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UNIVERSITY OF WASHINGTON
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DEVELOPMENT OF A SCUBA-BASED PROTOCOL FOR THE RAPID EVALUATION OF BENTHIC DEGRADATION DUE TO ORGANIC ACCUMULATION IN THE NEARSHORE SOFT-BOTTOM HABITAT OF PUGET SOUND

Susan A. Miller, Bruce S. Miller, Greg Jensen, and Harry B. Hill

Prepared for:

US Environmental Protection Agency
Region 10, Office of Puget Sound
Seattle, Washington

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Director

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KEY WORDS

Index of Biotic Integrity, SCUBA, organic accumulation, benthic degradation, invertebrates

INTRODUCTION

The development of explicit and efficient regulatory guidelines is critical to the effective implementation of criteria evaluations for ocean discharge. In order to be effective in assessing thousands of discharges, environmental assessment and monitoring of aquatic environments receiving point source discharges must (1) provide evaluative, interpretive and predictive information to decision-makers, (2) focus upon and measure only those parameters necessary for accurate environmental impact assessment, (3) generally follow standard procedures or protocols across ecoregions, (4) require moderate, cost-effective methods of monitoring accessible to small as well as large dischargers, and (5) provide results in a timely manner (preferably within 1 mo of monitoring).

Tiered or hierarchical environmental impact monitoring and assessment using SCUBA techniques potentially can meet the above requirements. Trained biologists utilizing SCUBA are able to combine direct observation with precise sampling to allow a staged, hierarchical assessment of measurements proceeding from observations of the epibenthic environment to sediment physiochemistry of infaunal community structure and functions. Monitoring programs can facilitate collection of all three sets of samples, yet enable analysis of these samples sequentially on an as-needed basis.

Direct diver observations would serve as the first monitoring tier, with observations of such parameters as sediment conditions, marine macrophytes, macroinvertebrates, and fishes providing an informational basis for classifying a site as undegraded, measurably degraded, or significantly degraded. Sites characterized as measurably or significantly degraded would be further assessed in a second tier of monitoring with the use of sediment samples to provide additional physiochemical and biological data.

Central to the application of hierarchical SCUBA-based environmental monitoring is the development of rigorous, quantitative protocols for the evaluation of impacts to the marine epibenthos. As with any scientific assessment technique, data must be collected and analyzed by knowledgeable individuals using familiar and appropriate methods. Assessment of data must produce results that are firmly grounded in an understanding of the ecological structure and processes of the system under evaluation.

In fact, environmental monitoring has received substantial consideration during the last decade. A number of articles, books, proceedings, and documents have addressed the design and implementation of effective and efficient monitoring programs (e.g., Cairns et al. 1977, Green 1979, Sheehan 1984, Karr et al. 1986, US Environmental Protection Agency 1988a, 1988b, 1989, 1990a, 1990b). Numerous conferences, symposia, and workshops have been conducted to consolidate our understanding and application of proper assessment and monitoring techniques (e.g., National Oceanic & Atmospheric Administration 1982).

In the assessment of the biological impacts of pollutant discharges to marine water, benthic macroinvertebrates are frequently studied (e.g., Gray 1979, Pearson et al. 1983, Bilyard 1987). Holistic approaches and indices that capture the response to stress of populations and communities are highly valued and applied widely (Cairns et al. 1977, McIntyre et al. 1984, Miller 1984, Sheehan 1984, Karr 1987). Particular value is attached to the identification of appropriate, simple, reliable, and robust indicators of marine pollution. In addition, identifying suitable reference stations for comparison with impacted sites is important; such regional "controls" can be established according to ecotypes within bioregions (Hughes and Larsen 1988).

The Index of Biotic Integrity (IBI) represents both a conceptual framework and a useful tool for biological monitoring (Karr et al. 1986). It is founded on the basis and need for evaluation of biological integrity generally conceived by biologists as "the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitat in the region" (Karr and Dudley 1981). The original IBI was designed to include a range of attributes of fish assemblages from three categories: Species richness and composition, trophic composition, and abundance and condition. Development of an appropriate IBI is a three-step process, the first of which is the identification of potential metrics that reflect biological attributes of the community and its response to changes in the degree of impact. These hypothesized metrics are then tested on sites with a wide range of impacts. Those metrics that can reliably measure changes in the community from a particular impact are then incorporated into a model IBI, which is then tested for its ability to measure the relative effects of impact on an independent set of sites.

We believe a marine IBI can provide a robust measure of ecological integrity that can be applied to sites of public and regulatory interest. If metrics can be identified that characterize the biotic integrity of the epibenthic communities/habitats, and an appropriate technique developed for measuring metrics, IBI values for such communities/habitats could be classified as undegraded, measurably degraded, and significantly degraded.

OBJECTIVES

The objectives of the present study are as follows:

- Identify metrics of the soft-bottom, 10- to 30-m depth marine habitat, that are potential indicators of the biological integrity of the epibenthos.
- Develop SCUBA-based underwater recording techniques for documenting these metrics of the soft-bottom, 10- to 30-m depth marine habitat.
- Evaluate an accurate and inexpensive SCUBA-based visual protocol for assessing the environmental impacts of organic deposition to the epibenthic community in soft-bottom marine waters 10 to 30 m deep.

Relevant Background Information

Underwater Recording Techniques

Many methods of rapid underwater recording have been developed. Hiscock (1987) describes audio recording, underwater photography, and video as reliable methods for underwater surveying. Currently, advances in low-light underwater video systems have increased their reliability and accuracy. Studies by Bergstedt and Anderson (1990) show that an unmanned underwater video recorder yielded acceptable estimates of a concentration of known objects along the bottom of Lake Huron. The author suggests that this method, with the use of line transects and a towed sled, would give acceptable estimates of the densities of objects on a lake bed.

Greene and Alevizon (1989) found that audio records of diver observations yielded more accurate data, and methods that use the human eye to identify species were more accurate than the methods utilizing video. Byers (1977) and Hiscock (1987) both have diagrams of audio recorders that were found useful in recording data underwater.

The use of photography for qualitative sampling of hard bottoms is well established (Bohnsack 1979). Hiscock (1987) showed that taking photographs of 22 contiguous quadrats of 0.03 m^2 and conducting a visual descriptive survey of the epibenthic macroinvertebrates in the same area both took about the same time to survey (5 h). Photographs recorded 29 taxa compared with 39 recorded by the descriptive visual survey, and 161 taxa were recorded by quantitative sampling of seven 0.1 m^2 quadrats, although this latter method required 35 d for sampling and identification. Photographs made percentage cover calculations easy, but excluded several large, widely distributed species. Photography is quick and offers a permanent record of the area sampled; however, it is limited when water turbidity is high or if species are large and well dispersed.

In a recent paper, Leonard and Clark (1993) compared point quadrat and video transect estimates of the cover of benthic red algae and concluded that video transects were inadequate for their purposes, primarily because of poor resolution and the extensive time needed for the lab analysis of the tapes. Another disadvantage given for video transects was an inability to sample layers of algae. Advantages for using videos were rapid data acquisition, cost effectiveness in situations where diver time is expensive, longevity of records, provision of qualitative site overview and perhaps quantitative sampling of some large organisms, and maximum data acquisition with limited dive time.

IBI (Metrics)

The Index of Biotic Integrity (IBI, Karr et al. 1986) can integrate information from individual, population, community, zoogeographical, and ecosystem levels into a single ecologically based index of the quality of a water resource. IBI is responsive to human-induced ecological impacts on energy source, water quality, habitat quality, flow regime, and biotic interactions. It relies on multiparameters and incorporates professional judgment in a systematic and sound manner, setting quantitative criteria that enable us to determine which habitat is "healthy" and which is "unhealthy."

For example, Karr and Kerans (1991) recently sought to develop an IBI for the Tennessee Valley Authority utilizing aquatic invertebrates. Metrics under consideration in this project included the following:

1. total taxa richness,
2. taxa richness of intolerant snails and mussels,

3. taxa richness of mayflies,
4. taxa richness of caddis flies,
5. percent of individuals as opportunistic *Cobicula* clams,
6. percent of individuals as oligochaetes,
7. percent of individuals as the two most abundant taxa,
8. percent of individuals as omnivores and scavengers,
9. percent of individuals as strict predators, and
10. total abundance.

Thus the IBI is a composite index that integrates attributes of communities, populations and individual organisms to assess biological integrity on the basis of accurate measures of relative abundance (Fausch et al. 1990). Its main advantages are that it is a broadly based ecological index, it is sensitive to different sources of degradation, and it produces biologically meaningful and reproducible results when applied by competent biologists. Its disadvantages are that its application requires at least moderate species richness and comprehensive historical background information, and that methods for setting some criteria are subjective. It also must be modified for different ecological regions, habitats and taxa, but modifications so far have retained the original ecological framework (e.g. Berkman et al. 1986, Miller et al. 1988).

Environmental Impact Assessments of Organic Deposition

Taub (1987) provides a list of "Trends Expected in Stressed Ecosystems." Changes in community structure that can be expected in a stressed system include an increase in the number of r-strategists, a decrease in the size of organisms, and a decrease in species diversity. The Pearson-Rosenberg Model (Pearson and Rosenberg 1978, Fig. 1) shows that along a gradient of increasing organic enrichment, total abundance increases until the level of pollution is too high, and that the number of species increases slightly in the "enriched" zone and then decreases steadily. These changes in the environment toward an increasing enrichment source may be visibly quantifiable, and as such a measure of the enrichment degradation, although the Pearson-Rosenberg Model is not considered generally reliable (Dr. James R. Karr, Univ. Washington Inst. Environmental Studies, pers. comm.). However, Mahnken (1993) indicates that in the literature there are many examples of studies that seem to verify the general nature of the Pearson-Rosenberg Model, and

> . . . demonstrated that macrobenthic communities respond in a similar manner to either natural or anthropogenic sources of enrichment whether the source is mariculture, domestic sewage, kraft millwastes, log dumping, seafood processing or natural cyclic changes in benthic enrichment (Thiel 1978, Gray 1979, Rhoads and Boger 1982, Swartz et al. 1985, Brown et al. 1987, Weston 1990).

Commercial salmon net farms can provide a classic example of organic deposition and have the additional advantage of having few if any associated toxics, unlike a sewage outfall or commercial outfall. These pen sites deposit organics on the benthos in the form of fish food both as uneaten pellets and feces. Weston (1990) surveyed infaunal macroinvertebrates around salmon net pens in Puget Sound, Washington, and found there was decreasing species richness with

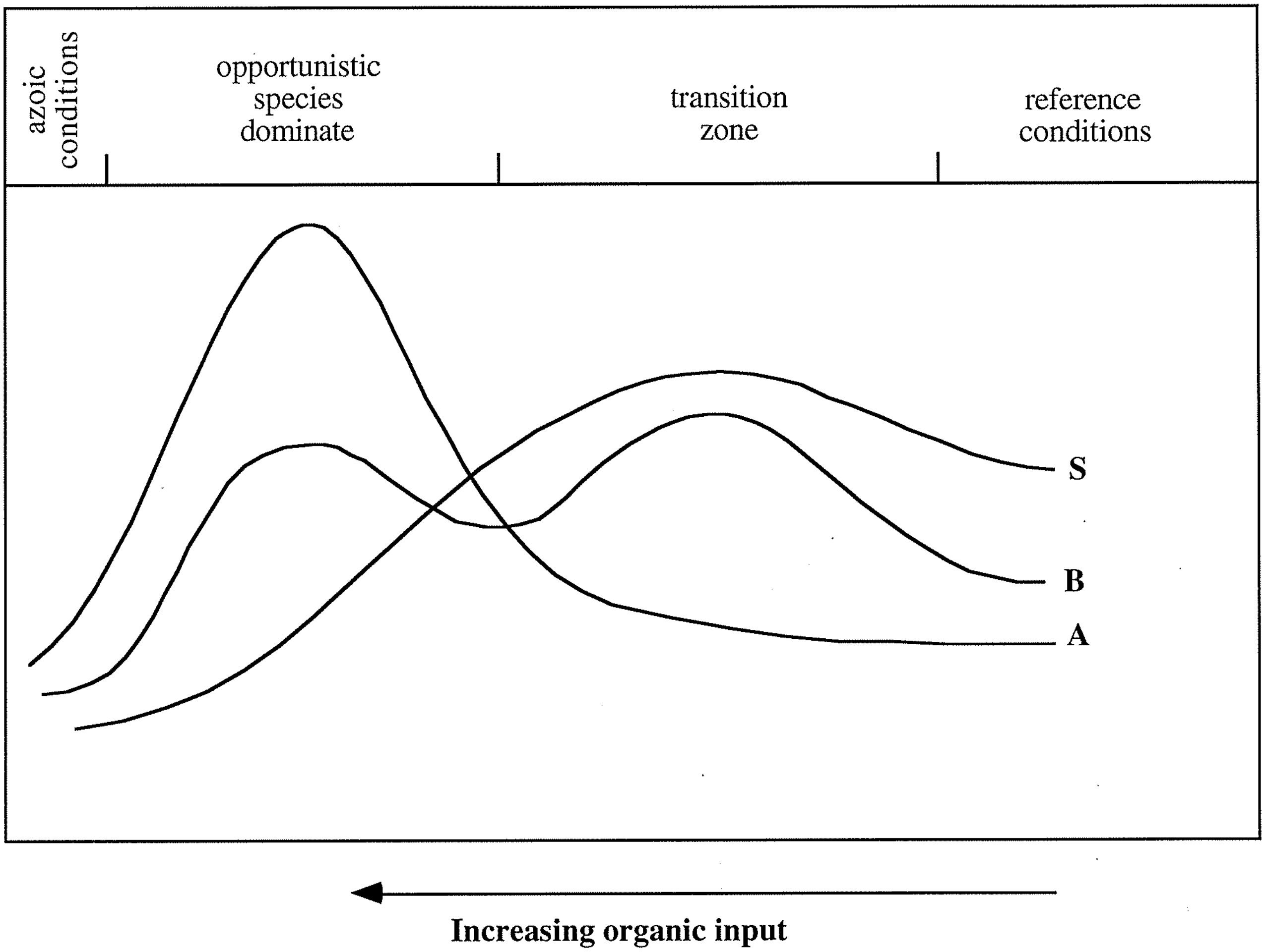


Figure 1. Expected trends along an organic enrichment gradient for number of species (S), biomass (B), and total macroinfaunal abundance (A) of a typical infaunal community. From Pearson and Rosenberg (1978).

increasing proximity to a source of organic deposition, similar to what is predicted by the Pearson-Rosenberg Model. In addition, an increase in the total number of individuals and a significant decrease in specimen size, with the loss of large bivalves, was recorded nearest to the source of organic deposition. Total community biomass decreased with increased proximity to the source of the deposition.

There appears to be only one long-term study of the impact of fish farming on the macrobenthos, namely the study by Tsutsumi et al. (1991) on the effect of sea bream (*Pagrus major*) farming on Tamoe Bay, Kyushu, Japan. This study began in 1966 and is continuing to this day, and has demonstrated dramatic changes in community structure and reduced abundance of benthic organisms as organic enrichment increased. Five years after the fish farm was established, the mollusc assemblage, which accounted for 70–98% of the abundance of the benthic community, was reduced to one species and replaced as the dominant by a polychaete assemblage. Twenty years later, polychaetes totally dominated (*Capitella capitata* densities exceeded

10,000 m^{-2}), and the entire cove was generally polluted from the farm, which resulted in oxygen depletion in the bottom waters and temporary defaunation in the summer. The timing of the changes in the macrobenthic communities and the organic pollution progression follow the Pearson-Rosenberg Model.

Recent work by Mahnken (1993) also generally reports results consistent with the Pearson-Rosenberg Model for organic enrichment. However, Mahnken's study is the only research that has followed the recovery of the benthos under a fish farm over an extended period of time (~2 yr) after the fish farm was removed. Pamatmat et al. (1973) had previously demonstrated that oxygen consumption under net pens may be extremely high but can return to normal 2 mo after the removal of the pens. Mahnken's (1993) results demonstrated that there was a dramatic increase in the number and abundance of species within the first 2.5 mo after removal of the net cages, and that subsequently the increase was slower but consistent. Although the abundance and richness of clam species fully recovered at the net pen site by the end of about 2 yr, for all other species the richness at the net pen site never reached the values for the reference site because rare species failed to reestablish. Mahnken was able to classify the recovery stages into four successional seres.

A rapid technique using SCUBA to evaluate the structure of undegraded subtidal macroinvertebrate communities (Himmelman 1991) was successful in identifying four major zonation patterns in the Gulf of St. Lawrence. Data collected on each dive consisted of substratum type, a ranked abundance for algae and non-predatory invertebrates, size of the large predatory species, and notes of any predation including size of both prey and predator. The distributional pattern generally corresponded to previously reported data for the area and was well described by Himmelman's SCUBA method. Himmelman found that this rapid technique was adequate to sample large invertebrates and discern differences in tier size structure.

METHODS AND MATERIALS

Indicator Metrics

Information was gathered on large, benthic organisms visible on the nearshore sea floor of Puget Sound, Washington. Marine benthic macroinvertebrates were considered as potential metric indicators of environmental integrity. Each of these indicators was evaluated based on whether they were (a) easily and accurately observed and identified, (b) common to Puget Sound and (at least seasonally) reliably present (or absent) at the undegraded (reference) site, and (c) indicative of degraded conditions.

Data characterizing the nearshore subtidal soft bottom habitat in Puget Sound are generally limited to samples from dredges, grabs, and core samples (Weston 1990). For this study we were interested in large invertebrates and fish species that for the most part are not found in grab samples, or if they are present, show an extremely low abundance. The authors' own knowledge, and personal interviews with Puget Sound biologists (C. Mahnken, National Marine Fisheries Service, Manchester, WA; J. Word, Battelle Marine Science Lab, Sequim, WA; R. Shimek, P.

Dinnel, Dinnel Marine Research, Kirkland, WA; R. Thom, Battelle Marine Science Lab, Sequim, WA; J. Armstrong, Environmental Protection Agency, Seattle, WA; J. Rensel, School of Fisheries; R. Anderson, Seattle Aquarium, Seattle, WA) familiar with the soft-bottom habitat were used in order to incorporate as many metrics in the general indicator list as possible. Additionally, several references of subtidal invertebrates and fishes (Hart 1973, Gotshall and Laurent 1979, Wingert and Miller 1979, Miller and Borton 1980, Kozloff 1987, Dethier 1990) were used. Those species and other metrics that occurred outside the geographical range, depth range, or habitat type, as well as those for which small size would make reliable surveys difficult, were eliminated from the list.

Testing of Underwater Recording Techniques

Several SCUBA techniques to evaluate soft bottom benthic condition were investigated using visual observations in conjunction with video, audio, and manual recording techniques. On the basis of discussions with the Washington State Department of Fisheries personnel familiar with the use of video censusing methods, and examples of the resolution possible from several currently available video systems, we discarded the use of video as neither readily available nor feasible as a censusing tool for benthic macroinvertebrates. To evaluate the potential for oral recording of dive observations, two dives were made using a full-face AGA mask with a microphone and tape recorder located within a housing attached to the divers tank.

Visual observations were greatly facilitated by the use of a Metrics Underwater Transecting Tool (MUTT). This device was designed to allow visual transects of a known area with a minimal amount of disturbance. The MUTT is inexpensively constructed using PVC pipe and plastic lids from 5-gal buckets (Fig. 2). Underwater, it has slightly negative buoyancy and is highly maneuverable so that it can be easily moved over obstructions such as debris piles, mooring cables, or pilings. The length of each transect can be varied up to 100 m or longer if desired. Data can be transcribed by individual sections along the total length in ≥5-m increments. The MUTT allows a dive team to survey two parallel transects, with the central spool and end wheels providing clearly visible reference points for maintaining a consistent survey width (Fig. 3a), which can be varied from 0.25–2 m on either side. For our survey, we designed paired transects of 1 m by 50 m with transcriptions every 5 m.

The MUTT gives a distinct visual reference for the edges of the transect, making it very clear which organisms fall inside or outside of the survey. Visual observations of all benthic macroinvertebrates seen, as well as any other relevant information, were recorded onto slates attached to the MUTT on either side of the center spool (Fig. 3b).

Survey dives were made over depths ranging from 10 to 30 m. The 10- and 20-m dives were well within the no-decompression diving limit for the average SCUBA diver. Times for each transect were recorded for use in calculating sampling area covered/dive. Due to the limits of no-decompression diving and in order to have an adequate amount of dive time at the 30-m depth to complete a 50-m total transect length, a NITROX mix was used in the tanks for the bulk of the dives, increasing the dive time available.

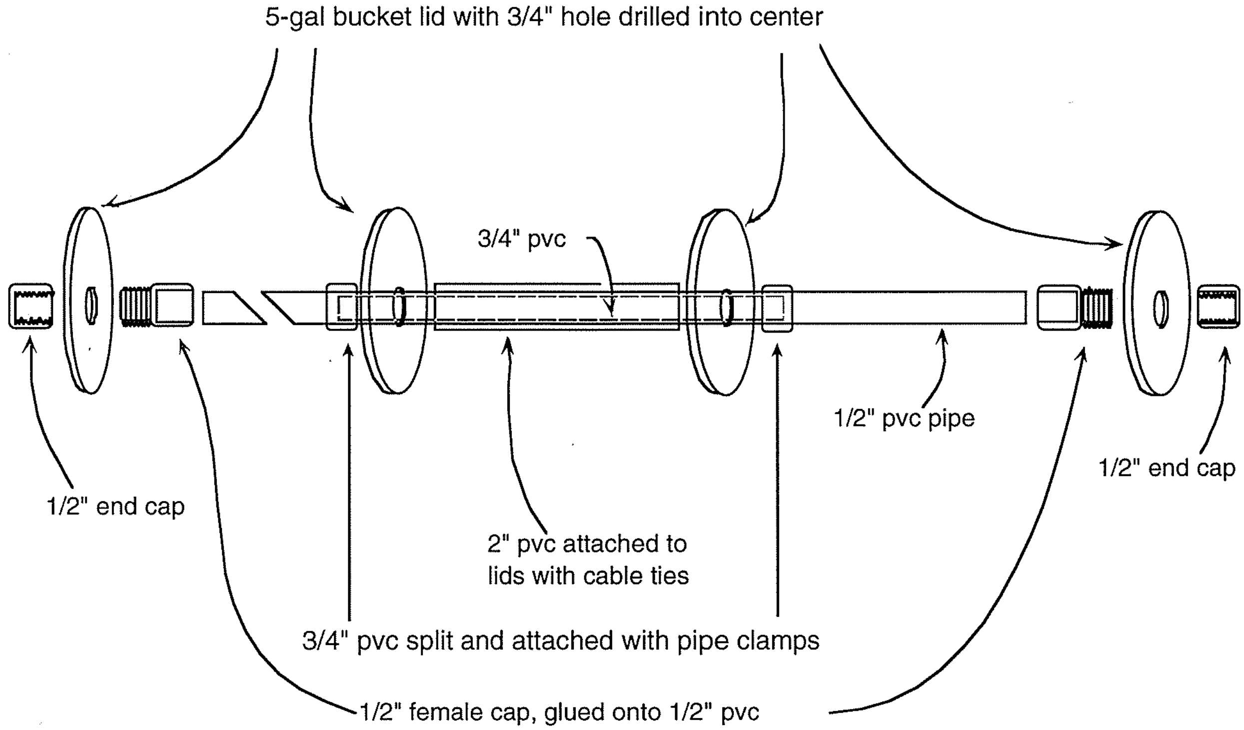


Figure 2. Metrics Underwater Transect Tool (MUTT). Length of between-wheel segments is 1 m. A transect line of any length can be attached to the center reel. All wheels, including the center reel, are free spinning. Total length is 2.25 m; the device is negatively buoyant.

Field Research Site Locations

A total of 71 dives were made at 10 locations within Puget Sound. University of Washington divers provided data for 54 of these dives; data for the remaining dives at one site were provided by the Environmental Protection Agency's (Region 10) dive team. Three locations were used as reference stations for this project: Alki Point, Seattle; Fay Bainbridge State Park, Bainbridge Island; and Picnic Point, Everett ; in addition, there were two test locations at commercial salmon net-pen facilities: Global Aqua at Clam Bay, and Birding Seafoods at Port Townsend (Fig. 4). Dives at the remaining five locations (Fort Ward State Park on Bainbridge Island, Seahurst and Golden Gardens in Seattle, the National Marine Fisheries Service experimental salmon net-pens at Manchester, and Scenic Beach in Hood Canal) were used for gear experiments or were under consideration for use as potential reference sites.

Reference Station 1—Alki Point. This long, sandy, northward-facing beach is fairly level throughout the intertidal zone and shallow subtidal. Once below the relatively narrow eelgrass bed, the slope increases dramatically, but the sediment composition remains unchanged. Alki Point served as an excellent site for many of the experiments in this project, because of its proximity to the University of Washington and the US Environmental Protection Agency's Region 10 offices. The sandy bottom and faunal distributions, as well as easy shore access, all added to the attractiveness of Alki as a reference and experimental site. Dives were made at depths from 10 to 30 m from December 1992 through July 1993.

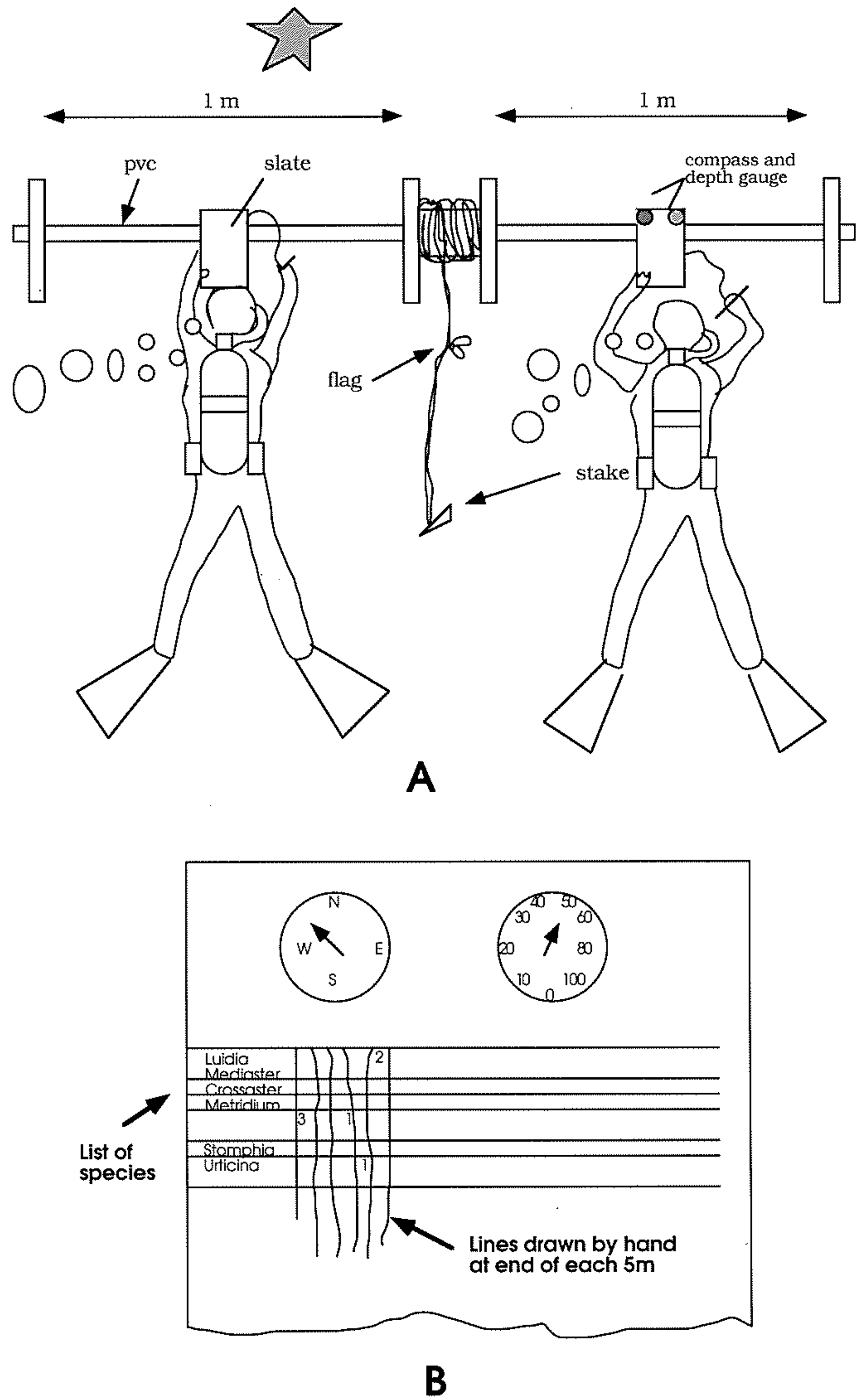


Figure 3. A. Metrics Underwater Transect Tool (MUTT) in use. B. Layout of slate.

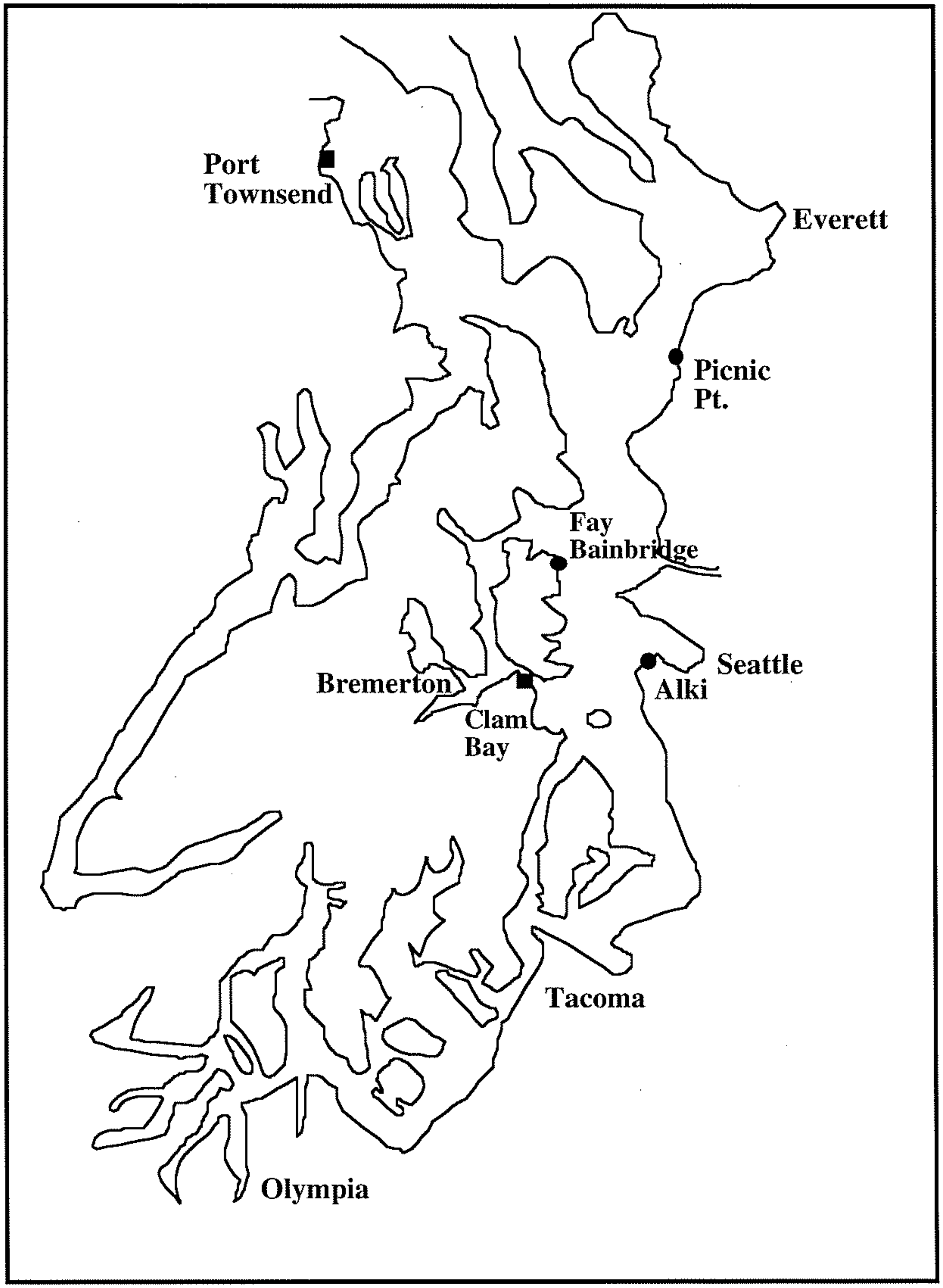


Figure 4. Locations of reference sites (●) and net pen sites (■).

Reference Station 2—Fay Bainbridge State Park. The subtidal transects at Fay Bainbridge State Park were noticeably siltier than the other two sites and generally had more woody debris. The fairly level sand beach has only a few small patches of eelgrass, then drops off suddenly to about 10 m before resuming a gentle slope. Located on the northeastern side of Bainbridge Island, Fay Bainbridge State Park has a faunal assemblage similar to Alki Point. Reference site dives were made here at depths from 10 to 20 m from April to June of 1993.

Reference Station 3—Picnic Point. This site has the greatest range of substrate types of the three reference areas. The intertidal area is very large and composed of a mixture of sand and cobble, and in front of the access the eelgrass beds are fairly small and patchy. At 10 m the substrate is sandy and appears to be well-worked by burrowing shrimp, with virtually no macroinvertebrates visible. From 15 to 20 m the bottom ranges from fairly pure sand to gravel mixed with sand. Reference site dives were made at Picnic Point at the 20-m depth only; no transects were made at the 10 m depth because of the abundance of burrowing shrimp and lack of macroinvertebrates. Dives were made at Picnic Point from June to July of 1993.

Net Pen Test Facility, Global Aqua, Clam Bay. Global Aqua operates two salmon net-pen facilities in Puget Sound: One is located near Orchard Rocks on the south end of Bainbridge Island, and the other is in Clam Bay, across Rich Passage from Orchard Rocks (Fig. 4). The Clam Bay facility historically produced 620 metric tons of salmon annually, with an estimated annual fecal and waste feed deposition of 250 t yr^{-1} (Weston 1990); however, the current facility is considerably smaller (Fig. 5) than the pen configuration for which Weston's estimates were originally calculated.

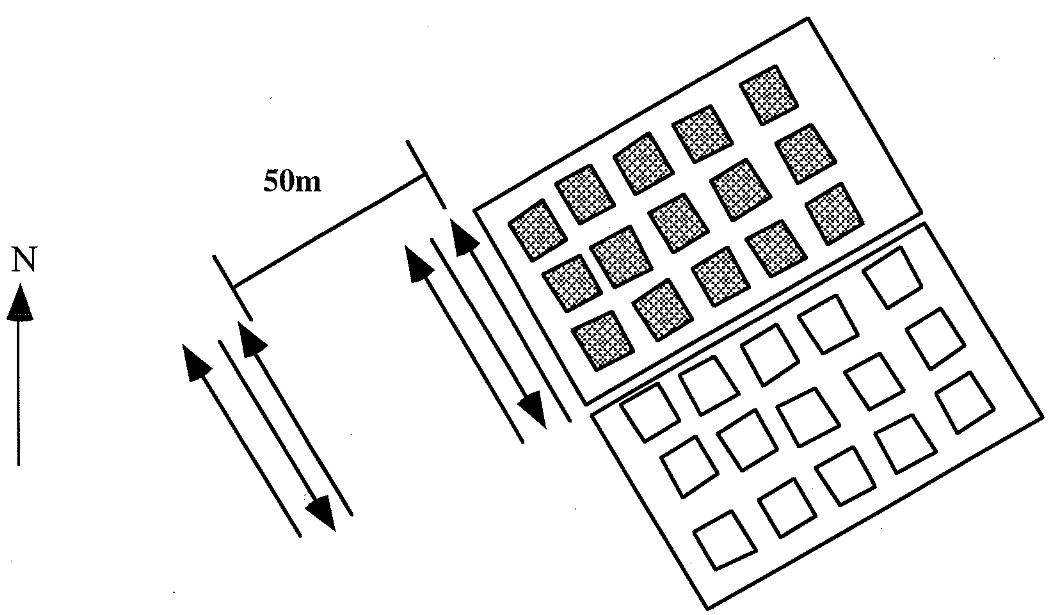


Figure 5. Global Aqua, aquaculture facility in Clam Bay, Washington. Depth on the transects was 20 m; the pen blocks were approximately 50 m X 75 m.

Net Pen Test Facility, Birding Seafoods, Port Townsend. Birding Seafoods recently installed two net-pens at the same Port Townsend location (Fig. 4) that was previously occupied by Paradise Bay Co. (using the same mooring buoys and cables). One block of pens became operational in January and the other in June 1993. University of Washington divers dove on the inland (western) edge of the more recently installed pen (Fig. 6), which is located in 10–20 m depths. The sediment at this facility was significantly different than that at the other sites, comprising a 99% silt/clay fraction. This facility is also notable for being located close to a paper-pulp mill.

Sampling Methods

Sediment Sampling. Sediment samples were taken from three randomly selected locations along transects at each reference and impact study site. Plastic jars (~120 cc) were used to scoop the top 6–8 cm of sediment and were tightly sealed, taking care not to lose any fine, flocculent material on the surface.

Samples were spread out in aluminum pie tins and placed in a drying oven at 50°C for 24 h, then shaken through a graded (0.063–2 mm) series of seven sieves for 15 min on a mechanical shaker. The contents of each sieve were placed into tared containers and weighed to the nearest 0.01 g on an electrobalence.

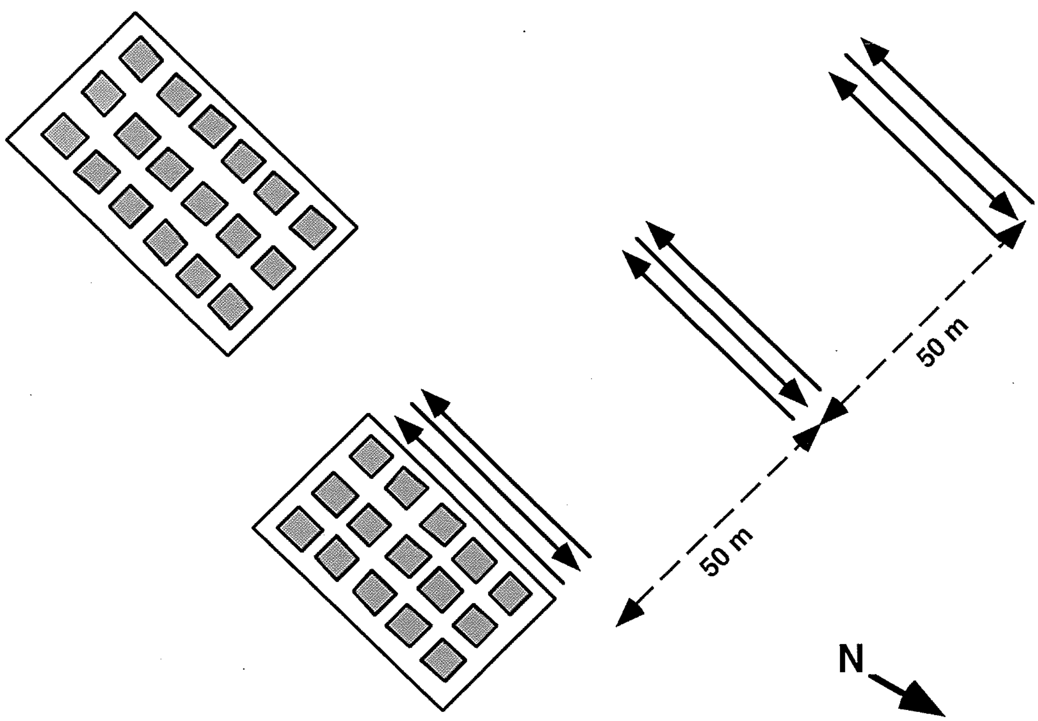


Figure 6. Birding Seafoods' aquaculture facility in Pt. Townsend, Washington. Transects at 17- to 9-m depths were run at distances of 0, 50 and 100 m from the edge of the northernmost pen block. Pen blocks were approximately 50 m X 75 m in length.

<u>Reference Site Sampling</u>. Transects were performed using the MUTT at depths from 10 to 30 m at the three reference stations using either a 1- or 2-m-wide strip transect method (Table 1). Observations collected utilizing the 2-m-wide transects were summed for each diver over the 50 m length (5-m sections were not recorded). In addition to the species data collected on these transects, information was recorded concerning height of tide, currents, and any unusual observations/occurrences for the dive.

All species seen were recorded on the diver's slate, except in the case of organisms considered too small (<2 cm) to be accurately sampled on a day with average visibility. Fish species were initially recorded but were determined to be attracted to the MUTT and therefore inaccurately censused utilizing this protocol. In cases where species were too numerous to accurately measure, counts were made up to 25 per 5 m^2 and recorded as >25. Sediment samples were taken at three randomly determined points along the first transect at each depth and site.

<u>Net Pen Site Sampling</u>. Three adjacent, 50-m transects were made at each site at distances of 0 and 50 m from the net-pen blocks, with an additional set of three transects at the 100-m distance at the Port Townsend site. Transects were started immediately adjacent to the pens and alternated in direction (Figs. 5 and 6). Sediment samples were taken at three randomly determined points within the first 50-m transect. In addition to species data, information was collected on the presence/absence of food pellets, *Beggiotoa* bacterial mats, and any pen-associated debris such as net piles, cables, etc.; data on these measures were recorded as presence/absence for each 5 m segment on each transect.

Methods of Data Analysis

<u>Sample Size</u>. To determine the sample size needed to accurately record at least 90% of the species present in a location, cumulative species totals for all transects were plotted against cumulative area sampled for the 10- and 20-m depths at Site 1 and 2.

<u>Interdiver Variability</u>. Interdiver variability was determined by having two divers simultaneously record their observation of the same 1-m X 50-m transects (in 5-m increments) for a total of 600 m^2. Paired Student's t-tests for means ($\alpha = 0.10$) and associated power calculations were performed on the individual counts of each species, species groups, and measures of species richness and total abundance for all transects. Shannon Diversity indices were also calculated and comparisons calculated on these diversities were made by using Hutcheson's t-test for equal diversities (Zar 1984).

Table 1. Depth, width, and number of 100-m^2 transects made at each of the references sites.

Width		2 m		1 m		
Depth		10 m	20 m	10 m	20 m	30 m
Study Site 1	Alki			11	11	10
Study Site 2	Bainbridge	6	6	10	10	
Study Site 3	Picnic Point				6	

Transect Width Comparisons. To determine if the data collected using both the 2-m-wide and 1-m-wide transects were comparable, we halved the counts for each species seen in the 2-m-wide transects, used a t-test to determine equal means, and then tested the data against that collected using 1-m transects for the same site and depth. In addition, the average abundance per 100 m^2 of each organisms was calculated and compared for each transect width (same site and depth).

Auto-correlation Tests. Auto-correlation tests (Neter et al. 1989) were performed on all species for which 1 m wide transect data was available for at least 20 continuous 5 m segments. Observations were pooled for both observers and autocorrelation function coefficients (acf) between segments up to $\sqrt{n+10}$ segments apart (n = number of observations, critical values = $2/\sqrt{n}$), were calculated. If no significant correlation was found at any distance between segments for all replicates for a species, site, and depth, segments were treated as independent. If any significant correlation existed between segments for a species, site, and depth for any replicate, then all samples were treated as non-independent for the largest number of significant lags in all the replicates.

Population Abundance. For species with no significant correlation at any lag, or where auto-correlation tests could not be run, abundance was calculated as the mean for all observations. Where the smallest area sampled was 100 m^2 (2-m-wide transects without 5-m sections), means and confidence intervals were calculated per 100 m^2. All other data were calculated per 5 m^2 and then standardized to 100 m^2 for comparison.

For all species that could not be considered independent for every 5 m section, only every n^{th} sample was used to calculate means and confidence intervals (where n = number of significant lags +1). The starting points for these subsamples were randomly assigned to the first or second data point.

Community Diversity. Both the Shannon diversity index for random samples and the Brillouin diversity index for non-random samples were calculated for all sites and depths. These diversities were converted to relative diversities and compared using Hutcheson's t-test for equal diversities (Zar 1984).

RESULTS

Individual Species as Possible Indicator Metrics

Rarer Species

The following species were encountered in <10% of reference station transects:

Nanaimo dorid, *Acanthodoris nanaimoensis*
Snow white dorid, *Archidoris odhneri*
Clown nudibranch, *Triopha catalinae*
Brachiopod, *Terabratalia transversa*

Scallop, *Chlamys* spp.
Octopus, *Octopus rubescens*
Oregon triton, *Fusitriton oregonensis*

Cryptic Species

Some species were frequently encountered but were either too cryptic or difficult to count accurately. The decorator crab *Oregonia gracilis* was very common at some sites, but its heavy camouflage of algae and other material made it very difficult to see unless it was moving. The dock shrimp *Pandalus danae* was exceptionally abundant in some areas, but actual numbers were hard to assess when groups milled about or moved along in front of the MUTT.

Potential Species Metrics

Potential metrics for use in a marine IBI can be found in Table 2. A species identification guide with underwater photos of metrics and a summary of significant characteristics was provided to the Environmental Protection Agency in Seattle as a separate item to this report. Additional information concerning the life histories and descriptions of these species can be found in the many guides available (e.g., Gotshall and Laurent 1979, Kozloff 1987). Other potential metrics are referred to in the Discussion section of this report.

Development and Validation of an Underwater Transect Protocol

Recording Technique

Use of the AGA full-face mask and underwater tape recording system was limited to two dives, at which time it was determined that no further testing was necessary. During the first dive at 10 m, a leak developed in the underwater housing, resulting in the loss of the tape recorder and the recorded information. The second dive was made without the recorder to test the new housing seal. On this dive it was determined that the use of the full-face mask (to operate the underwater recorder) required a significantly greater amount of air such that it severely limited the amount of dive time available. For reasons of time available per dive, associated safety concerns, and a general feeling that this equipment was not reliable or readily available for use by the average monitoring diver, subsequent surveys were limited to slate recordings and regular SCUBA equipment.

We also considered using an underwater video camera for permanent visual recordings of transects. However, reports from colleagues (primarily from the Washington State Department of Fisheries) indicated that the video resolution currently available was inadequate for our purposes. Additionally, our own testing (performed on a separately funded project) supported the finding that the resolution from video cameras would not allow accurate identification of the species we needed to survey.

The Metric Underwater Transect Tool (MUTT) proved reliable and easy to use, and provided all of the results which follow.

Table 2. Potential metrics for use in a marine IBI.

NUDIBRANCHS	
Striped nudibranch (*Armina californica*)	This distinctive nudibranch was nearly ubiquitous in 10- and 20- m transects at Alki and Fay Bainbridge State Park but was not found at Picnic Point, where its sole prey, sea pens, were also absent. Within a transect its distribution tended to be clumped, so adjacent 5-m sections should not be considered as independent samples for this species. Specimens occasionally bury, leaving only the anterior and posterior exposed, but their large size and unmistakable markings generally make them easy to count. This species is always associated with *Ptilosarcus gurneyi*, their sole prey.
Opalescent nudibranch (*Hermissenda crassicornis*)	Abundance of this species seems to vary seasonally more than other species. Specimens were abundant from February to April but absent in May and June; when the species reappeared in late July, all were much smaller than those seen earlier in the year. Seasonality would have to be taken into account if it were used as a metric.
White-lined dirona (*Dirona albolineata*)	This species was very infrequent in reference station transects and only recorded from Bainbridge and Picnic Point. This species also is abundant seasonally in some local areas, but may not be widespread.
Diomedes' triton (*Tritonia diomedea*)	This species was rare (4% of reference transects) and only seen at Bainbridge. However, it can be quite common seasonally in some areas.
SUMMARY	Nudibranchs seem highly seasonal and not highly abundant, with the exception of *Armina californica*, which depends on the presence of *P. gurneyi.*
BIVALVES	
SUMMARY	Separate counts were not kept for horse clams (*Tresus* spp.) and geoducks (*Panope abrupta*) because of the large numbers in each transect and the need to examine each siphon individually for an accurate species identification. Counts made of visible siphons and holes varied tremendously from one sampling period to another. Up to 50% of the geoducks in a bed can be buried without a trace at any one time (Washington Department of Fisheries, pers. comm.), and some of our transects suggest even higher proportions at times. Despite the difficulties of getting good quantitative abundance data on these species, they occurred in nearly all reference station transects and have been noted to be particularly sensitive to the presence of net pens (Mahnken 1993).

Table 2—cont.

SEA STARS	
Rose star (*Crossaster papposus*)	This seastar was recorded in 27% of the transects but was uncommon and typically observed as single individuals at the 20-m stations. It is fairly large and easily identified, but also relatively scarce.
Mottled star (*Evasterias troschelli*)	This large seastar was found in only 19% of the reference station transects. Although not abundant at any of the stations, this species (unlike *Crossaster*) is known to be exceptionally common in some areas. It generally prefers being on or near some sort of vertical relief such as pilings, so it may not be a useful metric species for soft bottoms.
Vermilion star (*Mediaster aequalis*)	Large specimens were common at Alki, but those at Picnic Point and especially Bainbridge were mainly juveniles. This species was easily recognized and generally abundant.
Spiny red star (*Hippasteria spines*)	This species was fairly common at Alki but was not recorded at the other two reference sites. Like the nudibranch, *A. californica*, it is wholly dependent on sea-pen beds.
Sunflower star (*Pycnopodia helianthoides*)	Sunflower stars are large and distinctive, and range over a wide variety of bottom types but generally avoid mud. Specimens were very infrequently found in our transects (6%).
Pink star (*Pisaster brevispinus*)	Pink stars were rarely found in our transects (4%), and although they can be fairly common they have not been observed in high densities on soft bottoms.
Sand star (*Luidia foliolata*)	This species can be quite common on sand or mud bottoms, but it was not particularly abundant at any of the reference sites, occurring in 23% of the transects. It is the only local seastar that buries, making it the easiest one to overlook.
Blood star (*Henricia* spp.)	*Henricia* was uncommon (17% of transects) and most of the specimens were fairly small. This seastar is usually associated with rockier bottoms.
Cushion star (*Pederast tessellates*)	Found only at Picnic Point, this species occurred in 33% of the transects. It was conspicuous and easily identified but was uncommon on soft bottoms.
Sun star (*Solaster stimpsoni*)	This seastar was only common at 20 m at Bainbridge, and not recorded from Alki. It is large and easily recognized, but usually occurs on rockier bottoms where its preferred prey (*Cucumaria*) is common.
Morning sun star (*Solaster damson*)	Recorded only from 20 m at Bainbridge and Picnic Point, this tertiary predator specializes on seastars and probably never occurs at high densities.
SUMMARY	Seastars are typically large and easily identified, although not generally abundant, and individual species of seastars were frequently observed at only one site. The total number of seastar species at a site, however, may be an indication of the relative health of a site.

Table 2—cont.

SEA CUCUMBERS	
California sea cucumber (*Parastichopus californicus*)	*Parastichopus* was found at all depths at Alki but not at the other two sites. Specimens found were large and abundant on soft substrates.
Cucumber (*Cucumaria piperata*)	A small cucumber found in huge aggregations at Picnic Point, it remains buried with only its tentacles visible. Small size, and dense clustering make counting difficult. It was not quantitatively sampled.
SUMMARY	Sea Cucumbers are highly visible but perhaps not easily quantifiable. Though not widespread at all sites, a presence/absence metric may be appropriate.
ANEMONES	
Plumose anemone (*Metridium* sp.)	This hardy anemone was present on virtually all transects but was usually associated with debris on the bottom or with other structures.
Swimming anemone (*Stomphia coccinea*)	Found at all three sites (31% of the transects), this small anemone was common only at Picnic Point. Like *Metridium* sp. it was attached to debris on the bottom, particularly small objects such as bottles.
Burrowing anemone (*Pachycerianthus fimbriatus*)	At reference sites, this anemone was found only at Alki at 20 m but was one of the most common species in the mud at Port Townsend. It can retract into the sediment but appears to do so only on rare occasions, and remains visible and countable.
Sand rose anemone (*Urticina columbiana*)	A large, obvious anemone found in all transects at Picnic Point and most 20-m transects at Bainbridge. It is not known to be common or widespread.
Snakelock anemone (*Cribrinopsis fernaldi*)	This species was recorded at 33% of the transects at Picnic Point; occasional specimens were seen outside transects at Alki. They are typically found in rocky areas, but are rare on soft bottoms.
SUMMARY	The sessile nature and size of sea anemones makes them easily identified, but many of the individual species were seen at only one site. Additionally, their tendency to be associated with debris may preclude them from use in a benthic IBI.
CRABS	
Blackeyed hermit (*Pagurus armatus*)	This was virtually the only species of hermit crab encountered in our transects, and it was found at all three reference sites. Many exceptionally large specimens were observed near the Global Aquaculture facility at Manchester. *Pagura* is large and fairly conspicuous inhabitant of open sand bottoms.

Table 2—cont.

CRABS—CONT.	
Dungeness crab (*Cancer magister*)	Dungeness crab were found in 21% of the reference station transects and were most common at Bainbridge. Buried specimens are easily overlooked, while partially buried ones will often run before they are sighted, leaving behind characteristic "smoking holes." This species lives at very high densities in some areas and may, like the following two species, be useful as part of a "Cancer guild" metric.
Graceful rock crab (*Cancer gracilis*)	Relatively uncommon in reference transects but extremely abundant on the *Beggiotoa* mats next to the Port Townsend net pens, this crab prefers muddier bottoms than *C. magister* and is much less tolerant of low salinities than is its larger relative.
Red rock crab (*Cancer productus*)	Although more characteristic of harder substrates, this species was slightly more common than either of the other two *Cancer* species in the reference transects.
SUMMARY	Crabs were highly mobile and relatively common. A "cancer guild" metric appears to have potential as an indicator of organic impact. Our sampling and observations at other sites throughout Puget Sound seem to indicate an inverse relationship between large *Pagurus armatus* and *Cancer* crabs. Large hermit crabs occur in areas with few or no *Cancer*, while only juvenile hermits are found in areas with large numbers of *Cancer*. Some possible hypotheses to explain this pattern include predation on hermits by *Cancer,* or an interaction between *Cancer* and moon snails, whose shells large *P. armatus* utilize exclusively.
MISCELLANEOUS	
Glassy sea squirt (*Ascidia paratropa*)	This was the only ascidian encountered on our transects that was both large enough and conspicuous enough to be accurately counted. It was recorded at Picnic Point and Alki but was extremely rare (total of only 8 specimens).
Sea pen (*Ptilosarcus gurneyi*)	Sea pens are a major component of the benthos at Alki and Bainbridge, supporting a number of specialist and generalist predators. At times it may fully retract into the sediment leaving little or no trace.
Moon snail (*Polinices lewisii*)	This large snail occurred in ~50% of the transects at Alki and 10% of them at Bainbridge; it was not recorded at Picnic Point but is exceptionally abundant in the intertidal there. *Polinices* are large and easily recognized, but more information is needed on the extent of subtidal populations.

Testing and Validation of the Transect Recording Technique at the Three Reference Stations

Interdiver Variability. Abundance and diversity measures, for all species, showed no significant difference between observers at the $\alpha = 0.10$ level (Fig. 7 and Appendix Table A.1). Post-dive interviews between observers determined that differences were primarily due to decreased mobility caused by two divers occupying the same transect and the hesitancy of the divers to examine organisms as closely as they might otherwise (so as not to bias the other diver's observations by picking up, or stopping to look at, an organism). Power calculations demonstrated that a sample size of 500 m^2 (50 5-m^2 segment pairs) provided >90% power to distinguish the differences found for all species except *Hippasterias* and *Pachycerianthus fimbriatus.* For these two species, where observations were limited to less than 5 individuals, power was still within an acceptable range (1-ß >0.75%).

Transect Width (1 m vs. 2 m). Data for the 2-m-wide transects were collected in April 1993, and all data for the 1-m-wide transects were collected in June 1993. Using the 2 m MUTT, we recorded seven species that were not observed during the 1-m transects, and two species recorded from 1-m transects were not seen in the 2-m transects. With the exception of one species of nudibranch (*Hermissenda*), all species not seen in the 2-m transects were represented by fewer than three individuals 600 m^{-2}. Results of Student t-tests of the data collected using the 1-m and 2-m MUTT surveys (Table 3), indicate that the average number of species seen in a 100-m^2

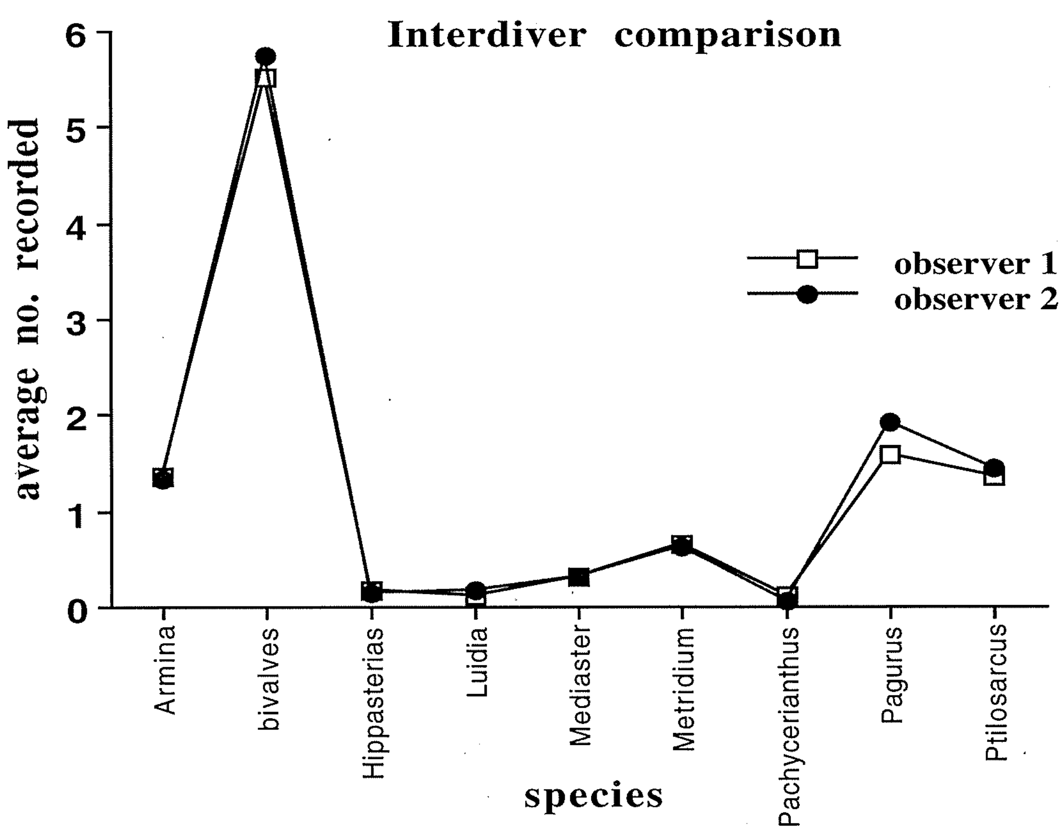


Figure 7. Interdiver variation at Alki Point, 10 m (500 m^2) for all species observed.

Table 3. Results of transect width comparisons for all species observed on either transect type (Fay Bainbridge State Park, 10-m depth). Student's t-test compares mean abundance estimates for each 100-m^2 transect (n = 6).

Species/measure	Observed on: 2 m trials	Observed on: 1 m trials	T-test results	Power
Armina californica	X	X	μ=μ	0.99
Bivalves	X	X	μ≠μ	
Cancer gracilis	X	X	μ≠μ	
C. magister	X	X	μ≠μ	
C. productus	X	X	μ≠μ	
Crossaster papposus	X			
Dirona albolineata	X			
Evasterias troschelli	X	X	μ=μ	0.97
Henricia spp.	X	X	μ=μ	0.32
Hermissenda crassicornis	X			
Mediaster aequalis	X			
Metridium sp.	X	X	μ=μ	0.99
Oregonia gracilis	X	X	μ=μ	0.52
Pagurus armatus	X	X	μ≠μ	
Pisaster brevispinus	X	X	μ=μ	0.97
Polinices lewisii	X	X	μ=μ	0.97
Ptilosarcus gurneyi	X	X	μ≠μ	
Pycnopodia helianthoides		X		
Solaster stimpsoni	X			
Scyra acutifrons	X			
Stomphia coccinea		X		
Tritonia diomedea	X	X	μ=μ	0.97
Urticina columbiana	X			
No. individuals	X	X	μ=μ	0.76
No. species	X	X	μ≠μ	

transect was significantly different between methods, although the number of individuals was not. Many species abundances were not different between the two transect types, but five crab species (all but *Oregonia gracilis*), bivalves, *Ptilosarcus gurneyi*, and the nudibranch *Hermissenda crassicornis* were different. *Hermissenda* were abundant for a few weeks, but were not seen after April at Fay Bainbridge State Park, so its difference is probably due to seasonal variations in abundances.

Autocorrelation Tests of Spatial Correlation for 5-m^2 Segments. Abundance of three species at Fay Bainbridge State Park was found to be correlated in adjacent 5-m^2 segments: bivalves, at three lags, acf = 0.490–0.826, critical value = 0.447; *Ptilosarcus gurneyi*, at two lags, acf = 0.611–0.510; and *Armina californica,* at one lag, acf = 0.691. Two species at Picnic Point were found to be correlated: *Hermissenda*, at one lag, acf = 0.431, critical value = 0.365, and *Stomphia*, at three lags, acf = 0.446–0.369. No other significant correlations were found for other stations or species.

Field Testing of the Protocol for the Rapid Evaluation of Organic Degradation

Sediment at All Sites

Sediments at the three reference sites were similar in composition (Fig. 8), consisting almost entirely of medium to very fine sand and confirming initial visual assessments. Sediment composition at the Global Aquaculture site mirrored that of the reference stations. Although statistical differences can be calculated between the various sites, medium and fine sands averaged over 88% of the dry weight of all the reference sites and at Global Aqua in Clam Bay. However, at Birding Seafoods in Port Townsend, virtually all material passed through the finest sieve, and the proportions of silt and clay within this fraction were not determined. Comparisons of sediment from 10, 20, and 30 m at Alki and 10 and 20 m at Bainbridge revealed essentially no change with depth (Fig. 9a, b), except for a tendency toward smaller grain size as depth increased.

Reference Stations

General Findings. A total of 45 species representing 36 families were seen at the three reference stations (Appendix Table A.2). These range from observations of one individual to totals of over 5,000 (bivalves) in all reference station transects combined. Of the 45 species, 58% were represented in 10% or fewer of the transects, 27% were >10% to ≤50%; and 7% were >50% to ≤75%. Four taxa were in more than 75% of the transects (*Armina californica* 77%, *Metridium spp.* 85%, *Pagurus armatus* 92%, and bivalves 96%).

Cumulative Species Curves. With the exception of the 10-m station at Alki, for which only 16 species were recorded, 20 to 24 species were recorded at each of the reference sites. Graphs of the cumulative species curves generated for each of the sites indicate that roughly 90% of the species at a given site will be encountered within the first 600 m^2 surveyed—six paired transects of 20 5-m^2 segments (Fig. 10a–c). An increase in areal survey coverage at Fay Bainbridge State Park from 644 m^2 to 1,520 m^2 (an additional six transects) accounted for only three additional species, all single individuals.

Average Abundances and Confidence Intervals. Calculated average abundances ranged from 0 to 798.6 100 m^{-2} (Appendix Table A.3). The highest calculated difference between the Shannon relative diversity value J' (random samples) and Brillouin's relative diversity value for J (non-random samples) was 0.003 for all sites and depths. This equates to a 1.5% or lower difference within the range of values (0.195–0.704) and was insignificant in the calculation of Hutcheson's t-test for differences between diversity indices.

Diversity measures for all the reference stations and sites were significantly different for all between-depth, and between-site comparisons (Table 4). Significant differences in the mean

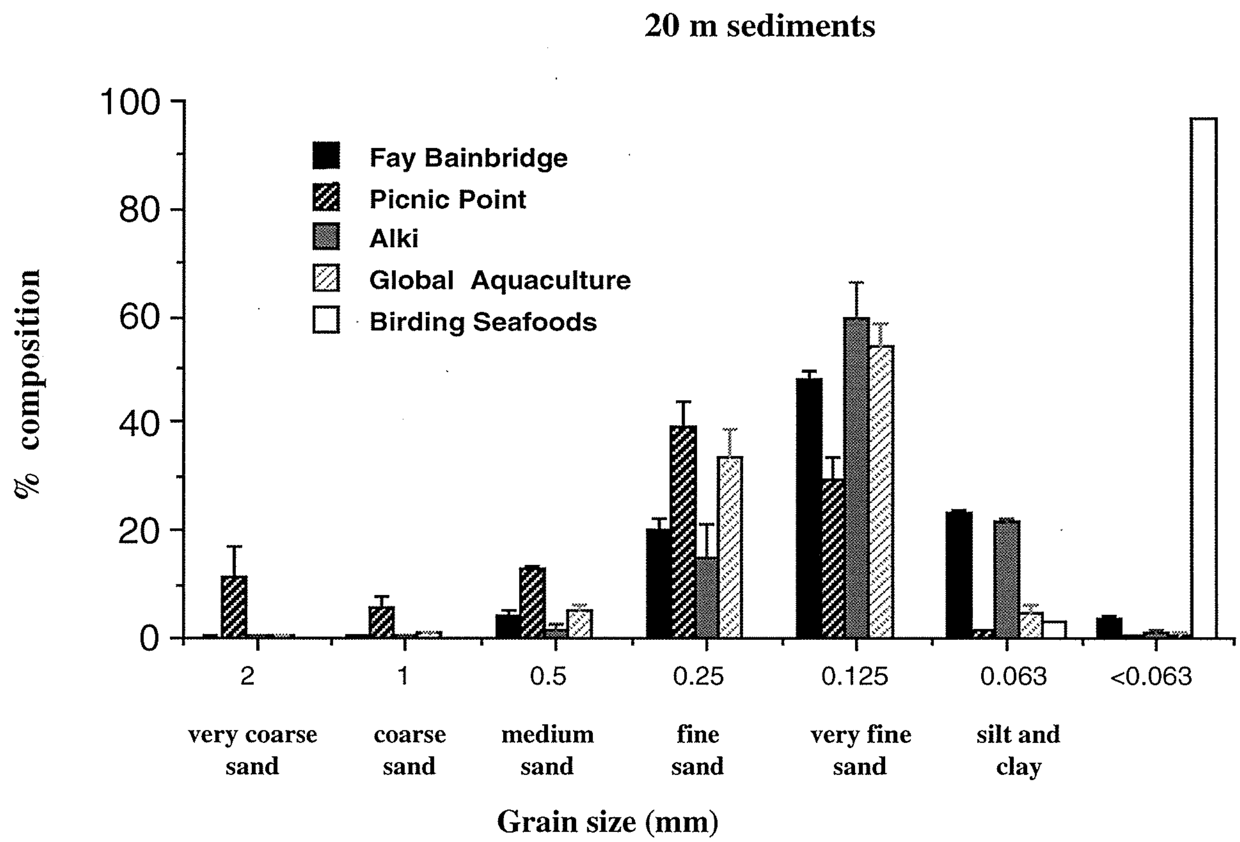


Figure 8. Average grain size for all reference and net pen sites, 20 m depth with one standard deviation shown.

number of species observed per 5 m^2 segment occurred only between the 20-m vs. 30-m and the 10-m vs. 30-m transects at Alki Point, and between Alki Point (10 m) and Fay Bainbridge State Park (10 m). The mean number of individuals seen per 5 m^2 segment was not significantly different in any of the between-depth comparisons, except Alki Point (10 m vs. 30 m). All of the between-site comparisons of mean number of individuals per 5 m^2 segment indicated significant differences.

Of the 45 species total seen at the reference stations, significant differences in between-depth abundances were found for eight different species, and for between-site abundances significant differences were found for six different species (Table 5). For all the comparisons between sites and depths, the ratio of the number of species seen at only one of the two sites or depths and the number of species seen at both sites or depths ranged from a high of 64% for Alki at 10 and 20 m, to a low of 28% for Alki at 20 m and Picnic Point at 20 m.

The between-site comparisons (20-m depth) for the percentage of individuals by trophic group (Fig. 11) show that at Alki and Fay Bainbridge State Park the most prominent species by number were suspension feeders, whereas at Picnic Point the most numerous species were predators. The three reference stations at 20-m depths are more uniform in the distribution of

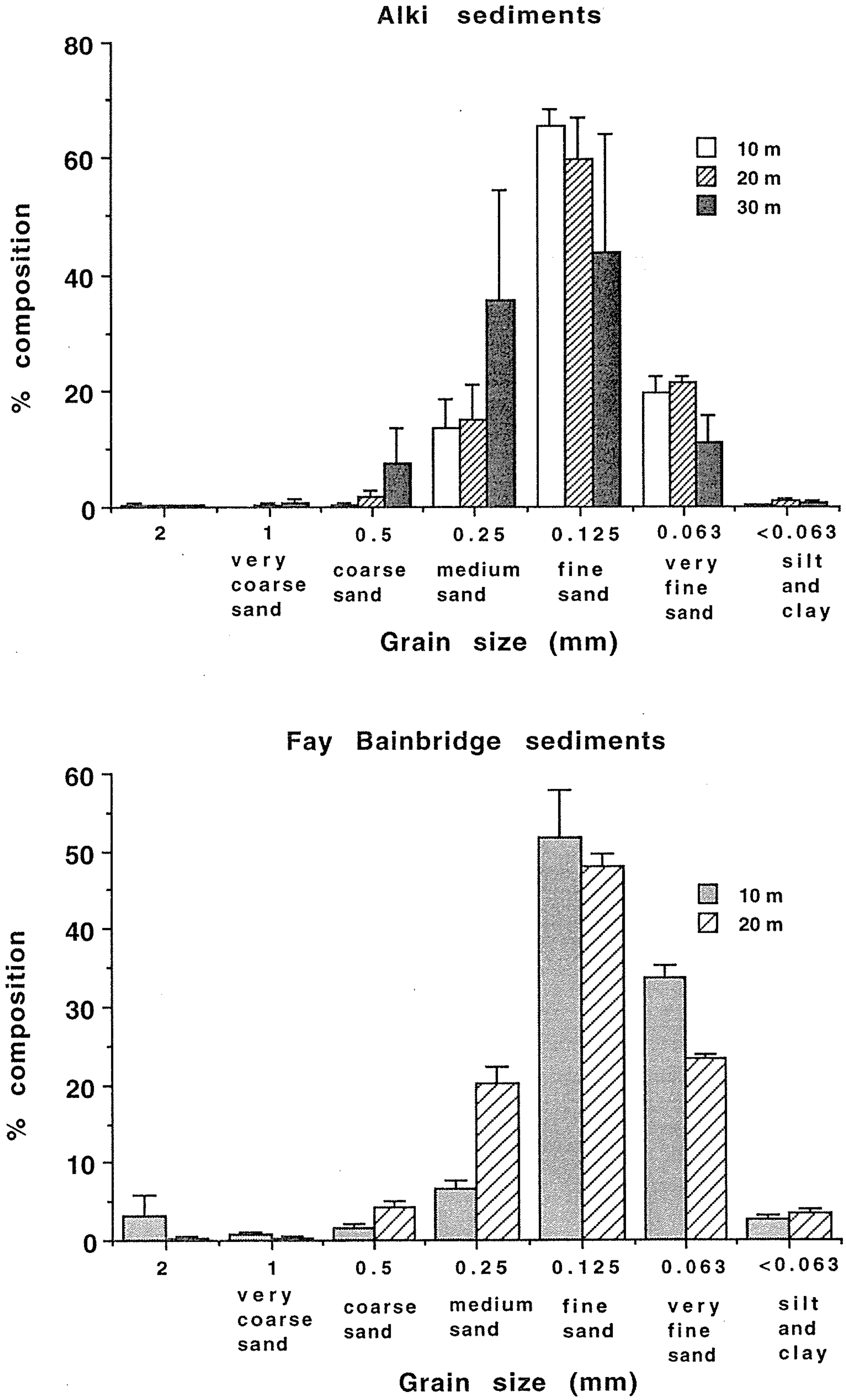


Figure 9. Average grain size for Alki, 10, 20, and 30 m (A), and Fay Bainbridge State Park, 10 and 20 m (B), with one standard deviation shown.

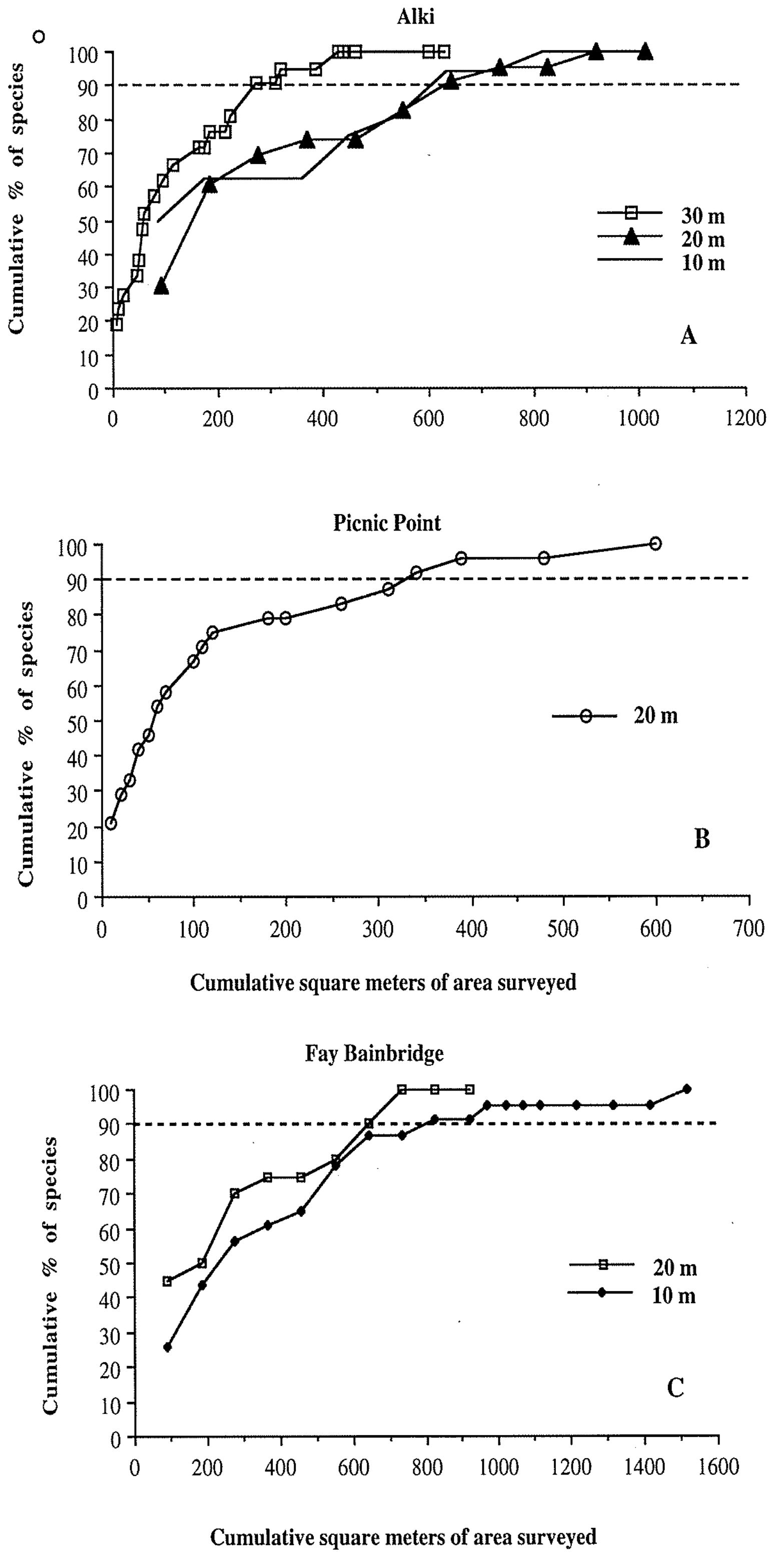


Figure 10. Cumulative number of species: (A) Alki, 10-, 20- and 30-depth; (B) Picnic Point, 20-m depth; (C) Fay Bainbridge State Park, 10-m depth (showing addition of three new species with an additional 876 m^2) and 20-m depth. Dashed line indicates 90% of the total number of species seen per site.

Table 4. Summary of the results of between-depth and between-site comparisons of species diversity, number of species, and number of individuals for all reference sites. BB = Fay Bainbridge State Park, PP = Picnic Point.

	Species diversity	Mean no. per 5 m^2		Species with significantly different abundances
		Species	Individuals	
Between-depth comparison				
Alki 10 m vs. 20 m	$J' \neq J'$	$\mu = \mu$	$\mu = \mu$	Bivalves, *Metridium* sp.
Alki 20 m vs. 30 m	$J' \neq J'$	$\mu \neq \mu$	$\mu = \mu$	*Eupentacta, H. crassicornis*
Alki 10 m vs. 30 m	$J' \neq J'$	$\mu \neq \mu$	$\mu \neq \mu$	Bivalves, *Eupentacta, H. crassicornis, M. aequalis, P. fimbriatus*
BB 10 m vs. 20 m	$J' \neq J'$	$\mu = \mu$	$\mu = \mu$	*A. californica, H. crassicornis, M. aequalis, P. gurneyi, U. columbiana*
Between-site comparisons				
10 m Alki vs. BB	$J' \neq J'$	$\mu \neq \mu$	$\mu \neq \mu$	Bivalves, *P. armatus*
20 m Alki vs. BB	$J' \neq J'$	$\mu = \mu$	$\mu \neq \mu$	*H. crassicornis, M. aequalis, Metridium* sp., *P. armatus*
20 m Alki vs. PP	$J' \neq J'$	$\mu = \mu$	$\mu \neq \mu$	Bivalves, *H. crassicornis, M. aequalis, S. coccinea*
20 m BB vs. PP	$J' \neq J'$	$\mu = \mu$	$\mu \neq \mu$	Bivalves, *Metridium* sp., *P. armatus, S. coccinea*

Table 5. Number of species observed at both depth or site and at either depth or site for between-station and between-site comparisons. BB = Fay Bainbridge State Park, PP = Picnic Point.

	No. of species		No. both ÷ no. either
	At both	At either	
Between-depth comparison			
Alki 10 m vs. 20 m	16	25	64%
Alki 20 m vs. 30 m	17	31	55%
Alki 10 m vs. 30 m	13	26	50%
BB 10 m vs. 20 m	14	28	50%
Between-site comparisons			
10 m Alki vs. BB	10	28	36%
20 m Alki vs. BB	12	34	35%
20 m Alki vs. PP	11	40	28%
20 m BB vs. PP	14	33	42%

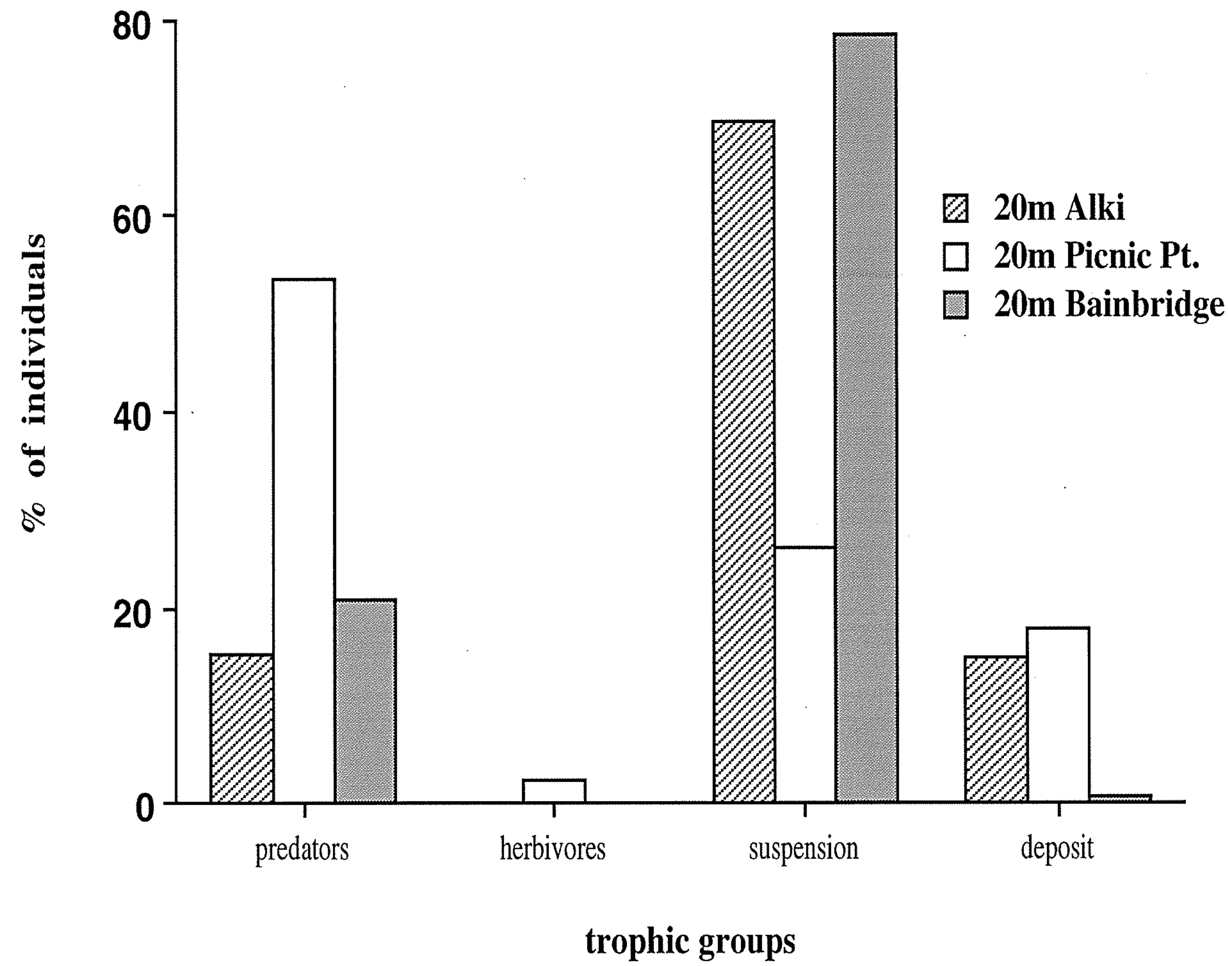


Figure 11. Between-site comparison of percentages of individuals by trophic group for all reference stations, 20 m.

trophic groups by species than by number of individuals, with predators being the largest group by species at all three stations and suspension feeders being the next largest group (Fig. 12). The total number of species ranged from 16 (Alki, 10 m) to 26 (Picnic Point, 20 m), and followed no trend with depth (Fig. 13).

Use of the Protocol at Two Salmon Net Pen Test Sites

A total of 20 species representing 17 families were seen at the two field test sites; of these, 8 species were seen only at Port Townsend, and 3 were only seen at Global Aqua (Appendix Table A.4). Of the 20 species, 39% were in less than 10% of all transects, 32% were in 10–24% of all transects, 16% were in 25–50% of all transects, 13% were in more than 50% of all transects. The most common species were *Metridium* sp. and *C. gracilis*, both of which were in 60% of all transects.

A comparison of the number of species falling into each of five large taxa groups for each of the two net-pen sites (total species seen at all distances from the net-pens) and an average for the

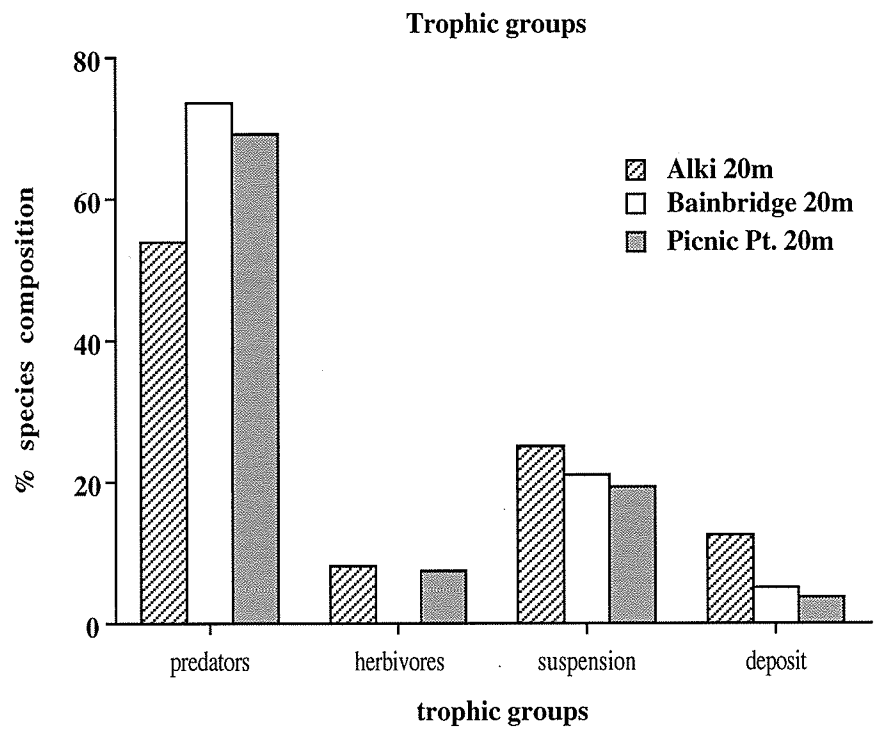


Figure 12. Between-site comparisons of the percentage species composition by trophic groups for all reference sites, 20 m.

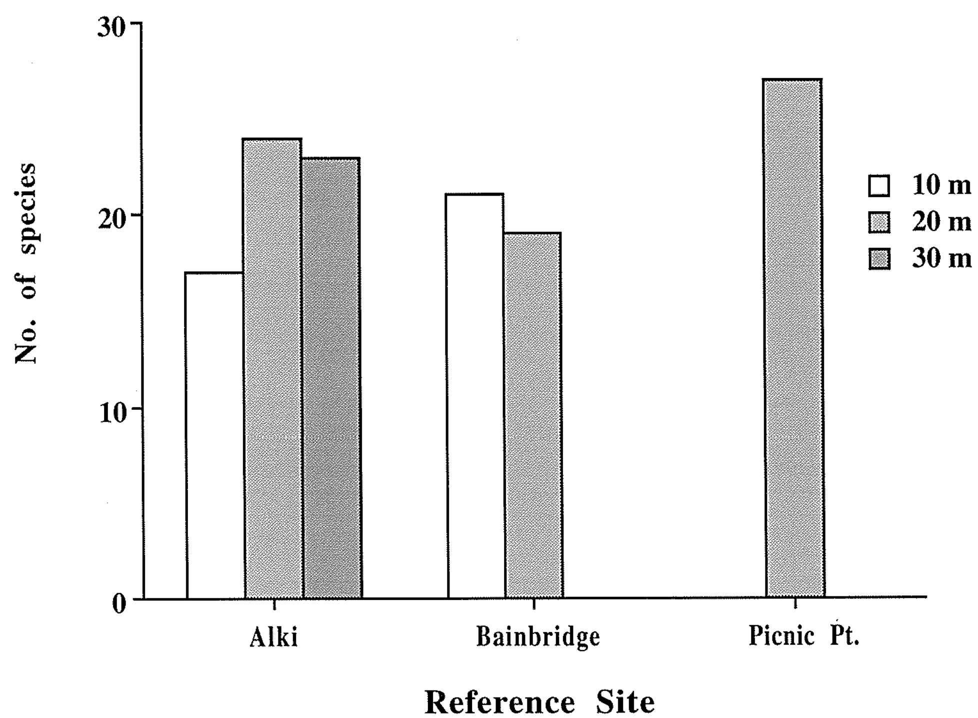


Figure 13. Total number of species seen on all transects, for all references sites and depths.

three reference stations (all 20 m transects) were made (Fig. 14). With the exception of the groups Crustacean and Other, the mean from all reference sites showed a higher number of species than either of the two net-pen sites, the largest difference being in the number of echinoderm species seen (four to five at the net-pens compared with an average of more than nine at the reference stations).

When comparing the percentage of species in each of five large taxa groups for both net-pen sites, we noted a decrease in the relative percentage of echinoderms and mollusc species at the net-pen sites and an increase in the number of crustaceans (Fig. 15a, b).

Global Aqua

Average abundance per 5 m^2 was calculated for every species seen at the field test stations. For all but one species (*Parastichopus californica*), there were statistically no differences between stations at the 0-m and 50-m distances. Presence of food pellets and the average number of species per segment were both statistically different, while the average number of individuals was not (Appendix Table A.5).

Significant differences for diversity and the number of individuals per 5 m^2 segment were not found between the 0- and 50-m distances at Global Aqua; however, the average number of species per 5 m^2 segment was found to be significantly different (Table 6). Global Aqua had the

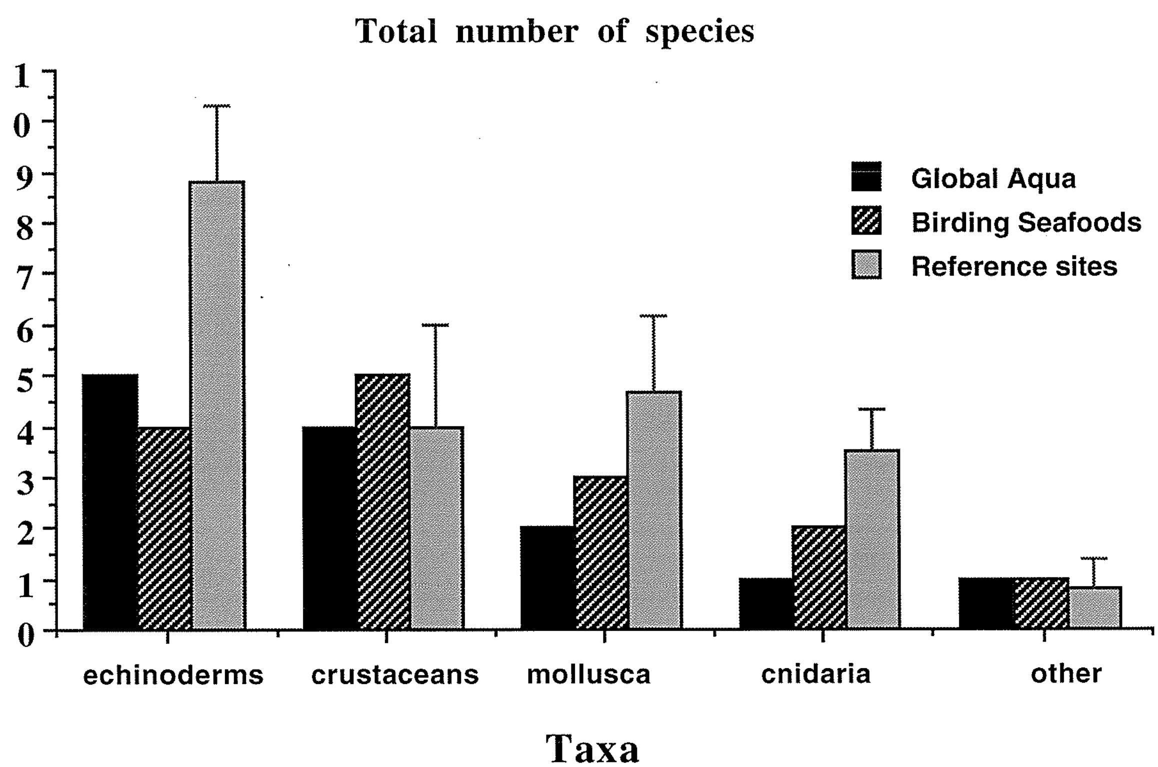
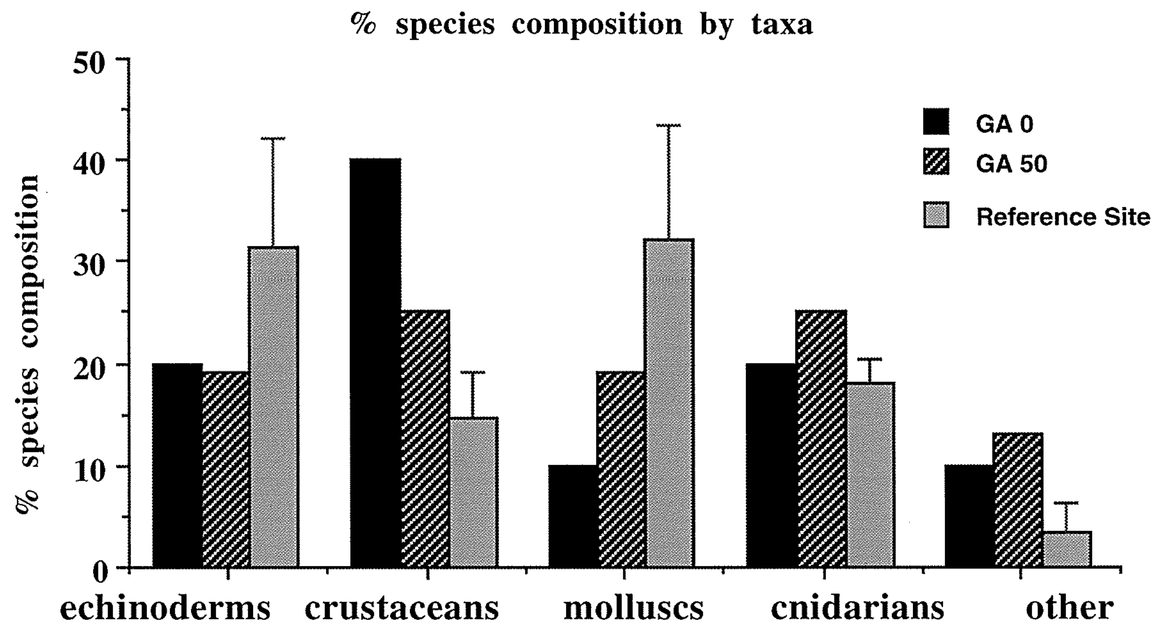


Figure 14. Total number of species seen in each of five large taxa groups at net-pen sites (all distances combined) with mean number of species seen at all reference sites combined. Bars show the 95% confidence interval for the reference means.



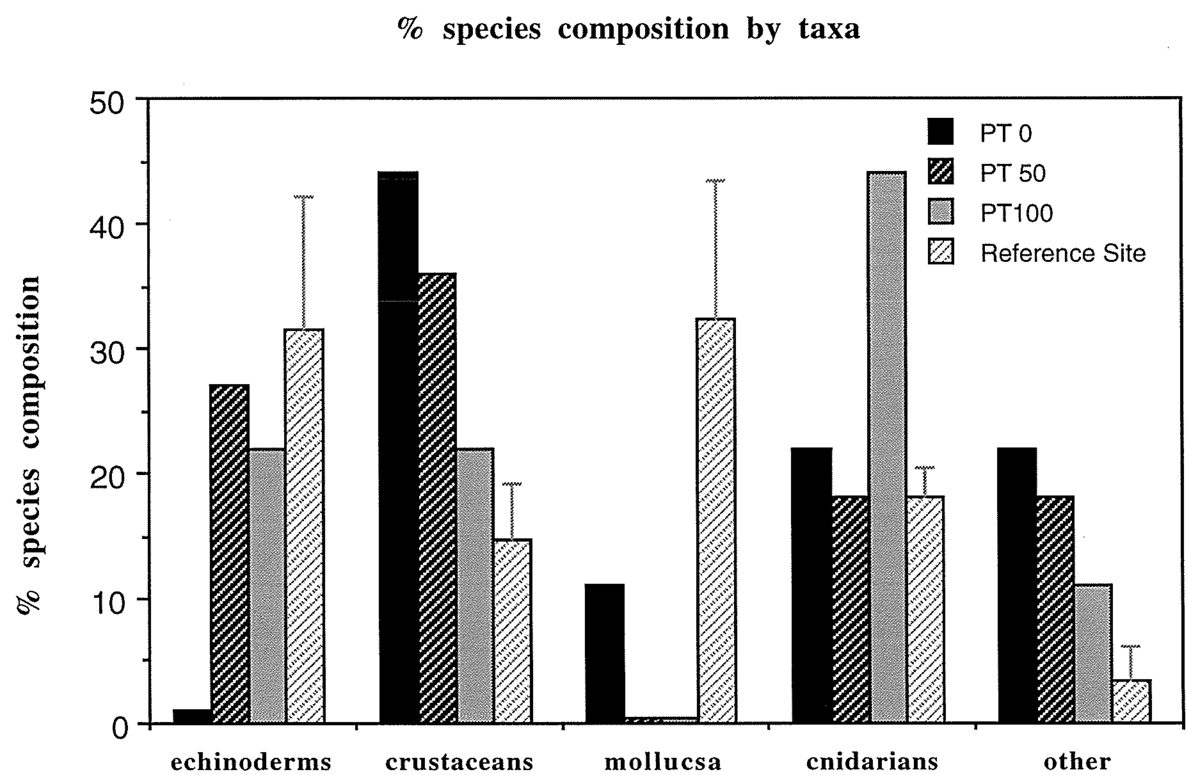


Figure 15. Percentage species composition in each of five large taxa for (A) Global Aqua, Clam Bay and (B) Birding Seafoods, Pt. Townsend, at 0-, 50- and 100-m stations. Reference site means (10-and 20-m depth stations combined) are shown with 95% confidence intervals. GA = Global Aqua, PT = Port Townsend.

Table 6. Summary of the results of between-distance comparisons and t-tests of all net pen sites for all distances. J' indicates diversity in Hutcheson's t-test for equal diversities.

Between-distance comparisons	Diversity	Species per 5 m^2	No. ind. per 5 m^2	# of species at both	# of species at either	# both ÷ no. either
GA 0 vs. 50 m	$0.10<p<0.20$	$\mu \neq \mu$	$\mu = \mu$	7	19	37%
PT 0 vs. 50 m	$J' \neq J'$	$\mu \neq \mu$	$\mu \neq \mu$	6	14	43%
PT 50 vs. 100 m	$p > 0.50$	$\mu \neq \mu$	$\mu \neq \mu$	7	13	54%
PT 0 vs. 100 m	$J' \neq J'$	$\mu \neq \mu$	$\mu \neq \mu$	4	13	31%

lowest ratio of species seen at both distances to species seen at either, with only 7 of the total 19 species (37%) seen at Global Aqua showing up at both 0 and 50 m from the pens.

Birding Seafoods

For most species abundances (95%), there were no differences between stations at the 0-m, 50-m or 100-m distances at Port Townsend. The presence of *Beggiotoa* was significantly different between the 0-, 50-, and 100-m stations; *C. gracilis* was also significantly different between the 0-m and 50-m stations, and the 0-m and 100-m stations; however, no differences could be shown between the 50-m and 100-m stations for this species. Presence of food pellets, diversity, and the average number of species per 5 m^2 segment were all statistically different between 0 m and 50 m, and 0 m and 100 m, with no difference between 50 m and 100 m (Appendix Table A.5). While the total number of species seen at each distance was essentially the same, the average number of species per segment was found to be statistically different between all stations (Table 6).

DISCUSSION

Potential Indicator Metrics

While the total number of species seen at each of the reference stations was high (45), the percentage of those species that were consistently seen at every site and depth was small. Only 15% of the species sighted at any of the reference sites was observed on 50% or more of the transects. Three of the four species that were seen with the greatest frequency (75% of all transects) have other characteristics that may affect their usefulness as direct measures. For example, the presence of *A. californica* and *P. gurneyi* is correlated since *A. californica* feeds solely on *P. gurneyi*. *Metridium* sp., were observed on nearly every transect but were usually associated with debris or other structures that allow *Metridium* sp. to avoid direct contact with the benthos. Bivalves were also found in nearly every transect, but actual counts of bivalves using the developed method may vary considerably and be inaccurate.

At present we have an insufficient database to establish an IBI for benthic marine invertebrates. However, our sampling has provided considerable insight into the species and species groups that are either inappropriate or have potential usefulness in such an application. Table 7 below gives an overview of (1) those metrics that we hypothesize may have value in a marine benthic macroinvertebrate IBI for evaluating areas affected by organic deposition, and (2) associated qualities of those metrics that make them attractive. The process for developing a functioning IBI will involve careful hypothesis testing of each of these metrics to determine their ability to indicate the health of the community of interest. Many of these metrics measure the same species or trophic group, but at different scales. Each of these metrics should be evaluated to determine which hold the most potential for accurate assessments of community changes associated with increased impact.

Table 7. Overview of potential metrics found in this study, including an evaluation of these metrics in four categories: ease of identification, ease of quantification, an estimate of variance, and the trend that was observed at the two net-pen sites.

Potential metric species, trophic groups, and measures	Easy to identify	Easy to quantify	Variance*	Trend as deposition increases
1. Total no. of species	X	X	High	Decrease
2. No. species/segment	X	X	Moderate	Increase
3. Shannon's Diversity	X	X	Moderate	Increase
4. Total no. of individuals	X	X	High	Increase
5. No. of individuals per segment			Moderate	Increase
6. % of individuals as the most abundant taxa	X	X	High	No trend
7. % segments with bivalves present	X	X	Low	Decrease
8. % of segments w/*Beggiotoa*	X		Low	Increase
9. % of segments w/food pellets	X		High	Increase
10. % of segments w/rare species†		X	Low	No trend
11. % of segments with sea cucumbers	X	X	High	No trend
12. % of species as *Cancer sp.*	X	X	Low	Increase
13. % of species as Cnidarians	X	X	Low	Decrease
14. % of species as Crustaceans	X	X	Moderate	Increase
15. % of species as Echinoderms	X	X	High	No trend
16. % of species as mobile	X	X	Moderate	Increase
17. % of species as predators	X	X	Moderate	Increase

*Estimates of the level of variation are based on coefficients of variation (CV); CVs <30% = low, ≥30% and <70% = moderate, and >70% = high.

†A rare species is considered any species observed in <10% of all transects at the reference sites in this study.

Development of a Metric Underwater Transect Tool

This study did not encompass comprehensive comparisons of the accuracy of video and/or photography for evaluation of benthic degradation. However, either of these techniques, particularly videography, show potential applications in this type of survey although the equipment we tested had inadequate resolution for use in our work. Use of audio recording may also prove to be accurate and effective, but for the scope of this study it was felt that audio recording had both practical, financial, and safety limitations for the "average monitoring diver" who may be using this protocol.

The development of the Metrics Underwater Transect Tool proved to be most instrumental to the success of this project. In the past, visual SCUBA surveys have been hampered by visibility estimations and sampling width calculations. The MUTT provided both a non-permanent transect length marker (that did not require any set up time before surveys) and a clear boundary for the survey width such as can be found in quadrat sampling. The bulkiness of the MUTT was considerably reduced when the paired transect widths were reduced to 1 m each. The resulting 2.2-m MUTT was both more portable in transport and more maneuverable in use. After several dives it became easy to use the MUTT. The slates were held by the divers while surveying and the MUTT could be steered by the slates, allowing both hands for recording information. The negative buoyancy and the attached slates also allowed the divers to set the MUTT down and not be required to hold on to any equipment. The stake proved a suitable anchor and retrieval was only a problem at sites with considerable debris. This problem was solved by having the divers ascend with the MUTT and rewind the line from the surface.

Testing of the Use of the Metrics Underwater Transect Tool

Diving Depth Range

Dives at 10 and 20 m were easily performed within acceptable diving limits. Two divers could survey up to 200 m^2 in one 20-m depth dive, and at least two of these dives could be made per day; thus the minimum suggested area (600 m^2) for a given depth can be covered in a day and a half. The 30-m dives were considerably more complex, both in planning and in the gear and training required. Time constraints at 30 m severely limited the distance and thus the maximum survey area per dive and reduced the number of dives per day. The accuracy and dependability of this type of monitoring will be limited to the abilities of the divers that make the surveys. It may be unreasonable to expect the average monitoring diver to perform accurate surveys at 30-m depths: We recommend that the diving depth range to be limited to 0–25 m.

Interdiver Variability

A major factor in the accuracy of this protocol lies in the hands of the survey divers, and while the interdiver variability tests were encouraging, a continuing training program would seem wise in addition to thorough initial training and testing procedures (see Appendix B, Quality Control/Assurance). In the future the use of high-resolution underwater video equipment could replace divers, thus eliminating observer bias and depth limitations and providing a permanent visual record of the surveys. However, at present that does not appear feasible because of financial limitations.

Transect Width, 1 m vs. 2 m

Both 2-m and 1-m transects were used in various parts of this project. Initial trials at Alki Point indicated that 2 m was not too large an area to adequately survey under "normal" circumstances. On dives at Alki, visibility was never a problem, and at the 10- and 20-m stations the number of organisms found in a given area was small enough to make accurate counts, with a 2-m transect. However, in situations with decreased visibility or areas with more complex structure (algae or large numbers of sea pens, etc.) 2 m was too wide, and the 1-m MUTT was definitely preferable. The University of Washington divers found the 1-m MUTT to be more versatile and (after practice) found that it did not significantly decrease the area that could be covered in a dive. Since visibility can change with each day and site, we recommend the 1-m MUTT be used as the standard. The comparisons of the 1- and 2-m MUTT transect data gave ambiguous results. Some measures showed no difference between methods, and other showed differences that are most likely due to the temporal changes of organisms but also could be due to differences in the scales at which different species are distributed. Four of the five crab species surveyed had significantly different mean abundances for the two widths, suggesting that the 1- and 2-m MUTT may sample highly mobile species differently. Bivalves and *Ptilosarcus* had significantly different mean abundances, although the highly variable visibility of these two organisms is most likely the cause of this difference.

Since the 1 m MUTT can be deployed at a faster rate, it is possible that mobile species do not have as much time to move out of the survey area and thus are included more frequently. To maintain comparable data sets, different widths should not be considered interchangeable within a site. Although both the 1- and 2-m MUTT were fairly easily maneuvered underwater, the 1-m MUTT is more versatile and easily moved around such underwater obstacles as the netting and mooring cables typically found under net pens. For these reasons we recommend use of the 1-m MUTT as the standard sampling tool.

Tests of Spatial Correlation of 5-m^2 Segments

When subunits or subsamples of a larger "fixed" sampling unit are taken, the subunits are generally considered to be correlated in some way and are not considered independent replicates unless further statistical testing proves them to be uncorrelated. Autocorrelation analyses are commonly used in time series and geostatistical studies to test for serial correlation of the same sampling unit in time or space. The autocorrelation function (acf) enables us to compute the average correlation (r^2) between sampling units at different distances, or lags, from one another. By plotting the acf against distance (or number of lags), one can determine the spatial correlation structure of a data series. For each species we used the acf to determine the distance at which correlation between abundance estimates in 5-m^2 segments of our 100-m^2 transects could be considered equal to zero. For most of the species observed, these autocorrelation tests revealed that adjacent segments were not correlated and so could be considered independent sampling units (replicates). For those species whose adjacent segments were correlated, bivalves, *P. gurneyi*, *A. californica*, *H. crassicornis*, and *Stomphia coccinea*, mean and variance estimates were calculated from segments known to be independent. This necessitated some loss of data, but avoided the complications of calculating and summing all covariances for each transect and subtracting them from the variance estimate using all the data as would have been required. Because $Var(\hat{X}) = Var(X_1)/n + \sum_j^{\neq k}(Cov(X_j, X_k))$, it is desirable to eliminate covariance in a data set for two reasons. First, if covariance exists within a data set, but that covariance is not known, it inflates variance estimates and makes the sampling design inefficient (higher variance than necessary). The higher the variance, the more samples that are required to detect a given desired difference. Secondly, the calculation of even simple summary statistics is greatly complicated by covariance. In the future it may be possible to reduce the amount of data collected so that only data from independent segments are collected.

For each site and depth, we usually had two to three transects of data on which to conduct the autocorrelation tests, resulting in two to three independent estimates of the acf for each species. While it would have been acceptable to use the mean correlation values for each distance in determining the distance at which segments could be considered independent, because of the small sample size we used the most conservative estimate (largest distance) of this distance. More data of this nature need to be collected to investigate both how the entire acf varies with depth, site, and season (i.e., how spatial patterns vary with site, depth, and season), and how individual autocorrelation coefficients (r^2) are distributed for each distance lag (within site, depth, season); until then we recommend that autocorrelation tests be conducted whenever sampling takes place before standard distances to independence are used for any species.

Field Testing of the Protocol for the Rapid Evaluation of Benthic Organisms

All three reference sites and Global Aqua were similar in sediment composition, consisting of mainly medium to very fine sand. No change in sediment composition with depth was apparent for the reference sites. However, grain size analysis indicated that the sediments at Birding Seafoods were distinctly different from the sediments at the other sites, and this distinction was apparent even during the transect dives. The sediments taken at Birding were 100% silt and clay fraction, compared with less than 5% at all other sites. This distinction should certainly be kept in mind when comparing organism data from Birding Seafoods with data from other sites. In addition, Birding Seafoods facility is situated near a pulp-paper mill, which may be affecting the sediment and organisms in the bay.

Reference Stations

<u>Cumulative Species Curves</u>. Using cumulative species curves for each of the three reference sites, we determined that 600 m^2, when laid out in paired 50-m^2 transects, were sufficient to observe 90% of the species at a location. Areas that were surveyed over relatively short time frames (1 mo, Picnic Point, 20 m; 1 wk, Alki, 30 m) showed substantially sharper curves than our stations at Alki (10 and 20 m), where transects were done over a 3-mo period. Because of seasonal variations, surveys spanning longer periods (but of the same total area) would be ex-

pected to show a less pronounced curve in the addition of new species and in general show a higher number of species seen overall.

Average Abundances and Confidence Intervals. Within-site depth comparisons indicate that while the distribution of many species is stratified by depth (36%–50%), the average number of species, average number of individuals, and abundances of most species (observed at both depths) were not significantly different among depth strata. The biggest difference was seen at Fay Bainbridge State Park (10 m vs. 20 m), where 5 of the 14 species seen at both depths had significantly different abundances.

Within-depth comparisons among sites did show significant differences in number of species and species abundances. Species seen at only one site comprised between 58%–72% (Alki 20 m vs. Picnic Point 20 m) of the total number of species seen at both sites, and the majority of species seen at both sites had significantly different abundances despite the fact that sediment type and depth were similar for both sites.

Between-site comparisons of percentage of individuals by trophic group indicated that trophic groups have similar relative importance at the Alki and Bainbridge reference sites. Picnic Point's percentage of individuals by trophic group would likely be more similar to the other two reference sites had we been able to count the large numbers of *Cucumaria piperata* (suspension feeders) present on several transects at this site. The reference stations have very similar relative numbers of species (% species composition), total number of species observed, and number of species per trophic group.

The differences between these measures at our reference sites, despite controlled depth, grain size, and general regional area, suggest that geographical location of the reference sites be considered very important for future surveys. While the depths of the surveys should be held consistent within a net-pen site, the location of the reference site should be adjacent to the net-pen site (as opposed to the use of regional references sites). Ideally, transects could be run parallel to the pens at increasing distances until no difference exists between adjacent transects, with the resulting distance being the reference station.

Using trophic groups as metrics rather than individual species abundances for comparisons among sites appears to be a more reliable measure for assessing environmental "health" of net-pen sites. Individual species may not be observed at all sites, and using a more robust indicator, such as trophic group, allows more flexibility in the actual species present but still will provide information about the structure of the community.

Use of the Protocol at Two Salmon Net-Pen Test Sites

The two field test sites showed a marked decrease in the number of species observed (20 compared with 45), and of these only 13% were in 50% or more of the transects (the highest being 60%). In comparing the number of species in each of five taxa (echinoderms, crustaceans, mollusca, cnidaria, and other) for each of the two net pen sites and an average from all the reference sites, we found a generally equal decline in all taxa groups with the exception of crustaceans. The number of crustacean species was the same or greater at the net-pen facilities than the reference mean, suggesting that crustaceans as a group would be attracted to the organic deposition from the pens. This was obvious to the divers because of the large numbers of *Cancer* sp. individuals and *Pandalus danae* shrimp observed at both net-pen sites. The use of crustaceans as a positive indicator of organic degradation should therefore be considered. Echinoderms showed the largest change (two to three species at the net pens and an average of nine at the reference sites), suggesting that echinoderms as a group are less tolerant of the deposition and should be examined more thoroughly as indicator species. In fact, the only species to show a significant difference in abundance between the 0- and 50-m stations at Global Aqua was *Parastichopus californica*, suggesting that echinoderms were perhaps the most affected taxa at this site.

While very little *Beggiotoa* or food pellets were observed at Global Aqua, they were both more widespread at Birding Seafoods, Pt. Townsend. The presence of *Beggiotoa* and food pellets was significantly higher as the stations neared the edge of the pens. *Beggiotoa* is a distinct indicator of anaerobic decomposition and seems to be a very good measure of the effects of deposition, and food pellets also seem to be a good direct indicator of organic deposition.

We have found that this SCUBA-based survey method worked well at reference sites with no debris, and at the net-pen sites with considerable underwater obstructions. A relatively large area could be covered in the time limits available with SCUBA, and the autocorrelation associated with this specific pattern of transecting was a factor for only a few species. The original objectives for this study were to identify metrics of the soft bottom, 10 to 30 m depth marine habitat, that are potential indicators of the biological integrity of the epibenthos; to develop SCUBA-based underwater recording techniques for documenting these metrics of the soft bottom, and to evaluate a SCUBA-based visual protocol for assessing the environmental impacts of organic deposition to the epibenthic community in soft bottom marine waters 10 to 30 m deep.

The development of the MUTT as well as the investigation of the survey technique has led to the identification of seventeen hypothesized metrics that have potential as indicators of the biotic integrity of the epibenthic community. Each of these metrics will need to be further evaluated as the next step in the development of a functioning Marine Benthic IBI. Once these metrics have been evaluated and the ability of each to assess organic deposition determined, a functioning IBI can be developed and tested on a range of areas under the influence of organic deposition. This Marine Benthic IBI can then be used as a rapid evaluation tool for these areas, giving regulatory agencies the ability to assess the effects of organic material deposition quickly, accurately and inexpensively.

Recommendations for Future Studies

Because of time constraints on the project, only three reference sites and two net pen sites could be surveyed. In order to prepare a preliminary Index of Biotic Integrity for marine macroinvertebrates, a considerably larger number of both reference and net-pen sites in a larger range of sediments types would need to be surveyed. However, even with this data, regional reference sites do not appear to be an acceptable means of comparison. An alternate protocol to using local reference sites would be more appropriate and more accurate.

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APPENDIX A

DATA TABLES

Table A.1. Interdiver variation comparison results, with mean abundances by species, taxonomic groups, and other measures for two observers on the same transects (1 m X 50 m) for a total of 600 m^2. N = no. 5-m^2 segments used to tabulate comparisons (where the minimum possible was 10 and the maximum possible was 50 depending on the number of segments in which a species was observed by either diver. Only groups with more than one species are shown. Diver A showed a higher count in 48% of all the measures combined.

	N	Diver A mean	Diver B mean	Average diff.	Var.	t	P (T≤t) two-tail	Power
By species								
Armina californica	40	1.35	1.33	0.03	0.54	0.22	0.83	0.93
Bivalves	42	5.52	5.74	-0.21	16.71	-0.34	0.74	0.98
Hippasteria spinosa	30	0.17	0.13	0.03	0.03	1.00	0.33	0.79
Luidia foliolata	30	0.10	0.17	-0.07	0.06	-1.44	0.16	< 0.99
Mediaster aequalis	20	0.30	0.30	0	0	0	1.00	1
Metridium sp.	40	0.65	0.63	0.03	0.18	0.37	0.71	0.91
Pachycerianthus fimbriatus	20	0.10	0.05	0.05	0.05	1.00	0.33	0.76
Pagurus armatus	40	1.58	1.93	-0.35	1.98	-1.57	0.12	< 0.99
Ptilosarcus gurneyi	20	1.35	1.45	-0.10	0.09	-1.45	0.16	< 0.99
By groups								
Anemone	40	0.70	0.65	0.05	0.20	0.70	0.49	0.84
Sea stars	40	0.30	0.28	0.07	0.13	1.00	0.32	0.70
Other measures								
No. individuals	50	8.36	8.82	-0.46	15.27	-0.83	0.41	0.99
No. species	50	2.28	2.38	-0.10	0.66	-0.87	0.39	< 0.99
Total species seen		9	9					
Shannon Diversity Index		0.637	0.635			0.056	0.524	

Table A.2. Percentage of 100-m^2 transects in which each species was observed, by site and showing totals for all reference sites.

	All sites		Alki Point			Bainbridge State Park		Picnic Point	Total Ref.
Depth	10 m	20 m	10 m	20 m	30 m	10 m	20 m	20 m	10 & 20
No. transects	21	27	11	11	10*	10	10	6	48
No. species/site	29	42	17	25	23	22	20	27	47
			Percentage of transects						
Acanthodoris nanaimoensis		4						17	2
Archidoris odhneri		4						17	2
Armina californica	100	59	100	91	40	100	60		77
Ascidia paratropa		19		9				67	10
Bivalves	95	96	91	100	100	100	100	83	96
Brachiopod		4						17	2
Cancer gracilis	14	7				30		33	10
Cancer magister	19	22				40	50	17	21
Cancer productus	33	33			10	70	70	33	33
Cancer sp.	5					10			2
Chlamys spp.		4		9					2
Cribrinopsis fernaldi		7						33	4
Crossaster papposus	10	41	9	55	20	10	40	17	27
Cucumaria piperata		7		18	10				4
Dermasterias imbricata					10				
Dirona albolineata	14	7				30		33	10
Eupentacta		4		9	80				2
Evasterias troschelli	19	19	18	27	30	20	10	17	19
Fusitriton oregonensis		4		9					2
Henricia sp.	10	22			30	20	30	50	17
Hermissenda crassicornis	48	81		55	100	100	100	100	67
Hippasteria spinosa	24	26	45	64	70				25
Lebbeus sp.		4						17	2
Luidia foliolata	14	30	27	45	70			50	23
Mediaster aequalis	33	70	45	100	100	20	60	33	54
Metridium sp.	76	93	64	82	60	90	100	100	85
Oregonia gracilis	19	19	18	9		20		67	19
Octopus rubescens		4					10		2

Table A.2—cont.

	All sites		Alki Point			Bainbridge State Park		Picnic Point	Total Ref.
				Percentage of transects—cont.					
Pachycerianthus fimbriatus		19		45	70				10
Pagurus armatus	100	85	100	91	100	100	70	100	92
Pandalus danae		4		9	10				2
Parastichopus californicus	10	11	18	27	40				10
Pisaster brevispinus	5	4			10	10	10		4
Polinices lewisii	33	19	55	45	20	10			25
Pteraster tessulatus		7						33	4
Ptilosarcus gurneyi	81	33	64	9		100	80		54
Pycnopodia helianthoides	5	7	9	9	30		10		6
Strongylocentrotus droebachiensis	5	7	9	9				17	6
Scyra acutifrons	5					10			2
Solaster dawsoni		19					40	17	10
Solaster stimpsoni	19	33				40	80	17	27
Stomphia coccinea	10	48	18	55	10		20	83	31
Triopha catalinae		4						17	2
Tritonia diomedea	10					20			4
Unid. nudibranch			9						2
Urticina columbiana	5	48				10	70	100	29
Urticina crassicornis					40				

*Transect lengths for 30 m Alki vary from 30 to 100 m.

Table A.3. Average abundances with 95% confidence intervals for all reference site species and species diversity indices. Blank spaces indicate that no observations were made for that species at that site or depth; n = no. paired 50 m^2 transects at each station.

Average Abundances ± C. I. in 100 m^2	Alki 10 m n=11	Alki 20 m n=11	Alki 30 m n=126*	BB St. Pk. 10 m 2 m wide n=10	BB St. Pk. 20 m n=10	BB St. Pk. 10 m n=120*	Picnic Pt. 20 m n=120*
Acanthodoris artemesia							0.17±0.33
Archidoris odhneri							0.17±0.33
Armina californica	21.06±14.82	4.74±3.50	11.27±10.74	31.63±17.75	2.93±2.82	12.76±12.76	
Bivalves	2.69±1.27	92.39±28.99	40.79±20.68	94.24±53.83	110.3±54.05	471.3±182.5	7.50±2.89
Boltenia		0.20±0.45					
Brachiopod							0.17±0.33
Cancer gracilis				0.76±1.04		0.33±0.66	0.50±0.57
C. magister				0.54±0.66	0.76±0.64	3.50±1.53	0.17±0.33
C. productus			0.16±0.56	1.41±1.16	1.52±1.05	0.33±0.46	0.67±0.65
Cancer sp.				0.11±0.25			
Chlamys spp.		0.10±0.22					
Cribrinopsis fernaldi							0.33±0.46
Crossaster papposus	0.10±0.22	0.69±0.50	0.48±0.96	0.11±0.25	0.76±0.97		0.17±0.33
Cucumaria piperata		0.99±1.54	0.16±0.56				
Dermasterias imbricata			0.16±0.56				
Dirona albolineata				0.65±0.98			0.67±0.65
Eupentacta		0.10±0.22	798.6±620.5				
Evasterias troschelli	0.20±0.30	0.30±0.35	0.63±1.11	0.33±0.52	0.11±0.25	0.33±0.46	0.33±0.46
Fusitriton oregonensis		0.20±0.45					
Henricia sp.			0.63±1.11	0.22±0.33	0.43±0.54	0.17±0.33	0.50±0.57
Hermissenda crassicornis		1.98±1.78	17.14±11.37	10.11±4.43	27.39±12.70		14.92±6.96
Hippasterias spinosa	1.29±1.14	1.28±1.14	1.75±1.95				
Lebbeus sp.							0.17±0.33
Luidia foliolata	0.30±0.35	1.19±1.02	2.54±2.25				0.83±0.73
Mediaster aequalis	0.79±0.75	7.21±1.73	8.57±4.66	0.43±0.75	2.17±1.94		1.00±0.79
Metridium spp.	3.27±2.89	4.15±2.45	3.81±3.79	3.80±2.30	38.26±10.94	1.67±1.37	8.00±2.50
Oregonia gracilis	0.20±0.30	0.10±0.22		0.22±0.33		1.17±0.85	1.33±1.12
Octopus rubescens					0.11±0.25		

Table A3—cont.

Average Abundances ± C. I. in 100 m^2	Alki 10 m n=11	Alki 20 m n=11	Alki 30 m n=126*	BB St. Pk. 10 m 2 m wide n=10	BB St. Pk. 20 m n=10	BB St. Pk. 10 m n=120*	Picnic Pt. 20 m n=120*
Pachycerianthus fimbriatus		1.09±1.33	3.17±3.12				
Pagurus armatus	15.45± 7.22	20.55± 9.98	26.19± 8.97	3.70± 1.43	1.30± 0.96	1.00± 0.79	11.17± 2.96
Pandalus danae		0.10±0.22	0.32±0.79				
Parastichopus californicus	0.10± 0.22	0.20± 0.30	8.89± 21.08		0.11± 0.25		
Pisaster brevispinus			0.16±0.56	0.11±0.25	0.11±0.25	0.17±0.33	
Polinices lewisii	0.99±0.84	1.68±1.64	0.48±0.96	0.22±0.49		0.33±0.46	
Pteraster tessulatus							0.50±0.57
Ptilosarcus gurneyi	36.32± 42.73	0.30± 0.66		118.8± 45.62	16.09± 16.52	24.67± 16.05	
Pycnopodia helianthoides	0.10±0.22	0.10±0.22	0.48±0.96		0.11±0.25	0.17±0.33	
Solaster dawsoni					0.65±0.66		0.50±0.74
Strongylocentrotus droebachiensis	0.10±0.24	0.10±0.22					0.17±0.33
Solaster stimpsoni				0.43±0.40	3.37±2.87		0.17±0.33
Scyra acutifrons				0.11±0.25			
Stomphia coccinea	0.20±0.30	0.89±0.73	0.16±0.56		0.33±0.52	0.33±0.46	8.00±4.63
Triopha catalinae							0.17±0.33
Tritonia diomedea				0.22±0.33		0.33±0.46	
Urticina columbiana				0.11±0.25	3.15±2.42		4.33±1.76
Urticina crassicornis			0.16±0.56				
Diversity = J'	0.545	0.424	0.223	0.445	0.479	0.195	0.704
No. species seen	8.55± 2.25	11.91± 1.48	14.67± 2.27	10.30± 0.83	11.50± 1.94	10.83± 2.04	13.83± 4.32
No. individuals seen	87.8± 56.1	158.8± 39.3	1081.4± 618	269.4± 60.8	212.1± 63.9	484.7± 71.6	63.2± 7.3

*These averages were calculated for 5-m^2 and converted to 100 m^2; values for n are based on the number of 5-m^2 samples.

Table A.4. Percentage of 50-m^2 transects within which each species was observed for the two field net-pen sites.

	Global Aqua		Pt. Townsend		
Distance from pen	0 m	50 m	0 m	50 m	100 m
No. transects (50 m^2 each)	6	6	6	6	6
Species	11	17	10	11	9
	% of transects		% of transects		
Anthopleura artemesia		17			
Beggiotoa	50	83	100	33	
Bivalves		17	17		
Cancer gracilis	33	33	100	83	50
Cancer productus	17	33	17		
Dirona albolineata	17	17			
Epiactis prolifera					17
Eupentacta				17	17
Evasterias troschelli	50	50			
Food pellets	50	67	50		
Metridium sp.	50	83	83	50	33
Oregonia gracilis				33	
Pachycerianthus fimbriatus			67	67	50
Pagurus armatus	33	50	33	17	17
Pandalus danae	17	17	33	33	
Parastichopus californicus		33		17	17
Pisaster ochraceous	17	17		17	
Polinices lewisii		17			
Stomphia coccinea		17			
Tellemesus		17	33		
Terrebellid				17	67
Urticina crassicornis	50	83			17

Table A.5. Average abundance and 95% confidence intervals for all field net-pen site species with diversity index values. Blank spaces indicate that no observations were made for that species at that site or depth; n = no. paired 5-m^2 transects at each station.

Average abundances ± C. I. in 5 m^2	G. Aqua 0 m μ ± C. I. n=60	G. Aqua 50 m μ ± C. I. n=60	PT 0 m μ ± C. I. n=60	PT 50 m μ ± C. I. n=60	PT 100 m μ ± C. I. n=60
Anthopleura artemesia		0.02 ± 0.04			
Beggiotoa	0.50 ± 0.13	0.28 ± 0.13	0.77 ± 0.11	0.07 ± 0.07	
Bivalves		0.15 ± 0.24	0.02 ± 0.03		
Cancer gracilis	0.03 ± 0.05		2.73 ± 0.79	0.23 ± 0.13	0.08 ± 0.07
C. productus	0.03 ± 0.05	0.02 ± 0.04	0.05 ± 0.06		
Dirona	0.02 ± 0.03				
Epiactis prolifera					0.02 ± 0.03
Eupentacta		0.02 ± 0.04		0.02 ± 0.03	0.02 ± 0.03
Evasterias	0.17 ± 0.11				
Food pellets	0.50 ± 0.13	0.22 ± 0.12	0.23 ± 0.11		
Metridium spp.	4.27 ± 1.07	3.28 ± 1.07	0.20 ± 0.18	0.12 ± 0.12	0.05 ± 0.06
Oregonia gracilis		0.02 ± 0.04		0.03 ± 0.05	
Pachycerianthus fimbriatus			0.27 ± 0.16	0.13 ± 0.12	0.10 ± 0.08
Pagurus sp.	0.10 ± 0.09	0.13 ± 0.10	0.03 ± 0.05	0.02 ± 0.03	0.02 ± 0.03
Pandalus danae	20.32 ± 2.48	14.58 ± 3.63	0.03 ± 0.05	0.05 ± 0.06	
Parastichopus californicus		0.17 ± 0.15		0.02 ± 0.03	0.02 ± 0.03
Pisaster ochraceous	0.02 ± 0.03			0.02 ± 0.03	
Polinices lewisii		0.02 ± 0.04			
Stomphia		0.02 ± 0.04			
Tellemesus		0.02 ± 0.04			
Terrebellid				0.02 ± 0.03	0.13 ± 0.10
Urticina crassicornis	0.20 ± 0.10	0.25 ± 0.18			0.02 ± 0.03
J' of the transect sums	**0.52**	**0.44**	**0.85**	**0.81**	**0.52**
No. species seen	**3.33 ± 0.32**	**2.45 ± 0.39**	**2.45 ± 0.26**	**1.15 ± 0.28**	**0.63 ± 0.20**
No. individuals seen	**26.27 ± 2.97**	**19.27 ± 4.08**	**4.65 ± 0.83**	**1.32 ± 0.31**	**0.68 ± 0.22**

APPENDIX B: RECOMMENDED PROTOCOL FOR THE RAPID (SCUBA) EVALUATION OF THE LEVEL OF ORGANIC DEGRADATION IN PUGET SOUND AT THE NEARSHORE SOFT-BOTTOM HABITAT

Susan A. Miller, Bruce S. Miller, Greg Jensen, and Harry B. Hill

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USE AND LIMITATIONS

Examples of Recent Studies of Benthic Community Changes along an Organic Enrichment Gradient (Salmon Feces and Excess Feed)

Two recent studies of organic degradation are summarized to illustrate the types of benthic studies that are commonly conducted, the kinds of sampling equipment that are frequently used, and the kinds of conclusions that have been drawn from such studies.

Using core sampling techniques, Weston (1990) surveyed infaunal macroinvertebrates around salmon net-pens in Puget Sound, Washington. Weston found there was decreasing species richness and total community biomass, with an increase in proximity to a source of organic deposition. In addition, an increase in the total number of individuals, significant decrease in specimen size, and the loss of large bivalves was recorded nearest to the source of organic deposition.

Even more recently, Mahnken (1993) studied benthic faunal recovery and succession after removal of a marine fish farm in Puget Sound. Quarterly samples for 2 yr were taken by two divers positioning a Van Veen grab on the sea floor initially beneath the net pens; after the net pens were removed, samples were taken in relationship to a buoy that marked the center of the "footprint" of the former net pen site. The entire grab sample was screened (0.1-mm mesh), stained, sorted into main taxonomic groups, and then distributed to specialists for species identification and enumeration, a process which proved to be very time consuming, difficult, and expensive. Traditional ecological indices (e.g., the Shannon-Weiner index, H') were used to analyze the data, which indicated that there was a dramatic increase in the number and abundance of species within the first 2.5 mo after removal of the net cages; subsequently, the increase was slower, but consistent. By the end of the study (nearly 2 yr), the species richness of the numerically dominant species at the study and control sites was equal. However, with the exception of the clam population (which recovered completely in 17.5 mo), the abundance of species at the farm site never recovered to that of the control/reference site. Rare species were unable to reestablish themselves within the time frame of this study, demonstrating that longer times may be required for full recovery of these sites.

STUDY DESIGN CONSIDERATIONS

Project Objectives

The particular project objective must be clearly understood in order to properly utilize this Protocol for a particular evaluation. However, in general the objective is to carry out a fast, accurate, and inexpensive evaluation that assesses the environmental impact of organic deposition to the epibenthic community in soft-bottom marine waters at depths from 10 to 30 m.

Sampling Schedule

The frequency of sampling is determined by study-specific objectives. Monitoring environmental impacts that occur over short time scales requires frequent sampling, usually on a weekly basis. Long-term and cumulative impacts may require only yearly or lower frequency sampling. It is always ideal (but often not possible) to sample a site before the impact occurs so that a baseline value is obtained.

In general, we recommend only daylight sampling, and when possible we recommend summer sampling when results of using this Protocol appear to be distinctive. It is important that reference sites and target sites be sampled at the same time of day (afternoon or morning) and that they be sampled during the same season of the year.

Habitat Coverage

There is a real diversity of habitats in Puget Sound (Fig. B1). The present Protocol is only currently valid for the nearshore and shallow offshore soft-bottom habitat, from 10 to 30 m in depth.

Replication

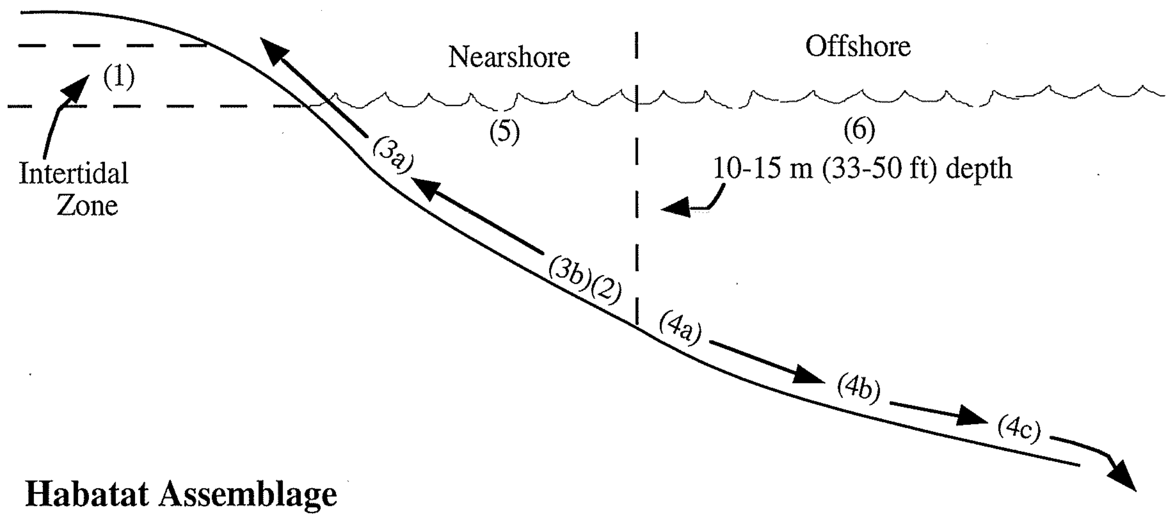
The Metrics Underwater Sampling Tool (MUTT) allows two immediately adjacent divers to record metrics simultaneously on adjacent, 1-m swaths. Besides providing an opportunity to obtain two adjacent samples simultaneously, the MUTT also provides an excellent training situation for observers and it allows a direct comparison of variability between observers. The method of evaluating observer differences is given in the Quality Assurance/Quality Control section of this Protocol.

One question of obvious importance is how many transects need to be surveyed to characterize a site. We plotted the cumulative percentage of species against the cumulative square meters sampled and found that after 6 paired 100 m^2 transects of bottom substrate had been surveyed, 90% of the species in a given area had been observed.

RECOMMENDED EQUIPMENT

Single depth transects, between 10 and 30 m, should be run using the MUTT by SCUBA divers (Fig. B.2 and Appendix B.1).

The MUTT device was designed to allow visual transects of a known area with minimal disturbance; it is inexpensive, using PVC pipe and plastic lids from 5-gal buckets. Underwater it has slightly negative buoyancy and is highly maneuverable so that it can be easily moved over obstructions. MUTT allows a dive team to survey two parallel transects, each 1 m wide by 50 m long, in 5 m increments with the central spool and end wheels providing clearly visible reference points for maintaining a consistent survey width.



Habatat Assemblage

(1) Intertidal rock-cobble fish assembalge

(2) Nearshore rocky reef assemblage

(3) Nearshore soft-bottom fish assemblage
- (3a) Intertidal to 5 m (0-16 ft) depth
- (3b) 5-10 m (16-33 ft) depth

(4) Offshore soft-bottom fish assemblage
- (4a) 10-30 m (33-100 ft) depth
- (4b) 30-70 m (100-230 ft) depth
- (4c) >70 m (>230 ft) depth

(5) Nearshore pelagic fish assemblage

(6) Offshore pelagic fish assemblage

Figure B1. Puget Sound marine habitats and fish assemblages. Source: Wingert and Miller (1979), Miller et al. (1990).

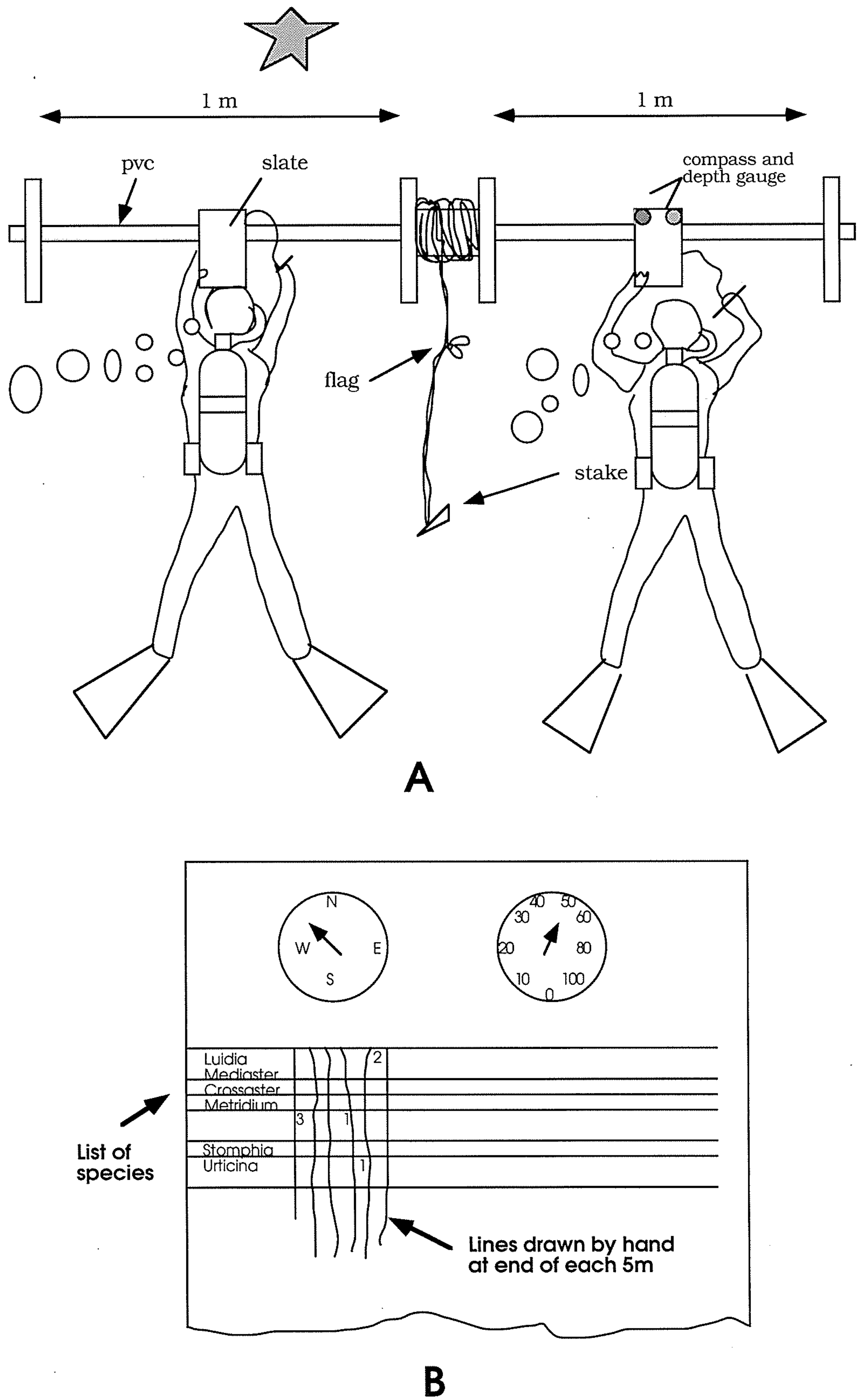


Figure B.2. A. Metrics Underwater Transect Tool (MUTT) in use. B. Layout of slate.

MUTT construction (see Appendix B.1)

Materials needed:

- Four 5-gal bucket lids (~12" in diameter)
- One 10' length of 1/2" schedule 40 (600 psi) PVC pipe
- One 10" piece of 3/4", 125 PVC
- One 8" piece of 2", 200 psi PVC
- Two 1/2" threaded end caps
- Two 1/2" threaded end fittings
- Two 3/4" hose clamps
- Eighteen 4" cable ties
- ~170 ft. of 1/4" nylon rope
- One 15" piece of 3/4" or 1" pipe or conduit for a stake
- flagging tape

For slates:

- Two ~10" X 12" pieces of flat stock 1/4" or (preferably) 3/16" PVC
- Two 8" pieces of 1", 200 psi PVC
- Six small screws
- C-type universal plastic pipe cement
- E-type purple primer

The 3/4" PVC fits like a sleeve around the main axle and allows the spool to spin freely, while the 2" pipe provides structural support and its larger diameter makes rewinding much faster. To build the spool, drill holes in the center of two of the bucket lids just large enough to force through the 3/4" PVC. Center the 2" pipe on each lid and mark where the holes for cable ties will be drilled. There will be eight ties on each end; each will be threaded through a hole in the lid, then through the PVC, and back down through a second hole in the lid and into the head of the cable tie. The spool is held in position by the two hose clamps. We recommend using these in conjunction with small, slotted pieces of PVC so that the spool does not rub against the metal clamps.

The holes in the two outside lids should be just large enough to allow the lids to spin freely on the threaded part of the end fittings. Once the end cap has been screwed to the proper tightness, a small hole can be drilled through both fittings and a cable tie used as a cotter pin to prevent the threaded cap from loosening.

Only one of the end fittings should be glued into place, so that the spool can be removed and the MUTT easily broken down for storage or transport. The other side can be held in place by a cable tie in the same manner as the end caps are held in place.

The rope must be pre-soaked and dried before it is measured and marked, as we found that even "non-shrinking" line can shrink by as much as 10%. The flagging tape marking each 5-m section can be tightly tied on to the line and secured with thin strips of duct tape; if divers are having trouble spotting the flags, the rope can be spray painted 0.5–1 m before each marker.

Two additional holes should be drilled near the outer margins of the spool lids to hold the stake in place when transporting the MUTT. This will prevent the line from unwinding when swimming with the device, and works well as a handle for carrying it on land.

The clips used to hold the slates on the MUTT are made from 1", 200 psi PVC. A 1/2" slot is cut along the entire length using a cutting wheel on a drill, and the piece attached with PVC cement and screws. We highly recommend that at least one of the slates has a compass and depth gauge attached, and that each diver carry a spare pencil.

FIELD PROCEDURES

Metrics

Before diving, the possible metrics should be listed on the slates (Fig. B.2) on separate lines using a permanent marker. More space should be allotted for those species or groups that may be extremely abundant (e.g., sea pens, bivalves). It is critically important that all divers involved in the survey be familiar with the organisms or be paired with a partner who can render assistance if questions arise.

There are a number of benthic marine macroinvertebrate species (metrics) that might make good indicators of soft-bottom benthic degradation at particular sites, and we have listed and profiled those species (Appendix B.2). However, in our experience many of the possible metrics were not useful for reasons of abundance, visibility, etc. At this time, the following appear to hold the most promise as metrics for use in evaluating marine soft bottoms in the 10–30 m depth range:

Total # of species
species/segment
Shannon's Diversity
Total # of individuals
# of individuals per segment
% of individuals as the most abundant taxa
% segments with bivalves present
% of segments w/*Beggiotoa*
% of segments w/food pellets
% of segments w/rare species
% of segments with sea cucumbers
% of species as *Cancer sp.*
% of species as Cnidarians
% of species as Crustaceans
% of species as Echinoderms
% of species as mobile
% of species as predators

DEPLOYMENT OF THE MUTT

Whenever possible, transects should be done while moving into the current for maximum visibility. At the desired depth the stake is pushed firmly into the sediment at about a 60° angle, typically about half of its length but variable depending on the consistency of the substrate; it must be anchored firmly enough to pull line off of the reel but not so deep that it cannot be dislodged by a hard pull on the other end. Once the stake is in place the MUTT is rolled forward until the first flag appears, at which point the divers begin the visual survey (Fig. B.3). All target invertebrates >~1 cm in length or diameter are recorded with hatch marks on the slates, and at the end of each 5 m section a line is drawn to mark off that section on the slate. If large numbers of a particular species are present, it is usually easier to maintain a running mental count for each section and record the number rather than using hatch marks. Only organisms having half or more of their body within the transect are counted, and colonial animals such as hydroids, sponges, and bryozoans are not counted and only noted if abundant. The presence of *Beggiotoa* and food pellets is also noted; algae is not recorded.

The MUTT can be rolled over relatively firm substrates, but on exceptionally silty bottoms any contact between the spool and the sediment will obscure the 5 m markers. Under these conditions the MUTT is best "flown" 15–20 cm above the bottom, with the divers controlling their buoyancy to minimize disturbance.

Divers should be positioned slightly toward the outside wheel, such that their angle of view makes it easy to watch for the 5-m markers and use their slate to push the MUTT forward. As soon as a marker comes off the reel the first diver to see it should stop the MUTT and give it a firm shake to get the other diver's attention. Once both divers have recorded their numbers and drawn a line on their slate, the transect is resumed.

When the end of the transect is reached, one diver dislodges the stake with a firm pull on the line. A long, steady pull works far better than sudden jerks, which tend to be absorbed by the springiness of the line. Once the stake is free, the second diver rewinds the line by spinning the spool (Fig. B.4) while the first diver feeds the line toward the spool and assures a fairly level wind. When fully rewound, the stake is once again planted and the process repeated.

Depending on the number of organisms present, an entire transect and rewinding takes 10–20 min, and two to four transects can be completed per dive at depths of 10 or 20 m. If time or air is limited, the final line retrieval can be done from the surface, but divers must take care not to get tangled in the line while ascending.

Data are transcribed onto sheets after each dive and the numbers double checked; the slates are then cleaned off with household cleanser or an eraser.

Figure B.3. UW diver demonstrating the use of the Metrics Underwater Transecting Tool (MUTT), survey width of 1 m; also shown is the modified slate with depth gauge, compass, and dive timer.

Figure B.4. Diver winding transect line by hand (center reel is free spinning), a 50 m line takes 3–4 min to rewind, but can be done on the surface.

LABORATORY PROCEDURES

Sediment Analysis

Sediment samples should be taken from three randomly selected locations along transects. Plastic jars (~120 cc) with lids are used to scoop the top 6–8 cm of sediment, after which they are tightly sealed, taking care not to lose any fine, flocculent material on the surface.

Samples should be spread out in aluminum pie tins and placed in a drying oven at 50°C for 24 hr, then shaken through a graded series of sieves for 15 min on a mechanical shaker. The contents of each sieve are then placed into tared containers and weighed to the nearest 0.01 g on an electrobalance.

QUALITY ASSURANCE/QUALITY CONTROL

QA/QC Control in the Field

Diver Training in the Use of the MUTT and the Identification of Nearshore Benthic Marine Life

Before using this protocol, divers should be required to participate in some form of diver training and be required to display reasonable accuracy and consistency of observation. One of the most important discoveries in the preliminary stages of this project was the need for consistency within and between diver observations. Statistical comparisons become irrelevant if the data collected are inaccurate, biased, or of inadequate detail.

Training should begin with a general familiarization of the species likely to be seen. Use of the "Metrics Species Guide" developed for this project as well as other field guides is recommended. Once a basic recognition is developed for the organisms, field trials are needed. Observations of beginning divers using this protocol demonstrated the need for good communication skills between dive teams as well as a thorough understanding of the protocol and sampling design. The dual-observer nature of the protocol is well suited for impromptu assistance from one diver to another, and whenever possible during training, more experienced divers/biologists should be paired with less experienced divers. Divers should continue to train in species recognition and count accuracy until such time that an inter-diver variability test shows no statistical difference between two divers (see next paragraph).

The inter-diver variability test should consist of a minimum of 600 m^2 of simultaneously surveyed area, with data recorded in 5-m sections to provide comparison units. Student t-tests (Zar 1984), $\alpha = 0.20$, should be performed for each species with more than 10 individuals represented in all transects (600 m^2). For each 50-m transect, every species seen at least once by either observer should be represented in the t-test by 10 pairs of observations (e.g., if a species was seen once by only one observer, t-test data for that transect should consist of nine 0,0 pairs and one 0,1). All species measures, total number of individuals, and number of species should pass at this α level; additionally there should be no difference between the total number of species seen by each observer. If the majority of these statistical results show no difference, the power of each test is ≥ 0.80, and no single measure failed at <0.05 , then the divers should be considered adequately trained.

Tests of inter-diver variability are not tests of accuracy. It is possible that a pair of divers will both be underestimating one or more type of organisms, such that although they are not accurately counting the species they are not significantly different from each other. The nature of the comparisons in this protocol merely test consistency between divers. To test diver sampling error would require use of high resolution video and other measures of true abundance (other sampling methods or many replicates by the same diver) that were beyond the scope of this project. Comparability among divers is adequate for the purposes of testing the protocol. Methods for testing the comparability of different dive teams still need to be developed.

Diver communication and consistency are highly important in data analysis. Measures such as number of species are easily affected by one diver recording organisms to a lower level of taxonomic classification. The data from the other diver must then be transformed to the same level of classification in order to be pooled and used for comparisons, which may result in the loss of some data.

QA/QC in the Laboratory and the Office

We found that the most common errors were in the transcription process. It was important for each diver to transcribe his/her own notes onto a data sheet (see Figure B.5). This eliminated any errors in misinterpretation of a species name or count. Additionally, we found that a subsequent check of all data entered into the computer against the original data sheets, by a third party, eliminated further errors. Duplicates of all data are stored separately in both paper and diskette form.

After each dive, the divers compared species identifications and any questionable taxonomic identifications were reduced to a less specific taxa. For example, if a diver saw a rare nudibranch but was not positive about the species identification, that species would perhaps be transcribed as "unidentified nudibranch," with a note as to the original proposed species identification.

DATA REPORTING REQUIREMENTS

Data reporting requirements should be defined by the project manager before the study design can be considered complete. It may be useful to report both raw and summarized data for particular project objectives.

Reports of raw data should include the following information for each sample:

Project name
Collection date
Station identifier (name, code)

Sampling gear (e.g., MUTT 1 + 1 m)
Transect number
Station depth
Transect attributes (transect divisions, distance covered, duration)
Species names and numbers seen
Sub-sampling method, if any (e.g., no counts when over 25 per unit area, etc.)
Remarks (e.g., abnormalities, disease, any QA/QC problems)

The recording sheet used for this project provided space for each diver to record observations from one 100-m transect divided into 5-m^2 units. The names of the most frequently observed species were preprinted on the recording sheet, with space available for additional species names (Figure B.5).

Standard computerized codes and formats should be followed to facilitate data management and exchange. The Puget Sound Ambient Monitoring Program has published a modified NODC format for use in Puget Sound (PSWQA 1988), and its use is recommended to facilitate the comparability of data from various studies in Puget Sound.

Diver:______________ Date:______________ Transect Number:______________
Location:____________ Time in:____________ Tide Height:___________
Depth:_____________ Time Out:______________

Species	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
Starfish																				
Crossaster																				
Dermasterias																				
Evasterias																				
Henricia																				
Hippasterias																				
Luidia																				
Mediaster																				
Pisaster																				
Pycnopodia																				
Solaster dawsoni																				
Solaster stimpsoni																				
Crabs																				
Cancer gracilis																				
C. magister																				
C. productus																				
Pagurus sp.																				
Clams																				
Horse clam																				
Panope																				
Piddock clam																				
Nudibranchs																				
Armina																				
Dirona																				
Hermissenda																				
Tritonia																				
Anemones																				
Metridium																				
Pachycerianthus																				
Stomphia																				
Urt. columbiana																				
Urt. crassicornis																				
Miscellaneous																				
Eupentacta																				
Pandalus danae																				
Parasticopus																				
Polinices																				
Ptilosarcus																				
S. droebachiensis																				

Figure B.5. Recording sheet used for this project. Species listed are those most commonly seen at our reference sites. Space is provided for one 100 m transect with 5 m^2 sections recorded.

APPENDIX B LITERATURE CITED

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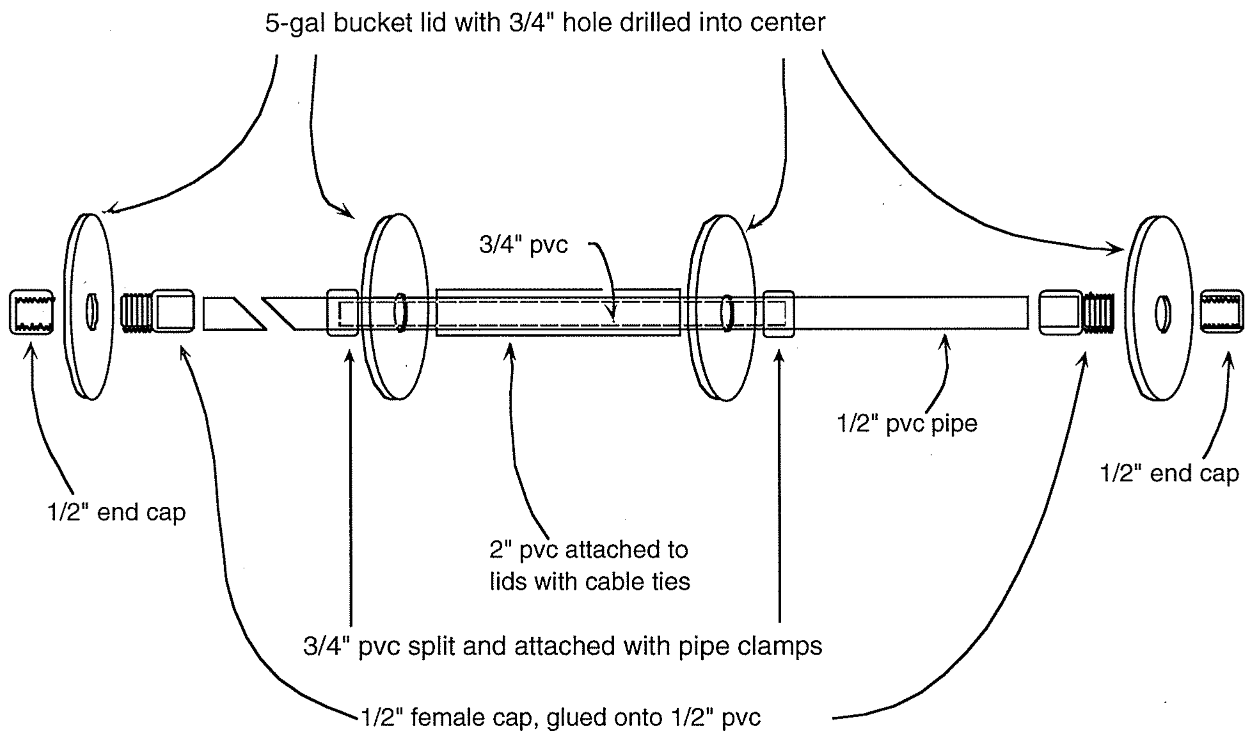
APPENDIX B.1

DETAIL OF THE METRICS UNDERWATER TRANSECTING TOOL



Length of between-wheel segments is 1 m. A transect line of any length can be attached to the center reel. All wheels, including the center reel, are free spinning. Total length is 2.25 m; the device is negatively buoyant.

APPENDIX B.2

PROFILE OF POTENTIAL METRICS TO USE IN RAPID EVALUATION COMPARISONS

Crossaster papposus	**Rose star**
Identification	Usually has 8–11 arms; dorsal surface with concentric rings of bright colors. Diameter to 0.3 m.
Life History	Spawning peaks in March–April, producing large eggs that hatch into nonfeeding larvae (Strathmann 1987).
Distribution	Circumpolar, extending south to Washington; intertidal to 1200 m (Lambert 1981).
Trophic interactions	Feeds primarily on nudibranchs; also eats small sea pens (Birkeland 1974). Preyed upon by *Solaster dawsoni*.
Metrics	
Advantages	Fairly large, easily identified.
Disadvantages	Often not very abundant.

Dermasterias imbricata	**Leather star**
Identification	A five-armed sea star with a smooth, slimy surface and leathery texture; color a mottled reddish brown, often with greenish markings. Diameter to 0.3 m.
Life History	Probably spawns in spring and summer, producing planktotrophic larvae (Strathmann 1987).
Distribution	Sitka, Alaska to La Jolla, California; intertidal to 91 m (Lambert 1981).
Trophic interactions	Feeds primarily on anemones but also preys on sea cucumbers, ascidians, and even sea urchins (Lambert 1981). It is eaten by *Solaster dawsoni* (Morris et al. 1980).
Metrics	
Advantages	Easily identified.
Disadvantages	Usually uncommon on soft substrates.

Evasterias troschelli	**Mottled star**
Identification	A five-armed sea star that is extremely variable in color, but usually mottled. Arms long, slender, with slightly "pinched in" appearance at their junction with the oral disk. Diameter to about 0.5 m.
Life History	Small eggs free-spawned March to June, producing planktotrophic larvae (Strathmann 1987).
Distribution	Pribilof Islands to Monterey, California; intertidal to 71 m (Lambert 1981).
Trophic interactions	Feeds primarily on bivalves and barnacles (Mauzey et al. 1968). Preyed upon by *Solaster dawsoni*.
Metrics	
Advantages	Large, easily identified.
Disadvantages	Somewhat incidental on soft bottoms; primarily found on cobble and on pilings.

Hippasteria spinosa	**Spiny red star**
Identification	A bright red, extremely spiny five-armed sea star. Diameter to ~0.3 m.
Life History	Probably free-spawns large eggs in spring/summer; larvae are non-feeding (Strathmann 1987). Juvenile recruitment extremely limited because they require small sea pens, which are very patchy in distribution (Birkeland 1974).
Distribution	Kodiak, Alaska to southern California; 10–512 m (Lambert 1981).
Trophic interactions	Feeds almost exclusively on the sea pen *Ptilosarcus*, selecting the largest specimens. Adults have no known predators (Birkeland 1974).
Metrics	
Advantages	Large, easily identified.
Disadvantages	Distribution patchy because entirely dependent on beds of adult sea pens.

Luidia foliolata	***Sand star***
Identification	A five-armed, slender, flexible sea star that is often partially buried in the sediment. Color brownish or gray; diameter to 0.6 m.
Life History	Reproductive season not known; eggs small and produce planktotrophic larvae (Strathmann 1987).
Distribution	Southeast Alaska to San Diego; intertidal to 613 m (Lambert 1981).
Trophic interactions	Feeds mostly on brittle stars in Oregon (Carey 1972) but primarily a predator on clams and sea cucumbers in Puget Sound (Mauzey et al. 1968).
Metrics	
Advantages	Large species typical of soft bottoms.
Disadvantages	Sometimes difficult to see due to burial.

Mediaster aequalis	**Vermilion star**
Identification	A very stiff, flattened, bright red or orange sea star with five broad arms; diameter to 0.2 m.
Life History	Spawns primarily in spring; large eggs produce non-feeding larvae that settle on *Phyllochaetopterus* tubes; can delay settlement at least 14 mo if the preferred habitat is not available (Birkeland et al. 1971).
Distribution	Chignik Bay, Alaska to southern California; 0–293 m (Lambert 1981).
Trophic interactions	Omnivorous, feeding on sea pens and other sessile animals and also on drift algae; also feeds on surface deposits and detritus from mud (Mauzey et al. 1968).
Metrics	
Advantages	Large and common on soft bottoms. One of the few shallow-water, macroscopic surface deposit feeders in our area.
Disadvantages	Easily confused with a relatively rare species, *Gephyreaster swifti*, which is a pale pinkish-orange in color but can appear similar when viewed at depth.

Pisaster brevispinus	**Giant pink star**
Identification	A large (to 0.64 m) five-armed sea star. Invariably pale pink color.
Life History	Probably spawns locally in spring and summer. Eggs are small, producing planktotrophic larvae (Strathmann 1987).
Distribution	Sitka, Alaska to Santa Barbara; intertidal to 102 m (Lambert 1981).
Trophic interactions	Feeds primarily on clams; is able to dig up large clams such as geoducks and horseclams, which are inaccessible to other species of sea stars (Van Veldhiuzen and Phillips 1978).
Metrics	
Advantages	Very large and easily distinguished from other sea stars; typical of soft bottoms.
Disadvantages	None.

Pycnopodia helianthoides	**Sunflower star**
Identification	A large, soft-bodied sea star with 15–24 arms; color variable. Diameter to 0.8 m.
Life History	Small eggs are free-spawned in March-July (Strathmann 1987)
Distribution	Aleutians to San Diego, from the low intertidal to 435 m (Lambert 1981).
Trophic interactions	Preys upon a wide range of organisms including clams (Paul and Feder 1975), urchins, and sea cucumbers; specimens on sand reportedly feed primarily on the barnacle *Balanus crenatus* (Mauzey et al. 1968). Adults largely immune from predators except for the sea star *Solaster dawsoni.*
Metrics	
Advantages	Large, easily identified, high trophic level.
Disadvantages	None.

Solaster dawsoni	**Morning sun star**
Identification	A uniformly light brown to pale orange sea star with 8–13 (usually 11–12) arms; diameter to 0.4 m.
Life History	Free-spawning in March-June; planktonic larvae.
Distribution	Alaska to Monterey; intertidal to 420 m (Lambert 1981).
Trophic Interactions	Feeds on a variety of other sea stars. Believed to be a keystone species in sea pen beds, where it controls the populations of *Mediaster* and *Crossaster* which could otherwise decimate the beds. The spiny red star *Hippasteria* is believed to benefit since it is entirely dependent on sea pens and is not preyed upon by *S. dawsoni* (Birkeland 1974).
Metrics	
Advantages	Large and easily identified, it is especially important in its role as a tertiary predator.
Disadvantages	Relatively uncommon on soft bottoms. Birkeland (1974) found densities of only 1 per 400 m^2 in sea pen beds near Golden Gardens in Seattle.

Solaster stimpsoni	**Sun star**
Identification	A ten-armed sea star, orange or yellowish in color with a blue or purple stripe running down each arm. Diameter to 0.46 m.
Life History	Spawns fairly large eggs in March-June, which develop into planktonic larvae that may not feed (Strathmann 1987). Juveniles found among tubes of the polychaete *Phyllochaetopterus*.
Distribution	Bering Sea to Oregon; intertidal to 610 m (Lambert 1981).
Trophic interactions	Predator on sea cucumbers, primarily *Cucumaria*, and is itself the favored prey of its congener, *S. dawsoni*.
Metrics	
Advantages	Large, easily identified.
Disadvantages	Primarily found on rocky bottoms.

Parastichopus californicus	***California sea cucumber***
Identification	Color typically a dark reddish-brown; dorsal surface darker and covered with conical projections. Length to 0.6 m.
Life History	Eggs and sperm are free-spawned in late spring through summer (Cameron and Fankboner 1986). Planktonic development lasts from 65–125 d, with larvae settling out at a length of 0.25 mm (Cameron and Fankboner 1989). The gut, gonad, circulatory system, and respiratory trees are lost by atrophy every year in the fall (Fankboner and Cameron 1985) and regenerated 1–3 mo later (Swan 1961).
Distribution	British Columbia to Baja California (Morris et al. 1980).
Trophic interactions	A surface deposit feeder that uses sticky tentacles to collect detritus (Cameron and Fankboner 1984). Adults are probably preyed upon by the sunflower star *Pycnopodia*, whose contact elicits a "swimming" response from the cucumber (Margolin 1976). Supports a small commercial fishery in Puget Sound.
Metrics	
Advantages	Large; easily identified; does not bury.
Disadvantages	Commercially fished.

***Metridium* spp.**	**Plumose anemone**
Identification	Distinguished by its dense, frilly tentacles. Color generally white, brown, or orange. Generally called *M. senile*, but probably a complex of 3 different species (Strathmann 1987); the large form typically found on soft bottoms in deep water is undescribed.
Life History	*M. senile* reproduces both sexually and asexually (forming clones by pedal laceration); *M. exilis* by binary fission; and the undescribed *Metridium* sp. reproduces sexually, spawning eggs in late summer (Strathmann 1987).
Distribution	Polar seas to Santa Catalina Is., California; to at least 30 m (Gotshall and Laurent 1980).
Trophic interactions	Filter feeder on plankton and detritus; occasionally captures large jellyfish. Preyed on by the sea star *Dermasterias* and the nudibranch *Aolidea papillosa*; small specimens eaten by kelp greenling (G. Jensen, Univ. Washington School of Fisheries, Seattle, WA, pers. obs.).
Metrics	
Advantages	Large, easily identified.
Disadvantages	Requires some object or structure to attach to.

Pachycerianthus fimbriatus	**Burrowing anemone**
Identification	Extremely long, slender, graceful tentacles, light colored and often with brownish bands. Can retract into a tube that may extend above the sediment.
Life History	Reproduction has not been studied. Larvae found in spring plankton (Strathmann 1987). Life span may be 10 yr or more (Morris et al. 1980).
Distribution	British Columbia to San Diego, California (Gotshall and Laurent 1980).
Trophic interactions	Feeds primarily on zooplankton, and is eaten by some nudibranchs (esp. *Dendronotus*) (Wobber 1970).
Metrics	
Advantages	Common, typical organism of local soft bottoms; fairly large; easily identified.
Disadvantages	Could be missed if retracted.

Stomphia coccinea	**Swimming anemone**
Identification	An orange or white and orange anemone with stout, short tentacles.
Life History	Unknown.
Distribution	Common throughout Puget Sound (pers. obs.).
Trophic interactions	Predator on invertebrates and fish. Well known for its "swimming" response to being touched by the leather star *Dermasterias*, a predator of sea anemones (Robson 1961).
Metrics	
Advantages	Easily identified.
Disadvantages	Often somewhat small; more typical of rockier areas and only found attached to objects.

Urticina columbiana	**Sand rose anemone**
Identification	The largest anemone surveyed in this study, reaching a diameter of over 0.3 m. Column usually has shell fragments adhering to it and is reddish with rows of white beads. Tentacles long, graceful, and white or pale pink in color.
Life History	Unknown.
Distribution	British Columbia to Baja California; 12 to 45 m (Gotshall and Laurent 1980).
Trophic interactions	Predator on fish and invertebrates.
Metrics	
Advantages	Large; easily identified; typical of soft bottoms.
Disadvantages	Never very abundant.

Urticina crassicornis	**Christmas anemone**
Identification	Tentacles fairly short and stout, with pointed tips and some banding. Column extremely variable in color, from solid yellow to bright red with green markings.
Life History	Appear to spawn April-May; larvae planktonic and do not feed until 2 wk after settlement (Strathmann 1987).
Distribution	Alaska to Carmel, California; intertidal and subtidal (Morris et al. 1980).
Trophic interactions	Predator on small fish and invertebrates. Preyed on by the leather star *Dermasterias* and the nudibranch *Aolidea papillosa*.
Metrics	
Advantages	Large, easily recognized.
Disadvantages	Primarily found in rocky areas and relatively uncommon on sand/mud; requires hard object to attach to.

Ptilosarcus gurneyi	**Sea pen**
Identification	A type of soft coral forming tall, fleshy, yellowish-orange colonies; unmistakable in form.
Life History	Eggs are free-spawned in March and April (Birkeland 1969) and develop into nonfeeding, demersal larvae (Chia and Crawford 1973) that settle on sand. "Individual" sea pens live 10–15 yr (Birkeland 1974).
Distribution	Gulf of Alaska to southern California; 8–70 m (Gotshall and Laurent 1980).
Trophic interactions	Filter-feeder on small zooplankton. Preyed upon by several species of sea stars and nudibranchs, some which specialize on sea pens (Birkeland 1974).
Metrics	
Advantages	Large, easily identified; typical soft-bottom form.
Disadvantages	Periodically retracts into the sediment, irrespective of time or conditions (Birkeland 1974).

Polinices lewisii	**Moon snail**
Identification	Large, rounded snail with a white shell that is largely obscured by the animal when it is active; although the foot can be four times the volume of the shell it can be fully retracted once all the water has been expelled.
Life History	Eggs are fertilized using stored sperm, and laid between two layers of sand in rubbery "sand collars" deposited primarily from April-September. Larvae are weak swimmers and settle on *Ulva* within a day of leaving the collar (Strathmann 1987).
Distribution	Vancouver Island to Baja California; intertidal to 150 m (Morris et al. 1980).
Trophic interactions	Predator on clams, which are drilled (Bernard 1967, Hopper 1972).
Metrics	
Advantages	Large, easy to identify.
Disadvantages	Can be overlooked when buried; usually more abundant in very shallow water (low intertidal).

Armina californica	**Striped Armina**
Identification	Dorsal surface smooth, with longitudinal light stripes on a dark background. Length to 50 mm.
Life History	Egg masses found year-round (Morris et al. 1980)
Distribution	Vancouver Island to Panama; shallow subtidal to 230 m.
Trophic interactions	In Puget Sound feeds exclusively on sea pens. No known predators; although *Solaster* and *Crossaster* have been observed attacking this species, they "freeze" in mid-attack and allow the nudibranch to crawl away (Birkeland 1974).
Metrics	
Advantages	Fairly large, easily identified.
Disadvantages	Can be very inconspicuous when buried; distribution entirely dependent on sea pen beds.

Dirona albolineata **White-lined Dirona**

Identification Dorsal surface covered with large, translucent, leaf-like cerata with white edges. Length typically to about 40 mm but can get much larger.

Life History Eggs found December-January and March-August (Strathmann 1987).

Distribution Vancouver Island to La Jolla, California; intertidal to 37 m (Morris et al. 1980).

Trophic interactions Primarily a predator on small snails, which are cracked and eaten (Robilliard 1971). They are preyed upon by *Crossaster*.

Metrics

Advantages Easily identified.

Disadvantages Small; seems to be somewhat seasonal in appearance.

Hermissenda crassicornis **Opalescent nudibranch**

Identification A small nudibranch with prominent frontal "horns" and numerous long, slender cerata on the dorsal surface, usually with orange or gold tips. Blue lines along side of foot and down midline. Length to 80 mm.

Life History Mating and egg laying occurs year-round in Puget Sound; egg strings are often attached to algae or eelgrass (Morris et al. 1980). The larval period lasts at least 34 d but can be extended if the hydroids they settle on are not available; the postlarval lifespan averages 130 d (Harrigan and Alkon 1978).

Distribution Sitka, Alaska to Baja California; intertidal to 35 m (Morris et al. 1980).

Trophic interactions A predator and scavenger, feeding on a wide range of organisms including its own species. Considered a significant predator on sea pens in Puget Sound. Preyed upon by the rose star, *Crossaster* (Birkeland 1974).

Metrics

Advantages Easily identified.

Disadvantages Small, somewhat seasonal (most abundant in summer [Birkeland 1974]).

Tritonia diomedea	**Diomedes' triton**
Identification	Color pink or pinkish-white. Frontal margin with numerous projections; two prominent rows of gills down back. Length to 150 mm (Behrens 1980).
Life History	Spawns year-round but primarily in the spring and summer. Larvae planktotrophic, settling out in 34–41 d in the laboratory; sexual maturity is reached in 272 d (Kemph and Willows 1977).
Distribution	Aleutian Islands, Alaska to Panama (Behrens 1980).
Trophic interactions	Feeds on sea pens (Strathmann 1987) and is probably preyed on by the rose star *Crossaster*.
Metrics	
Advantages	Easily identified; typical soft-bottom species.
Disadvantages	Appears to be seasonal and has been overcollected in some areas.

Panope abrupta	**Geoduck**
Identification	Only the tip of the siphon is visible in situ. It can be distinguished from the horseclam or gaper by its smooth, clean appearance; it lacks the horny plates present on the horseclam which tend to have algae, hydroids, or other organisms growing on them. The geoduck's siphons also do not have the small "tentacles" at the openings like those of horseclams.
Life History	Free-spawning March-July (Andersen 1971), producing planktotrophic larvae that settle after 3–5 wk (Goodwin and Bease 1989). Sexual maturity reached in 3–4 yr at a shell length of 100 mm (Washington Department of Fisheries/Washington Department of Natural Resources 1985). Average age of 40–60 yr; maximum reported 146 yrs. (Goodwin and Bease 1989).
Distribution	Southeastern Alaska to Baja California; low intertidal to 110+ m (Goodwin and Bease 1989).
Trophic interactions	Suspension feeder on phytoplankton and detritus. Juveniles eaten by crabs, fish, and sea stars; adults immune to most predators but some dug up by the large pink sea star *Pisaster brevispinus* and the sunflower star *Pycnopodia* (Sloan and Robinson 1983).
Metrics	
Advantages	Common on soft bottoms.
Disadvantages	Easily overlooked when retracted into sediment, and also cannot be distinguished from horseclams when retracted.

Tresus capax **and** *T. nuttallii*	**Gaper**
Identification	Only siphon tips visible. Recognized by horny pads (often with attached organisms) at end of siphon and small "tentacles" at openings of siphon.
Life History	*T. capax* free-spawns during February–May (Bourne and Smith 1972); *T. nuttallii* year-round but primarily in summer months (Clark et al. 1975). Larvae settle in 30–60 d; sexual maturity is reached at about 70 mm and 3 yr of age (Bourne and Smith 1972; Clark et al. 1975). Maximum life span 15–20 yr (Wendell et al. 1976.).
Distribution	*T. capax* from Kodiak Island, Alaska to San Francisco; *T. nuttallii* British Columbia to Baja California. Intertidal to 30 m.
Trophic interactions	Suspension feeders on phytoplankton and detritus. Preyed on by large sea stars, *Cancer* crabs, and moon snails (Wendell et al. 1976).
Metrics	
Advantages	Common on soft bottoms.
Disadvantages	Hard to see and impossible to identify when retracted into sediment.

Cancer gracilis	**Graceful rock crab**
Identification	Often mistaken for a small Dungeness crab. Can be distinguished by the lack of serrations on the upper surface of the claw and the white edging on the carapace teeth. Carapace width to 91 mm.
Life History	Mating occurs in summer and fall, followed by egg extrusion in winter and hatching in the spring (Knudson 1964). Larvae pass through five zoeal stages (Ally 1975); megalopae are often found in association with large jellyfish.
Distribution	Prince William Sound, Alaska to Baja California; intertidal to 174 m (Morris et al. 1980).
Trophic interactions	Feeds primarily on small barnacles and bivalves (Hart 1982), and is eaten by many species of fish. Occasionally taken by sport fishermen.
Metrics	
Advantages	Typical of muddy bottoms; high trophic level.
Disadvantages	Confusion with *C. magister*; sometimes runs when approached; can be inconspicuous when buried although it doesn't usually bury as deeply as *C. magister*.

Cancer magister	**Dungeness crab**
Identification	A large, light brown crab with white tipped claws bearing serrations along the upper margin. Carapace width rarely exceeds 190 mm.
Life History	Mating occurs in spring and early summer (MacKay 1942); females extrude and fertilize eggs in the fall using stored sperm. Eggs hatch in late winter, with larvae passing through five planktotrophic zoeal stages before settling out as a megalopae (Poole 1966).
Distribution	Pribilof Islands, Alaska to Santa Barbara, California; intertidal to 230 m (Morris et al. 1980).
Trophic interactions	Adults feed primarily on clams but also prey on smaller crustaceans and fish (Butler 1954). In turn, they are eaten by octopus and a variety of fish species, and support important commercial and sport fisheries.
Metrics	
Advantages	Large and easily identified; typical of sandy substrates.
Disadvantages	Often buries in the sediment leaving only the eyes and antennae exposed; when not buried they tend to run when approached; commercially exploited.

Cancer productus	**Red rock crab**
Identification	Distinguished by its brick-red coloration and the black fingers of its claws. Carapace width to 180 mm.
Life History	Mating occurs in spring and summer; eggs are extruded in the fall and winter and hatch in the spring (Knudson 1964; Strathmann 1987). Larvae pass through five zoeal stages (Trask 1970) before settling out as megalopae in July-August (Strathmann 1987).
Distribution	Kodiak, Alaska to San Diego, California; intertidal to 79 m (Morris et al. 1980).
Trophic interactions	Predator on clams and other mollusks; also feeds on barnacles and smaller crabs. Commonly sought by sport fisherman, and is also eaten by otters, octopus, and cabezon and other fish.
Metrics	
Advantages	Large and easily identified; high trophic level.
Disadvantages	As noted for the other two *Cancer* crabs, it too tends to bury but usually remains more visible than the other species.

Pagurus armatus **Blackeyed hermit**

Identification
One of the largest and most common of our local hermits, this species typically inhabits moon snail shells and is easily distinguished by its large, erect black eyes.

Life History
Planktonic larvae are released in the spring; passing through four zoeal stages before settling out as a megalopae.

Distribution
Unalaska, Alaska to San Diego, California; intertidal to 146 m (Hart 1982).

Trophic interactions
Primarily a surface deposit feeder but an occasional scavenger and predator. Predators unknown but probably include octopus and large *Cancer* crabs.

Metrics

Advantages
Easily recognized and common; one of the few large deposit feeders.

Disadvantages
Small juveniles are usually abundant and can easily be overlooked, especially when partially buried.