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Monitoring Coastal Habitats at Herring Bay

STUDY ID NUMBER: 10

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LEAD AGENCY: US Forest Service

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INCLUSIVE DATES OF STUDY PLAN: March 1, 1991-February 29, 1992

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II. INTRODUCTION:

As a part of the Coastal Habitat program, the US Forest Service, through the University of Alaska Fairbanks (UAF), established an experimental field station in Herring Bay, Knight Island, during 1990. The purpose of the station is to provide a research platform for intertidal Natural Resource Damage Assessment (NRDA) and Restoration-related studies.

During the summer of 1990, UAF implemented separate studies on 15 pairs of oiled and non-oiled sites in Herring Bay. These ongoing studies have initially applied to assessment of damages; however, four studies are directly related to the feasibility of intertidal restoration and monitoring of natural recovery.

One set of studies examines presence/absence differences in population dynamics between common intertidal species on impacted and reference sites. Results from these studies will summarize a checklists of species, and compare differences and densities per unit area.

A second group of studies examines settlement between oiled and non-oiled surfaces. Results from these studies compare density information for each species examined, per unit area, between oiled and control sites. A third study examines succession of algae at 104 study plots in Herring Bay.

A forth group of studies consists of a series of experiments which will monitor the ability of the dominant intertidal macrophyte, Fucus, to recover from the effects of the oil spill or spill clean-up efforts in Herring Bay. In addition we will be monitoring all algal species in the intertidal zone for rates of natural recover from spill damage.

For NRDA, these studies will provide immediately useful data regarding impacts associated with the *Exxon Valdez* spill. The continuance of these and other monitoring programs in 1991 will contribute to the base of knowledge concerning restoration feasibility, especially with respect to determining a rate of recovery to pre-impact conditions.

III. OBJECTIVES:

1. Compare between oiled and control sites abundance per unit area of intertidal invertebrates with limited dispersal capabilities. Based upon the data generated in 1990, compare 1991 field data between oiled and control site pairs. If differences between replicated site pairs indicate signs of a "normalization" (or no significant differences between populations), then forecast a "rate" at which populations might be expected to be similar.

2. Compare the success of settlement by barnacles and algae (e.g. *Fucus* sp.) between oiled and non-oiled substrates. Based upon data generated from the 1990 and 1991 seasons, estimate the length of time required before oiled substrates show no significant differences in colonization success by barnacles.

3. At the termination of the limpet grazing experiment in the 1990 field season, an opportunity was presented to gain an understanding of temporal algal succession within Herring Bay. Several questions regarding algal succesion in general are relevant to possible impacts from oil. These questions include:

   A. Can a basic algal succession for Herring Bay be defined?

   B. Based upon the number of sites chosen for study, is there a difference in algal succession (and thus, species composition) between reference and impacted sites?

   C. Is there a difference in algal succession between areas that are simply scraped free of all algae, vs. the application of a killing agent (such as bleach)?

4. Monitor the natural restoration and recovery of the intertidal algae, with special emphasis on Fucus in Prince William Sound. Sub-objectives are:

   A. The relative fecundity of Fucus plants will be determined to ensure that the existing populations have the capacity to "re-seed" the damaged areas.

   B. Fucus population dynamics will be monitored in oiled and unoiled areas in order to assess natural restoration rates.

   C. Algal recolonization and Fucus growth rates will be measured to asses how long it may take for the intertidal algal species to recover to pre-spill levels.

IV. METHODS:

This section defines each study conducted at the experimental station in 1991, and identifies the methods used. Standard Operating Procedures for these studies are listed as Appendix 1 & 2 under OTHER INFORMATION.

A. Sampling Methods:

Site Characterizations

Study sites are characterized through the establishment of three random transects along the site perpendicular to the water line, starting at Mean High High Water (MHHW). Quadrats were located randomly within the first three meters of vertical fall along each transect. In each quadrat, presence/absence data for all invertebrates and algae were recorded, as well as determination of percent cover. Also, data for temperature and salinity were collected at each of the study sites on a weekly basis.
Refer to the 1991 SOP in item **X** for modifications to this study.

1. Population Dynamics of certain invertebrate species

This study has been designed to examine the differences in numbers and recruitment of certain invertebrates with limited dispersal capability, between oiled and non-oiled sites. Limpets are included in this monitoring study because of their likely importance as grazers to community structure.

Materials and Methods, Population Dynamics:

Permanent plots were established at five pairs of sites: three sheltered rocky and two pairs of sheltered coarse grained environments. These plots were established at three meters of vertical fall along six randomly placed transects across the site length, establishing a total of 18 study plots per site. Quadrat dimensions were 20 X 50 cm. Within each of these permanent plots, all limpets, *Nucella* spp., *Littorina sitkana* and *Leptasterias hexactis* were counted. Also, using a 1 m semicircle adjacent to and centered at the left of the 20 X 50 cm quadrat, the nearest of each of these species was measured and recorded.
Refer to the 1991 SOP in item **X** for modifications to this study.

2. Settlement Studies

A. Barnacles

Within Herring Bay, certain oiled shorelines still possess heavy accumulations of dried tar, especially in the upper intertidal zone, where desiccation and baking by sunlight has resulted in an asphalt condition of the oil. Established colonies of barnacles were obviously impacted along many of these areas.
A study was implemented which examined whether the presence of such tar reduces the settlement capability of cyprid barnacles relative to cleaned areas within a tarred substrate.

Materials and Methods, Barnacles:

Two oiled sites and two reference sites of similar character were selected in Herring Bay for this study. Sites 1641A and 1342D are oiled vertical faces located on the southern end of a small island, in the lower center of Herring Bay. All sites have vertical faces where barnacles presently exist, or in the case of sites that were heavily oiled and treated, having many skeletons still attached to the substrate. Sites 1641B & 1642C are non-oiled reference sites in the southeastern cove of Herring Bay. All sites had high densities of the barnacle *Semibalanus balanoides*.

At each site, paired 10 X 10 cm plots were established. One member of each pair was scraped and brushed to remove all visible tar (or barnacles in the cases of the non-oiled sites). The length of each site was measured, and the number of planned pairs divided into the site length. The first plot was placed randomly, within the first segment, and subsequent plots were placed at equal distances from the first. A coin was flipped to determine which 100 cm^2 area of the first pair to scrape. The subsequent scraped plots were then alternated.

The sites were periodically examined for barnacle settlement, as well as germlings of the alga *Fucus gardneri*. The number of barnacle juveniles and germlings were recorded during each inspection. Each 100 cm^2 area was also photographed. Refer to the 1991 SOP in item **X** for modifications to this study.

B. Settlement on oiled and non-oiled substrates

A second study also examined differences in settlement of marine invertebrates and algae between oiled and non-oiled substrates. However, the substrates used in this second study were rocks retrieved from an oiled shoreline in Herring Bay, as well as rocks treated with fresh North Slope crude oil, taken from the T/V *Exxon Valdez* last year. The objectives of this experiment were to examine differences in: a) the percent cover of barnacles and macro algae; b) the number of individuals per unit area; and c) the presence/absence of invertebrate species on oiled and non-oiled substrates placed within various sites.

Materials and Methods, Substrate Transplants:

On a beach lying along the western arm of Herring Bay, seventy two oiled rocks of similar size were collected and returned to the laboratory. These rocks represent a substrate coated with 1 year old *Exxon Valdez* Prudhoe Bay Crude (EV). All rocks were

collected and packed in boxes and separated by aluminum foil, so that the rocks would not touch one another.

Upon return to the laboratory, all rocks were laid out and one-half of each rock was cleaned with the solvent, Methylene Chloride ($MeCl_2$), with the exception of 8, which were left completely oiled to serve as strata for a weathering analyses of the oil.

After each half was thoroughly cleaned, the rocks were allowed to dry. "Top" and "bottom" of each rock was determined with regard to symmetry and morphology. The "top" was assigned to the surface with the least irregularity. When dry, each rock received a unique identification number and was marked with an indelible marker. Each rock was measured with calipers for total length, and length of the cleaned and oiled sides, and each rock was then photographed.

Also, 72 rocks of approximately the same size were collected from a geologically similar, but non-oiled beach. Half of each rock surface was dipped in fresh Prudhoe Bay Crude (PB) until a "tarred" coating was achieved. These rocks were allowed to dry and were handled in a manner identical to the EV rocks.

In addition to the rocks, 72 clay tiles were incorporated into the experiment. The tiles, being uniform in surface texture and aspect, served as substrate heterogeneity controls for the rocks. Thirty six of these clay tiles were oiled with fresh PB oil and the other 36 remained clean. The tiles were placed side-by-side in the field as oiled and un-oiled pairs.

At each of the experimental sites, rocks and tiles were placed randomly at the 2 m elevation contour. Control rocks (i.e. rocks which were unoiled, but had half of the surface treated with $MeCl_2$) were also placed at each site to test for use of the $MeCl_2$ solvent. Each site received an identical number of rocks and tiles representing the following experimental conditions:

The basic experimental unit has been left in the field indefinitely, and consists of 3 EV rocks, 3 PB rocks and six pairs of tiles. The additional rocks were placed to be destructively sampled at three separate time periods. These time periods were mid summer 1990, early fall 1990, and mid spring, 1991.

After placement of all substrates in the field, settlement by barnacles and macro algae on each surface was recorded. Counting involved use of a 3 cm X 3 cm quadrat. The quadrat edge was placed at the midpoint of the line separating the oiled and un-oiled portions of the rock. Where possible, individual

species were identified, counted and recorded. Rocks were photographed at a fixed focal length to incorporate the quadrat.

Throughout this substrate transplant experiment, the chemical composition of crude oil will change over time. Consequently, the "thickness" of oil coating of the substrates will gradually decrease. Thus, a procedure was developed to quantify a rate of change in the oil's character. The procedure employed a gravimetric analysis of an area of oil extracted by $MeCl_2$. Therefore, completely oiled EV and PB rocks were also placed in the field as controls for taking samples for this oil "weathering" analysis.

The oil weathering analysis entailed a $MeCl_2$ extraction of a 3 X 3 cm area on each of the control rocks, using a pre-weighed absorbent material. This absorbent material was then placed in a pre-weighed vial. Each vial was opened and stored at room temperature, and allowed to dry. The absorbent material was then reweighed. The sample vials were refilled with $MeCl_2$, and refrigerated for Gas Chromatography/Flame Ionization Detection analysis (GC/FID). Refer to the 1991 SOP in item **X** for modifications to this study.

3. Algal Succession:

Because eight study sites were chosen for studies of limpets grazing (four pairs of impacted and reference sites) and the termination of the experiment involved removal of the fences and all algae within each 625 cm area, a platform was provided to easily examine algal species succession at multiple plots.

At the termination of the limpet grazing study, fences from two elevation contours were removed. Only the marine epoxy used to hold the fences in place was left to serve as a marker for each of the algal succession study plots.

All algae was removed from each 625 cm area. A coin was flipped to determine if the odd or even numbered fences would receive application of sodium hypochlorite (bleach).

Sessile invertebrates, such as barnacles were not removed from the 625 cm area. A photograph of each experimental area was taken (with photolabels in each frame).

Each study plot will be revisitied to assess algal species composition. At the time of visitation, percent cover of each plot by separate species will be determined by a point grid method. Also, photographs of each plot will also be taken, and a

voucher specimen of each plant observed within each plot will be collected. Voucher specimens will be sent to UAF for taxonomic identification. Finally, numbers and species of invertebrate grazers found within the 625 cm area will also be counted.

This process will be repeated until four time series plots have been established for both the scraped and bleached plots at each contour. These study plots will be monitored quarterly.

4. Fucus and algae Recovery

Experiment 1 - Fucus Reproductive Potential and Egg Viability. The Exxon Valdez oil spill may have affected the ability of intertidal plants to produce sufficient, viable reproductive cells to repopulate the natural habitat. This experiment will assess the relative fertility of Fucus in oiled and control sites by measuring the rate of egg release from randomly selected receptacles. In addition the viability of the released eggs will be monitored. Because of the many factors that can affect the release of eggs from the conceptacles, this experiment must be conducted at the field camp to help reduce the variation in the data. This is a continuation of experiments conducted this past field season.

Plants for this study will be collected from the top three meters at each site. The same sites and plots will be used for this experiment as those used for the population dynamics study. Two semicircles of 0.5 m radii will be used to select two plants at each 20 cm x 50 cm quadrat. There will be a maximum of 36 plants selected at each site (2 per quadrat x 6 quadrats per tidal range x 3 tidal ranges). The left semicircle will have its origin (point from which the radius is extended) on the left edge of the 20 cm x 50 cm quadrat, 25 cm down from the top edge. The semicircle will be concave to the quadrat. The right semicircle will be a mirror image of the left semicircle, with its origin on the right edge of the quadrat, 25 cm below the quadrat marker on the transect line. To select each plant, a search will be made in each semicircle for the Fucus plant which is nearest to the origin of the semicircle and which has receptacles. Receptacles are defined as inflated tips of the Fucus branches which contain conceptacles (note that the conceptacles may be in varying stages of development). If no plants within the semicircle contain receptacles, then a zero will be recorded for the number of eggs released for the sample. At each subsequent sampling, two new semi-circles will be used which will be located along the same contour on each side of the quadrat and displaced 0.5 m farther from the origin of the last sampled semicircles in directions away from the quadrat.

The selected plants are removed intact from the substrate and placed in numbered plastic bags. Plants should be kept separated by site location and tidal range. Plants should be collected

from the oiled and its paired control sites on the same day.

At the laboratory one receptacle is selected from each plant by the following method. An arbitrary receptacle is selected on the plant, and a random integer between 1 and 10, inclusive, is generated on a calculator. Using this receptacle as number '0', count out the number of adjacent receptacles corresponding to the selected random integer. When counting, proceed in one direction, counting neighboring receptacles on neighboring branches. Count only receptacles and not sterile branch tips. The receptacle with the number corresponding to the random integer is the selected receptacle for egg release. Trim away the non-selected part of the plant, leaving only the receptacle. Blot this receptacle dry with paper towels then rinse for a few seconds with fresh water. Re-dry the receptacle thoroughly and place between dry paper towels. Set at 8-10 C in the dark for 24 hours. After weighing the receptacle to 0.01 g, immerse it in 15 ml of cold, sterile seawater in a 60 x 15 mm petri dish. Cover the dish and set it in an incubator at 8-10 C, with a photoperiod of 16:8 (L:D), at 50-80 micromoles/m^2/s light.

After 48 hours remove the receptacle and return the dish to the incubator. After an additional 48 hours, remove the dish from the incubator and add Calcofluor solution, followed by 5% formalin 30 minutes later. Prepare the dish for shipment to JCFOS where the eggs will be counted and analyzed according to the procedure published in the CHIA SOP.

This experiment will be performed at three paired oiled and control rocky protected and two paired coarse textured sites in Herring Bay.

Experiment 2 - Fucus Population Dynamics

The population structure of the intertidal zone is often affected by disturbances. A disturbance such as a former clearing is evidenced by a preponderance of smaller plants in the population. This experiment will monitor the population structure of Fucus as it is affected by oiling or clean-up efforts. The plots selected for this experiment will be the same as those used for the recruitment studies in the intertidal invertebrate studies at Herring Bay. The sites include 4 sheltered rocky oiled sites and their matched control sites and 2 coarse gravel oiled sites with their paired controls. There are 6 transects per site with 3 quadrat locations per transect. The procedure for the location of these transects and quadrats is identical to that used in the stratified sampling done in the Comprehensive Assessment of Injury to Coastal Habitats surveys. However, only one 20 cm x 50 cm quadrat will be used at each quadrat location and will be placed with the upper right-hand edge of the quadrat located at the quadrat location point on the transect line. This experiment is a continuation from last field season.

At each quadrat, size-frequency distribution will be determined by measuring all visible Fucus plants for total length without removing the plants from the substrate. For each plant the reproductive status and the condition of the plant will be recorded using the scheme outlined for the intertidal plant transect studies. Percent cover of all organisms, bare rock, and oil will be estimated with a 50-point grid placed over the quadrat. Drift algae will be removed before any data are taken. If necessary, a small sample of the surface oil will be removed with a knife and examined under a microscope to ensure that it is actually oil. The measurements will be repeated at least 3 times during the summer field season. All plots will be photographed at all sampling dates.

Experiment 3 - Fucus Germling Growth

This experiment, slightly modified from that performed last year will be conducted in four segments established along a transect line extending along the contour of the site at the 1.0 and 2.0 m of vertical fall, at the top of the Fucus zone. The experiment will measure the growth rates of Fucus germlings in oiled and unoiled sites. Small settling plates will be seeded at JCFOS with Fucus eggs at an average density of 144 eggs per square cm. These plates will be shipped to Herring Bay and paired seeded and unseeded control plates will be placed in random order at the first and second meter vertical drop at three pairs of oiled and control sites. Separate sets of plates will be deployed at 4 to 6 week intervals.

Fucus plants, >1mm, growing on the plates will be photographed, enumerated and measured in the field to assess recruitment and growth throughout the summer. At the end of the summer, the plates will be photographed, removed from the rock surfaces, and plants will be enumerated and measured in the lab.

Detailed SOP's will be necessary for all aspects of this field work and will be generated during the month of March prior to the field season. The SOP's will be similar to those developed last year and published as part of the operational plan for the Coastal Habitat Injury Assessment project.

Temperature and Salinity:

Stations adjacent to all 1990 study sites were monitored weekly for temperaure and salinity at the 0, 0.5 and 1 through 5 m depth contours around MHHW, using a CTD meter. This procedure will be continued through the 1991 season.

B. Citations:

Dayton, P. K. 1971. Competetion, disturbance and community

organization: the provision and subsequent utilization of space in a rocky intertidal community. *Ecol. Monogr.* 41:351-389.

While it is probable that many components of these methods have been employed by other reasearchers previously, no additional citations were specifically referred to in developing the above procedures.

C. Standard Operating Procedure Requirements:

As identified at the beginning of this section, the SOP's for the experimental field station are presented as appendices 1 & 2 under item X, OTHER INFORMATION.

D. Quality Assurance and Control Plans.

1. Data Collection and Analysis.

For each of the studies identified in this plan, detailed data collection forms have been developed (see Appendix 1 and Section VI-B, Sample and Data Archival). The original, completed forms are maintained in a notebook while at the experimental station. These same notebooks are then stored at the University upon termination of each field season. In addition to the field data forms, the experimental station maintains the field data in an automated database. The contents of this database are also transferred to the UAF data management department.

2. Sample Collections and Labelling.

All hydrocarbon samples collected by the experimental field station in Herring Bay, are done so according to the sample collection procedures and chain-of-custody requirements discussed in the Analytical chemistry group technical services document #1, NMFS, Auke Bay, Alaska, entitled "State/Federal damage assessment plan analytical chemistry collection and handling of samples". Other samples (i.e. invertebrates) not used for hydrocarbon analyses are collected at the experimental station for voucher purposes. These samples are maintained at the experimental station, and transported to UAF upon termination of the field season.

V. DATA ANALYSIS:

The experiments described in this plan have been designed largely for standard parametric and non-parametric statistical analyses (i.e. Student's T-test, Wilcoxin Rank sum test, Sign test, Mann Whitney U-test, analysis of variance). Replication in the selection of study site pairs is the basis for analytical power.

The products to be generated from these experiments will be in digital and non-digital form. Report results will include

graphics in the form of maps, graphs, tables and figures.

VI. SCHEDULES AND PLANNING:

A. Data and Report Submission Schedule.

Data collection and information management will be conducted simultaneously while at the field station. Because the station is computer-equipped, data entry will be performed routinely. Consequently, the availability of raw data will be immediate upon termination of the 1991 field season. Report writing and submission will be completed within 60 days of the sampling season's termination.

B. Sample and Data Archival.

The sample and archival system for this research is documented as follows:

1. This study Plan, with all approved revisions

2. All SOPs related to the Herring Bay experimental field station are included in appendices 1 & 2

3. A complete set of 1990 field data from the Herring Bay Experimental field station is on file at the University of Alaska Fairbanks. Sediment hydrocarbon samples, and limpet tissue hydrocarbon samples are stored at the Auke Bay Marine Laboratory as part of the hydrocarbon sample pool

4&5. All records, logs, summaries, and reports used during 1990 are also on file at the University of Alaska Fairbanks. Samples of the data log sheets used for each study are provided as Appenidx 3, under item **X**.

C. Management Plan

The management plan for the experimental field station is straight forward. The sampling season will run from April 15 to September 15, 1991. Refer to the Logistics section below for a discussion of facilities. As most experiments described here are in place, their frequent monitoring is the primary requirement. Five resident field staff will monitor all invertebrate studies. Workable tides for these studies lie around the 0.0 tide level from MHHW. There are brief periods during each month where adequate low tides and daylight will not correspond. Each study site will be monitored twice weekly, on average, unless otherwise specified in the SOP.

D. Logistics

The presence of the experimental field station within Herring Bay makes access to the study sites ideal. This station (a floating barge) is currently in place and permanently moored within safe harbor in Herring Bay, and meets all Coast Guard safety regulations. Most of the field equipment is already on board. Initiating the 1991 sampling season will require transport of personnel and some equipment to the station. Throughout the season, the facility will receive food and supplies on a weekly basis. All study sites are located within Herring Bay, and are accessed via small gas-powered skiffs.

VII. BUDGET: (in thousands of dollars)

Personnel:	70
Equipment:	5
Barge Charter:	130
Indirect costs:	40
TOTAL COST:	$245

VIII. PERSONNEL QUALIFICATIONS:

Principal Investigator: Raymond C. Highsmith, Ph.D.

Raymond C. Highsmith is an Associate Professor of Marine Sciences in the Institute of Marine Science, School of Fisheries and Ocean Science of the University of Alaska Fairbanks.

Principal Investigator: Michael S. Stekoll, Ph.D.

Michael S. Stekoll is an Associate Professor with the University of Alaska, Southeast with a joint appointment with the School of Fisheries and Ocean Sciences, University of Alaska, Fairbanks. He has had extensive experience in conducting marine macrophyte research along the coast of Alaska, including research on intertidal Fucus populations in Bristol Bay, subtidal and intertidal marine macrophytes around St. Lawrence Island, and kelp research in SE Alaska. He also has published in the area of the effects of oil pollution on marine organisms in Alaska.

Project Leader: Anthony J. Hooten

Andy Hooten is currently enrolled in the doctoral program of biology at George Mason University in Fairfax, Va. He received his master of science degree in zoology from the University of Georgia in 1985. Mr. Hooten served as project leader aboard the Herring Bay experimental field station during the 1990 season. Prior to affiliation with the Coastal Habitat Program, he served

as an ecologist for the State of Alaska, Department of Environmental Conservation Oil Spill Response Center.

Project Leader: Peter van Tamelen

Peter van Tamelen is nearing completion of the Ph.D. degree in Zoology from Oregon State University under the direction of Bruce Menge. He has had extensive experience in research in intertidal ecology especially with respect to algal zonation and succession. He served as the project leader for the Herring Bay algal studies during the 1990 field season.

IX. REFERENCES:

Dayton, P. K. 1971. Competetion, disturbance and community organization: the provision and subsequent utilization of space in a rocky intertidal community. *Ecol. Monogr.* 41:351-389.

Knight-Jones, E. W. 1953. Laboratory experiments on gregariousness during setting in *Balanus balanoides* and other barnacles. J. Exp. Biol. 1953 30: 584-598.

Mann, K.H. and R.B. Clark. 1978. Long-term effects of oil spills on marine intertidal communities. J. Fish. Res. Board Can. (35) 791-795.

Nelson-Smith, A. 1977. Recovery of some British rocky seashores from oil spills and cleanup operations. In: Recovery and restoration of damaged ecosystems.

Southward, A.J. and E.C. Southward. 1978. Recolonization Cornwall after use of toxic dispersants to clean up the Torrey Canyon spill. J. Fish. Res. Board Can.35: 682-706.

Strathmann, R. R. and E. S. Branscomb. 1979. Adequacy of cues to favorable sites used by setting larvae of two intertidal barnacles. In: Reproductive ecology of marine invertebrates. Ed. Stephen E. Stancyk. Bell Baruch Library in Marine Science. Univ. South Carolina Press, Columbia, S.C. 1979.

X. OTHER INFORMATION:

Refer to Appendices 1 & 2 for the 1990 and 1991 Standard Operating Procedures.

APPENDIX 1

1990 Invertebrate Standard Operating Procedures
Herring Bay Experimental Field Station

COASTAL HABITAT INJURY ASSESSMENT: 1990 Standard Operating Procedures for Intertidal Invertebrate Field Experiments in Herring Bay, Price William Sound, Alaska

The experiments described in these Standard Operating Procedures (SOPs) are an addition to the Injury Assessment process. The presence of a base camp within Herring Bay allows repeated access to study sites to test specific hypotheses which pertain to possible ecological impacts from the Exxon Valdez Oil Spill. Herring Bay was selected as the general location to conduct these studies, due to the heavy levels of oiling experienced, the protected nature of the bay, which provides opportunities for locating non-oiled "control" sites, and the potential use of two "set-aside" sites, which have been left untreated for long term studies.

The selection of study sites is the single most important step for these experiments. Of all the habitat types defined in Prince William Sound, these experiments will be performed in Sheltered Rocky and Sheltered Coarse Gravel environments. These habitat types were selected largely because they represent the most common habitats within Prince William Sound.

In selecting sites for experiments, a range of potential site combinations include the following conditions:

A. A set aside site;
B. A non-oiled "control" site;
C. A mechanically treated site;
D. A bioremediated site.

A "set aside" is defined as a site that did not receive treatment from Exxon's cleanup effort. "Control sites", are sites which are either truly non-oiled, or have received such a light degree of oiling, that they presently can be determined as "cleaned". "Mechanically treated" sites are defined as those areas which received one or a combination of hand wiping and washing with various water temperatures and pressures. Finally, "bioremediated sites" are those areas which have received an application of Inipol, the chemical used by Exxon to accelerate bacterial degradation of oil.

Based on the above combinations, the original matrix created for site selection appears as follows:

Set Aside	Control	Oiled Mechanical	Oiled Bioremediated.

This matrix represents sites within one habitat type and serves as one unit for applying experimental treatments. Therefore, replication for any experiment conducted in Herring Bay requires selection of four additional sites to contain the same treatments. Ideally, the matrix listed above would be replicated three times for all experiments in sheltered rocky

habitat types and twice in coarse gravel habitats.

However, after surveys of sites in Herring Bay, and review of data from the Exxon/Federal/State spring shoreline assessments, it is evident that division of sites within the above categories cannot be clearly determined. Virtually all of the segments identified by Exxon and ADEC in Herring Bay have received bioremediation treatments, in addition to any mechanical cleaning, or will have bioremediation occurring during the 1990 treatment season. Furthermore, only one set-aside site of the sheltered rocky habitat type is located in Herring Bay and cannot be replicated within the general vicinity of northern Knight Island.

Therefore, the matrix of site selection can only be stated as impacted versus non-impacted. This results in use of a dual matrix which can be replicated several times, but site selection cannot differentiate between mechanically treated and bioremediated conditions. Thus, for application of the studies presented in this SOP, the modified matrix appears as follows:

IMPACTED	NON-IMPACTED
Oiled	Non-oiled
Mech. Cleaned	"Control" sites

Site Selection.

Careful attention will be given to matching pairs of sites, such that the only differences lie in their oiled and non-oiled conditions. This pairing will include similarity in substrate composition, slope, directional and solar aspect, wave exposure, and common biological communities. In considering pairs of sites to be selected, the following procedures will be conducted and information recorded:

1. Each site considered will be verified by Latitude/Longitude coordinates and compass bearings;
2. The length of the site will be measured at the MHHW line (observed at the base of the Verrucaria zone);
3. The substrate character of the site will be defined (rocky, boulder, coarse grained or a combination of these) and their relative percentages of each;
4. The solar aspect of the site will be determined by compass bearing and recorded;
5. The wave energy/exposure of the site (H,M,L) will be observed and recorded;
6. A detailed drawing of each site will be made; and will include the above factors and major life zones;
7. A video and still photographs will be taken of the site (Attachment I).

Site Characterizations.

In addition to the general description and mapping of each study site, information on the percent cover of major algal and invertebrate species, as well as presence-absence of all intertidal species will provide a basic understanding of community structure and species abundance and distribution (Fig. 1). This information will contribute to a better site description and comparison between study sites.

Each study site will be characterized using the transect and quadrat SOPs of the Coastal Standard Operating Procedure Number 2, and Intertidal Standard Operating Procedure Number 1 (ISOP), with the exception of the differences contained in the following procedures:

1. Because of available working time and the protected nature of Herring Bay, the 35 degree criterion for site workability is rejected, because some of the sites selected can be set up and surveyed by boat (ISOP #1, pg 20). . Given the steep nature of these slopes in excess of 35 degrees, the quadrat cannot be allowed to "rest naturally on the substrate". Rather, the quadrat will be allowed to "fit the frame to the slope", but will have to be held in place until the quadrat can be permanently marked with the rotohammer.

2. Each site will be measured based upon obvious traits in site composition, such as changes in substrate character or shoreline topography, or breaks/discontinuity of the intertidal zones of interest. In general, sites will range in length from 15 to 100 M. Each site will have the start and finish of measurement marked with a "medallion" of marine epoxy placed at the base of the *Verrucaria* Zone. Measurement of the site will occur along the edge of the supratidal, at the base of the *Verrucaria* zone. Care will be taken to avoid impacts to the study site; staff will traverse the site in the supratidal zone and in the walking pathway defined for each transect.

3. Note: These rules apply to workable beach lengths, i.e., subtract the beach length of unworkable sections (other habitat types) present within the habitat type from the total beach length of the habitat type before applying these rules.

After the length of the site is determined, divide the total workable beach length of a habitat by the number of transects to be established in the habitat (3 transects are divided into this total length). Call this number X. Essentially, this divides the habitat into intervals of equal length X; one transect will be located in each

interval. Multiply X by a random number. Call this number Y. The first transect will be located Y meters from the left end of the habitat (when facing the beach from the sea). If 3 transects are to be established in the habitat, they should be positioned X meters and 2X respectively, to the right of the first transect.

4. Quadrat size is 80x50 cm. Collect data for the presence/absence of all species;

5. Collect data for percent cover of sessile invertebrates and algae. Follow ISOP #1; remove all drift *Fucus* from both 40 X 50 cm areas. Remove the *Fucus* canopy from the right half of the left 40 X 50 cm quadrat. Photograph both halves per ISOP #1, 1990 Quadrats.

6. A swath survey for macro invertebrates will be conducted pursuant to ISOP # 2.

7. With item H of ISOP #1 (limpet/mussels/*Fucus* semicircle, only limpet data will be collected at this site. Aside from the biological data collection specified here, all other biological collections identified in ISOP #1 are ignored.

8. Collect Hydrocarbon data pursuant to Coastal Habitat SOP #4.

8. Move to the next quadrat and repeat steps 4-7.

These transects will be used for site characterization for presence/absence of all species, and percent cover of macro algae, barnacles and mussels. Figure 2 shows the layout of sampling procedures for site characterization.

Transplanting of oiled and non-oiled substrates.

The objectives of this experiment are to examine differences in: a) the percent cover of barnacles and macro algae; b) the number of individuals per unit area; and c) the presence/absence of species on oiled and non-oiled substrates placed within the various sites identified.

Seventy two oiled rocks are to be collected from a heavily oiled site within Herring Bay. These rocks will be of the same size and geological type and will represent substrate coated with 1 year old Exxon Valdez Prudhoe Bay Crude (EV). Also, 72 rocks of the same size and geological type will be collected from the same or a geologically identical, but unoiled beach. All rocks collected are to be packed in boxes or coolers and separated by aluminum foil so that the rocks do not touch one another.

Upon return to the laboratory, all rocks will be laid out and one half of each rock will be cleaned with Methylene Chloride ($MeCl_2$), with the exception of 8. These 8 will be left totally oiled to serve as strata for samples of oil weathering analysis. Also, 8 of the non-oiled rocks will have their entire surface cleaned with $MeCl_2$ to serve as controls for use of the solvent. Care should be taken when handling this solvent. Solvent resistant gloves should be worn at all times and work should be conducted in a well ventilated area. Cleaning of each rock will involve either dipping one half of the rock in several baths of $MeCl_2$ or hand-wiping or both. After this half is thoroughly cleaned, the rocks will be allowed to dry. "Top" and "bottom" of each rock will be determined with regard to symmetry and morphology. The "top" will be assigned to the surface with the least irregularity. When dry, each rock will receive a unique identification number with an indelible marker. Each rock will then be measured with calipers for total length, and the length of the cleaned sides. These data will be recorded on a data form (Fig. 3). Each rock will also be photographed and the frame number will be logged on the data form.

For the non-oiled rocks collected, half of each of these rocks surfaces will be dipped with fresh Prudhoe Bay Crude (PBC) until a "tarred" coating is achieved. These rocks will be allowed to dry. Again, each of these freshly oiled rocks will be measured for total length and clean length and photographed.

In addition to the rocks, a total of 72 clay tiles will also be incorporated into the experiment to be placed among the rocks. Half of these (36) will be oiled with fresh PBC and the other half will remain clean. These tiles will be placed in the field in oiled and unoiled pairs.

At each of the experimental sites, rocks and tiles will be placed at the 2 m elevational contour. Each site will receive an identical number of rocks and tiles representing the following experimental conditions (Fig. 4):

> The basic experimental unit is six rocks and six pairs of tiles. Three of the rocks are from the original oiling by EV and cleaned with $MeCl_2$. The remaining three rocks were one-half treated with fresh PBC. A pair of clay tiles consists of one tile treated with fresh PBC and one unoiled tile. In addition to this basic experimental unit, there are 3 sets of 3 rocks representing EV and 3 sets of 3 rocks representing fresh PBC. These are placed in the field as destructive samples, with each set to be retrieved at three separate time periods. These time periods are mid summer 1990, early fall 1990, and mid spring, 1991. Finally, at each site, one completely oiled rock with EV and one with fresh PBC and one entirely cleaned rock

with MeCl, will serve as sources for weathering analysis and cleaning control for MeCl, respectively. Refer to Attachment 2 for the SOP for oil weathering analysis.

For the purposes of this SOP, each of these experimental units (33 total per site) is referred to as a "batch" of rocks and tiles. This "batch" will represent all substrate combinations to be located at one site. Using a random numbers generator on a calculator, each uniquely identified rock (i.e. those freshly painted with PBC and last year's coating with EV) will be assigned to a "Batch". These batches of rocks and tiles will then correspond to numbered poker ships, and each chip will be drawn and assigned to the matrix of study sites. Each batch of rocks and tiles will be boxed and taken to their respective sites.

Once in the field, a uniform substrate at the 2 meter elevation contour of each site will be chosen, video taped and mapped. Establishment of the second meter of vertical fall is pursuant to IOP#1.

The marine epoxy used to glue the rocks to the rock substrate is a 1:1 mixture of a two-part compound. Working time of the epoxy is approximately 30 minutes.

It is important to ensure that all rocks and tiles are placed along the same elevation contour. Furthermore, the substrate should be scraped free of most barnacles and macro algae to ensure a firm setting of the epoxy to the substrate. Once the specific location is established, poker chips will be placed into a bag, shaken and drawn to determine whether an EV or PBC, tile pair, or control rock will be placed first. After this determination, the placement of these units will be alternated based upon the order drawn, until each batch is completely laid out. Obviously, those units with lessor replication will be exhausted first, eventually resulting in alternation only between EV and PBC rocks. This procedure is to be repeated at each site.

After placement, each site will be visited once a week during low tide series. Observations as to settlement by barnacles and macro algae on rock surface and surrounding settlement conditions will be recorded. Also, individual species will be identified, counted and recorded. Counting will involve use of a 3 cm X 3 cm quadrat. The quadrat edge is placed at the midpoint of the line separating the oiled and unoiled portions of the rock. Rocks will be photographed at a fixed

focal length to incorporate a 5 cm X 10 cm area. From left to right along the site, the first 3 rocks of EV, the first 3 rocks of fresh PBC, the clay tiles, and all control rocks are to remain affixed indefinitely, and will be examined in the field and through macro photography. However, the remaining rocks are to be retrieved from left to right at 3 separate time series. These time series will occur in mid-summer, 1990, early fall 1990, and spring of 1991. After field examination and photography, these rocks will be returned to the laboratory and organisms that settled will be counted. Counting will involve either a point method for settlement or a magnified transect and micro photographing of the rock surface. Also, scraping of the rock surface and preparation of slides for micro algae and diatom identification may be attempted.

Limpet Grazing

At each of eight sites, algal beds will be identified and measured at two tidal contours (i.e. meters of vertical fall). Because treatment of oiled sites by Exxon resulted in removal of the upper elevational zones (particularly *Fucus*), the first elevational contour is defined at the control sites. Fences will be placed at the first meter of vertical fall contour within the *Fucus* zone based. As an example, if the *Fucus* begins at MHHW, then the transect is placed at the 1m contour. This procedure will be replicated at the contour of the impacted shoreline, where mechanical treatment such as high pressure-hot water washing, may have removed large concentrations of *Fucus* cover. This first contour at the impacted sites will also be verified by evidence of *Fucus* holdfasts (plant bases) or stipes, occuring within the contour.

The objective for the second elevational contour was to examine grazing in an algal dominated zone other than *Fucus* (i.e. *Cladophora*). The oil-impacted sites are used to determine placement of fences. Because Exxon was instructed to treat the shorelines down to only the mid-intertidal zone, all fences will be established at the beginning of this *Cladophora* zone, where impacts from treatment activities can be observed. The elevational contour of this observed zone is averaged within the site, and all fences are placed along this selected contour, regardless of the variation of algal bed density throughout the contour level. For example, if *Cladophora* zone and observed impacts begin at 2.25 m, then the location of fences will be established in this zone. This procedure dictates the second contour at control sites. Consequently, the second contour level may vary between pairs, but is identical at each control and impacted pair.

Once the algal beds are measured, subtract 0.25 meters on either side to ensure the sample area is buffered. Each location should preferably be homogenous in substrate composition, such as an algal bed on a rock outcrop.

Eight fences will serve as a base unit. With the 2 tidal heights, this will constitute 16 fences per site (Fig. 5) Fences will be structured within the substrate by marine epoxy and hard ware cloth. Fences will be 25 X 25 cm in area, and will be randomly placed within each site. The length of each algal bed is measured and the appropriate buffer subtracted. Eight fences are divided into the resulting length, giving a segment length, and this number is multiplied by a random value. The resulting value establishes location of the first transect. Subsequent fences are placed one segment length from the precious fence. Each fence is placed at the transect mark at the selected elevation contour. Place the fence at this contour with the upper left corner of the fence serving as the marked point. As an example, a 25 meter algal bed minus 0.25 meters on each side equals a 24.5 meter workable area. Eight fences divided into this length equals 3 meters per fence. Three times the random value 0.98 equals 2.94 meters for the first fence. Therefore, the 2nd - 8th fences are placed every 3 meters after 2.94 m. Should a fence location be determined as unworkable, a rule is established to move the location 25 cm to the left until the first workable area is encountered. "Unworkable" is defined as an area that is not uniform for placement of a 25 X 25 cm fence.

Use of marine epoxy for anchoring the fences will require cleaning small strips within the algal beds to ensure proper adhesion of the epoxy. This should be done with as little disturbance to each site as possible so that the interior to each fence still retains all of its original algal cover. After the epoxy begins to set, place the fencing into the epoxy base and tie the corners with wire. Also, construct a small lip to prevent escape and entry of limpets over the fence.

After these fences are established at each site, large numbers of limpets from the genera *Lottia* and *Tectura* will be collected at a site away from all study areas. No individuals less than 10 mm in shell length will be used for this study, based upon difficulty in differentiation among genera below this size. The selected individuals will be measured, weighed and sorted in to genus and size classes, and uniquely identified and marked. The method of marking will involve a miniature tag (i.e. electronic wiring number labels & epoxy) glued to the shell of the limpet.

Based upon an average density of limpets determined by transects, a representative limpet from each genera and size class will be selected to form a "batch" of limpets (X). To

exemplify, if the transects disclose a mean density of six limpets per unit area, then six limpets of representative size and species distribution constitutes a single "batch" of limpets. These "batches" will serve as the base unit for placing numbers of limpets in fences. Each batch will be placed into customized containers and kept in fresh seawater for holding.

A randomized selection of fence treatments will occur by use of poker chips representing the cells of the following experimental matrix:

	2X	X	X/2	0
ALGAE				
NO ALGAE				

This process will be re-randomized at each site.

The batches of limpets will be numbered, and poker chips will serve as representatives for assigning groups to the fences at each site. All batch-numbered poker chips will be placed in a bag and drawn randomly to be assigned to each of the fences and treatments. Batches will be assigned according to the above matrix. For the X/2 treatments, one batch of limpets will be divided between two fences.

For those fences selected for algal removal, the entire 625 cm area within the fences will have all algae, herbivores and limpet predators removed. The algae will be bagged and returned to the laboratory for wet and dry weight measure. With the remaining cages, all mobile invertebrates (i.e. *Littorina*, *Nucella*) will be removed, and total percent algal cover, as well as percent cover by algal species will be estimated and determined by a point grid method. Also, a photograph will be taken of each fenced area.

Limpets will then be placed in each fence according to their batch assignments. The fences will be revisited during low tide periods on a weekly basis. Numbers of surviving individuals will be recorded; dead limpets will be removed and their shells measured (Fig. 6). Also, new recruits will be recorded, but removed. The total percent algal cover and percent cover by algal species will again be determined by a point grid method.

This experiment will be run throughout the summer, or until all limpets have died. Survivors will be remeasured and reweighed. At the termination of the experiment, all algae will be removed and bagged, identified in the laboratory, and a final wet and dry weight will be measured. When the experiment is terminated, all fence material will be removed from each site.

Population Dynamics of Limpets, Littorina, Nucella and *Leptasterias hexactis*.

The objectives of this study are to monitor differences in numbers of limpets, *Littorina*, *Nucella*, and *Leptasterias hexactis*, between impacted and non-impacted sites.

Transect heads will be established pursuant to Coastal Habitat operating procedures number 1 (Fig 7). The length of each site will be divided by 6 and multiplied by a random number to locate transect heads. Quadrats will be located randomly along each transect as in Site Characterizations. Permanent quadrats will be established within the first, second and third meters of vertical fall along each of these six transects. Use of a rotohammer will mark the upper left and bottom right of each quadrat.

Quadrat dimensions are 20X50 cm. Once in place, count all limpets, *Littorina*, *Nucella*, and *Leptasterias hexactis* within the quadrat and record this data on the data form (Fig. 8).

Measure a 1 m semicircle adjacent to and centered at the left of the 20X50 cm quadrat. Measure the distance from the center of the base of the semicircle to the closet limpet, *Littorina*, *Nucella*, and *Leptasterias hexactis* or 1.0 m if none of these species are found in the semicircle.

ATTACHMENT 1

Videography of Sites

Each site selected will be recorded on videotape during a low tide period. This record will serve as an aid to site description.

Each "Control" site and its oiled counterpart is filmed. Panning of each site from a skiff will occur from left to right. Site number and the experiments being conducted on the site is recorded on the audio portion of the tape.

After the distant filming of a given site, an on-shore filming of the 0 meter mark if taken. The video camera then pans to the right to view as much of the site as possible. The aspect of shooting is from the upper intertidal.

If the site is of significant length (i.e. 50 m or greater, or heterogeneous in shoreline topography) the video camera will record intermittent markings (i.e. z-spar epoxied meter marks) of the site, while panning to the right of the mark, and then back to the left to show reference to the beginning of the site.

Finally, the end of the site is videotaped, showing the end site mark, with total meters and site number marked in an epoxied "medallion" placed at the base of the Verrucaria zone. The video camera then pans across the entire site, back toward the zero meter mark.

ATTACHMENT 2

Settlement on Oiled and Un-oiled Substrates:
Oil Extraction for "Weathering" Analysis.

Throughout this substrate transplant experiment, the chemical composition of crude oil will change over time. Consequently, the "thickness" of oil coating of the substrates will gradually decrease. The purpose of this procedure is to quantify a rate of change in the oil's character. This procedure employs a simple gravimetric analysis of an area of oil, extracted by Methylene Chloride ($MeCl_2$).

Several of the substrates placed in the field are solely for the purpose of these extractions. On a monthly basis, a 3 cm^2 area of both an EV rock and PBC rock will be wiped with a methylene chloride saturated wipe (i.e. kimwipe). This wipe will be pre-weighed and placed in a small vial containing 25 ml of $MeCl_2$.

After wiping, the wipe will be returned to the laboratory. Al $MeCl_2$ will be allowed to evaporate, and the wipe will be reweighed and re-stored in the same vial with an additional 25 ml of $MeCl_2$. This vial will be refrigerated for possible Gas Chromatography/Flame Ionization Detection (GC/FID) analysis.

FIGURE 1.

Form Used in Recording Site Characterization Data

CHIA/UAF HERRING BAY EXPERIMENTAL STATION

Site Characterization Log Sheet

Date:_____ Site Number:_____

Time:_____ Site Name:_____

Weather: sunny partly cloudy cloudy rain snow

(circle)

Samplers: M. Derenoff G. Hollowell A. Hooten T. Lewis

G. Reedy F. Roddy C. Sullivan P. Van Tamelen _____

(circle)

Transect Number:____

Quadrat # ____ Procedural Checklist

___ photograph left 40x50 area with drift algae removed (_____ roll and frame #)

___ remove Fucus from right 40x50 cm area

___ photograph right quadrat (_____ roll and frame#)

___ determine total percent cover _____%(left 20x50)

_____%(right 20x50)

___ complete species checklist on reverse of this page

___ measure nearest limpet within 1 meter:_____ (enter 1m if none)

___ collect five nearest limpets (see reverse)

Quadrat # ____

___ photograph left 40x50 area with drift algae removed (_____ roll and frame #)

___ remove Fucus from right 40x50 cm area

___ photograph right quadrat (_____ roll and frame#)

___ determine total percent cover _____%(left 20x50)

_____%(right 20x50)

___ complete species checklist on reverse of this page

___ measure nearest limpet within 1 meter:_____ (enter 1m if none)

___ collect five nearest limpets (see reverse)

Quadrat #____

___ photograph left 40x50 cm area with drift algae removed (_____ roll and frame #)

___ remove Fucus from right 40x50 cm area

___ photograph right quadrat (_____ roll and frame#)

___ determine total percent cover _____%(left 20x50)

_____%(right 20x50)

___ complete checklist on reverse of this page

___ measure nearest limpet within 1 meter:_____ (enter 1m if none)

___ collect five nearest limpets (see reverse)

ACE 6604625

SPECIES CHECKLIST

ALGAE	Q1	Q2	Q3
Acrosiphonia arcta			
Audinella purpurea			
Blidingia chaudefaudii			
Cladophera sericea			
Cryptosiphonia woodi			
Devaleraea ramentacea			
Dumontia contorta			
Endocladia muricata			
Enteromorpha linza			
Enteromorpha intestinalis			
Fucus gardneri			
Fucus sp.			
Gloiopeltis furcata			
Halosaccion glandiforme			
Hildenbrandia rubra			
Mastocarpus papillatus			
Myelophycus intestinalis			
Neorhodomela aculeata			
Ondontalia floccosa			
Phycodrys rigii			
Pilayella washingtoniensis			
Polysiphonia senticulosa			
Prasiola borealis			
Ptilota pectinata			
Ralfsia fungiformis			
Rhodomela subfusca			
Scytosiphon lomentaria			
Ulothrix implexa			
Ulva fenestra			
Yendonia crassifolia			
Corallines: articulated ___ encrusting ___			

INVERTEBRATES	Q1	Q2	Q3
Anthropleura artemisia			
Balanus glandula			
Chthalamus dalli			
Halobisium accidentale			
Kathanna sp.			
Ligia sp.			
Littorina scutulata			
Littorina sitkana			
Lottia borealis			
Lottia pelta			
Lottia sp.			
Macoma balthica			
Mytilus edulis			
Modiolus rectus			
Nereis sp.			
Nucella emarginata			
Nucella lamellosa			
Polychaeta sp.			
Searlesia dira			
Semibalanous balanoides			
Semibalanus cariosus			
Siphonaria thersites			
Spirorbis sp.			
_____			
_____			
_____			

limpet data from reverse

Q3

	species	length	weight
1.			
2.			
3.			
4.			
5.			

Q1

	species	length	weight
1.			
2.			
3.			
4.			
5.			

Comments:

Q2

1.			
2.			
3.			
4.			
5.			

Signature: _____

ACE 6604626

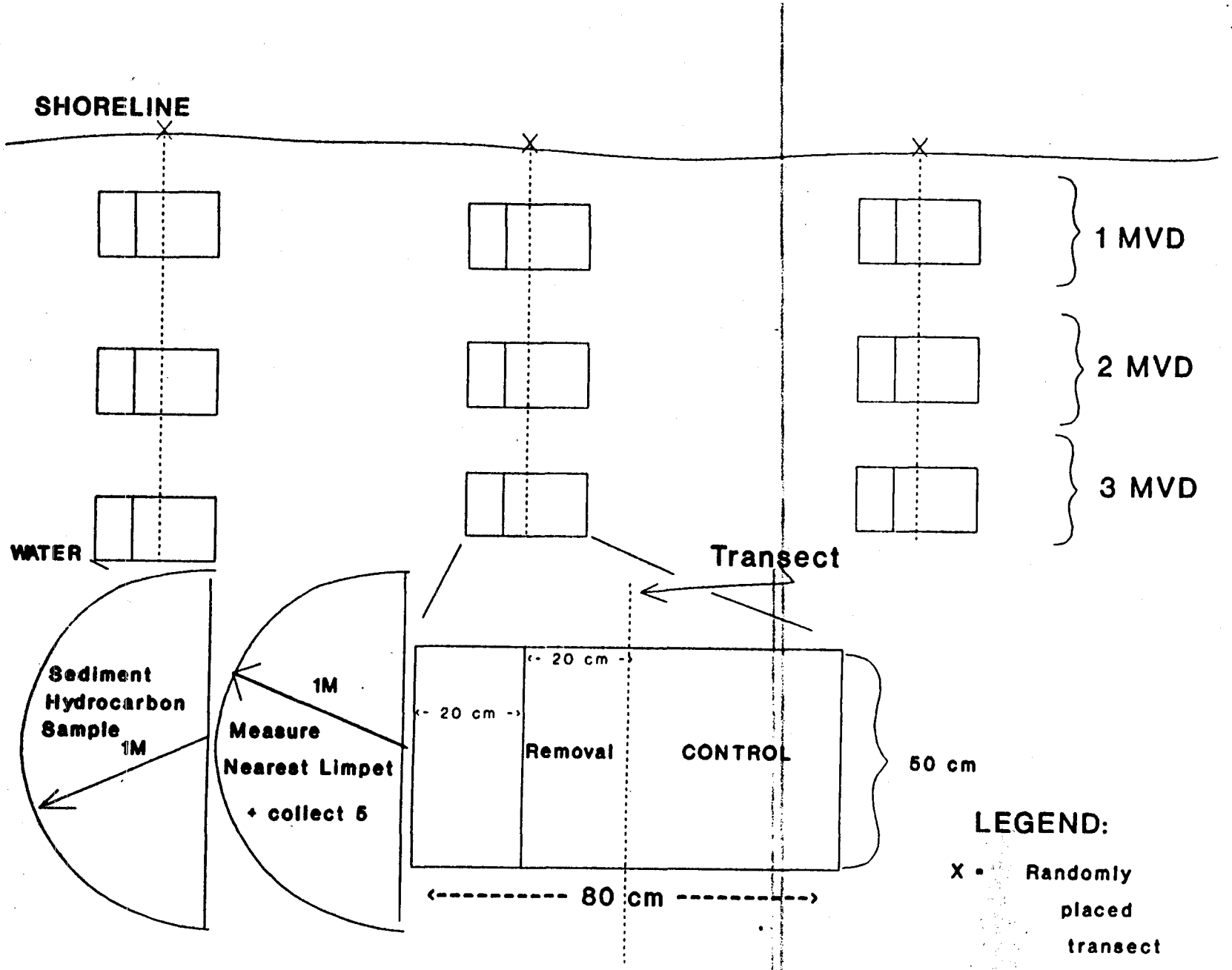

Figure 2. Placement of quadrats for Site Characterizations

FIGURE 3.

Form Used for Recording Settlement Data on Oiled and Non-Oiled Substrates.

CHIA/UAF HERRING BAY EXPERIMENTAL STATION

RXΔ Log Sheet

Date: 11 July 90 Site Number: 1723 X

Time: 1017 Site Name: Barnacle PL

Weather: sunny (partly cloudy) cloudy rain snow (circle)

Samplers: M. Derenoff G. Hollowell A. Hooten T. Lewis G. Reedy

F. Roddy (C. Sullivan) P. Van Tamelen (circle) _____

Legend: Unit #'s EV = 1+ year old Exxon Valdez crude oil
PB = fresh Prudhoe Bay crude oil
T = tile pair (oiled vs. non-oiled)

** NOTE: Report all data as O/U for oiled vs. non-oiled substrate.**

Counts done in 33mm² subsection of units.

Unit Number	New Settlement? O/U (y/n)	# Barnacle Juveniles	# Fucus Germlings	Other (define)	Roll and Frame #
1. T1	n/n	0	-		X:24 (cont.) 10,11,12
2. PB 40	n/n	0			13,14,15
3. PBOW	~		~	~	16
4. EV 60	y/y	3/7			17,18,19
5. T2	n/n	0			20,21,22,23,
6. PB 65	y/y	80/38			X:25 1,2,3 (both)
7. C1723X M	y/y	254/269			4,5,6
8. EV 9	y/y	25/32			7,8,9
9. T3	y/y	3/18			10 Non oiled
10. PB 20	y/y	171/250			11,12,13
11. C1723X EV OW					14
12. EV 12	y/y	103/37			15,16,17
13. T4	y/y	4/3			18 Non-oiled
14. PB 55	y/y	3/6			19,20,21
15. EV 45	y/y	223/331			22,23,24

Pics: O. Ch

ACF 6604629

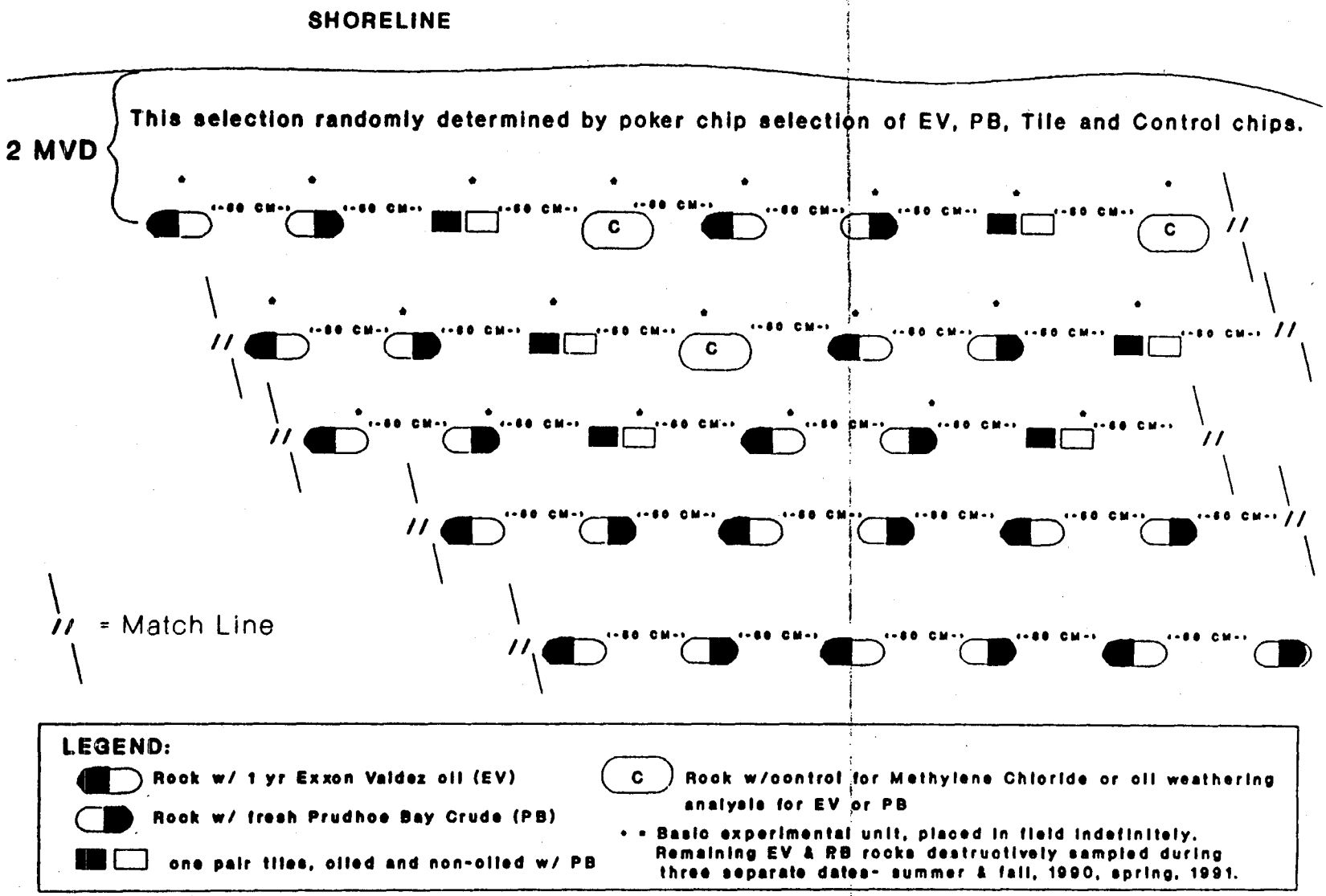

Figure 4. Placement of oiled and non-oiled substrates.

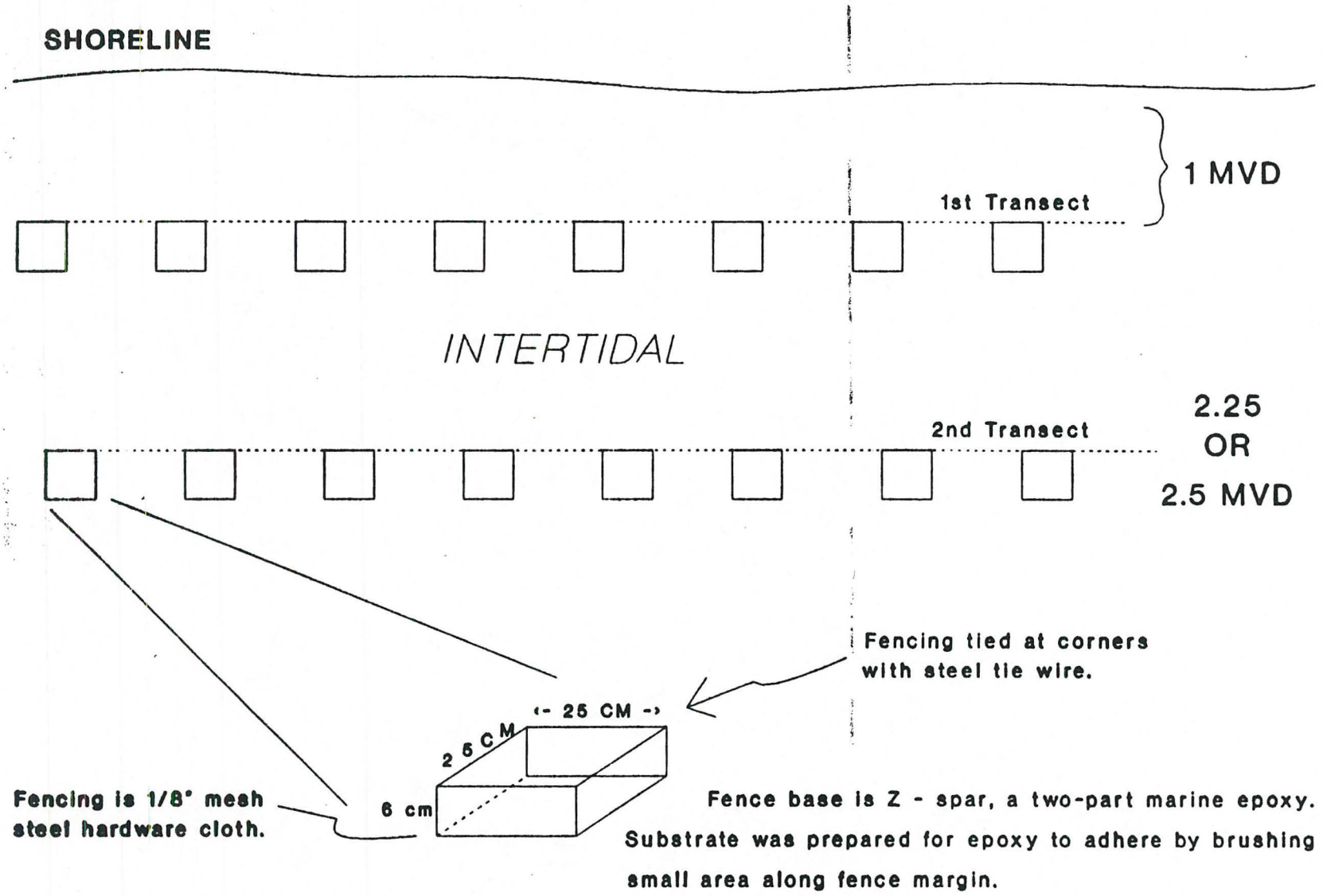

Figure 5. Placement of fences for Limpet Grazing Experiment.

FIGURE 6.

Form Used for Recording Limpet Grazing Data

CHIA/UAF HERRING BAY EXPERIMENTAL STATION

Limpet Grazing Log Sheet

Date: 7-13 Time: 1100 Site Name: Waikiki

Contour: 2.0 MVD Site Number: 1311X

Weather: sunny (partly cloudy) cloudy rain snow (circle)

Samplers: M. Derenoff G. Hollowell A. Hooten (T. Lewis) (G. Reedy)

F. Roddy C. Sullivan P. Van Tamelen _____ (circle)

Comments:

number last time missing

1 AX (5) Limpets remaining: 326 (1)

Algae present	Estimated % cover	# dots covered	Total Algal Cover:
1. Fc.y	(10 + 15).5 = 12.5 %	17/100 = 17%	42/100 = 42%
2. Cd.s	(5 + 10).5 = 7.5 %	10/ = 10%	
3. Mg.i.	(3 + 3).5 = 3 %	3/ = 3%	comments:
4. Rd.s.	(3 + 7).5 = 5 %	11/ = 11%	1 L.p removed
5. Palmaria	(<1 + <1).5 = <1 %	1/ = 1%	5 overlapping
6. Hb.r.	(1 + 2).5 = 1.5 %	5/ = 5%	
7.	(+).5 = %	/ = %	
8.	(+).5 = %	/ = %	

2 2X (4) Limpets remaining: 192, 232, 505, 282, 562, 506, 425, 260, 230, 644, 149, 547 (12)

Algae present	Estimated % cover	# dots covered	Total Algal Cover:
1.	(+).5 = %	/ = %	/ = %
2. Ø	(+).5 = %	/ = %	
3.	(+).5 = %	/ = %	comments:
4.	(+).5 = %	/ = %	removed 1 L.p.
5.	(+).5 = %	/ = %	removed 1 N.I.
6.	(+).5 = %	/ = %	
7.	(+).5 = %	/ = %	
8.	(+).5 = %	/ = %	

3 Ø Limpets remaining

Algae present	Estimated % cover	# dots covered	Total Algal Cover:
1. Hb.r.	(2 + 1).5 = 1.5 %	Ø/198 = %	Ø/198 = Ø%
2.	(+).5 = %	/ = %	
3.	(+).5 = %	/ = %	comments:
4.	(+).5 = %	/ = %	removed 1 N.I.
5.	(+).5 = %	/ = %	removed 1 sea star
6.	(+).5 = %	/ = %	
7.	(+).5 = %	/ = %	
8.	(+).5 = %	/ = %	

4 X/2 (1) Limpets remaining: Ø

Algae present	Estimated % cover	# dots covered	Total Algal Cover:
1. Hb.r.	(1 + 3).5 = 2 %	4/100 = 4%	4/100 = 4 %
2. Cd.s.	(<1 + <1).5 = <1 %	Ø/ = 0 %	
3.	(+).5 = %	/ = %	comments:
4.	(+).5 = %	/ = %	
5.	(+).5 = %	/ = %	
6.	(+).5 = %	/ = %	ACE 6604633
7.	(+).5 = %	/ = %	
8.	(+).5 = %	/ = %	

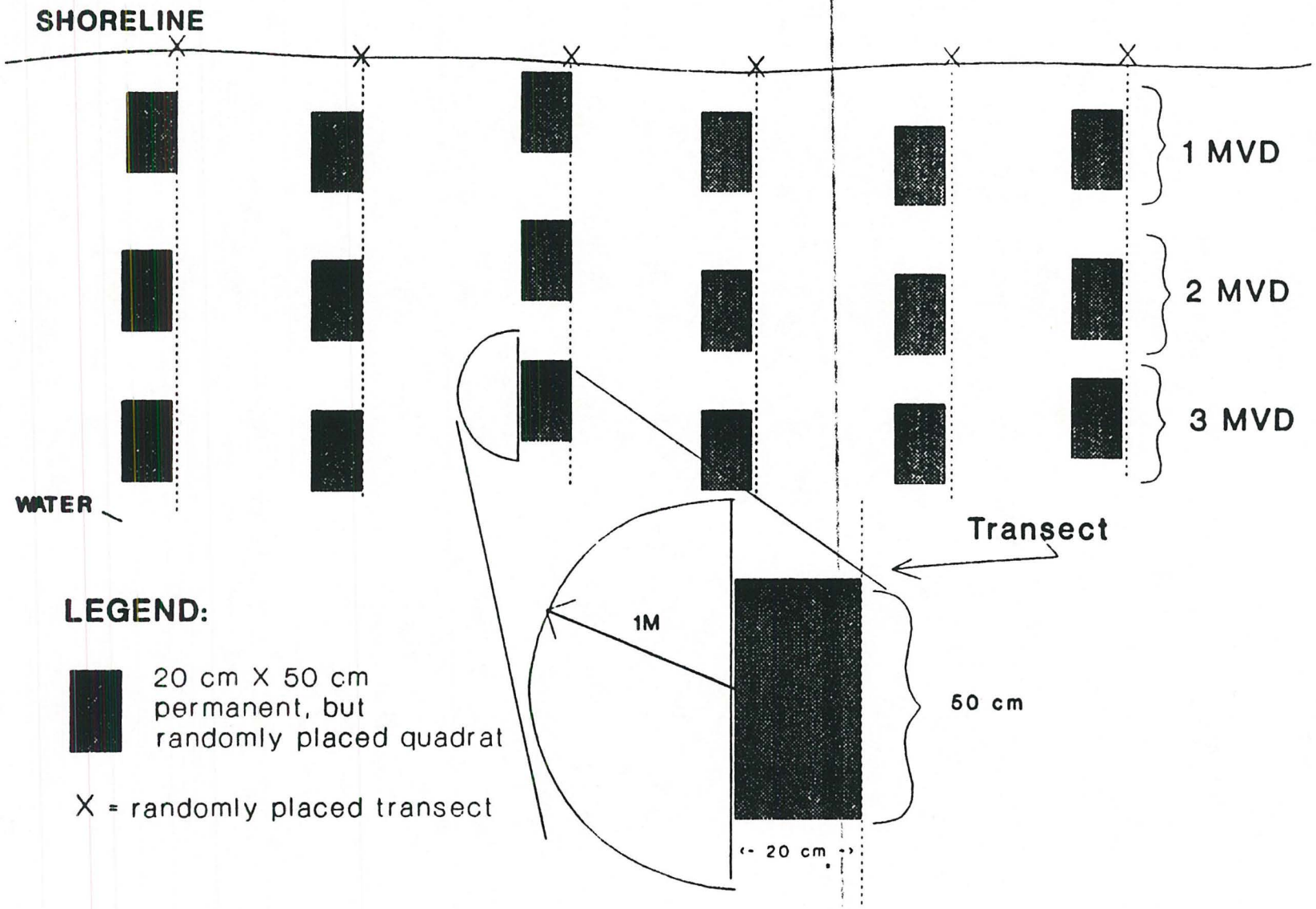

Figure 7. Placement of Permanent Quadrats for Population Dynamics of Limpets, Littorina and Nucella

FIGURE 8.

Form Used for Recording Invertebrate Population Dynamics Data

CHIA/UAF HERRING BAY EXPERIMENTAL STATION
Invertebrate Population Dynamics

Site Name: Anchor Beach Date: 08/22/90 Signature: MD
Site Number: 2834X Time: 8:08am and (10:43a) (comments on reverse)

Weather: (sunny) partly cloudy cloudy rain snow
(circle)

Samplers: (M. Derenoff) G. Hollowell A. Hooten T. Lewis G. Reedy
C. Sullivan F. Roddy P. Van Tamelen ______
(circle)

		# of Lottia	# of T. persona	# of T. scutem	# of unknown limpets	Σ of all limpets	# of N. emarginata	# of N. lamellosa	Σ of all Nucella	# of L. sitkana	# of L. hexactis	distance to nearest Littorina	distance to nearest limpet (L., T.p. or T.s.) (UNK.)	distance to nearest Nucella (N.r. or N.l.)
transect 1	1m drop 1		3			3			0	2	0	4.5cm	19cm T.p.	>1m
	2m drop 2	1	11		3	15			0	0	0	>1m	6cm T.p.	60cm Ne.
	3m drop 3		1			1			0	0	0	>1m	38cm L.p.	>1m
transect 2	1m drop 4		-			0			0	14	0	10cm	43cm L.p.	>1m
	2m drop 5		3			3			0	8	0	38cm	1cm T.p.	>1m
	3m drop 6	6	10		61	77			0	0	0	36cm	4cm T.p.	94cm Ne.
transect 3	1m drop 7					0			0	1	0	26cm	>1m	>1m
	2m drop 8		2			2			0	4	0	4cm	44cm T.p.	>1m
	3m drop 9					0			0	12	0	4cm	29cm T.p.	>1m
transect 4	1m drop 10					0			0	5	0	7cm	>1m	>1m
	2m drop 11	1	1			2			0	7	0	6cm	35cm L.p.	>1m
	3m drop 12				1	1			0	20	0	9cm	21cm T.p.	>1m
transect 5	1m drop 13					0			0	3	0	9.5cm	23cm T.p.	>1m
	2m drop 14		2			2			0	12	0	3.5cm	7cm T.p.	>1m
	3m drop 15	4	8		15	27			0	2	0	>1m	14cm T.p.	4.7cm Ne.
transect 6	1m drop 16					0			0	1	0	37cm	56cm T.p.	>1m
	2m drop 17					0			0	13	0	3cm	7cm L.p.	>1m
	3m drop 18	1			15	16			0	3	0	>1m	22cm UNK.	43cm Ne.

#2: Pilayella/Cladophora lying over rocks.
#9: drift plants covering 40% of Q.

ACE 6604636

APPENDIX 2

1991 Invertebrate Standard Operating Procedures
Herring Bay Experimental Field Station

COASTAL HABITAT INJURY ASSESSMENT:
Standard Operating Procedures
Intertidal Invertebrate Field Experiments
Herring Bay, Price William Sound, Alaska: 1991

These 1991 Standard Operating Procedures (SOP) serve as a supplement to the 1990 barge SOP. A copy of the 1990 SOP is appendix 1. Any changes, or exceptions to studies from 1990 are identified herein.

Site Selection:

Additional sites may be selected to increase replication of some of the 1990 studies. The selection of sites will be based upon availability, to effectively match both impacted and control pairs. Any additional site selection in 1991 will be implemented pursuant to the 1990 SOP.

Population Dynamics of Limpets, Littorina, Nucella and Leptasterias.

The population dynamics monitoring as described in the 1990 SOP will be continued at all 10 sites. For 1991, size class determination of each species examined will be included in the normal census. When quadrats are conducted at each study site, Limpets, *Littorina sitkana*, *Nucella* spp. and *Leptasterias hexactis* will not only be counted, but each count will be divided into size classes for each of these species. If possible, additional site pairs for population dynamic studies will be established.

Transplanting of oiled and non-oiled substrates.

With the 1990 experiment, the last time-series sample is to be retrieved in April. The remaining experimental units will be left in the field indefinitely and monitored during the 1991 season. However, as a supplement to this original experiment, new red clay tile pairs (both tarred and cleaned) will be added to each site in a manner identical to the procedure defined in the 1990 SOP. Six pairs of tarred and cleaned tiles will be replaced at each study site, with one pair destructively sampled each month, and quantified for barnacle settlement in the laboratory. At each site, four pairs will be destructively sampled over the course of the field season, and two pairs will remain on site through the duration of the experiment. These tiles will be placed in those identical locations where time series units were removed during 1990. These locations can be easily determined from the permanent units remaining at each site, and will preserve the original random assignment of units. Units remaining in the field will continue to be quantified pursuant to the 1990 SOP.

Also, one additional oiled tile placed at each site will serve as a source for oil weathering analysis. Refer to Attachment 2 of the 1990 SOP for the oil weathering analysis procedures.

Mussel Densities:

As a result of the CHIA intertidal sampling in 1989 and 1990, an increase in the abundance of mussels at oiled sites has become evident. This may be explained by certain species of filamentous algae recolonizing at oiled sites free from grazing pressure, and recruiting juvenile mussels benefitting from these algae, which serve as anchoring substrate. The following experiment tests this hypothesis by clearing small areas and excluding all but algae and mussels and monitoring mussel density over time.

Three pairs of oiled and control sites with evidence of mussel populations will be selected for this experiment. At each study site three transect heads will be randomly selected pursuant to the methods identified for transect selection (1990 SOP). At each of these points, an area at the 1.5 meter contour (from MHHW) will be selected. This area, 0.5 m in radius, will be cleared of all algae and invertebrates. A circular fence of 1/8" mesh steel hardware cloth (10 cm in height) and marine epoxy will be constructed around the boundary of the cleared area.

Each site will be monitored three times each week to ensure that all grazers/predators are removed from the fence. Once each month, a random dot grid will be placed over the fence and a patch of filamentous algae 5 x 5 cm will be selected and removed from each fence. This sample will be placed in a whirlpak bag and returned to the laboratory. Each sample will be analyzed for mussel content, and the total number of mussels in each sample will be counted. Counting will involve use of a dissecting scope and a hand-held counter. Counting of each sample will be repeated until the coefficient of variance of the mean does not exceed 0.05. If too many mussels are present in the sample, then a subsample of the original will be quantified as above.

Site Characterizations:

Site characterizations will remain a lower priority compared to the specific experiments conducted at each of the study sites. Each of the sites originally selected during the 1990 field season will again be characterized pursuant to the 1990 SOP, and will be visited three times during the beginning, middle and end of the field season. However, no new additional sites that may be selected for expansion of any of the detailed experiments will be added to the site characterization process.

Barnacle recolonization:

This experiment was not an original part of the 1990 SOP. Therefore, materials and methods used during 1990 are presented in this SOP, and a discussion to expand this experiment in the 1991 field season follows.
Within Herring Bay, certain oiled locations have heavy accumulations of dried tar, especially in the upper intertidal zone, where desiccation and baking by sunlight have resulted in an asphalt condition of the oil. The purpose of this study was to examine whether the presence of tarred upper intertidal areas reduces the settlement capability of barnacle larvae relative to cleaned areas within a tarred substrate. Further, does the presence of oil reduce the survival of barnacle juveniles, and how do such differences compare to barnacle settlement at reference sites?

Two oiled sites and two reference sites of similar character were selected in Herring Bay for this study in 1990. The oiled sites selected have vertical rock faces where barnacles presently exist, or in the case of sites that were heavily oiled and treated, having skeletons still attached to the substrate. The non-oiled reference sites chosen had high densities of the barnacle *Semibalanus balanoides*.

At each site, paired 10 X 10 cm plots were established. One member of each pair was scraped and brushed to remove all visible tar (or barnacles in the cases of the non-oiled sites). The length of each site was measured, and the number of planned pairs divided into the site length. The first plot was placed randomly, within the first segment, and subsequent plots were placed at equal distances from the first. A coin was flipped to determine which 100 cm^2 area of the first pair to scrape. The subsequent scraped plots were then alternated.

The sites were periodically examined for barnacle settlement, as well as germlings of the alga, *Fucus gardneri*. The number of barnacle juveniles and germlings were recorded during each inspection. Each 100 cm^2 area was also photographed.

During the 1991 field season, this experiment will be expanded to include three additional study site pairs. Each site will be measured and prepared in a manner identical to that described above. All sites will continue to be monitored, counted and photographed during 1991.

Grazing by Limpets:

Based upon results obtained in 1990, this study will be discontinued.

Algal Succession:

At the termination of the limpet grazing experiment, an opportunity was presented to gain an understanding of temporal algal succession with Herring Bay. Several questions regarding algal succesion in general are relevant to possible impacts from oil. These questions include:

1. Can a basic algal succession for Herring Bay be defined?

2. Based upon the number of sites chosen for study, is there a difference in algal succession (and thus, species composition) between reference and impacted sites?

3. Is there a difference in algal succession between areas that are simply scraped free of all algae, vs. the application of a killing agent (such as bleach)?

Because eight study sites were chosen for the limpet study (four pairs of impacted and reference sites) and the termination of the experiment involved removal of the fences and all algae within each 625 cm area, this provided a platform to easily examine algal species succession at multiple plots.

At the termination of the limpet grazing study, fences from two elevation contours were removed. Only the marine epoxy used to hold the fences in place was left to serve as a marker for each of the algal succession study plots.

All algae was removed from each 625 cm area. A coin was flipped to determine if the odd or even numbered fences would receive application of sodium hypochlorite (bleach). The results from this random selection are listed as follows:

Site #	Contour:		Date conducted
1411C	upper:	Odd	9-10-90
1411C	lower:	Odd	9-10-90
1311X	upper:	Even	9-10-90
1312C	upper:	Even	9-10-90
1312X	upper:	Odd	9-11-90
3811C	upper:	Even	9-12-90
3611X	upper:	Odd	9-13-90
1713C	upper:	Even	9-14-90
1713X	upper:	Even	9-14-90
1251X		Odd	9-15-90
1251C		Even	9-16-90
1231X		Even	9-15-90
1231C		Even	9-17-90
1852C		Odd	9-18-90

Sessile invertebrates, such as barnacles were not removed from the 625 cm area. A photograph of each experimental was taken (with photolabels in each frame).

Each study plot will be revisitied to assess algal species composition. At the time of visitation, percent cover of each plot by separate species will be determined by a point grid method. Also, photographs of each plot will also be taken, and a voucher specimen of each plant observed within each plot will be collected. Voucher specimens will be sent to UAF for taxonomic identification. Finally, numbers and species of invertebrate grazers found within the 625 cm area will also be counted.

This process will be repeated until four time series plots have been established for both the scraped and bleached plots at each contour. These study plots will be monitored quarterly.

Temperature and Salinity:

Stations adjacent to all 1990 study sites were monitored weekly for temperaure and salinity at the 0, 0.5 and 1 through 5 m depth contours around MHHW, using a CTD meter. This procedure will be continued through the 1991 season.