrate will be cooked and shucked at a later time. Once specimens have been cooked and shucked, each valve for each specimen is labeled with a specimen number unique to that specimen. Care will be taken to keep specimens from different sampling quadrates separate until specimen numbers have been assigned and recorded for each sampling quadrate.

The sample of 100 specimens from each transect will provide 300 samples from each beach and 1200 clams for each level of beach impact. Sample size for growth is based on the difference between mean shell height for age i and age i+1 clams, variance in shell height for age i+1 clams, probability of making a type I error equal to .01 and probability of making a type II error equal to .05 (Netter and Wasserman 1985). Data for mean shell height and variance in shell height was taken from Paul and Feder (1973) and applied to areas outside Prince William Sound where information on littleneck clam growth is absent from the literature. The sample size used for detecting difference in growth at age of clams between impact levels was the same as used for Prince William Sound. The sample size used for detecting difference in growth at age was estimated at 261-275 clams for each impact level (Appendix D) which was rounded up to 300 clams. This sample size was probably larger than needed for areas outside Prince William Sound but was maintained to offset any differences in growth that may occur between areas inside Prince William Sound and outside Prince William Sound.

Data Analysis

To address objective A1 (hydrocarbons in bivalve tissues), an analysis of variance will be performed on the hydrocarbon content of clam samples among sites. The results of this test will be related to the level of sediment impact. To address objective A2 (hydrocarbons in sediments), an analysis of variance will be used to test for differences in hydrocarbon content in sediment between sites. Differences in sediment hydrocarbon content will verify that control sites (areas of no oil impact) are in fact "controls".

To address objective C, the proportion of dead clams among sites will be subjected to analysis of variance, and the results related to level of oil contamination of sediments. Statistical differences in injury rates (within specific categories of injury as outlined in the histology guidelines) between impact areas will be evaluated using chi-square analysis (Objective B).

To provide baseline (pre-impact) information on variance in growth at age among sites, an analysis of variance on growth parameters from clams between areas will be conducted. Growth parameters will be determined for various growth curves, such as Gompertz, von Bertalanffy, or polynomial equations. Growth parameters will be presented for the most appropriate growth models only. These beach sites will be resampled after 4 years. An analysis of variance on growth parameters obtained from fitting algorithms for clam growth after impact (1990-1994) will be compared to growth parameters for clam growth prior to impact (approximately 1979-1989) to resolve impact of oil contamination on growth (Objective D). Graphics will be used to display differences in growth among areas over time, including growth curves (size at age) and growth increment at age by year for each beach.

Statistics for analysis of variance will also be computed for comparisons of hydrocarbon content and proportions of dead clams. Appropriate statistics for non-linear and polynomial curve fitting will be computed to evaluate the strength of the fit, including mean square, sum of squares, degrees of freedom, etc. Variances will be estimated for all means computed, such as mean growth at age, mean proportion dead, etc.

A chi-square test will be used to determine if a change in the numbers of young-of-the-year clams has occurred between years and impact levels (Objective E). Trend analysis of the number of young-of-the-year clams will be used to detect potential recruitment failures associated with oil impact.

To address Objective F, all data will be analyzed to determine degree of damage to a stock. Appropriate restoration or mitigation measures will be made. This may include restrictions on human usage to reduce mortality rates or may include the need for continued monitoring of stocks.

SCHEDULES AND REPORTS

Date(s)	Activity
August 1989	Phase I field sampling, lower Cook Inlet
September 1989	Phase I field sampling, Kodiak
September-December 1989	Data entry and analysis
December 1989	Preliminary report on impacts of oil on clams (will not include pre- and post-impact comparisons for growth).
Spring 1990	Phase II field sampling.

PROJECT BUDGET

Line Item	Category	Budget
100	Personnel Services	$ 30.4
200	Travel	$ 2.6
300	Contractual	$ 67.5
400	Commodities	$ 6.0
500	Equipment	$ 2.3
700	Grants	$ 0
Total		$ 108.8

FUNDED PERSONNEL

Class	PCN	PFT_mm	SFT_mm
FB I	11-	2	
FT II	11-		1

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Clark, B. Personal communication. National Marine Fisheries Service, Environmental Conservation Division, 2725 Montlake Boulevard East, Seattle, WA.

Meyers, T. Personal communication. Alaska Department of Fish and Game, Fisheries Rehabilitation and Economic Development Division, P.O. Box 3-2000, Juneau AK.

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Paul, A.J. and H.M. Feder. 1973. Growth, recruitment, and distribution of the littleneck clam, Protothaca staminea in Galena Bay, Prince William Sound, Alaska. Fishery Bulletin 71(3):665-677.

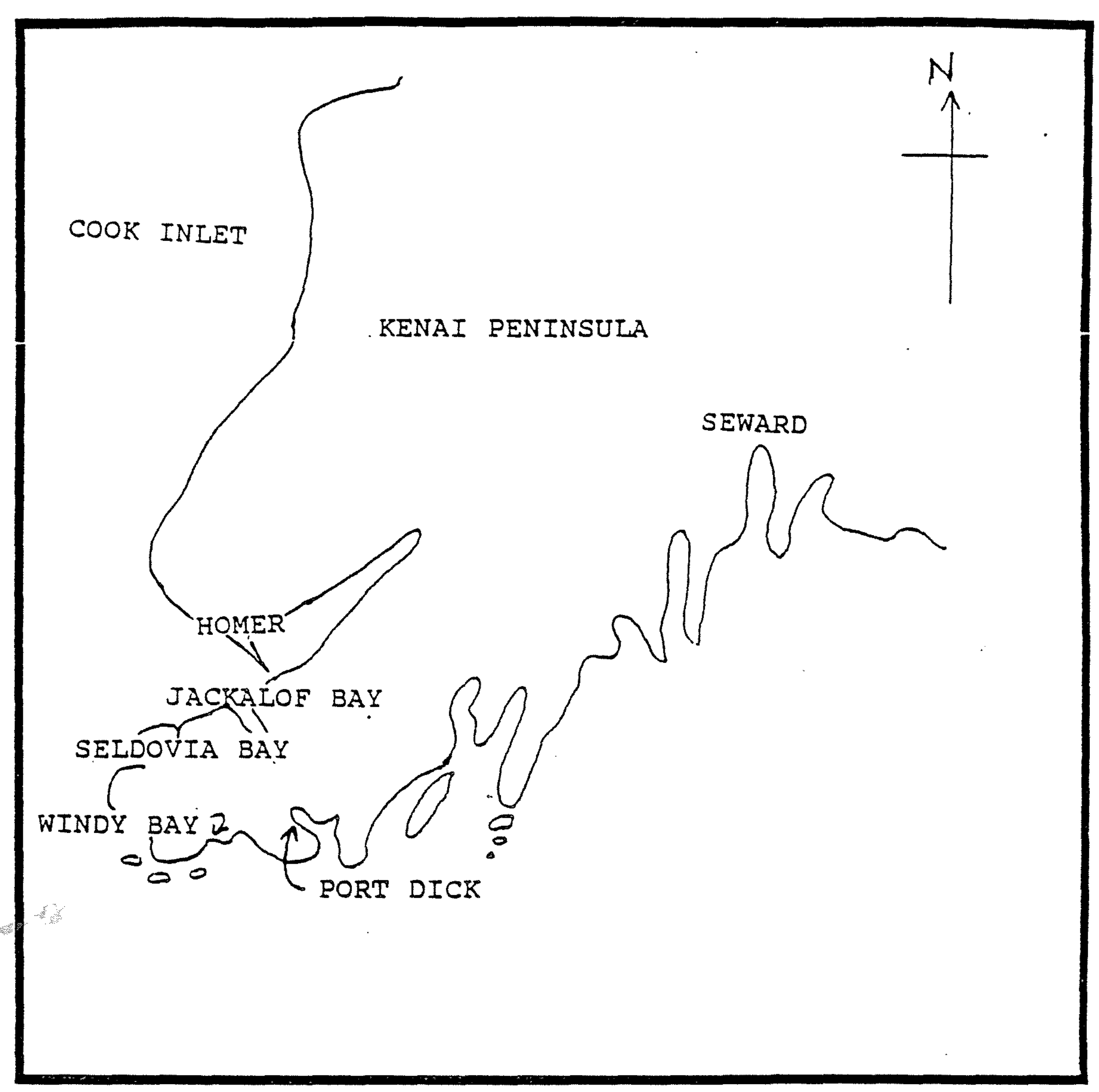


Figure 1. Sampling sites on the lower Kenai Peninsula coast - Jackalof Bay, Seldovia Bay, Windy Bay, and Port Dick.

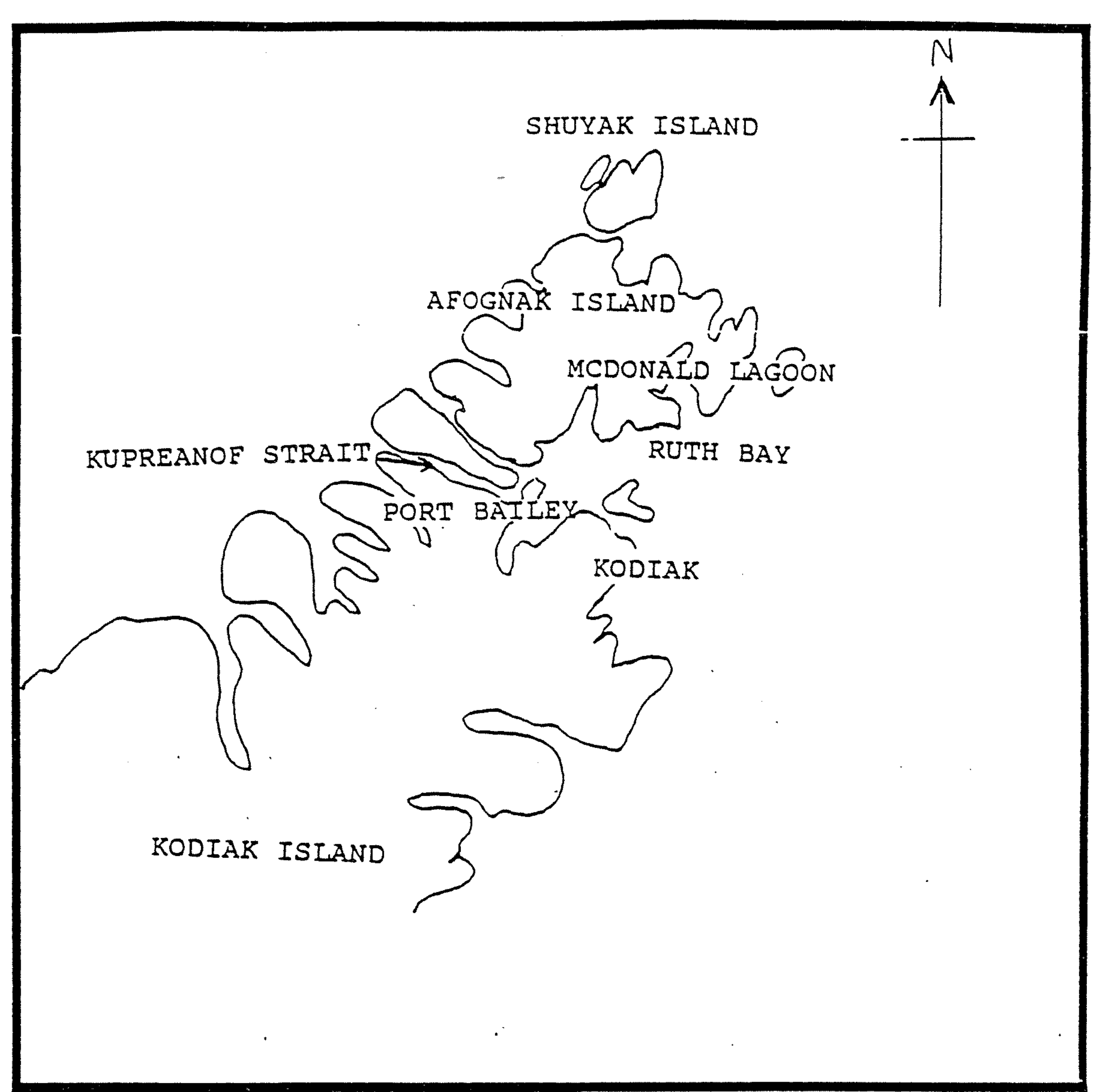


Figure 2. Sampling sites around Kodiak Island - Ruth Bay, McDonald Lagoon, Kupreanof Sraight, and Port Bailey.

ADF&G BIVALVE OIL PROJECT SITE DESCRIPTION FIELD FORM

Site Name ____________________ Date ____________________

Latitude ____________________ Longitude ____________________

Skipper Name ____________________

Vessel Name ____________________ Vessel Ak Number ____________________

Vessel Description ____

Sampling Team	Agency
____________________	____________________
____________________	____________________
____________________	____________________
____________________	____________________

	Code	Description
Waves	___	______________________________
Weather	___	______________________________

Air Temp __________ Sea Temp __________ Salinity __________

	%	Code	Description
Beach Substrate	___	___	______________________________
	___	___	______________________________
	___	___	______________________________

Beach Slope __________ Beach Orientation (eg N-NW) __________

NOAA Reference Point __________ NOAA Low Tide Height __________

Film Role Number __________ Photogragh numbers __________ __________

Waves

Code	Description
1	Glassy
2	Rippled
3	Wavelets
4	Slight 2-4'
5	Moderate 4-8'
6	Rough 8-13'
7	Very Rough 13-20'

Weather

Code	Description
1	Clear
2	Partly Cloudy
3	Overcast
4	Fog or Thick Haze
5	Showers
6	Squalls
7	Drizzle
8	Rain
9	Rain and Snow
10	Snow
11	Blizzard

Substrate

Code	Description
1	Mud-Silt
2	Clay
3	Sand
4	Granule (2-4mm)
5	Pebble(4mm-3cm)
6	Rock Fragments (3-6cm)
7	Cobble Shingle (6-15 cm)
8	Rock (15-25 cm)
9	Boulder(>25 cm)

ADF&G BIVALVE OIL PROJECT
TRANSECT DESCRIPTION FORM 2

Site Name ____________________ Date ____________________

Latitude ____________________ Longitude ____________________

	Transect 1	Transect 2	Transect 3
Bottom of Transect Tidal Height	______	______	______
Top of Transect Tidal Height	______	______	______
Middle of Transect Tidal Height	______	______	______
Length of Transect to Quadrant 3	______	______	______
Length of Transect to Quadrant 4	______	______	______
Length of Transect to Quadrant 5	______	______	______
Length of Transect to Quadrant 6	______	______	______
Total Length of Transect	______	______	______

Distance Between Top of Transect 1 and 2: ______ 2 and 3: ______

Total Width of Sampling Site ______

	Total Number of Clam Shells	Number of Recently Dead Clam Shells
High Tide Line 30 Meter Transect	______	______

Site Name ______________________ Date ______________________

Latitude ______________________ Longitude ______________________

SAMPLE COLLECTION - CHECK THE BOX IF THE SAMPLE WAS COLLECTED

	Transect 1		Transect 2		Transect 3	
SEDIMENT HYDROCARBON	Yes	#Q	Yes	#Q	Yes	#Q
composite sediment sample 1 & number of quadrants sampled	☐	___	☐	___	☐	___
composite sediment sample 2 & number of quadrants sampled	☐	___	☐	___	☐	___
composite sediment sample 3 & number of quadrants sampled	☐	___	☐	___	☐	___

	Transect 1			Transect 2			Transect 3		
LITTLENECK CLAM HYDROCARBON	Yes	#S	#Q	Yes	#S	#Q	Yes	#S	#Q
composite hydrocarbon sample, number of specimens sampled, number of quadrants sampled & size range	☐	___	___	☐	___	___	☐	___	___
	________			________			________		
BUTTER CLAM HYDROCARBON									
composite hydrocarbon sample, number of specimens sampled, number of quadrants sampled & size range	☐	___	___	☐	___	___	☐	___	___
	________			________			________		
COCKLE HYDROCARBON									
composite hydrocarbon sample, number of specimens sampled, number of quadrants sampled & size range	☐	___	___	☐	___	___	☐	___	___
	________			________			________		

	Transect 1			Transect 2			Transect 3		
LITTLENECK CLAM NECROPSY	Yes	#S	#Q	Yes	#S	#Q	Yes	#S	#Q
composite necropsy sample, number of specimens sampled, number of quadrants sampled & size range	☐	___	___	☐	___	___	☐	___	___
	________			________			________		
BUTTER CLAM NECROPSY									
composite necropsy sample, number of specimens sampled, number of quadrants sampled & size range	☐	___	___	☐	___	___	☐	___	___
	________			________			________		
COCKLE NECROPSY									
composite necropsy sample, number of specimens sampled, number of quadrants sampled & size range	☐	___	___	☐	___	___	☐	___	___
	________			________			________		

CLAM MORTALITY FORM 4

Site Name ______________________ Date ______________________

Latitude ______________________ Longitude ______________________

	Transect 1		Transect 2		Transect 3	
	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
SAMPLING QUADRANT 1						
Littleneck clam	___	___	___	___	___	___
Butter clam	___	___	___	___	___	___
Cockle	___	___	___	___	___	___
SAMPLING QUADRANT 2						
Littleneck clam	___	___	___	___	___	___
Butter clam	___	___	___	___	___	___
Cockle	___	___	___	___	___	___
SAMPLING QUADRANT 3						
Littleneck clam	___	___	___	___	___	___
Butter clam	___	___	___	___	___	___
Cockle	___	___	___	___	___	___
SAMPLING QUADRANT 4						
Littleneck clam	___	___	___	___	___	___
Butter clam	___	___	___	___	___	___
Cockle	___	___	___	___	___	___
SAMPLING QUADRANT 5						
Littleneck clam	___	___	___	___	___	___
Butter clam	___	___	___	___	___	___
Cockle	___	___	___	___	___	___
SAMPLING QUADRANT 6						
Littleneck clam	___	___	___	___	___	___
Butter clam	___	___	___	___	___	___
Cockle	___	___	___	___	___	___
SAMPLING QUADRANT 7						
Littleneck clam	___	___	___	___	___	___
Butter clam	___	___	___	___	___	___
Cockle	___	___	___	___	___	___

LITTLENECK CLAM GROWTH AND AGE FORM 5

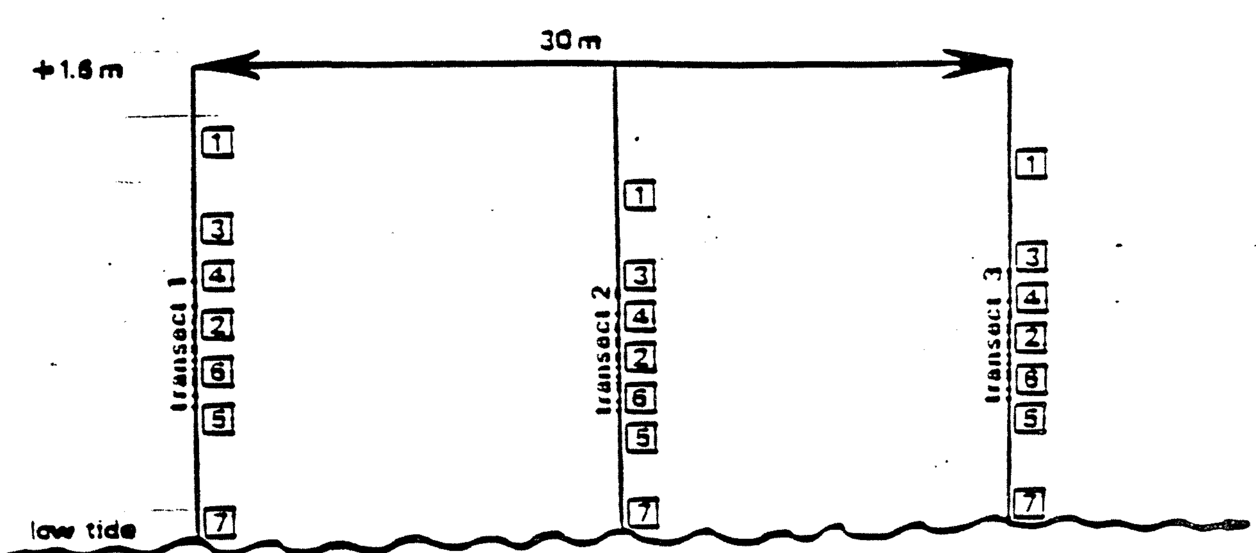
Site Name ________________ Date ________________

Latitude ________________ Longitude ________________

	Transect 1	Transect 2	Transect 3
QUADRANT 1, number of specimens	____	____	____
& specimen numbers	____ ____	____ ____	____ ____
QUADRANT 2, number of specimens	____	____	____
& specimen numbers	____ ____	____ ____	____ ____
QUADRANT 3, number of specimens	____	____	____
& specimen numbers	____ ____	____ ____	____ ____
QUADRANT 4, number of specimens	____	____	____
& specimen numbers	____ ____	____ ____	____ ____
QUADRANT 5, number of specimens	____	____	____
& specimen numbers	____ ____	____ ____	____ ____
QUADRANT 6, number of specimens	____	____	____
& specimen numbers	____ ____	____ ____	____ ____
QUADRANT 7, number of specimens	____	____	____
& specimen numbers	____ ____	____ ____	____ ____

Appendix [illegible].

high tide



Note - the tidal height of sampling quadrant 1 may vary at each transect.

HISTOLOGICAL SAMPLE PREPARATION FOR BIVALVE MOLLUSCS - ADF&G FRED PATHOLOGY

NOTE: Only live or moribund bivalves will be suitable for processing. Histopathological changes caused by toxic chemicals are often very subtle at best. Tissues in dead bivalves autolyze very quickly and will mask these changes. Do not collect and process dead bivalve molluscs.

1. The fixative to be used is Bouin's solution (formula attached).

2. The volume of fixative should be 10 times the volume of the tissue. This is important since any less fixative may result in tissue autolysis and worthless samples.

3. The sample size per site and species will be 20 bivalves, live or moribund.

4. Bivalves less than 6 cm in length (shucked) can be fixed whole by dropping into preservative. Animals must be shucked cleanly from the shell by severing adductor muscles (diagram) prior to fixation. Discard the shell unless there is some type of shell deformity or otherwise abnormal valve. In such a case the shell should be included and attached to the donor animal by wrapping both in gauze.

5. Larger bivalves will need about 3 incisions (anterior, mid, posterior) made across the surface of the animal about midway through the tissues. Do not cut completely through the animal so that individual specimens remain intact and tissues do not become mixed.

6. Tissue and shell abnormalities must be noted on a necropsy field sheet (attached) respectively numbered for a particular animal (bag in gauze and label if necessary). If no abnormalities within the 20 specimens are observed then a single field sheet will suffice for that sample series. The field sheet(s) will also contain the label information below and must accompany the samples in a zip loc bag.

7. A label with bivalve species, size range and life stage, date of sample, location of sample and contact person's name, address and telephone number must be placed within each of the sample jars.

8. Do not mix samples of different species within the same jar of fixative. Each species requires a separate sample jar(s).

9. Place sample jars and zip loc bag containing sample data into a suitable shipping package with adequate packing material to prevent breakage. Plastic jars or containers for fixative and samples work best. Be sure lids are on tight and do not leak.

10. Mail to FRED Fish Pathology Lab: 333 Raspberry Rd., Anchorage 99502 (907-267-2244) or P.O. Box 3-2000, Juneau 99802 (907-465-3577).

11. Notify the fish pathology lab prior to sample shipment so that samples may be expected and tracked en route.

1. Any questions regarding sample preparation should be directed to:

Dr. Ted Meyers
Principal Fish Pathologist III
ADF&G, FRED Division
Juneau Pathology Lab
P.O. Box 3-2000
Juneau, AK 99802 (907) 465-3577

HISTOLOGICAL SAMPLES OF FISH, BIVALVES,
PATHOLOGY, ADF&G

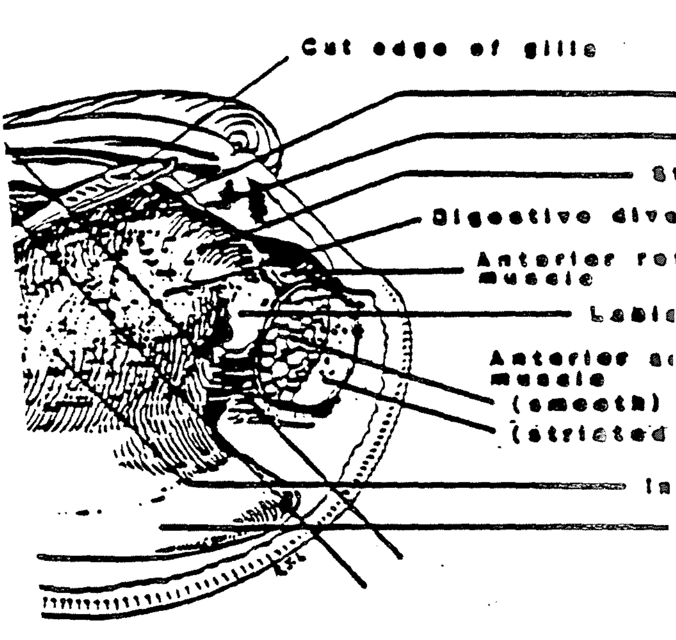
us solution*

730.0 ml

250.0 ml

50.0 ml

into 1000 ml distilled water with the aid of
and use the supernatant fluid.



tion. The bold parallel lines show the
s for cross-section should be taken

NECROPSY FIELD DATA SHEET FOR HISTOLOGICAL SAMPLES, FRED PATHOLOGY, ADF&G

COLLECTOR/ADDRESS/TELEPHONE #

SPECIES

NUMBER SPECIMENS IN SAMPLE

SIZE RANGE

LIFE STAGE

DATE OF COLLECTION

LOCATION OF COLLECTION (SITE NAME OR NUMBER)

ABNORMALITIES OBSERVED PER SPECIMEN NUMBER

Sample size estimation for detecting differences in growth (height) of Protothaca staminea between impacted and non-impacted sites in Prince William Sound

Age	Δ $m_{i+1}-m_i$	σ_{i+1}	Δ/σ	α =.10 1-β=.90 n	α =.05 1-β=.95 n	α =.01 1-β=.95 n
0-1	.84	.33	2.55	5	7	9
1-2	.67	.70	.96	22	32	43
2-3	2.28	.73	3.12	4	5	7
3-4	1.91	.63	3.03	4	5	7
4-5	2.17	1.87	1.16	15-22	21-32	29-43
5-6	3.15	3.26	.97	22	32	43
6-7	5.77	3.99	1.45	11	15	20
7-8	7.07	4.07	1.74	8	12	16
8-9	3.36	1.96	1.71	8	12	16
9-10	3.53	1.83	1.93	7	9	12
10-11	2.15	2.25	.96	22	32	43
11-12	2.94	1.69	1.74	8	12	16
				N=136-143 site	N=194-205 site	N=261-275 site
				N= 45-48 transect	N= 65-68 transect	N= 87-92 transect

Recommended sample size/transect is 100 specimens.

CONFIDENTIAL DRAFT

Injury to Crabs Outside Prince William Sound

Fish/Shellfish Study Number 22

Charles E. O'Clair[1], J. Lincoln Freese[1] and William Donaldson[2]

1. National Marine Fisheries Service, Auke Bay Laboratory, Auke Bay, Alaska.

2. Alaska Department of Fish and Game, Kodiak, Alaska.

Lead Agency - National Marine Fisheries Service

Cooperating agencies - Alaska Department of Fish and Game

Cost of Proposal - $111.5K

Dates of Study Plan - September 1 to October 30, 1989

	Signature	Date
Principal Investigators:	C E O'Clair	10/12/89
	[signature]	10.12.1989
	William Donaldson	10/13/89
Organization Leaders:	[signature]	10/12/89
Financial Officers:	Robert R. Simpson for Debi Rathbone	10/12/89

Injury to Crabs Outside Prince William Sound

INTRODUCTION

The sensitivity of crabs to contamination by petroleum hydrocarbons has been documented in a number of studies. Crabs exposed to sublethal concentrations of petroleum hydrocarbons can suffer early-postmolt autotomy of limbs, behavioral disorders and reduced reproductive output (Karinen and Rice 1974, Krebs and Burns 1977, Karinen et al. 1985 and Malan 1988). Responses of crabs to petroleum hydrocarbons may depend on sex or reproductive state. Krebs and Burns (1977) found a greatly reduced proportion females in populations of the fiddler crab, *Uca pugnax*, at oiled stations in Buzzards Bay, Massachusetts. Jackson et al. (1981) found reproductively active ghost crabs, *Ocypode quadrata*, to be more sensitive to the water soluble fraction of Kuwait crude oil than crabs not in reproductive condition. Dungeness crabs may be particularly susceptible to oil contamination because they occupy nearshore habitats where they frequently burrow into benthic sediments. If oil becomes incorporated in shallow subtidal sediments it persists and can affect crab populations for several years after an oil spill (Krebs and Burns 1977, Boehm et al. 1987).

This project addresses the need to determine, quantitatively, adverse impacts on populations of Dungeness crab outside Prince William Sound as a result of the Exxon Valdez oil spill. The project will provide data on hydrocarbon levels in sediments of habitats occupied by crabs as compared to levels in the tissues of the crabs in contaminated and uncontaminated areas near Kodiak Island. It will also provide biological data on fecundity, reproductive capacity and distribution and relative abundance of the crabs. These data will permit the assessment of short-term losses caused by contamination of harvestable crabs and long-term impacts owing to adverse effects on crab reproduction. The data will also contribute to the long-term data base for management of fisheries and assessment of future oil spills.

Products of this project will include estimates of the amount of petroleum hydrocarbons taken up by the tissues of crabs inhabiting areas with contaminated sediments, estimates of the impact of hydrocarbons taken up by crab reproductive tissues on crab fecundity and reproductive capacity, and identification of possible contamination pathways from sediments to crab reproductive tissues and developing eggs.

OBJECTIVES

A. Determine the levels of hydrocarbons, if present, in Dungeness crabs in oiled and non-oiled sites in lower Cook Inlet and near Kodiak Island.

B. Assess reproductive condition of crabs in oiled and non-oiled areas by measuring such variables as fecundity, egg loss, condition and development and by measuring larval production of ovigerous females collected in oiled and non-oiled areas.

C. Determine the incidence of limb loss and of abnormalities in newly formed crab exoskeletons.

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

METHODS

The study will be conducted at 11 sites with populations of Dungeness crab near Kodiak Island and in lower Cook Inlet. Eight sites will be in areas that have received oil from the Exxon Valdez; three will be reference sites (Table 1). Dungeness crab will be sampled with Dungeness pot gear. Three strings of 10 pots each will be set along the depth contour where crab are most abundant. Pots will soak for 24 hours. Crab distribution will be isolated by setting strings of pots at multiple depths. Pot soak time will be decreased from 24 hours to 6 hours during the initial search phase. The time each pot is set and pulled, the depth at which the pot is set and the species composition of the catch of each pot will be recorded. On a separate form sex, carapace width, presence or absence of an egg clutch and external physical condition of all crab will be recorded.

A total of 30 live female crab[1] will be sampled from each site during each sampling period. Three samples of 10 crab each will be selected from the pot catches. To form one sample, one female crab will be selected from the catch of each pot in a string of 10 pots. Each of the three samples, therefore, includes 10 females from each of the three strings of 10 pots. If a pot contains no female crab a crab will be taken from the group of females in another, randomly selected pot.

1. Determination of sample sizes for all variables covered by this project depends on estimates of sample size for comparable variables by Margaret C. Murphy in the Project Operational Plan on "The effects of hydrocarbons on reproduction in Dungeness crab."

The specimen number, carapace width, fresh weight, clutch description and physical condition of each female crab in the samples will be recorded. Three crab will be randomly selected from the 10 crab in each sample, measured and sacrificed for ovaries. Three ovaries will equal one composite hydrocarbon sample. One composite hydrocarbon sample of eggs is taken by clipping a small portion of the egg clutch (4 g = 1/3 of pleopod) from the right fifth pleopod of each of the three crab. The left fifth pleopod will then be removed from each of the ten crab to estimate egg development, egg mortality and egg fouling. The 7 remaining crab from the sample of 10 will be returned to the sea.

Composite samples of ovaries or eggs will be placed in 16 oz glass jars rinsed with methylene chloride or heated to 440°C for 4 h. Jars containing samples will be placed immediately in a freezer and kept frozen until hydrocarbon analysis is begun. Pleopods will be placed in 8 oz jars to which enough 5 % neutral-buffered formalin is added to cover the egg sample. All jars will be labeled PWS-89-site name (abbreviated)-julian date-sample type (o=ovary, h=hepatopancreas, e=egg, vs=sediment)-sample number.

Sediment samples will be collected at all sites. One van Veen grab will be taken at the center of each string of crab pots. Using a metal core tube and spatula four cores will be removed from randomly selected points within each grab. The subsamples will be combined to form one sample per grab. All samples will be taken from the surface (top 0-2 cm) of the sediment contained in a grab. Each subsample will be transferred to a sample jar using the spatula. The core tube and spatula will be washed, dried and rinsed with methylene chloride between sampling periods. Sample jars will be baked at 440°C or rinsed with methylene chloride prior to use. The jars will be fitted with teflon lined caps that are also rinsed with methylene chloride prior to use. Sediment samples will be frozen immediately after collection. Appropriate blanks will be collected at each site. Chain of custody procedures will be followed (Appendix A).

Physical oceanographic data will be collected at each site during each sampling period using an instrument that measures conductivity, temperature and density with depth (CTD). Data is recorded by the CTD every 2 seconds as it is lowered to the bottom and raised to the surface. The CTD will be deployed once at each site during each sampling period.

In April 1990, 15 live ovigerous crabs will be collected from each site and transported to the NMFS Auke Bay Laboratory. Crabs will be individually marked with Petersen disc tags. Tag number, carapace width, fresh weight and external physical condition of each crab will be recorded. The crab will be held individually in flow-through tanks until larval release. Crab

will be fed a mixed diet of locally available prey including shrimp shrimp, clams and mussels. Water temperature, salinity and general condition of crabs will be regularly monitored. Timing of larval release, number of live and dead larvae and larval swimming ability will be recorded to estimate larval production and viability.

Definitive analysis of the chemical composition of petroleum hydrocarbons in the sediments, tissues and eggs will be accomplished in the laboratory with gas chromatography/mass spectrometry as directed by the Analytical Chemistry Quality Assurance/Quality Control Group. The types of analyses to be performed on the samples will be determined by the Analytical Chemistry Group and will include 1) TPH/GC and PNA/SIM characterization of oil in marine sediments and crab tissues, 2) total organic carbon on selected samples, and 3) size fraction analysis on representative sediment samples. Prescreening analyses of collected samples will occur prior to full GC/MS analysis in areas of low liklihood of oiling. Details of the methods used in the chemical analyses are recorded under the Quality Assurance Program.

DATA ANALYSIS

The number of specimens required for one hydrocarbon analysis depends on the amount of tissue available in a crab and the need for a composite sample. Three Dungeness crab are enough to provide 15 g of ovarian tissue. One pleopod from an average clutch would provide 15 g of crab eggs, but a sample representative of more than one crab is desireable. Therefore egg clips from the clutches of three crab will be combined to form a composite sample for hydrocarbon analysis. Three hydrocarbon samples from each site are the minimum needed to detect contamination between oiled and non-oiled sites (see operational plan for Fish/Shellfish Study Number 14)

A sample size of 30 crab was estimated to be an adequate number to determine differences in reproductive output between impact levels based on data from Dungeness crab at log transfer facilities (LTF) in Southeast Alaska (O'Clair and Freese 1988). Sample size for reproductive capacity is based on the difference between mean fecundity and mean worm infestation at 7 paired control sites and LTF sites, variance in fecundity and infestation by pair, probability of making a type I error equal to .05, and probability of making a type II error equal to .05 (Neter et al. 1985, Table A-10). Data for mean fecundity and infestation level were taken from O'Clair and Freese (1988). Sample size for detecting difference in mean fecundity and infestation level between impact levels was estimated at 27 crab for fecundity and 26 crab for infestation level. The sample size was rounded up to 30 crab.

All data will be tested for heteroscedasticity with Bartlett's test or equivalent. Data will be reported as means and 95% confidence intervals calculated according to a standard formula (Sokal and Rohlf 1981). Parametric statistics (analysis of variance and Scheffe's a posteriori test) will be used to test for differences in means between oiled and non-oiled sites if underlying assumptions of the parametric procedures are met, otherwise nonparametric tests (eg. the Kruskal-Wallis test) will be employed. Variables to be tested will include hydrocarbon concentrations in Dungeness crab tissues, the reproductive parameters of Dungeness crabs, crab larval production and viability and hydrocarbon content of sediments in crab habitat.

Further multivariate statistics (eg. analysis of covariance, rank correlation coefficients, discriminant analysis) will be computed if the above summary statistics indicate relationships may exist between Dungeness crab hydrocarbon content, reproductive capacity, sediment hydrocarbon content, and physical oceanographic factors.

SCHEDULES & PLANNING

Sampling began in late September 1989. Sampling will continue in spring and late summer of 1990. The timetables for data compilation, analysis and report writing depend on the date of completion of the chemical analyses of the sediments and the crab tissues and eggs which analyses are funded under Technical Services Study Number 1. The Technical Services Committee controls the reporting schedule for chemical analyses. Estimates of crab fecundity, egg mortality, egg fouling and infestation level of egg predators on Dungeness crab eggs will be completed six months following sample collection. Dates of completion of data compilation, analysis and report writing for the 1989 sediment sampling will be 4, 8, and 12 months respectively after the date of completion of the chemical analyses.

BUDGET

	Salaries	Travel	Contracts	Supplies	Equipment	Total
Alaska Department of Fish and Game						
	10K	1K	0K	0K	0K	$11K
National Oceanic and Atmospheric Administration						
	30.0K	7.5K	25.0K	31.0K	7.0K	$100.5K

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Table 1

Location of sites for the study of injury to crabs outside Prince William Sound in 1989.

Location	North Latitude ° ' "	West Longitude ° ' "	Date
OILED SITES			
Missak Bay	58 08 11	154 19 44	27 Sep
Kuliak Bay	58 12 08	154 16 29	28 Sep
Kukak Bay	58 20 30	154 11 40	29 Sep
Hallo Bay	58 26 56	154 02 53	29 Sep
Cape Chiniak	58 30 52	153 54 33	28 Sep
Muskomee Bay	58 04 16	153 06 48	2 Oct
Sharatin Bay	57 47 47	152 46 58	2 Oct
Kizhuyak Bay	57 43 49	152 56 13	3 Oct
REFERENCE SITES			
Uganik Bay East Arm	57 41 19	153 28 34	1 Oct
South Arm	57 37 30	153 30 32	1 Oct
Terror Bay	57 43 35	153 12 59	1 Oct

Appendix A.

STANDARD OPERATING PROCEDURES
FOR SAMPLING BENTHIC SEDIMENTS

INTERTIDAL SEDIMENTS

1. Choose an area of intertidal beach having a substrate as homogeneous as possible with particle sizes of 2 mm or less. The area must be large enough to accomodate a 30 m transect. Lay the transect parallel to the water's edge within the designated area.

2. Choose 8 random distances along the transect from a random number table or pocket calculator.

3. Three samples of substrate will be collected at each station (= transect). Each sample will represent a composite of 8 subsamples, each subsample having been taken at one of the 8 randomly selected points. Using a metal core tube and spatula or metal scoop remove approximately 10 g of sediment from the upper 2 cm of substrate at one of the 8 randomly selected points on the transect and place in a properly cleaned 4 oz jar. Repeat the procedure for two more jars collecting 10 g of sediment from adjacent patches of substrate and placing it in each of the two additional jars.

4. Repeat the procedure described in 3 for the 7 remaining points on the transect.

5. At one station per site a sample blank (handled in the same way as the sediment samples except without receiving any sediment) will be taken.

6. Label, seal (with custody control seal) and freeze sediment samples and blank as soon as possible after collection.

7. Proper cleaning procedure for sampling implements and jars.

Sampling implements - All sampling implements will be washed with soap and water, rinsed, dried, rinsed with methylene chloride and if not used immediately wrapped in clean aluminum foil that has been rinsed with methylene chloride. The cleaning procedure will be performed before each transect is sampled.

Jars - If sample jars have not come from the supplier cleaned to EPA specifications they will be baked for four hours at 440° C or rinsed with methylene chloride. Sample jars will have teflon-lined lids rinsed with methylene chloride or will be capped with aluminum foil rinsed with methylene chloride before the lid is replaced after sample collection.

SUBTIDAL SEDIMENTS

Diver collected

Sampling will be conducted as described above for intertidal sediments with the following modifications.

1. Lids will be closed on sample jars on the surface before divers descend to the bottom to prevent contamination by petroleum hydrocarbons floating on the surface of the water.

2. Care must be taken to avoid contamination of dive mitts/gloves with petroleum hydrocarbons.

Remote sampling by van Veen grab.

1. The grab or corer the interior surfaces of which have been cleaned and rinsed with methylene chloride will be lowered to the bottom and activated to enclose a sample of substrate and then retrieved. The surface of the water will be checked visually for sign of contamination by petroleum hydrocarbons (such as an oil sheen) before the grab is lowered or retrieved through it. If any indication of oil is observed the vessel will be moved to a visually clean area.

2. When the grab is brought to the surface and placed on deck after having taken a sample the sample will be subsampled using a stainless steel core tube and spatula. The location of the subsamples will be determined randomly. Four subsamples will be taken from each sample and placed in a 4 oz cleaned jar. Three samples will be taken at each station. Subsamples of different grabs will be placed in separate jars.

Samples will be labeled, sealed and frozen as soon as possible after being collected.

3. Sampling implements and jars will be cleaned as described in the section on intertidal sediments above.

SUBMERSIBLE SEDIMENTS

1. Wash 100 micron mesh bags in Alconox detergent and rinse thoroughly in hot water daily.

2. Wash sampling hose with Alconox detergent and rinse thoroughly with hot water daily. (A plastic suction hose was used June 27-July 5, and a teflon suction hose used July 5-July 15.)

3. Wash the stainless steel suction pump daily with methylene chloride.

4. Use sterile gloves to attach and detach sample bags from submersible, and process sediment samples for freezing.

5. Label samples with date, area, method of sampling, name of sampler, depth of sample, and sample number.

Qualifications of Project Leader

CHARLES E. O'CLAIR S.S. No.: 012-32-1851

Personal: Born May 29, 1941; Ayer, Massachusetts

Education: University of Massachusetts, B.S., Zoology, 1963
University of Washington, Ph.D., Fisheries, 1977

Experience: 1977 - present: Fishery Biologist (Research), National Marine Fisheries Service, Auke Bay Laboratory, Juneau, Alaska. Research experience includes five years of field and laboratory work on the effects of oil pollution on benthic invertebrates in conjunction with the Outer Continental Shelf Energy Assessment Program followed by seven years of research on the ecology and behavior of Dungeness, king, and tanner crab in relation to the management of these species.

1983-1987: Affiliate Assistant Professor of Fisheries, School of Fisheries and Science, University of Alaska, Juneau.

Selected Publications:

O'Clair, C. E. and S. D. Rice. 1985. Depression of feeding and growth rates of the seastar _Evasterias troschelii_ during long-term exposure to the water-soluble fraction of crude oil. Mar. Biol. 84:331-340.

O'Clair, C. E. and S. T. Zimmerman. 1987. Biogeography and ecology of intertidal and shallow subtidal communities. pp. 305-344. _In_ The Gulf of Alaska: Physical Environment and Biological Resources. D. W. Hood and S. T. Zimmerman, Eds. National Technical Information Service, Springfield, Virginia.

O'Clair, C. E. and J. L. Freese. 1988. Reproductive condition of Dungeness crabs, _Cancer magister_, at or near Log Transfer Facilities in Southeastern Alaska. Marine Environ. Res. 26:57-81.

Morado, J. F., A. K. Sparks and C. E. O'Clair. 1988. A preliminary study of idiopathic lesions in the Dungeness crab, _Cancer magister_, from Rowan Bay, Alaska. Marine Environ. Res. 26:311-318.

O'Clair, C. E., R. P. Stone and J. L. Freese. (in press). Movements and habitat use of Dungeness crab and the Glacier Bay fishery. Proc. Second Glacier Bay Science Symposium, 1988.

NMFS/ADF&G DUNGENESS OIL PROJECT SITE DESCRIPTION DATA FORM

Site Name ______________________ Date ______________________

Latitude ______________________ Longitude ______________________

Skipper Name ______________________

Vessel Name ______________________ Vessel Ak Number ______________

Vessel Description ____

Sampling Team	Agency
______________________	______________________
______________________	______________________
______________________	______________________
______________________	______________________
______________________	______________________

	Code	Description
Waves	___	______________________________________
Weather	___	______________________________________

Air Temp ______________________ Sea Temp ______________________

Wind Speed ______________________ Wind Direction ______________________

Time of Low Tide ______________ Low Tide Height ______________

Beach Orientation ______________

String #	Time Set	Begin Depth	End Depth	Time Pull	Comments
___	______	______	______	______	______________
___	______	______	______	______	______________
___	______	______	______	______	______________

Film Role Number ________ Photograph Numbers ________ ________

Waves

Code	Description
1	Glassy
2	Rippled
3	Wavelets
4	Slight 2-4'
5	Moderate 4-8'
6	Rough 8-13'
7	Very Rough 13-20'

Weather

Code	Description	Code	Description
1	Clear	7	Drizzle
2	Partly Cloudy	8	Rain
3	Overcast	9	Rain and Snow
4	Fog or Thick Haze	10	Snow
5	Showers	11	Blizzard
6	Squalls		

NMFS/ADF&G DUNGENESS OIL PROJECT CATCH COMPOSITION DATA FORM

Site Name ______________________ Date ______________________

Latitude ______________________ Longitude ______________________

String #	Pot #	Transect #	Sex	Specimen #	Use	Width	Weight	Shell Age				Egg Color Code	% Fullness	Durometer Reading	
								S	N	O	VO				

Specimen Use Codes: Blank=not used, H=hydrocarbon, P=pleopod, N=necropsy
Shell Age Codes: S=soft, N=new, O=old, VO=very old

Appendix [illegible]

NMFS/ADF&G DUNGENESS OIL PROJECT DIVER TRANSECT DATA FORM

Site Name ______________________ Date ______________________

Latitude ______________________ Longitude ______________________

Recorder ______________________ Transect Number ______________________

Direction of Transect ______________ Visibility ______________________

	Diver 1	Diver 2
Name		
Time Down		
Time Up		

Meters	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
Depth																					
Slope																					
Substrate																					
Zone																					
# of Crab																					
Specimens																					
Sediment																					
Meiofauna																					

Zone: 1=intertidal 2=subtidal

Substrate:

Code	Description
1	Mud-Silt
2	Clay
3	Sand
4	Granule (2-4mm)
5	Pebble (4mm-3cm)
6	Rock Fragments (3-6cm)
7	Cobble Shingle (6-15 cm)
8	Rock (15-25 cm)
9	Boulder (>25 cm)

NMFS/ADF&G DUNGENESS OIL PROJECT CTD, SEDIMENT AND MEIOFAUNA DATA FORM

Site Name ____________________ Date ____________________

Latitude ____________________ Longitude ____________________

Transect # or String #	Zone	Sample #	Transect Distance, Distance off shore or Loran Coordinates		CTD Time	CTD Depth

Zone:
1=intertidal
2=subtidal

Transect # or String #	Zone	Sample #	Transect Distance, Distance off shore or Loran Coordinates		Sediment Time	Sediment Depth	Meiofauna ime	Meiofauna Depth

N.M.F.S. Prince William Sound Oil Assessment Chain of Custody Form

VAL-89- _Project ______________________

Serial #

Page ______ of ______

NMFS Auke Bay Fisheries Laboratory
Box 210155, Auke Bay AK 99821
For information contact Sid Korn
(907) 789-6021 or 789-6000

NOTE: Use ballpoint pen, waterproof ink (eg Rapidograph) or fine-tip waterproof marker

Date Collected	Sample # (collector's)	Assigned # (leave blank)	Type (tissue, water, sediment, etc)	Location Collected	Latitude	Longitude	Remarks

Continue on back of page →

Chain of Custody

Samples collected by ______ (print name) of ______ (agency) ______ (signature) ______ (date)

Relinquished by ______ (signature) ______ (date) at ______ (place) ______ (comments)

Recieved by ______ (print name) of ______ (agency) at ______ (place) ______ (signature) ______ (date)

Relinquished by ______ (signature) ______ (date) at ______ (place) ______ (comments)

Recieved by ______ (print name) of ______ (agency) at ______ (place) ______ (signature) ______ (date)

Relinquished by ______ (signature) ______ (date) at ______ (place) ______ (comments)

Recieved by ______ (print name) of ______ (agency) at ______ (place) ______ (signature) ______ (date)

Qualifications of Project Leader

CHARLES E. O'CLAIR S.S. No.: 012-32-1851

Personal: Born May 29, 1941; Ayer, Massachusetts

Education: University of Massachusetts, B.S., Zoology, 1963
University of Washington, Ph.D., Fisheries, 1977

Experience: 1977 - present: Fishery Biologist (Research), National Marine Fisheries Service, Auke Bay Laboratory, Juneau, Alaska. Research experience includes five years of field and laboratory work on the effects of oil pollution on benthic invertebrates in conjunction with the Outer Continental Shelf Energy Assessment Program followed by seven years of research on the ecology and behavior of Dungeness, king, and tanner crab in relation to the management of these species.

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O'Clair, C. E. and S. T. Zimmerman. 1987. Biogeography and ecology of intertidal and shallow subtidal communities. pp. 305-344. *In* The Gulf of Alaska: Physical Environment and Biological Resources. D. W. Hood and S. T. Zimmerman, Eds. National Technical Information Service, Springfield, Virginia.

O'Clair, C. E. and J. L. Freese. 1988. Reproductive condition of Dungeness crabs, *Cancer magister*, at or near Log Transfer Facilities in Southeastern Alaska. Marine Environ. Res. 26:57-81.

Morado, J. F., A. K. Sparks and C. E. O'Clair. 1988. A preliminary study of idiopathic lesions in the Dungeness crab, *Cancer magister*, from Rowan Bay, Alaska. Marine Environ. Res. 26:311-318.

O'Clair, C. E., R. P. Stone and J. L. Freese. (in press). Movements and habitat use of Dungeness crab and the Glacier Bay fishery. Proc. Second Glacier Bay Science Symposium, 1988.

September 12, 1989

STATE/FEDERAL DAMAGE ASSESSMENT PLAN

ANALYTICAL CHEMISTRY

COLLECTION AND HANDLING OF SAMPLES

FOR AGENCY USE ONLY
NOT FOR RELEASE
ATTORNEY WORK PRODUCT

1. Introduction

In response to the release of more than 10 million gallons of crude oil into Prince William Sound, the State of Alaska and four Federal Agencies, the Departments of Agriculture, Commerce and Interior and the Environmental Protection Agency are acting together to assess the damages to the natural resources. Authority for this action is provided by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and the Clean Water Act (CWA).

A damage assessment requires documentation of the exposure of the resources to oil released from the EXXON VALDEZ, identifying which resources were injured by that exposure, measuring the magnitude of the adverse affects on each resource over time and assigning economic values for that injury. Once this is done, monetary compensation can be sought from the potentially responsible parties to restore and/or replace the injured resources.

Recovery of monetary damages may involve civil court actions. It will then be necessary to prove that the samples were collected in a scientifically approved manner and that the samples were protected from outside contamination (non-incident related) and accidental mix-ups during handling and analyses. It is, therefore, extremely important that every sample be readily identified and their location and analytical status known and documented at all times.

This document and the associated training sessions, were prepared to assist field personnel in collecting samples that will provide scientifically sound and legally defensible data to support the State/Federal Natural Resource Damage Assessment for the EXXON VALDEZ oil spill.

2. Record Keeping and Documentation

Standard operating procedures (SOPs) for all sampling procedures, including chain of custody procedures; sampling protocols; cleaning and preparation of sample collection and storage devices; and labeling, handling, and sample

Errors in field logbooks or other records are corrected by drawing a single line though the error, entering the correct information and signing and dating the correction. Never erase an entry or any part of an entry.

Do not remove pages from the logbook.

Completed logbooks and field data sheets are returned to the Team Leader or their representative to be archived in a central location under chain-of-custody procedures until the Trustees indicate that they may be released.

3. Sample Identification and Labelling

A tag or label identifying the sample must be completed and attached to each sample. Waterproof (indelible) marker must be used on the tag or label. The minimum information to be included on the tag are the sample identification number, the location of the collection site, the date of collection and signature of the collector (who, what, where & when). This information and any other pertinent data such as the common and scientific names of the organism collected, the tissue collected and any remarks are recorded in the logbook. Field sample data sheets, photographs, any pertinent in-situ measurements (such as temperature, salinity, depth) and field observations are recorded in the logbook.

The location of the sampling site is determined with the aid of USGS grid maps, NOAA charts or navigational systems such as LORAN C. The site locations should be plotted on a chart of appropriate scale and photocopies incorporated into the logbook. In addition, a clear, detailed descriptive location as well

water. They must however, be solvent-rinsed as described above before use.

Glass jars may be cleaned by heating to 440°C for a minimum of 1 hour.

Clean glassware should be stored inverted or tightly capped with either solvent-rinsed aluminum foil or teflon-lined caps.

The dull side of the aluminum foil should be the side that is solvent-rinsed. Pre-cleaned squares may be stored with the clean sides folded together.

All equipment that comes in contact with the sample such as dredges or dissecting equipment must be solvent-rinsed before contacting each sample. Equipment should be steam-cleaned or washed with soap and hot water at the end of each day or between sampling locations.

5. Sampling Procedures

The method of collection must not contaminate the samples. Do not collect any subsurface samples through surface slicks. Do not collect any samples with oil-fouled equipment, such as nets or dredges. Do not touch or collect any sample with your bare hands.

Sample container volume must be appropriate to sample size; fill the jar to just below the shoulder. Overfilled jars will break when they freeze; underfilled jars will allow the sample to dry out.

ing the contents with your hands. Total weight, volume (measured or calculated), length, width and contents weight must be recorded for each egg. Bile is collected by removing the gall bladder, puncturing it with a scalpel fitted with a new #11 blade, and collecting the contents in a 4 mL amber glass vial.

6. Sample Preservation and Holding Time

Samples must be kept cool, i.e. on ice.

Samples that are to be frozen, sediment and tissue, should be frozen quickly and rapidly. That is, these samples should be frozen as soon after collection as possible and the freezing process should be rapid.

Frozen samples must be kept frozen, at -20°C or less, until extracted or prepared for analysis. Repeated freezing and thawing of samples can destroy the integrity of the samples resulting in questionable data or the loss of data.

6.1 Water - All water samples must be immediately extracted with methylene chloride or preserved with HCl to pH<2. If preserved, water samples are stored in the dark at 4°C and extracted within 7 days. All extracts must be stored in the dark in air tight chemically clean containers until analysis.

6.2 Sediment and Tissue - Samples should not be extracted until immediately before analysis; if there is a lag between sample extraction and sample analysis, extracts must be stored in air tight containers kept in the dark at 4°C.

legal action.

The field sampler is personally responsible for the care and custody of the samples collected until they are transferred under chain-of-custody procedures.

A sample is considered in "custody" if:

it is in your actual physical possession or view;

it is retained in a secured place (under lock) with restricted access

or it is placed in a container and secured with an official seal(s) such that the sample cannot be reached without breaking the seal(s)

Evidence tape or sample seals are used to detect unauthorized tampering of samples following sample collection. The seal must be attached in such a way that it is necessary to break it in order to open the container. Seals must be affixed to the container before the samples leave the custody of sampling personnel.

All samples must be accompanied by a chain-of-custody record or field sample data record (Figure 1). When samples are transferred from one individual's custody to another's, the individuals relinquishing and receiving the samples will sign and date the chain of custody record. This record documents the transfer of custody of samples from the sampler to another person or to a specified analytical laboratory.

Shipping containers must be custody-sealed for shipment. The seal must be signed before the container is shipped. The chain-of-custody record must be dated and

signed to indicate any transfer of the samples. The original chain-of-custody record accompanies the shipment; a copy is retained by the sample shipper. If samples are sent by common carrier, copies of all bills of lading or air bills must be retained as part of the permanent documentation.

Whenever samples are split, a separate chain-of-custody record is prepared for those samples and marked to indicate with whom the samples are being split.

HISTOPATHOLOGY TECHNICAL GROUP

Justification/Concern

Histopathology is an important tool used in determining mechanisms of death and sublethal effects caused by infectious agents and toxic substances. A definitive diagnosis often does not result from histological examination, but can give strong support to other positive measurements. Tissues deteriorate (autolyze) rapidly after an animal dies; therefore, to be of value, any sample taken for histological evaluation as part of the damage assessment of the Exxon Valdez oil spill must be collected, preserved, and processed under strict guidelines.

Introduction

This committee was established to serve as an ad-hoc advisory and technical control group that reports to the Management Team. Its specific function is to serve as a control point for all laboratory aspects of histopathological analysis associated with the Exxon Valdez oil spill assessment program. This includes the development of detailed sampling protocols, appropriate training of field personnel in collecting samples, review of all histological sampling proposed and identification of effort duplication, establishment of a secured repository for all histology samples for storage until processing, oversee archiving and inventory of collected samples, qualification evaluation of potential subcontractors to be hired for processing and interpretation of histology samples, quality control assurance in all work performed, advice on chain-of-custody guidelines, and development of budget estimates to accommodate the required histopathological analyses.

TABLE OF CONTENTS - Histopathology Technical Group

1. Sample collection and preservation protocols
2. Processing and interpretation protocols
3. Quality assurance in field collection of samples and in interpretation of results
4. Repository for samples and inventory procedures
5. Chain-of-custody guidelines
6. Subcontracting for histopathology work
7. Finfish and shellfish mortality assessments
8. References
9. Appendices

1. Sample Collection and Preservation Protocols

Standard protocols for necropsy and preservation of tissue samples (including a materials list and catalog numbers) for histopathology described in the appendices shall be used throughout the oil spill assessment studies. Different protocols have been designed to accommodate the different groups of animals to be encountered in the assessment studies. Necropsy procedures are included for:

Appendix 1: Finfish
Appendix 2: Bivalve molluscs
Appendix 3: Brachyuran and crab-like Anomurans; i.e., King crabs)
Appendix 4: Shrimp
Appendix 5: Marine and terrestrial mammals
Appendix 6: Migratory and nonmigratory waterfowl

Paired sampling of animals from oiled versus non-oiled sites will be done for comparative purposes. Histopathological sampling should be done during any observed acute episodes of mortality or morbidity to determine the cause of death or abnormality. These types of samples are the most valuable in assessing acute toxicity affects and will be the most likely samples collected for birds and mammals due to their high visibility in the impacted areas. Because of the low visibility of fish and shellfish, many histology samples will consist of random collections in impacted and control areas with little prior obvious indication of morbidity or mortality.

Any histological processing of samples collected from apparently normal shellfish should be performed after results of parallel hydrocarbon sampling is known; i.e., positive hydrocarbon results may merit further histopathology studies. This would not be advisable for fish and other higher animals that possess an active mixed function oxidase (MFO) liver enzyme system which could metabolize hydrocarbons to other compounds providing negative hydrocarbon results, but potential toxicological lesions. Other enzyme function analyses being performed, such as on bile, may show an activated MFO system in exposed fish and higher animals. Consequently, histology and hydrocarbon samples, as well as other appropriate samples, such as bile, for metabolite and enzyme function analyses, should be taken from the same animal when possible. If certain fish and shellfish are too few or small, subsampling other animals from the same site at the same time will be necessary.

2. Processing and Interpretation Protocols

Histopathology assessment of birds and mammals will be done primarily on tissues from clinically affected animals using established criteria of cellular degenerative and necrotic changes recognized by any board-certified veterinary pathologist.

Histopathological analysis of finfish and shellfish tissues will include the criteria above as well as indices established in the Amoco Cadiz oil spill studies (Haensly et al. 1982; Berthou et al. 1987) to allow some quantification of potentially subtle degenerative changes in tissue histology of otherwise clinically normal animals. Briefly these indices include:

a. Mean concentration of mucus cells per mm^2 of gill lamellae (fish).
b. Mean concentration of mucus cells per mm of epidermis in 10 fields (fish).
c. Average epidermal thickness in mm measured in 10 fields (fish).
d. Mean concentration of macrophage centers per mm of liver.

e. Mean concentration of hepatocellular vacuolation due to fatty degeneration (fish).
f. A mean and total tissue necrosis index (invertebrates).
g. Histological gonadal index (invertebrates).
h. Differences in prevalences and intensities of incidental lesions caused by infectious agents (fish and invertebrates).

3. Quality Assurance in Field Collection of Samples and in Interpretation of Results

Field Collection

Veterinary personnel trained in sample taking should be utilized for on-site necropsies of birds and mammals in order to ensure adequate quality control and standardized sample collection in these less familiar and more complex species. The same high standards should be attainable in fish and invertebrates if sample collection is done by trained finfish and shellfish biologists. A fish pathologist and technician will be available to train field personnel and assist in necropsy and preservation of finfish and shellfish samples at collection sites.

Sample collection from migratory birds and sea otters should be coordinated with the U.S. Fish and Wildlife Service National Wildlife Health Laboratory in Madison, Wisconsin. Collection of samples from nonmigratory birds and other marine mammals could be coordinated with the Alaska State Veterinary Laboratory in Anchorage. Finfish and shellfish samples can be coordinated through the on-site fish pathologist and the ADF&G, Fisheries Rehabilitation, Enhancement and Development (FRED) Division Juneau Fish Pathology Laboratory.

Interpretation of Results

Quality control of all processed work will require independent blind reading of subsampled histology slides by two different laboratories.

Tissues with known lesions will be included periodically in groups of tissue samples for blind reading and determination of competency in interpretation.

4. Repository For Samples And Inventory Procedures

A common repository for storage of all histology samples awaiting processing will be established at Anchorage in a secured building in compliance with chain-of-custody requirements. Samples received will be given a unique accession number to be cross-referenced with the project and original numbering assigned by the collector.

5. Chain-Of-Custody Guidelines

Due to the evidentiary nature of sample collecting investigations, the possession of samples must be traceable from the time the samples are collected until they are introduced as evidence in legal proceedings. To maintain and document sample possession, chain-of-custody procedures must be followed.

The field sampler will be personally responsible for the care and custody of the samples collected until they are transferred. All samples will be accompanied by a chain-of-custody record or field sample data record (Appendix 7). When samples are transferred from one individual's custody to another's, the individuals relinquishing and receiving will sign, date, and note the time on the record. This record documents the transfer of custody of samples from the sampler to another person and, ultimately, to a specified analytical laboratory.

Shipping containers will be custody-sealed for shipment. This procedure includes use of a custody seal such that the only access to the package is breaking the seal. The seal shall be signed before the sample is shipped. The chain-of-custody record will be dated and signed to indicate transfer. The original record will accompany the shipment and a copy will be retained by the sample collector. Whenever samples are split, a separate chain-of-custody record will be prepared for those samples and marked to indicate with whom the samples are being split.

If samples are being sent by common carrier, copies of all bills of lading or air bills must be retained as part of the permanent documentation.

6. Subcontracting for Histological Work

Subcontracting work for histopathology processing and interpretation should be coordinated through the Histology Technical Group which will determine if selected processors are qualified to do the work. Qualifications for mammal and avian samples will require a board-certified veterinary pathologist. Finfish and shellfish work will require individuals with a demonstrated publication record in the field of histopathology.

7. Finfish and Shellfish Mortality Assessments

Estimates of finfish and shellfish mortalities will be according to guidelines established for estimating fish kills contained in Part II (Fish Kill Counting Guidelines) of the Monetary Values of Freshwater Fish and Fish-Kill Counting Guidelines, American Fisheries Society Special Publication Number 13, 1982, including use of appropriate random sampling methods and tagged carcasses (Natural Resource Damage Assessments provided by CERCLA).

8. References

Bell, T. A. and D. V. Lightner. 1988. A Handbook of Normal Penaeid Shrimp Histology. The World Aquaculture Society, Baton Rouge, LA.

Berthou, F., G. Balouet, G. Bodennec, and M. Marchand. 1987. The occurrence of hydrocarbons and histopathological abnormalities in oysters for seven years following the wreck of the Amoco Cadiz in Brittany (France). Mar. Environ. Res. 23:103-133.

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Johnson, P. T. 1980. Histology of the Blue Crab, *Callinectes sapidus*: A Model for the Decapoda. Praeger Publ., New York.

Sparks, A. K. 1985. Synopsis of Invertebrate Pathology Excluding Insects. Elsevier Publ., New York.

9. <u>Appendices</u> (attached)

HISTOLOGICAL SAMPLE PREPARATION FOR BRACHYURAN AND ANOMURAY CRAB SPECIES

Histopathological Technical Group

NOTE: Only live or moribund crabs will be suitable for processing. Histopathological changes caused by toxic chemicals are often very subtle at best. Tissues in dead crabs autolyze very quickly and will mask these changes. Do not collect and process dead crabs. Keep crabs alive in containers of seawater or live wells if they must be transported to the processing site. Do not over-ice animals such that tissues freeze while in transit. Frozen tissues are worthless for histological examination.

1. The fixative to be used is 10% neutral buffered formalin solution (formula attached). Formalin should be handled wearing rubber or latex gloves.

2. The volume of fixative should be ten times the volume of the tissue. This is important since any less fixative may result in tissue autolysis and worthless samples. After 72 hours, the formalin should be poured off and replaced with 70% ethyl alcohol for storage and transport. This accomplishes an important objective; i.e., it prevents tissues from becoming too hard and brittle when stored in fixative for long periods. Also, the fixative poured off may be saved and strained of tissue fragments and used one more time for preserving other samples.

3. The sample size per site or species will be 20 crabs, live or moribund.

4. Prior to tissue collection, a blood smear should be prepared from each live crab. Insert a 1-cc syringe with a 20-gauge needle into the articular membrane of any walking leg. The third joint of either cheliped works best. Express a large drop of blood from the syringe onto one end of a clean, frosted-end glass slide and use another slide to make the smear as illustrated in the attached information. Allow to air dry, label the frosted end with an assigned crab number, and date and include in a small slide box with the samples below. An alternative method would be to pull off a walking leg and allow not more than 1-2 drops of blood to fall onto the slide.

   Be sure to not let salt water mix with the blood on the slide, as it will cause blood cell lysis. Note: King crab blood clots unbelievably fast, so make your smear quickly.

5. The chitinous exoskeleton of large crustacea prevents adequate penetration of any fixative by simple immersion. Consequently, major organs and tissues of crabs must be dissected out and dropped into fixative. This procedure is described by the following:

   a. The carapace over the visceral cavity of the crab must be removed using tin snips or bone snips, or otherwise heavy duty serrated scissors (Figure 2).

   b. Once the carapace is removed, the pigmented epidermis may come off attached or remain overlying the viscera. Snip a small 5-mm portion of the

k. In female Dungeness crabs, the paired seminal recepticles will be located below and on either side of the thoracic ganglion. Remove the right organ for fixation.

l. Remove both eyestalks and the cerebral ganglion (brain) appearing as a white, pea-sized organ located at the juncture of the eyestalks (Figure 7). This all can be removed as one piece by snipping out with a pair of scissors.

6. All tissues removed from a single crab should be placed into tissue processing cassettes, 4-5 tissue samples to one cassette. Each cassette must be labelled with the animal number from which the tissues were collected. Cassettes are then placed within the sample jar containing fixative.

7. Behavioral, external, and internal abnormalities must be noted on a necropsy field sheet (attached), respectively numbered for a particular pooled crab tissue sample. If no abnormalities within the 20 specimens from a site are observed, then a single field sheet for the sample series will suffice. These field sheets will also contain the label information below and must accompany the samples in a ziploc bag. Be sure to include tissue from a lesion if one is observed--this includes shell lesions as well.

8. A label with crab species, size range and life stage, date of sample, sample location, and contact person's name, address, and telephone number must be placed within each of the sample jars. Use a pencil with soft lead for labelling so that the writing remains legible.

9. Do not mix samples of different crab species within the same jar of fixative. Each species requires a separate jar(s).

10. Place sample jars and ziploc bag containing sample data into a suitable shipping package with adequate packing material to prevent breakage. Plastic jars or containers for fixative and samples work best. Be sure lids are tight and do not leak.

11. Mail to the FRED Division Fish Pathology Lab, ~~P.O. Box 3-2000, Juneau, Alaska 99802-2000; phone (907) 465-3577~~ AK Dept. of Fish and Game, 333 Raspberry Rd., Anchorage, AK 99518-1599; phone (907) 344-0541.

12. Notify the Fish Pathology Lab prior to sample shipment so that samples may be expected and tracked en route.

13. Follow proper procedures and include completed forms regarding chain of custody.

14. Any questions regarding sample preparation should be directed to:

Dr. Ted Meyers
Principal Fish Pathologist III
ADF&G, FRED Division
Juneau Fish Pathology Lab
P.O. Box 3-2000
Juneau, Alaska 99802-2000

Phone: (907) 465-3577

Fig. 2. The dorsal carapace. Lines A, B and C indicate where the carapace is cut before its removal.

Fig. 7. The hepatopancreas, testes, and vas deferens have been removed, revealing the anterodorsal brain 1 and the circumesophageal commissures 2. The large thoracic ganglion 3 lies medioventrally. Its central aperture is visible. In the living animal, the sternal artery passes through the aperture. Connective tissues have been removed in order to show the location of the antennal glands 4, which lie against the anteroventral face of the exoskeleton.

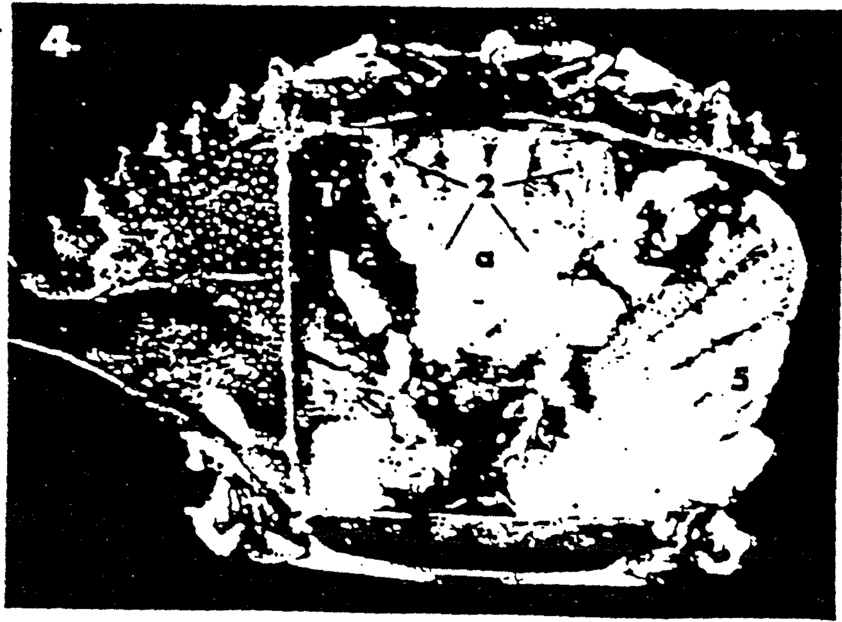

Fig. 4. The medial part of the carapace and much of the epidermis 1 have been removed. The large cardiac stomach 2 is visible. The [illegible] posterior diamond-shaped portion a. Two [illegible] are visible in the semitransparent heart 3. Intermingled hepatopancreas and [illegible] 4 and the gills 5 can also be seen.

NECROPSY FIELD DATA SHEET FOR HISTOLOGICAL SAMPLES
ADF&G, FRED Division Fish Pathology Lab

Collector/Address/Telephone #

Species

Number Specimens in Sample

Size Range

Life Stage

Date of Collection

Location of Collection (Site Name or Number)

Abnormalities Observed Per Specimen Number

Qualifications of Project Leader

CHARLES E. O'CLAIR S.S. No.: 012-32-1851

Personal: Born May 29, 1941; Ayer, Massachusetts

Education: University of Massachusetts, B.S., Zoology, 1963
University of Washington, Ph.D., Fisheries, 1977

Experience: 1977 - present: Fishery Biologist (Research), National Marine Fisheries Service, Auke Bay Laboratory, Juneau, Alaska. Research experience includes five years of field and laboratory work on the effects of oil pollution on benthic invertebrates in conjunction with the Outer Continental Shelf Energy Assessment Program followed by seven years of research on the ecology and behavior of Dungeness, king, and tanner crab in relation to the management of these species.

1983-1987: Affiliate Assistant Professor of Fisheries, School of Fisheries and Science, University of Alaska, Juneau.

Selected Publications:

O'Clair, C. E. and S. D. Rice. 1985. Depression of feeding and growth rates of the seastar *Evasterias troschelii* during long-term exposure to the water-soluble fraction of crude oil. Mar. Biol. 84:331-340.

O'Clair, C. E. and S. T. Zimmerman. 1987. Biogeography and ecology of intertidal and shallow subtidal communities. pp. 305-344. *In* The Gulf of Alaska: Physical Environment and Biological Resources. D. W. Hood and S. T. Zimmerman, Eds. National Technical Information Service, Springfield, Virginia.

O'Clair, C. E. and J. L. Freese. 1988. Reproductive condition of Dungeness crabs, *Cancer magister*, at or near Log Transfer Facilities in Southeastern Alaska. Marine Environ. Res. 26:57-81.

Morado, J. F., A. K. Sparks and C. E. O'Clair. 1988. A preliminary study of idiopathic lesions in the Dungeness crab, *Cancer magister*, from Rowan Bay, Alaska. Marine Environ. Res. 26:311-318.

O'Clair, C. E., R. P. Stone and J. L. Freese. (in press). Movements and habitat use of Dungeness crab and the Glacier Bay fishery. Proc. Second Glacier Bay Science Symposium, 1988.

CONFIDENTIAL

STATE/FEDERAL RESOURCE DAMAGE ASSESSMENT
DETAILED STUDY PLAN

Project Title:	INJURY TO ROCKFISH, HALIBUT, AND LINGCOD ALONG THE LOWER KENAI PENINSULA
Study ID Number:	Fish/Shellfish Study Number 23
Lead Agency:	State of Alaska, ADF&G; Sport Fish Division
Cooperating Agencies:	Federal: USFS State: DNR
Principal Investigator:	Kelly Hepler, Fishery Biologist
Assisting Personnel:	Andrew Hoffmann, Fishery Biologist Tom Brookover, Fishery Biologist Two Fishery Technicians
Date Submitted:	September 25, 1989

	Signature	Date
Principal Investigator:	Kelly Hepler	10/9/89
Supervisor:	Doug McBride	10/9/89
OSIAR Senior Biometrician:		
OSIAR Program Manager:	[signature]	10/13/89
OSIAR Director:		

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INTRODUCTION

The goal of this project is to determine whether the Exxon-Valdez oil spill will have a measurable impact on stocks of rockfish, *Sebastes sp.*, of the lower Kenai Peninsula (LKP). These waters received large amounts of crude oil after the oil slicks moved out of Prince William Sound. Assemblages of rockfish occur along the Gulf coast and support a significant recreational fishery out of Seward. Most of the annual harvest is supported by five species of rockfish, although several other species are harvested in small numbers. Of particular importance are: yelloweye rockfish *S. ruberrimus*; dusky rockfish *S. ciliatus*; and black rockfish *S. melanops*. Unlike many species of marine fish, demersal rockfish complexes are relatively sedentary, residing near rocky reefs and boulder fields. Rockfish are long-lived, recruitment is low, and the potential for long-term stock decline due to habitat degradation is high. The potential impact of the oil spill on various nearshore assemblages is dependent upon location of various "rockpiles" and the potential uptake of various contaminants will be related to the level of oil contamination and food web characteristics of these various reefs.

Data Base

Only limited baseline data are available for rockfish populations of the LKP. Rockfish were studied as part of a study of nearshore fish assemblages during the early 1980's (Morrison 1982). These investigations provided descriptions of selected rockfish populations including estimates of species composition, density, and length and age composition. Sampling primarily occurred in ten major sites from Gore Point to Cape Pudget. Additional baseline data were obtained from local charter operators who identified historical fishing sites.

Experimental Design

It is hypothesized that several detrimental impacts on these species could result from the presence of crude oil in marine waters including: (1) reduced survival; (2) reduction in reproductive success; and (3) accumulation of sublethal levels of toxic petrochemical by-products that could render the fish inedible. To test whether there will be a measurable impact on these stocks, selected locations in each of two treatments will be sampled: oiled and non-oiled areas.

The principal objectives of the project are to document presence/absence of: (1) rockfish in areas where rockfish are known to have previously occurred; and (2) oil contamination in rockfish and/or the substrate. Our primary assumption is that there is a difference in exposure to oil for fish stocks from each of the two treatments. Evidence from the literature indicates that the greatest observable impact of oil related activities occur in the littoral zone (National Academy of Sciences 1985); an area which supports populations of rockfish.

A measurable detrimental impact on these stocks of rockfish may result in a loss to the sport fishery. The status of the sport fishery will be investigated through: (1) an ongoing postal survey (Mills 1988) and (2) an onsite creel survey of selected LKP fishery access ports (OSIAR Study FS #6).

OBJECTIVES

1. Document the presence or absence of rockfish in 12 locations of the Lower Kenai Peninsula.

2. Document the presence or absence of oiled rockfish in 12 locations of the Lower Kenai Peninsula.

3. Document the presence or absence of oiled substrate in 12 locations of the Lower Kenai Peninsula.

4. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified (to be accomplished upon completion of the project).

METHODS

This operational plan addresses both the long range study design of this project and work that has already been accomplished as part of the initial fishery impact assessment work. Throughout the remainder of this document, work that has already been initiated will be identified. Because of the immediacy of the initial fishery impact assessment work, some of the experimental design had not been fully developed and certain aspects of the sampling were not initiated. Changes in sampling design will be identified for initiation in the future.

Study Design and Data Collection

Twelve sites will be sampled across two treatments: oiled and non-oiled areas (Table 1). Most of these sample sites correspond to areas which have received previous study (Morrison 1982). Morrison established transects and sampled for density by species. The remaining sample sites were selected after consulting with local charter operators. This information will provide the basis for determining the historic presence or absence of rockfish. Sampling will be conducted during at least two surveys over the course of the summer. The surveys will occurr in the same months as did Morrison's work (1982).

At each location, transects will be established and sampling will be conducted along the majority of the reef with longline gear and/or jigging. The exact location of each transect will be identified with LORAN and/or latitude/longitude coordinates at each end of the transect. In addition to coordinates, depth along the transect and a compass reading from the starting to ending point will be recorded. In

addition, samples of more pelagic species will be collected by jigging. If fish are present, species composition will be estimated for comparison with Morrison's dive and jigging surveys (1982). Species identification will be accomplished using the methods of Kramer and O'Connell (1988). Any catches of lingcod or halibut will also be noted.

Sampling for oil contamination will be accomplished as follows. A total of 10 rockfish from each sampling location will be collected for hydrocarbon testing. Each entire fish will be wrapped in aluminum foil, marked with an evidence seal, and frozen. Upon return to port, the following samples will be collected for hydrocarbon analysis: gallblader, stomach, pyloric caeca, liver, and muscle. Procedures to prevent hydrocarbon contamination, as specified by the National Marine Fisheries Service Auke Bay Lab, will be followed for each fish: (1) hands and sampling gear will be washed with soap and water; (2) dissection tools will be rinsed in methylene chloride; (3) samples of each tissue will be individually stored in certified hydrocarbon-free sampling jars; and (4) samples will be frozen. Samples will not be touched by human hands or any petrochemical product (i.e. plastic). These samples will be transferred to National Marine Fishery Service for analysis. Additionally, any moribund fish that are found will be examined for the presence of tar balls in the stomach and all contaminated samples will be handled in the prescribed chain of custody procedures.

The presence/absence of oil at each sampling site will be documented with a Remote Operating Vehicle (ROV). This work is covered under another project ("Undersea Observations to Support Benthic Fishery Damage Assessment). Visual (video tape) records will be collected along transects within rockfish sampling areas. Presence of oil, general distribution, occurrence of rockfish, depth, substrate type, turbidity, temperature, and salinity will be recorded. Transect density will be increased where evidence of oil is found. If oil is observed, subsequent sampling will be initiated to collect a sample for verification. ROV work was only accomplished during the mid-June survey.

Data Analysis

Data analysis will be limited to a simple expression of presence or absence; percent of sample contaminated; and degree of contamination (ppm of hydrocarbons). Proportions of all nonoiled sites with rockfish will be compared to the proportions of all oiled sites with rockfish on a species by species basis. Hydrocarbon contamination will be detected 99% ($\alpha = .01$) of the time if over 37% of the population is contaminated at the selected sample size of 10.

SCHEDULES

A schedule of tasks to be completed during 1989 is as follows:

Task	Dates
Sampling: 1st Survey	Late June
2nd Survey	Mid September
Data Analysis and Report Preparation	9/15-3/15

REPORTS

Results of these study efforts will be reported to the Division of Oil Spill Impact Assessment and Restoration. Upon completion of litigation, these data will be published as either an Alaska Department of Fish and Game, Sport Fish Division, Fishery Data Series report or in the fisheries literature.

BUDGET SUMMARY

A line item breakdown of project costs for the period beginning April 1, 1989, and ending February 28, 1990 is as follows:

Line Item	Category	Cost (thousand $)
100	Personnel	34.2
200	Travel	5.0
300	Services	52.7
400	Commodities	3.5
500	Equipment	13.0
	Total	108.4

LITERATURE CITED

Kramer, D.E. and V.M. O'Connell. 1988. Guide to Northeast Pacific Rockfishes Genera Sebastes and Sebastolobus. University of Alaska Marine Advisory Bulletin No. 25.

Mills, M.J. 1988. Alaska statewide sport fisheries harvest report. Alaska Department of Fish and Game, Fishery Data Series No. 2.

Morrison, R. 1982. Trip report: outer district rockfish survey June 1-10, 1982. Alaska Department of Fish and Game Lower Cook Inlet Data Report No. 82-6. 20 pp.

National Academy of Sciences 1985. Oil in the Sea: Impacts, Fates, and Effects. National Academy Press, Washington DC. 601 pp.

Table 1. Sampling locations for rockfish along LKP, 1989.

Location	LORAN
Gore Point[1]	12884/31756
Port Dick[1]	12375/31728
Nuka Passage[1]	12470/31720
Front Point	12459/31737
Aligo Point	12915/31800
Harris Bay	12948/31838
Outer Island[1]	12607/31729
Granite Island	12925/31824
Seal Rocks[1]	12884/31756
Aialik Cape	13025/31820
Cape Fairfield	13290/31855
Cape Pudget	13330/31840

[1] Oiled sites

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STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DETAILED STUDY PLAN

Project Title: SHELLFISH AND GROUNDFISH TRAWL ASSESSMENT OUTSIDE PRINCE WILLIAM SOUND

Study ID Number: Fish/Shellfish Study Number 24

Lead Agency: National Oceanic and Atmospheric Administration; State of Alaska, ADF&G, Commercial Fisheries Division

Cooperating Agency(ies): None

Principle Investigator: Usha Varanasi, NMFS, Seattle
Principle Investigator: Gary Stauffer, NMFS, Seattle
Principle Investigator: William Nippes, ADF&G, Kodiak
Principle Investigator: Al Kimker, ADF&G, Homer

Assisting Personnel: See Next Page

Date Submitted: October 12, 1989

	Signature	Date
Principal Investigator, NMFS:	[illegible]	10/9/89
Supervisor, NMFS:	[illegible]	10/11/89
Principal Investigator, NMFS:	[illegible]	10/11/89
Supervisor, NMFS:	[illegible]	10/11/89
Principal Investigator, ADF&G:	[illegible]	10/9/89
Supervisor, ADF&G:	[illegible]	10/9/89
Principal Investigator, ADF&G:	[illegible]	10/10/89
Supervisor, ADF&G:	[illegible]	10/9/89
OSIAR Senior Biometrician:		
OSIAR Program Manager:	[illegible]	10/12/89
OSIAR Director:		

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I. OVERVIEW

INTRODUCTION

Oil spilled from the Exxon Valdez March 24, 1989 traveled out of Prince William Sound south and west along the Kenai Peninsula to the Aleutian Islands. Spilled oil was documented in both nearshore and offshore waters. In the area north of Kodiak, water currents divided and carried a portion of the oil north into Cook Inlet, another portion went west into Kamishak Bay, and the remainder went south to Kodiak Island and the Alaska Peninsula. Oil spread over 400 miles away from the spill site and impacted open ocean and nearshore waters throughout a geographically varied region.

To assess any damage from this oil to a variety of groundfish and shellfish species, a multi-agency project with three component studies was created to collect specimens for hydrocarbon and metabolite analysis, to study biological and biochemical effects, to estimate population abundance of important groundfish and shellfish species, and to collect age composition and other biological data on these species. A multi-faceted project was required due to the sheer size and varied nature of the area and natural resources to be studied.

The three components of this study include: A.1.: Alaska Department of Fish and Game (ADF&G) trawl surveys that target shellfish and groundfish in Lower Cook Inlet, embayments and other coastal areas of Kodiak Island and as far west as Unimak Island; A.2.: a portion of the NMFS triennial trawl survey that targets Gulf of Alaska groundfish species ranging from nearshore regions to the continental slope; and B: National Marine Fisheries Service (NMFS) sampling of groundfish and shellfish aboard the R/V Fairweather in nearshore waters of Prince William Sound, lower Cook Inlet, Kodiak Island, the Kenai Peninsula and Alaska Peninsula. The integration of the three major components of this study is important for fully evaluating any impacts of the oil spill on the extremely valuable marine fishery resources of the western Gulf of Alaska.

This multidisciplinary project is designed to determine both short and long-term effects of oil on economically and ecologically important species. Short-term effects will be assessed through testing levels of hydrocarbons and metabolites in stomach contents, bile and tissues of fishes, in hepatopancreas and tissues of crustaceans and by testing hydrocarbon levels in sediments. Fish will also be analyzed to assess pathology and reproductive dysfunction due to oil exposure. Long term effects of oil will be assessed by monitoring population levels, age composition, and growth rates. Analyses of these data will test for declining stock levels, high mortality of the 1989 year class, or changes in growth

rates that may be related to oil exposure. The potentially confounding effects of predation, fishing effort, and environmental variability will be reduced by coupling the short-term and long-term effects. For example, detection of short-term reproductive malfunctions, if any, would assist in determining causal mechanisms of year-class failures that may occur over the long term. As another example, cause and effect could be established by coupling data on tissue damage revealed by histology with data on reduced growth or increased mortality.

OVERALL OBJECTIVES

A. Measure abundance of Tanner crab, red king crab, halibut, pollock, sablefish, and other commercially important species.

B. Determine age composition for primary species.

C. Determine the incidence of abnormalities in tissues and organs in fish and shellfish captured in oiled areas and whether such abnormalities result in adverse changes in the viability of the resource.

D. Catalog specific areas from the outer Kenai Peninsula to the Aleutian Islands where fishery resources show the bioaccumulation of petroleum compounds and their metabolite derivatives.

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

GENERAL METHODS

Objectives A and B will be addressed through population assessment surveys of groundfish and shellfish throughout the Gulf of Alaska from Prince William Sound to Unimak Island. Sampling will be conducted using fishing boats towing poly-Noreastern (NMFS) or 400 mesh Eastern (ADF&G) otter trawls, depending on the target species. Tows are placed according to stratified sampling designs most appropriate to the specific area fished. The sample designs link ADF&G and NMFS trawl surveys together in one comprehensive assessment of groundfish and shellfish stocks in the impacted areas outside Prince William Sound. The design also allows comparisons to historic assessment surveys in the Gulf of Alaska conducted by both resource agencies. Trawling for population assessment will take place during August, September, and October of 1989. Abundance estimates will be calculated using the area swept

methods.

Objective C and D will be addressed through the NMFS sampling and analysis program. The majority of samples for hydrocarbon analysis and biological effects studies will be collected by NMFS personnel aboard the R/V Fairweather. These samples include juvenile and adult specimens from oiled and non-oiled nearshore waters of Prince William Sound, lower Cook Inlet, Kodiak Island, the Kenai Peninsula and Alaska Peninsula. A limited number of samples from adult specimens not available in the nearshore sampling will be collected by ADF&G in lower Cook Inlet and by NMFS in offshore waters of the Gulf of Alaska. A variety of groundfish and shellfish species will be collected which represent various habitat types and trophic levels as well as those of high economic value. Otter trawls, beach seines, gillnets, and long lines will be used to collect samples. Samples will be collected from the R/V Fairweather during mid-May to late-September. Sampling by NMFS and ADF&G trawl surveys in the Gulf of Alaska and Lower Cook Inlet will occur during October.

Objective E will be addressed through the combined interpretation of results and the three component projects. Mitigation and restoration will be achieved through the fishery management process for stocks where injury is identified.

II. COMPONENT STUDIES

ABUNDANCE AND DISTRIBUTION

A. 1. Alaska Department of Fish and Game

Introduction

The Alaska Department of Fish and Game multispecies trawl surveys are one component of a three component study aimed at assessing damage done to shellfish and groundfish stocks outside of Prince William Sound by Prudhoe Bay crude oil spilled from the Exxon Valdez.

This component of the study assesses impact on shellfish and groundfish populations in Lower Cook Inlet, Shelikof Strait, waters off Kodiak Island and embayments along the Alaska Peninsula. Major stocks of groundfish and shellfish occur in these areas which support commercial fisheries. Fisheries include pot fisheries for king, Tanner, and Dungeness crab; trawl and pot fisheries for shrimp; and longline, trawl, and jig fisheries for halibut, pollock, sablefish, Pacific cod, flatfish, rockfish and others. These stocks are an important part of the economies of Alaska and Washington.

It is hypothesized that the oil spill could adversely affect these populations in two ways. First, most benthic species go through a planktonic stage which ranges widely through the water column. It is well documented that these plankton are extremely sensitive to the water soluble fractions of crude oil (Mecklenburg et al., 1977, Wells and Sprague, 1976). A multispecies trawl survey permits assessment of shellfish and groundfish abundance and distribution. Data from this project will allow detection of reduced year-class strength consistent with the timing of the oil spill. Secondly, adult populations could be impacted through movement of oil to the benthic environment causing direct mortality on groundfish and shellfish through immediate toxic effects, contamination of prey, direct ingestion of tar balls, injury to tissue and malfunction of organs. Data from this study will document incidence of tissue abnormalities and bioaccumulation of petroleum compounds for Tanner crab, pollock and flathead sole in lower Cook Inlet and in any area covered by this survey where oil is found on the bottom or on fish or shellfish. Establishing actual degree of damage to these stocks by oil is required to advise the public on the full scope of impact on the commercial fishing economy due to the spill. Determining the level of damage to these stocks is also necessary so that it can be factored into future management decisions on harvest levels.

Objectives

A1. Estimate species composition and abundance of dominant groundfish and shellfish species, including Tanner crab, king crab, halibut, sablefish, Pacific cod, flathead sole, pollock, arrowtooth flounder, and other important groundfish (incl. rockfish species) and shellfish species caught in bottom trawls in Lower Cook Inlet, Shelikof Strait, waters off Kodiak Island and embayments along the Alaska Peninsula and determine impacts of oil contamination on abundance of shellfish and groundfish stocks.

B1. Determine age composition in lower Cook Inlet for primary commercial species (rex Sole, Dover sole, arrowtooth flounder, and pollock such that the estimated proportion for each age is within ±5% of the true value 90% of the time.

D1. Document and catalog areas found in Lower Cook Inlet Shelikof Strait, waters off Kodiak Island and embayments along the Alaska Peninsula where oil occurs on the bottom or occurs on or in bottom dwelling fish and shellfish.

E1. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

This project is one component of a three component study. Letters (A1-E1) refer to overall objectives this component is designed to address. Objectives C and D listed for Project Number 24 in the State/Federal Natural Resource Damage Assessment Plan for the Exxon Valdez Oil Spill August 1989 are to be met through the two components of this study completed by NMFS.

Methods/Data Analysis

The ADF&G multispecies trawl survey is similar to trawl surveys used for oil spill impact assessment in Prince William Sound by ADF&G/NMFS. Similar surveys are used by NMFS in the Bering Sea and Gulf of Alaska to assess populations for fishery management purposes. Parks and Zenger (1979) report a similar project done by NMFS in Prince William Sound. Ronholt et al. (1978) document historical surveys by NMFS and its predecessor, BCF, done in the Gulf of Alaska. The dominant species caught by value or volume are expected to be Tanner crab, king crab, halibut, sablefish, pollock, Pacific cod, flathead sole, rex sole, Dover sole, arrowtooth flounder and various rockfish.

Abundance, species composition, and other biological attributes of the catch will be estimated for the entire area covered by the trawl survey. Sampling in future years and/or post-stratification of the sample area into oiled and non-oiled areas will allow comparison of changes in abundance, species composition, age composition, or other biological parameters that may be related to the oil spill.

Study Design

Lower Cook Inlet:

The trawl survey in lower Cook Inlet will be conducted October 2-12, 1989 aboard the R/V Pandalus of ADF&G. If weather permits additional fishing will take place during the period October 19 - 24. Sampling will occur at predetermined stations during each survey period. Each station will be sampled once during the survey.

Stations to be fished are selected by a stratified random sampling plan. Lower Cook Inlet was divided into 9 areas based on geographical location and depth as follows:

1. Inner Kachemak Bay (shallow)
2. Outer Kachemak Bay (shallow)
3. Outer Kachemak Bay Deep (deep)
4. North Central (shallow)
5. South Central (deep)
6. North Kamishak (shallow)
7. South Kamishak (shallow)
8. South Kamishak Deep (deep)
9. South West Kamishak (shallow)

Strata are based on depth ranges of 10 to 50 fathoms for shallow strata and 50 fathoms or greater for deep strata. Geographic areas were selected according to available information on distribution of shellfish and groundfish throughout Lower Cook Inlet. These areas are shown in Appendix A. Figure 1.

Size of each area in square nautical miles was calculated (Appendix A, Table 1). Each of these areas is divided into a grid of square stations that are 2.5 minutes of latitude by 5 minutes of longitude (2.5 nautical miles square, or 6.25 square nautical miles). Trawl sites were randomly selected from the grid of stations in each area using a random number table. Each station will be sampled once during the survey.

The trawl survey is anticipated to take a minimum of 14 days. If three tows can be accomplished each day for 14 days, a total of 42 tows will be completed. A minimum of three stations will be sampled (3 tows completed) in each area yeilding 27 preassigned

tows (9 areas times 3 tows per area equals 27 tows). The remaining 15 tows are allocated based on the relative number of square nautical miles in each area (Appendix A. Table 2). Effort allocation for a total of 42 tows presented in Appendix A, Table 3 is based on the assumption of having good weather during the full survey period.

A standard 400 mesh eastern otter trawl (Appendix B, Figure 1) will be fished for 30 minutes at a speed of 2 knots for a tow length of approximately 1 nautical mile. Tows will be placed at random within the station when possible. All tows will be made during daylight hours.

For each station to be fished, the vessel first would go to the station center and search for 20 to 30 minutes for trawlable bottom. If no trawlable bottom was found, the vessel would proceed to the location of the nearest successful haul. At the location of the nearest successful haul, the vessel would again search 20 to 30 minutes for trawlable bottom and presumably trawlable bottom would be found. If trawlable bottom is found while travelling from the station location to the location of the nearest successful haul, a haul would be made. If no trawlable bottom was found at the station location, enroute to the location of the nearest successful haul, or at the location of the nearest successful haul, the vessel would move to the next station.

Kodiak Island and Alaska Peninsula:

The trawl survey in the waters off Kodiak Island and in bays along the Alaska Peninsula bays will be conducted during August, September, and October 1989 aboard a chartered commercial fishing vessel. The Alaska Department of Fish and Game annually conducts benthic trawl surveys in the Kodiak and Alaska Peninsula areas for shellfish and groundfish assessment. The study sites selected for this project represent additional coverage for the 1989 survey year with the intent being to provide more complete coverage of targeted stocks for oil damage assessment.

Each offshore survey area was divided into approximately 5 nm square stations and each inshore or bay area divided into 2.5 nm square stations. Considerable variation occurs in the size of some offshore and most bay staions because of land boundaries. Each station will be sampled once during the survey. Exact Station location assignments are depicted in Appendix A, Figures 2-9. Trawl placement within stations will be randomly chosen except that untrawlable bottom will be avoided. All tows will be made during daylight hours. The survey is estimated to take 37 fishing days. Additional survey coverage is distributed as follows:

District	Area	# of Tows	# of Days
Chignik	Chignik	28	5
	Kujulik	11	2
	Mitrofania	16	3
	Ivanof	19	4
Total		74	14
South Peninsula	Stepovak	10	2
	Balboa/Unga	15	2
	W. Nagai Straits	8	2
	Kennoys Is.	6	1
	Beaver Bay	6	1
	Pavlof/Volcano	39	5
	Cold Bay/Belkofski	24	4
	Morzhovoi	25	4
	Sanak Island	8	2
Total		141	23
Grand Total		215	37

A standard 400 mesh eastern otter trawl (Appendix B, Figure 1) will be fished on the bottom for approximately 30 minutes to cover 1.0 nautical mile.

Data Collection

Station information including location (latitude and longitude), depth, time and duration of tow, direction of tow, weather, surface temperature, and sea conditions, etc. will be recorded by the vessel skipper on a paper form (Appendix C. Figure 1).

Shellfish Samples:

Sample size for Tanner, king, and Dungeness crabs will be 100% of the catch because the numbers are expected to be fairly low. Weight and number of all crabs by species will be recorded (Appendix C., Figure 2). For king Tanner, and Dungeness crabs sex, size (carapace length or width), shell age and shell condition will be recorded. For female crabs of these species, stage of maturity (juvenile or mature) and relative egg clutch size and stage of egg development will be recorded. Degree of black mat infestation will be recorded for all crabs (Appendix C. Figure 3).

In lower Cook Inlet, weight and number of each species for all other invertebrate species will be recorded (Appendix C, Figure 2).

In Kodiak, miscellaneous invertebrates are included in a subsample of the catch remaining after all large fish, Tanner crab, king crab and Dungeness crab are removed from the catch. This subsample consists of two fish baskets filled with miscellaneous fish and invertebrates. This subsample is sorted by species and the weight and number of each species is recorded (Appendix C, Figure 2).

All shellfish data will be recorded on paper forms and later entered into the R:base database in ADF&G area offices.

Groundfish Samples:

Sample size for groundfish in lower Cook Inlet will be 100% except in the case of extremely large catches where the total tow is weighed and then split into a subsample (e.g. one half or one fourth). In this case the subsample results are expanded to the weight of the entire tow. For smaller catches weight and number of fish by species will be recorded. Length, weight, and sex of a sample of approximately 200 specimens of each target species will be recorded for each tow (Appendix C, Figure 4). Target species are halibut, sablefish, Pacific cod, pollock, flathead sole, arrowtooth flounder and rockfish. See Appendix D for a detailed discussion of finfish processing for lower Cook Inlet.

In lower Cook Inlet weight and number of non-target species will be recorded. Length, weight, and sex will be recorded for a randomly selected sample of 200 specimens of the dominant species from a tow if it is not one of the target species listed above.

In Kodiak, all halibut and skate are measured and returned to the sea as soon as possible. Length to weight conversion tables are used to determine total weight of these species in the catch (Appendix E). Large fish such as Pacific cod, rockfish, sablefish, or dogfish are sorted completely from the catch and the weight of each species is measured. Weight of large fish is recorded on the form presented in Appendix C, Figure 2. Miscellaneous fish are included in a subsample of the catch remaining after all large fish, Tanner crab, king crab and Dungeness crab are removed from the catch. This subsample consists of two fish baskets filled with miscellaneous fish and invertebrates. The subsample is sorted by species and the weight and number of each species is recorded (Appendix C, Figure 2). See Appendix E for a detailed discussion of groundfish processing for Kodiak.

All groundfish data will be recorded on a paper form and later entered into the R:base database in the ADF&G area offices.

Age Composition (otoliths):

A random subsample of 500 rex sole, Dover sole, arrowtooth flounder, and pollock will be collected for otolith extraction in

lower Cook Inlet. Sample size per species was set to simultaneously estimate proportions by age when sampling from a multinomial population such that the probability will be at least 1-a (precision) that all of the estimated populations will be simultaneously within 5 percentage points (accuracy = 0.05) of the true population age proportions. The largest sample size for a = 0.1 occurs when there are only 3 age classes present in equal proportions and guarantees at least this level of precision and accuracy for any number of age classes and proportions. This "worst" case sample size (Thompson, 1987) of 403 ageable otoliths will be used to estimate the age composition with the desired level of precision and accuracy in the absence of a better estimate of population proportions. An unreadable rate of 25% was estimated for a sample size of 500.

Sampling for otoliths will be spread over a number of tows and represent the entire trawl survey. For widely distributed species such as arrowtooth flounder or rex sole, 20 otoliths per tow should result in the sample size objective being met. For pollock whose distribution is more patchy, 50-75 otoliths should be collected when large catches are made.

Flatfish otoliths will be stored in normal glycerin solution. Pollock otoliths will be stored in 50% ethyl alcohol. All will be labled in a manner consistent with the NMFS triennial Gulf of Alaska survey (component A.2.). The form for otolith collection is Appendix. C. Figure 4.

Hydrocarbon and Histological Samples:

Ten hydrocarbon samples will be taken from each area in lower Cook Inlet for the following species and tissues:

a. Tanner crab muscle, hepatopancreas, eggs
b. Pollock muscle, viscera, bile
c. Flathead sole muscle, viscera, bile

Ten adult specimens of each species will be randomly selected from all tows in an area. Hydrocarbon samples from the above species will supplement collections of adult specimens not available to nearshore sampling by the R/V Fairweather. A sample size of 10 from each area will enable detection of a difference of 2.0 standard deviations in hydrocarbon content between areas with the probablility of making a type I and type II error equal to 0.05 and 0.1, respectively.

At each station in lower Cook Inlet, fish of the target species, halibut, sablefish, Pacific cod, pollock, flathead sole, arrowtooth flounder and rockfish, will be sampled for the presence of tarballs in the stomach and intestinal tract. At a minimum, five individuals of each species sampled for otoliths (rex sole, Dover

sole, arrowtooth flounder, and pollock) will be examined for presence of tar balls.

Histological samples and hydrocarbon samples will be randomly selected from the catch at stations in lower Cook Inlet, Kodiak, and Alaska Peninsula where tarballs or other oil contaminants are found in stomachs, on crab setae, or on the net. If hydrocarbon and histological samples are taken as a result of oil contamination, then hydrocarbon and histological samples will be taken from an additional station where target species are uncontaminated to represent a control.

Ten replicate samples will be taken of the following species and tissues from any site with evidence of oil contamination:

a. Pollock muscle, viscera, bile
b. Tanner crab muscle, hepatopancreas, eggs
e. Flathead sole muscle, viscera, bile

Total number of samples will be dependent on the number of stations where tarballs or other oil contaminants are found in stomachs, on crab setae, or on the net. Post stratification will allow comparison of samples from oiled and non-oiled areas.
Species to be sampled for histology are pollock and pink shrimp. Twenty animals of each species will be sampled at each sampling site selected according to the criteria stated above. Total number of samples will be dependent on the number of stations where tarballs or other oil contaminants are found in stomachs, on crab setae, or on the net. Post stratification will allow comparison of samples from oiled and non-oiled areas. Samples sizes and species were recommended by T. Meyers (ADF&G, personal communication).

Data forms for tarball sampling and hydrocarbon and histological sampling are found in Appendix C, Figures 5 and 6. Procedures for hydrocarbon and histological sampling are described in Appendices F and G. Chain of custody procedures are described in Appendix H.

Data Analysis

Area-swept methods will be used to estimate abundance and density by depth and geographic strata to address objective A1. Trawl catch data will be analyzed to generate abundance estimates with operational computer statistical programs utitlized for population estimates from annual Gulf trawl surveys (Blackburn et. al., 1989). Absolute abundance will be estimated in numbers for each target crab species by size, relative age, and sex. Absolute abundance in weight will be estimated for each target fish species by size. Absolute abundance in weight will be estimated by species for miscellaneous shellfish and groundfish.

Negative impacts of oil contamination on abundance of shellfish and

groundfish stocks can be determined by testing for differences in species composition and abundance with categorial data analysis techniques and other multivariate statistics.

Data can be post-stratified to determine impact levels based on presence of oil contaminants and mapping information from the coastal habitat project. Analysis of variance (parametric) or a Kruskal-Wallis test (non-parametric) will be used to test for differences between years and impact levels for: 1) average number of mature female crab; 2) average relative clutch size of female crab; 3) average stage of egg development; 4) average number of abnormal egg clutches; and 5) hydrocarbon content of tissues.

Multivariate statistics may be used to identify more complex associations among biological and physical parameters between oiled and non-oiled areas.

Age composition of primary commercial species (objective B1) available to the trawl net can be addressed with age frequency diagrams. Size frequency distribution of crabs by species, sex and relative age and size frequency distribution for target species of groundfish will also be examined.

Areas found in Lower Cook Inlet, Shelikof Strait, waters off Kodiak Island and embayments along the Alaska Peninsula where oil occurs on the bottom or occurs on or in bottom dwelling fish and shellfish will be documented and cataloged to address objective D1.

To address objective E1, all data will be analyzed to determine degree of damage to stocks and suggest appropriate restoration or mitigation measures. This may include restrictions on human harvest to reduce mortality rates and may include the need for continued monitoring of stocks.

Analyses will be conducted in the area offices of ADF&G following each survey using microcomputers running R:base applications. Statistical analyses will be conducted on microcomputers using SAS, MathCAD, Stats-Plus or other appropriate software by ADF&G personnel.

Schedule/Personnel

Schedule:

Date(s)	Activity
August - October, 1989	Kodiak trawl survey
October 2 - 13, 1989	Lower Cook Inlet trawl survey period 1
October 19 - 23, 1989	Lower Cook Inlet trawl survey period 2
November - December, 1989	Data entry and analysis
December, 1989	Preliminary report on shellfish and groundfish trawl assessment outside Prince William Sound.

Actual length of survey periods may vary due to weather.

Personnel:

Name	Role	Mo
Al Kimker	Principal Investigator	nf*
Rich Gustafson	Fishery Biologist	3.0
Lee Hammarstrom	Fishery Biologist	nf
Bill Bechtol	Fishery Biologist	nf
Bill Nippes	Principle Investigator	nf
Dave Jackson	Fishery Biologist	2.0
Robert Popken	Fish and Wildlife Technician	1.25
Ken Vartan	Fish and Wildlife Technician	1.25
Mike Villegas	Fish and Wildlife Technician	0.70
Jeff Query	Fish and Wildlife Technician	2.0
Dan Moen	Fish and Wildlife Technician	2.0

* nf: personnel not funded by this project

Budget

Line Item	Category	Budget
100	Personnel Services	34.5
200	Travel	2.3
300	Contractual	196.5
400	Commodities	10.5
500	Equipment	52.0
Total		295.8

A.2. National Marine Fisheries Service

Introduction

As early as 1948, abundances of commercially important fish stocks have been assessed in the Gulf of Alaska using trawl surveys (Ronholt et.al., 1978). The design and execution of standardized National Marine Fisheries Service trawl surveys has been ongoing since 1984 on a triennial schedule. The third triennial trawl survey is scheduled for 1991. Survey methods have been standardized and the sampling design has been tailored to reflect the variety of environmental conditions in the Gulf.

The Gulf of Alaska triennial survey area covers the groundfish stocks from nearshore to the upper continental slope from the U.S.-Canada border at Dixon entrance to 170° W in the eastern Aleutian Islands. The trawl survey time series provides a data base to assess changes in the distribution and abundance of groundfish in the central Gulf of Alaska before and after the Exxon oil spill. This component of the project 24 study plan is a late summer, early fall trawl survey of the central Gulf of Alaska designed to assess population abundance of dominant groundfish species using area-swept methods of estimating fish abundance. Standardized sampling techniques and data analysis procedures developed by the Resource Assessment and Conservation Engineering Division (RACE) of the Alaska Fisheries Science Center, Seattle WA., will be followed. The survey will be conducted aboard the chartered fishing vessel Pelagos from approximately 1 September to 26 October.

Objectives

The NMFS trawl survey component of Project 24 is designed to address Objectives A and B and support objective E for the dominant commercial groundfish species. The project objectives under A and B are:

A1. Estimate abundance, variance and confidence intervals of the dominant groundfish species that would allow test comparisons with previous triennial survey estimates for alpha = 0.05.

A2. Map geographic distributions of dominant groundfish stocks for preliminary spatial comparisons and analysis.

B1. Make otolith collections for pollock, rock sole, flathead sole and rex sole using standard RACE sampling criteria.

The age determination and analysis for age composition from ADF&G and NMFS otolith collections will be scheduled in 1990 if Project 24 is funded after February 1990.

Methods/Data Analysis

This survey will sample the central Gulf of Alaska area extending westward from 147° W longitude to 154° W longitude on the southeast side of Kodiak Island and southward to 57° 30' N latitude in Shelikof Strait. This is approximately one-third of the area surveyed in triennial years, due to fewer available vessel days in 1989.

Only about 40 of the 55 charter days are expected to be available for fishing. It is probable that no more than 150 tows can be executed in 40 days. In previous triennial surveys this area has been allocated between 260 and 320 stations.

The survey area was divided into seven strata based primarily on habitat type and depth. Strata with the highest productivity and highest station density are the 1-100 m nearshore and bays, 101-200 m gullies and 101-200 m slope. Strata with medium productivity and sampling density are the 201-300 m gullies and Shelikof Deep while those with lowest productivity and sampling density are the 1-100 m shelf, 101-200 m shelf and 201-300 m slope. The latter three strata were allocated only as many tows to allow minimum coverage and provide an adequate estimate of variance of CPUE. Regions deeper than 300 m will not be surveyed.

Station Selection

Allocating effort:

The following table shows the approximate percentage of expected sampling effort to be placed in each stratum type. The column labeled "Number of Tows" gives the percentage of effort in actual numbers of tows. These allocations were based on the area of each stratum type and the importance of each with respect to detecting oil effects and estimating the abundance of important groundfish species. In the last two columns those numbers that are not enclosed by parentheses were expected after allocations were made but before stations had been assigned. Those numbers within parentheses are the allocation after the sample sites had been selected.

Stratum Type	Relative Productivity	Percent	Number of Tows
1- 100 m nearshore and bays	highest	30 (33.5)	45 (53)
101 - 200 m gullies	highest	20 (18.4)	30 (29)
101- 200 m slope	highest	10 (9.5)	15 (15)
201- 300 m gullies and Shelikof Deep	medium	11 (12.0)	17 (19)
101- 200 m shelf	lower	17 (15.2)	28 (24)
1- 100 m shelf	lower	9 (8.2)	13 (13)
201- 300 m slope	lower	3 (3.2)	5 (5)
greater than 300 m	--	0	0
Total		100	153 (158)

Locating the tow sites:

Once the allocations were made, the tow sites were selected in each stratum type systematically. Randomness was preserved by using a random number generator to select the starting point. This procedure was employed in order to insure a broad coverage of the area.

A grid of points separated by five miles north, south, east, and west, and strata boundaries were superimposed on a map of the survey area. Only points on the grid were considered as candidates for tow sites. The number of grid points in each stratum type was counted and then divided by the number of stations that had been assigned. This produced the interval between sample sites, called here the "selection interval". For example, if there were 100 grid points in a stratum type and 25 tows assigned to it, then a tow site was located every fourth grid point. A starting point was selected randomly from within a set of points equal in number to the selection interval and located as far to the south and west as possible. From the starting point the interval count was initiated first to the north until no more potential grid points could be found for that stratum type then the count continued southward on the next column of grid points to the east. The counting direction alternated column by column regardless of whether or not a column was devoid of site candidates.

Some strata types presented complications to this procedure for counting the selection interval. The 0 - 100 m nearshore stratum and the 101 - 200 m gully stratum both exist in multiple, narrow bands running southwest to northeast on both sides of Shelikof Strait and on the east side of Kodiak Island. Thus by counting up and down columns of grid points more than one band was crossed.

Station reassignment:

Special problems were presented by lower Cook Inlet. The entire lower reach of the inlet is classed in the 1 - 100 m nearshore stratum type. This results in an extremely broad area that has tenuous or no proximity to shoreline to be included for nearshore sample allocation. As a result lower Cook Inlet consumed many stations that had been intended for sampling in nearshore waters. Based on results of the 1984 and 1987 triennial surveys, portions of lower Inlet are only marginally productive and since more tows were desired in the truly nearshore and bay areas, the sampling density was decreased and effort reassigned to these areas. This was considered to be a legitimate mitigation of the systematic process since the point of that process was to provide as thorough a coverage as possible.

The resulting sampling plan consists of 158 tows located throughout the central Gulf area (see Appendix I, Figure 1).

Extra sampling time or increased weather days:

Little trawl sampling effort has been undertaken in many of the bays and fjords in the central Gulf during past triennial surveys. To increase our database on the variance structure of fish density in these areas for future survey planning, special sites will be sampled when the opportunity arises.

Sites for these tows are to be determined by the Field Party Chief with advice from the vessel operator. The objectives to be considered in choosing bays and tow locations within bays are to cover bays throughout the central Gulf area, and to map fish densities within each chosen bay. These special bay tows will be given a code that differentiates them from tows belonging to the stratified systematic design. That being so, the special tows will be chosen within each bay to provide thorough coverage of the bay, with secondary concern being given to randomness. The bays themselves are to be selected in a way that allows coverage of as many as possible. Thus if the vessel must run from weather, it may be best to hide in a bay that is a little further away so that tows can be made there, rather than to run to a nearer bay that has already been covered.

While inside a bay or protected water, if the weather is too harsh to spend prolonged periods out on deck, short tows will be made to gather data on species composition. Simply recording what species are there and obtaining a measure of their relative density will provide extremely valuable data, although not precise CPUE estimates.

Vessel operations

Fishing operations will be carried out during daylight hours (approximately 0700 - 1900). A poly-Noreastern trawl will be fished on the bottom for 30 minutes (Appendix J, Figure 1). Tow time starts after ensuring that the net has contacted the bottom. Most tows will be made with SCANMAR net mensuration gear on the nets, along with a net Sonde unit, mounted on the headrope. For those tows made without using SCANMAR equipment, 3-8 minutes depending on depth of tow, will be required for the net to achieve equilibrium. The standard towing speed is 3 knots. If untrawlable bottom is encountered at a pre-selected station, an alternative site will be sought within a one nautical mile radius of the station. If an alternate station cannot be found within a reasonable time, the search will be abandoned and the next station attempted.

Trawl mensuration

SCANMAR net mensuration systems will be aboard during the entire survey. This equipment includes sensors which attach to the trawl and a microcomputer system to read mensuration data from the sensors. The mensuration equipment will be used on as many tows as possible, but will not be used on tows judged to have a high risk of losing valuable scanmar equipment. The SCANMAR systems will be operated according to the RACE SCANMAR field manual.

On deck sampling procedures

Catch estimation and sampling procedures will follow standard RACE Division survey procedures for initial handling, sorting and weighing of the catch and additional sampling for biological data such as length frequencies and age collections (Bakkala et.al., 1985). Complete descriptions of the standard procedures are described in the 1984 and 1987 Gulf of Alaska triennial survey Scientific Operations Plans.

Initial handling of the catch:

Catches weighing less than 2,500 lb. will be emptied directly onto the sorting table, sorted, weighed, and counted by species. If the catch exceeds 2,500 lb., the catch is split as follows: the codend is weighed with the load cell, then emptied into the deck bin with the retainer and cargo nets in place, and the empty codend weighed again. The technique of subsampling with a cargo net will follow the procedure prescribed in Highes, 1976. If the load cell is not available, the weight of each split can be estimated using the volumetric method: the proportion of the amount of the sub-sample to the total volume in the deck bin is determined by measuring the level of fish in the splitting bin before and after removing the subsample. The weight of the sub-sampled portion is then multiplied by this proportion to obtain the weight of the entire catch. These weights are recorded on the on-deck sampling form, and the method used for determining catch weight is recorded on the haul position form (Appendix K, Figures 1 and 2).

Sorting and weighing the contents of the sampling table:

The catch will be sorted by species, filling baskets as needed. For invertebrates that are difficult to identify to the species level, broad categories (e.g., octopus, jellyfish, snails, starfish, crabs, invertebrate-unidentified) will be used.

While weighing the catch components, the number caught for each species will also be determined. For species with only a few specimens, this can be done by direct count. For those species where a length-frequency sample is taken, the sample weight and number will be used to automatically estimate the total number in the catch while the catch data is being entered on the computer. For those species which have a large number of individuals but which are not sampled for length frequencies, a subsample of the

catch (usually 1-2 baskets, or about 100 fish) should be weighed, counted, and the results recorded on the On-Deck Sampling Form (Appendix K, Figure 3). The weights of baskets for which there are counts will be recorded in the "subsample" column while weights of baskets without counts go in the "non-subsample" column of the On-Deck Form. These sample numbers will also be expanded automatically by the computer as the catch data is entered.

Catches from "unsatisfactory" performance hauls (ripped nets, hangups, etc.), as well as satisfactory performance hauls, will be sampled for species composition and should have an On-Deck Form and a Haul-Position Form completed for them. Sample all species in these catches for numbers and weight.

Biological sampling:

The following species are identified as priority species for biological sampling: pollock, rock sole, flathead sole and rex sole. Lengths, age structures and individual weights will be collected for each of these species and data recorded on the species catch and specimen forms (Appendix K, Figures 4 and 5). Length frequency data will be taken for all major species caught after these primary species have been processed and recorded on the length frequency form (Appendix K, Figure 6).

Length data:

Length frequencies will be collected for all species which occur in the catch in numbers of 10 or more. The species with top priority for lengths are the 4 priority species listed above, and following that, any major species caught in that tow. Length frequencies will always be taken by sex, with the exception of Pacific halibut or non-commercial species such as rattails which may at times constitute a large portion of the catch, but which are not presently managed.

Halibut length frequencies:

The first collection from a tow will be to select out all halibut (sample 100% in the case of a split), take length frequency measurements, and return them overboard alive. Halibut weights are obtained through a length-weight relationship available in the data entry program.

Crab length frequencies:

Length frequencies for king and tanner crabs, (Paralithodes sp. and Chionoecetes sp.) will be taken for 100% of crabs caught in the tow, (i.e. sample the crabs before sub-sampling a large tow, as with halibut). King crab lengths are taken by measuring carapace length, and tanner crab lengths by measuring carapace width.

Age data

Pollock:

Because pollock is a dominant species in the central Gulf of Alaska, a random sample of otoliths from 30 pollock will be collected from stations where pollock are abundant.

Rock sole, flathead sole, rex sole:

For these less abundant species, the standard RACE stratified sampling scheme for age structures will be followed. For each survey leg, an otolith collection will consist of five individuals per sex per one cm length interval. The collections should be made from fish taken over a broad geographical area, so no more than two individual otoliths are taken per sex per length interval per tow. If a species is very prevalent then no more than one individual otolith will be taken per sex per length interval per tow. Individual weights will be taken at the time otoliths are collected as with pollock.

Otolith samples will be excised from fish and stored using the same methods outlined in the appendices of the ADF&G component study. Age compositions of the sampled fish populations will be estimated using standard analytical methods developed by the Seattle, WA. NMFS Ageing Unit. Otolith collections made during the ADF&G and NMFS surveys will be submitted to the Ageing Unit by January 1990 to be assigned annual reading priority. Given the work load of the Unit, the age reading of the collections is expected to be completed late in 1990 if funding for the Project 24 survey components is continued past the first year.

Fish collection for hydrocarbon analysis:

Ten each of the following species will be collected and frozen: pollock, Pacific cod, sablefish, arrowtooth flounder, flathead sole, rex sole, Dover sole, rock sole, Pacific halibut and chinook salmon. Each specimen will be individually wrapped and labeled for future analysis.

At sea data entry

The catch and haul data from each tow will be recorded onto RACE data forms during the workup of each trawl catch. The data from these forms will be entered into the RACEBASE computer data system prior to the end of each day using two microcomputers on board. The data will be stored both in the standard RACE data books in hard copy and on computer hard disks and floppy disks. The data are to be coded and recorded according to the specifications contained in the 1989 RACE ADP Code Book (Attached).

Data Analysis

All analytical methods follow the general procedures for estimating abundance used during previous RACE Division bottom trawl surveys of the U.S. west coast, the Aleutian Islands, Bering Sea and the Gulf of Alaska. A description of these methods and the survey limitations which may bias the outcome of samples obtained by trawl sampling is discussed by Bakkala et. al., 1985.

Generally, the catch per unit of effort (CPUE) is calculated at each station by dividing the catch of each species (kg) by the area swept by the trawl (nm^2). Mean CPUE's for a stratum are calculated by dividing the sum of all standardized CPUE's (kg/nm^2) for a species by the total number of stations within the stratum. The overall mean CPUE for all subareas combined is calculated using area-weighted CPUE's. The standing stock (biomass) in each stratum is approximated by expanding the mean CPUE for each stratum by its respective area.

Length distributions within each stratum are estimated by weighting each length frequency sample by the size of its respective catch. The overall length distribution for a stratum or the total survey area is obtained by weighting the distribution from each stratum by the appropriate square mileage value. Once the age collections have been analyzed, age specific biomass estimates will be calculated. The completed age data will not however be available until sometime in 1990.

Schedule/Personnel

Schedule:

Date(s)		Activity
Jul-Sept	1989	Project planning and charter vessel procurement
Sept 1- 27	1989	Trawl survey, leg 1
Sept 28- Oct 26	1989	Trawl survey, leg 2
Sept 28- Nov 15	1989	Data entry and verification
Nov - Dec	1989	Data processing and analysis
Dec	1989	Preparation of status report n
population abundances		
Jan	1990	Final report on results of 1989 survey

Personnel:

Name	Role	Mo
Gary Stauffer	Project Leader	1.2
Eric Brown	Chief Scientist	4.8
Peter Munro	Statistician/Field Party Chief	4.8
Ron Payne	Sampling Logistics	1.2
Lynn Faughnam	Sampling	2.4
William Flerx	Sampling	2.4
Harold Zenger	Sampling	2.4
Virginia Molenaar	Sampling/Data Base Mgr.	2.4
Sherrie Wennberg	Data Editing	1.2
David King	Logistics	1.2
Rod Gonzalez	Marine Mammal Observer**	

* Temporary Personnel funded by project 24 budget
** Part of marine mammal study 1 and 2

Budget

Object Class	Catagory	Budget
11	Labor	5,000
	Overtime	25,000
12	Benefits	800
21	Travel	7,000
22	Transportation	100
23	Rent	275,000
26	Supplies	80,000
31	Equipment	4,100
	Total	397,000

B. EXPOSURE TO OIL AND ITS EFFECTS

Assessment of Oil Spill Impacts on Fishery Resources: Measurement of hydrocarbons and their metabolites, and their effects, in important species.

Introduction

For the initial phase of the study, this project component will focus on important species in oil-impacted nearshore areas of the Gulf of Alaska. The fish and shellfish species collected off shore will be analyzed for oil exposure after the data on abundance and distribution (Component A) are evaluated. The nearshore species to be studied include juvenile salmonids and Dolly Varden which inhabit many of the intertidal areas, adult salmon which pass through intertidal and subtidal waters, and groundfish and shellfish species which live in subtidal areas in close association with bottom sediments. A comprehensive damage assessment program is needed to document the uptake of petroleum hydrocarbons by fish shellfish species from a range of habitats. For example, fish can take up petroleum hydrocarbons (aromatic and aliphatic) from water, food or sediment. Then, the aromatic hydrocarbons (AHs) are metabolized by the liver into derivatives that can cause a variety of adverse effects. However, because of metabolism, direct measurement of tissue concentrations of parent AHs generally does not provide a useful indicator of exposure of fish to petroleum AHs from the environment (Varanasi et al. 1989a). To estimate the exposure of fish to petroleum AHs, metabolites of these compounds can be measured in the bile (Krahn et al. 1984, 1986a, b, c). The usefulness of this method in measuring exposure of fish to spilled oil has been demonstrated by a study done during the 1984 Columbia River oil spill (Krahn et al. 1986a).

A comprehensive damage assessment program also needs to estimate any adverse effects associated with oil exposure. Changes at the tissular, cellular and subcellular levels in response to crude oil exposures are often observed (National Academy of Sciences 1985). Examples of changes after exposure of fish to oil-contaminated sediments include increases in mixed-function oxygenase enzyme activities, liver hypertrophy and fatty liver in winter flounder (<u>Pseudopleuronectes americanus</u>) (Payne et al. 1988) and the occurrence of hepatocellular lipid vacuolization in English sole (<u>Parophrys vetulus</u>) (McCain et al. 1978). Certain AHs (e.g., benzo[a]pyrene) are known carcinogens in rodents (Lutz 1979). Moreover, studies with several groundfish species in urban estuaries show that, of the xenobiotic chemicals in sediments, AHs are most strongly associated with high prevalences of liver lesions, including neoplasms (Malins et al. 1984, Myers et al. 1987, Black et al. 1983, Varanasi et al. 1987).

AHs, especially those with 4-5 benzene rings, generally exhibit

their toxicity after metabolism. Liver enzymes of fish including aryl hydrocarbon hydroxylase, (AHH) convert certain AHs into reactive intermediates (such as epoxides and diol epoxides) that bind to DNA and proteins. Additionally, modification of DNA by chemicals may result in a host of toxic effects in exposed organisms. A sensitive assay of such DNA damage has been developed recently and is known as ^{32}P-postlabeling (PPL) assay (Reddy et al. 1984). The PPL method is currently the most sensitive technique available for directly detecting genotoxicity of environmental contaminants and has been shown to be applicable to fish species (Varanasi et al. 1989b, c). In addition to its high sensitivity, a major advantage of this method is that it directly measures metabolites of xenobiotic compounds, such as AHs, covalently bound to DNA in target tissues (e.g., liver, gonads).

In the initial phase of this study, we intend to sample in areas affected by the oil spill (see site maps) to include species listed in Table 1 and to measure conversion products of petroleum hydrocarbons (e.g. metabolites, DNA adducts) in selected species. Both nearshore and offshore species will be collected; but in the initial phase, emphasis will be placed on the nearshore species because of the greater potential that these biota were affected by the oil spill. The large number of sites selected and diverse species sampled make this damage assessment project particularly comprehensive. Additionally, during this first year, we plan to measure biochemical effects such as changes in liver enzyme activities and DNA damage to provide information on early signs of hydrocarbon-induced stress in a variety of species. Only by employing a number of state-of-the art chemical and biochemical methods will analytical data be obtained to document the degree of exposure and extent of effects of petroleum hydrocarbons on economically and ecologically important fish species.

Based on the information obtained in the initial phase, in the future we plan to measure the concentrations of petroleum hydrocarbons in sediments and in certain tissues (e.g., stomach contents, liver, muscle) of biota from the spill area. In addition, frequency of liver lesions (Myers et al. 1987) and several indicators of reproductive impairment, such as inhibited ovarian maturation (Johnson et al. 1988) and failure to spawn (Casillas et al. 1989), will be measured. We will then incorporate all this information for important Alaskan fish species from Components A and B into simulation models for use in estimating oil spill impacts on fishery resources.

Objectives

(Initial phase: March 1989-February 1990. Letters below refer to the General Objectives described in the Overview Section.)

C1. To estimate the biochemical effects (e.g., induction of hepatic AHH activity or increased binding of petroleum

hydrocarbon metabolites to hepatic DNA of petroleum hydrocarbons in a variety of species from oiled and nonoiled habitats such to detect a statistical difference in levels of effects with a = 0.05 and b = 0.10.

D1. To sample selected economically and ecologically important fish species at a large number of sites in the Gulf of Alaska, including several in Prince William Sound, to obtain samples for a comprehensive chemical and biochemical evaluation of the effects of exposure of fish to petroleum hydrocarbons.

D2. To determine the level of metabolites of petroleum hydrocarbons in a variety of species from oiled and nonoiled habitats such to detect a significant difference in bile concentrations with a = 0.05 and b = 0.10.

Methods/Data Analysis

Study Design

Samples of biota collected from a large number of selected sites will make this damage assessment program particularly comprehensive. Sites will be located in potentially oil-impacted areas and unimpacted sites in the the Gulf of Alaska (lower Cook Inlet and along the Kenai Peninsula, Alaska Peninsula, and Kodiak Island) and in Prince William Sound. Juvenile and adult salmon, and Dolly Varden will be sampled in intertidal areas, whereas Pacific halibut, Pacific cod, pollock, yellowfin sole, rock sole, flathead sole, and Tanner crab will be sampled in subtidal areas. Salmon and halibut were selected primarily because of their economic importance, and the other fish species were selected because of their wide geographical distribution and year-round residency in the sampling areas. The crab species will also be sampled as an example of economically important shellfish. Surface sediment samples for establishing levels of petroleum hydrocarbon residues will be collected at all sites.

Initially, petroleum exposure by fish will primarily be assessed by measuring concentrations of metabolites of petroleum aromatic compounds in bile and activities of liver enzyme (AHH). These types of measurements are necessary because petroleum hydrocarbons in fish are rapidly metabolized to compounds that are not detectable by routine chemical analyses. AHH activity in fish is generally due to a single cytochrome P-450, apparently cytochrome P-450IA1 (Varanasi et al. 1986, Buhler and Williams 1989). Measurement of hepatic AHH activity will provide a very sensitive indicator of contaminant exposure of sampled animals (Collier and Varanasi, 1987). Moreover, the induction of AHH activity indicates not only that contaminant exposure has occurred, but also that

biological changes have occurred as a result of the exposure. In addition to measuring AHH activity, cytochrome P-450IA1 will be directly quantitated in liver samples by an immunochemical method recently developed at the University of Bergen (Collier et al., 1989). Direct quantitation of the P-450IA1 has the advantage that this method can be used on archived samples and samples frozen at non-cryogenic temperatures (> -80° C), thus allowing for future comparisons to be made between data collected in this Damage Assessment Program and data from other sample collection programs, if samples from the other programs are subjected to the same immunochemical quantitation techniques.

Genetic damage will also be measured in selected liver samples by estimating levels of petroleum hydrocarbon metabolites bound to DNA using ^{32}P-postlabeling (PPL) analysis of DNA adducts. The PPL method is currently the most sensitive technique available for directly detecting genotoxicity of environmental contaminants (Varanasi et al. 1989b, c). In addition to its high sensitivity, a major advantage of this method is that it directly measures metabolites of xenobiotic compounds, such as AHs, covalently bound to DNA. Moreover, it can be applied to archived samples, with the same potential benefits as discussed above for the immunochemical quantitation of cytochrome P-450IA1.

Collection areas:

Sampling activities for Component B will be conducted at approximately 60 sites along the path of the oil spill (see the site maps, Appendix L, Figures 1-4). Among the sites in Prince William Sound are nonoiled sites in Port Valdez and Port Gravina and petroleum-exposed sites off Knight Island, Evans Island, and Naked Island (see site map of Prince William Sound). Sites outside Prince William Sound include: Resurrection Bay; Gore Point; Kachemak Bay; Kukak Bay; Kamishak Bay; Shuyak Island; Chignik Bay; and Balboa Bay (see the site maps of Lower Cook Inlet and the Gulf of Alaska adjacent to the Kenai and Alaska Peninsulas).

<u>Sample Collection</u>

Sample collection for Component B will be performed from the NOAA Ship FAIRWEATHER at water depths of approximately 10 to 320 meters. The coordinates and depths of each site will be recorded. At each site, sediment samples will be collected with a box corer, Van Veen or Smith-McIntyre grab. Sediments will be stored at - 20°C.

Fish will be collected with a bottom trawl, long-line gear, gill nets, or beach seines. Bottom trawls will be performed with an otter trawl (7.5 m opening, 10.8 m total length, 3.8 cm-mesh in the body of the net, and 0.64 cm-mesh in the liner of the cod end). Tows will be of 5 to 15 minutes duration. In order to reduce contamination of the catch by free oil, trawling will avoid areas of surface films or slicks. If a net is fouled by subsurface or

bottom oil, it will be replaced (or cleaned, if possible) and a new area for trawling will be selected. Other fish sampling gear appropriate to the species and conditions will also be deployed.

Individuals of selected target fish species will be sorted and examined for externally visible lesions; up to 30 fish of selected species will be measured, weighed, and necropsied; and selected tissue samples (including liver, stomach contents, muscle) will be excised and frozen at -20°C for future hydrocarbon analyses. Samples of liver will also be preserved in Dietrich's fixative (Gray 1954) for histopathological examination. Bile samples will be collected and stored at -20°C. Blood samples from selected sexually mature fish will be collected, centrifuged and the plasma stored at -20°C for future measurement of estradiol and vitellogenin. Ovaries will be preserved in Davidsons fixative for future histological examination (Johnson et al. 1988). Portions of the liver to be used for AHH and PPL analyses will be preserved in liquid nitrogen on board the ship and then returned to the laboratory and frozen at -80°C. Appendix M, Table 1 contains a summary of the fish species, capture methods, and types of analyses to be conducted.

Laboratory Analyses

Bile Metabolite Assay:

Samples of bile will be injected directly into a liquid chromatograph and a gradient elution conducted using a Perkin-Elmer HC-ODS column with a gradient of 100% water (containing 5µL acetic acid/L) to 100% methanol (Krahn et al. 1984, 1986a, b, c). Two fluorescence detectors are used in series. The excitation/emission wavelengths of one detector are set to 290/335 nm, where metabolites of naphthalene (NPH) fluoresce. Excitation/emission wavelengths of the other detector are set to 260/380 nm, where metabolites of phenanthrene (PHN) fluoresce. The total integrated area for each detector is then converted (normalized) to units of either NPH or PHN that would be necessary to give that integrated area.

Liver Aryl Hydrocarbon Hydroxylase (AHH) Analysis:

Hepatic microsomes are prepared essentially as described by Collier et al. (1986). AHH activity is assayed by a modification of the method of Van Cantfort et al. (1977) as described by Collier et al. (1986), using ^{14}C-labeled benzo[a]pyrene as the primary substrate. All enzyme assays will be run under conditions in which the reaction rates are in the linear range for both time and protein.

DNA Damage:

The ^{32}P-postlabeling assay will be conducted using a procedure

described by Varanasi et al. (1989b, c). Briefly, the procedure involves digesting the DNA from liver tissue into nucleotides, labeling the nucleotides with ^{32}P, and separating the labeled nucleotides with thin-layer chromatography.

Quality Assurance and Control Plans

Bile Analytes:

Quality assurance procedures for bile analyses will include NPH and PHN calibration standards; and the calibration standard will be analyzed after every 6 samples and the RSD will be reported. In addition, one blank sample and two "bile pool" reference material (control material) is analyzed daily. The concentrations of analytes should be within ±2 s.d. of the established concentrations in control material(s). Replicate analyses will be performed on approximately 10% of the samples.

AHH Activity:

Quality assurance procedures for AHH measurements include duplicate zero-time and boiled enzyme blanks for each set of assays. Each sample will be run in duplicate and those samples showing > 10% difference between duplicates will be repeated.

DNA Damage:

Procedures for the ^{32}P-postlabeling assay involves (1) hydrolysis of isolated DNA to normal and adducted deoxyribonucleotide monophosphates, (2) butanol extraction of the DNA hydrosylate to concentrate the adducts, (3) labeling of the adducts using ^{32}P-phosphate, (4) thin-layer chromatographic separation of the normal nucleotides from the adducts, separation of individual DNA adducts from one another, and separation of individual DNA bases from one another, and (5) determining the level of adducts present in the isolated DNA.

Quality assurance procedures for the ^{32}P-postlabeling assay include (1) the use of salmon testes DNA for measuring the efficiency of DNA hydrolysis, and as a sample blank and reference standard, (2) the use of 7R,8S,9S,10R-(N^2-deoxyguanosyl-3'-phosphate)-7,8,9,10-tetrahydrobenzo(a)pyrene (BaP-dG) as an internal standard to measure both the efficiency of the adduct enrichment and the efficiency of the enzyme-mediated transfer of the ^{32}P-phosphate from ATP to the adducts, (3) the use of the ^{32}P-labeled BaP-dG monophosphate standard will be used as a chromatography calibration standard in a separate analysis, rather than an internal standard, because of the interference of the standard with the measurement of the unknown adducts, and (4) the use of 2'-deoxyguanosine-3'-monophosphate standard to measure the efficiency of the enzyme-dependent labeling of the normal nucleotides by ^{32}P-phosphate from ^{32}P-ATP.

Data Analysis

Statistical Tests:

The relative concentrations of contaminants (metabolites) in fish bile at the study sites will be compared statistically using GT2 comparison intervals (Gabriel, 1978, Sokal and Rohlf, 1981). Where significant differences among concentrations are found, the a-value will be understood to be < 0.05. To determine whether the prevalence of each type of biological effect (AHH or DNA damage) measured in each of the fish species is statistically uniform among the sites, the G test for heterogeneity (Sokal and Rohlf, 1981) will be performed.

Analytical Methods:

Where possible, non-parametric statistical tests will be employed to avoid assumptions that the data are normally distributed. Non-parametric tests give highly reliable results. The principal non-parametric tests that will be used are Spearman rank correlation, which has about 0.91% of the power of product-moment correlation when the parametric assumptions are met (Zar, 1984), and the heterogeneity-G statistic. Spearman rank correlation will be used for estimating uptake of petroleum hydrocarbons from oiled and non-oiled habitats when an independent measure of contamination (e.g., sediment PAH level) is available. In addition, logistic regression (appropriate where the outcome variable is binomial) will be used to model the prevalences of pathological conditions in relation to contamination.

Cohen (1977) will be used for computations of statistical power.

Products:

Reports will contain information on the concentrations of metabolites of petroleum hydrocarbons in bile, the activity of AHH in liver and the results of DNA damage in selected species. Data will be submitted in the form of tables or distribution maps, and all data will be stored in computerized data management programs. The data management formats were designed in cooperation with the National Oceanographic Data Center (NODC), and are compatible with the NODC data storage systems.

Schedule/Personnel

<u>Data Submission Schedule</u> (see Milestone Chart, Appendix N)

<u>Sample and Data Archival</u>

Samples for chemical analyses will be stored frozen at - 80° C for at least three years, or until disposal is authorized by the Trustees.

<u>Personnel:</u>

Name	Role	Task	Mo	Total Cost (K)
Varanasi, Dr. Usha	Principal Invest.(PI)	(1) Project	2.0	4.9
Chan, Dr. Sin-Lam	Co-PI	Management.	2.0	14.7
Stein, Dr. John E.	Co-PI		2.5	13.2
Landahl, Dr. John T.	Co-PI		2.0	7.5
MacLeod, Jr., Dr. William D.	Supervis. Res. Chemist		0.5	3.4
Townsend, Ms. Dorothy	Admin. Officer		1.5	4.1
Perry, Ms. Shirley	Secretary		1.5	3.4
Tajon, Ms. Ruth	Admin. Supp. Clerk		1.5	2.7
Fuller, Mr. Steven	Secretary		1.5	2.4
Clark, Jr., Dr. Robert C.	Co-PI	(2) Site	4.0	23.5
Krahn, Dr. Margaret M.	Co-PI	Sampling*	4.0	21.7
Gronlund, Mr. William D.	Fishery Biologist		4.0	16.2
Myers, Mr. Mark S.	Supervis. Fish. Biol.		1.5	6.3
Weber, Mr. Doug	Supervis. Fish. Biol.		2.0	12.2
Plesha, Mr. Paul	Fishery Biologist		4.0	14.4
Hagen, Ms. Jennifer	L.Biotechnician		5.0	8.9
Haley, Mr. Craig	Fishery Biologist		2.0	3.6
Hanson, Ms. Karen	Pathologist		3.0	5.4
Eberhart, Ms. Bich-Thuy	Biochemist		2.0	6.0
Johnson, Ms. Lyndal	Histopathologist		2.0	5.4
Olson, Mr. O. Paul	Histopathologist		3.0	9.8
Willis, Ms. Maryjean	Biotechnician		2.5	4.5
Stehr, Ms. Carla	Histopathologist		3.0	11.2
Carrasco, Mr. Ken	Histopathologist		3.0	6.6
Ylitalo, Ms. Gina	Chemist		4.0	8.9
Collier, Dr. Tracy K.	Co-PI	(3) AHH/PPL	3.5	15.5
Reichert, Dr. William	Biochemist	Analyses	3.5	16.9
Nishimoto, Dr. Marc	Biochemist		3.5	11.6
Hom, Mr. Tom	Biochemist		2.5	8.0
Eberhart, Ms. Bich-Thuy	Biochemist		6.0	18.2
Blood, Ms. Ethel	Biotechnician		5.5	17.7

*includes overtime

[a]Personnel and other costs for hydrocarbon analyses (bile metabolites for Phas 1) will be given separately to Technical Services Study 1 Hydrocarbon Analyses

Budget

Line Item	Category	Cost
100	Salaries	318.8
200	Travel	24.7
300	Contractural services	13.4
400	Supplies and materials	56.3
500	Equipment	39.2
TOTAL		452.4

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IV. APPENDICES

APPENDIX A

Appendix A

Table 1. Square nautical miles by geographic Area and Depth Strata Lower Cook Inlet OSIA Trawl Survey, September, 1989

Depth strata	Inner Kachemak	Outer Kachemak	North Central	South Central	North Kamishak	South Kamishak	Southwest Kamishak	Total
	Area							
Shallow 10-50 fm	35.742	194.040	551.703	0.000	453.917	219.456	219.934	1674.792
Deep 51-100 fm	0.000	17.340	0.000	356.465	0.000	364.272	0.000	738.077
Total	35.742	211.380	551.703	356.465	453.917	583.728	219.934	2412.869

Table 2. Proportion of Lower Cook Inlet Survey Area by geographic Area and Depth Strata, Lower Cook Inlet OSIA Trawl Survey, September, 1989

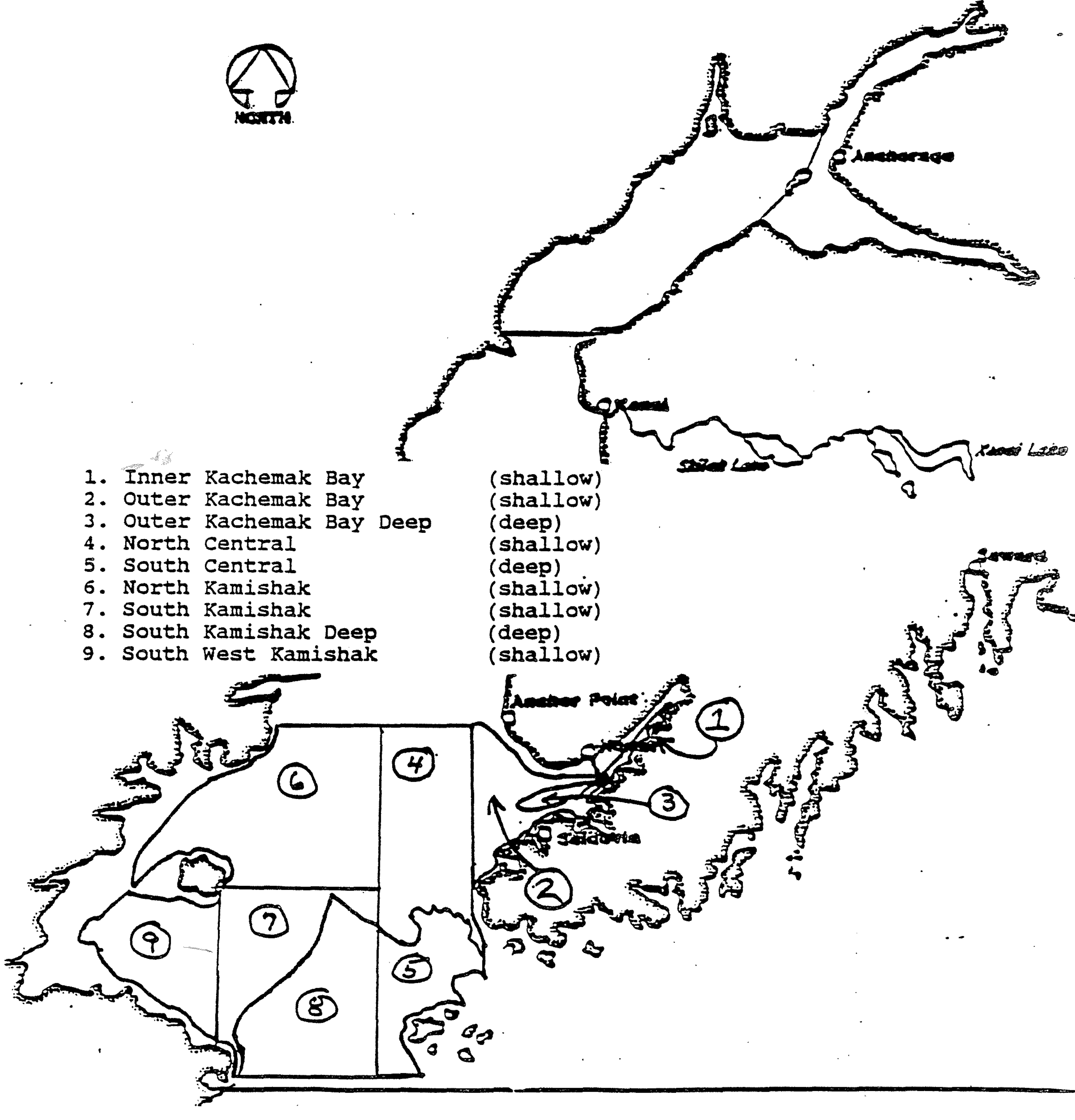
Depth strata	Inner Kachemak	Outer Kachemak	North Central	South Central	North Kamishak	South Kamishak	Southwest Kamishak	Total
	Area							
Shallow 10-50 fm	1.48%	8.04%	22.87%	0.00%	18.81%	9.10%	9.12%	69.41%
Deep 51-100 fm	0.00%	0.72%	0.00%	14.77%	0.00%	15.10%	0.00%	30.59%
Total	1.48%	8.76%	22.87%	14.77%	18.81%	24.19%	9.12%	100.00%

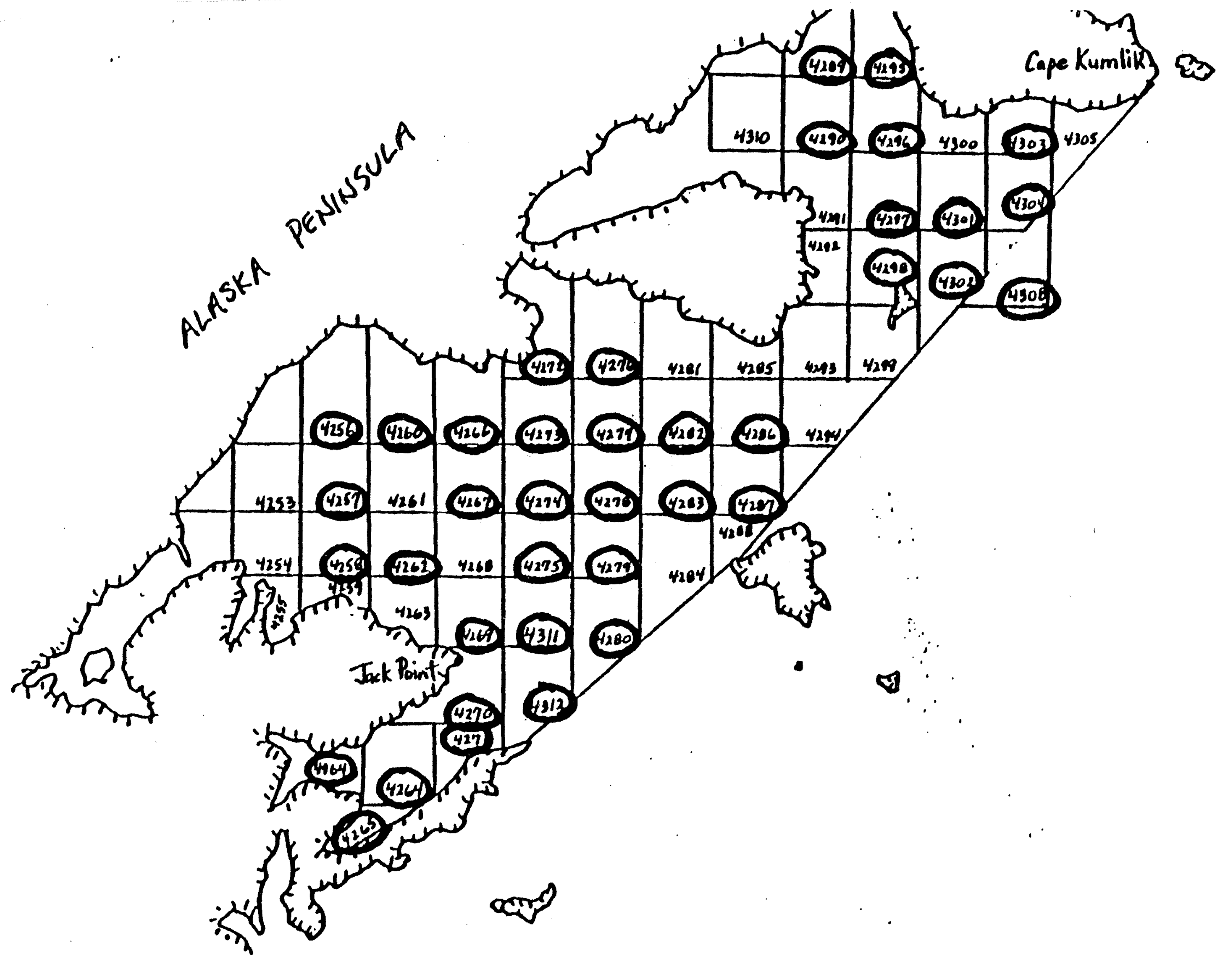
Table 3. Sample size in number of tows by geographic Area and depth Strata, Lower Cook Inlet OSIA Trawl Survey, September, 1989 Total number of days towed equals 14.

Depth strata	Inner Kachemak	Outer Kachemak	North Central	South Central	North Kamishak	South Kamishak	Southwest Kamishak	Total
	Area							
Shallow 10-50 fm	3	4	7	0	6	4	4	29
Deep 51-100 fm	0	3	0	5	0	6	0	13
Total	3	7	7	5	6	10	4	42

Appendix A, Figure 1

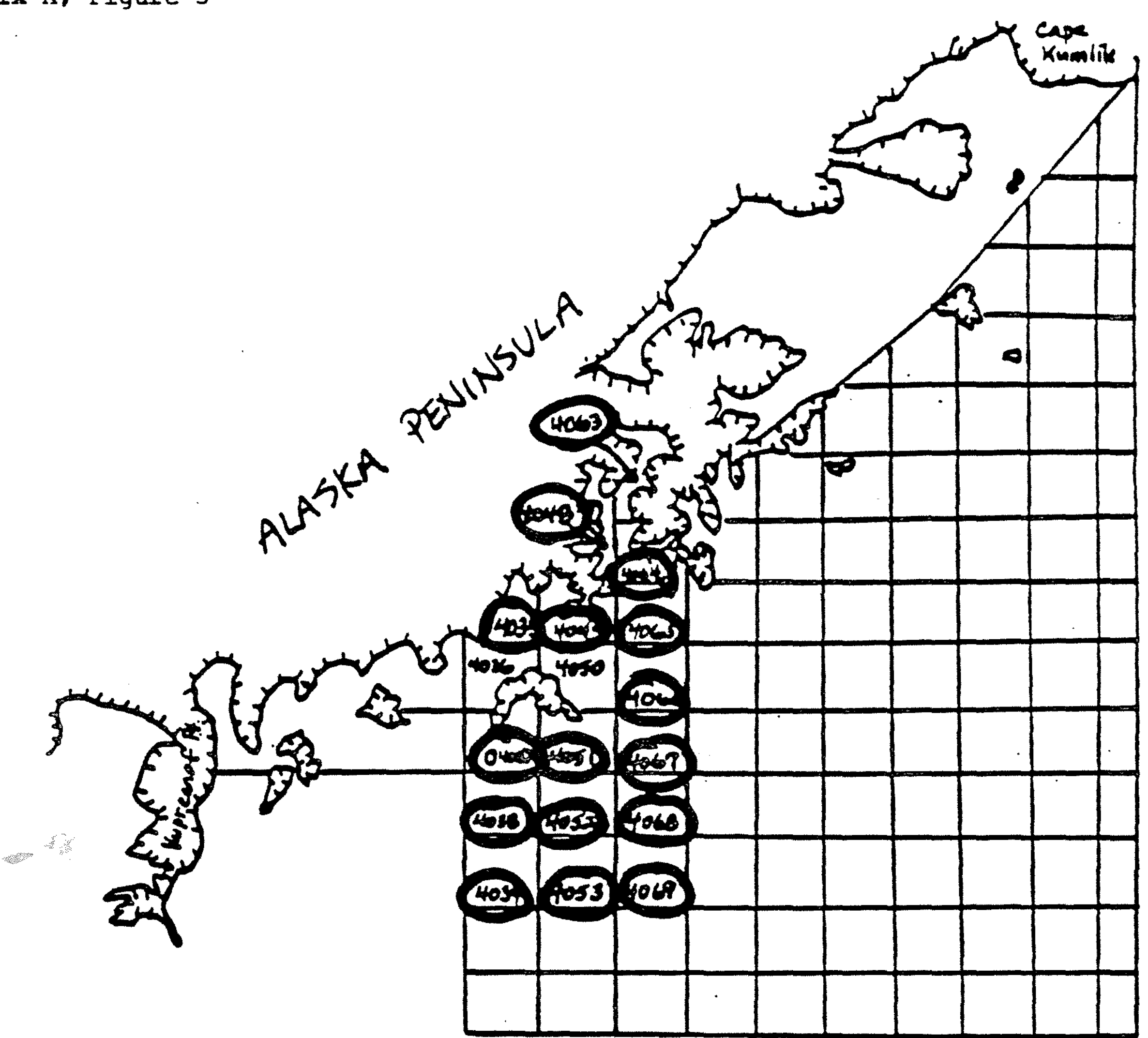
LOWER COOK INLET



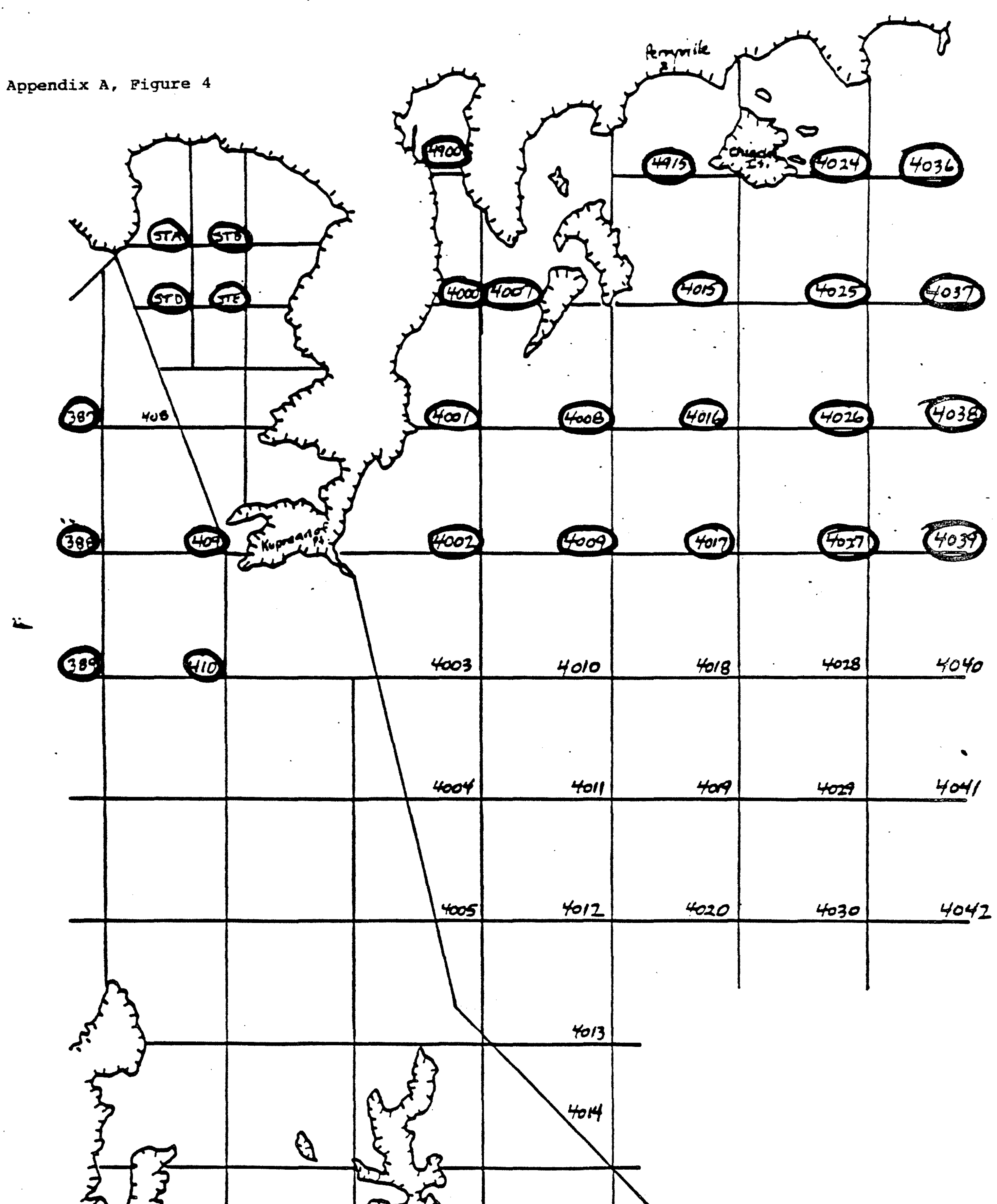


Appendix A, Figure 2

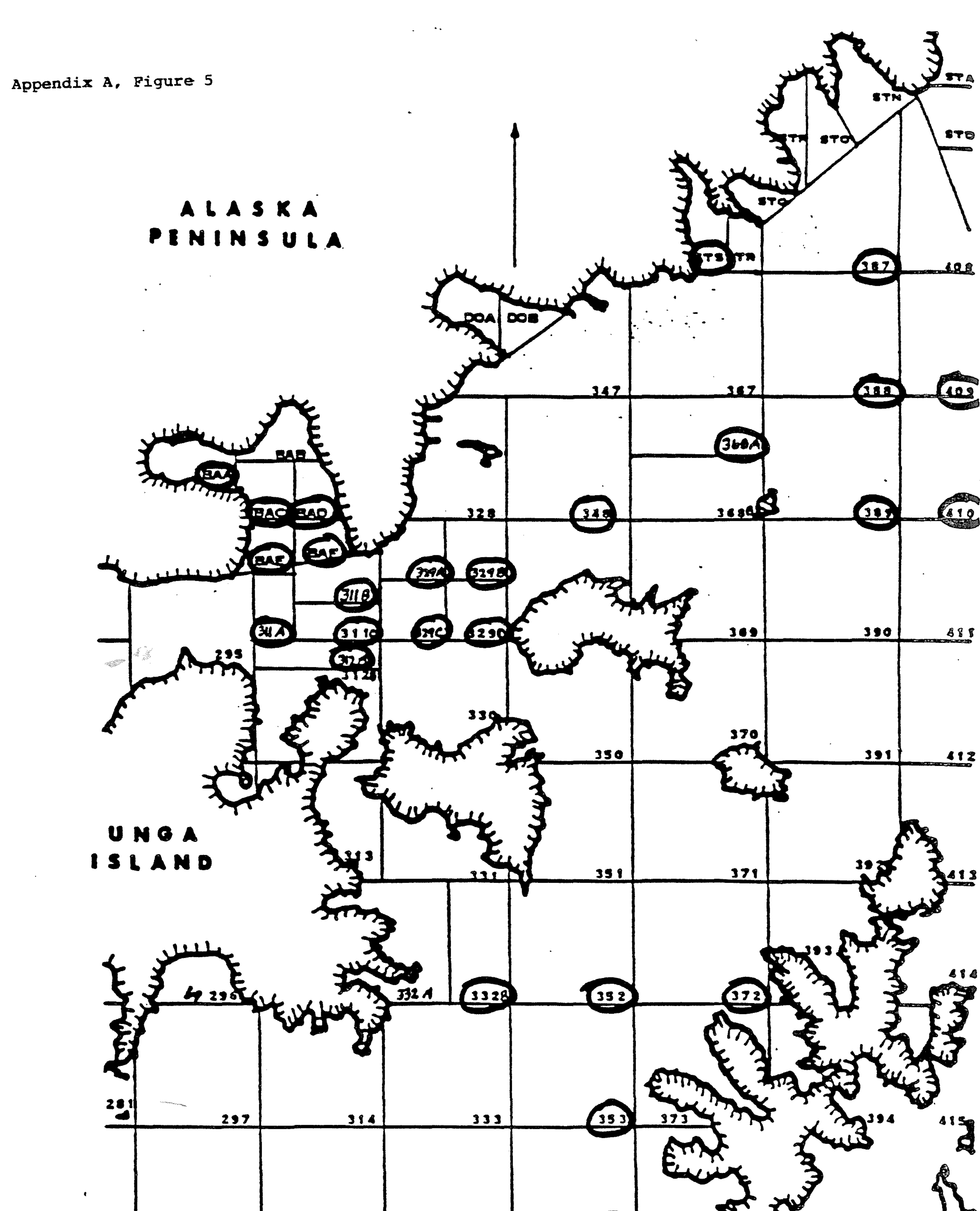
Appendix A, Figure 3



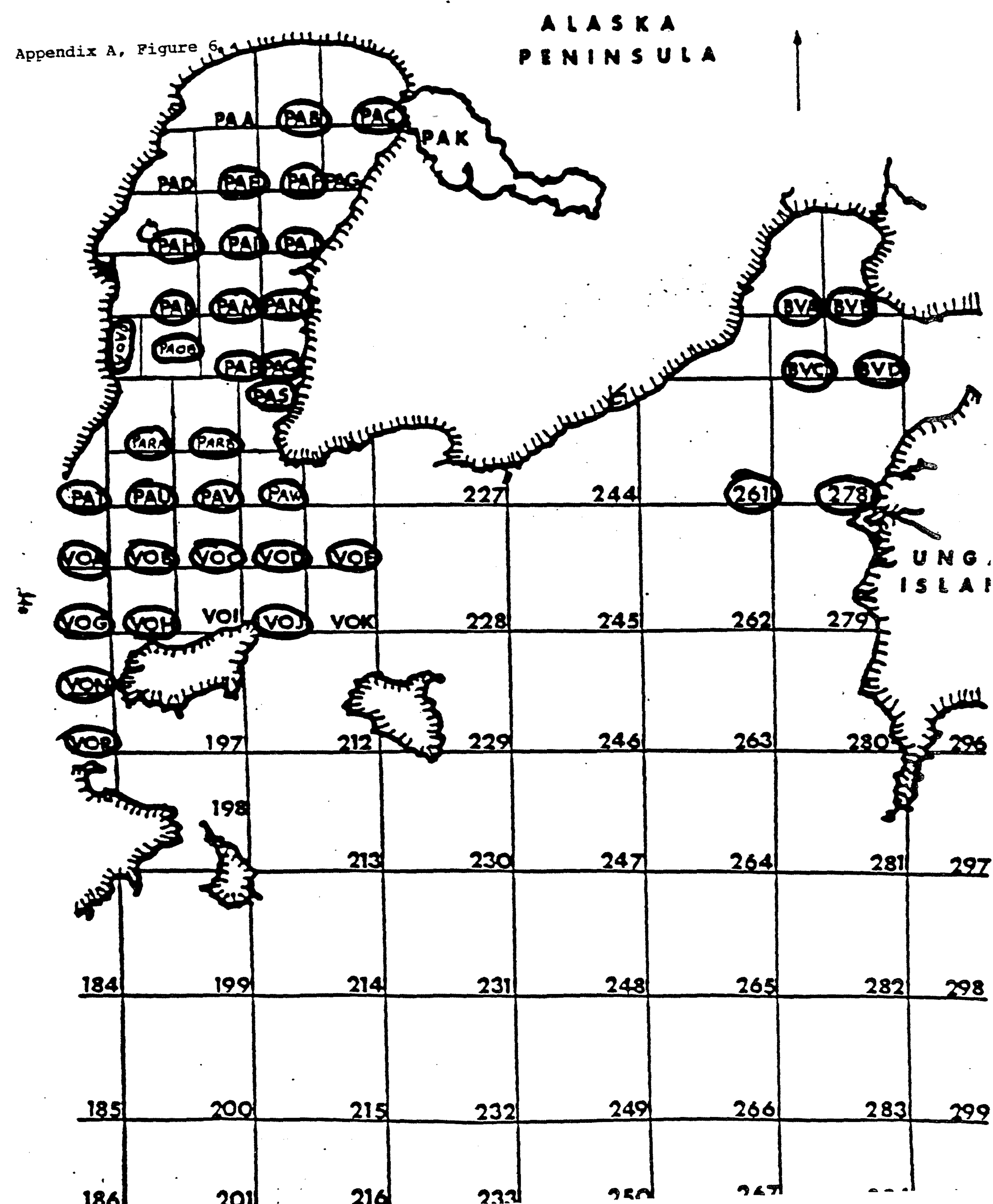
Appendix A, Figure 4

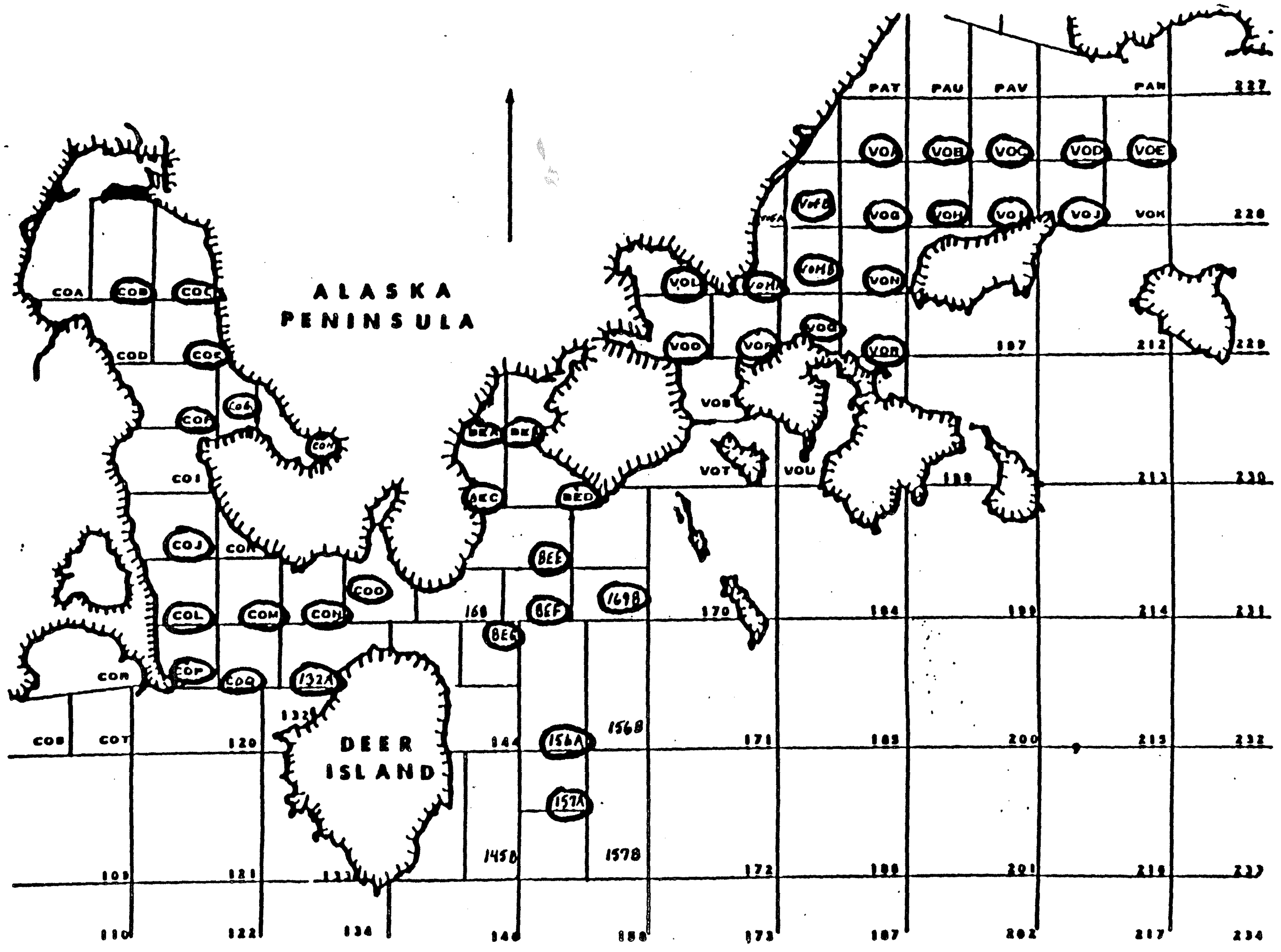


Appendix A, Figure 5

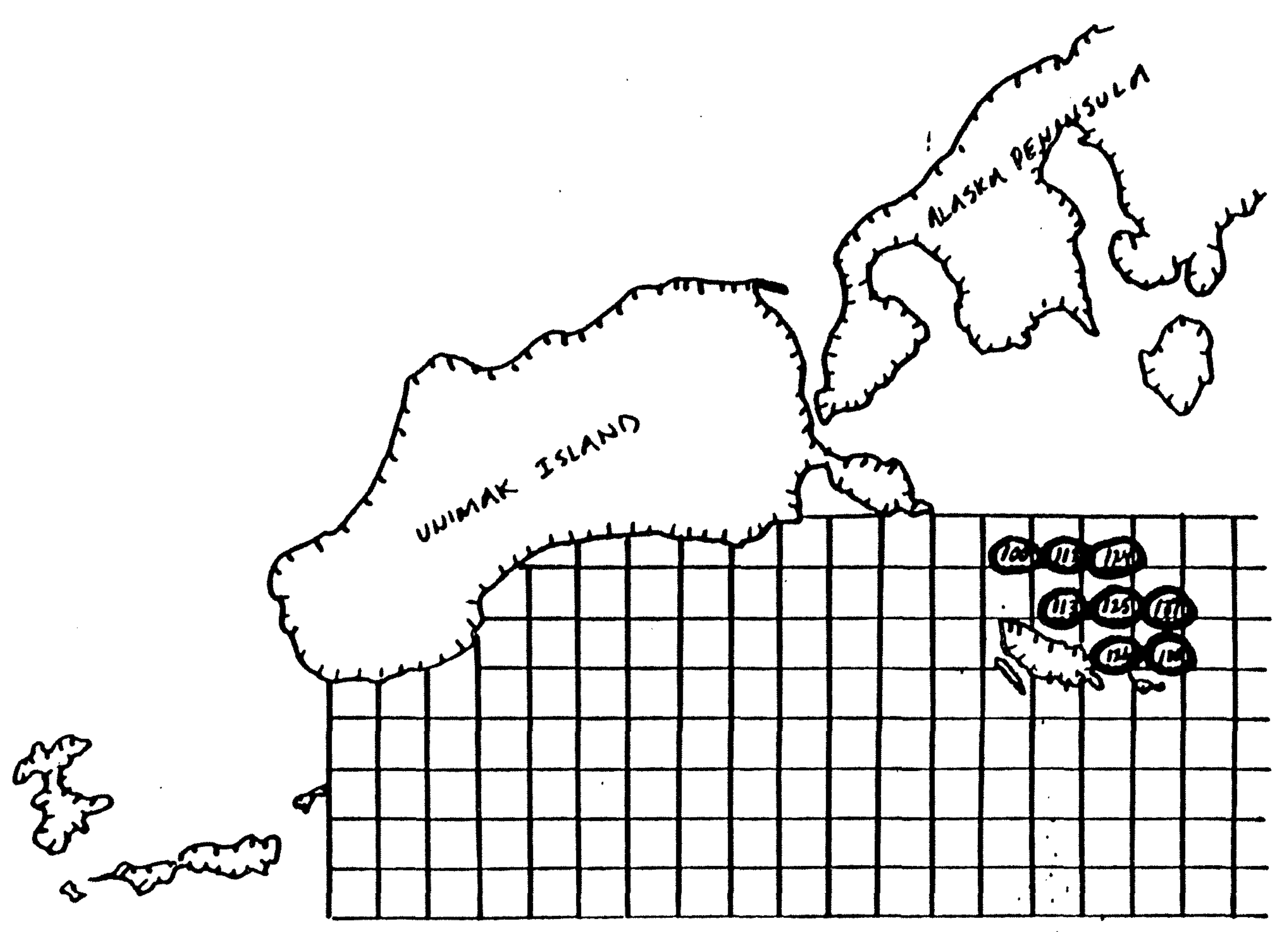


Appendix A, Figure 6

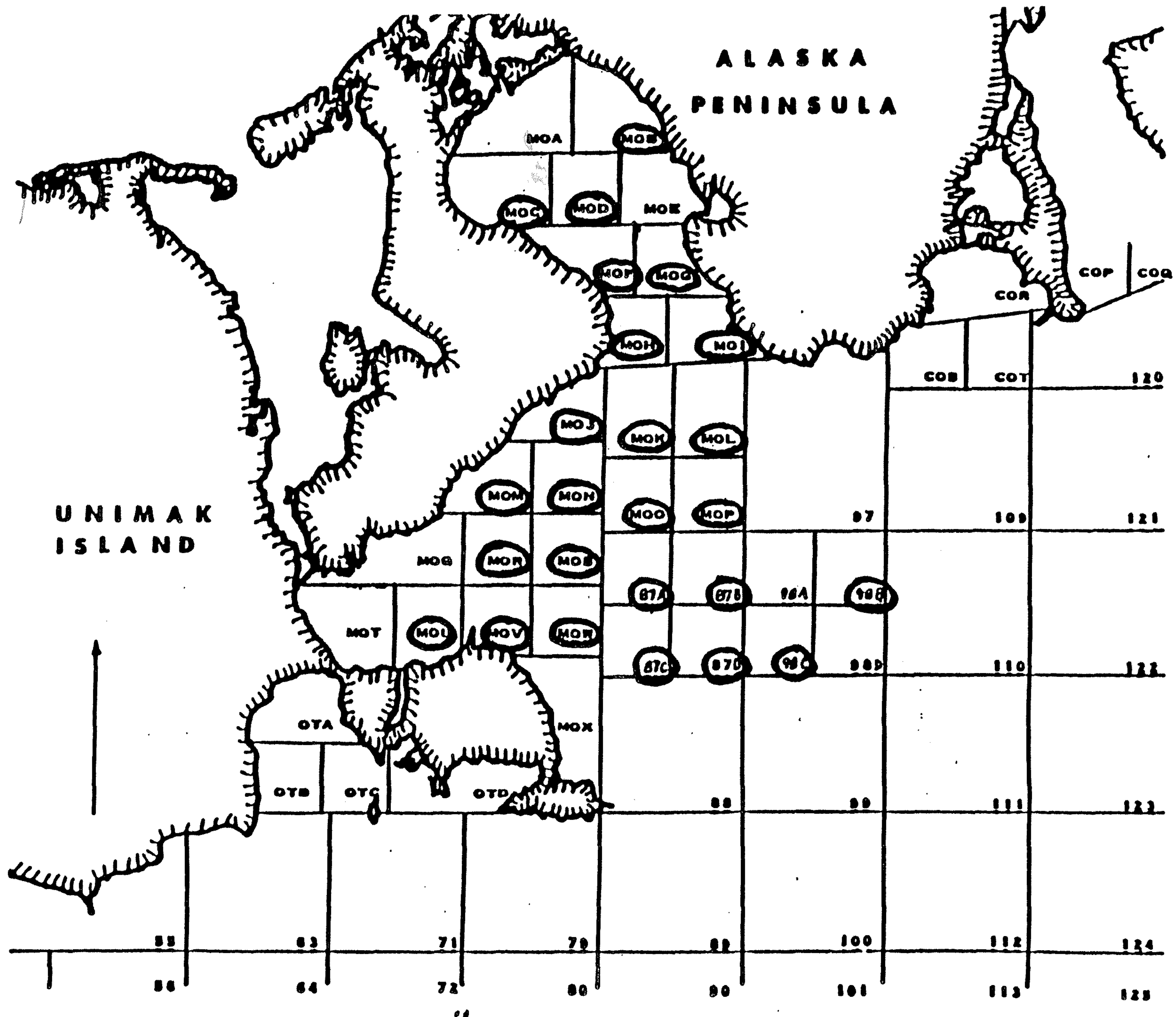




Appendix A, Figure 7



Appendix A, Figure 8



Appendix A, Figure 9

APPENDIX B

APPENDIX B. TRAWL GEAR SPECIFICATIONS

NET: 400 MESH EASTERN OTTER TRAWL

Footline:

1. 95' footline of 1/2" cable wrapped with 7/16" corsair line and 3/8" chain attached snug to footline every 10".

Headline:

1. 70' headline of 3/8" cable wrapped with 3/8" poly with 18 @ 8" aluminum floats with ears, attached about every 4 feet.

Web:

1. 42 thread 4" mesh in the wings and body.
2. 60 thread 3-1/2" mesh in the intermediate.
3. 18 thread 1-1/4" mesh cod end liner extending 3 feet beyond cod end.
4. 120 thread 3-1/2" mesh cod end
5. 3-1/2" 6.00 mm P.E. web chaffing gear around cod end.

Other:

1. Flymesh on back end of body of net, both ends of intermediate, and front end of cod end. This is for attachment of the body of the net to the intermediate with a zipperline and for attachment of the intermediate to the cod end with a similar zipperline arrangement.

2. The intermediate is straight 110 open meshes around and 120 meshes deep attached to the body of the net with a flymesh zipperline configuration. The other end of the intermediate with 1 flymesh every two meshes, attached to cod end with a zipperline with the same flymesh configuration. The end of the cod end will have a 2" x 3/8" galvanized ring for every 4 meshes.

3. 3/4" poly-dac rib lines with eyes at intermediate and cod end seams.

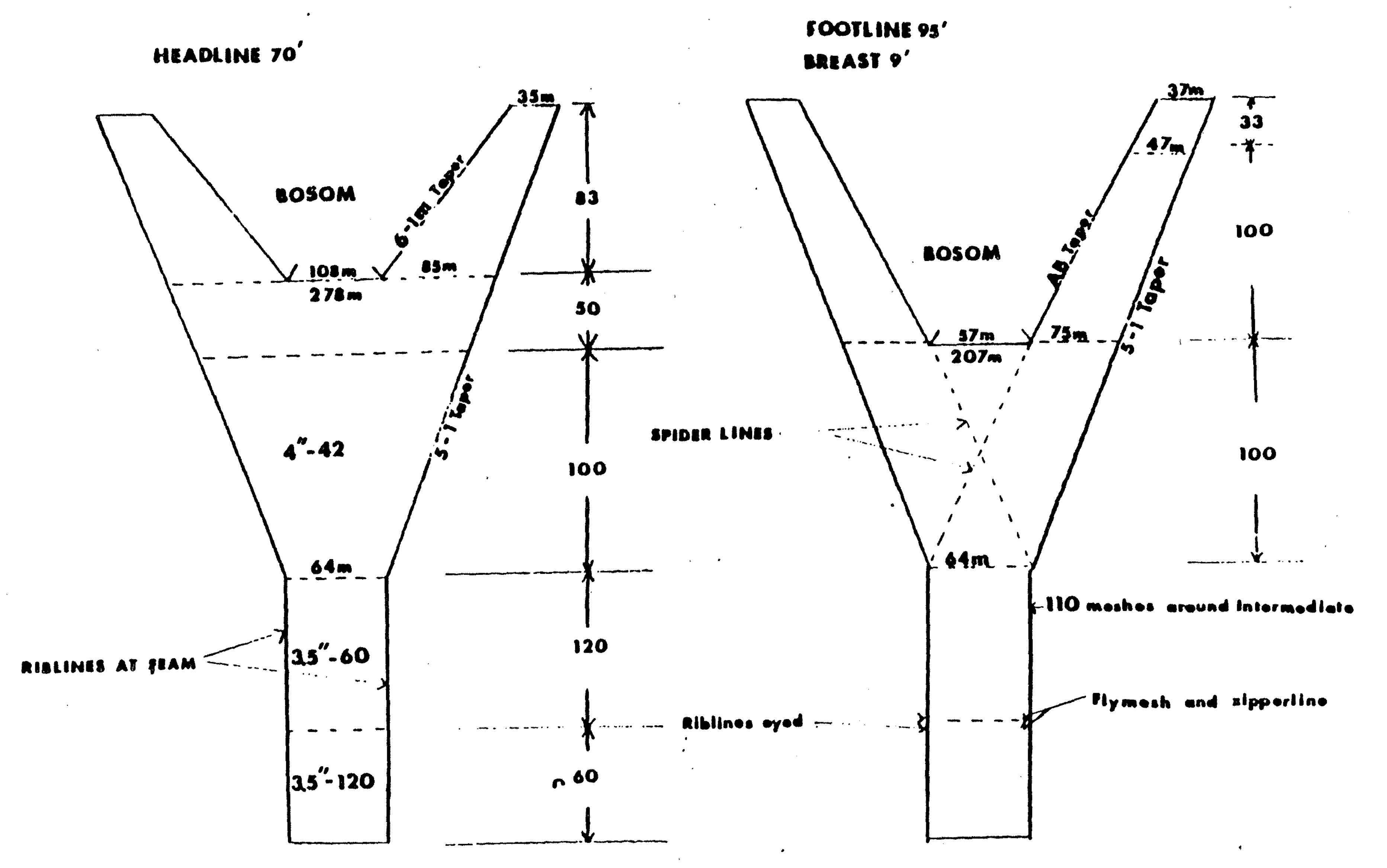
4. 1/2' poly-dac spider lines from corners of bosom across belly of net.

5. 5 @ 4" x 1/2" splitting strap rings sewn to cod end 21 meshes from end of cod end. These rings should be evenly spaced around the net.

6. A 1/2" poly bolsh line will be attached to the foot line and web should be drop hung from the bolsh line. The bolsh line should be attached to the footline every 8".

DOORS: NOR'EASTERN TRAWL SYSTEMS INC. NOR'EASTERN ASTORIA VEE DOOR

1. 5'x 7'; 800 lbs. each


HEADLINE 70'
35m
BOSOM
6-1m Taper
83
108m
85m
278m
50
5-1 Taper
4"-42
100
64m
120
RIBLINES AT SEAM
3.5"-60
3.5"-120
60
FOOTLINE 95'
BREAST 9'
37m
33
47m
100
BOSOM
AB Taper
5-1 Taper
57m
75m
207m
SPIDER LINES
100
64m
110 meshes around intermediate
Flymesh and zipperline
Riblines eyed

APPENDIX C

APPENDIX C **FIGURE 1.**

ALASKA DEPARTMENT OF FISH AND GAME
TRAWL SURVEY
SKIPPER TRAWL RECORD

Skipper's Name ______ Survey Area ______

CRUISE NUMBER	HAUL NUMBER	REGION	SURVEY AREA	STRATUM	STATION NUMBER	VESSEL CODE	DATE month	day	year
1	5	8	9	11	13	17	19	21	23

STARTING POSITION		Compass Heading (Magnetic)	TRAWL TIME START	END	Elapsed (min.)	Dist Towed (nm)
25 1 (1) LATITUDE: 26 degrees mins. secs	- LONGITUDE: 33 degrees mins. secs	40 0	43 :	:	47	49
25 2 (2) LORAN C: 26 position x	33 position y					

DEPTH (fathoms) Maximum	Minimum	Avg.	WEATHER Cloud	Sea	Swell	SCOPE (fathoms)	GEAR PERF.
51	54		57	58	59	60	63

Skipper's Comments (gear problems, snags, weather, tides, etc.):

57. CLOUD COVER	CODE	58. SEA STATE (feet)	CODE	59. SWELL (feet)	CODE
Clear	1	0 - 2	1	0 - 2	1
1/8 obscured	2	2 - 4	2	2 - 4	2
1/4 obscured	3	4 - 6	3	4 - 6	3
3/8 obscured	4	6 - 8	4	6 - 8	4
1/2 obscured	5	8 - 10	5	8 - 10	5
5/8 obscured	6	10 - 12	6	10 - 12	6
3/4 obscured	7	12 - 14	7	12 - 14	7
7/8 obscured	8	14 - 16	8	14 - 16	8
Completely overcast	9	Over 16	9	Over 16	9

63. GEAR PERFORMANCE

GEAR PERFORMANCE	CODE	GEAR PERFORMANCE	CODE
Gear performance satisfactory	01	Mudded down	26
Gear performance unsatisfactory	20	Telemetry malfunction	50
Doors nonfunctional (crossed, collapsed)	21		
Net nonfunctional (collapsed, torn, twisted, etc.)	22		
Hung up	23		
Trawl upside down	24		

APPENDIX C FIGURE

ALASKA DEPARTMENT OF FISH AND GAME

TRAWL SURVEY — STATION CATCH RECORD

p ___ of ___

Survey Area ____________ Name ____________ Number ____________ Station Number

Cruise No. [ ][ ][ ][ ]
Haul No. [ ][ ][ ]
Region/Survey Area [ ][ ][ ]

(1) Gross Catch Wt. - lbs. ____________

(2) Tare - lbs. ____________

(3) Net Catch Wt. - lbs. (1-2) ____________

(4) Fish, Rocks, etc. weighed directly ____________

(5) Wt. from which subsamble is taken-lbs. (3 or 3-4) ____________

(6) Subsample Wt. - lbs. ____________

(7) Raising Factor (5÷6) ____________

INCIDENTAL SPECIES	SPECIES CODE	(8.) Wt. in Subsamp. lb.	(9.) No. in Subsamp.	(10.) Weight in Total Catch lbs. (8 x 7)	Mean Weight lbs. (8 ÷ 9)
Cod	2 1 7 2 0				
Flathead Sole	1 0 1 3 0				
Pollock	2 1 7 4 0				
Arrowtooth flounder	1 0 1 1 0				
Halibut	1 0 1 2 0				
Total Incidental Species					
Debris (kelp, mud, sticks, rocks)					

Halibut lengths (mm) ____________

APPENDIX C **FIGURE 3.**

CRAB DATA FORM

SPECIES ____________
SEX ____________
VESSEL
DATE

STATION NUMBER
POT ORDER
BUOY NUMBER
TRAWL HAUL NUMBER
SAMPLING FACTOR

PAGE ___
OF ___

	SPECIES	SEX	CARAPACE LENGTH (MM)	LEGAL Y=YES	CARAPACE WIDTH (MM)	SHELL CONDITION	BLACK MAT Y = PRESENT	EGGS % CLUTCH FULLNESS	EGGS DEVELOPMENT	EGGS CLUTCH CONDITION	Juvenile J=yes	COMMENTS
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												

INSTRUCTIONS

SPECIES
1-L. aequispina
2-P. camtschatica
3-P. platypus
6-C. bairdi
7-C. opilio
9-C. magister

SEX
1-male
2-female

SHELL CONDITION
0-soft
1-new

EGG DEVELOPMENT
1-uneyed eggs
2-eyed eggs

CLUTCH CONDITION
1-dead eggs not apparent
2-dead eggs <20%
3-dead eggs >20%
4-barren with clean "silky" setae
5-barren with "matted" setae

APPENDIX C, FIGURE 4

SPECIMEN FORM

HAUL YR [| | | |]

PAGE ____

VESSEL (1 2 3) [| |] ______________ CRUISE (5 6 7) [| |] HAUL (9 10 11) [| |]

STRATUM (13 14 15) [| |] SPECIES CODE (17 18 19 20 21) [| | | |] SPECIES NAME ______________

FREQUENCY (36) [1] SUBSAMPLE TYPE (48) [ ] WEIGHT DETERMIN. (49) [ ] AGE STRUCTURE (50) [ ] AGE DETERMIN. (51) [ ]

MATURITY TABLE (59 60) [|] YOUR NAME ______________ DATE ______________

SEX (23)	MATUR (25)	LENGTH IN MM. (28 29 30 31)	WEIGHT (GRAMS) (37 38 39 40 41)	AGE (45 46)	SPECIMEN NUMBER (53 54 55 56 57)	(62 63 64 65 66)	(68 69 70 71 72)

Appendix C, Figure 5

MULTISPECIES TRAWL SURVEY
PWS OIL IMPACT ASSESSMENT PROJECT
TARBALL SAMPLING FORM

Vessel ______________ Cruise ______________
Date ______________ Tow Number ______________
Area ______________ Sampler ______________

SAMPLE COLLECTION - CHECK BOX IF OBSERVATION WAS MADE AND INDICATE # OBSERVED

Species		Tarballs # sampled/	# with tarballs		Stomach contents (optional) # crab/ species	other contents/ comments
Arrowtooth flounder	☐	____	____	☐	____	____
Dover Sole	☐	____	____	☐	____	____
Rex Sole	☐	____	____	☐	____	____
Pollock	☐	____	____	☐	____	____
(other) ____	☐	____	____	☐	____	____
____	☐	____	____	☐	____	____
____	☐	____	____	☐	____	____

Appendix C, Figure 6

MULTISPECIES TRAWL SURVEY
PWS OIL IMPACT ASSESSMENT PROJECT
HYDROCARBON AND NECROPSY SAMPLE FORM

Vessel ______________ Cruise ______________

Date ______________ Tow Number ______________ Gear ________

Latitude ______________ Longitude ______________ Agency ________

SAMPLE COLLECTION - CHECK BOX IF SAMPLE WAS COLLECTED AND INDICATE SAMPLE

Species/Tissue	Hydrocarbon Yes	Hydrocarbon Sample #			Necropsy Yes	Necropsy Sample #
Pollock/muscle	☐	______	______	______	☐	______
Pollock/viscera	☐	______	______	______		
Pollock/bile	☐	______	______	______		
S.Shrimp/muscle	☐	______	______	______	☐	______
P.Cod/muscle	☐	______	______	______		
P.Cod/viscera	☐	______	______	______		
P.Cod/bile	☐	______	______	______		
Flthed sole/mscle	☐	______	______	______		
Flthed sole/vscra	☐	______	______	______		
Flthed sole/bile	☐	______	______	______		
Tanner Crab/mscle	☐	______	______	______		
Tanner C/hepat.	☐	______	______	______		
Tanner Crab/egg	☐	______	______	______		
Sablefish/muscle	☐	______	______	______		
Sablefish/viscera	☐	______	______	______		
Sablefish/bile	☐	______	______	______		
(other) ______	☐	______	______	______		
______	☐	______	______	______		
______	☐	______	______	______		
______	☐	______	______	______		

APPENDIX D

14.10.1

APPENDIX D

Catch Sampling
and
Data Recording Manual

Gulf of Alaska Groundfish Subtask
RACE Division
Northwest and Alaska Fisheries Center

January 1982

PROCESSING THE CATCH

Initial Handling

Methods employed to handle trawl catches can vary, depending upon the size of the catch. The methods presented are tried and proved but are not the only methods. The primary concern is to obtain data on the total catch or a random sample of the total catch.

A. Catches of 8,000 pounds

1. If there is a load cell on board, the easy solution is to weigh all splits subtracting each time the weight of the trawl to obtain the total catch weight. Two or three splits, such as the first and last, or first, middle and last, are selected for biological sampling, depending upon the size of the catch and the size of the deck bin on the vessel being used. When the selected splits are in the deck bin, the sample will be further subsampled using the methods described in the section for catches from 2,000-8,000 pounds. In the Gulf of Alaska catches of this magnitude will usually consist of pollock or a mixture of pollock and other roundfish. When the catch is principally of one species with the same size composition throughout the cod end or a mixture of roundfish, the above selection of splits will usually provide an adequate sample. If, however, there are substantial numbers of "heavy" fish such as flatfish, sablefish, or Atka mackerel in the catch, the above system will probably not prove adequate as a disproportionate number of these fish will end up in the last split. If these circumstances occur, the splits selected for biological sampling from the forward portion of the net must be handled and sorted separately from the last split and then finally combined to obtain the total catch.

2. When a load cell is not available, acceptable results can usually be obtained by counting the number of splits and weighing the components of one or two splits which were selected for sampling. As in Section One above, you may have to treat the last split separately if there is a good showing of flatfish in the catch. The total catch weight is then obtained by assuming that each split is identical and use the weights of the sample splits.

3. When you are working aboard a large vessel, a third alternative is available. First estimate the total catch from the size of the cod end which you can see completely when it is hauled up on deck. Then dump the entire catch on deck. This will usually provide an opportunity to observe the species composition and possible clumping. The subsample can then be selected by filling the desired number of baskets directly from the deck. Be sure to select baskets from all portions of the catch.

B. Catches from 2,000-8,000 pounds

The catches in this size range are generally much easier to handle with the maximum size depending upon the capacity of the checker or deck bin aboard the vessel. The typical processing system of a catch greater than 2,000 pounds is diagrammatically shown in Figure 1. First you must estimate the total amount of the catch or weigh the splits with a load cell, so you will know what percentage to select for a subsample. Then the deck bin is lined with the cargo net and adjusted to line that portion of bin necessary to give a resulting subsample of approximately 2,000 pounds. The cargo net must be positioned so that it divides the deck bin athwartship. If the total catch is 6,000 pounds, you would adjust the cargo net to line 1/3 of the deck bin while for a 4,000 pound catch you would adjust the cargo net to line 1/2 of the deck bin, etc. Following the technique developed by Hughes (1976), it is vitally

important that the cod end be positioned so that the top and bottom of the trawl are facing athwartship prior to dumping the catch. Proper orientation of the cod end can be facilitated by tying a short marker line to the top and bottom of the cod end.

1. If a load cell is available the splits can be individually weighed and net weight subtracted to obtain the total catch weight. When the catch is in the deck bin, the cargo net is lifted by the boom removing the subsample from the deck bin and dumping it on the sorting tables.

2. If a load cell is not available the total catch weight can be obtained by leveling off the catch after it has been dumped into the deck bin. Measure the total volume of the catch by bin boards; that is, does the catch fill 1-1/2 or 2-1/2 or 2-3/8 bin boards. After removing the subsample and placing it in the sorting table, again level the remaining catch in the deck bin and record how many boards the catch now fills. After the weight of the subsample is obtained the total catch weight is obtained by extrapolation from the weight of the subsample.

C. Catches of 2,000 pounds or less

Catches in this weight range are dumped directly onto the sorting table.

Sorting and Weighing the Catch or Subsample

The first step in processing the catch or the subsample thereof on the table is to sort the dominant fish species in a manner to provide a random sample. Place three or more baskets on the sorting table, depending on the total species weight, and fill them simultaneously with the most dominant species. In filling the baskets, each person rotates from basket to basket, putting one fish in each basket. When the baskets are filled, repeat this procedure with another set of three baskets until the dominant species is

completely sorted. Other species can be sorted into separate basket sets at the same time the dominant species is being sorted. If no single species is highly dominant in the catch, sets of two or single baskets can be used for any species. Place the filled basket sets or single basket aside in the order they are removed from the table. Do not combine partially filled baskets. In most situations, it is usually convenient to weigh the baskets as they are removed from the table and before they are set aside for further processing.

Sort all species, including invertebrates, and determine the weight and number of each species for the catch or that portion of the catch processed and record on the on-deck sampling form. For species with only a few specimens, this can be done by direct count. For those species where a length-frequency sample is taken, the sample weight and number will be used to estimate the total number in the catch. It is not necessary to do this in the field--the expansion will be done by computer at the Center from the sample data supplied on the length-frequency form. For other species where length-frequencies are not taken, but the number of individuals is large, a subsample should be weighed and counted and recorded on the on-deck sampling form. These sample numbers will also be expanded by computer. It is not necessary to get sample or total counts on miscellaneous species for all hauls. When time is not available, don't bother with these counts. Numbers will be expanded by strata and all that is required for this computation is a good estimate of mean individual weight within each stratum. A further expansion of the weights and numbers to adjust them to the total catch will be necessary in those cases where only a portion of the catch was processed. This will be done in the field. For those invertebrates that are difficult to identify to species, they may be grouped into one of the following categories

Octopus unidentified	Starfish unidentified
Jellyfish "	Clams "
Squid "	Hermit crabs "
Snails "	Other invertebrates

Hermit crabs should be weighed in the shell. It is not necessary to weigh empty shells. See the section on Data Records for an example of how to record catch data.

Biological Sampling of Fish and Shellfish

A. Species

Species of fish from which biological data is desirable are listed below:

Pacific cod (*Gadus macrocephalus*)

Pollock (*Theragra chalcogramma*)

Rex sole (*Glyptocephalus zachirus*)

Dover sole (*Microstomus pacificus*)

Rock sole (*Lepidopsetta bilineata*)

Flathead sole (*Hippoglossoides elassodon*)

Halibut (*Hippoglossus stenolepis*)

Sablefish (*Anoplopoma fimbria*)

Pacific ocean perch (*Sebastes alutus*)

Atka mackerel (*Atheresthes stomias*)

Snow crab (*Chionoecetes bairdi*)

King crab (*Paralithodes camtschatica*)

It is anticipated that only three or four species will be available in any one haul in sufficient numbers to take biological data. The field party chief will decide the species and number of specimens from which to take data based on need and the time available.

B. Selecting a random sample

The next step in processing the catch is to reduce the number of baskets (set aside during the sorting procedures) to a random subsample of from 200-250 fish of other species (300 fish for Pacific cod) which will be processed for biological data. Handle juvenile pollock (<20 cm) as a separate sample from the adults. With a little experience, the number of baskets to be filled at one time to acquire these sample sizes will be apparent. Randomly select one row from the 3-5 basket sets of fish. If the subsample is too large because too few baskets were filled at one time, the subsample can be further reduced by selecting baskets from the front, middle, and end of the row to obtain the subsample for processing. Another procedure is to dump the subsample on the table and resort into baskets a second time, using the number of baskets needed and randomly selecting one of the two sets a second time.

After the subsample is selected, the unused baskets of fish can be discarded overboard after their weights have been recorded. Weigh and count all species not requiring further processing.

C. Length-frequency samples for fish species

Take length-frequencies from as many of the 13 commercially-important species as time permits from each tow. Normally only four or five of these species will be present in any given haul. If time is not available to take frequencies from all commercially-important species of fish present, take frequencies from species having the highest priority. If necessary, separate length-frequency samples can be taken for adult and juvenile pollock and the length for each group recorded on separate forms--only about 30 juvenile pollock need to be measured. If possible, determine the sex of juveniles. Record the weight of the length-frequency sample and total weight of the adults and juveniles in the catch on the length-frequency forms.

Using plastic strips, length-frequencies will be taken at all stations where target species are captured using the random subsample of 200-250 fish previously selected. In the case of halibut, all specimens will be measured using a metric tape for large specimens. Frequencies will be reported by sex, recording lengths for one sex on the upper part of the plastic strip and the other sex on the lower half. Lengths will be recorded by centimeter interval and measured from the tip of the snout to the end of the middle rays of the caudal fin.

The plastic length-frequency strip is attached to the measuring board using thumb tacks. The first line on the measuring board is 9.5 centimeters from the front of the board so that when the first "0" interval on the length-frequency strip is properly aligned on this mark, the lengths tallied in this interval will represent those fish that are 10 cm long. Length-frequency measurements should be transferred to length-frequency forms daily.

Sex determination will be made by opening the abdominal cavity. Flatfish species can best be sexed by observing the shape of the reproductive gland. The ovary is generally triangular with a long tail lobe which extends posteriorly. If mature, eggs are generally visible within the ovary. Male testes do not have the tail lobe and are white in mature specimens. Some species of flatfish can be sexed without making an incision by holding them up against a light and observing the presence or absence of the long tailed lobe of the ovary (see data code book).

In Pacific ocean perch, which are oviparous, the ovaries of immature females appear yellow in color and are firm in texture. Mature females will have embryos at various stages of development and will appear red or gray in color. Mature males' testes should be whitish-colored (see Appendix I).

The gonads of pollock and Pacific cod should appear quite similar to one another. Mature females will have large grayish ovaries full of eggs while the male testes will be white and composed of many leafy lobes. The smaller immature specimens can be separated by the shape of the gonad. In the case of pollock, rather small fish of less than 20 cm can be sexed by the presence or absence of ovaries. The ovaries are oblong and clear to reddish, and appear toward the back of the body cavity. The absence of ovaries identifies a male (see data code book).

If sexes cannot be separated in the case of small specimens, record their length measurements as unsexed.

See the section on Data Records below for the method of recording length-frequencies.

D. Length-frequency samples for crabs

Length-frequency samples will be selected in the same manner as for the fish species. King crab are measured by length, from the posterior edge of the carapace. Tanner crabs are measured by width, measured at the widest point on the lateral-posterior lobes of the carapace. Both king and Tanner crabs can be sexed by the shape of the abdominal flap. Females of both specie have wide abdominal flaps, while the males are narrow. Shell condition will recorded for all Tanner crabs. Incidence of black mat disease and infections due to chinoclastic bacteria will be noted.

E. Age structure samples

Otolith or scale samples will be collected from the principal demersal fish species captured during the cruise. A random sample will be taken from each station where a species is a dominant form. If time does not allow aging samples for all species captured, the field party chief will select the species in order of priority.

Vials held in polystyrene boxes will be used to store the otoliths and paper envelopes for scale samples. Otoliths for a length-sex category will be stored in a single vial. Each container vial will have a label giving the species, sex, length groups, and vessel. Keep a permanent record of the number of otoliths by sex-centimeter group for each completed sample and return this record to the Center.

Scales rather than otoliths will be taken from Pacific cod. See Appendix III for methods of collecting scales and otoliths.

Use Specimen Data forms for recording otolith data. See the section on Data Records below for example of data required on the form.

F. Length-weight

To establish length-weight relationships for the various species, weights and lengths for individual fish will be taken. This can be a sample independent of the otolith and length-frequency or the same sample. The sample will be random with lengths and weights taken from all specimens in the sample. Stomach contents should be removed prior to taking the weight measurement. Weights will be determined on a triple-beam balance or during periods of rough weather by hand-held spring scales. When possible, a K-TRON electronic balance will be used.

G. Maturity

An effort will be made to determine the maturity status of all species in order to define spawning areas and times. The timing of the survey should be during the spawning period for some species. Use Specimen Data forms for this purpose (see the section on Data Records below for the method of filling out forms).

Maturity determinations will be estimated by visual observation and will be somewhat subjective because of the lack of precise guidelines for the classification of gonads. Five classifications (except for rockfish) will be used--immature, maturing, spawning, spent, and sexually inactive (see ADP code book for criteria or Appendix II for rockfish criteria). Attempt to photograp each classification in color for as many species as possible.

Data Records

A. On-deck sampling form

This form will be used for initial recording of catch and sample data. It will be kept as a permanent record, although most of the data it contains will be transcribed onto the Trawl Catch Form (Figure 4) after the haul or at the end of the day. An example of how data should be recorded on this form is shown in Figure 2.

For each species, the individual basket weights will be recorded for the total catch or that proportion of the catch processed. Individual basket weights will be summed and the total weight for each species recorded. Also, record the total number in the sample, or a subsample weight and number, if the total number for any species is large. It is not necessary to count or record the number of fish in a length-frequency subsample, since this will be done by computer.

B. Haul-position and species catch form

Entries on the trawl catch form are described in the ADP codebook (Page 3). On the front side of this form, information for each haul will be recorded pertaining to the location, depth, duration and distance of the haul and weather and sea conditions (Figure 3). Much of this information will com-

from the bridge, and the field party chief should make arrangements with the Captain for the most convenient time and method of acquiring this information.

When recording loran readings for the start and end positions, be sure to use the correct loran rates as shown in the ADP Code Book (Table 2. Pg. 32). Geographic start and end positions will be plotted on the appropriate hydrographic chart or mylar overlay and by loran plotter if available.

The distance fished will be determined by the best available method. If a tow deviates from a straight line, indicate in the "remarks" section of the Haul-Position form and calculate distance fished from the loran plotter. The duration of tow which is computed as the difference between the Equilibrium and Haul time will also be measured by the best available method. A net sonde unit or other electronic on-bottom indicator is required to determine the moment the gear reaches and leaves the bottom. This corresponds to the Equilibrium and Haul times on the Haul-Position form (Table 1). If these devices are not available, the duration of the tow will be measured as the difference between when the winch brakes have been set (Time Out = Equilibrium Time) and when the winches begin retrieving the trawl gear (Haul Time = Time gear leaves bottom). All times will be local to the survey area and noted on each haul form.

Table 1.--Definition of times used on the Haul-Position form.

Time Start	--	Time when the trawl begins to be pulled off the deck. Always use local time indicating time zone and daylight or standard time.
Time Out	--	Time when the amount of cable specified for the haul is out and the brakes on the trawl winch have been set.
Equilibrium Time	--	Time when the gear reaches bottom. Would be same as Time out without on-bottom indicator. Duration is computed as the difference between the Equilibrium time and the Haul time.
Haul Time	--	Time when trawl winches begin retrieving the gear. With on-bottom indicator would be the time when gear leaves bottom.
Time In	--	Time catch is on deck.

C. Length-Frequency form

Data from the length-frequency strips will be transcribed to these forms in addition to some haul identification data, the weight of the length-frequenc subsample, and the total catch weight for the species (Figure 5).

D. Specimen data form

All length-weight, length-fecundity, and length-maturity data collected from individual fish will be transcribed on this form. Entries will be made using the ADP and species code book. At the top of the form enter columns 1-7, vessel, cruise, and haul. Columns which are not applicable will be left blank. See Figure 6 for an example of a completed form.

The field party chief will return all original data to Seattle personally. It is important that data forms be completely filled out, the information

coded, and entries checked prior to the completion of each leg. It is suggested that this work be kept up on a daily basis if possible. When data forms have been double-checked, initial upper right-hand corner of page and date.

Immediately upon return to the laboratory, data will be punched onto cards for further processing and analysis.

LITERATURE CITED

Hughes, S.E.

1976. System for sampling large trawl catches of research vessels. J. Fish. Res. Board Can. 33(4): 833-839.

1:4.10.19

APPENDIX II

COLLECTION AND STORAGE OF OTOLITHS AND SCALES

The clearness of the age marks on otoliths and scales depends greatly on the collection and storage procedures used. The following notes are intended to be of assistance to those who have had little or no practical experience in the collection of age structures. Also, current information is summarized on the age structures and storage media preferred by the Age Determination Unit for the various species in the North Pacific Region (Table 1).

Removal of the Age Structure From the Fish

OTOLITHS

The method to remove the otoliths is to cut open the head (Fig. 1), exposing the cavities in which the otoliths are located (Figs. 2-3). A knife is usually sufficient to make the cut, although a hacksaw may be useful for large specimens. The otoliths are in the otic capsule, a cavity at the base of the skull. A few exploratory cuts and probings in the skull cavities will usually be necessary to get the "feel" for the location of the cut and to find the otoliths. Frequently, the otoliths can be quite difficult to locate.

There are six otoliths in the otic capsule, three on each side. The sagitta (Fig. 4), by far the largest and usually the only one readily visible is the one that is collected. Its size varies with the species and the size of the fish and will range from about as small as a grain of rice to as large as 4 cm.

The otolith is easily removed with tweezers or the fingers. Rinse the otolith in running water or in a bucket to remove slime and tissue. Then, store it in the appropriate media (Table 1) and container. Small paper

envelopes are usually used when storage is dry, and glass or plastic vials, leak-proof compartmented boxes, or plastic envelopes are used when storage is wet. The otoliths usually must be identified so that date of collection, area, and other information can be related to them. It is very important to have a clear understanding of the scheme used to identify the otoliths being collected. A mistake in the numbering sequence or procedure used to relate the otolith to associated biological and time-area data can make a collection useless. If at all possible, practice the entire procedure on a few fish of each type (flatfish and roundfish) before attempting to process large samples.

SCALES

At present scales are collected from Pacific cod and lingcod. The procedure is to take a scrape sample (see below) of about 50 scales from A (the preferred zone), or B (next preferred zone), on either the right or left side of the fish (Fig. 5). If scales are missing from these zones take them from any location (zone C). The scales are usually stored in a coin envelope.

SAMPLING PROCEDURE

1. Examine fish and select zone A, B, or C. RECORD ZONE on envelope or data sheet.
2. Wipe the area to be sampled with a sponge, paper towel, or cloth. This is to minimize contamination of the sample with scales of other fish.
3. Using any thin edged instrument (knife, scalpel), scrape within the zone in an anterior direction (toward the head).
4. Wipe off inside the coin envelope the scales that adhere to the instrument. Be certain the envelope is properly labeled.
5. Remove excess scales from instrument before sampling the next fish.

REMARKS

These instructions may be modified from time to time. The best age structure and storage media has not been determined for some species. On occasion, determined by the biologist in charge, two different age structures or both otoliths may be collected.

These instructions are based on ideal sampling conditions. It is recognized that strict adherence to the methods will sometimes be impossible or impractical. Keep a record of the deviations from instructions so that the effect can be evaluated.

Table 1.--Age structure and storage media for flatfish and roundfish.

Type of Fish	Age Structure	Storage Media
Roundfish		
Pacific cod	Scale	Dry in envelope
Lingcod	Scale	Dry in envelope
Rockfish, pollock, sablefish, and other roundfish	Otolith from right side[1]	50% Ethyl Alcohol
Flatfish	Otolith from eyed side[1,2]	Glycerin solution

1 - If preferred otolith is damaged, take from other side.

2 - Take both otoliths from Greenland halibut (turbot) and take both otolith from all flatfish when possible.

NECROPSY FIELD DATA SHEET FOR HISTOLOGICAL SAMPLES

ADF&G, FRED Division Fish Pathology Lab

Collector/Address/Telephone #

Species

Number Specimens in Sample

Size Range

Life Stage

Date of Collection

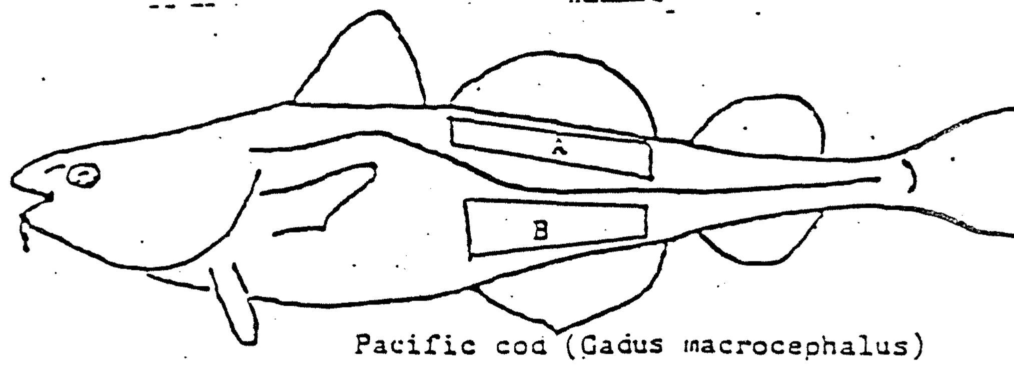
Location of Collection (Site Name or Number)

Abnormalities Observed Per Specimen Number

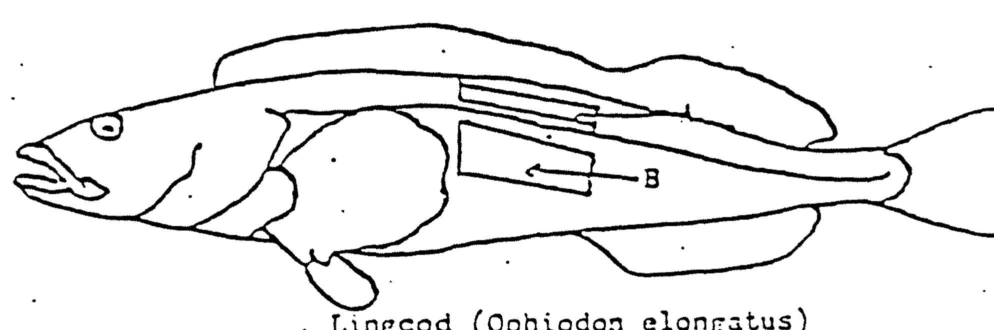
APPENDIX 2: BIVALVE MOLLUSC SAMPLING PROCEDURES

	FLATFISH	ROUNDFISH	ROCKF
Top view			
Side view			
	Yellowfin sole (Limanda aspera)	Walleye pollack (Theragra chalcogramma)	Pacific Ocea (Sebastodes a

Fig. 4. Diagrammatic sketches giving top and side view of representa of flatfish, roundfish, and rockfish.



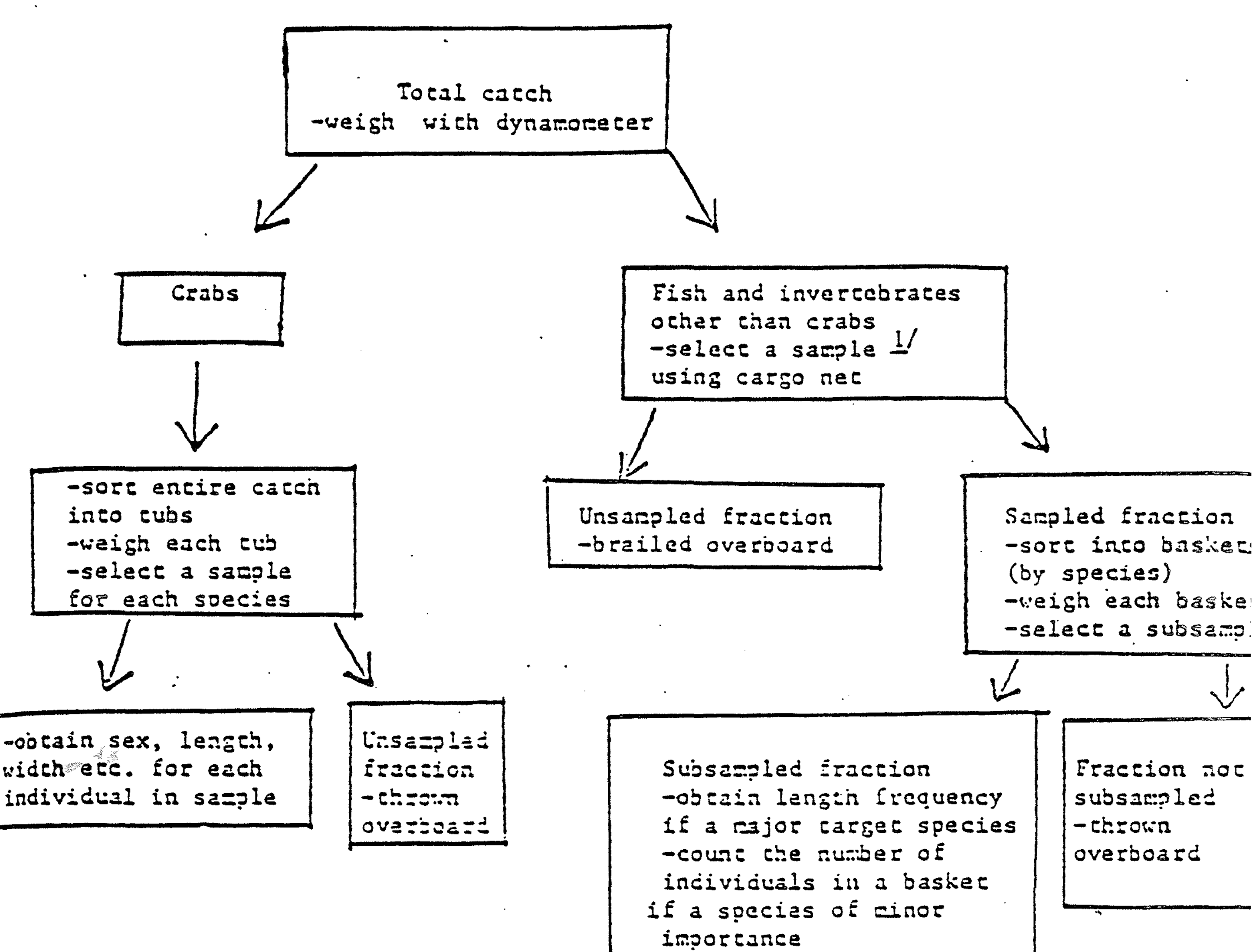
Pacific cod (Gadus macrocephalus)



Lingcod (Ophiodon elongatus)

Fig. 5. Body location for collecting scales from Pacific cod and Lingcod. A is preferred zone and B is next preferred All other zones are zone C.

Figure 1.--Typical Processing of a Catch Greater Than 2500 lbs.



1/ This step is ommitted if the catch is less than about 2500 lbs.

APPENDIX E

Appendix E

KODIAK CRAB TRAWL SURVEY

SHIPBOARD SAMPLING PROCEDURE

ALASKA DEPARTMENT OF FISH AND GAME

KODIAK, ALASKA

JUNE 1988

Precruise Preparation

I. Check all lines and nets for wear and measure to be sure they conform to specifications.

II. Draw up a list of trawling locations for each area to be surveyed. Make sure navigational charts are clearly marked so that the captain can easily locate trawl stations. It would help if the trawl location list could be prioritized to encompass the least amount of running time between tows and in case stations have to be cut due to time restraints.

III. Assemble all gear:

a. Tool box (see Equipment List No. 1).
b. Trawl gear (see Equipment List No. 2).
c. Sampling gear (see Equipment List No. 3).

IV. Load vessel - the vessel crew will rig the gear.

Information for Skipper

The skipper should be given all navigational charts that he will need to conduct the survey, a list of trawl locations and the forms for the trawl record.

The crew leader should explain (or provide a written explanation) the sampling program to the captain, explain station grids if they are drawn on the navigational charts, describe the strata needed and the reasoning behind the strata selected. In other words, if the skipper understands what you are trying to accomplish and how you are approaching the survey, he can better use his expertise to enhance the success of the survey.

The crew leader should also go over the skipper trawl record

(Appendix C)
form (Figure 1) with the captain pointing out the data needed for analysis of the survey catches. You _must_ have correct "haul number....year....starting position....compass heading....distance towed." The other data is also important and should not be minimized, but the above information is _absolutely essential_ if the results of a tow are to be used! The crew leader should spot check the observations noted on the skipper's forms daily to catch errors which can diplomatically be brought to the attention of the skipper. Specific instructions to the skipper trawl record included in appendix 1.

Inform the skipper that all tows should be approximately 30 minutes and should cover exactly one nautical mile (approximately 2 knot towing speed depending on the fishing characteristics of the vessel). Of course, tows may be less than 30 minutes if the gear is pulled early to avoid hanging up or does hang up. Record the exact distance towed on the skipper form. Trawl locations should be as near to the center of a target area as is practicable.

Survey vessels with video plotters should be programed to give a constant readout of distance towed. Where possible each tow should be recorded on a cassette tape for future reference. If a hard copy plotter is available each tow should be recorded on paper for future reference.

If a tow is terminated due to gear hang up, the crew leader should observe the catch and nature of the damage to the net and decide whether that tow is acceptable or should be retaken. If the catch looks to be affected by the gear problems, a different trawl location within the target area can be selected after consulting with the skipper on bottom conditions. A similar decision will have to be made if the tow catch appears to have been affected by objects caught in the trawl (e.g. crab pots, large amounts of rocks or mud, etc.) Be sure to get the correct distance towed recorded on the skipper's form for short tows that

are deemed acceptable.

Handling the Catch

All catch weights and specie composition of the catch should be recorded on the station catch record form (Appendix C) (Figure 2 and Appendix 2). As the net is brought up to the stern, the net should be shaken to force the crabs from the intermediate down to the codend portion of the net.

Upon retrieval of the net, the catch should be weighed with a crane scale. If rough weather does not permit a weight to be taken, the total weight of the catch must be estimated. The catch weight will be recorded on the station catch record form. The catch should be dumped on deck or in a sorting bin if available. If a weight of the catch is taken, then a tare weight for the net should be recorded at this time. All halibut should be measured and returned to the sea as soon as possible. Skates will also be measured as directed in length-weight conversion appendix (Appendices 3 & 4). Large fish such as graycod, rockfish, blackcod, or dogfish should be sorted completely from the catch with a weight determined of each species.

All Tanner crab and king crab should be removed from the catch and separated by sex. The remainder of the catch (fish and miscellaneous invertebrates) should be subsampled with the rest shoveled overboard. This subsample should consist of two fish baskets filled with fish to be set aside until the crab are worked up. The total crab weight should then be determined and recorded. Remember to tare the scales used with the proper basket weight. Record all Tanner crab and king crab weights in the comments section of the station catch record form until they can be totaled and recorded in the proper column. At this time all crab should be measured and shell-aged using the proper sampling procedure for each specie of crab. Record this information on a crab data form. (Appendix C) Figure 3 is an example with

this will depend on how "safe" the clipboards are while on deck. If the clipboards cannot be placed in a dry location out of the wind, you may want to bring inside each tow's deck forms as the work is done.

When you have finished taking the required data from a sample, ALWAYS take that series of forms off the clipboards and take them to a safe location. This cannot be stressed too much!!

All spaces on all forms used at a station should be completed. A daily station summary, Appendix 8, will be kept for the entire survey. Sum totals need not be completed until the end of each day, but should not be neglected more than one day since the back log can quickly get out of hand. This is also a good time to recheck the forms, to be sure that all the blanks are filled in for the data-entry people. Having all forms completed as soon as possible after a tow also helps to decrease the likelihood of error.

Subsampling:

The amount of time you have to work up a catch depends on the amount of running time to the next tow location. You should have at least one hour before the next tow is completed. During that time, two persons can take and record about 500 measurements. The best data situation occurs when all crabs captured in a tow are measured. In the rare instances when you cannot accomplish this before retrieving the subsequent tow, the crab catch can be subsampled. The crab subsampled should all be of the same species, sex and shell condition. Estimate the total number of individuals, then pick a subsampling fraction that would allow for at least 200 individual measurements. Be sure to note the sampled fraction on the crab data form. You should also take into consideration the time necessary to

collect supplementary data (such as size at maturity work or other studies) unless you can put those individuals aside so the data can be collected when you are not busy (of course, this can only be done when you are able to sacrifice the crab used). The important thing to keep in mind if you do set crabs aside is to label them and put them in a location where they will not be confused with subsequent crab catches brought on board.

Data Collection - Specific Forms

CRAB DATA FORM (Appendix C, Figure 3)

The Crab Data Form is used for recording all crab measurements. A separate form should be used for each sex and specie of crab. Tanner crab measurements (carapace width) are taken at the widest point of the carapace, between the spines, using a Vernier caliper (Appendix 6). Measurements should be rounded off to the nearest millimeter. Note immature females with a "J" in 17th column as shown in Figure 3.

Shell-aging is problematic for Tanner crabs. It is, to a large extent, subjective and shell condition may vary in individuals of the same year class from different sampling locations. The best way to sort out the newshell, oldshell, and very oldshell crabs captured in a tow is to make a pile for each category. Any ambiguity between age groups should become visible if this is done. Examining crabs individually does not give you a basis for comparison. It may take a bit more time to sort into shell age categories before measuring, but the resulting greater accuracy probably merits the effort. A series of photographs are included which may help in distinguishing shell ages. However, the background used in the photograph is probably not similar to an onboard background and lighting was ideal during the photo sessions. You will be dealing with a greater range in lighting (from heavy cloud cover or even fog through bright sunlight). Keep this in mind!

Carapace length is the measurement used on king crab and is taken from the posterior edge of the right orbit to the posterior median edge (usually a notch) of the carapace using a Vernier caliper.

Shell-aging is not as difficult for king crab as it is for Tanner crabs in that skip molting does not occur to the same degree. A photograph has been included to show the general appearance of the newshell, oldshell, and very oldshell crabs (see attached).

EQUIPMENT LIST NO. 1
Tool Box - Inventory

Swivels:
- 1/2" - 8 ea.
- 5/8" - 3 ea.
- 5/16" - 2 ea.
- 3/4" - 2 ea.

Shackles:
- 3/8" - 10 ea.
- 1/2" - 10 ea.
- 5/8" - 12 ea.
- 3/4" - 2 ea.
- 1 1/4" - 1 ea.
- 7/16" - 2 ea.
- 3/16" - 1 ea.

Hammerlocks:
- 1/2" - 8 ea.
- 5/8" - 4 ea.

Rings:
- 3/8" - 6 ea.
- 1/4" - 6 ea.
- 4" - 3 ea.

Misc:
- Split Links - 2 ea.
- G-hooks - 6 ea.
- D-rings - 6 ea.
- Codend Clip - 2 ea.
- 12" Crescent Wrench - 2 ea.
- Large Screwdriver - 2 ea.

EQUIPMENT LIST NO. 2
Trawl Gear

Nets - 400 eastern otter trawl (Appendix 8).
Spare web - 25 lbs. 4" mesh.
Dandylines (3 complete sets) and cables.

10 f. singles - 6 ea. (5/8" galvanized wire rope)
15 f. doubles - 12 ea. (1/2" galvanized wire rope, 6 X 19)
24 ft. transfer cables - 4 ea. (3/8" galvanized wire rope), length may be adjusted to suit particular survey vessel
Door cables - 4 ea. (5/8 galvanized wire rope)
Codend clips with 30 ft Sampson line, 5/8" - 2 ea.
Spare splitting strap - 2 ea.
Lazyline - 2 ea.
Sampling table - if not supplied by vessel.
Trawl doors, 5' x 7' Astoria - 1 pr. (or 2 pr. if Ron K. is skipper)

Seine Twine:

120 thread - 2 spools
60 thread - 4 spools
36 thread - 4 spools
18 thread - 2 spools

Net Needles:

Large, No. 14 - 2 ea.
Medium, No. 816 - 3 ea.
Small, No. 814 - 2 ea.

Electricians Tape - 5 rolls

EQUIPMENT LIST NO. 3
Sampling Gear

Measuring tools:
- Port-a-Weigh Crane Scale - 2 ea.
- Tape Measure - 3 ea.
- Small Caliper - 1 ea.
- Crab Caliper - 3 ea.
- 200 lbs. Cap. Scale - 2 ea.
- 20 lb. Cap Scale - 2 ea.
- Sippican Bathythermograph Expendible Probes
- Trayco tubs - 3 ea.
- Garbage can, 25 gal - 2 ea.
- Fish Baskets - 24 ea.
- Wash Tubs - 12 ea.
- Fish Shovels - 2 ea.
- Knife - 2 ea.
- Scissors - 1 ea.
- WD 40 - 1 lg. can
- Never Seize - 1 can
- Joy Soap - 1/2 gal.
- Garbage Bags (Heavy) - 1 box
- Scrub Brush - 2 ea.

References:
- "Alaska Saltwater Fishes and Other Sea Life" D. Kessler
- "Pacific Fishes of Canada" J. Hart
- "Tanner Crab Trawl Survey Shipboard Sampling Procedures"
- "National Marine Fisheries Serv. Species Code Book"
- "1987 Westward Region Crab Survey Results"
- "Sippican Bathythermograph Instruction Manual"

Chemical Fixatives:
- Formaldehyde - 1 gal.
- Bouins Solution - 3 pts.
- Sodium Borate - 1 8 oz. jar
- Assorted Sample Jars
- Disecting Kit - 1 ea.
- Foreceps - 2 ea.
- Squeeze Bottles - 2 ea.
- Specimen Bags - 2 pkgs.

Office material:
- Write-in-Rain book - 1 ea.
- Writing Pads - 12 ea. (1 pkg)
- Erasers - 2 ea.
- Felt Markers, Black - 2 ea.
- Felt Markers, Red - 1 ea.
- Pencils - 2 boxes
- Desk Mount Pencil Sharpener- 1 ea.
- Clipboards, 6 ea.
- Scotch Tape - 2 rolls
- Masking Tape 1 roll
- Paper Clips - 1 box
- Lg. Manila Envelopes - 10 ea.
- Collection Labels - 100 ea.
- Rubber Bands - 1 box
- Calculator with tape - 1 ea.
- Sampling forms -
  - Skipper Trawl Record
  - Trawl Station Catch Record
  - Crab Data Forms
  - Daily Summary Forms
- Navigational Charts
- Survey Charts w/current Station Plan

Personnel Gear:
- Survival Suit - 1 sci crew member w/user responsible for condition and maintenance
- Raingear - 1 pr/sci crew mbr.
- Gloves - 2 pr/sci crew mbr.

Skipper Trawl Record Documentation

This form records each haul: area, data, position, time trawled, depth, length of tow and weather.

Column Heading	Columns	Contents
Cruise No.	1-4	Sequential number by year.
Haul No.	5-7	Beginning with 1, each drag is numbered in sequence throughout each trip whether the haul was successful or not.
Haul Location	8-16	8 - Region code (see codes) 9-10 - Survey codes (Appendix) 11-12 - Strata-Consult cruise plan. 13-16 - Station No. Consult cruise plan.
Vessel Code	17-18	Consult codes Appendix.
Date	19-24	19-20 - Month. 21-22 - Day. 23-24 - Year.
Starting Position	28-39	Coordinate Code: 25 - Use either (1) Lat-Long or (2) Loran C. 26-32 - Latitude (degrees, mins., and secs.) or second Loran C reading. 33-39 - Longitude (degrees, mins., and secs.) or second Loran C reading.
Compass Heading	40-42	Magnetic.
Trawl Time	43-48	43-46 - Using 24 hour clock. 47-48 - Elapsed time of tow (mins.).
Haul Depth	51-56	51-53 - Maximum depth (f). 54-56 - Minimum depth (f).
Weather	57-59	57- Cloud 58- Sea Consult codes on data sheet 59- Swell
Scope	60-62	In fathoms, how much cable out.
Gear Performance	63-64	Fill out for each haul. Consult codes on data sheet. A written description of any problem is desirable.

APPENDIX F

Appendix F

September 12, 1989

STATE/FEDERAL DAMAGE ASSESSMENT PLAN

ANALYTICAL CHEMISTRY

COLLECTION AND HANDLING OF SAMPLES

FOR AGENCY USE ONLY
NOT FOR RELEASE
ATTORNEY WORK PRODUCT

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   - 5.2 Water
   - 5.3 Sediment
   - 5.4 Tissue

6. SAMPLE PRESERVATION AND HOLDING TIME
   - 6.1 Water
   - 6.2 Sediment and Tissue

7. SAMPLE SHIPPING

8. CHAIN-OF-CUSTODY PROCEDURE

1. Introduction

In response to the release of more than 10 million gallons of crude oil into Prince William Sound, the State of Alaska and four Federal Agencies, the Departments of Agriculture, Commerce and Interior and the Environmental Protection Agency are acting together to assess the damages to the natural resources. Authority for this action is provided by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and the Clean Water Act (CWA).

A damage assessment requires documentation of the exposure of the resources to oil released from the EXXON VALDEZ, identifying which resources were injured by that exposure, measuring the magnitude of the adverse affects on each resource over time and assigning economic values for that injury. Once this is done, monetary compensation can be sought from the potentially responsible parties to restore and/or replace the injured resources.

Recovery of monetary damages may involve civil court actions. It will then be necessary to prove that the samples were collected in a scientifically approved manner and that the samples were protected from outside contamination (non-incident related) and accidental mix-ups during handling and analyses. It is, therefore, extremely important that every sample be readily identified and their location and analytical status known and documented at all times.

This document and the associated training sessions, were prepared to assist field personnel in collecting samples that will provide scientifically sound and legally defensible data to support the State/Federal Natural Resource Damage Assessment for the EXXON VALDEZ oil spill.

2. Record Keeping and Documentation

Standard operating procedures (SOPs) for all sampling procedures, including chain of custody procedures; sampling protocols; cleaning and preparation of sample collection and storage devices; and labeling, handling, and sample

preservation and holding time must be written in detailed, clear, simple and easy to follow language.

Personnel must be knowledgeable and experienced in the described sampling techniques and must adhere to the SOPs.

Any changes in procedures must be recorded in detail in the field logbook. The log entry must include reasons that the change in procedure was unavoidable.

Field logbooks are issued by the Team Leader or their representative. The logbooks should be serially numbered, sturdy, bound books with sequentially numbered pages. Waterproof logbooks should be used if available.

Field data sheets, if used, must be consecutively numbered by project. The field data sheets must be referred to in entries in logbooks which reference, the precise data sheet involved and the relationship to specific data in the logbook noted.

All information pertinent to field activities, including descriptive notes on each situation, must be recorded in indelible marker in the field logbook. The information must be accurate, objective, up-to-date and legible. It should be detailed enough to allow anyone reading the entries to reconstruct the sampling situation. Additional information may be provided by field data sheets, sample tags or photographs.

Entries should be made in the logbook or on field data sheets with indelible marker at the earliest possible time. Notes should never be written on scrap paper and then transferred to the logbook.

Entries into field logbooks or field data sheets are signed or initialed, and dated by the person making the entry at the time of entry.

Each day's entries are closed out with a horizontal line, date and initial.

Errors in field logbooks or other records are corrected by drawing a single line though the error, entering the correct information and signing and dating the correction. Never erase an entry or any part of an entry.

Do not remove pages from the logbook.

Completed logbooks and field data sheets are returned to the Team Leader or their representative to be archived in a central location under chain-of-custody procedures until the Trustees indicate that they may be released.

3. Sample Identification and Labelling

A tag or label identifying the sample must be completed and attached to each sample. Waterproof (indelible) marker must be used on the tag or label. The minimum information to be included on the tag are the sample identification number, the location of the collection site, the date of collection and signature of the collector (who, what, where & when). This information and any other pertinent data such as the common and scientific names of the organism collected, the tissue collected and any remarks are recorded in the logbook. Field sample data sheets, photographs, any pertinent in-situ measurements (such as temperature, salinity, depth) and field observations are recorded in the logbook.

The location of the sampling site is determined with the aid of USGS grid maps, NOAA charts or navigational systems such as LORAN C. The site locations should be plotted on a chart of appropriate scale and photocopies incorporated into the logbook. In addition, a clear, detailed descriptive location as well

as the latitude and longitude, in degrees, minutes and seconds, of the collection site must be recorded in the logbook.

4. Sampling Equipment and Sample Containers

All sample containers must be either organic-free (solvent-rinsed) glass or organic-free (solvent-rinsed) aluminum foil. Lids for the glass containers must be lined with either teflon or solvent-rinsed aluminum foil.

Certified-clean glass jars are available from various vendors and if obtainable, may be used without cleaning.

Sample collection and storage devices are cleaned by washing with soap and hot water, rinsed extensively with clean water and then rinsed with either methylene chloride or acetone followed by pentane or hexane and allowed to dry before use.

First rinse: tap water, then re-rinse in distilled water.

Second rinse: methylene chloride or acetone

Third rinse (if acetone is used): pentane or hexane

The solvents (methylene chloride, acetone, pentane and hexane) used for cleaning sample collection and storage devices must be of appropriate quality for trace organic residue analysis and be stored in glass or Teflon containers, not plastic.

New glass jars or unused aluminum foil do no need to be washed with soap and

water. They must however, be solvent-rinsed as described above before use.

Glass jars may be cleaned by heating to 440°C for a minimum of 1 hour.

Clean glassware should be stored inverted or tightly capped with either solvent-rinsed aluminum foil or teflon-lined caps.

The dull side of the aluminum foil should be the side that is solvent-rinsed. Pre-cleaned squares may be stored with the clean sides folded together.

All equipment that comes in contact with the sample such as dredges or dissecting equipment must be solvent-rinsed before contacting each sample. Equipment should be steam-cleaned or washed with soap and hot water at the end of each day or between sampling locations.

5. Sampling Procedures

The method of collection must not contaminate the samples. Do not collect any subsurface samples through surface slicks. Do not collect any samples with oil-fouled equipment, such as nets or dredges. Do not touch or collect any sample with your bare hands.

Sample container volume must be appropriate to sample size; fill the jar to just below the shoulder. Overfilled jars will break when they freeze; underfilled jars will allow the sample to dry out.

HISTOLOGICAL SAMPLE PREPARATION FOR BIVALVE MOLLUSCS

Histopathology Technical Group

NOTE: Only live or moribund bivalves will be suitable for processing. Histopathological changes caused by toxic chemicals are often very subtle at best. Tissues in dead bivalves autolyze very quickly and will mask these changes. Do not collect and process dead bivalve molluscs. Keep molluscs alive in containers of seawater or live wells if they must be transported to the processing site. Do not over-ice animals such that tissues freeze while in transit. Frozen tissues are worthless for histological examination.

1. The fixative to be used is 10% neutral buffered formalin solution (formula attached). Formalin should be handled wearing rubber or latex gloves.

2. The volume of fixative should be ten times the volume of the tissue. This is important since any less fixative may result in tissue autolysis and worthless samples. After 72 hours, the formalin should be poured off and replaced with 70% ethyl alcohol for storage and transport. Replacement with alcohol will prevent tissues from becoming too hard and brittle when stored in fixative for long periods. Also, the fixative poured off may be saved and strained of tissue fragments and used one more time for preserving other samples.

3. The sample size per site and species will be 20 bivalves, live or moribund.

4. Bivalves less than 6 cm in length (shucked) can be fixed whole by dropping into preservative. Animals must be shucked cleanly from the shell by severing adductor muscles (diagram) prior to fixation. Discard the shell unless there is some type of shell deformity or otherwise abnormal valve. In such a case, the shell should be included and attached to the donor animal by wrapping both in gauze.

5. Larger bivalves will need about 3 incisions (anterior, mid, posterior) made across the surface of the animal about mid-way through the tissues. Do not cut completely through the animal so that individual specimens remain intact and tissues do not become mixed.

6. Tissue and shell abnormalities must be noted on a necropsy field sheet (attached), respectively numbered for a particular animal (bag in gauze and label, if necessary). If no abnormalities within the 20 specimens are observed, then a single field sheet will suffice for that sample series. The field sheet(s) will also contain the label information below and must accompany the samples in a ziploc bag.

7. A label with bivalve species, size range and life stage, date of sample, location of sample, and contact person's name, address, and telephone number must be placed within each of the sample jars. Use a pencil with soft lead for labelling so that the writing remains legible.

8. Do not mix samples of different species within the same jar of fixative. Each species requires a separate sample jar(s).

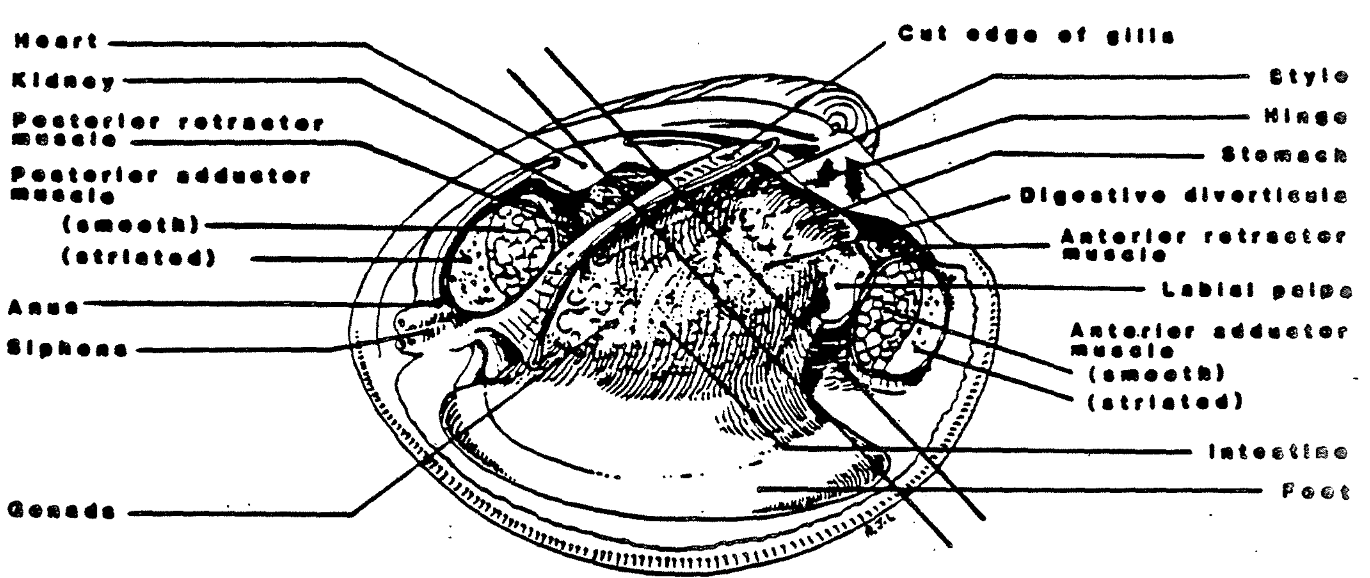


Fig. 44. Gross anatomy of hard clam. The bold parallel lines show the location where the tissue for cross-section should be taken (Illustration by A.J. Lippson).

NECROPSY FIELD DATA SHEET FOR HISTOLOGICAL SAMPLES
ADF&G, FRED Division Fish Pathology Lab

Collector/Address/Telephone #

Species

Number Specimens in Sample

Size Range

Life Stage

Date of Collection

Location of Collection (Site Name or Number)

Abnormalities Observed Per Specimen Number

APPENDIX 3: CRAB SAMPLING PROCEDURES

HISTOLOGICAL SAMPLE PREPARATION FOR BRACHYURAN AND ANOMURAY CRAB SPECIES

Histopathological Technical Group

NOTE: Only live or moribund crabs will be suitable for processing. Histopathological changes caused by toxic chemicals are often very subtle at best. Tissues in dead crabs autolyze very quickly and will mask these changes. Do not collect and process dead crabs. Keep crabs alive in containers of seawater or live wells if they must be transported to the processing site. Do not over-ice animals such that tissues freeze while in transit. Frozen tissues are worthless for histological examination.

1. The fixative to be used is 10% neutral buffered formalin solution (formula attached). Formalin should be handled wearing rubber or latex gloves.

2. The volume of fixative should be ten times the volume of the tissue. This is important since any less fixative may result in tissue autolysis and worthless samples. After 72 hours, the formalin should be poured off and replaced with 70% ethyl alcohol for storage and transport. This accomplishes an important objective; i.e., it prevents tissues from becoming too hard and brittle when stored in fixative for long periods. Also, the fixative poured off may be saved and strained of tissue fragments and used one more time for preserving other samples.

3. The sample size per site or species will be 20 crabs, live or moribund.

4. Prior to tissue collection, a blood smear should be prepared from each live crab. Insert a 1-cc syringe with a 20-gauge needle into the articular membrane of any walking leg. The third joint of either cheliped works best. Express a large drop of blood from the syringe onto one end of a clean, frosted-end glass slide and use another slide to make the smear as illustrated in the attached information. Allow to air dry, label the frosted end with an assigned crab number, and date and include in a small slide box with the samples below. An alternative method would be to pull off a walking leg and allow not more than 1-2 drops of blood to fall onto the slide.

   Be sure to not let salt water mix with the blood on the slide, as it will cause blood cell lysis. Note: King crab blood clots unbelievably fast, so make your smear quickly.

5. The chitinous exoskeleton of large crustacea prevents adequate penetration of any fixative by simple immersion. Consequently, major organs and tissues of crabs must be dissected out and dropped into fixative. This procedure is described by the following:

   a. The carapace over the visceral cavity of the crab must be removed using tin snips or bone snips, or otherwise heavy duty serrated scissors (Figure 2).

   b. Once the carapace is removed, the pigmented epidermis may come off attached or remain overlying the viscera. Snip a small 5-mm portion of the

k. In female Dungeness crabs, the paired seminal recepticles will be located below and on either side of the thoracic ganglion. Remove the right organ for fixation.

l. Remove both eyestalks and the cerebral ganglion (brain) appearing as a white, pea-sized organ located at the juncture of the eyestalks (Figure 7). This all can be removed as one piece by snipping out with a pair of scissors.

6. All tissues removed from a single crab should be placed into tissue processing cassettes, 4-5 tissue samples to one cassette. Each cassette must be labelled with the animal number from which the tissues were collected. Cassettes are then placed within the sample jar containing fixative.

7. Behavioral, external, and internal abnormalities must be noted on a necropsy field sheet (attached), respectively numbered for a particular pooled crab tissue sample. If no abnormalities within the 20 specimens from a site are observed, then a single field sheet for the sample series will suffice. These field sheets will also contain the label information below and must accompany the samples in a ziploc bag. <u>Be sure</u> to include tissue from a lesion if one is observed--this includes shell lesions as well.

8. A label with crab species, size range and life stage, date of sample, sample location, and contact person's name, address, and telephone number must be placed <u>within</u> each of the sample jars. Use a pencil with soft lead for labelling so that the writing remains legible.

9. Do not mix samples of different crab species within the same jar of fixative. Each species requires a separate jar(s).

10. Place sample jars and ziploc bag containing sample data into a suitable shipping package with adequate packing material to prevent breakage. Plastic jars or containers for fixative and samples work best. <u>Be sure lids are tight and do not leak.</u>

11. Mail to the FRED Division Fish Pathology Lab, ~~P.O. Box 3-2000, Juneau, Alaska 99802-2000; phone (907) 465-3577~~ AK Dept. of Fish and Game, 333 Raspberry Rd., Anchorage, AK 99518-1599; phone (907) 344-0541.

12. Notify the Fish Pathology Lab prior to sample shipment so that samples may be expected and tracked en route.

13. Follow proper procedures and include completed forms regarding chain of custody.

14. Any questions regarding sample preparation should be directed to:

Dr. Ted Meyers
Principal Fish Pathologist III
ADF&G, FRED Division
Juneau Fish Pathology Lab
P.O. Box 3-2000
Juneau, Alaska 99802-2000

Phone: (907) 465-3577

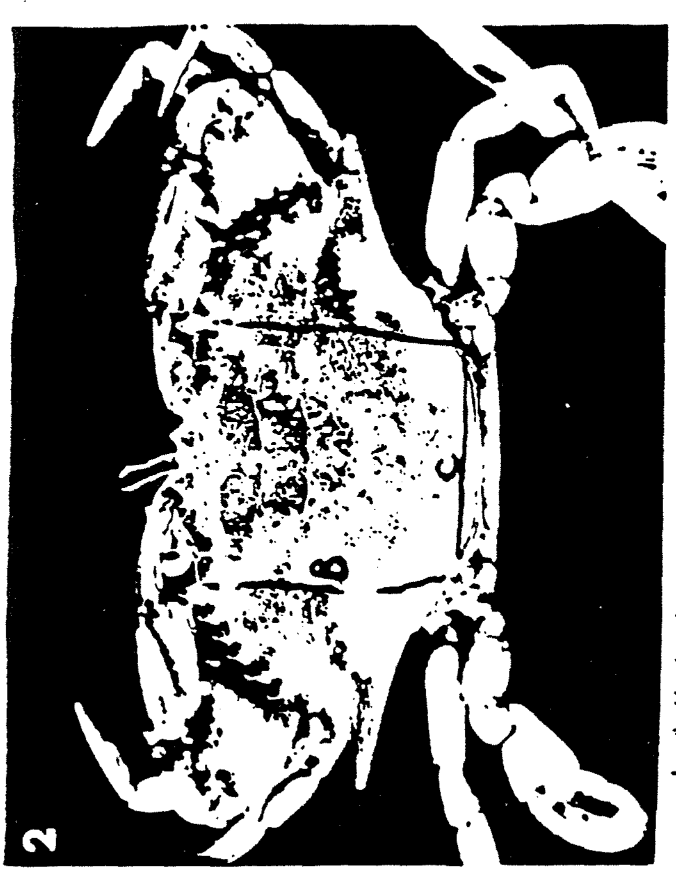


Fig 2. The dorsal carapace. Lines A-B and C indicate where the carapace is cut before its removal

Fig. 7. The hepatopancreas, testes, and vas deferens have been removed, revealing the anterodorsal brain (1) and the circumesophageal commissures (2). The large thoracic ganglion (3) lies medioventrally. Its central aperture is visible. In the living animal, the sternal artery passes through the aperture. Connective tissues have been removed in order to show the location of the antennal glands (4), which lie against the anteroventral face of the exoskeleton.

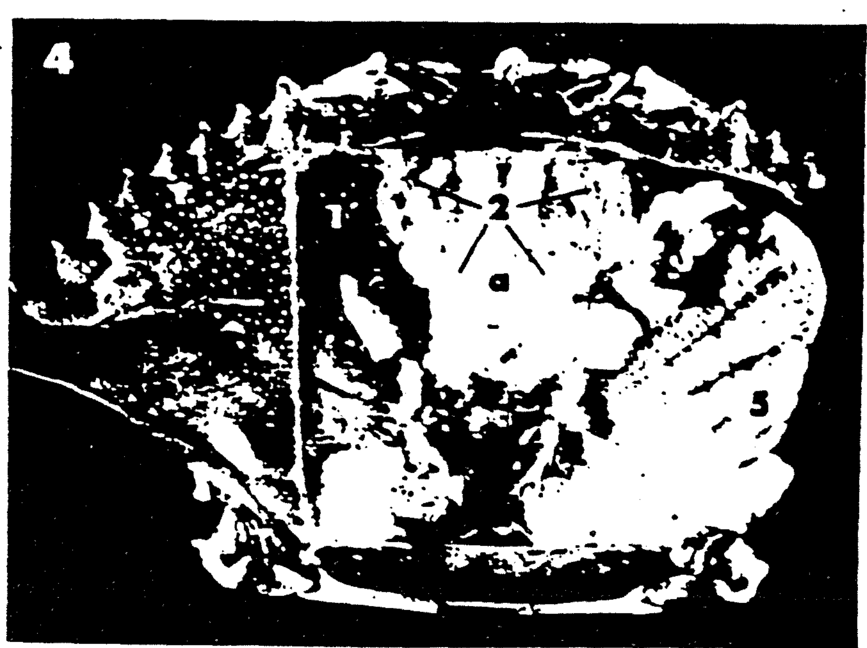


Fig 4. The medial part of the carapace and much of the epidermis (1) have been removed. The large cardiac stomach (2) is visible. The hemopoietic tissue lies on the posterior diamond-shaped portion (a). Two lateral ostia are visible in the semitransparent heart (3). Intermingled hepatopancreas and testes (4) and the gills (5) can also be seen.

NECROPSY FIELD DATA SHEET FOR HISTOLOGICAL SAMPLES

ADF&G, FRED Division Fish Pathology Lab

Collector/Address/Telephone #

Species

Number Specimens in Sample

Size Range

Life Stage

Date of Collection

Location of Collection (Site Name or Number)

Abnormalities Observed Per Specimen Number

APPENDIX 4: SHRIMP SAMPLING PROCEDURES

HISTOLOGICAL SAMPLE PREPARATION FOR SHRIMP

Histopathology Technical Group
(Taken from Bell and Lightner 1988)

NOTE: Only live or moribund shrimp will be suitable for processing. Histopathological changes caused by toxic chemicals are often very subtle at best. Tissues in dead shrimp autolyze very quickly and will mask these changes. Do not collect and process dead shrimp. Keep shrimp alive in containers of seawater if they must be transported to the processing site. Also, minimize the handling stress on the live shrimp to be preserved so that stress-mediated histological artifacts do not occur. Do not over-ice animals such that tissues freeze while in transit. Frozen tissues are worthless for histological examination.

1. The fixative to be used is 10% neutral buffered formalin (formula attached). Formalin should be handled wearing rubber or latex gloves.

2. The volume of fixative should be ten times the volume of the tissue. This is important since any less fixative may result in tissue autolysis and worthless samples. After 72 hours, shrimp specimens should be transferred to 70% ethyl alcohol for shipment and storage. This prevents tissues from becoming too hard and brittle when stored in fixative for long periods. Also, the fixative poured off may be saved and strained of tissue fragments and used one more time for other samples.

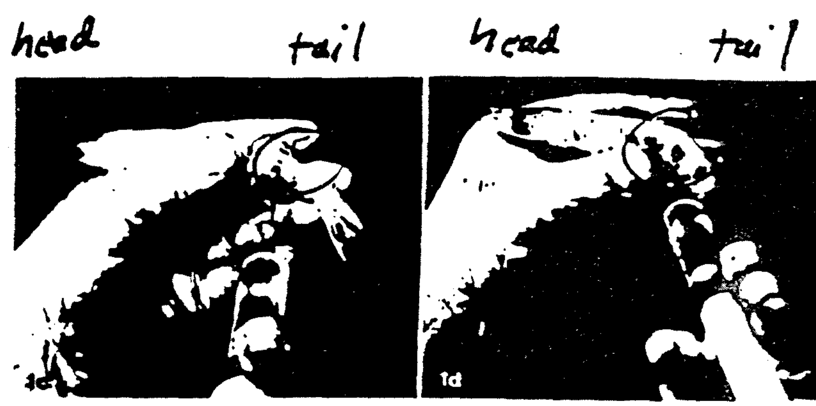
3. The sample size per site or species will be 20 shrimp, live or moribund.

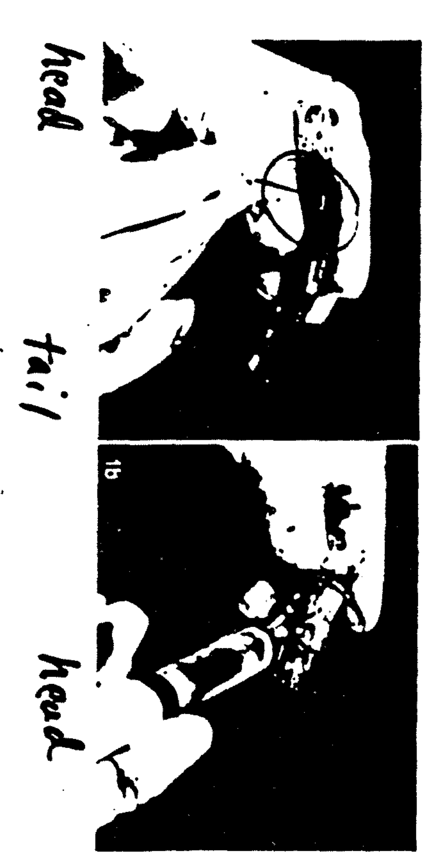
4. The chitinous exoskeleton of shrimp prevents adequate penetration of any fixative by simple immersion. Consequently, the fixative must be injected into strategic internal areas of each animal prior to dropping the whole shrimp into the fixative. Inject fixative into the living shrimp using a 10-ml syringe and appropriately sized needle, depending upon the size of the animal (small shrimp; i.e., small-gauge needle). This procedure is described by the following:

   a. First inject laterally into the hepatopancreas; i.e., cephalothorax region (Figure 1a).
   b. Then inject dorsally into the region anterior to the hepatopancreas; i.e., between the thorax and the eyestalks (Figure 1b).
   c. Inject the posterior abdominal region (Figure 1c).
   d. Inject the anterior abdominal region (Figure 1d).

   Inject more of the fixative into the hepatopancreas than the other sites but overall use about 5%-10% of the shrimp's body weight. All signs of life should disappear.

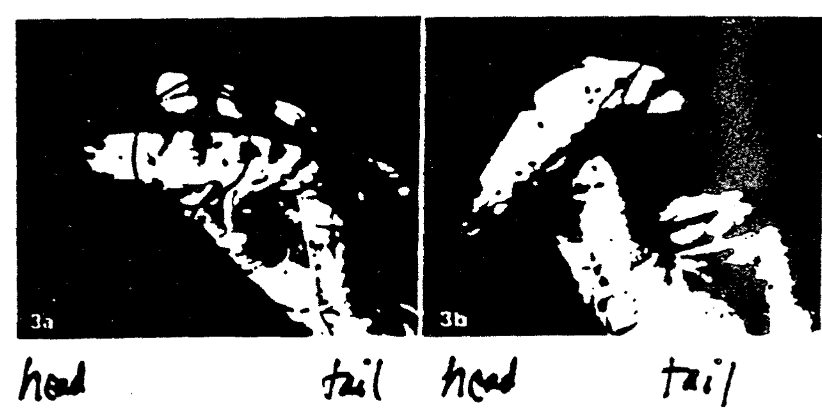
   e. Immediately after injection, slit the cuticle of the animal from the last (6th) abdominal segment to the base of the rostrum. The incision in the cephalothoracic region should be just lateral to the dorsal midline and that in the abdominal region should be mid-lateral (Figure 2). Do not cut too





4) Immediately following injection, slit the cuticle, with dissectin scissors, from the sixth abdominal segment to the base of th rostrum, paying particular attention not to cut deeply into th underlying tissue. The incision in the cephalothoracic regio should be just lateral to the dorsal midline, while that in th abdominal region should be approximately mid-lateral (Figure 2

5) Shrimp larger than 12 grams, should then be transversely slit onc at the abdomen/cephalothorax junction (Figure 3a) or again mid abdominally (Figure 3b).
6) Following injection, incisions and bisection/trisection, immers the specimen in the remainder of the fixative.



NECROPSY FIELD DATA SHEET FOR HISTOLOGICAL SAMPLES

ADF&G, FRED Division Fish Pathology Lab

Collector/Address/Telephone #

Species

Number Specimens in Sample

Size Range

Life Stage

Date of Collection

Location of Collection (Site Name or Number)

Abnormalities Observed Per Specimen Number

APPENDIX 5: MAMMAL SAMPLING PROCEDURES

Records

All carcasses for necropsy will be recorded in a necropsy logbook maintained in the necropsy area, utilizing a stanardized format.

Each carcass will be assigned an individual accession number identified in logbook. The case and accession numbers will be used to identify all tissue samples and records pertaining to the case. Each accession will also be assigned a FWS seisure tag number. The diagnostician will identify the location on the front side of the tag and fill in the top line on the reverse side of the tag to start the chain of custody. The tag becomes part of the permanent necropsy record. Any other identification numbers or markings must be clearly recorded in the logbook and necropsy record.

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.

Gross necropsy

A standard complete and detailed gross necropsy will be performed on sea otters in good postmortem condition.

Body weight of each carcass will be recorded; other weights and measurements should be taken when appropriate for documentation of necropsy findings.

Three minimum standardized tissue collection protocols will be followed for all carcasses as outlined below. The diagnostician should also collect additional pertinent samples as indicated by abnormalities noted during the necropsy exam.

Tissue collection for hydrocarbon analyses

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 PETROLEUM PRODUCTS!

Instrutments used to collect tissues for hydrocarbon analyses should be scrubbed in detergent and rinsed in acetone, then hexane if a chemical fume hood is available. These chemicals are toxic; avoid breathing fumes or contact with skin. If a hood is not available, rinse instruments in 70% ethanol. Do not touch tissue samples with gloves or hands. Select clean central portions of organs to sample to avoid inadvertent contamination. A minimum of 10 g. is required; a larger sample is preferred. A separate duplicate sample of each tissue should be taken.

(Reminder: If lesions are present, additional samples may be selected for laboratory workup at the discretion of the diagnostician.)

Samples should be 2 cm. thick in all dimensions if anatomically possible; intestine samples should be at least 10 cm in length and should not be opened longitudinally. Avoid crushing or puncturing the sample in order to maintain a clean core in the specimen. Samples should be placed individually in a whirl-pac bag, indelibly labelled with the case and accession numbers, species, tissue type, and laboratory (bacteriology, virology or parasitology). Samples should be chilled immediately and stored frozen, preferably at ultra-low temperatures, as soon as possible.

Parasite specimens removed from tissue can be fixed in 10% buffered formalin in containers indelibly marked with the case and accession number, species, and tissue site in which the parasite was found. Formalin-fixed parasites can be stored at room temperature.

DRAFT

Specimen Storage Log

(Use separate sheets for freezer vs. cabinet-stored specimens.)

Case Number	Species	Number x Tissue Stored	Date Stored	Date Removed

To: TED MEYERS
ADF&G
FRED DIVISION
FAX # 465-4168

FROM: Dr. Larry Duffy
Institute of Arctic Biology
UofA - Fairbanks
FAX - 474-7204

SAMPLING AND PRESERVING SPECIMENS FOR LABORATORY EXAMINATION

All specimens collected for laboratory examination should be labeled. The label should be easy to find and information should include the animal it came from and its sex and age, the date of collection, locality, and name of the person who collected it.

1. BLOOD SERUM

If possible, a blood sample should be taken for future serologic tests. This can be done only if the animal has very recently died or if it is euthanized for examination. Blood should be collected in blood tubes that do not contain anticoagulant; if these are unavailable, a clean glass jar may be used. Depending on the animal, the blood can be acquired by cutting ~~a~~ a ~~jugular~~ vein ~~in the neck~~, draining it from the heart, or, if these sources fail, from any large blood vessel containing blood that is cut during the postmortem examination. It is important to prevent bacterial contamination of whole blood samples because bacteria reproduce rapidly in blood serum and destroy its diagnostic value. At lease 20 ml should be collected.

Whole blood should be preserved by refrigeration, not by freezing. If it can be delivered to a laboratory within 48 hours, no further processing is necessary. If transport time will be greater than 48 hours, serum (the clear fluid portion) should be removed from the clot (the clumped blood cells). Frequently this can be accomplished by carefully pouring part of the serum into a second container. An eye dropper or pipette will serve better than pouring to transfer serum.

APPENDIX 6: AVIAN SAMPLING PROCEDURES

Necropsy Protocol for Birds

Records

All carcasses for necropsy will be recorded in a necropsy logbook maintained in the necropsy area, utilizing a stanardized format.

Each carcass will be assigned an individual accession number identified in logbook. The case and accession numbers will be used to identify all tissue samples and records pertaining to the case. Each accession will also be assigned a FWS seizure tag number. The diagnostician will identify the location on the front side of the tag and fill in the top line on the reverse side of the tag to start the chain of custody. The tag becomes part of the permanent necropsy record. Any other identification numbers or markings must be clearly recorded in the logbook and necropsy record.

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.

Gross necropsy

A standard complete and detailed gross necropsy will be performed on birds in good postmortem condition.

Body weight of each carcass will be recorded; other weights and measurements should be taken when appropriate for documentation of necropsy findings.

Three minimum standardized tissue collection protocols will be followed for all carcasses as outlined below. The diagnostician should also collect additional pertinent samples as indicated by abnormalities noted during the necropsy exam.

Tissue collection for hydrocarbon analyses

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 PETROLEUM PRODUCTS!

Instrutments used to collect tissues for hydrocarbon analyses should be scrubbed in detergent and rinsed in acetone, then hexane if a chemical fume hood is available. These chemicals are toxic; avoid breathing fumes or contact with skin. If a hood is not available, rinse instruments in 70% ethanol. Do not touch tissue samples with gloves or hands. Select clean central portions of organs to sample to avoid inadvertent contamination. A minimum of 10 g. is required; a larger sample is preferred.

(Reminder: If lesions are present, additional samples may be selected for laboratory workup at the discretion of the diagnostician.)

Samples should be 2 cm. thick in all dimensions if anatomically possible; intestine samples should be at least 10 cm in length and should not be opened longitudinally. Avoid crushing or puncturing the sample in order to maintain a clean core in the specimen. Samples should be placed individually in a whirl-pac bag, indelibly labelled with the case and accession numbers, species, tissue type, and laboratory (bacteriology, virology or parasitology). Samples should be chilled immediately and stored frozen, preferably at ultra-low temperatures, as soon as possible.

Parasite specimens removed from tissue can be fixed in 10% buffered formalin in containers indelibly marked with the case and accession number, species, and tissue site in which the parasite was found. Formalin-fixed parasites can be stored at room temperature.

APPENDIX 7: CHAIN-OF-CUSTODY RECORD

ADF&G Prince William Sound Oil Assessment Chain-of-Custody Form

VAL-89-

SERIAL #

ADF&G, FRED Division
Fish Pathology Lab
P.O. Box 3-2000
Juneau, AK 99802-2000
Phone: (907) 465-3577

Page _____ of _____

Note: Use ballpoint pen, waterproof ink (e.g., Rapidograph) or fine-tip waterproof marker

Date Collected	Sample # (collector's)	Assigned # (leave blank)	Type (tissue, water, sediment, etc.)	Location Collected	Latitude	Longitude	Remarks
5							
10							
15							
20							

(continue on back of page)

CHAIN OF CUSTODY

Samples collected by ____________ (print name) of ____________ (agency) ____________ (signature) ______ (date)

Transferred to ____________ (print name) of ____________ (agency) at ____________ (place) ____________ (signature) ______ (date)

Transferred to ____________ (print name) of ____________ (agency) at ____________ (place) ____________ (signature) ______ (date)

Transferred to ____________ (print name) of ____________ (agency) at ____________ (place) ____________ (signature) ______ (date)

Transferred to ____________ (print name) of ____________ (agency) at ____________ (place) ____________ (signature) ______ (date)

Transferred to Ted Meyers of ADF&G at Juneau ____________ (signature) ______ (date)

APPENDIX 8: MATERIALS LIST

IRP FIELD SUPPLIES AND EQUIPMENT

Item	Check	Quantity	Vendor and Cat #
Microscope slides			Baxter, #M6146
Syringes - 1 cc			Baxter, #S9501-18
Syringe needles, 22 gx, 1-1/2 in			Baxter, #89549-22J
Syringe needles, 20 gx, 1-1/2 in			
Scissors, 6-1/2 in			Baxter, #D2655-1A
Scissors, 5 in			
Scissors, 4-1/2 in			VWR, #25608-203
Dissecting forceps, 5-1/2 in			VWR, #25718-100
Dissecting forceps, 4-1/2 in			VWR, #25715-043
Specimen forceps, 8 in			
Tissue forceps, 4-1/2 in			Baxter, #D2567-1A
Hemostat, 5 in			Baxter, #D2680-1A
Utility scissors			
Bone cutters			Baxter, #D2576
Scalpel handle, no. 6			
Scalpel handle, no. 5			
Scalpel blades, <no. 20			
Scalpel blades, no. 12			
Scalpel blades, no. 10			Baxter, #D2865-10
Dissecting tray			
Tissue cassettes			Baxter, #M7321-33
10% Formalin w/ Na acetate			
Lab markers			
2L Nalgene Bottles			Baxter, #B7541-64
Marker II's			Baxter, #P1220
Slide boxes			VWR, #48450-006
Single-edge blades			
Formalin (37%)			Baxter, #5016-4NY
Sodium Acetate			VWR, #JT3450-11
Prepared 10% buffered formalin			Baxter, #H121-4NY

Project Title: Histopathological Studies of Injury Assessment for Birds, Mammals, Finfish, and Shellfish Exposed to the Oil Spill in Prince William Sound

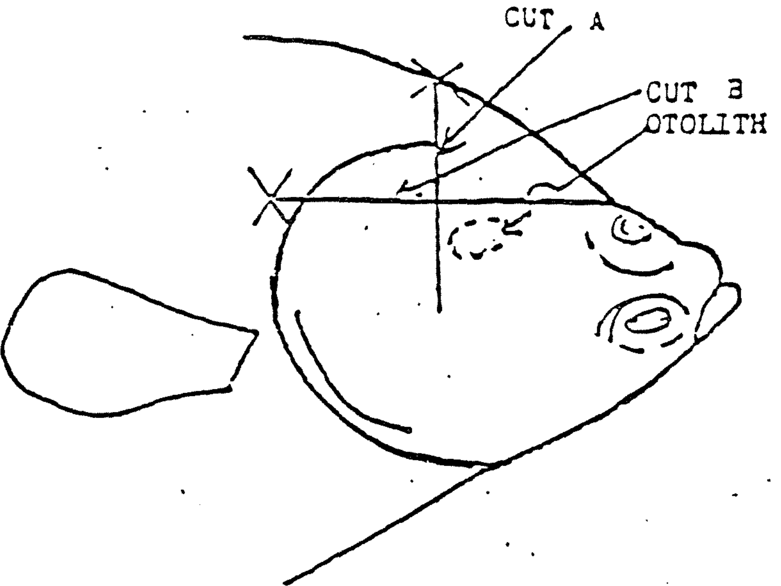
Brief Flowsheet for Field Sample Collection

1. Collect animals from a specific site.
2. Necropsy.
3. Take tissue samples for the following analyses from the same animal, if possible, or from the same subset in the collection.
   a. Hydrocarbon analysis
   b. Histology
   c. Other (?)
4. Send hydrocarbon sample set to the National Marine Fisheries Service Auke Bay Laboratory in Juneau for storage and later analysis.
5. Send histology sample set to the Alaska Department of Fish and Game, FRED Division repository in Anchorage for storage and later analysis.
6. Other samples, if any, are sent to their respective storage site for later analysis.
7. Don't forget chain-of-custody paperwork to accompany each sample set.

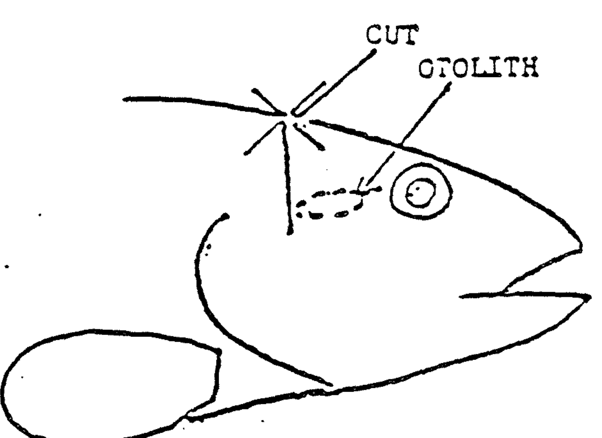
APPENDIX H

CLYCERIN SOLUTION FOR FLATFISH OTOLITHS

Mix 1/4 tsp Thymol crystal into 1/4 tsp ethol alcohol (95% strength, not denatured). In a separate container mix 375 ml of glycerol with 375 ml of slightly warm or tepid water. Finally mix the thymol solution into the water-glycerol solution.



FLATFISH



ROUNDFISH AND ROCKFISH

Fig. 1 Approximate location of the otoliths (sagitta) and the cut f the removal of otoliths from flatfish, roundfish and rockfis (Use either cut A or Cut B for flatfish)

At least one field blank and replicate sample should be taken for each collection site, batch of samples or 20 samples taken. (A field blank is a sample container opened in the field, closed and stored as if it contained a sample. A replicate sample is a second sample from the same site.) Rinsate blanks should be taken if appropriate.

5.1 Water - The method must be described or adequately referenced in sampling SOPs. Recommended sample size is 1-4 liters depending on the analytical methodology.

Water samples for volatiles analyses should be taken in 40 ml amber vials with no head space or bubbles.

5.2 Sediment - Any accepted methods of collecting undisturbed surface sediment samples such as box cores, hand corers, or grabs may be used. The method must be described or adequately referenced in sampling SOPs. Recommended sample size is 10-100 grams (a 4 oz. jar).

5.3 Tissue - Organisms to be analyzed for petroleum hydrocarbons should be freshly killed or recently dead. Decomposed organisms are rarely of any value for analysis.

Whole organisms may be stored in solvent-rinsed glass jars or wrapped in solvent-rinsed aluminum foil.

Tissue sections may be taken either on site from freshly killed organisms or in the laboratory from carefully collected and preserved - cold or frozen - whole organisms. Tissue should include flesh and internal organs, especially liver. Recommended sample size is 10-15 grams.

Tissue samples need to be protected from external contamination at time of collection. Contents of the intestinal tract, external slime coating, contaminated collecting utensils, etc. are all potential sources of contamination when collecting internal tissue samples.

All instruments used in handling samples must be made of a non-contaminating material (e.g. stainless steel, glass, teflon, aluminum) and solvent-rinsed between each sample collection.

Instruments used for exterior dissection must not be used for internal dissection.

Avoid hand contact with tissue sample.

Collect stomach and intestinal tract last.

Bird eggs are wrapped in solvent-rinsed aluminum foil and transported by any convenient means that will prevent breakage. They should be opened or refrigerated as soon as possible. Eggs are opened by cutting them with a solvent-rinsed scalpel or by piercing the air cell end and pouring/pulling the contents out. Avoid including pieces of egg shell with the contents or touch-

ing the contents with your hands. Total weight, volume (measured or calculated), length, width and contents weight must be recorded for each egg. Bile is collected by removing the gall bladder, puncturing it with a scalpel fitted with a new #11 blade, and collecting the contents in a 4 mL amber glass vial.

6. Sample Preservation and Holding Time

Samples must be kept cool, i.e. on ice.

Samples that are to be frozen, sediment and tissue, should be frozen quickly and rapidly. That is, these samples should be frozen as soon after collection as possible and the freezing process should be rapid.

Frozen samples must be kept frozen, at -20°C or less, until extracted or prepared for analysis. Repeated freezing and thawing of samples can destroy the integrity of the samples resulting in questionable data or the loss of data.

6.1 Water - All water samples must be immediately extracted with methylene chloride or preserved with HCl to pH<2. If preserved, water samples are stored in the dark at 4°C and extracted within 7 days. All extracts must be stored in the dark in air tight chemically clean containers until analysis.

6.2 Sediment and Tissue - Samples should not be extracted until immediately before analysis; if there is a lag between sample extraction and sample analysis, extracts must be stored in air tight containers kept in the dark at 4°C.

7. Sample Shipping

All samples, except water samples, must be kept frozen throughout the shipping process.

Samples must be packaged to prevent breakage. Glass jars should be individually wrapped so that they will not contact each other if padding shifts in transit (which styrofoam chips do). Bubble wrap or the divided boxes that new jars are shipped in work well. Pack samples in insulated containers (e.g. ice chests) with enough frozen mass to remain frozen in transit.

It is the responsibility of the sample shipper to arrange for sample receipt. Do not send samples off without arranging for pickup and storage.

To insure that samples are not compromised, shipment should not be initiated later in the week than Wednesday nor should samples be shipped in any week in which there is a holiday.

Shipments must comply with Department of Transportation regulations.

8. Chain-of-Custody Procedure

Samples must be kept in such a manner that they cannot be altered either deliberately or accidentally. Any indication that a sample has been subjected to tampering or physical alteration could disqualify it as evidence for possible

legal action.

The field sampler is personally responsible for the care and custody of the samples collected until they are transferred under chain-of-custody procedures.

A sample is considered in "custody" if:

it is in your actual physical possession or view;

it is retained in a secured place (under lock) with restricted access

or it is placed in a container and secured with an official seal(s) such that the sample cannot be reached without breaking the seal(s)

Evidence tape or sample seals are used to detect unauthorized tampering of samples following sample collection. The seal must be attached in such a way that it is necessary to break it in order to open the container. Seals must be affixed to the container before the samples leave the custody of sampling personnel.

All samples must be accompanied by a chain-of-custody record or field sample data record (Figure 1). When samples are transferred from one individual's custody to another's, the individuals relinquishing and receiving the samples will sign and date the chain of custody record. This record documents the transfer of custody of samples from the sampler to another person or to a specified analytical laboratory.

Shipping containers must be custody-sealed for shipment. The seal must be signed before the container is shipped. The chain-of-custody record must be dated and

signed to indicate any transfer of the samples. The original chain-of-custody record accompanies the shipment; a copy is retained by the sample shipper. If samples are sent by common carrier, copies of all bills of lading or air bills must be retained as part of the permanent documentation.

Whenever samples are split, a separate chain-of-custody record is prepared for those samples and marked to indicate with whom the samples are being split.

N.M.F.S. Prince William Sound Oil Assessment Chain of Custody Form

VAL-89-

Project ____________________

Serial #

Page ______ of ______

NOTE: Use ballpoint pen, waterproof ink (eg Rapidograph) or fine-tip waterproof marker

NMFS Auke Bay Fisheries Laboratory
Box 210155, Auke Bay AK 99821
For information contact Sid Korn
(907) 789-6021 or 789-6000

Date Collected	Sample # (collector's)	Assigned # (leave blank)	Type (tissue, water, sediment, etc)	Location Collected	Latitude	Longitude	Remarks

Continue on back of page

Chain of Custody

Samples collected by ______________ (print name) of ______________ (agency) ______________ (signature) (date)

Relinquished by ______________ (signature) (date) at ______________ (place) ______________ (comments)

Recieved by ______________ (print name) of ______________ (agency) at ______________ (place) ______________ (signature) (date)

Relinquished by ______________ (signature) (date) at ______________ (place) ______________ (comments)

Recieved by ______________ (print name) of ______________ (agency) at ______________ (place) ______________ (signature) (date)

Relinquished by ______________ (signature) (date) at ______________ (place) ______________ (comments)

Recieved by ______________ (print name) of ______________ (agency) at ______________ (place) ______________ (signature) (date)

APPENDIX G

Appendix G

HISTOPATHOLOGY TECHNICAL GROUP FOR OIL SPILL ASSESSMENT STUDIES IN PRINCE WILLIAM SOUND, ALASKA

Member Organizations:

U.S. Department of the Interior
U.S. Department of Commerce
Alaska Department of Fish and Game

Group Members:

Dr. Theodore R. Meyers (chair), ADF&G, Juneau, AK
Dr. J. Christian Franson, USF&WS, Madison, WI
Dr. Roger Lee Herman, USF&WS, Leetown, WV
Dr. Bruce B. McCain, NOAA/NMFS, Seattle, WA
Dr. Albert K. Sparks, NOAA/NMFS, Seattle, WA

APPENDIX 1: FINFISH SAMPLING PROCEDURES

HISTOLOGICAL SAMPLE PREPARATION FOR FISH

Histopathology Technical Group

NOTE: Only live or moribund fish will be suitable for processing. Histopathological changes caused by toxic chemicals are often very subtle at best. Tissues in dead fish autolyze very quickly and will mask these changes. Do not collect and process dead fish. Keep fish alive as long as possible during transport to the site of necropsy. Do not over-ice fish such that tissues freeze while in transit. Frozen tissues are worthless for histological examination.

1. The fixative to be used is 10% neutral buffered formalin (formula attached). Formalin should be handled wearing rubber or latex gloves.

2. The volume of fixative should be ten times the volume of the tissue. This is important since any less fixative may result in tissue autolysis and worthless samples. After 77 hours, the formalin fixative may be poured off and replaced with 70% ethyl alcohol for storage and transport. This accomplishes an important objective; i.e., it prevents tissues from becoming too hard and brittle when stored in fixatives for long periods. Also, the fixative poured off may be saved and strained of tissue fragments and used one more time for other samples.

3. The sample size per site or species will be 20 fish, live or moribund.

4. Fish less than 3 cm may be fixed whole by dropping into preservative.

5. Fish 4 cm-10 cm should have the belly slit with a scalpel or scissors, the intestine detached at the vent, and the internal organs pulled out slightly for proper fixative penetration.

6. Larger fish (11 cm-20 cm) will require on-site excision of 0.5-cm sections of major tissues and internal organs (attached diagram) as listed. Do not send whole fish.

   Excise: Whole head detached from just behind the opercular opening, liver, spleen, GI tract (anterior intestine, stomach, pyloric cecae, posterior intestine, and rectum), air bladder, kidney (anterior and posterior), heart, gonads. Also, take a 0.5-cm square of musculature and attached skin intersected by the lateral line midway between the head and tail on the right side of the fish. Take a second 0.5-cm section of muscle and skin from the body wall covering the viscera from the right side of the fish.

   Organs and tissue samples from a single fish should be placed in tissue processing cassettes, 4 to 5 tissue samples to one cassette. Each cassette must be labelled with the animal number from which it was taken. Place cassettes in a jar of fixative.

   Fish larger than 20 cm will also require that 0.5-cm portions of each major organ be utilized (if larger than 0.5 cm) and the whole head will be eliminated from the sample. In this case the first right gill arch must be excised and fixed before

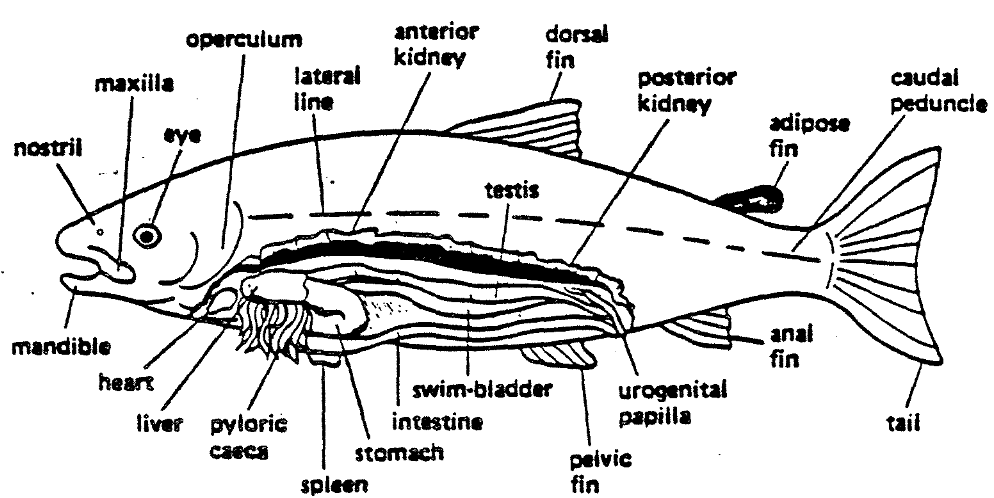


Fig. 2.6. Diagram of the basic anatomy of a salmonid fish.

Appendix H

EQM&LO CHAIN-OF-CUSTODY PROCEDURES

Chain-of-Custody is necessary if there is a possibility that the conclusions based upon analytical data will be used in litigation. The components of chain-of-custody are : sample seals, a field log book, chain-of-custody record, and the Request for Laboratory Services (RLS); the procedures for their use are described in the following sections.

Due to the evidentiary nature of samples collected during enforcement investigations, possession must be traceable from the time samples are collected until they or their derived data are introduced as evidence in legal proceedings. To maintain and document sample possession, chain-of-custody procedures are followed.

Admissibility of Analyses as Evidence. To be admissible as evidence, samples must be proved conclusively to be in an appropriate person's possession until the analyses resulting therefrom have been introduced as evidence. Rigid controls must be maintained to establish a chain-of-custody for the samples from the time of sampling until ultimate disposition of the particular case.

CUSTODY DEFINITION

A sample is under custody if:

If it is in your possession, or
It is in your view, after being in your possession, or
It was in your possession and you locked it up, or
It is in a designated secure area.

1. Evidence tape or sample seals are used to detect unauthorized tampering of samples following sample collection up to the time of analysis. The seal must be attached in such a way that it is necessary to break it ~~in order~~ to open the container. Seals must be affixed to containers before the samples leave the custody of sampling personnel.

2. Samples must be kept in such a manner that they cannot be altered wether deliberately or accidentally. Until the samples can be sent to the laboratory they should be kept in a cool, dark, dry place. Refrigeration, freezing or other chemical method of preservation are usually required. Chemical preservatives are added at the laboratory.

   Any indication that a sample has been subjected to tampering or physical alteration could disqualify it as evidence for possible legal action. Therefore, the instructions given herein must be followed strictly.

opening. A evidence tape is placed on the openings of the shipping container, signed and dated.

Sample tags and custody forms must be legible and filled out using waterproof, non-fading ink. Secure individual sample containers or group of sample containers using tamperproof evidence tape or seals.

4. Maintain an up-to-date Field Data Record Logbook. Record field measurements and other pertinent information necessary to refresh the sampler's memory if, later on, he/she takes the stand to testify regarding his/her actions during the evidence gathering activity. Maintain a separate set of field notebooks for each survey; store them in a safe place where they can be protected and accounted for at all times.

5. The field sampler is responsible for the care and custody of the collected samples until they are properly dispatched to the receiving laboratory, or turned over to an assigned custodian. The field sampler should verify that each container is in his/her physical possession or in his/her sight at all times, or is locked so that no one can tamper with it.

6. Colored slides or photographs are often taken to show the outfall sample location and any visible water pollution. Written documentation on the back of the photo should include the photographer's signature, and the time, date and site location. These photographs can be used as evidence, and are handled by chain-of-custody procedures to prevent alteration.

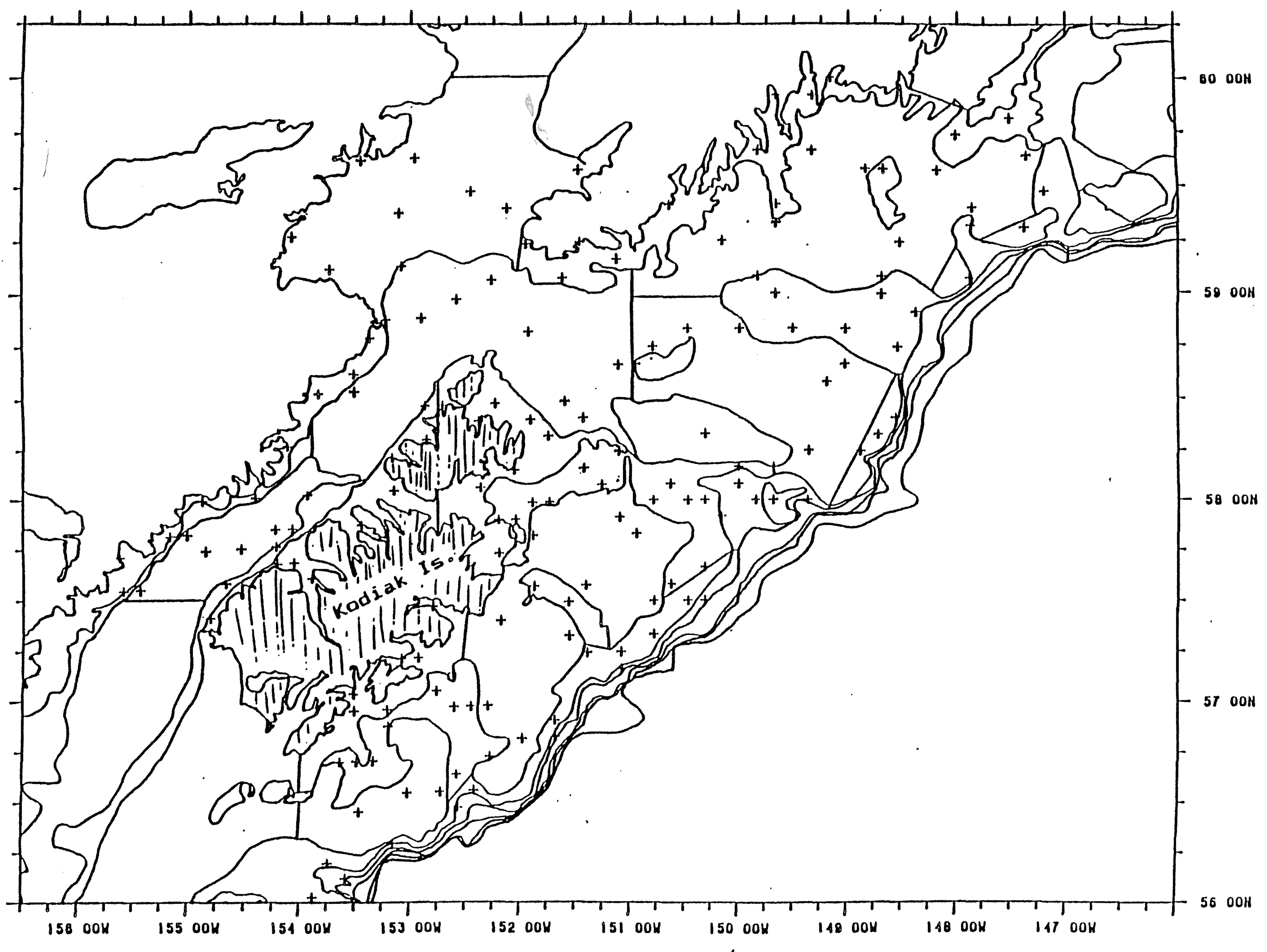
TRANSFER OF CUSTODY AND SHIPMENT

1. Samples are accompanied by a Request for Laboratory Services which has a chain of custody section. When transferring the possession of samples, the individual relinquishing and receiving the samples will sign, date and note the time. This record documents sample custody transfer from the sampler, often through another person, to the laboratory Sample Custodian.

2. Ensure that samples are properly packed in shipping containers (for example, ice chests) to avoid breakage. Ensure that shipping containers are sealed for shipment to the laboratory.

3. If the package is sent by the US mail, ensure that it is sent with a return receipt. If the package is hand-delivered, note that it was hand carried in the method of shipment block in the chain of custody record. Send field receipts from the post office and bills of lading to the laboratory custodian for retention as part of the permanent chain of custody documentation.

APPENDIX I



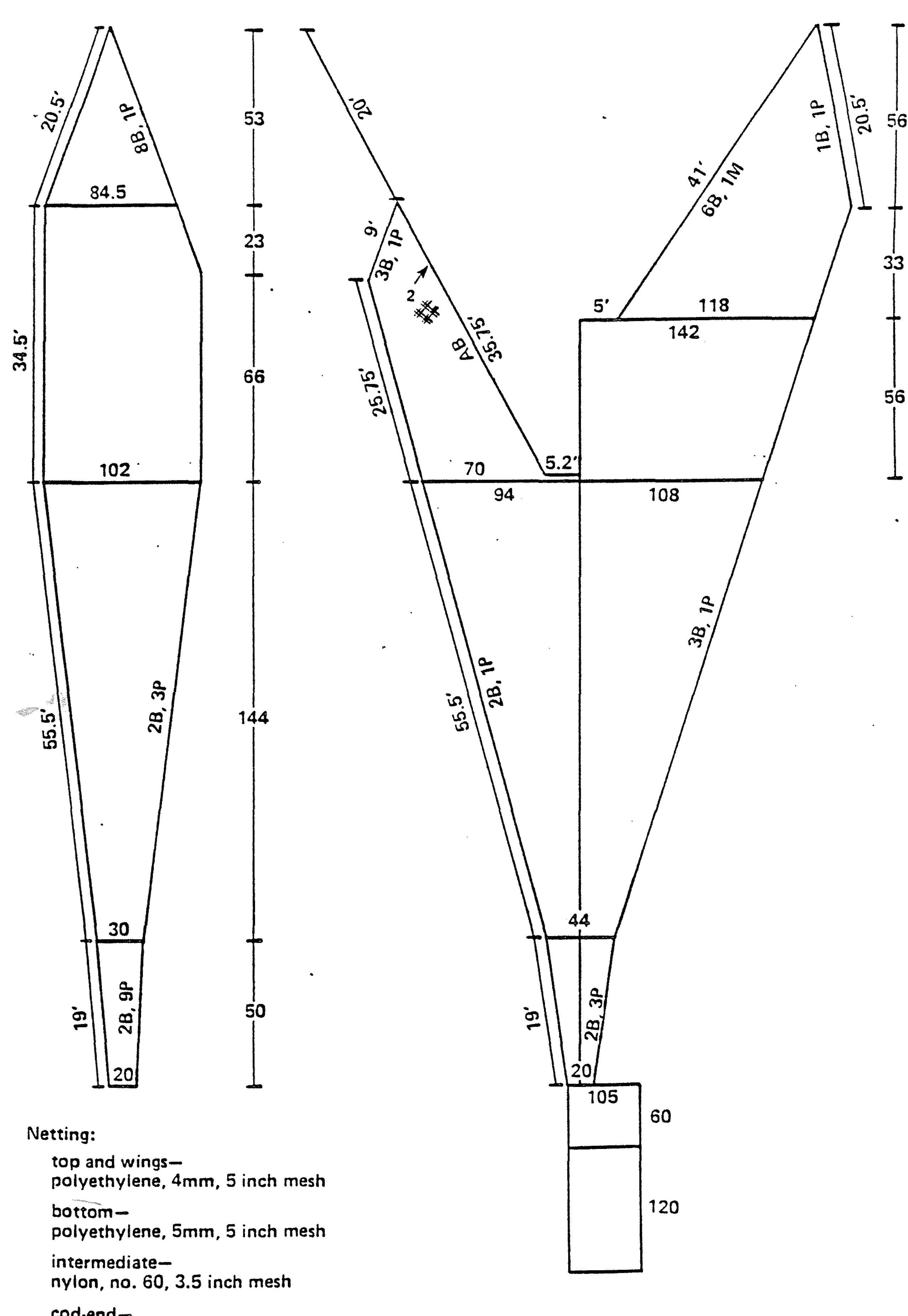
APPENDIX I

Figure 1. Gulf of Alaska 1989 survey area with sampling station locations.

APPENDIX J

APPENDIX J, FIGURE 1

POLY-NOREASTERN



Netting:

top and wings—
polyethylene, 4mm, 5 inch mesh

bottom—
polyethylene, 5mm, 5 inch mesh

intermediate—
nylon, no. 60, 3.5 inch mesh

cod-end—
nylon, no. 96, 3.5 inch mesh

APPENDIX K

APPENDIX K, FIGURE 1

ON-DECK SAMPLING FORM – SUBSAMPLE CALCULATIONS

Vessel ________________ Cruise ________________ Haul No. ________________

Split Weights	1.	2.	3.	4.	5.	Total
Dynamometer						
– Bag weight						
– Debris removed before subsampling						
= Animal Weight						

Calculation Of Percent Processed

1. In cases where the proportions of fish and crab processed are the same:

$$\text{Percent of fish and crab processed} = \frac{\text{weight of fish and crab processed}}{\text{total weight of catch}} \times 100\%$$

$$= \frac{\qquad\qquad}{\qquad\qquad} \times 100\%$$

= [%]

2. In cases where some items in the catch are completely processed, but only a fraction of the fish are processed:

(a) Total weight of catch (A) = ____________

(b) Weights of items completely processed: Crab = ____________

Halibut = ____________

Other = ____________

Total (B) = ____________

(c) Total weight of all items (including debris) processed in groundfish subsample (C) = ____________

(d) Percent of fish processed $= \frac{C}{A-B} \times 100\% =$ [%]

APPENDIX K, FIGURE 2

HAUL-POSITION FORM

POSITION FORM

VESSEL [2 3 4] ________ CRUISE [6 7 8] HAUL [10 11 12]

YEAR [14 15] MONTH [16 17] DAY [18 19] YOUR NAME ____________

	LATITUDE DEGREE	MIN.	LONGITUDE DEGREE	MIN.
POSITION START...	20 21 22 (+)	23 24 26 27	28 29 30 31	32 33 35 36
END.....	37 38 39 (+)	40 41 43 44	45 46 47 48	49 50 52 53

"+" = WEST
"-" = EAST
COL. 28 & 45

	RATE	FIRST READING	RATE	SECOND READING
LORAN START	55 56	58 59 60 61 62 64 65	67 68	70 71 72 73 74 76 77
LORAN END...	79 80	82 83 84 85 86 88 89	91 92	94 95 96 97 98 100 101

---------- DUP. COL. 1-36 FROM ABOVE

HAUL FORM

GEAR DEPTH WTD. AVG. (FM) [37 38 39 40] MIN ________ MAX ________

BOTTOM DEPTH WTD. AVG. (FM) [41 42 43 44] MIN ________ MAX ________

TIME STANDARD USED ________

TIME START ________ OUT ________

EQUIL. HOUR [46 47] DURATION (HRS.) [48 49 51 52] EQUILIBRIUM ________

HAUL ________

DISTANCE FISHED (N.M.) [54 55 57 58] HAUL TYPE [60 61] IN ________

STRATUM [62 63 64] WTD. AVG. TRACE DEPTH (FM) [65 66 67 68] MIN ________ MAX ________ DESCR. ________

WATER TEMPERATURES (DEGREES C.) SURFACE [70 71 73] GEAR [75 76 78] METHOD ________

BOTTOM TYPE [83 84] DESCR. ________

WIRE OUT (FM) [85 86 87 88] GEAR TYPE [94 95 96] DOOR + ACCES. [97 98 99]

SUBSAMPLE METHOD [101]

PERFORMANCE [103] ________ WEATHER, SEA CONDITIONS ________

REMARKS

DP-001 86-01-07

APPENDIX K, FIGURE 3

ON-DECK SAMPLING FORM — SPECIES COMPOSITION

Page ______ Of ______

Vessel ______________ Cruise ______________ Haul No. ______________

Date __________ Haul Type --- Regular Other (circle) Surface (bucket) Temperature __________ C°

1. If the trawl sample was subsampled, check box here ☐ and then enter data and computations on back side of page.

2. If the same portion of all species was processed, enter the percent here ☐☐☐☐ %

Otherwise enter percents for each species on next lines ↓

Species name and code	Non-sub-sample basket weights	Sub-sample basket weights	Sub-sample numbers	Perce

79-3-10

When data have been recorded on terminal, mark an 'X' here ◯

APPENDIX K, FIGURE 4

SPECIES CATCH FORM

DP-002 83-01-15

VESSEL [1 | 2 | 3] CRUISE [5 | 6 | 7] HAUL [9 | 10 | 11]

YOUR NAME ______________________

SPECIES NAME	CODE (13 14 15 16 17)	WEIGHT TO A TENTH LB (19 20 21 22 23 . 25)	NUMBER (27 28 29 30 31 32)	COMMENTS

APPENDIX K, FIGURE 5

SPECIMEN FORM

VESSEL [1 2 3] ____________ CRUISE [5 6 7] HAUL [9 10 11]

STRATUM [13 14 15] SPECIES CODE [17 18 19 20 21] SPECIES NAME ____________

FREQUENCY [36: 1] SUBSAMPLE TYPE [48] WEIGHT DETERMIN. [49] AGE STRUCTURE [50] AGE DETERMIN. [51]

MATURITY TABLE [59 60] YOUR NAME ____________ DATE ____________

23 SEX	25 MATUR	28 29 30 31 LENGTH IN MM.	38 39 40 41 42 WEIGHT (GRAMS)	45 46 AGE	53 54 55 56 57 SPECIMEN NUMBER	62 63 64 65 66	68 69 70 71 72

APPENDIX K,
FIGURE 6

DP-003 83-01-15

LENGTH-FREQUENCY FORM

VESSEL [1 2 3] ________ CRUISE [5 6 7] HAUL [9 10 11]

DATE ________ TOTAL WEIGHT (LB.) ________ SUBSAMPLE WEIGHT (LB.) ________

SPECIES CODE [13 14 15 16 17] SPECIES NAME ________

YOUR NAME ________ SUBSAMPLE TYPE [32] LENGTH TYPE (IF NOT FORK LEN.) [33 34]

19 20 21 22 23	26 27 28	30	19 20 21 22 23	26 27 28	30	19 20 21 22 23	26 27 28	30
LENGTH IN MM.	FREQUENCY	SEX	LENGTH IN MM.	FREQUENCY	SEX	LENGTH IN MM.	FREQUENCY	SEX
0			0			0		
0			0			0		
0			0			0		
0			0			0		
0			0			0		
0			0			0		
0			0			0		
0			0			0		
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0			0			0		
0			0			0		
0			0			0		
0			0			0		

APPENDIX L

APPENDIX L, FIGURE 1

148° 147° 146°

61° 40' 20'

Valdez
Glacier Is
Bligh Is
Naked Is
Cordova
Hawkins Is
Hinchinbrook Is
Seal Rk.
Knight
Grn
Montague Is

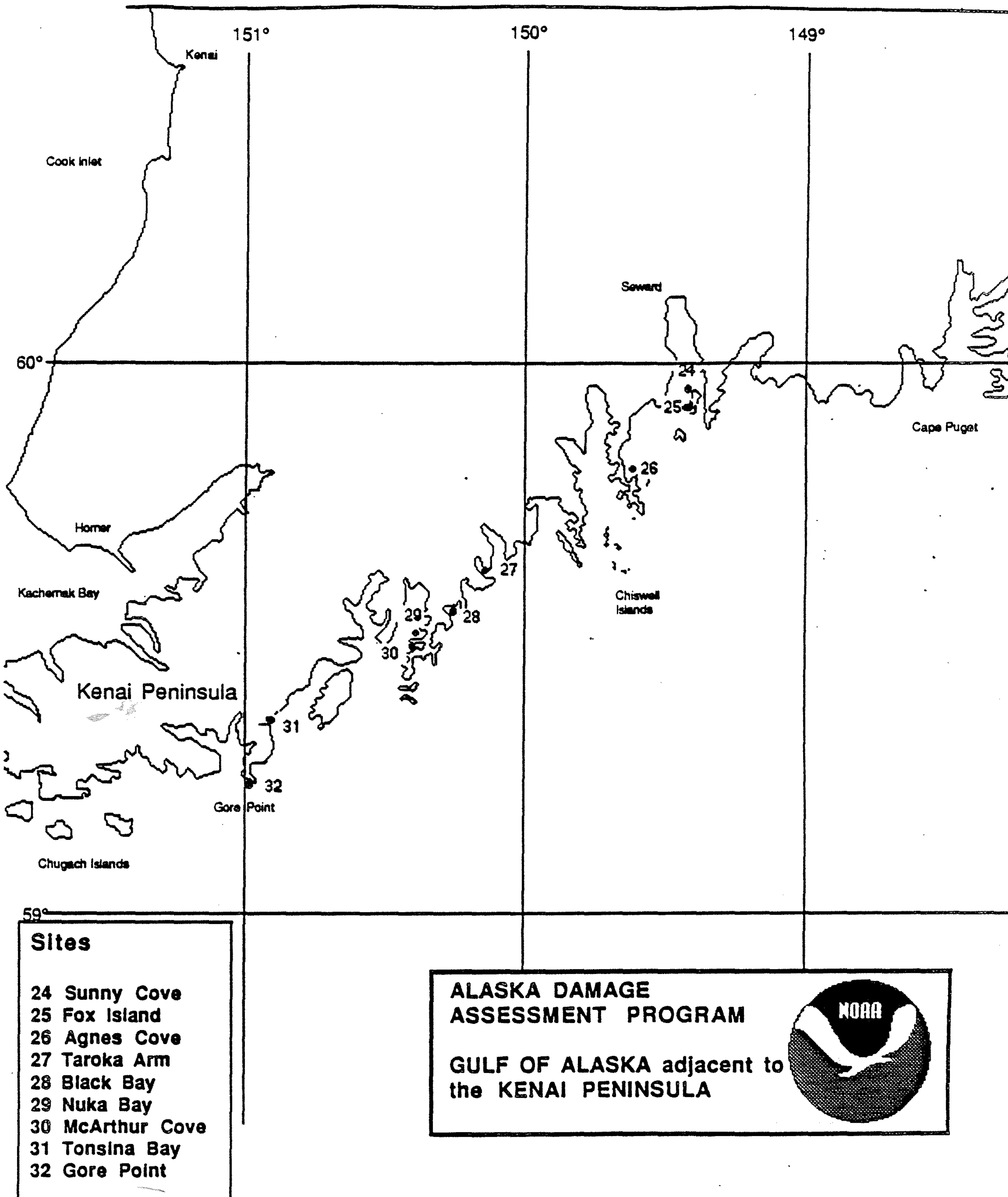
Sites

1 Port Valdez
2 Columbia Bay
3 Bligh Island
4 Olsen Bay
5 Knowles Bay
6 Cabin Bay
7 Outside Bay
8 Northwest Bay
9 Disk Island
10 Smith Island
11 Herring Bay
12 Eshamy Bay
13 Bay of Isles
14 Rocky Bay
15 Green Island
16 Snug Harbor
17 Mummy Island
18 Shelter Bay
19 Iktua Bay
20 Sleepy Bay
21 Sawmill Bay
22 Evans Island
23 Fox Farm

ALASKA DAMAGE ASSESSMENT PROGRAM

PRINCE WILLIAM SOUND

APPENDIX L, FIGURE 2



APPENDIX L, FIGURE 3

ALASKA DAMAGE ASSESSMENT PROGRAM

LOWER COOK INLET

NOAA

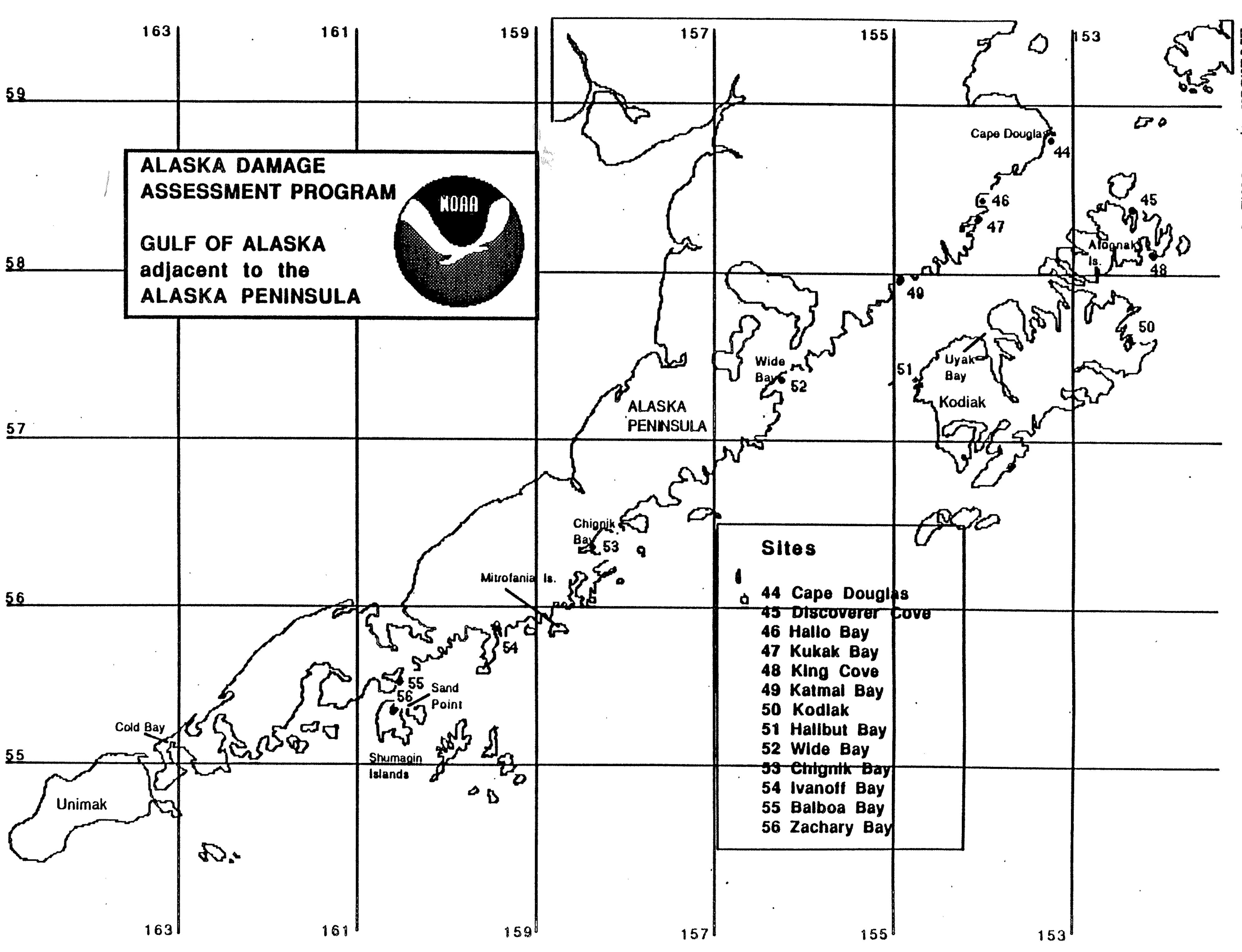
152° 151° 153° 60° 59°

East Foreland
Kenai
Kalgin Is
Tuxedni Bay
Chinitna Bay
Anchor Point
Homer
37
Kachemak Bay
38
36
Seldovia
Augustine Is
Port Graham
English Bay
Nuka Bay
39
Kamishak Bay
Port Chatham
34
35
33
Gore Pt
32
40
Chugach Is
42
41
Barren Islands
Cape Douglas
Shuyak Is
43

Sites

32 Gore Point
33 Port Dick
34 Windy Bay
35 Chugach Bay
36 Seldovia Bay
37 Kachemak
38 Ursus Cove
39 Amakdedori Beach
40 Kamishak
41 Douglas Beach
42 Ushagat Island
43 Andreon Bay

APPENDIX L, FIGURE 4



APPENDIX M

Appendix M

Table 1. Fish species to be sampled and analyses to be performed (P = present study by 2/90; F= future study) in Fish/Shellfish Study 24.

Fish Species	Method of Capture	Types of Analyses[a] Bile	AHH	Tissue AHs	PPL	Path	Repro
Flathead sole	otter trawl	P	P	F[b]	P	F	
Yellowfin sole	otter trawl	P	P	F[b]	P	F	F[f]
Rock sole	otter trawl	P	P	F[b]			
Juvenile salmon[c]	beach seine	P[d]	P[d]	F[e]		F[d]	
Adult salmon[c]	gill net	P	P	F[b]	P		
Dolly Varden	beach seine/ gillnet	P	P	F[b]	P		F[f]
Pacific halibut	long line	P	P	F[b]			
Pacific cod	trawl	P	P	F[b]			
Pollock	trawl	P		F[b]			
sablefish	trawl	P		F[b]			
Tanner crab	trawl	P					

[a] Selected samples will be analyzed.

[b] Analyses of liver and muscle tissues will usually be conducted on samples from fish which have significant levels of bile metabolites or AHH activity.

[c] A number of salmon species were collected including pink, silver, chum and red.

[d] Bile, AHH, and histopathological analyses will be conducted only on fish of sufficient length.

[e] Fish that are too small for bile or AHH analyses will be composited and analyzed by routine tissue analyses.

[f] Including ovarian histology, plasma estradiol and alkaline-labile phosphate (vitellogenin).

APPENDIX N

MILESTONE CHART

	1989									1990	
	A	M	J	J	A	S	O	N	D	J	F
MAJOR MILESTONES											
Collect samples aboard NOAA ship FAIRWEATHER		X	X	X	X	Δ					
Analyses of bile for metabolites				X	X	X	X	X	X	X	X
Analyses of liver for AHH and P-450 activity							X	X	X	X	X
Analyses for DNA damage							X	X	X	X	X
Submission of report											Δ

Activity underway X
Completion date Δ

DRAFT

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT DETAILED STUDY PLAN

Project title:	Injury to Scallop Resources in Kodiak Waters
Study I D Number:	Fish/Shellfish study number 25
Lead Agency:	State of Alaska, ADF&G Fisheries Rehab, Enhancement and Development (FRED) Division
Cooperating Agencies:	Federal: NMFS
Principle Investigator:	Michael Kaill, Fishery Biologist
Assisting personnel:	Lorne White, Fishery Biologist Larry Peltz, Fishery Biologist One Fishery Biologist YTD
Date Submitted:	October 12, 1989

	Signature	Date
Principle Investigator:	[signature]	10/11/89
Supervisor:	[signature]	10/11/89
Consulting Biometrician		
OSIAR Senior Biometrician:		
OSIAR Program Manager:	[signature]	10/13/89
OSIAR Director:		

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DETAILED STUDY PLAN

Project title:	Injury to Scallop Resources in Kodiak Waters
Study I D Number:	Fish/Shellfish study number 25
Lead Agency:	State of Alaska, ADF&G Fisheries Rehab, Enhancement and Development (FRED) Division
Cooperating Agencies:	Federal: NMFS
Principle Investigator:	Michael Kaill, Fishery Biologist
Assisting personnel:	Lorne White, Fishery Biologist Larry Peltz, Fishery Biologist Chris Pace, Fishery Biologist
Date Submitted:	October 12, 1989

	Signature	Date
Principle Investigator:	__________	______
Supervisor:	__________	______
Consulting Biometrician	__________	______
OSIAR Senior Biometrician:	__________	______
OSIAR Program Manager:	__________	______
OSIAR Director:	__________	______

INTRODUCTION

The goal of this project is to measure the extent to which the Exxon-Valdez oil spill affected the scallop resources of the Kodiak area. Weathervane scallops form the basis of a valuable commercial fishery based primarily out of the Kodiak area. This resource may have been exposed to oil as a result of the Exxon Valdez oil spill. In addition, cooperative projects between the State of Alaska, the Japanese Government, the Kodiak City and Borough, and the Kodiak Native Association have pursued pilot projects on scallop mariculture in the Kodiak area for the past three years. The projects are located at sites around Kodiak Island. The participants of these efforts have spent a total of about $2 million. These programs, as well as the traditional weathervane dragger scallop fishery, have been put at risk by the Exxon Valdez oil spill.

This damage assessment project will assemble existing information to provide baseline data on growth and survival of scallops. In addition, test stations will be deployed in areas believed to be still exposed to hydrocarbon pollution. This will aid in determining of the extent of uptake during the spill, as well as effects of chronic exposure on growth and survival.

OBJECTIVES

1. Compare growth and survival of scallops cultured in oiled areas with growth and survival of scallops cultured in non-oiled areas.[1]

2. Measure hydrocarbon uptake, depuration, and recovery in scallops. The hypothesis is that the level of oil contamination in scallops is not related to the level of oil in a site. The experiment is designed to detect a difference of 1.4 standard deviations in hydrocarbon content with the probability of making a type I and type II error of 0.05 and 0.10, respectively.

3. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.[2]

---

1 This objective will not be conducted until April 1990 because this is the beginning of the scallop growing season and because uniform spat will not be available until that time.

2 Methods by which this objective will be completed will be addressed only after injury is documented.

METHODS/DATA ANALYSIS

This project will consist of three separate activities:

1. establish a data base on growth and survival of scallops for future reference and comparative purposes;

2. compare scallops in oiled and non-oiled environments to determine:
   a. uptake of hydrocarbons; and,
   b. growth and survival; and,

3. survey hydrocarbon contamination of scallops.

Data Base

There is an extensive data base on growth and survival of cultured scallops in Kodiak (State of Alaska et al. 1989). The first activity of this project will be to standardize the data in an R:Base format, develop growth and survival curves, and use this to evaluate ongoing performance of scallops in culture. The data available is for the pink and spiny scallops, *Chylamys rubida* and *C.hastata*, respectively.

Growth/Survival Comparisons

Scallop spat will be deployed in standard test units at three heavily oiled sites, three moderately oiled sites, and three non-oiled sites. Three test units will be randomly set at each sample site (Figure 1). Each test unit will be a standard 12 mm mesh, 10 layer Japanese lantern net rearing cage (Bourne et al. 1989). It will be anchored 1 m above the bottom in 10 - 30 m of water and suspended with a submerged "trawl" float. A ground line or other means of locating the unit will be attached to the cage. Each unit will be seeded with 18 to 20 mm scallop seed obtained from wild scallops. The seed will be provided by a local harvester of seed from an area which is known to be uncontaminated with oil. Seeding rates will be 50 animals per layer for a total of 500 animals per cage. One triplicate hydrocarbon sample (three 10 - 15 g samples) will be taken from the initial lot of scallop seed before it is deployed to stations in Kodiak waters.

Data and samples will be taken at each site at monthly intervals including: temperature, salinity, water transparency (productivity of plant material), number of live and dead scallops, and shell length (the greatest distance from the hinge) during the growing season (April - October/November). During the winter, samples will be taken every three months. All animals from every other layer will be measured; layers to be measured will be alternated during

each sampling period. The maximum number (before mortalities) to be sampled from each cage is 250 scallops (50 specimens X 5 layers), and from each site is 750 scallops (250 X 3 cages per site).

The number of live and dead scallops in each sampled level will also be counted. All dead shells will be measured to record approximate time of death. After being measured, all dead scallop shells will be discarded. All data will recorded on a field data form (Appendix A). Measurements will be made with a digital caliper, which allows direct input to the memory of a portable micro computer. Temperature will be recorded automatically with a solid-state temperature recorder (Ryan tempmentor brand) fastened to the cage at level five.

Twice a year (during June and December) a composite sample of 15 g of scallop tissue will be taken to evaluate hydrocarbon content. Scallops will be randomly selected from all layers to make up this sample. Hydrocarbon sampling and analyses will be as described in the hydrocarbon section.

Hydrocarbon Surveys

Existing Farms:

This part of the project will establish the extent of injury, and determine rates of recovery from that injury. Pink and spiny scallops will be sampled approximately bimonthly at two non-oiled stations, each of which have existing mariculture activity. The sample sites are:

Amook Island, in Uyak Bay
Trident Basin, near Kodiak.

An alternative site for one of the two will be Akhiok at Alltak Bay if costs, time, and logistics dictate. Sampling at these mariculture sites will be conducted by collecting six hydrocarbon samples from each site for a total of 12 hydrocarbon samples from non-oiled sites. These samples will function as controls. The six samples will be taken from six cages randomly selected within each site. Within each cage, six scallops will be selected at random from among the ten possible cage levels. Scallops between 4 and 7 cm in length will be selected to assure uniformity in scallop size. This is necessary because depuration rates of hydrocarbons may vary with scallop size.

Specimens for each hydrocarbon sample will be placed together in a factory sealed teflon coated glass jar. Each container will be labelled with the site name, longitude, latitude, date, species, name of sampler, "scallops" and "ADF&G". Samples will be frozen and transferred to the NMFS Auke Bay Laboratory following rigorous and routine Chain-of-Custody procedures.

Hydrocarbon samples of wild pink and spiny scallops will also be obtained on a bi-monthly basis by divers at Izhut Bay which is an oiled area. Divers will choose scallops randomly at three separate sample sites in Izhut Bay. Four hydrocarbon samples of six scallops each will be collected at each site by randomly selecting scallops in four discrete areas along the bottom. Scallops will be between 4 and 7 cm in length and stored and transferred in the procedure described above.

A total sample size of 12 composite samples (of six scallops each) per impact level will allow the detection of differences in hydrocarbon content of 1.4 standard deviations with α and β levels of 0.05 and 0.10, respectively.

Commercial Fishery:

Weathervane scallops Patinopecten caurinus (a deep water dwelling species) will be sampled on one occasion from the commercial fishery. If hydrocarbons are detected in this sample, additional sampling will be conducted to measure rates of depuration.

Six whole, unshucked scallops will be placed in a precleaned, hydrocarbon-free 16 oz. or 32 oz sample jar or rinsed aluminum foil. Samples will be collected to provide at least 15 g tissue which is the amount necessary for hydrocarbon analyses. Triplicate samples will be taken from the commercial catch.

Sampling methods will be identical to those given above which follow the general guidelines set forth by the NOAA Auke Bay Lab and the Hydrocarbon Technical Committee both in written material and in training sessions.

Growth/Survival Sites:

Three sediment samples sufficient for hydrocarbon analysis will be taken at the beginning and end of the project from the bottom below each station. Bottom sediment will provide a reasonable index of the hydrocarbon content of the general location in the water column inhabited by the suspended scallops. In total, nine (3 samples X 3 sites per impact level) hydrocarbon samples will be processed for each impact level before and after the study. These samples will be analyzed for hydrocarbon content and used as indicators of oil contamination in the vicinity of the cages. A sample size of nine sediment hydrocarbon samples for each of three impact levels will be an adequate number to detect a difference of 1.9 standard deviations in hydrocarbon content with α and β levels of 0.05 and 0.10, respectively. Samples will be collected according to established guidelines for quality assurance and quality control set out by the Analytical Chemistry Operations Plan. The appropriate Chain of Custody will be followed as well.

DATA ANALYSES

Growth/Survival Comparisons

To examine scallop survival, the proportion of dead scallops to live scallops will be subjected to analysis of variance by site and by oil impact levels.

Differences in incremental growth between treatment levels will be compared with any historic data on scallop growth in the Kodiak area. Growth parameters will be determined for various growth curves, such as Gompertz, von Bertalanffy or polynomial equations. Growth parameters will be presented for the most appropriate growth models only. An analysis of variance on growth parameters obtained from fitting algorithms for scallop growth during the study will be compared with growth parameters obtained from historical data. Analysis of variance will also be used to compare the growth parameters obtained at each treatment level to detect differences in growth parameters due to oil contamination level. Graphics will be used to display differences in growth among areas including length at age for each site.

Hydrocarbon Surveys

Analysis of variance will be used to test for significant differences in concentrations of hydrocarbons in sediments and scallop tissue between sites and impact levels. Differences in levels of tissue hydrocarbons over time will also be tested. In cases where the use of parametric statistics is not possible, nonparametric statistical methods will be used to ascertain differences between sites. Pairwise comparisons (Tukey or Student's *t*) can also be used for this purpose.

SCHEDULES AND REPORTS

Dates	Activity
October 1989 to February 1990	Bi-monthly hydrocarbon sampling, data base analysis. Preliminary report will be prepared by December 21.
May 1990 to October 1990	Monthly sampling of comparative test sites. Continued sampling of scallops exposed to oil spill to establish depuration rates.

PROJECT BUDGET[1]

Line Item	Category	Budget
100	Personnel Services	$ 12,300
200	Travel	$ 2,500
300	Contractual	$ 18,900
400	Commodities	$ 2,000
500	Equipment	$ 11,500
700	Grants	$ 0
Total		$ 47,200

[1] Budget is for all activities performed from March 27, 1989 to February 28, 1990.

FUNDED PERSONNEL

Class	PCN	Name	PFT_mm	SFT_mm
FB II		C. Pace		3.0

LITERATURE CITED

Bourne, N., C. A. Hodgson, and J. C. Whyte. 1989. A manual for scallop culture in British Columbia. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1694. 215 pp.

State of Alaska, Kodiak Area Native Association, and Overseas Fishery Cooperation Foundation of Japan. 1989. Final report of the scallop mariculture feasibility study, Kodiak Island, Alaska 1987 - 1988. 126 pp.

Appendix A. Field data form.

ADF&G KODIAK SCALLOP PROJECT

DATE ____________________ SITE ____________________

SUBSITE ____________________ CAGE NUMBER ______________

WATER TEMPERATURE ______ (degrees C) WATER SALINITY ______ 0/00

WATER TRANSPARENCY ________ (meters) WATER COLOR ____________

LAYER NUMBER _________ RECORDER NAME ________________

NUMBER OF LIVE SCALLOPS ____________________

NUMBER OF DEAD SCALLOPS (0 - 20 mm.) ____________________

NUMBER OF DEAD SCALLOPS (21 - 30 mm.) ____________________

NUMBER OF DEAD SCALLOPS (31 - 40 mm.) ____________________

LENGTH FREQUENCY

SCALLOP NUMBER	LENGTH	SCALLOP NUMBER	LENGTH	SCALLOP NUMBER	LENGTH

DRAFT **CONFIDENTIAL**

STATE/FEDERAL NATURAL RESOURCE DAMAGE ASSESSMENT
DETAILED STUDY PLAN

Project Title: Sea Urchin Injury -- Assessment of Impacts of oil on Green Sea Urchins, *Strongylocentrotus droebachiensis*, in the Kodiak Island area.

Study ID Number: Fish/Shellfish Study Number 26

Lead Agency: State of Alaska, ADF&G; Commercial Fish Division

Cooperating Agency: Federal: NMFS
State: DEC

Principal Investigator: William Donaldson, Fishery Biologist III

Assisting Personnel: Forrest Blau, Fishery Biologist II
Two Non-Perm Fisheries Technicians

Date Submitted: October 1, 1989

	Signature	Date
Principal Investigator:	William Donaldson	9-21-89
Supervisor:	[illegible]	9-21-89
OSIAR Senior Biometrician:	----------------------	------
OSIAR Program Manager:	----------------------	------
OSIAR Director:	----------------------	------

INTRODUCTION

The goal of this project is to determine if the Exxon-Valdez oil spill has or will have a measureable impact on populations of green sea urchins, Strongylocentrotus droebachiensis, in the Kodiak Island area.

Green sea urchins occupy intertidal and subtidal habitats which may become heavily contaminated by hydrocarbons from oil spills. An oil spill could cause concentrations of hydrocarbons lethal to urchins, render the roe product unmarketable, smother or contaminate their primary food source (kelp and algae on rocks), or result in reproductive failures due to injuries to ovaries, lowered egg production, or developmental deformities in the eggs. Furthermore, oil may be toxic to larvae, and result in fewer recruits to the population in the future. This study will evaluate potential effects by comparing data obtained from study sites representing a range of oil contamination. Data from this project will provide quantification of any harvest foregone due to the oil spill.

OBJECTIVES

1. To estimate the relative abundance of green sea urchins in oiled and non-oiled areas.
2. To estimate the roe production of urchins in oiled and non-oiled areas such that differences of ± 5 % can be determined between the two impact levels 95% of the time.
3. To estimate the incidence of abnormalities in ovarian development in urchins in oiled and non-oiled areas such that differences of ± 5 % can be determined between the two impact levels 95% of the time.
4. To estimate recruitment of young urchins, as a percentage of all urchins in the sample area, in oiled and non-oiled areas.
5. To estimate the toxicity of crude oil to urchin larvae.
6. Identify potential alternative methods and strategies for restoration of lost use, populations, or habitat where injury is identified.

METHODS/DATA ANALYSIS

This project will be conducted in two phases, phase 1: field work and phase 2: laboratory work. Objectives 1 through 4; abundance estimates, roe production estimates, ovarian development

abnormalities, and recruitment estimates will be conducted wholy or partially in the field while the larval bioassy work (objective 5) and a portion of the ovarian development work will be conducted in a laboratory. The larval bioassys will be performed by contract with a separate laboratory while the ovarian development work will be conducted by the ADF&G. Objective 6 will consist of recommendations based on the results of objectives 1-5.

Study Sites

Four oiled and four non-oiled areas of green sea urchin habitat containing urchins will be selected for study. Oiled habitat will likely be selected in the Uyak Bay area if concentrations of urchins are located. Otherwise alternative sites will be found in Larson Bay. Non-oiled habitat will be selected adjacent to the Kodiak road system in the Chiniak bay area. Likely sites are Burkers Lagoon, Mayflower Beach, Happy Beach and "the notch" at Gibson Cove. Specific site data to be recorded includes: site name, site orientation (N-NE etc.), latitude, longitude, dominate substrate composition in percent, surface and bottom water temperature and salinity at the beginning, middle and end of each transect, weather conditions and wave action (Appendix A, Form 1 and Form 2).

Sample Design

Four transect lines will be established from mean low water to minus 20 meters or for a maximum distance of 90 meters at each of the eight study sites. The sites will be sampled twice during the egg maturation period (October-January). Transects marked at one meter intervals will be established perpendicular to the water's edge and parallel to each other along the bottom where urchins occur. Distance between transects will be determined on site by the distribution of urchins with a minimum distance of 15 meters between transects. Scuba divers will survey each transect within three meters on either side of the transect mid-line. Depth, presence of oil, kelp and kelp condition (alive/dead/oiled/unoiled) will be recorded on underwater slates within every one meter interval. Health (alive/dead), size, and position of every urchin encountered along each transect will also be recorded.

Along each transect, a random sample of ten mature female urchins will be collected, and the diameter, live weight, and roe weight of these animals will be measured.

A random sample of ovaries from 10 urchins at each site will be prepared for histological examination for abnormalities (Appendix A, Form 3). Ovaries will be broken open and preserved in 10% formalin (Dr. Ted Meyers ADF&G personal communication). Three random composite samples of three ovaries each will be randomly selected from transects in each area for hydrocarbon analysis according to the procedures outlined in Appendix B.

In the first year of the study, 20 live urchins will be shipped to

a contractor for laboratory bioassay experiments on toxicity of oil to urchin larvae. The urchins will be transported in a dry condition in ice chests lined with kelp and maintained as close to normal ambient temperature as possible. Additional information on the bioassay techniques and sample methodology is presented in Appendix C.

In addition to the stated objectives, attempts will be made to inspect sea urchins from each major commercial fishing area for the presence or absence of oil.

Data Analysis

Survey areas will be stratified into those impacted and not impacted by oil. Statistics for analysis of variance will be computed to assess any differences in hydrocarbon content, incidence of ovary abnormalities, changes in relative abundance and any differences in parameters describing relationships between ovary weight and urchin diameter or ovary weight and total weight. Trend analysis of the relative abundance of young of the year urchins will be used to detect potential recruitment failures associated with oil impact. Multivariate statistics (e.g. log linear models) may be used to identify more complex associations among the biological and physical parameters between oiled and non-oiled areas.

SCHEDULES AND REPORTS

Date(s)	Activity
October - December 1989	Phase I field sampling. Transect sampling should occur at the beginning and end of this period.
November - December 1989	Data entry and analysis
December 1989	Preliminary report on impacts of oil on sea urchins.

PROJECT BUDGET

Line Item	Category	Budget
100	Personnel Services	$ 14.0
200	Travel	1.0
300	Contractural	61.0
400	Supplies	6.0
500	Equipment	3.0
		$ 85.0

PERSONNEL

Name	Role	Mo
Bill Donaldson	Fishery Biologist	1.0
Forest Blau	Fishery Biologist	1.0
Susie Byersdorfer	Fishery Biologist	5.0
Mo Lambein	Fisheries Technician	1.0
Dianne Carney	Fisheries Technician	1.0

LITERATURE CITED

Meyers, T. Personal communication. Alaska Department of Fish and Game, Fisheries Rehabilitation and Economic Development Division, P.O. Box 3-2000, Juneau, Ak.

Appendix A. Form 1.

ADF&G URCHIN OIL PROJECT SITE DESCRIPTION FIELD FORM

Site Name: ______________________ Date: ______________________

Latitude: ______________________ Longitude: ______________________

Recorder: ______________________

Sampling Team	Agency
______________________	______________________
______________________	______________________
______________________	______________________

	Code	Description
Waves	________	______________________
Weather	________	______________________

Air Temp ______ Surface Sea Temp ______ Surface Salinity ______

	%	Code	Description
Adjacent Beach Substrate	______	______	______________
	______	______	______________
	______	______	______________

Beach Slope __________ Beach Orientation (e.g. N-NW) __________

NOAA Reference Point __________ NOAA Low Tide Height __________

Film Role Number __________ Photogragh Numbers ________ ________

Code	Waves Description	Code	Weather Description	Code	Substrate Description
1	Glassy	1	Clear	1	Mud-silt
2	Rippled	2	Partly Cloudy	2	Clay
3	Wavelets	3	Overcast	3	Sand
4	Slight 2-4'	4	Fog or Thick Haze	4	Granule (2-4mm)
5	Moderate 4-8'	5	Showers	5	Pebble (4mm-3cm)
6	Rough 8-13'	6	Squalls	6	Rock Fragments (3-6cm)
7	Very Rough 13-20'	7	Drizzle	7	Cobble Shingle
		8	Rain	8	Rock (15-25cm)
		9	Rain and Snow	9	Boulder (>25cm)
		10	Snow		
		11	Blizzard		

Appendix A. Form 2.

Site Name: ______________________ Date: ____________________

Transect No. (1-4): _______________ Recorder: ________________

Time: _____________

	MLW	Midpoint	-20m
	X-------------------------	X-------------------------	X
1. S. Temp.	_____	_____	_____
2. B. Temp.	_____	_____	_____
3. Depth	_____	_____	_____
4. S. Salinity	_____	_____	_____
5. B. Salinity	_____	_____	_____
6. Visibility	_____	_____	_____

Transect Length (meters): ______________

Substrate (%): _________________ Description: _______________

_________________ _______________

_________________ _______________

***DRAW DETAILED MAP ON REVERSE.

Appendix A, Form 3

NECROPSY FIELD DATA SHEET FOR HISTOLOGICAL SAMPLES

ADF&G, FRED Division Fish Pathology Lab

Collector/Address/Telephone #

Species

Number Specimens in Sample

Size Range

Life Stage

Date of Collection

Location of Collection (Site Name or Number)

Abnormalities Observed Per Specimen Number

Appendix B

September 12, 1989

STATE/FEDERAL DAMAGE ASSESSMENT PLAN

ANALYTICAL CHEMISTRY

COLLECTION AND HANDLING OF SAMPLES

FOR AGENCY USE ONLY
NOT FOR RELEASE
ATTORNEY WORK PRODUCT

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4. SAMPLING EQUIPMENT AND SAMPLE CONTAINERS

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   5.1 General
   5.2 Water
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6. SAMPLE PRESERVATION AND HOLDING TIME
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   6.2 Sediment and Tissue

7. SAMPLE SHIPPING

8. CHAIN-OF-CUSTODY PROCEDURE

1. Introduction

In response to the release of more than 10 million gallons of crude oil into Prince William Sound, the State of Alaska and four Federal Agencies, the Departments of Agriculture, Commerce and Interior and the Environmental Protection Agency are acting together to assess the damages to the natural resources. Authority for this action is provided by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and the Clean Water Act (CWA).

A damage assessment requires documentation of the exposure of the resources to oil released from the EXXON VALDEZ, identifying which resources were injured by that exposure, measuring the magnitude of the adverse affects on each resource over time and assigning economic values for that injury. Once this is done, monetary compensation can be sought from the potentially responsible parties to restore and/or replace the injured resources.

Recovery of monetary damages may involve civil court actions. It will then be necessary to prove that the samples were collected in a scientifically approved manner and that the samples were protected from outside contamination (non-incident related) and accidental mix-ups during handling and analyses. It is, therefore, extremely important that every sample be readily identified and their location and analytical status known and documented at all times.

This document and the associated training sessions, were prepared to assist field personnel in collecting samples that will provide scientifically sound and legally defensible data to support the State/Federal Natural Resource Damage Assessment for the EXXON VALDEZ oil spill.

2. Record Keeping and Documentation

Standard operating procedures (SOPs) for all sampling procedures, including chain of custody procedures; sampling protocols; cleaning and preparation of sample collection and storage devices; and labeling, handling, and sample

preservation and holding time must be written in detailed, clear, simple and easy to follow language.

Personnel must be knowledgeable and experienced in the described sampling techniques and must adhere to the SOPs.

Any changes in procedures must be recorded in detail in the field logbook. The log entry must include reasons that the change in procedure was unavoidable.

Field logbooks are issued by the Team Leader or their representative. The logbooks should be serially numbered, sturdy, bound books with sequentially numbered pages. Waterproof logbooks should be used if available.

Field data sheets, if used, must be consecutively numbered by project. The field data sheets must be referred to in entries in logbooks which reference, the precise data sheet involved and the relationship to specific data in the logbook noted.

All information pertinent to field activities, including descriptive notes on each situation, must be recorded in indelible marker in the field logbook. The information must be accurate, objective, up-to-date and legible. It should be detailed enough to allow anyone reading the entries to reconstruct the sampling situation. Additional information may be provided by field data sheets, sample tags or photographs.

Entries should be made in the logbook or on field data sheets with indelible marker at the earliest possible time. Notes should never be written on scrap paper and then transferred to the logbook.

Entries into field logbooks or field data sheets are signed or initialed, and dated by the person making the entry at the time of entry.

Each day's entries are closed out with a horizontal line, date and initial.

Errors in field logbooks or other records are corrected by drawing a single line though the error, entering the correct information and signing and dating the correction. Never erase an entry or any part of an entry.

Do not remove pages from the logbook.

Completed logbooks and field data sheets are returned to the Team Leader or their representative to be archived in a central location under chain-of-custody procedures until the Trustees indicate that they may be released.

3. Sample Identification and Labelling

A tag or label identifying the sample must be completed and attached to each sample. Waterproof (indelible) marker must be used on the tag or label. The minimum information to be included on the tag are the sample identification number, the location of the collection site, the date of collection and signature of the collector (who, what, where & when). This information and any other pertinent data such as the common and scientific names of the organism collected, the tissue collected and any remarks are recorded in the logbook. Field sample data sheets, photographs, any pertinent in-situ measurements (such as temperature, salinity, depth) and field observations are recorded in the logbook.

The location of the sampling site is determined with the aid of USGS grid maps, NOAA charts or navigational systems such as LORAN C. The site locations should be plotted on a chart of appropriate scale and photocopies incorporated into the logbook. In addition, a clear, detailed descriptive location as well

as the latitude and longitude, in degrees, minutes and seconds, of the collection site must be recorded in the logbook.

4. Sampling Equipment and Sample Containers

All sample containers must be either organic-free (solvent-rinsed) glass or organic-free (solvent-rinsed) aluminum foil. Lids for the glass containers must be lined with either teflon or solvent-rinsed aluminum foil.

Certified-clean glass jars are available from various vendors and if obtainable, may be used without cleaning.

Sample collection and storage devices are cleaned by washing with soap and hot water, rinsed extensively with clean water and then rinsed with either methylene chloride or acetone followed by pentane or hexane and allowed to dry before use.

First rinse: tap water, then re-rinse in distilled water.

Second rinse: methylene chloride or acetone

Third rinse (if acetone is used): pentane or hexane

The solvents (methylene chloride, acetone, pentane and hexane) used for cleaning sample collection and storage devices must be of appropriate quality for trace organic residue analysis and be stored in glass or Teflon containers, not plastic.

New glass jars or unused aluminum foil do no need to be washed with soap and

water. They must however, be solvent-rinsed as described above before use.

Glass jars may be cleaned by heating to 440°C for a minimum of 1 hour.

Clean glassware should be stored inverted or tightly capped with either solvent-rinsed aluminum foil or teflon-lined caps.

The dull side of the aluminum foil should be the side that is solvent-rinsed. Pre-cleaned squares may be stored with the clean sides folded together.

All equipment that comes in contact with the sample such as dredges or dissecting equipment must be solvent-rinsed before contacting each sample. Equipment should be steam-cleaned or washed with soap and hot water at the end of each day or between sampling locations.

5. Sampling Procedures

The method of collection must not contaminate the samples. Do not collect any subsurface samples through surface slicks. Do not collect any samples with oil-fouled equipment, such as nets or dredges. Do not touch or collect any sample with your bare hands.

Sample container volume must be appropriate to sample size; fill the jar to just below the shoulder. Overfilled jars will break when they freeze; underfilled jars will allow the sample to dry out.

At least one field blank and replicate sample should be taken for each collection site, batch of samples or 20 samples taken. (A field blank is a sample container opened in the field, closed and stored as if it contained a sample. A replicate sample is a second sample from the same site.) Rinsate blanks should be taken if appropriate.

5.1 Water - The method must be described or adequately referenced in sampling SOPs. Recommended sample size is 1-4 liters depending on the analytical methodology.

Water samples for volatiles analyses should be taken in 40 ml amber vials with no head space or bubbles.

5.2 Sediment - Any accepted methods of collecting undisturbed surface sediment samples such as box cores, hand corers, or grabs may be used. The method must be described or adequately referenced in sampling SOPs. Recommended sample size is 10-100 grams (a 4 oz. jar).

5.3 Tissue - Organisms to be analyzed for petroleum hydrocarbons should be freshly killed or recently dead. Decomposed organisms are rarely of any value for analysis.

Whole organisms may be stored in solvent-rinsed glass jars or wrapped in solvent-rinsed aluminum foil.

Tissue sections may be taken either on site from freshly killed organisms or in the laboratory from carefully collected and preserved - cold or frozen - whole organisms. Tissue should include flesh and internal organs, especially liver. Recommended sample size is 10-15 grams.

Tissue samples need to be protected from external contamination at time of collection. Contents of the intestinal tract, external slime coating, contaminated collecting utensils, etc. are all potential sources of contamination when collecting internal tissue samples.

All instruments used in handling samples must be made of a non-contaminating material (e.g. stainless steel, glass, teflon, aluminum) and solvent-rinsed between each sample collection.

Instruments used for exterior dissection must not be used for internal dissection.

Avoid hand contact with tissue sample.

Collect stomach and intestinal tract last.

Bird eggs are wrapped in solvent-rinsed aluminum foil and transported by any convenient means that will prevent breakage. They should be opened or refrigerated as soon as possible. Eggs are opened by cutting them with a solvent-rinsed scalpel or by piercing the air cell end and pouring/pulling the contents out. Avoid including pieces of egg shell with the contents or touch-

ing the contents with your hands. Total weight, volume (measured or calculated), length, width and contents weight must be recorded for each egg. Bile is collected by removing the gall bladder, puncturing it with a scalpel fitted with a new #11 blade, and collecting the contents in a 4 mL amber glass vial.

6. Sample Preservation and Holding Time

Samples must be kept cool, i.e. on ice.

Samples that are to be frozen, sediment and tissue, should be frozen quickly and rapidly. That is, these samples should be frozen as soon after collection as possible and the freezing process should be rapid.

Frozen samples must be kept frozen, at -20°C or less, until extracted or prepared for analysis. Repeated freezing and thawing of samples can destroy the integrity of the samples resulting in questionable data or the loss of data.

6.1 Water - All water samples must be immediately extracted with methylene chloride or preserved with HCl to pH<2. If preserved, water samples are stored in the dark at 4°C and extracted within 7 days. All extracts must be stored in the dark in air tight chemically clean containers until analysis.

6.2 Sediment and Tissue - Samples should not be extracted until immediately before analysis; if there is a lag between sample extraction and sample analysis, extracts must be stored in air tight containers kept in the dark at 4°C.

7. Sample Shipping

All samples, except water samples, must be kept frozen throughout the shipping process.

Samples must be packaged to prevent breakage. Glass jars should be individually wrapped so that they will not contact each other if padding shifts in transit (which styrofoam chips do). Bubble wrap or the divided boxes that new jars are shipped in work well. Pack samples in insulated containers (e.g. ice chests) with enough frozen mass to remain frozen in transit.

It is the responsibility of the sample shipper to arrange for sample receipt. Do not send samples off without arranging for pickup and storage.

To insure that samples are not compromised, shipment should not be initiated later in the week than Wednesday nor should samples be shipped in any week in which there is a holiday.

Shipments must comply with Department of Transportation regulations.

8. Chain-of-Custody Procedure

Samples must be kept in such a manner that they cannot be altered either deliberately or accidentally. Any indication that a sample has been subjected to tampering or physical alteration could disqualify it as evidence for possible

legal action.

The field sampler is personally responsible for the care and custody of the samples collected until they are transferred under chain-of-custody procedures.

A sample is considered in "custody" if:

it is in your actual physical possession or view;

it is retained in a secured place (under lock) with restricted access

or it is placed in a container and secured with an official seal(s) such that the sample cannot be reached without breaking the seal(s)

Evidence tape or sample seals are used to detect unauthorized tampering of samples following sample collection. The seal must be attached in such a way that it is necessary to break it in order to open the container. Seals must be affixed to the container before the samples leave the custody of sampling personnel.

All samples must be accompanied by a chain-of-custody record or field sample data record (Figure 1). When samples are transferred from one individual's custody to another's, the individuals relinquishing and receiving the samples will sign and date the chain of custody record. This record documents the transfer of custody of samples from the sampler to another person or to a specified analytical laboratory.

Shipping containers must be custody-sealed for shipment. The seal must be signed before the container is shipped. The chain-of-custody record must be dated and

signed to indicate any transfer of the samples. The original chain-of-custody record accompanies the shipment; a copy is retained by the sample shipper. If samples are sent by common carrier, copies of all bills of lading or air bills must be retained as part of the permanent documentation.

Whenever samples are split, a separate chain-of-custody record is prepared for those samples and marked to indicate with whom the samples are being split.

N.M.F.S. Prince William Sound Oil Assessment Chain of Custody Form

VAL-89-

Project ____________________

Serial #

NMFS Auke Bay Fisheries Laboratory
Box 210155, Auke Bay AK 99821
For information contact Sid Korn
(907) 789-6021 or 789-6000

Page ______ of ______

NOTE: Use ballpoint pen, waterproof ink (eg Rapidograph) or fine-tip waterproof marker

Date Collected	Sample # (collector's)	Assigned # (leave blank)	Type (tissue, water, sediment, etc)	Location Collected	Latitude	Longitude	Remarks

Continue on back of page →

Chain of Custody

Samples collected by ____________ (print name) of ____________ (agency) ____________ (signature) ____ (date)

Relinquished by ____________ (signature) ____ (date) at ____________ (place) ____________ (comments)

Recieved by ____________ (print name) of ____________ (agency) at ____________ (place) ____________ (signature) ____ (date)

Relinquished by ____________ (signature) ____ (date) at ____________ (place) ____________ (comments)

Recieved by ____________ (print name) of ____________ (agency) at ____________ (place) ____________ (signature) ____ (date)

Relinquished by ____________ (signature) ____ (date) at ____________ (place) ____________ (comments)

Appendix C

FISHERIES RESEARCH INSTITUTE
School of Fisheries
University of Washington
Seattle, Washington 98195

Circular No. 85-3

METHODOLOGY AND ANALYSIS OF SEA URCHIN EMBRYO BIOASSAYS

by

Paul A. Dinnel and Quentin J. Stober

November 6, 1985

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METHODOLOGY AND ANALYSIS OF SEA URCHIN EMBRYO BIOASSAYS

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1.0 Introduction

Echinoderm gametes and embryos have long been favorite laboratory tools of the developmental biologist because of their size, ease of collection and handling, and cosmopolitan occurrences. Since about 1970, however, have sea urchin embryos been used in an applied manner for testing and monitoring toxicants in marine waters. Preliminary work and methodologies were first described by Kobayashi (1971a) and Hagström and Lönning (1973). Many investigators have since used variations of these early methods but a recent "standardized" methodology could not be found in the published literature. Thus, a practical guide to the use of sea urchin and sand dollar embryos in marine toxicity testing of effluents and sediment elutriates was needed.

The objective of this paper is to present a recommended protocol for conducting echinoderm (sea urchin and sand dollar) embryo bioassays. The recommended protocol is a synthesis of the methods used by a wide variety of investigators (see bibliography) and based on experiences gained in our own laboratory. It gives special attention to the use of Pacific Northwest species and the analysis of test end-points which can range from a coarse, subjective evaluation of embryo morphology to sophisticated biochemical meausrements of DNA or pigment syntheses.

It should be noted that embryo tests are but one of several life stages which can be used for assessing water or sediment quality. Sperm/fertilization assays have been used by a variety of workers and the methodology for this type of assay has recently been developed (Dinnel et al. 1982), refined and validated (Dinnel et al. 1983 and in manuscript), and

applied to Puget Sound sewage effluents (Dinnel et al. 1984). This test has also been used to assess sediment elutriate toxicity. However, present data recommends against the use of the sperm/fertilization test for sediment elutriates (Ross et al. 1984; Malins et al. 1985) until the effects of interfering factors (i.e., high control responses in chemically clean control sediments) are resolved.

Adult urchins also provide valuable tools for toxicology. Besides direct acute toxicity tests, adult urchins have been used in a variety of sensitive behavioral tests using "righting behavior" (Axiak and Saliba 1981) and defensive reactions of the pedicellarie to starfish extracts (Johnson 1979). A promising new use of adult animals is in a sublethal exposure of the adults and subsequent testing of the success of the next generation with sperm/fertilization and embryo assays using the sex products previously exposed *in vivo*. Adult sand dollar exposure to sediments with subsequent spawning and embryo analyses holds special promise for a sublethal test for contaminated sediments. A generalized life cycle diagram for sea urchins and sand dollars is presented in Figure 1.

2.0 Methodology for Conducting Sea Urchin and Sand Dollar Embryo Bioassays

2.1. Test Species:

Three species are recommended for use in the Pacific Northwest for routine bioassays. Purple sea urchins (*Strongylocentrotus purpuratus*) can be collected intertidally at low tide on the Pacific Coast of Washington and in the Strait of Juan De Fuca as far east as Crescent Bay on the northern Olympic Peninsula. Purple urchins are the species of choice in California and Oregon and are usually ripe from December through about March in Washington waters. Green sea urchins (*S. droebachiensis*) are also winter spawners (ripe from

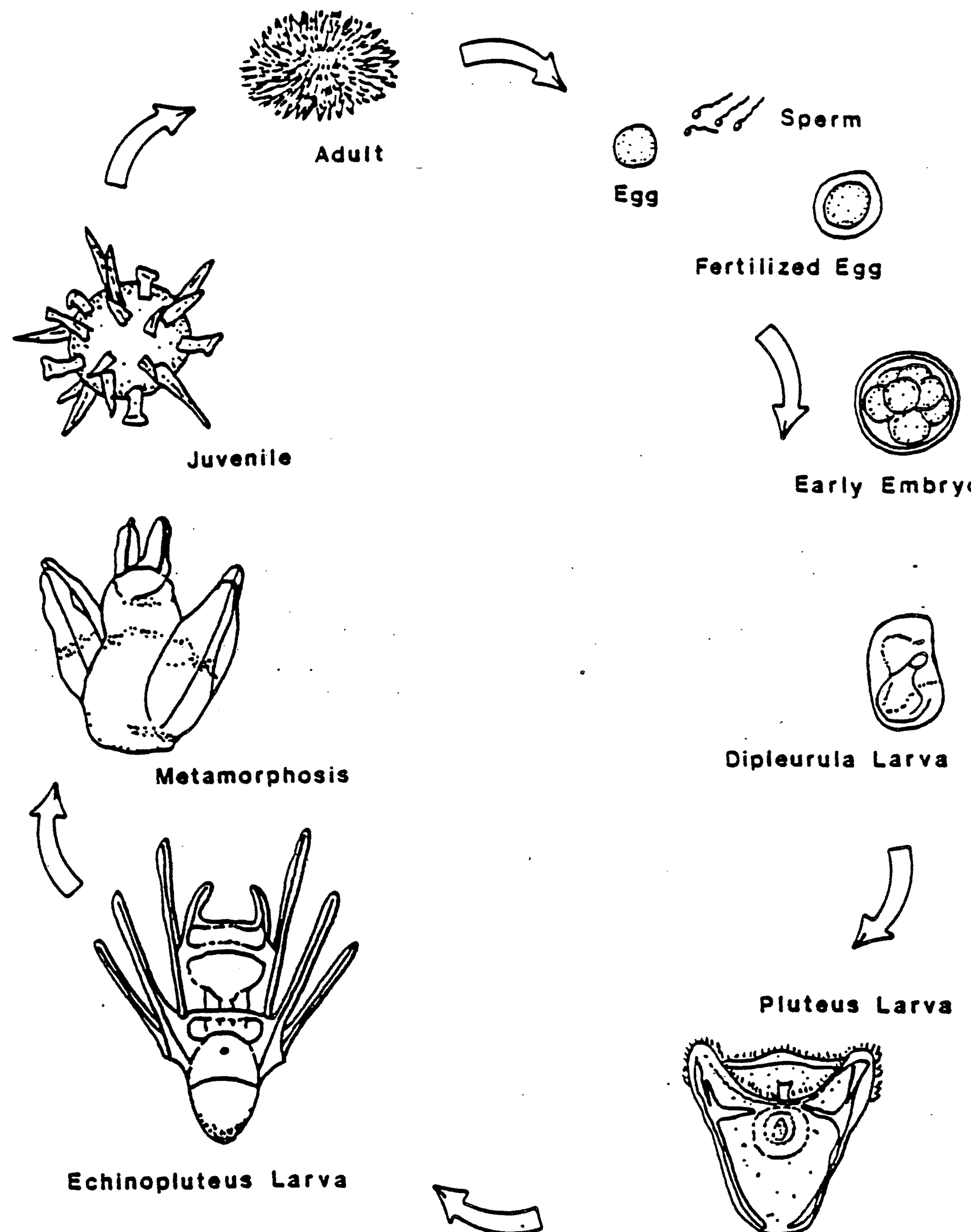


Figure 1. Diagram of the general life cycle of sea urchins and sand dollars. Adapted from Linfield et al. 1985.

about January through April) and are the dominant urchin in the inland waters of Washington, occurring in the shallow subtidal zone. Both of these species can be maintained in a ripe condition past their normal reproductive season by controlling temperature, light, food and salinity in a closed laboratory system. However, a better alternative is the use of sand dollars (Dendraster excentricus) which are summer spawners and normally ripe from approximately May through October. Sand dollars are plentiful on many beaches of the Washington Coast and the inland waters and can be easily collected intertidally at low tides.

2.2. Collection and Holding of Test Animals:

Sea urchins and sand dollars are best transported in a "dry" condition in ice chests lined with kelp or other moist material and maintained as close to their normal ambient temperature as possible. Immersion in water during transport should be avoided as epidemic spawning can occur.

Sea urchins can be held in a flowing seawater system if the seawater does not fluctuate greatly in temperature or salinity (minimum salinity 27 $^{o}/oo$). Many species of macroalgae (especially Nereocystis or Macrocystis) can be fed sea urchins several times a week although some species (e.g., Fucus) are not suitable.

Sand dollars are best held on a bed of sand in flowing seawater. Planktonic organisms in the seawater and detritus in the sand provide a reasonable source of food for this species. Caution must be observed with both urchins and sand dollars to avoid temperature shocks or changes in water levels (i.e., hydrostatic pressure fluctuations) as these can trigger spawning when the animals are approaching their natural spawn-out times.

2.3. Spawning:

Sexes are separate and indistinguishable prior to spawning except by needle biopsy. Hence, usually about three or four animals (or more depending on chance) are usually spawned at one time to assure a supply of both sperm and eggs. Urchins are easily spawned by inverting over a 150-250 ml beaker full of seawater and injecting 1 ml of 0.5 M potassium chloride (KCl) into the coelomic cavity through the peristomal membrane with a small syringe. Sand dollars should be handled in the same manner except that only 0.5 ml of KCl is injected at an angle through the oral opening. Ripe animals will usually start spawning within 5 minutes and be spawned out within about 20 to 30 minutes, an additional dose of KCl can be administered to "reluctant" animals but too forceful a spawning may yield over- or under-ripe gametes. Early in the season, spawned out animals can be returned to a holding tank (separate from unspawned animals) and can be expected to provide additional spawnings at 30-45 day intervals.

2.4. Gamete Quality and Handling:

Ripe eggs are normally round, uniform in size, free of excessive debris and slightly granular in consistency. Underripe eggs are obvious by the appearance of a clear spot (the germinal vesicle) in the cytoplasm of the egg. Overripe eggs will have a cytoplasm of inconsistant granularity, appear less circular and often be associated with increased debris and hesitant spawning. If the proportion of over or under-ripe eggs exceeds 10%, another female should be spawned. Problems with sperm quality are rare, lack of motility in seawater being the only good clue to a problem. A rare hermaphroditic urchin can produce both eggs and sperm and obviously must be discarded.

After spawning, the sperm should be mixed in the 150-250 ml of seawater

into which each individual was spawned to provide a stock solution. Water above the eggs should be decanted and the eggs "washed" in several changes of 500 to 1,000 ml of new seawater (≥27 ‰) by letting eggs settle to the bottom of the beaker between washes. Washing removes and dilutes any anti-fertilization compounds which may have been shed with the eggs.

2.5. Fertilization:

After the eggs have been washed at least twice, add approximately 0.5 ml of the sperm solution to the eggs in a 500 ml volume of seawater and mix lightly. Allow 10-15 minutes for fertilization and membrane elevation. Check for fertilization success in a subsample of eggs by observing the percent of eggs with a raised fertilization membrane (Figures 1 and 2). If fertilization success is less than 90%, add another aliquot of sperm. If fertilization success remains less than 90%, use a new batch of eggs. Fertilization with excess sperm densities should be avoided since this can lead to multiple egg fertilization (polyspermy) which causes abnormal development.

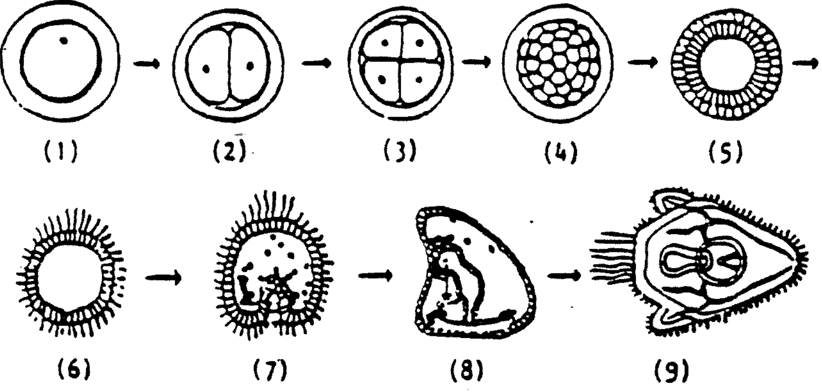
2.6. Inoculation of Test Solutions:

Following fertilization, the eggs are adjusted to an average density of 25,000 per ml (range 20,000 to 30,000) by diluting with seawater or by decanting surficial water if the starting density is less than 25,000 per ml. One ml of fertilized eggs (25,000) is then added to one-liter volumes of the test and control solutions contained in glass or polyethylene beakers.

2.7. Assessment of Embryo Density:

Mortality of embryos is one index that is routinely assessed at the end of a bioassay. Mortality is determined by comparing final embryo densities

Normal Development



Examples of Abnormal Development

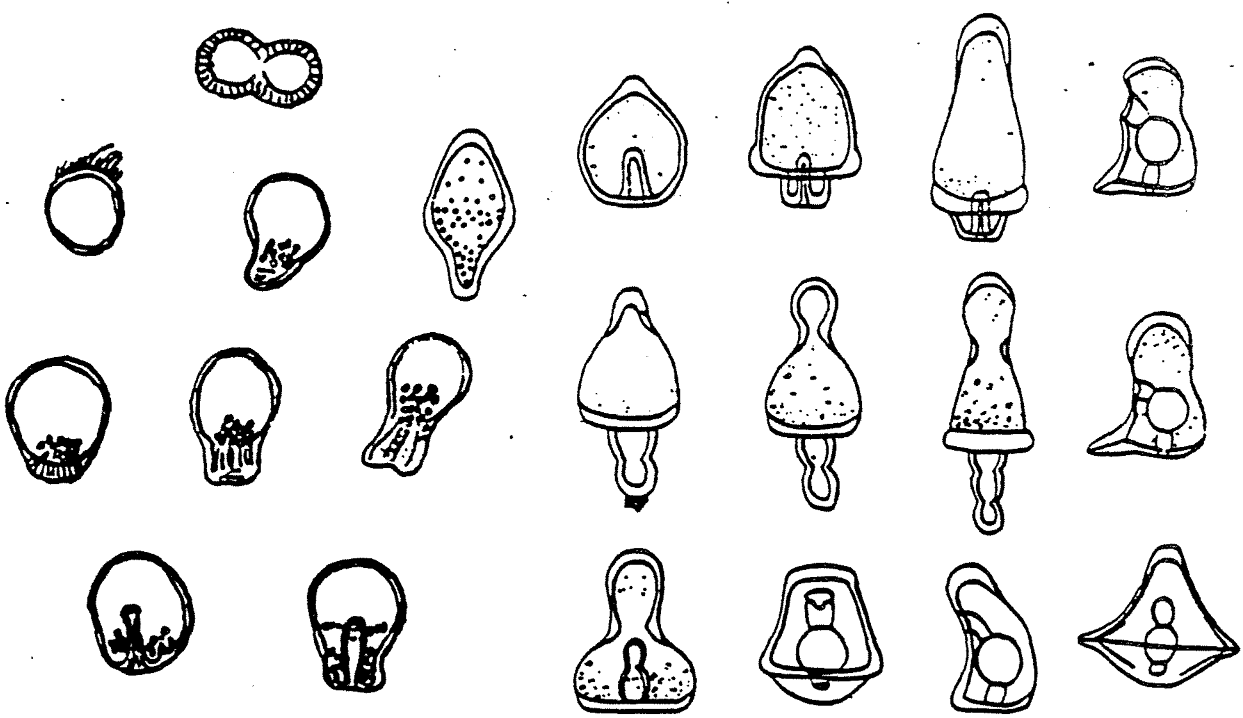


Figure 2. Some general examples of normal and abnormal sea urchin or sand dollar embryo development. For normal development: (1) fertilized egg, (2) and (3) early cleavage, (4) morula, (5) and (6) blastula, (7) gastrula, (8) prism, (9) pluteus. Adapted from Kinae et al. 1981; Timourian 1969; and Rulon 1956.

with initial densities. Hence, initial embryo counts are necessary for a base of comparison. These counts are obtained by subsampling 10 ml (~250 eggs) of egg solution from a minimum of three extra 1-liter beakers set up alongside the test beakers. All eggs are counted in each 10 ml subsample and the contents of the extra beakers discarded (or, if desired, maintained to check development as the test progresses).

2.8. Replication:

The degree of replication depends on the needs and manpower of each investigator. If replication is used (this is normally the case) an n=3 is the least number of replicates which should be used (replication of n=3 would mean 3 separate beakers for each test or control solution).

2.9. Exposure Conditions:

Salinity of the test and control solutions should be 30 ±3 °/oo. Salinities outside this range should be monitored with appropriate "salinity controls" to assure satisfactory embryo survival and development.

Exposure temperatures should reflect the normal ambient temperature for each respective species. Purple and green urchin embryos, being winter spawners, are best incubated at 8 to 10°C. Sand dollars are incubated at normal summer water temperatures of 12 to 16°C. Incubation at these temperatures are especially convenient since temperature control can simply be provided by a flow-through sea water system if available. Otherwise, a static water bath with thermoregulators will be required. If air incubation is used, the beakers should be covered with watchglasses to retard evaporation.

A normal light-dark cycle is preferred although there are no indications in the published literature that an all light or dark cycle leads to abnormal development.

2.10. Exposure Times:

Development times to the pluteus stage may be dependent on species to some extent, but the primary controlling factor in the rate of development is temperature. Embryos of tropical or sub-tropical urchins usually reach a full pluteus stage within 48 hours when temperatures are >20°C. Cooler water requires longer incubation times. Past experience in Puget Sound (Dinnel et al. 1983 and 1984) has led to incubation times of 72 hours for sand dollars (temp. = 12-16°C) and 96 hours for sea urchins (temp. = 6-10°C). Although we have not tried it, a compromise time and temperature of 72 hours at 12°C might be satisfactory for both sand dollars and urchins should a uniform exposure time be necessary.

2.11. Sample Fixation:

At the termination of the appropriate exposure times (check a subsample of the controls), each beaker is subsampled by mixing with a perforated plunger (easily made from plexiglas flatstock and tube) and withdrawing 10 ml of solution with an automatic pipette and pipette tip with an opening of at least 1 mm. The 10 ml subsamples are put into standard size (16 x 100 mm) test tubes, fixed with the addition of 1 ml of concentrated formalin, and sealed with corks. Caution must be observed when using formalin since this substance can be a serious source of glassware contamination if not contained. For this reason the use of a sealed repipette or similar device is required.

2.12. Sample Analyses:

Each sample is normally assessed for embryo mortality and abnormality. The supernatent above the egg pellet in each tube is carefully pipetted off (to remove most of the formalin - this reduces exposure to the counter), the

eggs resuspended in a small amount of clean seawater, and dumped into a Sedgewick Rafter cell. Rinse the test tube with one or two additional aliquots of seawater to assure that all eggs are added to the counting chamber.

Mortality is determined by counting all eggs in a sample and comparing with the initial densities at the time of inoculation (see Section 7). Determination of abnormality is a subjective measure which can vary between investigators, depending on degrees of abnormality. A very workable alternative used in our laboratory is to define an "abnormal embryo" as any embryo which fails to reach a reasonably well-defined pluteus stage. Normal and abnormal stages of embryo development are illustrated in Figures 2 and 3. An example of the type of results typical of an embryo bioassay is shown in Figure 4. This same data can also be used to generate EC50's (concentrations of toxicant causing a 50% reduction in normal development) using appropriate statistical procedures.

2.13. Additional Endpoint Measures of Embryo Development:

Investigations conducted during the last decade or two have led to the development of additional measures of embryo abnormalities. These additional measures help to reduce analysis time and subjectivity as well as increase the the sensitivity of the test, especially to genotoxic compounds.

One of the first refined measures of pluteus abnormality was the morphometric analysis by Heslinga (1976) where skeletal length of the pluteus arms were measured to provide an index of growth success. More recently, inovative biochemical assays have been designed to give measures of embryo growth success without having to screen thousands of individual embryos. Bay et al. (1983) have developed a way to rapidly quantify changes in echinochrome

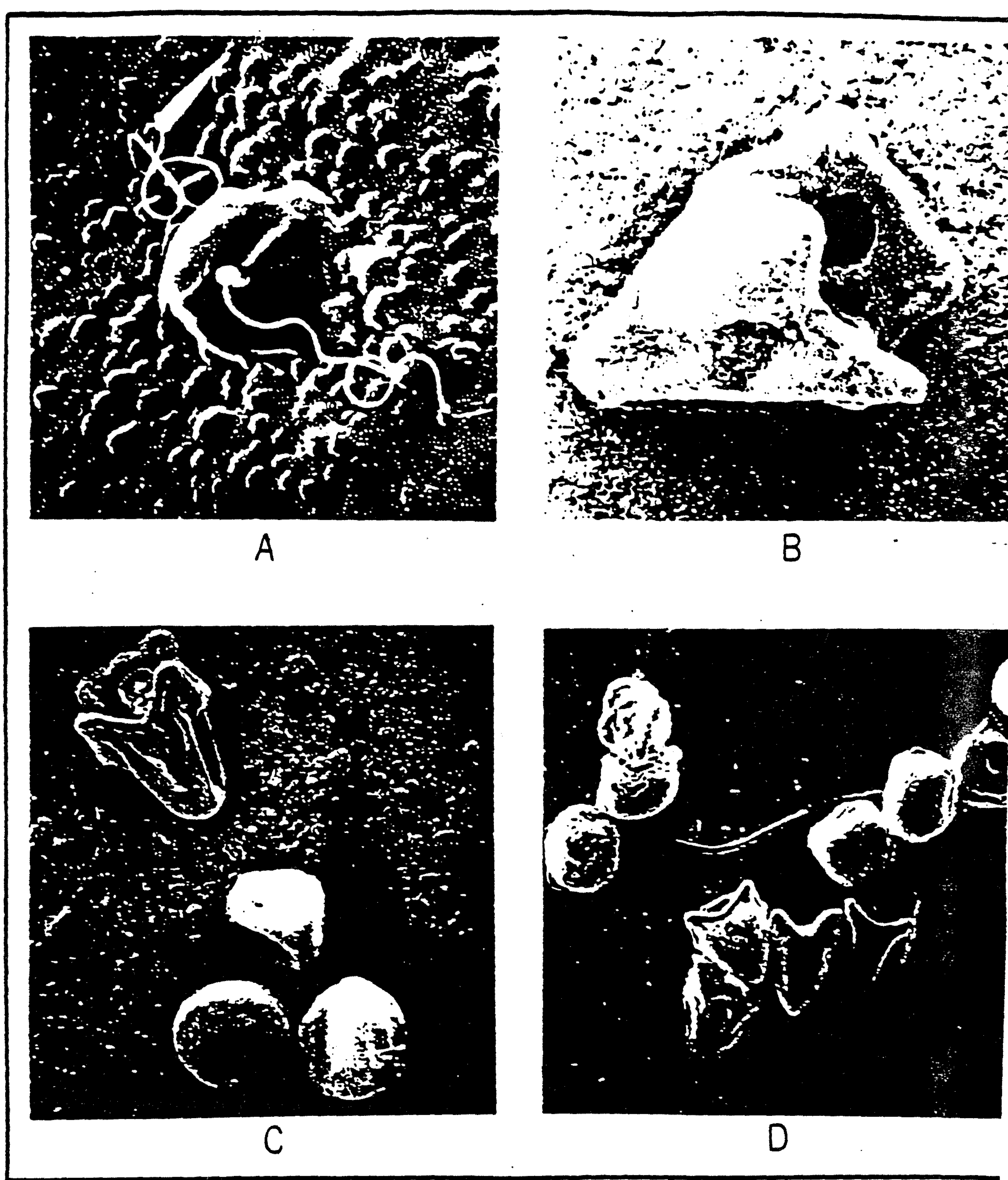


Figure 3. Scanning electron micrographs of sea urchin egg fertilization and development. (A) egg fertilization and initiation of lifting of the fertilization membrane, (B) normal pluteus, (C) and (D) normal and abnormal green sea urchin embryos following 96-hr exposures to silver (C) and endosulfan (D).

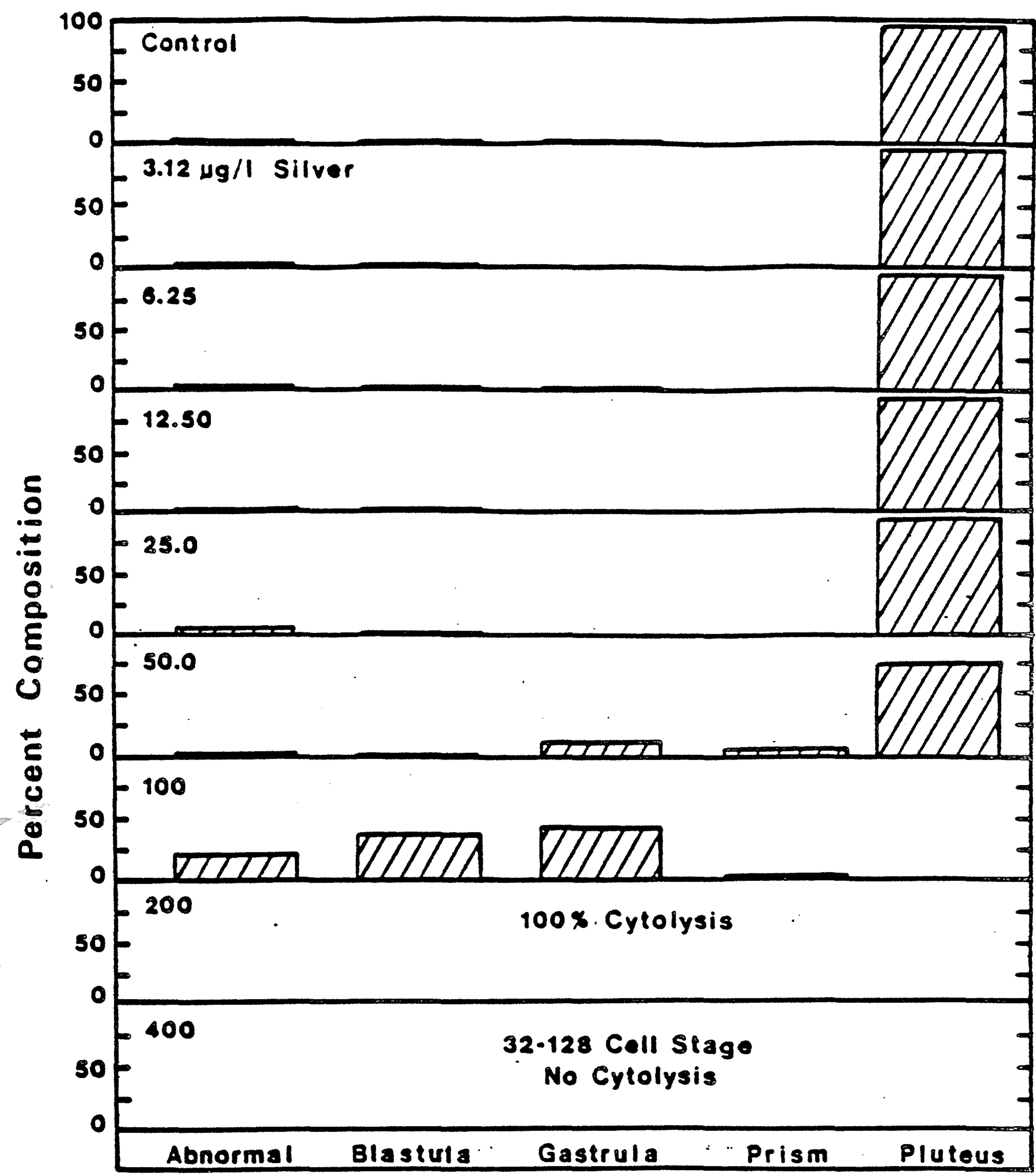


Figure 4. An example of the results of a sea urchin assay of a toxicant. This example illustrates the effects of 96-hour exposures of green sea urchin embryos to 0-400 µg /liter silver in seawater.

pigment synthesis in batches of embryos. The authors claim that this method "appears to be as good or better than the morphological examination technique since it is as sensitive, less variable and takes about 25% of the time required for morphological examination." A second biochemical method of quantifying embryo development is by measuring the degree of incorporation of radioactivity labled thymidine into growing embryos (Jackim and Nacci, in press). Inhibition of thymidine incorporation serves as an indicator of reduced DNA synthesis, and thus cell division.

A third category of embryo assessment looks at the subcellular impacts of genotoxic compounds. Hose and Puffer (1983) and Hose et al. (1983) have developed methodology for assessing mitotic abnormalities in preparations of embryos following in vivo or in vitro exposures of gametes or in vitro exposure of the embryos to toxicants.

2.14. Factors Favoring the Use of an Echinoderm Embryo Assay.

The Pacific oyster (Crassostrea gigas) embryo bioassay developed by Woelke (1972) has served as a biological water quality criterion in Washington State for approximately two decades. Use of this assay has documented regions of water quality impairment and served as an impetus for water quality reform. There is little question that oyster embryos have served as a valuable toxicological tool.

Echinoderm embryos have been shown to have a sensitivity very comparable to oyster embryos (Okubo and Okubo 1962; Dinnel et al. 1983) the following list of positive features.

1. Worldwide availability.
2. Year-round availability of ripe animals without laboratory conditioning.
3. Reliable and consistent gamete quality.

4. Quick and easy to spawn; may be spawned repetitively.

5. Embryos larger than oyster embryos; easier to assess.

6. Multiple endpoints for embryo assay, including genotoxicity and in vivo exposure of gametes.

7. Sea urchins and sand dollars native to Pacific Northwest, hence can be assayed at ambient seawater temperatures.

8. Other life stages of urchins also valuable for toxicological testing.

9. Extensive basic biological and toxicological data base available in the published literature.

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