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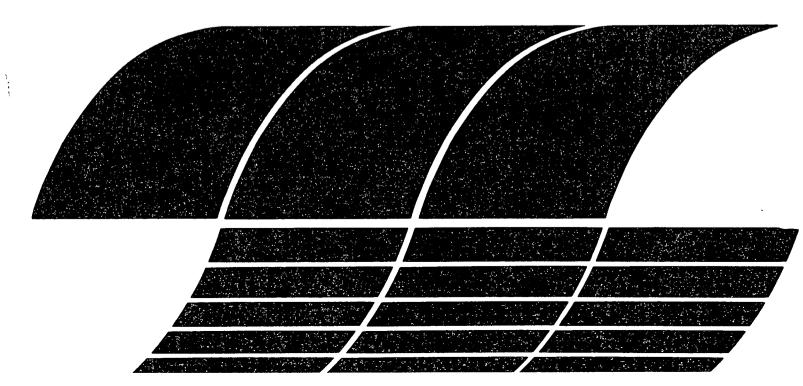
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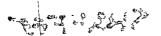
United States Environmental Protection Agency Office of Environmental Engineering and Technology Washington DC 20460 EPA-600/7-80-140 July 1980

Research and Development

Recovery of Strait of Juan de Fuca Intertidal Habitat Following Experimental Contamination with Oil

Interagency Energy/Environment R&D Program Report





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RECOVERY OF STRAIT OF JUAN DE FUCA INTERTIDAL HABITAT FOLLOWING EXPERIMENTAL CONTAMINATION WITH OIL

Second Annual Report Fall 1979 - Winter 1980

by

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Prepared for the MESA (Marine Ecosystems Analysis) Puget Sound Project, Seattle, Washington, in partial fulfillment of

EPA Interagency Agreement No. D6-E693-EN Program Element No. EHE625-A

This study was conducted as part of the Federal Interagency Energy/Environment Research and Development Program

Prepared for

OFFICE OF ENVIRONMENTAL ENGINEERING AND TECHNOLOGY OFFICE OF RESEARCH AND DEVELOPMENT U.S. ENVIRONMENTAL PROTECTION AGENCY WASHINGTON, D.C. 20460

March 1980

Completion Report Submitted to
PUGET SOUND ENERGY-RELATED RESEARCH PROJECT
MARINE ECOSYSTEMS ANALYSIS PROGRAM
ENVIRONMENTAL RESEARCH LABORATORIES

by

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FOREWORD

An anticipated increase in oil tanker traffic and proposals for construction of subsea pipelines in the Strait of Juan de Fuca and northern Puget Sound regions of Washington State are foreseen as part of the national energy development plans. These activities increase the opportunity for spillage of crude oil into the marine ecosystems of the region. The U.S. Environmental Protection Agency has supported studies dealing with biological characterizations, physical oceanography, trajectory modeling, pollutant monitoring, and fate and effects of oil in the region. These studies are being administered by NOAA's Marine Ecosystem Analysis (MESA) Puget Sound Project Office. The research reported here deals with recovery of intertidal and shallow subtidal communities in experimental habitats contaminated with Prudhoe Bay crude oil. The studies make comparisons in rate of recovery by communities in experimental coarse and fine sand habitat, and hard substrate habitat. They examine the role of vertical distribution of habitat in the tidal zone, site. type of substrate, season, and duration for recovery in field experiments.

ABSTRACT

This is a second year interim report on the effects of experimental oiling with Prudhoe Bay crude oil on recovery of intertidal infauna and epifauna of the Strait of Juan de Fuca, Washington State. It describes completed studies of the recovery of infauna as recovery rate relates to the experimental oiling, the site of study, tidal height, season of study, and duration of recovery. The report also describes the methods and initial results of studies of the effects from experimental oiling on epifauna colonization of hard substrates.

Full recovery is defined within the experimental framework for infauna as that composition and density of species which had colonized trays of untreated coarse substrate within the 15-month study period. The relevance of this definition is supported by presentation of data on composition and density of infauna at adjacent baseline stations as measured by other investigators. In terms of species composition, nearly full recovery of oiled substrates occurred in 15 months. For individual species densities, as well as overall abundance, however, oiled substrates had recovered only about one-half in 15 months. Total hydrocarbons in treated substrates were reduced from initial concentration by 85 and 97% for fine and coarse sediments, respectively, in 15 months. Based on rate of loss between 3 and 15 months, it is speculated that total hydrocarbons would have reached background levels in 18.5 months. Analyzed saturate compounds appeared to be lost from treated sediments at a rate similar to total oil. Analyzed aromatic compounds exhibited a much more rapid reduction in concentration than did saturate compounds or total oil.

As analyzed experimental variables, the site of study, tidal height, and sediment type, produced significant effects on the density if primary biological species. Overall, there were much higher densities at two feet below Mean Lower Low Water (MLLW) than at MLLW. Overall abundance appeared about equal between sediment types. Although not analyzed statistically, there appeared to be an order of magnitude higher density in the summer-fall experimental period than in the spring-summer experimental period.

The most severe effects from oiling on infauna density, as an expression of recovery, were seen for detritivorous and herbivorous species. The species for which significant effects on recovery were demonstrated were among those identified as having major trophic importance for a variety of bottom feeding fishes by other Strait of Juan de Fuca investigators.

The experimental oil treatment, while perhaps a "worst" case in the sense that the oil was mixed in sediment, was well within the concentration measured in sediments following some actual oil spills.

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INTRODUCTION

This is a second-year interim report on experimental studies of the effects of crude oil on recovery of intertidal communities of the Strait of Juan de Fuca. High interest in effects from oil and recovery of communities following oiling, stems from anticipated increases in tanker traffic, and superport and pipeline construction in the marine systems of the region. The interest is national in scope because the region is targeted as a through-point for crude oil shipment to midwestern markets.

Intertidal and shallow subtidal benthic communities are especially vulnerable to spilled oil because it is in their habitat that the surface of the water, on which oil floats, comes into intimate contact with the ground material, or substrate. Oil dissolved in water masses may have short-term toxic effects on marine organisms, but the substrates have been most clearly identified as sinks for spilled oil over longer periods of time (e.g., Michael et al., 1975; Straughan, 1978; Clark et al., 1973). There is variety in the substrate composition of the intertidal zone of the Strait of Juan de Fuca, but rock and mixed substrate consisting of rock and a matrix of mud, sand, or gravel predominate. because of longevity of predominate organisms, Nyblade (1979) has indicated that rock substrates will require the longest periods to recover (decades). However, the retention time for the oil itself is much longer in finegrained substrate. In certain conditions of spillage, this retention of oil may have an overwhelming influence on rate of recovery (Michael et al., 1975).

In particular cases, recovery of communities depends not only on organism longevity and substrate suitability but on a host of other factors including specific site, position with respect to tidal influence, season, and prevailing exposure. Additionally, recovery will depend on amounts of oil spilled, the types of cleanup measures used, and the degree of protection afforded critical populations, if these exist.

The overall objective in the present studies is to examine the influence of Prudhoe Bay crude oil on rate of recovery by intertidal and shallow subtidal communities in terms of substrate impairment by the oil as it relates to specific controllable variables (substrate type, site, tide level, season, and duration of recovery period). Information on the relative influence of these factors is pertinent to choices which may arise concerning physical protection of specific sites during a spill, application of remedial measures (chemical dispersion and cleanup) or siting of shore based facilities related to the through-point function.

To meet the overall objective, the studies have been divided into a number of tasks. The tasks associated with infaunal recovery are now complete and are as follows:

- A. Provide a time-series survey of species composition and total hydrocarbon concentration in experimentally prepared substrates.
- B. Measure the effect from Prudhoe Bay crude oil on reseeding by early colonizers as related to site and substrate grain size during a late summer early fall recruitment period.
- C. Measure the effect from Prudhoe Bay crude oil on reseeding by later colonizers one year after initial colonization during the late summer - early fall recruitment period.
- D. Measure at one experimental site the effect from Prudhoe Bay crude oil on reseeding by early colonizers during the late spring early summer season.
- E. Measure at one experimental site the effect from Prudhoe Bay crude oil on reseeding by early colonizers as related to tide height.

In addition, tasks have been initiated and some individual experiments have been completed for attached epifaunal studies on solid substrates as follows:

- F. Investigate the suitability of some artificial hard substrates for attachment in the exposed rocky intertidal of the Strait of Juan de Fuca for future experimental recovery studies.
- G. Investigate recovery of a rocky intertidal community in terms of larval reseeding rate for key species.

The following two tasks are scheduled for initiation during April 1980 and completion during August 1980:

- H. Investigate recovery of a commercial clam bed in terms of larval reseeding rate.
- I. Investigate recovery of a rocky intertidal community in terms of mortality and removal of key species.

CONCLUSIONS

Experimental studies of the effects of Prudhoe Bay crude oil on the recovery of infauna were conducted over a 15-month period. The studies involved field placement of oil-treated and untreated sediment trays at two sites, two tide levels, in two seasons and using two types of native sediment. At the conclusion of 15 months of field colonization, the numbers and kinds of animals in control trays closely resembled numbers and kinds of animals reported in similar habitat at two adjacent baseline stations. The similarity extended beyond overall numbers and kinds of animals since the relative distribution of numbers and kinds within the major taxa also closely paralleled that reported for baseline stations. From these data it is concluded that the 15-month control sediments were fully recovered and they reasonably represent what one would find by sampling uncontaminated sites on the Strait of Juan de Fuca with similar habitat.

Using the 15-month recovery of untreated sediments as a definition of full recovery, 3-month recoveries in similar sediment type, site and tide conditions were 69% for summer and 82% for fall in terms of numbers of species, and only 11% for summer and 18% for fall in terms of numbers of individuals. In 15 months, oil-treated sediments had recovered more than 90% in terms of numbers of species but had recovered only 48% in terms of numbers of individuals.

To protect the validity of statistical procedures, 13 species were selected as "primary" to evaluate effects on recovery in terms of individual species density. The numbers of individuals of the primary species comprised a very substantial proportion of all individuals in this study (78%) as well as at the Beckett Point baseline station (73%). They represented 33% of all individuals at the Jamestown baseline station for comparable conditions. Three-month control recovery for these primary species in terms of numbers of individuals closely paralleled that seen for all individuals (19% fall; 8% summer). The primary species represent the three major taxonomic categories quite well (polychaetes, crustaceans, mollusks).

Statistically significant effects on density (in this framework, recovery) were seen for individual primary species in each of the seasonal 3-month experiments and in the 15-month recovery period. The species affected were principally within the polychaete and crustacean groups. The mean differences were greater than those indicated for all species and indicate reductions in oil-treated sediments with one exception. The exception was a high density mollusk after 15-month recovery. Because of the differential response, here indicated by a statistically significant finding and elsewhere in the study indicated by mean differences, we

conclude that using total numbers of individuals to measure recovery from oil contamination will lead to highly conservative estimates of actual effects on recovery.

Effects from the oiling on recovery is strongly related to feeding type. Detritivorous and herbivorous species were almost universally influenced by the oiling. Carnivorous species were about evenly divided in their response to the oiling and, with one exception, no significant effect was seen on the recovery of a suspension feeder.

Although effects from oiling on recovery were found at each of the tide levels (MLLW and -2'- (.61m)), at each of the sites (Protection Island and Sequim Bay), in each of the sediment types (Sequim Bay native and Protection Island native), and in each of the seasons (spring-summer and summer-fall), these physical factors, nevertheless, influenced gross density in both treated and control sediments. Thus, much higher densities were found in the summer-fall season than in the spring-summer season; much higher densities were found at Sequim Bay than Protection Island; and much higher densities were found at -2' below MLLW as compared to MLLW tide level. Density related to sediment type tended to be species specific and was about equal overall. Studies designed to elicit effects on recovery in an actual oil spill event will, thus, need some form of experimental control for these variables.

Two species, because of their nearly ubiquitous occurrence within the recovery experiment and the north Puget Sound region, generally have been identified as good recovery indicators when used in an appropriate experimental framework. These species are the crustacean, Leptochelia dubia, and the polychaete, Exogone lourei. A taxonomic problem with the former and an important oil-resistant congener of the latter were identified.

Based on adjunct MESA studies of trophic relationships, it appears that the severity of influence on recovery of species in this study could be expected to have a deleterious effect on important fish populations, and that this effect would extend somewhat beyond the 15-month period studied here.

Because oil was mixed into sediment, the present case may be considered a "worst" case situation in terms of treatment severity. However, the sediment-borne concentrations of both total oil and analyzed aromatic and saturate compounds were well within the range of concentration reported for sediments exposed to actual spillages elsewhere. Initial target concentrations of 2000 ppm for summer-fall, 3-month recovery and 15-month recovery and 1000 ppm for spring-summer recovery were obtained. Reductions in total hydrocarbons were about 35% in three months for summer-fall regardless of sediment type. The comparable amount for MLLW in the spring-summer period was 43%. There was a slightly more rapid loss at the lower tide level (53%). At 15 months, total hydrocarbons were reduced by 85 and 97% for coarse and fine sediments, respectively. Based on rate of loss data between 3 months and 15 months, it is speculated that background levels would be reached in a total of 18.5 months. An important contribution to sediment infrared spectra (most likely due to biogenic materials and unrelated to oiling) was identified in analyses of control sediments after 15 months.

Capillary gas chromatography revealed a much more rapid loss of analyzed aromatic compounds than saturate compounds. There were seasonal differences in aromatic content of sediments. Aromatics were more than 80% reduced in the summer-fall, 3-month period and at background levels in the spring-summer, 3-month period. As a percentage of preliminary concentrations, analyzed saturate compounds closely paralleled concentrations of total oil as measured by infrared spectrophotometry.

RECOMMENDATIONS

The data in this study verify the utility of an experimental approach to measuring recovery of infauna. There are some specific studies using the approach which have high priority and are recommended based on the present findings.

In the realm of oil pollution research, the following should be investigated:

- 1. A less severe treatment with Prudhoe Bay crude oil should be used to bracket potential effects on recovery. The reduction in severity should relate to the method of applying oil and not necessarily the total amount used which, in the present case, was slight. Thus, an approach where oil is layered onto the surface of sediments, either from a seawater surface slick or direct surface application would be appropriate.
- 2. Comparative studies of the effects on recovery from processed petroleum products, i.e., light fuel oils and residual fuels should be undertaken especially in areas of the north Puget Sound region especially vulnerable to such spillage.
- 3. The relative severity on recovery effects following oil and oil dispersant combinations should be investigated using the present approach to assist decision making regarding the application of dispersants should spillage occur in our region.

The methods used in this study appear particularly suitable for investigation of other sediment-related pollutant problems in the Puget Sound region. Specifically, we recommend studies of the effects on recovery from:

- 1. Heavy metal contamination.
- 2. Synthetic organic contamination.
- 3. Dredge spoil contamination.
- 4. Wood fiber and by-product contamination.
- 5. Combinations of the above.

Since the methodological sensitivities are a function of the prevailing seed populations and the types of physical factors identified in this study, there is good reason to believe that the approach used here would be appropriate for these studies.

The present studies have clearly identified an urgent need for two further types of investigation to assist in interpretation of the effects demonstrated:

- 1. Basic life history studies of especially oil-sensitive primary species to include studies of behavior in response to pollutant contamination. Two questions need to be addressed: (1) what is the zonal distribution of the seed source? and, (2) where do organisms go that are absent from oil treated sediments?
- 2. Experimental studies of feeding relationships, particularly of bottom-feeding flatfishes. The experiments should have a field orientation.

MATERIALS AND METHODS

The approach in these studies used substrate units (trays of sediment or concrete bricks) which were: (1) initially free of organisms; (2) treated with oil (treated) or not (controls); and (3) allowed to colonize in a selected array of intertidal habitat conditions. The power of this approach lies in: (1) an equal starting point (organism free) for treated and untreated substrates; (2) the use of strict random procedures (as opposed to haphazard) for allocation of substrate units within and between treatments; (3) the inclusion of important environmental variables (site, tide level, substrate type, season) as treatment categories (receiving both oil-treated and untreated units); (4) the use of a replication scheme for which methodological sensitivity: (a) had been pre-evaluated (Vanderhorst et al., 1978); and (b) can be re-evaluated.

Balanced designs were used in the experiments reported here, and independent controls were utilized in each phase of each experiment; thus, a correct use of analysis of variance is indicated. This is in marked contrast to use of analysis of variance in field surveys for which time series data create dependencies between treatment categories and alter error probabilities in an undefinable manner. In contrast to field survey approaches, the present approach has a much smaller sampling requirement for two important reasons.

The reasons for a smaller sampling requirement in the present approach relate to: (1) habitat description; and (2) population characteristics. In a survey context, it is necessary to relate the contribution of each of these features to an index in which there is inherent interest (e.g., population density, community diversity, stock value). With respect to habitat description, an adequate survey with the aim of defining effect due to oiling must deal with the variance associated with mean index magnitude contributed by all or at least "representative" habitat features. In the case of population characteristics, the variance associated with mean index magnitude also includes elements relating to seasonal spawning cycles, competitive and predator-prey relationships in the water mass, and both passive and active migrations of larval forms. Crude, but conservative, estimates of contribution to variance of mean density by the latter features range (expressed as coefficient of variation) from 150% to several hundred percent of the mean in rock habitat of the Strait of Juan de Fuca, and 30% to more than 100% for infauna (based on data from Nyblade, 1979). Contribution to the habitat mean density is even greater for many species. Against this background, even rather substantial (and perhaps detectable by visual inspection) effects from oiling cannot be statistically validated with a practical number of samples. present approach avoids this problem by providing controls within each treatment category of interest.

INFAUNAL STUDIES

Details of the experimental methods including criteria for site selection, preparation and placement of substrates, chemical and biological characterization, and sampling rationale and procedures have been previously reported for the infaunal studies (Vanderhorst et al., 1979). In summary, native substrate was collected from two sites (Figure 1), brought to the laboratory, and given a repeated freezing-thawing treatment to kill macrofauna. Both native sediments were principally sand. At the time of collection, sediments dug from each of the experimental sites were thrown through a 13 mm (1/2-in) mesh hardward cloth screen to remove cobble and larger pebbles. Based on visual inspection, sediments from Protection Island were classified as "fine" and those from Sequim Bay were classified as "coarse," relative to each other. The evaluation of effect of sediment type in this report is, in fact, an evaluation of effect from native Protection Island sediment compared to native Sequim Bay sediment as represented by the respective initial collections. Samples for particle size analysis, which will more clearly place these relative sediment textures in perspective to the region as a whole, were taken and preserved at the time of initial sediment collection. Results of these analyses will be presented in the final report on this project.

Half of the substrate from each site was treated with Prudhoe Bay crude oil by mixing in a commercial cement mixing truck for Tasks A, B, and C, and a motor-driven portable cement mixer for Tasks D and E. Because of the difference in mixing method and availability of results from Task B, a target concentration of 2,000 ppm total oil was sought in the former case, and a target concentration of 1,000 ppm total oil was sought in the second. Total amounts of oil and concentrations of selected petroleum compounds were measured in treated and untreated sediments prior to field installation, at intervals between installation and completion, and upon completion of a given experiment. The other half of the substrate from each site served as control. It received the mixing just as the oiled substrate but did not receive an application of oil. The prepared substrates were placed in PVC trays (30 \times 15 \times 15 cm). bottom of the trays were provided with eight 2.5 cm diameter holes for drainage. Experimental substrates were retained in trays by placing a fiberglass screen over these holes. For Tasks A, B, C, and E, trays were buried with top surface flush with the ground surface at Mean Lower Low Water at each of the sites. For Task D, trays were buried in a similar fashion at -2 feet below Mean Lower Low Water at the Sequim Bay site. Field installations for Tasks A, B, and C were during August 1978. Field installations for Tasks D and E were during April 1979. Task B terminated in November 1978. Tasks D and E terminated in August 1979. Tasks A and C terminated in November 1979.

A relatively high amount of replication was used in both the placement and sampling of substrate units. This was based on a predesign study using similar units (Vanderhorst et. al., 1978). See Table 1 for list of sampling. This replication was done to permit evaluation of methodological sensitivity and to attain a quantitative measure of the density of individual species colonizing the trays. Because of interest in a large number of species and a limited number of independent units in even this rather large design, it was necessary to a priori select species of special interest for hypothesis testing with valid probability statements

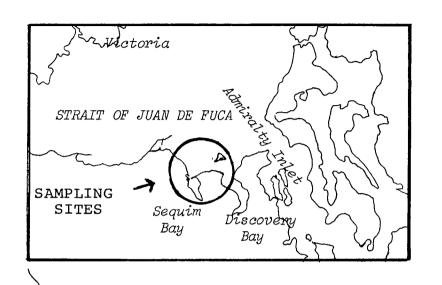
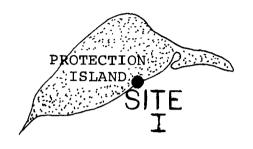


FIGURE 1. STUDY SITES



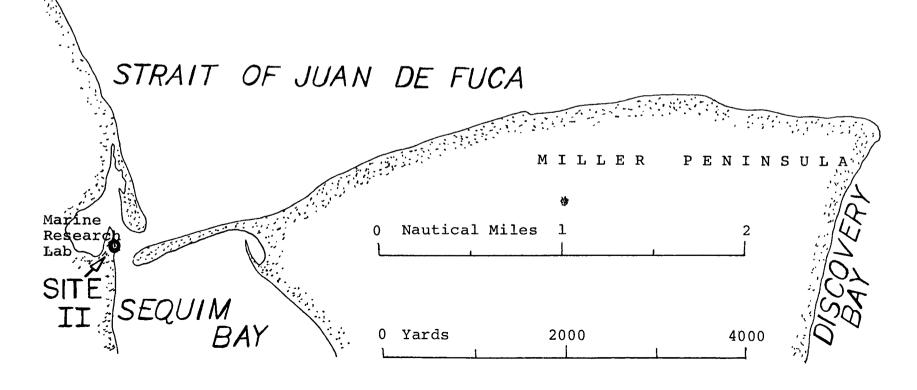


Table 1. Schedule of Sampling for Oil Recovery Experiments.

MONTH/YEAR	SITE/STATUS TIDE LEVEL ()	TASK	TREATMENT STATUS	SUBSTRATE TYPE	SÄMPLE TYPE	NUMBER TRAYS	NUMBER CORES
August/1978	Preliminary	ABC	Oiled	Coarse	Infrared	3	9
J	•				Gas chromat.	3	3
				Fine	Infrared	3	9
					Gas chromat.	3	3
			Unoiled	Coarse	Infrared	3	9 3
					Gas chromat.	3	3
				Fine	Infrared	3	9
					Gas chromat.	3	3
September/1978	Protection Is.	Α	Oiled	Fine	Infrared	1	1
	(0')				Biological	1	1
			Unoiled	Fine	Infrared	1	1
					Biological	1	1
	Sequim Bay	Α	Oiled	Coarse	Infrared	1	1
	(01)				Biological	1	1
			Unoiled	Coarse	Infrared	1	1
					Biological	1	1
October/1978	Protection Is.	Α	Oiled	Fine	Infrared	1	1
	(0')				Biological	1	1
			Unoiled	Fine	Infrared	1	1
					Biological	1	1
	Sequim Bay	Α	Oiled	Coarse	Infrared	1	1
	(0')				Biological	1	1
			Unoiled	Coarse	Infrared	1	1
					Biological	1	1
November/1978	Protection Is.	В	Oiled	Coarse	Infrared	3	9
	(0')				Gas chromat.	3	3
					Biological	5	35
				Fine	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35

Table 1. (Continued)

MONTH/YEAR	SITE/STATUS TIDE LEVEL ()	TASK	TREATMENT STATUS	SUBSTRATE TYPE	SAMPLE TYPE	NUMBER TRAYS	NUMBER CORES
November/1978	Protection Is.	В	Unoiled	Coarse	Infrared	3	9
	(0')				Gas chromat.	3	3
					Biological	5	35
				Fine	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
November/1978	Sequim Bay	В	Oiled	Coarse	Infrared	3	9
	(0')				Gas chromat.	3	3
					Biological	5	35
				Fine	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
			Unoiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
				Fine	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
December/1978	Protection Is.	Α	Oiled	Fine	Infrared	ĺ	1
	(0')				Biological	$\bar{1}$	ī
			Unoiled	Fine	Infrared	1	ī
					Biological	1	ī
	Sequim Bay	Α	Oiled	Coarse	Infrared	1	$\bar{1}$
	(0')				Biological	$\bar{1}$	ī
			Unoiled	Coarse	Infrared	1	$\bar{1}$
					Biological	$\bar{1}$	ī
January/1979	Protection Is.	Α	Oiled	Fine	Infrared	ĩ	ī
	(0')				Biological	ī	ī
			Unoiled	Fine	Infrared	$\bar{1}$	ī
					Biological	$\overline{1}$	i
	Sequim Bay	Α	Oiled	Coarse	Infrared	$\bar{1}$	î
	(0')				Biological	ī	î
			Unoiled	Coarse	Infrared	ī	1
					Biological	$\bar{1}$	ī

Table 1. (Continued)

MONTH/YEAR	SITE/STATUS TIDE LEVEL ()	TASK	TREATMENT STATUS	SUBSTRATE TYPE	SAMPLE TYPE	NUMBER TRAYS	NUMBER CORES
Apri1/1979	Sequim Bay	Α	Oiled	Coarse	Infrared	1	1
	(0')				Biological	1	1
Apri1/1979	Preliminary	D,E	Oiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
June/1979	Sequim Bay	Α	Oiled	Coarse	Infrared	1	1
	(0')				Biological	1	1
	Sequim Bay	D(A)	Oiled	Coarse	Infrared	1	1
	(0')				Biological	1	1
	Sequim Bay	Α	Unoiled	Coarse	Infrared	1	1
	(0')				Biological	1	1
	Sequim Bay	D(A)	Unoiled	Coarse	Infrared	1	1
	(0')				Biological	1	1
	Sequim Bay	E(A)	Oiled	Coarse	Infrared	1	1
	(-2')				Biological	1	1
	Sequim Bay	E(A)	Unoiled	Coarse	Infrared	1	1
	(-2')				Biological	1	1
July/1979	Sequim Bay	Α	Oiled	Coarse	Infrared	1	1
	(0')				Biological	1	1
	Sequim Bay	D(A)	Oiled	Coarse	Infrared	1	1
	(0')				Biological	1	1
	Sequim Bay	Α	Unoiled	Coarse	Infrared	1	1
	(0')				Biological	1	1
	Sequim Bay	D(A)	Unoiled	Coarse	Infrared	1	1
	(0')				Biological	1	1
	Sequim Bay	D(A)	Oiled	Coarse	Infrared	1	1
	(-2')				Biological	1	1
	Sequim Bay	E(A)	Unoiled	Coarse	Infrared	1	1
	(-2)				Biological	1	1

Table 1. (Continued)

ONTH/YEAR	SITE/STATUS TIDE LEVEL ()	TASK	TREATMENT STATUS	SUBSTRATE TYPE	SAMPLE TYPE	NUMBER TRAYS	NUMBER CORES
ugust/1979	Sequim Bay	А	Oiled	Coarse	Infrared	1	1
agas s, 20, 5	(0')				Biological	1	1
	,		Unoiled	Coarse	Infrared	1	1
					Biological	1	1
ugust/1979	Sequim Bay	D	Oiled	Coarse	Infrared	3	9
5	(0)				Gas chromat.	3	3
					Biological	5	35
			Unoiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
ugust/1979	Sequim Bay	E	Oiled	Coarse	Infrared	3	9
	(-2')				Gas chromat	3	3
				_	Biological	5	35
			Unoiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
eptember/1979	Sequim Bay	Α	Oiled	Coarse	Infrared	1	1
•	(0)				Biological	1	1
			Unoiled	Coarse	Infrared	1	1
					Biological	1	1
ctober/1979	Sequim Bay	Α	Oiled	Coarse	Infrared	1	1
	(0)				Biological	1	1
			Unoiled	Coarse	Infrared	1	1
					Biological	1	1
ovember/1979	Sequim Bay	С	Oiled	Coarse	Infrared	3	9
	(0)				Gas chromat.	3	3
	. ,				Biological	5	35
			Unoiled	Coarse	Infrared	3	9
					Gas chromat.	3	3
					Biological	5	35
						254	788

concerning statistical error. For these species we use a criterion of $\alpha \sim 0.01$ to deem "significant" effect on density. The maximum real probability for Type I error for any one of the seven species (Task B) was 7%, and for any one of the ten species (C, D, E) it was 10%. Task A data were outside the experimental framework. To meet the objectives of Tasks B, C, D, and E, three independent experiments were conducted and a priori selection of target species was made. These species were designated "Primary" species and consist of the following:

For Experiment I

(Task B):

Mollusks

Mysella tumida Transennella tantilla Lacuna sp.

Polychaetes

Platynereis bicanaliculata Armandia brevis Ophiodromus pugettensis Capitella capitata

For Experiment II

(Tasks D and E) and

For Experiment III

(Task C): Mollusks

Mysella tumida Protothaca staminea Lacuna variegata

Polychaetes

Platynereis bicanaliculata Armandia brevis Polydora socialis Exogone lourei

Crustaceans

<u>Leptochelia dubia</u> <u>Corophium ascherusicum</u> <u>Photis brevipes</u>

The basis for <u>a priori</u> selection for Task B rested on: (1) results from the predesign study (Vanderhorst et al., 1978); and (2) for two species, <u>Lacuna</u> sp., and <u>Capitella capitata</u>, reported perturbations following oil spills elsewhere. The basis for <u>a priori</u> selection of species in Tasks C, D, and E were: (1) results of Task B studies; and (2) examination of baseline data for nearby stations (Nyblade, 1979). The danger in <u>a priori</u> selection in any experiment involving field colonization is that selected species may not occur in the future experiment, or may be relatively unimportant constitutents. In general, this was not the

case for the species selected here. However, for one species of considerable commercial importance, <u>Protothaca staminea</u>, a low frequency of occurrence was a problem. That species is to be specifically addressed in a yet to be completed task (H).

In addition to the 13 species which were <u>a priori</u> selected to protect the validity of error probability statements, <u>quantitative</u> data were also collected on the density of nearly 200 other species which colonized Experiments I, II, and III. Analyses of variance were computed for these data for descriptive purposes. Since the densities of these many species cannot realistically be assumed to be independent from one another, we do not attribute statistical significance to the results obtained from these analyses. However, the mean densities computed for these species in the several treatment categories in each experiment do serve to better describe the communities involved and are included in the results on recovery of other community members.

<u>Sediment Extraction and Chemistry</u>

Sediment cores collected for chemical analysis were frozen immediately after collection. The frozen samples were thawed at room temperature and thoroughly mixed for subsampling. Twenty grams (wet) of sediment were placed in a 250 ml teflon-capped bottle with 20 g of anhydrous sodium sulfate and thoroughly mixed to hydrate the water from the sediment. To the samples for I.R. analysis, 50 ml carbon tetrachloride was added, and for capillary G.C. analysis 25 ml hexane were added. The bottles were shaken overnight on a reciprocal shaker. The solvents were decanted from the sediment. Carbon tetrachloride was extracted into a 25 ml scintillation vial, and hexane was extracted into a 50 ml graduated cylinder. The CC14 subsample was analyzed by infrared spectroscopy (Simard et al., 1952). The samples for G.C. analysis were extracted for an additional 2 hours with 25 ml hexane and decanted into the graduated cylinder with the first extraction. The sediment sample was then extracted with 5 ml volumes of hexane and decanted until the total extract volume was 50 ml. Twenty-five ml of the hexane extract was concentrated under nitrogen to 3 ml, and separated into aliphatic and aromatic fractions by silica gel chromatography (Warner, 1976). These fractions were concentrated to 1 ml and analyzed for individual hydrocarbons by capillary gas chromatography. Concentrations were calculated by using standard addition and internal standard compounds with comparison to authentic standards. For purposes of this report, concentrations of individual aliphatic and individual aromatic compounds were summed to represent the respective groups.

EPIFAUNAL RECOVERY STUDIES

In summary, the method involves four steps: (1) preconditioning of concrete construction bricks by placement in a flowing seawater system for two weeks; (2) treatment of one-half of the pool of conditioned bricks with Prudhoe Bay crude oil; (3) characterization of treatment severity by extraction of oil from bricks and chemical analyses; and (4) field testing in the intertidal zone at Site II (Figure 1). Independent experiments were conducted monthly commencing with October 1979.

Preconditioning of bricks in flowing sea water was done to leach out any foreign materials which might have been associated with the manufacture of the bricks and to allow some chance for colonization with a microflora.

Prior to the treatment phase, all bricks, both control and treated, received a thorough washing with a high-pressure hose both to remove unseen but possible incidental occurrences of settled macroorganisms and to aid in equalizing the effect of preconditioning on control versus treated bricks. For treated bricks, treatment lasted five days. During this time the control pool remained in the preconditioning tank. At the end of the treatment phase, the control bricks again received a wash with the high pressure hose.

The treatment of bricks with oil was designed to simulate repeated exposure of intertidal rock during shifts of the tide. The procedure was as follows. A pool of conditioned bricks was placed on the bottom of a rectangular tank $(0.54 \times 4.88 \text{ m})$. The tank was then filled with sea water (25 cm depth). Prudhoe Bay crude oil (20 ℓ) was poured on the surface of the sea water to provide a slick thickness of about 1 cm. Continuous inflow of clean sea water was provided (2 ℓ /min). Both the inflow and outflow of the sea water were subsurface to prevent disturbing the surface slick and to retain it in the tank. Twice each day, the inflow of sea water was discontinued and the seawater level reduced to a depth of 2 cm. The period of this reduced water level was for two hours at each treatment. During the periods of low water, the slick was in contact with the upper and side surfaces of the bricks. The twice daily regime was repeated for five days. At the end of five days, a surface outflow was provided, and the slick was washed into the laboratory oiltreatment facility. Clean seawater flow (2 l/min) was provided for a further 24 hours.

Chemical Characteristics of Bricks

Routine chemical characterization of the treatment severity has been based on 5 brick subsamples of the pool (see Table 2 for schedule). Analysis methods for both infrared spectrometry and capillary gas chromatography follow those previously reported (Vanderhorst et al., 1979). Three types of extraction procedures have been evaluated and two are routinely used. The first procedure involved washing whole wet bricks with 500 ml CCl4. The extraction efficiency was poor. The second procedure involves air drying bricks for a period of 48 hours before extraction with 500 ml CCl₄. This improved the extraction efficiency approximately 100%. To provide a better measure of the amount of oil actually "seen" by colonizing organisms, a two-part extraction procedure has been adopted. In this procedure the top surface of bricks are washed with 200 ml CCl4. The amount of oil in this extract is measured. The bricks are then air-dried for 48 hours and reextracted with 500 ml CCl4. The amount of oil in this extract is measured. Diluted samples of the extracts measured by infrared spectrophotometry are computed in terms of numbers of grams of oil per brick. Samples analyzed by capillary gas chromatography are reported in terms of number of milligrams per brick for individual compounds.

Table 2. Preparation of Units and Sampling Schedule for "On-going" Experiments on Effects of Oil on Recovery by Commercial Clams and Epifauna of Rocky Intertidal. 1

ASK/SITE	DATES	UNIT TYPE	PRELIMINARY	MLLW TIDE	+2 MLLW TIDE	TOTALS
H/Discovery Bay	5/80	Infrared			9-A-78-	
	·	Trays	6		1	6
		Cores	18			18
		Capillary GC				
		Trays	6 6			6 6
		Cores	6			6
	6/80	Infrared				
		Trays		2	2	4
		Cores		2 2	2 2	4
		Biological				
		Trays		2 2	2 2	4
		Cores		2	2	4
	7/80	Infrared				
	.,	Trays		2	2	4
		Cores		2 2	2 2	4
		Biological			_	•
		Trays		2	2	4
\	8/80	Infrared				
\		Trays		6	6	12
		Cores		18	18	36
		Capillary GC				
		Trays		6	6	12
		Cores		6	6	12
		Biological				
		Trays		10	10	20
		Cores		70	70	140

Note: Core profile on capillary GC and Biological Cores means that discrete samples will be 2X indicated number for 8/80 sampling.

Table 2. (Continued)

TASK/SITE	DATES	UNIT TYPE	PRELIMINARY	MLLW TIDE	+2 MLLW TIDE	TOTALS
G/Sequim Bay	9/79	Concrete				
		Infrared	10	10	10	30
		Capillary GC	10	10	10	30
		Biological ²		30	30	60
	10/79 - 8/80	The pattern for Capillary GC, gi			h month with exce	ption of
G/Rocky Point	5/80	Concrete Infrared	10	10 30	10 30	30 60
		Biological ²		30	30	80
	6/80	Concrete				
		Infrared	10	10	10	30
		Biological ²		30	30	60
I/Sequim Bay	4/80	Concrete				
		Infrared	10	10	10	30
		Capillary GC	4	4	4	12
		Biological ²	120	60	60	240
	5/80	Concrete				
	0, 00	Infrared		10	10	20
		Biological		30	30	60

Experiment balanced, i.e., for every treated sample a control sample is also indicated.
 Each of the biological units with this notation (with the exception of preliminary) should be multiplied by 5 for 5 daily observations within the month. Appropriate total unit samples are: Capillary GC, 60; Infrared, 532; Biological, 3328.

RESULTS AND DISCUSSION

THEAUNA RECOVERY

A Perspective

Since marine systems are dynamic and differ greatly from place to place, a definition of full recovery is nebulous. For purposes of these studies, we defined full recovery of the infaunal communities as that composition and density of organisms in native substrate control trays after 15 months colonization. The experimental results presented in the following subsections arrive at effects from oil on recovery by making direct comparisons in density of individual species in trays with and without oil treatment but identical in all other respects, i.e., preparation method, site, sediment type, beach location, tidal height, season, and duration of colonization. The purpose of this section is to demonstrate the relevance of the above definition of recovery in terms of baseline information available for nearby sites from related MESA studies (Nyblade, 1979). Also, summary data will be presented to give perspective to the relevance of using a defined full recovery in assessing impacts of oil on recovery.

Table 3 presents data from two MESA Puget Sound baseline stations geographically embracing the location of the present study and data from 15-month control coarse substrate at Sequim Bay. These data show the numbers of species of animals within the three principal taxonomic groups (polychaetes, crustaceans, mollusks), for all other taxonomic groups, and the total number of species. The MESA baseline data for Beckett Point (Discovery Bay) and Jamestown were derived from Appendix I (Nyblade, 1979) and apply to the Mean Lower Low Water (0') tide level for fall samples at those stations (collected October 1977). The data for the recovery study are based on enumeration of infauna from coarse sediment control trays at Mean Lower Low Water (0') collected November 1979, 15 months after placement at Sequim Bay. In a general sense the habitats are similar.

The total number of species for the three situations is reasonably similar. The indicated number for the recovery study is slightly lower, but one must keep in mind the sensitivity of data collection methods and annual influences. For example, if one consults the same category of samples for Jamestown during 1976 (Nyblade, 1978), a total of 57 species are indicated, or less than the recovery study. In each of the situations, polychaetes have the highest representation with from 43 to 56% of the total. There is quite a bit of difference in percent contribution by the other groups. Beckett Point and the recovery study were similar in having 21 to 25% contributed by mollusks, and relatively small contributions by the "other species" group (7 and 8% for Beckett Point and the recovery study, respectively). The recovery study and Jamestown had more nearly equal representation by crustaceans with 28 and 17%, respectively.

Table 3. Summary of Number of Species in Principal Groups of Animals from Baseline Studies (Nyblade, 1979) and 15-Month Controls in Recovery Experiment.*

apaup	NUMBER OF SPI	ECIES AND PERCENT ()	OF TOTAL
GROUP	BECKETT POINT	RECOVERY STUDY	JAMESTOWN
Polychaetes	52 (56)	25 (43)	41 (54)
Mollusks	23 (25)	13 (21)	6 (8)
Crustaceans	11 (12)	17 (28)	13 (17)
All Others	7 (7)	5 (8)	16 (21)
Totals	93 (100)	61 (100)	76 (100)

Numbers of species, or species richness, is strongly related to area sampled until enough area is covered to reach some "asymptote" mean number. Data from the recovery studies are conservative with respect to baseline. See text for details.

We conclude from these data that in crude form both the total number of species and the relative distribution of species in the principal taxonomic groups in the 15-month recovery study coarse sediment controls are similar to what might be expected in sampling a fully recovered nearby beach with similar physical attributes.

There are several good reasons which preclude quantitative comparisons to further refine the above judgment. First, year to year variation in numbers of species at a given site may be great. The difference cited above for the baseline studies at Jamestown between 1976 and 1977 (25% fewer in 1976) is not unusual. Our study was conducted during the succeeding year (1978). Jamestown and Beckett Point differ within the same year and season (17% fewer at Jamestown). Perhaps most important to the present discussion is the relationship of increases in numbers of species with area sampled. In general, this has been shown to be a logarithmic function for marine sediments. The baseline studies sampled about 2 times the area sampled in the recovery study. For this reason we believe the numbers of species in each of the categories of the recovery study are conservative estimates of the state of recovery.

Data for the same categories on numbers of individuals contributed by the principal groups are shown in Table 4. Both the baseline data and recovery study data have been normalized to a per m² basis. Distinctions closer than an approximate order of magnitude should not be made. For example, one polychaete species in the recovery study, Polydora socialis, contributed more than 49,000 individuals per m², roughly 48% of polychaetes or 40% of all individuals per m^2 . High density of this species was not an anomaly of the experimental design or sediment trays. The species is quite visible and literally covered the Sequim Bay beach during this period. Similar disproportions in contribution by a single species to total numbers can be found in the baseline information. For example, Oligochaeta spp. comprised 39% of individuals at Jamestown during the period of interest (Nyblade, 1979), and for Beckett Point, the tanaidacean, Leptochelia dubia, contributed 46% of all individuals. We believe that the data in Table 4 indicate high similarity between the numbers of individuals in taxonomic groups from the recovery study coarse controls and the baseline sites. They also make a case for study of recovery under controlled conditions on a species by species basis.

In the recovery study, each of the factors (site, tide level, sediment type, season, and duration of recovery) had independently allocated oiled and unoiled units. For this reason, a comparison of summary statistics on total numbers of individuals (Table 5) and total numbers of species (Table 6) is appropriate and adds perspective to the use of a "full recovery" concept in describing recovery.

Sequim Bay coarse sediment controls with 15 months intertidal colonization, defined above as fully recovered, showed a rank order of importance: (1) polychaetes; (2) crustaceans; (3) mollusks; and (4) "other species," both in terms of individuals (Table 5) and species (Table 6). This rank order of importance was frequently observed in the experiments although it does not consistently relate to the primary treatment categories (oiling status, sediment type, tide level, or site). Thus, in the three experiments this control rank order applied to 7 of the 15 remaining treatment categories in terms of individuals (Table 5) and 11 of the remaining 15 categories in terms of species (Table 6).

Table 4. Summary of Number of Individuals in Principal Groups of Animals from Baseline Studies (Nyblade, 1979) and 15-Month Controls in Recovery Experiment.

GROUP	INDIVI	INDIVIDUALS NORMALIZED TO M2				
	BECKETT POINT	RECOVERY STUDY	JAMESTOWN			
Polychaetes	18,640	102,590	41,956			
Mollusks	21,644	1,042	2,636			
Crustaceans	46,136	15,780	4,452			
All Others	10,692	472	1,416			
Totals	97,112	119,884	50,460			

Table 5. Group Density/m² in Oil Recovery Experiments.*

EXPERIMENT/CONDITION	POLYCHAETES	<u>INDIVI</u> CRUSTA <mark>CEAN</mark> S	DUALS/M ² MOLLUSKS	OTHERS	TOTALS
I. Fall 3-Month Recov Two sites (Protect at each site (Coar categories (Oiled;	ion Island; Sequ se-Fine); One ti	im Bay); Two de level (MLL	sediment ty	pes	
Protection Island					
Coarse Sediment					
Control	2,046	4,781	571	197	7,595
Oiled	807	866	256	0	1,928
Fine Sediment	7 207	2 160	0 570	0	7 140
Control	1,397	3,168	2,578	0	7,142 4,447
Oiled	669	1,181	2,597	0	4,44/
Sequim Bay Coarse Sediment					
Control	18,259	2,400	590	236	21,486
Oiled	6,316	1,180	571	59	8,126
Fine Sediment	,	,			,
Control	9,976	964	2,282	79	13,301
Oiled	14,973	433	2,322	79	17,807
II. Summer 3-Month Rec One site (Sequim I (MLLW; -2' below I	Bay); One sedime MLLW); Two oilin	nt type (Coar	se); Two ti	de level	S .
Sequim Bay - Coarse Sec	diment				
Minus 2' below MLLW	16 744	12 502	C C 3	0.25	20 012
Control Oiled	16,744 12,711	12,592 5,549	551 236	925 59	30,812 18,544
Mean Lower Low Water	12,711	5,545	230	33	10,544
Control	11,825	1,200	197	315	13,537
Oiled	5,549	394	98	20	6,060
III. Fall 15-Month Reco One site (Sequim I level (MLLW); Two	Bay); Two sedime	nt types (Coa	rse; Fine);	One tid	9. le
Sequim Bay, MLLW Coarse Sediment					
Control	102,590	15,780	1,043	472	119,886
Oiled	49,564	7,103	1,003	275	57,847
Fine Sediment		·			
Control	120,869	17,571	1,220	453	140,113
Oiled	94,405	9,602	1,358	433	105,797

Each mean presented based on n=35 cores (14.52 cm²) in groups of 7 with 5 replicate groups (trays).

Table 6. Summary of Numbers of Species in Oil Recovery Experiments.*

EXPERIMENT/CONDITION	POLYCHAETES	NUMBER CRUSTACEANS	R OF SPECIES MOLLUSKS		TOTALS
I. Fall 3-Month Recove Two sites (Protecti at each site (Coars categories (Oiled;	on Island; Sec e-Fine); One t	uim Bay); Two ide level (MLL	sediment ty	/pes	
Protection Island					
Coarse Sediment					
Control	12	12	6	1	31
Oiled	11	5	4	0	20
Fine Sediment	7.5	7.0	_		
Control	16	13	5	0	34
Oiled	13	9	3	0	25
Sequim Bay					
Coarse Sediment	0.7	1.4	^	c	40
Control Oiled	21	14	8	6	49
Fine Sediment	14	16	5	1	36
Control	18	15	6	Е	11
Oiled	16	6	4	5 1	44 27
One site (Sequim B	ay); One sedim	ent type (Coar	rse); Two ti	de levels	5
One site (Sequim B (MLLW; -2' below'M Sequim Bay - Coarse Sed Minus 2' Below MLLW Control Oiled	ay); One sedim LLW); Two oili	ent type (Coar	rse); Two ti	de levels	5
One site (Sequim B (MLLW; -2' below M Sequim Bay - Coarse Sed Minus 2' Below MLLW Control Oiled Mean Lower Low Water	ay); One sedim LLW); Two oili iment 27 19	ent type (Coam ng categories 25 21	rse); Two ti (Oiled; Und 7 3	de levels piled Cont 10 0	69 43
One site (Sequim B (MLLW; -2' below M Sequim Bay - Coarse Sed Minus 2' Below MLLW Control Oiled Mean Lower Low Water Control	ay); One sedim LLW); Two oili iment 27 19 21	nent type (Coan ng categories 25 21 14	rse); Two ti (Oiled; Und 7	de levels piled Cont 10 0	69 43
One site (Sequim B (MLLW; -2' below M Sequim Bay - Coarse Sed Minus 2' Below MLLW Control Oiled Mean Lower Low Water Control Oiled III. Fall 15-Month Reco One site (Sequim B level (MLLW); Two	ay); One sedim LLW); Two oili iment 27 19 21 15 very: Started ay); Two sedim	ent type (Coar ng categories 25 21 14 6 1 8/78; Complet	rse); Two ti (Oiled; Und 7 3 4 ted and Samparse; Fine);	de levels piled Cont 10 0 6 1 pled 11/79 One tide	69 43 44 26
One site (Sequim B (MLLW; -2' below M Sequim Bay - Coarse Sed Minus 2' Below MLLW Control Oiled Mean Lower Low Water Control Oiled III. Fall 15-Month Reco One site (Sequim B level (MLLW); Two	ay); One sedim LLW); Two oili iment 27 19 21 15 very: Started ay); Two sedim	ent type (Coar ng categories 25 21 14 6 1 8/78; Complet	rse); Two ti (Oiled; Und 7 3 4 ted and Samparse; Fine);	de levels piled Cont 10 0 6 1 pled 11/79 One tide	69 43 44 26
One site (Sequim B (MLLW; -2' below M Sequim Bay - Coarse Sed Minus 2' Below MLLW Control Oiled Mean Lower Low Water Control Oiled III. Fall 15-Month Reco One site (Sequim B level (MLLW); Two	ay); One sedim LLW); Two oili iment 27 19 21 15 very: Started ay); Two sedim	ent type (Coar ng categories 25 21 14 6 1 8/78; Complet	rse); Two ti (Oiled; Und 7 3 4 ted and Samp arse; Fine); noiled Contr	de levels piled Cont 10 0 6 1 pled 11/79 One tide	69 43 44 26
One site (Sequim B (MLLW; -2' below M Sequim Bay - Coarse Sed Minus 2' Below MLLW Control Oiled Mean Lower Low Water Control Oiled III. Fall 15-Month Reco One site (Sequim B level (MLLW); Two Sequim Bay, MLLW Coarse Sediment Control Oiled	ay); One sedim LLW); Two oili iment 27 19 21 15 very: Started ay); Two sedim oiling categor	nent type (Coar ng categories 25 21 14 6 1 8/78; Complet nent types (Coar ries (Oiled; Un	rse); Two ti (Oiled; Und 7 3 4 ted and Samparse; Fine);	de levels piled Cont 10 0 6 1 pled 11/79 One tide	69 43 44 26
One site (Sequim B (MLLW; -2' below 'M Sequim Bay - Coarse Sed Minus 2' Below MLLW Control Oiled Mean Lower Low Water Control Oiled III. Fall 15-Month Reco One site (Sequim B level (MLLW); Two Sequim Bay, MLLW Coarse Sediment Control	ay); One sedim LLW); Two oili iment 27 19 21 15 very: Started ay); Two sedim oiling categor	nent type (Coar ng categories 25 21 14 6 18/78; Complet nent types (Coar ries (Oiled; Un	rse); Two ti (Oiled; Und 7 3 4 ted and Samp arse; Fine); noiled Contr	de levels piled Cont 10 0 6 1 pled 11/79 One tide rol).	69 43 44 26
One site (Sequim B (MLLW; -2' below M Sequim Bay - Coarse Sed Minus 2' Below MLLW Control Oiled Mean Lower Low Water Control Oiled III. Fall 15-Month Reco One site (Sequim B level (MLLW); Two Sequim Bay, MLLW Coarse Sediment Control Oiled	ay); One sedim LLW); Two oili iment 27 19 21 15 very: Started ay); Two sedim oiling categor	nent type (Coar ng categories 25 21 14 6 18/78; Complet nent types (Coar ries (Oiled; Un	rse); Two ti (Oiled; Und 7 3 4 ted and Samp arse; Fine); noiled Contr	de levels piled Cont 10 0 6 1 pled 11/79 One tide rol).	69 43 44 26

Each number is aggregate of species in 35 cores (14.52 cm 2 /core) distributed 7 cores per tray in 5 replicate trays.

Some of the exceptions to the rank order of importance pattern are of interest. In terms of numbers of individuals it never applied to Protection Island due primarily to the fewer number of polychaete individuals found there (Table 5). In sharp contrast, the numbers of species always followed the above rank order of importance at Protection Island (Table 6). Interestingly, in Experiment II, the summer tide level experiment at Sequim Bay, the oiled sediments at each of the tide levels followed the fully recovered pattern for individuals while the control sediments did not.

In coarse, oiled sediments, there was a reduction of the total number of individuals (Table 5) as compared to unoiled coarse sediments in every instance. In only one case (Experiment I, Protection Island) did this reduction result in a change in the "full recovery" ranking. A disproportionate reduction in polychaete numbers placed that group second in importance for this case. Total number of individuals in oiled fine sediments was also reduced in comparison to unoiled controls in the 15-month data (Experiment III), and 3-month data (Experiment I) for Protection Island. Three-month fall data at Sequim Bay (fine sediments) did not follow this pattern.

For the 3-month recovery data (Experiment I), it is apparent that the site of experimentation had quite an appreciable effect on both the numbers of individuals per m² (Table 5) and numbers of species (Table 6). Overall, Protection Island had a much smaller number of species and individuals. Protection Island had 31 versus 49 species for Sequim Bay for coarse sediment controls, and about a third as many individuals. For fine sediment, native to Protection Island, the differences are not quite so great with 34 species versus 44 species, and about half as many individuals. The overall data trend for both species and individuals is set by the polychaetes. Protection Island had a greater number of individual crustaceans (slightly fewer species) and about the same number of individuals and species of mollusks. Protection Island was lower in the contribution by "other species" both in terms of species and individual numbers.

The type of sediment (coarse versus fine) also had an influence on the total number of number of individuals and species. For the fall 3-month experiment (I) there were a greater number of individuals per m^2 in coarse sediment controls as compared to fine at each of the sites. For oiled sediments, the situation was exactly reversed. In the 15-month recovery experiment (III) there were a greater number of individuals per m^2 in fine sediments as compared to coarse. This was true in both the oiled and unoiled condition.

The tide level experiment (II) in Sequim Bay-coarse sediment reveals important differences in recovery reflected by numbers of individuals (Table 5) and numbers of species. The minus two feet below MLLW level had substantially more species and individuals. The trend in this case is consistent among all control groups.

Data on Tables 5 and 6 also permit a comparison of 3-month recovery periods in the spring-summer, and late summer-fall. The appropriate

comparison is Sequim Bay-coarse sediment controls from Experiment I to Mean Lower Low Water controls in Experiment II. The total number of species and individuals was slightly greater in the fall recruitment period. The greater number of species was contributed by the mollusks. The greater number of individuals pervades all groups with the exception of "other species." The data are consistent with the baseline study findings (Nyblade, 1979) for Beckett Point and Jamestown which also indicate fewer species and individuals in summer collections versus the fall.

The data presented above indicate that attempts to evaluate the effects of oil on recovery of infaunal communities in terms of a one-condition "fully recovered" situation, or indeed to baseline data since these too are available (Tables 3 and 4), will be strongly confounded by tide level, site, sediment grain size, and possibly season. The data do, however, illustrate that, where these factors can be fixed by experimental design, a valuable perspective on recovery effects emerges. To clarify this position, data are presented on Sequim Bay-coarse sediment from Mean Lower Low Water, indexed as a percentage to the 15-month control substrates of like kind.

From Table 6, recovery relative to 15-month unoiled controls in terms of total number of species, was 70 to 80% in 3-month periods. For oiled substrates the corresponding range is from 40 to 60%. Within the taxonomic groupings, crustaceans and polychaetes were 80% recovered in 3-month controls for either season. Mollusks were relatively less recovered, although quite variable (20 to 60%). Overall, in 15 months, oiled substrates were 92% recovered relative to unoiled controls in terms of numbers of species.

Numbers of individuals (Table 5) were much less fully recovered for all categories than were species, except for mollusks. Three-month control recovery was from 11 to 18%. Fifteen-month oiled coarse substrate recovery was slightly less than one-half of controls. The 3-month oiled substrate recovery of polychaetes and crustaceans was only 2 to 7% and 21 to 45% for the 15-month recovery.

The data presented in Tables 5 and 6 indicate that there were clear effects from the oiling on degree of recovery, in terms of numbers of species, and that it had largely been compensated for by the end of 15 months. These data further indicate that there were marked effects from oiling on the total number of individuals at the end of three months and that these effects from the oiling still amounted to roughly half the numbers of individuals by the end of 15 months. Interpretation of the importance of effects on numbers of individuals can only come from an analysis of individual species populations because of the highly disproportionate contribution of individual species to total numbers (previously discussed) and the fact that while the numbers of some species are undoubtedly reduced by the oiling, others may be increased.

Recovery of Primary Species

From the preceding section, the recovery of total numbers of individuals required more time and was apparently more strongly influenced by

the oiling compared to recovery of numbers of species. Because we wish to have statistically valid probability statements in hypothesis tests concerning the effects of oil, site, sediment type, and tide level, and want valid comparisons of season and duration of recovery, subsets of the total number of species were preselected and designated as primary. data in this section relate to the question of how well the primary species represent all species in terms of numbers of individuals. By way of clarification, 13 species were designated as primary species. Seven of these were designated as primary for Experiment I (site, sediment type, oil variables); ten were designated as primary for Experiments II (season, tide level, oil variables) and III (sediment type, oil variables, 15-month duration). In some cases, designating the same species for the two groups as primary accounts for the total of 13 rather than 17 species. Of the 13 species, eight contributed to available data for Beckett Point, and eight contributed to available data for Jamestown (Nyblade, 1979). These were not the same eight species for the site cases, so that the combined data for Beckett Point and Jamestown included 12 of the 13 primary species.

On Table 7, the numbers of individuals per m² for all primary species are shown as a percentage of total numbers of individuals per m² for baseline sites (Nyblade, 1979) and 15-month-coarse sediment control data in the recovery study. Overall, the primary species encompassed 73% of individuals per m² reported for Beckett Point, 78% of all individuals per m^2 for the recovery study, and 33% of total individuals per m^2 for Jamestown. In other words, they comprised a very substantial contribution of all individuals for Beckett Point and our "fully recovered" experimental They comprised about a third of total individuals per m² for Jamestown. While this is not as great, it is still a large contribution. Within the various taxonomic categories, primary species account for 97% of crustacea, 92% of mollusks and 36% polychaetes reported for Beckett For Jamestown, primary species contributed 29% for polychaetes, 40% for crustacea, and 97% for mollusks. For the recovery study, they represented 76% of polychaetes, 90% of crustacea, and 51% of mollusks. We conclude from these data that effects on the density of primary species will indeed alter indicated recovery in terms of total numbers of individuals.

To illustrate the contribution of density for primary species within a recovery framework, Table 8 lists the numbers of individuals per m², the percent of final control (recovery) for numbers of individuals per m², in coarse sediment, MLLW experimental situations, and the percent that the primary species contributed to means for all species in a given experimental situation. The data indicate a recovery pattern for primary species similar to that presented for total species. Fifteen-month recovery in coarse oiled substrates is 48 and 38% for polychaetes and crustaceans, respectively, and 48% overall. Mollusks show an "over-recovery". i.e., 174% of controls. Three-month recovery was 8 and 19% for controls; and was 5 and 7% for oiled substrates.

Table 7. Numbers of Individuals for "Primary" Species as a Percentage of All Individuals for Beckett Point and Jamestown (from Nyblade) and Recovery Study Situations.*

CDOLID		% OF ALL INDIVIDUALS				
GROUP	BECKETT POINT	RECOVERY STUDY	JAMESTOWN			
Polychaetes	36	76	29			
Mollusks	92	51	97			
Crustaceans	97	90	40			
All Others	0	0	0			
Totals	73	78	33			

Proportions of individuals/m² computed from Appendix I (Nyblade, 1979), Fall 1977, +0' tide level, for Beckett Point and Jamestown. Data from Recovery Study, November 1979, Sequim Bay, coarse sediment controls.

Table 8. Density (No./ m^2), Recovery (% Final Control), and Representation (% Respective Total), for Primary Species in Recovery Experiments at Sequim Bay/MLLW/Coarse Sediment.

	3-MONTH FALL			3-MONTH SUMMER		NTH FALL
	0il	Control	0i1	Control	0il	Control
OLYCHAETES						
No./m ² (%) Final Control % Respective Total	5706 (7) 90	16055 (20) 88	4623 (6) 83	7064 (9) 60	37994 (48) 77	78378 (100) 76
RUSTACEANS						
No./m ² (%) Final Control % Respective Total	511 (4) 43	1220 (9) 51	236 (2) 60	610 (4) 51	5351 (38) 75	14127 (100) 90
OLLUSKS						
No./m ² (%) Final Control % Respective Total	472 (89) 83	393 (74) 69	59 (11) 60	177 (33) 90	924 (174) 97	531 (100) 51
OTALS FOR ALL GROUPS						
No./m ² (%) Final Control % Respective Total	6690 (7) 82	17688 (19) 82	4919 (5) 81	7850 (8) 58	44271 (48) 77	93036 (100) 78

To this point we have presented data and discussed recovery of individual numbers in terms of numbers per m² to facilitate general comparisons with the baseline studies and the recovery study. All data which follow are in the original units (numbers of individuals of a category per tray (experimental unit)). More explicitly, this is an average of the total numbers of individuals of a given category removed from 5 groups of 7 cores per tray. We do this to preclude inflating the statistical error. Further, to better meet assumptions of normality and discrete as opposed to continuous statistical distributions, for each analysis of variance performed on the counts, supplemental analyses were performed on the transformations of raw data, $\ln (x + 1)$ and $\sqrt{x + 1}$. In general, the "significance" attributed to results did not differ when these transformations were made. They will be mentioned again only in cases where computed differences in "significance" did arise. Obviously, intuitive interpretation of effects on the transforms would be much more obscure.

Experiment I. Site, Sediment, Oil Effects, 3-Month Recovery--

Mean density for primary species in Experiment I is shown on Table 9. A cursory examination of these data indicate that higher densities and more decided differences between experimental conditions are seen for polychaetes. In general, polychaetes were more abundant at the Sequim Bay site as opposed to Protection Island. Ophiodromus pugettensis was entirely absent at Protection Island. Densities of the remaining polychaete species were considerably less there. The three mollusk species appeared more equitably distributed at the two sites. For the most abundant mollusks, the small bivalves, Mysella tumida and Transennella tantilla, there appeared to be substantially greater numbers in the finer sediment, independent of site and oil treatment.

We made null hypotheses that individual densities for the primary species would be equal at the two sites, in the two sediment types, and in oiled and unoiled trays. The computed probability for Type I statistical error (significance level from Analysis of Variance) in rejecting these hypotheses are tabulated on Table 10. We preselected a rejection criterion of 0.010 to deem "significant" effects. Since we are testing hypotheses on seven species, the maximum real probability for error is 7% in this experiment. Based on this criterion, we reject the hypotheses that densities at Sequim Bay and Protection Island are equal for Ophiodromus pugettensis, Platynereis bicanaliculata, and Armandia brevis. Furthermore, we reject the hypotheses that Mysella tumida and Transennella tantilla have equal densities in the two sediment types. With respect to effects from oil treatment on density, we reject the hypotheses that densities for Platynereis bicanaliculata and Armandia brevis are equal in oiled and unoiled trays.

In light of the results of these hypotheses tests, a reexamination of the mean densities on Table 9 can provide insight into the general recovery picture in terms of individual numbers previously discussed. Recall from Tables 5 and 8, that 3-month fall recovery in terms of numbers of individuals was quite low. For primary species (Table 8) it amounted overall to 7 and 19% for oil-treated and untreated trays, respectively.

Table 9. Mean Density of Primary Species (No./tray) in Experiment I.*

		MOLLUSKS		POLYCHAETES #
EXPERIMENTAL CONDITIONS	Lacuna sp.	Mysella . tumida	Transanella tantilla	Ophiodromus pugettensis Armandia brevis Platynereis bicanaliculata Capitella capitata
Protection Island				
Coarse Control Oiled	1.2	2.4 0.4	1.4 1.8	0 7.4 2.4 0.8 0 1.0 0.2 0.6
Fine Control Oiled	1.6 1.4	7.4 10.2	16.4 14.8	0 2.6 0.8 1.6 0 1.4 0.8 0.6
Sequim Bay				
Coarse Control Oiled	2.4 0.6	1.4 2.6	0.2 1.6	0.4 78.6 16.0 17.0 1.2 11.2 6.8 17.0
Fine Control Oiled	1.6 1.2	7.2 8.8	13.4 13.4	0.2 57.2 9.6 11.2 0.6 2.2 4.6 134.2

^{*} This experiment included: Two sites (Protection Island; Sequim Bay); Two sediment types (Coarse; Fine); One tide level (MLLW); Two oiling categories (Oiled and Unoiled Control). 3-Month Recovery (Started 8/78; Completed and Sampled 11/78). Means are based on 5 replicates per condition (7 cores per replicate). Hypothesis test for main effects on Table 10.

Table 10. Hypothesis tests for Density of Primary Species in Oil Recovery Experiment I.

PRIMARY SPECIES	PROBABILITY FOR ERROR	IN REJECTING SEDIMENT ²	THE HYPOTHESES
Lacuna sp.	0.353	0.353	0.042
Mysella tumida	0.879	0.000*	0.178
Transennella tantilla	0.334	0.000*	0.973
Ophiodromus pugettensis	0.000*	0.459	0.220
Platynereis bicanaliculata	0.000*	0.079	0.004*
Capitella capitata	0.142	0.334	0.305
Armandia brevis	0.000*	0.014	0.000*

¹ The site hypothesis is: Density in trays at Protection Island equals density in trays at Sequim Bay.

 $^{^2}$ The sediment hypothesis is: Density in trays containing coarse sediment is equal to density in trays containing fine sediment.

³ The oil hypothesis is: Density in trays receiving oil treatment is equal to density in trays not receiving oil treatment.

^{*} We reject the indicated hypothesis with a maximum real probability for error of 7%.

The primary species "significantly" affected by oil are among those of highest density within the group. Armandia brevis had the highest density in coarse sediments from which we drew the general recovery picture (previous section). In fine, oil-treated sediments at Sequim Bay, density of \underline{A} . \underline{brevis} was far exceeded by $\underline{Capitella}$ $\underline{capitata}$. The latter species, an opportunistic polychaete for which density has been suggested as an indicator of pollution generally, and oil pollution specifically, demonstrated a high lack of consistency in its experimental pattern of density with the one exception. That is, that it, like the other polychaetes, was apparently more prevalent at Sequim Bay. Because of relatively high contributions to total numbers of individuals by species like Capitella capitata, and the high density mollusks, Mysella tumida, and Transennella tantilla, whose density was unrelated to oil treatment in this experiment, total number of individuals even among primary species, will tend toward a conservative estimate of the effects of oil on recovery. Because of "significant" site effects on density, neither Ophiodromus pugettensis nor Platynereis bicanaliculata, should be deemed as general indicators of recovery following a spillage of oil.

Experiment II. Tide Level, Oil Effects, 3-Month Recovery--

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Density of primary species in the setting of Experiment II is shown in Table 11. Generally, densities for the primary species were greater at the lower tide level, with the outstanding exception of the high density polychaete, Exogone lourei. Consistently lower densities in oiled versus unoiled sediments are observable for half of the primary species including, Mysella tumida, Corophium ascherusicum, Leptochelia dubia, Exogone lourei, and Polydora socialis.

From the hypotheses tests, Table 12, tide level differences are deemed significant for Leptochelia dubia, Armandia brevis, Platynereis bicanaliculata, and Polydora socialis. Significant density differences due to oil treatment were found for Corophium ascherusicum, Leptochelia dubia, Exogone lourei, and Polydora socialis. For each of these species, mean density was greater in control than in oiled trays (Table 11).

Seasonal aspects are of interest, but are in most cases, confounded. Lacuna sp., Mysella tumida, Platynereis bicanaliculata, and Armandia brevis were primary species common to Experiments I and II. Among those species, Platynereis bicanaliculata, and Armandia brevis were deemed to have significant effects on density due to site and oiling in Experiment I, and due to tide level in Experiment II. Nonalignment of these factors should therefore be excluded in seasonal comparison. Because of dependencies in treatment description data and possible differences in the oil treatment itself (p. 57), Analysis of Variance was not used to describe seasonal differences. For the appropriate comparison (coarse sediment, control, mean lower low water), the mean density (Tables 9 and 11) appears to be an order of magnitude lower for these two species during the summer season as compared to fall. The mollusk density (Mysella tumida, Lacuna sp.) appears inconsistent with respect to season.

Table 11. The Mean Density of Primary Species in Experiment II (Tide Level, Oil, 3-Month Recovery, Summary).

	MLL	MEAN NUME	BERS/TRAY MINUS	21
PRIMARY SPECIES	CONTROL	OIL	CONTROL	OIL
MOLLUSKS				
Mysella tumida	1.6	0.2	2.8	1.6
Protothaca staminea	0	0	0	0
<u>Lacuna</u> sp.	0	0.4	0.2	0
CRUSTACEANS				
Corophium ascherusicum	1.0	0.6	3.0	0.6
Photis brevipes	0.6	0	0.8	5.0
<u>Leptochelia</u> <u>dubia</u>	4.6	1.8	106.6	40.2
POLYCHAETES				
Armandia brevis	6.0	0.2	43.6	56.6
Exogone lourei	38.0	9.0	16.2	10.2
Platynereis bicanaliculata	0	0	0.8	1.4
Polydora socialis	2.6	0.4	32.0	11.4

Means based on 5 replicates per condition (7 cores per replicate). Analyses of Variance for main effects on Table 12.

Table 12. Hypothesis Tests for Density of Primary Species in Experiment II (Spring-Summer, Tide, Oil).

PRIMARY SPECIES PROBABILITY	FOR ERROR IN REJECTI	NG THE HYPOTHESIS OIL ²
MOLLUSKS		
Mysella tumida	0.092	0.092
Protothaca staminea	1.000	1.000
<u>Lacuna</u> sp.	0.536	0.536
CRUSTACEANS		
Corophium ascherusicum	0.031	0.005*
Photis brevipes	0.178	0.344
Leptochelia dubia	0.000*	0.000*
POLYCHAETES		
<u>Armandia</u> <u>brevis</u>	0.000*	0.640
Exogone lourei	0.049	0.002*
Platynereis bicanaliculata	0.003*	0.357
Polydora socialis	0.000*	0.004*

 $^{^{1}}$ The tide level hypothesis is: Density in trays at MLLW is equal to density in trays at $^{-2}$ below MLLW.

 $^{^2}$ The oil hypothesis is: Density in trays receiving oil treatment is equal to density in trays not receiving oil treatment.

^{*} We reject the indicated hypothesis with a maximum real probability for error of 10%.

Experiment III. Sediment, Oil Effects, 15-Month Recovery--

Mean primary species densities in Experiment III are shown on Table In this 15-month framework, the most easily observable phenomenon is the marked increase in density for most species, especially crustaceans and polychaetes, in contrast to the 3-month recovery period (Tables 9 and There are greater numbers of individuals in unoiled sediments as compared to oiled in both coarse and fine sediment for all of the crustacean species, and all of the polychaete species with one exception (Polydora socialis in fine sediments). One mollusk, Lacuna sp., also had lesser numbers in oiled sediments. Another mollusk, Mysella tumida, consistently had a greater number of individuals in oiled versus control sediment. Hypothesis tests in this experiment revealed no significant differences due to sediment type (Table 14). Differences in density due to oiling were significant for Mysella tumida, Armandia brevis, and Platynereis bicanaliculata. The $\sqrt{x+1}$ transform of density for Corophium ascherusicum was significantly different in oiled versus unoiled sediment. This was the only case in which data transformation changed "significance" designation.

Substrate Recovery Indicators for the Strait of Juan de Fuca.

We have shown in the preceding section that the 13 primary species contain a substantial part of the total number of individuals in fully recovered experimental trays and from nearby baseline stations. Further, while the preponderance of effects on density from the oil treatment were reductions compared to appropriate controls, in the case of the high density mollusk, Mysella tumida, there was, in fact, a significantly greater density in oiled substrates as compared to controls. For the 3-month recovery data presented, there is also an indication (non-significant) that Capitella capitata may have greater density in oiled than in non-oiled substrates. Thus, while the total number of individuals in the primary species follows total numbers of individuals quite well, there is an inconsistent but conservative effect for both the total number of individuals and total number of individuals of primary species due to species responding differently to the oil treatment.

Two of the primary species, Exogone lourei and Leptochelia dubia, were excellent indicators of recovery in this study and would seem to be good region-wide indicators as well. It should be emphasized that this is not in the sense of "indicator species" concepts where some measure of the density for these species alone would be indicative of recovery. Rather, the two species alone, used in a proper experimental design implemented at the time of some future oil contamination and backed with appropriate chemical analyses, would provide a good measure of substrate suitability for recovery in terms of numbers of individuals. Because of high replication needs, the limitation of interest to the two species would result in substantial savings in sampling effort. The merits of these species are: (1) a relatively high sensitivity to the effects of the oil treatment; (2) a relatively high methodological sensitivity; and (3) a rather ubiquitous distribution in the Strait of Juan de Fuca and northern Puget Sound region. The data in this section are provided to document these features. Before proceeding, however, we briefly digress to two taxonomic considerations.

Table 13. The Mean Density of Primary Species in Experiment III (Sequim Bay, 15-Month Recovery, Sediment Type, Oil).

	COAF	NE		
PRIMARY SPECIES	CONTROL	OIL	CONTROL	OIL
MOLLUSKS				
Mysella tumida	3.4	7.2	6.8	12.4
Protothaca staminea	0	0.6	0.4	0.2
Lacuna sp.	1.0	0.8	1.0	0.0
CRUSTACEANS				
Corophium ascherusicum	34.8	11.6	38.8	0.8
Photis brevipes	5.6	0.8	3.6	0.6
Leptochelia dubia	103.2	42.0	99.0	87.4
POLYCHAETES				
Armandia brevis	48.8	22.2	69.6	10.6
Exogone lourei	323.4	197.2	512.4	279.6
Platynereis bicanaliculata	32.8	9.0	40.2	3.6
Polydora socialis	322.3	83.6	224.1	294.9

Means based on 5 replicates of group's of 7 cores per group. Hypothesis tests shown in Table 14.

Table 14. Hypothesis Tests of Density of Primary Species in Experiment III (15-Month Recovery, Sequim Bay, Sediment, 0il).

PROBABILITY PRIMARY SPECIES	FOR ERROR IN REJECT SEDIMENT ¹	ING THE HYPOTHESIS OIL ²
MOLLUSKS		
Mysella tumida	0.012	0.007*
Protothaca staminea	1.000	0.514
Lacuna sp.	0.465	0.278
CRUSTACEANS		
Corophium ascherusicum	0.790	0.027 ³
Photis brevipes	0.533	0.038
Leptochelia dubia	0.485	0.225
POLYCHAETES		
Armandia brevis	0.767	0.000*
Exogone lourei	0.108	0.039
Platynereis bicanaliculata	0.913	0.004*
Polydora socialis	0.266	0.349

¹ The sediment hypothesis is: Density in trays with coarse sediment is equal to density in trays with fine sediment.

 $^{^2}$ The oil hypothesis is: Density in trays receiving oil treatment is equal to density in trays not receiving oil treatment.

³ ANOVA on square-root transform computed "significant" according to our criteria.

^{*} We reject the indicated hypothesis with a maximum real probability for error of 10%.

The polychaete, <u>Exogone lourei</u>, has an abundant congener, <u>E. gemmifera</u>, in our region. Although they are distinctive, it may be quite important to make that distinction (see p. 43). The crustacean, <u>Leptochelia dubia</u>, has a confusion of names. It is often called <u>L. savignyi</u>. It is beyond the scope of this study to provide the necessary investigation of nomenclature to assign an appropriate name. We have followed the lead of Nyblade (1979) who calls the species <u>L. dubia</u>, apparently using keys of Light, Smith and Carlton (1975). Taxonomic keys provided by Kozloff (1974), perhaps more widely used in the region, give the name <u>L. savignyi</u>. Thus, some authors who provide the comparative data may use this name. Unusually high numbers of this species in a wide variety of situations in our region certainly warrant studies to clarify its nomenclature.

Although neither of the above species was preselected as a primary species for Experiment I (the site comparison 3-month fall recovery), they were, in fact, important contributors to all segments of that experiment. Table 15 provides the mean density information for the two species in that experiment. In general, the pattern of density follows that discussed in Experiments II and III. Fine sediment information is not conclusive. Exogone lourei was less dense at Protection Island. Even there, however, the species consistently occurred in all treatment categories. Leptochelia dubia was even more abundant at Protection Island than at Sequim Bay, and shows the oil treatment trends as clearly for Protection Island as those shown for Sequim Bay.

Seasonal mean density for Mean Lower Low Water of Exogone and Leptochelia at the adjacent baseline stations (Nyblade, 1979) is shown in Table 16. Both the species are at least moderately abundant on a year-round basis. The data from Nyblade (1979), Webber (1979), and Smith and Webber (1978), indicate a predominantly lower intertidal and shallow subtidal abundance pattern for the two species. For stations on the west shore of Whidbey Island, Webber (1979) listed Leptochelia savignyi and Exogone sp. among dominant species in the shallow subtidal zone. As far away as Drayton Harbor in the north, Leptochelia savignyi was identified as an important constituent of the low intertidal zone by Smith and Webber (1979). The species was used by these same authors to illustrate site differences between Cherry Point, where it was rarely found, and Shannon Point, where it was an extremely common constitutent. Simenstad et al. (1980) found L. dubia, as a member of the epibenthic zooplankton, in high numbers at stations west of Port Angeles but, inexplicably, not at stations east of Port Angeles.

The relative spatial and seasonal ubiquity of these species indicates to us that sources of these species would likely remain stable well beyond any envisioned immediate impact zone due to oil contamination. Given proper experimental control they should reflect very well the degree of recovery from oil contamination.

Recovery of Other Community Members

Taken alone, the data on primary species from the two preceding sections might be construed as an anomalous indicator of the general community recovery in terms of numbers of individuals. Therefore, the computed densities for other community constituents are reported here for

Table 15. Mean Density for Recovery Indicators in Experiment I.

	MEAN NUMBERS/TRAY ¹		
EXPERIMENTAL CONDITION	Exogone	Leptochelia	
Protection Island			
Coarse Sediment			
Control	1.0	32.0	
Oiled	0.2	5.8	
Fine Sediment			
Control	0.6	14.8	
Oiled	0.8	5.4	
Sequim Bay			
Coarse Sediment			
Control	51.2	9.6	
Oiled	21.8	5.0	
Fine Sediment			
Control	12.4	1.6	
Oiled	5.0	2.2	

 $^{^{1}}$ Mean density based on 5 replicates of groups of 7 cores for each mean.

Table 16. Estimated Density for Recovery Indicator Species at Adjacent Baseline Sites. 1

LOCATION/SEASON	NUMBERS PE Exogone lourei	R SQUARE METER Leptochelia dubia
Beckett Point		
Spring 1977	720	9670
Summer 1977	8920	1798
Fall 1977	3104	44632
Winter 1977	1600	26372
Jamestown		
Spring 1977	5220	810
Summer 1977	2560	1492
Fall 1977	5180	1760
Winter 1977	3432	2080

¹ Data from Nyblade (1979) +0' Tide Level.

comparison in recovery. For reasons previously discussed (p. 9), we cannot be certain of the error probabilities for these species. For this reason we simply present the mean numbers of individuals per square meter. To preclude perhaps needless repetition of the same point, we include only data from Experiment I, Sequim Bay, and Experiment III in this section. A complete data list and Analyses of Variance will be included in the final report.

On Table 17 are the densities of polychaetes from Sequim Bay trays, Experiment I. The trend in the data is quite clear. With the notable exception of \underline{E} . $\underline{gemmifera}$, the congener to $\underline{Exogone}$ \underline{lourei} , the species indicate reduced densities in oiled substrates as compared to controls. Although for a few of these species the differences were computed to be significant (alpha = 0.05), we do not select species here and attribute significance. The central point is that the data do not contradict the generalizations made about effects of oil generally from the data on primary species in the preceding sections. It appears that the effect of the oiling was to reduce the numbers of polychaetes on a rather broad spectrum basis.

Data for the individual crustacean species is a little less clear-cut (Table 18). Where larger numbers of individuals are involved, e.g., ostracods (undetermined species), Melita dentata, amphipods (undetermined species), the trend of reduced numbers in oiled substrates seems clear. The ambiguity which arises when lesser numbers of individuals are involved is undoubtedly related to methodological sensitivity. Data on the primary species does appear to fairly represent the response of crustaceans.

The gastropod mollusks, like the primary species, do not seem to show a pattern dependent on oil treatment (Table 19). Among bivalves, Clinocardium sp. was consistently more dense in control trays. From analysis of variance, that difference was computed to be significant. We do not attribute statistical significance to this indicated effect for previously stated reasons. However, the cockles have some importance as recreational clams; since the result seems to be contrary to the generalization that the mollusk density is not affected by oiling, some further investigation of the species is warranted.

Also shown in Table 19 are the densities in Experiment I for the "Other Species" category. There are no highly abundant, highly sensitive species indicated in these data for this group.

Comparable data sets for densities of nonprimary species after 15-months recovery are on Tables 20 through 22. The striking feature of Table 20 relating to polychaete density is the large increase, relative to 3-month recovery, not only in the number of polychaete species but also in the estimated densities for individual species. Overall, numerical superiority resides with the control substrates as compared to oiled, an observation consistent with the data on primary species. In contrast to the 3-month recovery data (Table 17) where a preponderance of oiled substrates had indicated densities of zero, the oiled substrates in this case do show an appreciable amount of recovery for most species. It is interesting, although not necessarily significant, that the congener of our suggested recovery indicator species, Exogone gemmifera, in this

Table 17. Mean Density of Nonprimary Species (No./m 2) of Polychaetes in Experiment I Sequim Bay Example.*

	CON	TROL	0110	FD	
SPECIES/FAMILY	COARSE	FINE	COARSE	FINE	
DORVILLEIDAE Dorvillea gracilis	60	20	0	0	
GLYCERIDAE Hemipodus borealis	20	60	0	0	
GONIADIDAE Glycinde polygnatha	20	0	0	0	
NEPHTYIDAE Nepthys sp.	30	60	0	0	
ONUPHIDAE <u>Nothria</u> <u>elegans</u>	20	20	0	60	
PHYLLODOCIDAE Phyllodoce (Anaitides) maculata	40	0	20	0	
POLYNOIDAE Harmothoe imbricata Framothoe sp.	20 0	20 20	0 0	0 0	
SYLLIDAE Exogone gemmifera	0	0	40	220	
CAPITELLIDAE Notomastus sp.	0	0	0	20	
MALDANIDAE Axiothella rubrocincta	280	20	40	20	
SPIONIDAE Boccardia sp. Nerine cirratulus Polydora californica Polydora sp.	160 40 0	20 0 40	0 20 0	0 0 0	
Prionospio sp. Rhynchospio arenicola Spio filicornis Spionid undet.	0 20 740 0	20 0 500 20	0 0 320 0	0 0 20 60	
STERNASPIDAE Sternaspis fossor	20	0	0	0	
TOTALS	1470	820	440	400	

^{*} This experiment included: Two sites (Protection Island; Sequim Bay); Two sediment types at each site (Coarse; Fine); One tide level (MLLW); Two oiling conditions (Oiled; Unoiled Control). 3-Month Recovery (Started 8/78; Completed and Sampled 11/78). For convenient comparison to Experiment III (Table 20) only Sequim Bay data are shown. Species density is rounded to nearest 10. Each mean based on 5 replicates with 7 cores each replicate.

Table 18. Mean Density of Nonprimary Species (No./m²) of Crustaceans in Experiment I Sequim Bay Example.*

SPECIES/GROUP	CONT COARSE	FINE	OI COARSE	LED FINE
OSTRACODA Ostracoda undet.	360	100	140	40
COPEPODA Copepoda undet.	120	160	20	100
AMPHIPODA Ampithoe sp. Caprella undet. Corophium sp. Melita dentata Melita sp. Orchestoidea sp. Parallorchestes sp. Paraphoxus sp. Photis sp. Pontharpinia sp. Pontogeneia inermis Pontogeneia sp. Amphipoda undet.	0 0 0 340 60 20 60 20 0 0 20	20 20 0 0 60 0 20 0 20 0	20 0 40 60 100 0 20 80 0 20 40 60	0 0 0 0 0 0 0 20 0 0
DECAPODA Crangon sp. Heptacarpus paludicola Pandalus platyceros Spirontocaris prionota Spirontocaris sp.	0 20 0 0 0	40 0 20 20 20	20 0 0 0 20	0 0 0 0 0
REPTANTIA Pagurus hirsutiusculus Pinnotheres sp. TOTALS	20 0 1100	0 0 640	0 <u>20</u> 660	0 0 200

^{*} This experiment included: Two sites (Protection Island; Sequim Bay); Two sediment types at each site (Coarse; Fine); One tide level (MLLW); Two oiling conditions (Oiled; Unoiled Control). 3-Month Recovery (Started 8/78; Completed and Sampled 11/78). For convenient comparison to Experiment III (Table 21) only Sequim Bay data are shown. Species density is rounded to nearest 10. Each mean based on 5 replicates with 7 cores each replicate.

Table 19. Mean Density of Nonprimary Species (No./ m^2) of "Others" and Mollusks in Experiment I Sequim Bay Example.*

SPECIES/GROUP	CONTI	ROL	OILE	D
	COARSE	FINE	COARSE	FINE
OTHER SPECIES				
HYDROZOA <u>Sertularella</u> sp. <u>Sertularia</u> sp. Hydrozoa undet.	60	0	40	0
	20	0	0	0
	20	20	0	0
NEMERTEA Tubulanus sp. Emplectonema gracile Paranemertes peregrina Nemertea undet.	40	20	0	0
	0	0	0	20
	0	0	0	20
	80	40	20	40
MOLLUSKS				
GASTROPODA Alvania compacta Littorina sp. Margarites sp. Solariella sp.	20	0	0	0
	0	60	40	20
	0	0	60	0
	20	20	0	0
BIVALVIA Clinocardium sp. Psephidia lordi	120 	20 0	0 0	0 0
TOTALS	400	180	160	100

^{*} This experiment included: Two sites (Protection Island; Sequim Bay); Two sediment types at each site (Coarse; Fine); One tide level (MLLW); Two oiling conditions (Oiled; Unoiled Control). 3-Month Recovery (Started 8/78; Completed and Sampled 11/78). For convenient comparison to Experiment III (Table 22) only Sequim Bay data are shown. Species density is rounded to nearest 10. Each mean based on 5 replicates with 7 cores each replicate.

Table 20. Mean Density of Nonprimary Species (No./ m^2) of Polychaetes in Experiment III.*

	CO	NTROL	OIL	 ED
SPECIES/GROUP	COARSE	FINE	COARSE	FINE
DORVILLEIDAE Dorvillea rudolphi Protodorvillea gracilis	60 260	160 120	80 60	40 40
GLYCERIDAE Glycera americana Hemipodus borealis	0 125	0 140	20 120	40 120
GONIADIDAE Glycinde armigera Goniada brunnea	400 20	400 20	20 0	20 0
LUMBRINEREIDAE Lumbrinereis zonata	80	460	80	360
NERIDAE Nereis vexillosa	220	160	100	80
ONUPHIDAE Nothria elegans	300	460	200	500
PHYLLODOCIDAE Eteone longa Eumida bifoliata Phyllodoce (Anaitides) macula	40 1580 ata 500	0 1540 380	60 400 480	100 620 660
POLYNOIDAE Halosynda brevisetosa Harmothoe imbricata	0 280	100 200	0 260	600 80
SYLLIDAE Exogone gemmifera Trypanosyllis gemmipara Trypanosyllis ingens Trypanosyllis sp.	1540 40 0 0	2180 0 0 0	1500 0 0 20	2320 0 200 0
CIRRATULIDAE <u>Cirratulus cirratus</u> <u>Tharyx</u> <u>multifiliis</u>	80 360	3160 120	20 20	280 520

Table 20. (Continued)

CONTROL		OILED	
COARSE	FINE	COARSE	FINE
600	200	40	420
000	360	40	420
0	20	60	0
40	160	240	380
580	580	400	160
0	40	0	0
600	560	980	2700
0	20	0	0
0	20	0	0
7785	11380	5160	10240
	COARSE 680 0 40 580 0 600 0 0	COARSE FINE 680 380 0 20 40 160 580 580 0 40 600 560 0 20 0 20	COARSE FINE COARSE 680 380 40 0 20 60 40 160 240 580 580 400 0 40 0 600 560 980 0 20 0 0 20 0

^{*} This experiment included: One site (Sequim Bay); Two sediment types (Coarse; Fine); One tide level (MLLW); Two oiling conditions (Oiled; Unoiled Control). 15-Month Recovery (Started 8/78; Completed and Sampled 11/79). Comparable data for 3-month recovery on Table 17. Species density is rounded to nearest 10. Each mean based on 5 replicates with 7 cores each replicate.

Table 21. Mean Density of Nonprimary Species (No./ m^2) of Crustaceans in Experiment III.*

SPECIES/GROUP	COARSE	NTROL FINE	COARSE	FINE	
OSTRACODA Ostracoda undet.	0	120	60	40	
NEBALIACEA Nebalia pugettensis	40	200	80	120	
CUMACEA Cumella vulgaris	60	40	20	20	
AMPHIPODA Ampithoe lacertosa Ampithoe sp. Aoroides columbiae Caprella laeviuscula Caprella undet. Ischyrocerus anguipes Orchomene pacifica Paraphoxus sp. Pontogeneia inermis Amphipod undet.	0 20 0 40 20 680 0 560 20	100 200 20 60 60 580 40 360 60	210 20 0 20 20 100 0 80 0	420 0 0 0 0 20 0 60 20 0	
DECAPODA Heptacarpus sitchensis Heptacarpus taylori	0 0	20 520	0 220	0 120	
REPTANTIA Hemigrapsus nudus Pinnixia occidentalis Pinnixia schmitti Pinnixia tubicola Telemessus cheiragonus Upogebia pugettensis	0 20 380 0 0 40	0 0 560 20 20	20 0 640 0 0	0 0 320 0 0	
TOTALS	1900	2980	1550	1240	

^{*} This experiment included: One site (Sequim Bay); Two sediment types (Coarse; Fine); One tide level (MLLW); Two oiling conditions (Oiled; Unoiled Control). 15-Month Recovery (Started 8/78; Completed and Sampled 11/79). Comparable data for 3-month recovery on Table 18. Species density is rounded to nearest 10. Each mean based on 5 replicates with 7 cores each replicate.

Table 22. Mean Density of Nonprimary Species (No./ m^2) of "Others" and Mollusks in Experiment III.*

SPECIES/GROUP	CON' COARSE	TROL FINE	OIL COARSE	ED FINE
OTHER SPECIES				
HYDROZOA Obelia sp. Hydrozoa undet.	4 0 0	40 20	40 0	0 0
NEMERTEA Baseodiscus sp. Emplectonema gracile Nemertea undet.	20 40 160	0 60 200	20 20 160	0 80 260
ECHINODERMATA Holothuroid undet. MOLLUSKS	0	20	60	0
GASTROPODA Alvania compacta Bittium sp. Littorina sitkana	240 20 20	120 0 0	0 10 20	0 20 0
BIVALVIA Clinocardium nuttallii Clinocardium sp. Macoma inflatula Macoma sp. Mya sp. Mytilus edulis Saxidomus nuttallii	20 40 100 20 20 20 20	60 60 40 0 0	0 20 20 0 0 0	0 20 20 0 0 0
TOTALS	780	620	370	400

^{*} This experiment included: One site (Sequim Bay); Two sediment types (Coarse; Fine); One tide level (MLLW); Two oiling conditions (Oiled; Unoiled Control). 15-Month Recovery (Started 8/78; Completed and Sampled 11/79). Comparable data for 3-month recovery on Table 19. Species density is rounded to nearest 10. Each mean based on 5 replicates with 7 cores each replicate.

independent experiment again shows slight numerical advantage in oiled substrates.

The 15-month recovery densities for crustaceans (Table 20) excluding primary species, do not show an increase in numbers of species over 3 months (Table 18) as did the polychaetes. Overall, numerical superiority is in favor of unoiled substrates. The difference is mainly attributable to differences in density in fine substrates. We do not see any strong exceptions in the data to refute the finding that crustacean numbers are reduced in oiled substrates compared to controls based on primary species. As for the polychaetes, the effects of oiling seem to be a rather broad spectrum on the recovery of crustaceans.

The 15-month recovery data for mollusks (Table 22) indicate a greater number of species, especially in coarse control substrates than for 3-month recovery (Table 19). Relatively low density prevails throughout. For the two species, Macoma inflatula and Clinocardium sp., the mean differences due to oiling are interesting, because of the consistency in pattern. For Clinocardium, this is a second independent experiment in which the same trend was observed. There is likely a real effect on the density of Clinocardium due to oiling. This experiment is the first in which the genus Macoma has been observed. The data here reported are not conclusive but are consistent with reports of oil sensitivity by Macoma (Shaw et al., 1976). The overall data on mollusks suggest that quantitative differences in density due to oiling may require substantially longer recovery experiments.

The "Other Species" category, also shown in Table 22, does not indicate a marked increase in number of species from the 3-month recovery data (Table 19). Like the earlier experiment, there do not seem to be any highly abundant or oil-sensitive species in this group.

Trophic Mode of Primary and Selected Other Species.

In experiments of the sort reported here we are, in effect, measuring the suitability of the substrate habitat. A central assumption is that the organisms for colonization are available in "normal" numbers for all of the experimental substrates, control or oiled, on an equal basis. In cases where there are demonstrable differences in recovery, data from the experiment itself can tell us nothing about why organisms are at a lower density in oiled versus unoiled substrates of like character or, indeed, the fate of the "absent" organisms. It is a certainty that organisms must eat to grow and survive. Therefore, trophic modes may play a central role in determining substrate suitability. This section deals with the trophic modes of primary species and a few examples from the nonprimary group.

We are fortunate in having available a classification of feeding level for each of the primary species from related MESA studies (Simenstad et al., 1979). These data, excerpted from Simenstad et al. (1979) are displayed in Table 23. There is one very clear trend from the data. No organism classified as a suspension feeder was deemed to have significantly reduced density in oiled substrates in our experiments. To the contrary, one species, Mysella tumida, was deemed to have greater density in oiled substrates. It is interesting that this finding includes the amphipod

Table 23. Trophic Levels of Primary Species in Oil Recovery Experiments.

GROUPS/SPECIES	TROPHIC LEVELS
MOLLUSKS	
Mysella tumida	Suspension feeder on phytoplankton
<u>Transennella</u> tantilla	Suspension feeder on phytoplankton
Lacuna sp.	Herbivore on microalgae
CRUSTACEANS	
Corophium ascherusicum	Detritivore
Photis brevipes	Suspension feeder on detritus; detritivore
Leptochelia dubia	Detritivore; carnivore on small benthic
POLYCHAETES	
Armandia brevis	Detritivore
Exogone lourei	Detritivore
Platynereis bicanaliculata	Herbivore; macroalgae
Polydora socialis	Detritivore; carnivore on zooplankton
Capitella capitata	Detritivore
Ophiodromus pugettensis	Carnivore on annelids, tanaids and cumaceans

Data excerpted from Simenstad et al., 1979.

crustacean, Photis brevipes, since the amphipods as a group are usually considered to be highly sensitive to the effects of oiling. With the exception of Capitella capitata, each of the species classified as a detritivore was found to have significantly reduced density in oiled substrates in at least one of the experiments, and sometimes two (Corophium ascherusicum, Experiments II and III; Armandia brevis, Experiments I and III). The only one of the primary species classified as a carnivore, Ophiodromus pugettensis, was not significantly reduced in oiled substrates in any experiment. The results on herbivores indicate some relation to feeding mode also. Lacuna sp., an herbivore on microalgae, had consistent, but nonsignificant, reduced densities in Experiments I and III, and inconsistent occurrence in Experiment II. The polychaete, Platynereis bicanaliculata, an herbivore on macroalgae, had significantly reduced density in Experiments I and III.

The foregoing data can be considered as "hard" data with clear-cut results. It is tempting to delve into the data on nonprimary species for further substantiation or refutation, but the cautionary note must be made that we do not have verifiable probability statements on the errors for hypothesis tests of effects indicated by mean differences in density.

If we examine the mean data on polychaetes (Table 20), 13 species are classified as carnivores. For 3 of these, there appears to be distinctly reduced density in oiled substrates as compared to controls (Protodorvillea gracilis, Eumida bifoliata, Glycinde armigera). Only 3 species are classified as herbivores (Lumbrinereis zonata, Nereis vexillosa and Nothria elegans). For each of these species the mean densities are slightly higher (N. vexillosa distinctly higher) in unoiled as compared to oiled substrates. Nine of the species are classified as detritivores. Of these, three have much higher mean density in controls (Cirratulus cirratus, Axiothella rubrocincta, Spio filicornis). Two species had distinctly higher density in oiled substrates (Rhynchospio arenicola, Thelepus crispus), and 4 species were inconsistent. We conclude that the data on primary species reflects the overall relationship of feeding mode and oil effects reasonably well.

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The data on crustaceans (Table 21) is much less clear on the effects from oil generally as earlier stated. However, there are distinctly higher densities in controls for the detritivores, <u>Cumella vulgaris</u> and <u>Paraphoxus</u> sp. There are also distinctly more <u>Ischyrocerus</u> <u>anguipes</u> in controls. The group to which this latter species belongs receives various feeding level designations by Simenstad et al. (1979), i.e., suspension feeders on detritus, herbivores and detritivores. These data then, however insufficient, are consistent with the generalizations on feeding mode derived from primary species.

The data on mollusks and "other species" are even more severely limited. It is of interest that the apparent effect on Macoma inflatula, a deposit feeder, fits a feeding mode picture quite well while the apparent effect on Clinocardium, for which we have more confidence, is in contradiction to the general picture. The latter is a suspension feeder on phytoplankton.

Relevance of Findings to Upper Trophic Levels.

An examination of species and functional groups with major importance to upper trophic levels (Table 4, Simenstad et al., 1979) reveals the presence of 7 of the 13 primary species in this study. It is, therefore, pertinent to attempt to evaluate the relevance of effects on recovery, as elicited here, to effects on upper trophic levels, principally fishes. Criteria for deeming major trophic importance, used by Simenstad et al. (1979), included that the designated species or functional group: (1) provides the majority of energy sources for consumer organisms at some time; (2) provides important conversion or transfer of organic matter to trophic levels where it is available to higher level consumers, or (3) holds "keystone" roles in structuring the composition of the community and the directions and rates of food web energy flow.

The following primary species from the present study are on the list (Simenstad et al., 1979) so defined: Lacuna sp., Platynereis bicanaliculata, Transennella tantilla, Photis brevipes, Ophiodromus pugettensis, Leptochelia dubia, and a member of a family defined, Spionidae, Polydora socialis.

Of these, significant effects on density were indicated for Platynereis bicanaliculata, Leptochelia dubia, and Polydora socialis. These particular species are indeed important ones in the present study since jointly they contributed 62,980 individuals per m² in coarse sediment controls (15-month recovery) out of a total of 119,884 (53%).

Based on these data alone, one would expect impact on the appropriate upper trophic levels for at least 15 months following an oil contamination of the magnitude used in this study.

Although one could pose the specific counter arguments: (1) that reductions in these particularly high density species will be replaced by other species with similar proclivities toward high density; or (2) these are small species and in spite of high density comprise small biomass, we see no evidence in any of the experimental data to support such arguments. Rather, we see a broad spectrum reduction in density across polychaetes, crustaceans, and even mollusks with detritivorous and herbivorous feeding modes. In the case of small versus larger individuals, the data for Nereis vexillosa (Table 20), a rather large polychaete, seems to indicate that biomass as well as density may be substantially reduced.

Based on an extensive amount of stomach analysis and synthesis of published information, Simenstad et al. (1979) have constructed composite food webs for several shallow sublittoral habitats of the northern Puget Sound region. It is our judgment that the Sequim Bay experimental site in the present study is best represented by the composite constructed for protected sand/eelgrass shallow sublittoral habitat. In this, as in all the composite food webs constructed by Simenstad et al. (1979), with the exception of the neritic web, detritus forms the base of the web. We have included (Figure 2) a copy of the composite web for this habitat. The trophic groups containing primary species for which significant effects due to oiling were demonstrated are indicated in Figure 2 by solid fill boxes. Trophic groups containing nonprimary species for which the mean density data suggest a detrimental effect on density are indicated in Figure 2 by boxes with cross-hatch marking. The reader should keep in

Significant effects due to oiling in Recovery Experiments.

Effects due to oiling indicated by mean differences between control and oiled substrates.

PROTECTED SAND-GRAVEL/EELGRASS SHALLOW SUBLITTORAL FOOD WEB

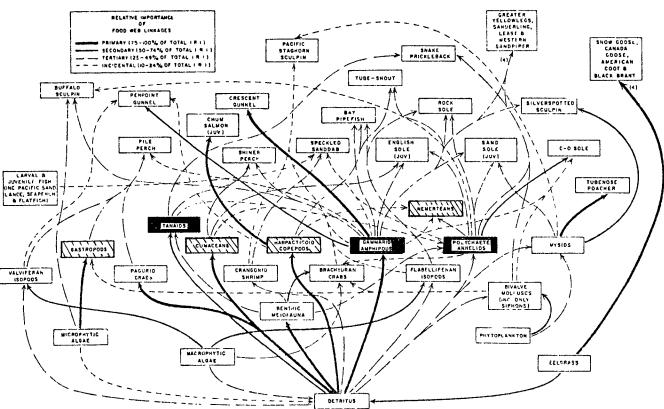


FIGURE 2. COMPOSITE FOOD WEB CHARACTERISTIC OF PROTECTED SAND/ EELGRASS, SHALLOW SUBLITTORAL HABITATS IN NORTHERN PUGET SOUND AND THE STRAIT OF JUAN DE FUCA.

mind that other groups in the food web may contain species susceptible to oiling which were not sampled in the present study.

The clearest and most widespread effects on density from the oiling in the present study were on detritivorus tanaid crustaceans, amphipod crustaceans, and polychaete annelids. One would expect reductions in density for the constituent species to result in a shortage of food and reduced growth for bottom feeding fishes principally using these groups. Contributions to the food web are expressed in terms of an index of relative importance (IRI, Simenstad et al., 1974). From the composite web, the gammarid amphipods contribute, at least on a tertiary basis, to a wide variety of fishes (25-49% of IRI). They are a primary food source for the crescent gunnel (75-100% of IRI). The principal users of the polychaete annelids are among the flatfishes and include the English sole, rock sole, sand sole, and C-O sole. For the C-O sole they are indicated as a secondary source (50-75% of total IRI). The tanaids are principally tertiary contributors (25-49% of IRI) to snake prickleback and shiner perch and contribute incidentally to carnivorous polychaetes. There is now some evidence that oil spillage may, in fact, result in reduced growth for young flat fish in a real-world situation (Desaunay, 1980). This study, conducted following spillage of the AMOCO CADIZ, indicated a marked decrease in growth for a young year class of a bottomfeeding flatfish.

Severity of Treatment in Infauna Experiments.

Characterization of the oil treatment was in terms of large numbers of samples analyzed by infrared spectrophotometry (IR) and capillary gas chromatography (CGC) (see Table 1 for schedule).

The oil treatments applied in Experiments I and III were equivalent. A target concentration of 2000 ppm total oil was sought. Calculated total oil (IR) in sediments was determined by substracting mean concentrations measured in control substrates from mean concentrations in oil-treated substrates in the appropriate treatment categories. shows total oil data for substrates sampled prior to placement in Experiments I and III (preliminary), Experiment I (3-month fall recovery), and Experiment III (15-month fall recovery). Total oil concentrations slightly in excess of 1700 ppm in both fine and coarse sediments were reasonably close to the target concentration. For Experiment I (3-months) initial concentrations were reduced by 34 and 35% for fine and coarse sediment, respectively, leaving total oil concentrations of slightly in excess of 1100 ppm at the termination of this experiment. By the end of Experiment III (15-months), total oil concentrations were reduced by 87 and 85% for fine and coarse sediments, respectively, leaving total oil concentrations of between 200 and 300 ppm at the termination of this experiment. Thus, there are no differences indicated for retention of oil by fine and coarse sediments using the IR methods. The marked increases in control substrate IR of CCl₄ extractable organics by the end of 15 months are of Initially, control substrates gave IR readings of 1 or 2% of oil-treated substrates. By the end of 3 months the controls gave readings of 2 and 3% (primarily derived from reduction in oil-treated concentrations). At 15 months, the controls represented 35 and 36% of oil-treated concentrations. This increase is due primarily to increases in the controls themselves. The increase in control IR measurements probably relates

Table 24. Comparisons of Total Oil (IR) in Experiments I and III, at Sequim Bay. $^{\rm 1}$

SEDIMENT/TREATMENT	PRELIMINARY	CONCENTRATION (ppm) 3-Month EXPERIMENT I	15-Month EXPERIMENT III
COARSE			
Treated	1758 ± 252	1154 ± 260	391 ± 43
Control	21 ± 8	32 ± 15	136 ± 52
Total Oil	1737	1122	258
FINE			
Treated	1794 ± 66	1183 ± 153	366 ± 44
Control	37 ± 10	24 ± 7	132 ± 54
Total Oil	1757	1159	234

¹ Each mean presented based on 9 core analyses. Standard deviations shown are between trays (n = 3 per mean).

to an increase in organic matter over the longer time period. The fact that the relative variability in control IR data is not greatly increased at 15-months versus 3-months (Table 24) indicates to us that cross contamination is not a likely source for these increased readings. Subsampling analyses and Protection Island data have been previously reported. The total oil concentrations at Protection Island did not significantly (p=0.05) differ from the Sequim Bay data.

A time series plot of total oil IR (controls subtracted) is on Figure 3. A loss of oil from substrates from the initial concentration to the 3-month concentration appears to have taken place early (perhaps in the first month) and, thereafter, the loss of total oil appears to follow a linear course.

The comparable data for Experiment II, spring (3-month recovery in coarse substrates) are shown in Table 25. In this case the target concentration of 1000 ppm was achieved. Three month losses of total oil were 43 and 53% for Mean Lower Low Water and minus two feet tide levels, respectively. From these data the losses at both tide levels appear a little more rapid than for the comparable fall experiment. Also, the lower tide level appears to have lost oil a little more quickly than the higher. For the tide level differences indicated, mean differences are not significant. It is inappropriate to statistically compare the apparent seasonal difference.

The time series course of total oil concentration for Experiment II is shown in Figure 4. The intermediate data, as did the final mean data, indicate a more rapid loss of oil at the lower tide level. pattern in total hydrocarbon concentrations in Experiments I and III (Figure 3), one can derive a rate of loss over the 15-month period. loss of total hydrocarbons was more rapid at the beginning of the experiment, averaging 12% per month of the initial concentration. For the entire 15-month period, the average was 5.6% per month. For the period between 3 and 15 months, the indicated loss appears to be relatively stable at a rate of 4.2% per month. In terms of concentration data, this amounts to roughly 71 ppm per month. At that rate of loss, sediments might be expected to reach background levels in terms of IR measurements in a further 3.5 months, i.e., a total of 18.5 months. Obviously, because of a greater lack of homogeneity in substrate in a real-world situation, and because oil would be distributed over a wider area, the data are not directly translatable. They do, however, provide a conservative estimate and give a framework for assessing the time to full recovery for environmental conditions similar to our experiment.

Means of summed analyzed saturate and aromatic compounds in Experiments I and III are shown in Table 26. Losses of both of these compound groups were somewhat more rapid than were losses of total hydrocarbons. Again, we took the approach of substracting out "background" as indicated by concentrations in unoiled control units. Initial concentrations of the analyzed saturate and aromatic compounds were somewhat higher in coarse substrates as compared to fine. Reductions of 81 and 86% are seen in coarse substrate in 3 months. At 15 months the comparable losses are 95 and 98% of initial concentrations, or nearly complete. In the case of fine substrates, losses were slower during the first three months, amounting

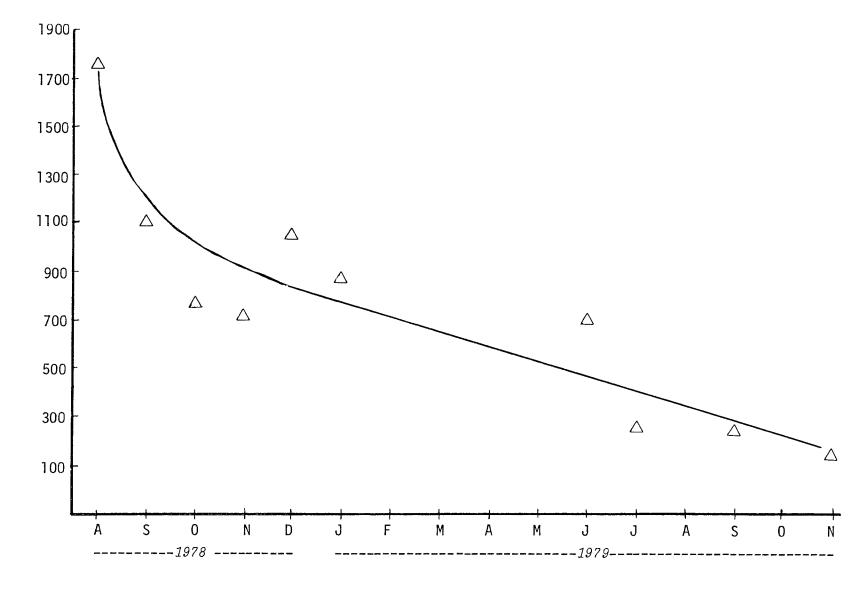


FIGURE 3. CONCENTRATIONS OF TOTAL OIL IR (CC14 EXTRACTABLE ORGANICS IN OIL TREATED SUBSTRATES MINUS CC14 EXTRACTABLE ORGANICS IN CONTROL SUBSTRATES).

Table 25. Comparisons of Total Oil (IR) in Experiment II at Sequim Bay. $^{\rm 1}$

TREATMENT	PRELIMINARY	CONCENTRATION (ppm) MLLW	MINUS 21
Treated	1069 ± 144	612 ± 99	503 ± 16
Control	21 ± 8 ²	17 ± 6	15 ± 2
Total Oil	1048	595	488

 $^{^{1}}$ Each mean presented based on 9 core analyses. Standard deviations shown are between trays (n = 3 per mean).

² These control data are from Experiment I.

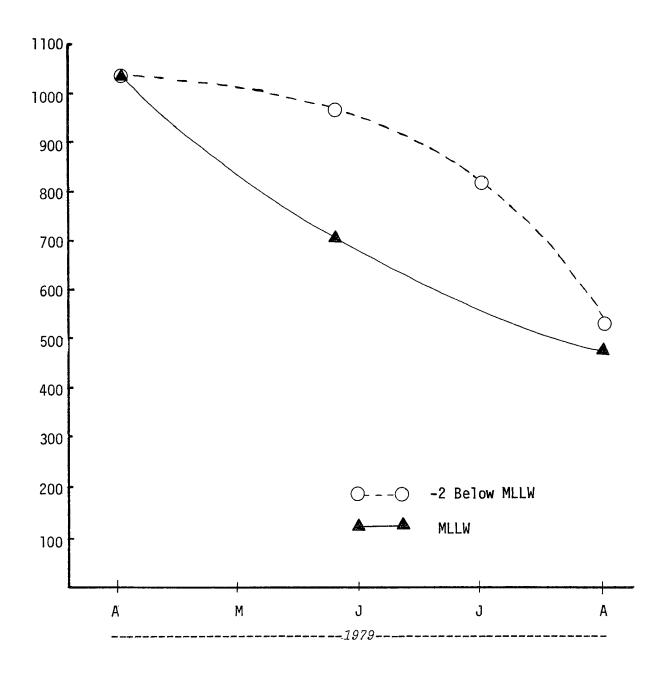


FIGURE 4. CONCENTRATIONS OF TOTAL OIL IR (CC14 EXTRACTABLE ORGANICS IN OIL TREATED SUBSTRATES MINUS CC14 EXTRACTABLE ORGANICS IN CONTROL SUBSTRATES), EXPERIMENT II.

Table 26. Means of Summed Analyzed Saturate and Aromatic Compounds (Capillary GC) in Experiments I and III at Sequim Bay.

SEDIMENT/TREATMENT	PRELIM SATURATES	INARY AROMATICS	MEAN CONCENTE 3-MONT SATURATES	RATIONS (µg/g) TH (I) AROMATICS	15-MONTH (III) SATURATES AROMATICS		
	SATURATES	ANOMATICS	3/10///123	711011111100			
Coarse Sediment							
Oil Treated	157	19.5	30.7	2.8	8.1	0.4	
Control	0.58	0.17	0.73	0.09	0.42	0.08	
Total Due to Oiling	156.52	19.33	29.97	2.72	7.68	0.32	
Fine Sediment							
Oil Treated	121.6	17.6	52.1	5.1	3.3	0.1	
Control	0.53	0.14	0.43	0.08	1.9	0.1	
Total Due to Oiling	121.07	17.46	51.57	5.02	1.40	0.0	

Each mean presented based on analysis of one core from each of three experimental trays receiving the treatment indicated. Analysis of variance and measurement sensitivity in Vanderhorst et al. (1979).

to 57 and 71% for saturates and aromatics, respectively. By 15 months, the losses were virtually complete in fine substrates (98% for saturates and 100% for aromatics).

In Experiment II, the spring-summer tide level experiment (Table 27) commensurate with the reduced target and total oil concentrations, the saturates were a little less than one-half of that measured in preliminary data for Experiments I and III. Inexplicably, the analyzed aromatic concentrations were considerably lower than expected. In this experiment, aromatic concentration was about 4% of saturate concentration as compared to 12% (Table 26) of saturates in Experiments I and III. The losses of measured saturate compounds for this spring-summer, 3-month period, are comparable to rates of loss indicated in the fall experiment (I), amounting to 83% of initial concentration at MLLW and 87% at the -2' tide level. Measured aromatic compounds were at background concentrations in 3 months at each of the tide levels.

RECOVERY ON HARD SUBSTRATES

There is a clear difference in status between the infaunal studies just reported and the recovery on hard substrates reported here. The former studies are complete and conclusions are warranted. The hard substrate studies are under way as of this writing. The points addressed in this section relate to Task F, a testing of attachment method for hard substrates in the exposed rocky intertidal, and Task G, a series of one-month duration, independent experiments on the effects of oil on recovery of communities on hard substrates. Task H, commercial clam bed recovery, and effects of key species removal and oil on recovery will be reported in a subsequent report.

For Task F, polyvinylchloride (PVC), asbestos, and concrete plates were attached to conglomerate rock at Observatory Point on the Strait of Juan de Fuca to evaluate plate survival and colonizing characteristics. Attachments were made in the spring following the severest winter storm activity with the intent of following gross colonization of the differing materials. Triplicate plates of each type were attached to individual PVC backing (60 cm x 30 cm, .25" thickness). The backing was bolted to individual wooden frames. The wooden frames were attached to a northern exposed face, approximately 45° inclination, of intertidal rock approximately 2' above MLLW.

Types of attachment within the plate systems included: (1) wooden frames attached to intertidal rock using masonry nails (2.0") driven with a powder actuated impact gun; (2) PVC backing bolted to wooden frames; (3) concrete plates attached to PVC backing by gluing PVC strips (.25") thick, 5" wide) on edge to form a 30 cm x 30 cm border on the PVC backing. These strips were glued with PVC cement. Holes (.25") were drilled in the border strips to form "key" ways, and concrete was poured directly into the bordered area. (4) Asbestos plates, cut (30 cm x 30 cm) from $\sim .25"$ thick asbestos sheet were glued directly to the PVC backing with contact cement.

Table 27. Means of Summed Analyzed Saturate and Aromatic Compounds (Capillary GC) in Experiment II at Sequim Bay. 1

SEDIMENT/TREATMENT	PRELIM SATURATES	INARY AROMATICS		RATIONS (µg/g) LLW AROMATICS	-2" BELC SATURATES	OW MLLW AROMATICS
Coarse Sediment						
Oil Treated	70.5	2.6	12.4	0.11	9.2	0.12
Control	0.58^{2}	0.17 ²	0.41	0.10	0.38	0.11
Total Due to Oiling	69.92	2.43	11.99	0.01	8.82	0.01

¹ Each mean presented based on analysis of one core from each of three experimental trays receiving the treatment indicated.

 $^{^{2}}$ These control values from Experiment I preliminary analysis.

All attachments had failed at the first observation, with the exception of one wooden frame with bolted on PVC backing.

Species Composition and Density in Monthly Experiments

Monthly experiments for Task G were completed through May 1980. Data from the first four monthly experiments (October-January) concerning recovery of epifauna on hard substrates are presented. The counts of organisms at daily intervals during the first five days of each month resulted only in random occurrences of "crawl-on" type organisms. Enumeration of organisms after 30 days of colonization resulted in some specific patterns of abundance within the major taxomonic categories.

The same major taxonomic categories dominated the hard substrates as were previously seen in the infauna experiments (polychaetes, crustaceans, mollusks). Data for mean number of individual polychaetes for the first four monthly experiments are in Table 28. Polychaetes occurred principally in the October experiment and at the Mean Lower Low Water tide level. On the average, slightly more polychaetes were observed on control substrates as compared to oiled, but the standard deviations are quite large and no significance can be attributed to the difference. Without exception, the polychaete species were ones observed commonly in the infauna experiments. Two of the seven species were ones which were designated as primary species in the infaunal studies. One of these, Platynereis bicanaliculata, was the predominate polychaete observed in this hard substrate experiment, and dominates the density pattern shown in Table 28.

Table 29 shows the mean numbers of individual crustaceans found in the first four experiments. Both the numbers of individuals and species (10) were higher than for polychaetes. While the highest numbers of individuals follow the same patterns as observed for polychaetes (principal occurrence during October and at Mean Lower Low Water), there were measurable densities at the higher tide level and during the winter months. These densities relate to the contribution of one species, the isopod, Exosphaeroma sp. and may account for the apparently anomalous result of higher densities on control substrates at Mean Lower Low Water, and lower densities on control substrates at the +2' tide level.

Density of individual mollusks is shown in Table 30. The mollusks were represented by 7 species, perhaps a preliminary indication that this group will have more importance here than was seen in the infauna. The individual numbers, however, are quite low throughout the experiments and the brick to brick variation is high.

These preliminary data indicate to us that even the relatively high replication used (15 substrate units per treatment category) is not sufficient to allow us to distinguish treatment effects of the magnitude which may be indicated by mean differences between oiled and unoiled substrates. The amount of variation seen is high but somewhat less than that reported for baseline studies at rocky sites (Nyblade, 1979). The general patterns of distribution in densities are consistent with what is known about annual recruitment patterns and tide height distribution for the involved species. It may be that the spring and early summer experiments will prove more useful in distinguishing effects from the oiling, if these exist.

Table 28. Mean Numbers of Individual Polychaetes in Hard Substrate Oil Recovery Experiments. (One-month exposure periods, oil administered at the start of each monthly experiment)

EXPERIMENTAL CONDITIONS	0ct	ober	NUMBERS 0 Nov	ember		= 15 BRICKS) ember	January		
	MEAN	(S.D.)	MEAN	(S.D.)	MEAN	(S.D.)	MEAN	(S.D.)	
Mean Lower Low Water									
Oiled	5.8	(5.1)	0.5	(0.8)	0.0	(0.0)	0.0	(0.0)	
Control	7.0	(6.3)	0.5	(0.5)	0.0	(0.0)	0.1	(0.3)	
Plus 2' above MLLW									
Oiled	0.1	(0.3)	0.3	(0.5)	0.1	(0.3)	0.0	(0.0)	
Control	0.9	(1.8)	0.2	(0.4)	0.1	(0.3)	0.0	(0.0)	

Species Included: Platynereis bicanaliculata; Harmothoe imbricata; Thelepus sp.; Armandia brevis; Nothria elegans; Halosynda brevisetosa; Family Spionidae.

Table 29. Mean Numbers of Individual Crustaceans in Hard Substrate Oil Recovery Experiments. (One-month exposure periods, oil administered at the start of each monthly experiment).

EXPERIMENTAL CONDITIONS	Oct MEAN	ober (S.D.)	Nov MEAN	ember (S.D.)	Dec MEAN	ember (S.D.)	Jan MEAN	uary (S.D.)
Mean Lower Low Water	A REAL PROPERTY OF THE PROPERT						***************************************	***
Oiled	58.6	(38.0)	34.1	(21.6)	8.3	(3.6)	2.8	(2.2)
Control	86.7	(75.0)	21.9	(22.4)	9.9	(4.9)	1.1	(1.1)
Plus 2' above MLLW								
Oiled	20.2	(13.7)	27.3	(18.6)	4.9	(3.0)	0.1	(0.3)
Control	6.7	(5.5)	10.8	(9.64)	4.3	(3.0)	0.5	(0.6)

Species Included: Exosphaeroma sp.; Melita sp.; Corophium sp.; Ampithoe sp.; Jassa sp.; Parallorchestes ochotensis; Caprella laeviuscula; Leptochelia dubia; Cancer sp.; Pinnixia sp.

Table 30. Mean numbers of individual molluscs in hard substrate oil recovery experiments. (One-month exposure periods, oil administered at the start of each monthly experiment).

EXPERIMENTAL CONDITIONS	October			ember		= 15 BRICKS) ember	January	
	MEAN	(S.D.)	MEAN	(S.D.)	MEAN	(S.D.)	MEAN	(S.D.)
Mean Lower Low Water								
Oiled	0.4	(0.7)	0.3	(0.5)	0.0	(0.0)	0.0	(0.0)
Control	0.9	(1.2)	0.1	(0.3)	0.0	(0.0)	0.0	(0.0)
Plus 2' above MLLW								
Oiled	0.7	(0.9)	0.3	(0.5)	0.3	(0.6)	0.1	(0.3)
Control	0.5	(0.5)	0.3	(0.6)	0.3	(0.5)	0.0	(0.0)

Species Included: Lacuna sp.; Clinocardium nuttallii; Mytilus edulis; Cooperella subdiaphana; Transennella tantilla; Protothaca staminea; Odostomia sp.

Severity of Treatment on Hard Substrates.

The conventional expression of treatment severity in terms of weight/ weight or volume/weight ratios commonly used for sediment or water exposures has little meaning in regard to hard substrates. The methods used in this study result in measurements of total hydrocarbon weight on a per brick basis or, in the case of top surface extraction, on surface area basis. The data for whole brick extractions are on Table 31. Since the extraction methods differed during the first two monthly experiments, overall comparisons cannot be directly made. For the first experiment (October), preliminary samples taken immediately post treatment were appreciably higher in the total amount of oil per brick than were 30-day MLLW samples. The indicated loss of oil during this period is 86%. For the November and January experiments, however, the total amount of oil present after 30 days represented a much higher percentage of the initial concentration with 30-day reductions being 16 to 55%. Although variations related to method and brick-to-brick variability in treatment are high enough to preclude conclusions regarding the effect of tide level, or in fact, 5-day versus 30-day losses of oil, these data, nevertheless, indicate that the whole brick retains substantial amounts of oil for the entire 30-day periods of these experiments. Recovery of known applied amounts of oil to bricks indicated that the whole brick extraction method recovered 66% of applied oil.

As part of attempts to make the analyzed oil relate to surface exposure conditions, top surface extractions were used in addition to the whole brick extractions in the November, December, and January experiments, as well as those which have followed. The data on these extractions are on Table 32. These data indicate much greater percentage losses for the 30-day period, ranging from 76 to 96% of the initial concentrations. The 5-day data from December and January, taken after extraction procedures were standardized, indicate that most of this loss probably takes place during the first 5 days. These data have the added advantage that they may be transformed into surface area estimates of oil coverage by multiplication with an appropriate factor.

The two types of data presented here give diametrically opposed views of "best" approaches to study recovery in the rocky intertidal zone. In natural situations, if oil is retained in pores or cracks for a significant period of time, as would seem to be indicated by these early data on whole brick extractions, then future studies had best emphasize highly controlled treatment and development of standardized extraction and analytical methods. If, on the other hand, the very short retention times indicated by the top surface extractions are typical, a greater emphasis needs to be placed on understanding the natural recovery rates. This is an extremely important distinction since the feasibility of the former types of studies appears to be quite limited with presently available methods, while studies of the latter type are not only quite feasible but well under way in our region. It is anticipated that the studies reported here will aid in making that distinction.

Table 31. Mean Total CCl_4 -Extractable Organics (Grams/Brick) for Whole Brick Extractions in Recovery Experiments.

	Octobon			TOTAL HYDROCARBONS (GRAMS/BRICK) November December Januar							·V	
MEAN		Method ¹	MEAN								Method	
3.47	(1.24)	W	4.06	(1.19)	W	3.09	(1.02)	D	2.91	(0.90)	D	
	-		1.76	(0.57)	W	1.97	(1.07)	D	2.86	(1.63)	D	
	-		4.80	(3.38)	М	3.64	(2.00)	D	2.01	(1.15	D	
0.49	(0.16)	W	3.41	(1.56)	D				1.92	(0.83)	D	
	-		2.43	(1.17)	D				1.32	(0.51)	D	
	3.47	3.47 (1.24) - - 0.49 (0.16)	MEAN (S.D.) Method ¹ 3.47 (1.24) W 0.49 (0.16) W	MEAN (S.D.) Method¹ MEAN 3.47 (1.24) W 4.06 - 1.76 - 4.80 0.49 (0.16) W 3.41	October MEAN (S.D.) Method ¹ MEAN (S.D.) 3.47 (1.24) W 4.06 (1.19) - 1.76 (0.57) - 4.80 (3.38) 0.49 (0.16) W 3.41 (1.56)	October (S.D.) Method¹ MEAN (S.D.) Method 3.47 (1.24) W 4.06 (1.19) W - 1.76 (0.57) W - 4.80 (3.38) M 0.49 (0.16) W 3.41 (1.56) D	MEAN October (S.D.) Method¹ November (S.D.) Method MEAN 3.47 (1.24) W 4.06 (1.19) W 3.09 - 1.76 (0.57) W 1.97 - 4.80 (3.38) M 3.64 0.49 (0.16) W 3.41 (1.56) D	October	MEAN October (S.D.) Method¹ November (S.D.) Method December (S.D.) Method 3.47 (1.24) W 4.06 (1.19) W 3.09 (1.02) D - 1.76 (0.57) W 1.97 (1.07) D - 4.80 (3.38) M 3.64 (2.00) D 0.49 (0.16) W 3.41 (1.56) D	MEAN October (S.D.) Method¹ November (S.D.) Method December (S.D.) Method MEAN 3.47 (1.24) W 4.06 (1.19) W 3.09 (1.02) D 2.91 - 1.76 (0.57) W 1.97 (1.07) D 2.86 - 4.80 (3.38) M 3.64 (2.00) D 2.01 0.49 (0.16) W 3.41 (1.56) D 1.92	MEAN October (S.D.) Method¹ November (S.D.) Method December (S.D.) Method January (S.D.) 3.47 (1.24) W 4.06 (1.19) W 3.09 (1.02) D 2.91 (0.90) - 1.76 (0.57) W 1.97 (1.07) D 2.86 (1.63) - 4.80 (3.38) M 3.64 (2.00) D 2.01 (1.15 0.49 (0.16) W 3.41 (1.56) D 1.92 (0.83)	

Methods differed in some experimental conditions; symbols indicate: W = extraction using "wet" brick directly from sea water; D = extraction from dry brick, air-dried 48 hours before extraction; M = mixture of methods, part wet-part dry, means indicate total amount of material extracted (bricks/mean).

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Table 32. Total Hydrocarbons (Grams/Brick) Extracted From Top Surface of Hard Substrates. 1

EXPERIMENTAL CONDITIONS	Nov	TOT/		BONS (GRAMS/ ember		anuary
	MEAN	(S.D.)	MEAN	(S.D.)	MEAN	(S.D.)
Preliminary						
Post Treatment	0.82	(0.33)	1.04	(0.47)	1.60	(0.51)
<u>Five-Day</u>						
MLLW	0.49	(0.10)	0.07	(0.05)	0.08	(0.05)
+2'	0.89	(0.72)	0.07	(0.03)	0.32	(0.60)
<u>Thirty-Day</u>						
MLLW	0.03	(0.02)	-		0.39	(0.18)
+21	0.07	(0.03)	_		0.32	(0.12)

 $^{^{1}}$ All extractions from top surface made using "wet" bricks (N = 5 bricks/mean).

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