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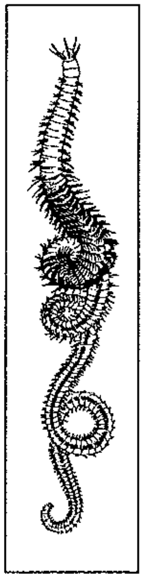
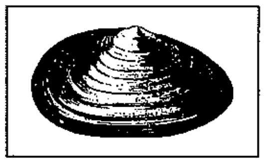
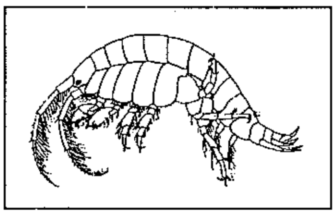
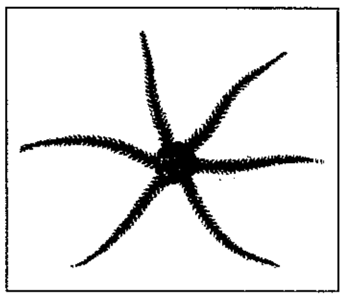
ENVIRONMENTAL SERVICES

Recommendations for Assessing Adverse Benthic Effects in Puget Sound









Prepared for

Washington Department of Ecology
Sediment Management Unit
Olympia, Washington

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Dr. Thomas Ginn served as moderator at the National Benthic Experts Workshop, which was conducted as part of this study. The following individuals were members of the experts panel at the workshop:

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Dr. Peter Chapman	EVS Consultants, Vancouver, British Columbia
Dr. Robert Diaz	Virginia Institute of Marine Science, Glouster Point, Virginia
Dr. Jeff Hyland	Arthur D. Little, Boston, Massachusetts
Ms. Nancy Musgrove	Roy F. Weston, Inc., Seattle, Washington
Dr. Rick Swartz	U.S. Environmental Protection Agency, Newport, Oregon
Mr. Bruce Thompson	Aquatic Habitat Institute, San Francisco, California

INTRODUCTION

Regulatory assessments of adverse environmental impacts are frequently based on the monitoring of benthic invertebrates. These sediment-dwelling organisms, being sedentary, respond to both short-term acute and long-term chronic anthropogenic stresses (e.g., dredging, contaminated sediments) at a single geographic point and are useful indicators of environmental conditions. Collection of data on the abundance and community composition of benthic organisms has therefore been an important component in Puget Sound monitoring and discharge permitting studies as well as remedial investigations. Regulatory and monitoring programs within Puget Sound that rely on the use of benthic invertebrates for impact assessment include the Washington Department of Ecology (Ecology) Sediment Management Standards source control and cleanup programs, the Puget Sound Ambient Monitoring Program, the Puget Sound Dredged Disposal Analysis (PSDDA) program, and the U.S. Environmental Protection Agency (EPA) Superfund and Urban Bay Action programs.

Analysis and interpretation of benthic invertebrate data can be complicated, however, by several factors. Abundance and species composition of benthic communities exhibit an inherent natural variability in both space and time. Year-to-year and seasonal changes in the abundance of benthic organisms and species composition can be dramatic. Significant differences in the benthic communities of two locations separated by only a few hundred meters can also occur. The detection of adverse effects associated with anthropogenic activities can be obscured by these naturally occurring differences in benthic community composition. In many cases, knowledge of the sensitivity or tolerance of individual species to specific contaminants is not known. Information on the presence or absence of indicator species or pollution-sensitive species could be a valuable tool in assessing impacts, but detailed analytical studies, which are required to identify sensitive or indicator species, have not been widely performed in Puget Sound. An additional complicating factor in the use of benthic invertebrates as indicators of adverse environmental impacts is the absence of consistent, broadly accepted methods for analyzing benthic community data. Investigators of benthic communities have used widely different approaches to analyzing benthic data, reflecting their specific study objectives or personal preferences. These differences extend from the use of different endpoint variables (e.g., total abundance, major taxa abundance, species abundances, species richness, derived indices) to the use of divergent statistical methods (e.g., graphical comparisons, analysis of variance [ANOVA], multivariate techniques).

This report is the result of a joint effort by EPA, the Puget Sound Water Quality Authority, and Ecology to address the issues that complicate the use of benthic communities as indicators of adverse environmental impacts. The specific objectives of this study are 1) to identify and evaluate the technical adequacy of selected methods used to assess adverse effects on benthic communities in marine sediments, 2) to evaluate the current interpretive methods used in Puget Sound programs to identify adverse effects, and 3) to develop recommendations for improving the current benthic interpretation methods used in Puget Sound programs to identify adverse effects.

To meet these objectives a workshop composed of nationally recognized benthic ecologists was held on February 25, 1993. Prior to the workshop, the available information on methods used for identifying adverse benthic effects was compiled (Weston 1993; see Appendix A). Recommendations for improving the techniques used in Puget Sound programs to interpret benthic community data were provided by the experts panel (see Appendix B).

REGULATORY USE OF BENTHIC COMMUNITY DATA

Numerous studies have been conducted in Puget Sound during the last 30 years in which benthic invertebrate data have been collected and analyzed (Appendix A, Table 1). If the benthic data were collected for regulatory programs, these data sets were generally assembled by collecting 4–5 replicate van Veen grab samples at stations located within the study area and at a reference location. Benthic organisms were enumerated either to the major taxa level (e.g., molluscs, crustaceans, annelids, echinoderms) or to the species level. Determinations of adverse benthic effects have been based on pair-wise statistical comparisons between each study area station and a reference location. If any study area station demonstrated a 50-percent reduction in the abundance of any major taxon relative to the reference area and if the reduction was statistically significant (t-test, $\alpha < 0.05$), then the station was considered to be adversely affected.

Historical establishment of the 50-percent reduction criteria was based on several factors. First, previous studies have shown naturally occurring 2-fold changes in the abundance of major taxa over a 1-year seasonal cycle. Therefore, decreases in abundance that are >50 percent are considered to be outside the range of natural seasonal variability. Second, statistical power analyses of historical benthic data show that, with five replicate samples per station and an α value of ≤ 0.05, the minimum difference that can be detected in major taxa abundance between a study area and a reference station is generally about 50 percent. Except in cases of very low natural variability, detecting changes of <50 percent would require more than five replicate samples and would thereby increase the overall cost of the study.

The basis for using major taxa as the endpoint for assessing adverse benthic effects has been the need for a reliable screening tool that is broadly applicable in a regulatory context. Using major taxa abundance has the following advantages:

- **Unbiased taxonomic identifications**—Not all taxonomists agree on the identification of all species. In addition, some families of organisms can consistently be identified to the species level while other identifications depend on the level of expertise of the taxonomist. There are frequent disagreements about whether to use only data identified at the species level in assessments of adverse effects or to also include those data sets that have been identified at the taxonomic level of family or order. It is easy, however, to place organisms into major taxa categories. By using major taxa as an endpoint, consistency across studies can be achieved.

- **Amenable to statistical comparisons with reference conditions**—Major taxa abundance can be compared statistically with reference conditions using either *t*-tests or ANOVA. In some habitats, the abundance of all but a few dominant species is very low, with individual species absent from most of the replicate samples. The number of replicate samples needed to statistically detect reductions in the abundance of these species is prohibitively large. By selecting major taxa, an endpoint that can be consistently applied in all habitats and communities is available.

- **Clear indication of adverse effects**—Whereas there can be considerable technical or regulatory disagreement about whether the reduction in the abundance of a single species is significant, few can argue that the reduction in abundance of a major taxon is not an adverse effect.

Currently, several benthic sampling programs in Puget Sound have adopted the 50-percent reduction approach to screening for adverse benthic effects. These programs include the Ecology Sediment Management Section, Urban Bay Action Programs in Elliott Bay and Everett Harbor, and PSDDA. Specific details on each of these programs are included in Appendix A.

A survey of benthic interpretation methods that are used in other states was conducted as part of a study by Weston (1993) (Appendix A). EPA Region 9 (California), the Southern California Coastal Water Research Project, the California Water Resources Control Board, the Florida Department of Environmental Regulations, the Virginia Water Control Board, and scientists at Old Dominion University and Virginia Institute of Marine Sciences were contacted and asked to provide details on the methods that they are currently using to analyze benthic invertebrate data. No two organizations followed the same procedures, and none of the organizations had adopted the approach used in Puget Sound regulatory programs (i.e., if there was a 50-percent reduction in major taxa abundance, then an adverse effect had occurred). However, all the organizations contacted did identify benthic samples to the lowest possible taxonomic level (usually species level). The analytical techniques used by the out-of-state organizations varied from cluster and discriminant function analyses to nonparametric statistical trend analyses. Some programs also relied on single-value indices, such as diversity.

CASE STUDY OF HISTORICAL DATA

As a preparatory step to the benthic workshop, the historic benthic data sets for Puget Sound were studied. Analysis of benthic community data in many of these historical studies relied on commonly used statistical methods to identify trends or impacts (e.g., ANOVA among stations or over time using community indices such as indicator species abundance, individual species abundance, total abundance, major taxa abundance, biomass, infaunal index [Word 1979, 1980], species richness, and species diversity and dominance). Many of the studies in the historical data sets also relied on comparing potentially impacted stations with reference areas to identify adverse impacts.

Based on the results of the agency survey, selected statistical methods were applied to a specific data set from Puget Sound to test the effectiveness of each method or index to identify adverse impacts. Analytical methods for evaluating benthic community indices are described in Appendix A. The results of the Puget Sound case study provided the basis of the discussions among experts at the benthic workshop.

REVIEW OF EVERETT HARBOR CASE STUDY

Analytical methods were selected in Weston (1993) to apply to the historical data set from Puget Sound. The analytical methods were used to test the effectiveness of each method or index to identify adverse impacts. Numerous historical data sets were appraised for use as a case study for the expert panel's discussions. The data set from the 1986 Everett Harbor Action Program investigation (PTI and Tetra Tech 1988a) was selected for the case study. Benthic infaunal data were collected from six stations located along a transect in the East Waterway of Everett Harbor. Habitat characteristics within the study area were not homogeneous. Because habitat attributes can affect benthic community structure, stations were grouped by their relative similarity in percent sediment fines for some of the subsequent analyses to help detect potential contaminant effects on the benthos.

Reference area data were collected from three stations at Port Susan during the PTI and Tetra Tech (1988a) study. These reference area sediments, which were primarily coarse-grained, were compared with the Everett Harbor stations that were composed of coarse-grained sediments. Because the Everett Harbor samples did not have homogeneous grain-size, a fourth reference station, which was sampled during the 1985 Elliott Bay Action Program (PTI and Tetra Tech 1988b), was also included in the case study to represent reference communities at fine-grained habitats.

Richness, total abundance, major taxa abundance, relative abundance of pollution-tolerant and sensitive taxa, community composition based on numerically dominant taxa, the infaunal index, and several measures of diversity were used in Weston (1993) as indicators of deleterious changes in community structure. ANOVA and multivariate techniques were used to interpret whether the changes were due to anthropogenic activities in Everett Harbor. An in-depth discussion of the case study is provided in Appendix A (Weston 1993).

EVALUATION OF THE EVERETT HARBOR CASE STUDY RESULTS

All statistical methods and indices used to evaluate differences among stations in the case study prepared by Weston (1993) appeared to be adequate in detecting differences between highly stressed communities versus unstressed communities, but some techniques provided more objective measures of community health for moderately stressed benthic communities. Comparisons of results using different indices are presented in Table 1.

Results of the Everett Harbor case study indicated that the benthic communities at inner harbor stations (Stations EW-01, EW-04, and EW-07) were highly stressed relative to outer harbor stations (Stations EW-10, EW-12, and EW-14) and Port Susan reference areas. Station EW-10 appeared to be moderately stressed relative to reference, and Stations EW-12 and EW-14 appeared to be relatively unstressed. (A map of station locations is provided in Appendix A.) The results of this case study evaluation were supported by the sediment bioassays conducted during the PTI and Tetra Tech (1988a) investigation.

TABLE 1. COMPARISON OF TEST RESULTS IN IDENTIFYING ADVERSE BENTHIC EFFECTS

	Conclusion Based on Numeric Criteria				Conclusion Based on Best Professional Judgment						
Station	Total	Crustacean Abundance	Mollusc Abundance	Polychaete Abundance	Richness	H′	J and D	SDI	ITI	Pollution-Sensitive Taxa (%)	Pollution-Tolerant Taxa (%)
EW-01	*	<50%*	<50%*	<50%*	*	stressed	stressed	stressed	stressed	stressed	stressed
EW-04	*	*	<50%*	*	*	stressed	stressed	stressed	stressed	stressed	stressed
EW-07	*	<50%*	<50%*	<50%*	*	unstressed	moderately stressed	moderately stressed	stressed	stressed	moderately stressed
EW-10	NS	*	<50%*	*	*	stressed	stressed	stressed	stressed	stressed	stressed
EW-12	*	*	NS	NS	NS	unstressed	moderately stressed	moderately stressed	unstressed	unstressed	unstressed
EW-14	NS	*	<50%*	NS	NS	unstressed	unstressed	unstressed	unstressed	stressed	unstressed

Source: Weston (1993)

Note:
- * - significantly different compared with reference
- <50% - less than 50 percent of the reference abundance
- NS - not significantly different compared with reference

EXPERTS PANEL DELIBERATIONS AND TECHNICAL ISSUES

A panel of benthic ecology experts was assembled to address the technical issues related to detecting adverse benthic effects in Puget Sound. Key criteria used in selecting the members of this panel were that they 1) have been recognized as national experts in the field of benthic invertebrate ecology, 2) have prior experience in evaluating adverse environmental impacts on benthic invertebrate community structure, and 3) have demonstrated abilities to integrate technical information into a regulatory setting. The six panelists were:

- **Dr. Peter Chapman** (EVS Consultants, Vancouver, British Columbia)—Dr. Chapman's has a doctorate in benthic ecology. He serves as an advisor to both the United States and Canadian federal governments for environmental biomonitoring assessment policy and protocol. Dr. Chapman co-developed the internationally accepted sediment quality triad concept for determining pollution-induced degradation in aquatic habitats. He has published numerous papers on taxonomy and benthic ecology and is a peer reviewer for journals in this subject area.

- **Dr. Robert Diaz** (Virginia Institute of Marine Science, Glouster Point, Virginia)—Dr. Diaz has a doctorate in benthic ecology. He specializes in the application of numerical methods in benthic ecology. Dr. Diaz has managed and performed more than 30 studies that evaluated adverse effects on marine benthic organisms on the eastern and gulf coasts of the United States. In addition, Dr. Diaz has published more than 50 papers that deal with marine ecology.

- **Dr. Jeff Hyland** (Arthur D. Little, Boston, Massachusetts)—Dr. Hyland has a doctorate in biological oceanography. Dr. Hyland's research interests include statistical methodology as applied to benthic invertebrate sampling and the interpretation of natural vs. anthropogenic patterns. He has been the associate director of marine studies at the University of California at Santa Barbara and was the project leader for benthic studies in the Santa Maria Basin off the California coast.

- **Ms. Nancy Musgrove** (Roy F. Weston, Inc., Seattle, Washington)—Ms. Musgrove's degree is in fisheries. Her master's degree was based on studies of Puget Sound benthic invertebrates. She has been active in the analysis and interpretation of benthic invertebrate data in Puget Sound for 10 years. Ms. Musgrove is the author of Weston (1993).

- **Dr. Richard Swartz** (EPA, Newport, Oregon)—Dr. Swartz has a doctorate in marine science. Dr. Swartz is familiar both with environmental conditions within Puget Sound and national issues related to the use of benthic invertebrates as indicators of environmental conditions. He has conducted studies extending over many years on the effect of marine discharges on the benthic invertebrates of the Southern California Bight.

- **Dr. Bruce Thompson** (Aquatic Habitat Institute, San Francisco, California)—Dr. Thompson has a doctorate in benthic ecology. He has participated in numerous studies investigating the effects of point-source discharges on the benthic infauna of coastal marine waters. Dr. Thompson recently worked with a team of experts to evaluate the effectiveness of multivariate statistical techniques in defining areas of adverse environmental conditions on the southern California coastline.

The experts panel was convened to provide recommendations for improving the methods used in programs in the Puget Sound region to interpret benthic community data. Discussions among the six panelists focused on the identification of adverse effects and advantages and disadvantages of key benthic interpretive methods. The objectives of the workshop were as follows:

1. Identify and summarize the technical methods used in regulatory programs to assess benthic community effects
2. Evaluate the adequacy of effects endpoints and analytical methods with respect to identifying benthic effects
3. Provide recommendations for improving the selection, analysis, and interpretation of benthic effects endpoints used in the management of Puget Sound sediments.

Two types of technical issues were addressed by the experts panel. The first issue was the selection of suitable benthic endpoints. Candidate endpoints identified in Weston (1993) and by the experts panel were endpoints that are based directly on the total number of benthic invertebrates in samples (i.e., indicator species abundance [both pollution-sensitive and pollution-tolerant species], individual species abundance, total benthic invertebrate abundance, major taxa abundance, and biomass) and endpoints that are computed or derived from the abundance counts (i.e., infaunal index, species richness, and species diversity and dominance). The second issue considered by the panel was the selection of appropriate data analysis methods that should be applied to the selected endpoint(s).

Overall panel discussions and the conclusions reached by the experts panel regarding each of the above items are described in the following sections. Results from case studies performed by applying the above-mentioned endpoints and several analytical techniques (Appendix A, pages 33–37) are included.

BENTHIC ENDPOINTS

Nine benthic endpoints are described in Weston (1993). These endpoints were modified and discussed by the experts panel. A discussion of these benthic endpoints is provided in the following sections. A summary of the evaluation criteria and candidate endpoints is shown in Table 2. The evaluation criteria that were applied to benthic endpoints were as follows:

- **Sensitivity**—Can the method be used to correctly discern areas with adversely affected benthos?
- **Objectivity**—Is individual judgment required for interpretation of the results?
- **Ease of Interpretation**—Can the index or results be related to an effects endpoint that is interpreted the same by everyone? Is it understandable in a regulatory context?
- **Reference Area Comparison**—Is a defined reference area required for comparison?
- **Cost**—What is the relative cost and the cost effectiveness of the index or analytical method?

The overall rating (high, medium, low) of each endpoint relative to these evaluation criteria is shown in Table 2. The overall ratings reflect an attempt to reach consensus among the entire experts panel. In many cases, there was divergence of opinion regarding the usefulness of specific endpoints. Accordingly, each expert was requested to submit their individual rating of endpoints for inclusion in Appendix B. The range of individual ratings from all experts is also provided in Table 2.

A distinction was made during the workshop between primary endpoints (i.e., those that are calculated and interpreted as a primary means of interpreting data) and secondary endpoints (i.e., those endpoints that contribute information to the analysis of adverse effects, but are more supportive or confirmatory). Each endpoint was assigned to one of these two categories when there was general agreement among the panelists.

Indicator Species Abundance

Indicator species represent a specific subset of all species present which, because of *a priori* considerations, are important indicators of environmental conditions. These species can include those organisms that are known to be pollution-sensitive or pollution-tolerant or those organisms that could be expected to occur in nonimpacted habitats. For interpretative purposes, the presence of pollution-tolerant species is not as important as the presence of pollution-sensitive species or species that are normal inhabitants of unpolluted habitats. The use of indicator species as a benthic endpoint

TABLE 2. COMPARISON OF BENTHIC COMMUNITY MEASUREMENTS WITH EVALUATION CRITERIA

	Evaluation Criteria[a]								
	Sensitivity		Objectivity		Ease of Interpretation		Reference Area Comparison	Cost Effectiveness	
Endpoint	Range[b]	Overall[c]	Range[b]	Overall[c]	Range[b]	Overall[c]	Overall[c]	Range[b]	Overall[c]
Direct Measurements									
Indicator species abundance[d]	H to H+	H	H[e]	H	H– to H	H	Yes	M to H	M
Individual species abundance	M to H+	H	M+ to H	H	M to M+	M+	Yes	M to H	M
Total abundance	M– to H	M	M– to H	M	M– to H	M+	Yes	M– to H	H
Major taxa abundance	L to M	M	L to M	M	L to M+	M+	Yes	M to H	H
Biomass	L to H	M	M– to M	M	L to H	M	Yes	L to M	L+
Indices									
Infaunal index	M to H+	H	M– to H	H–	L to M+	M	Yes	M to H+	H
Species richness	M to H	H	M to H+	M	M to H	M+	Yes	L to H–	H
Species diversity and dominance	L to H[f]	L	L to H[f]	L	L– to H[f]	L	Yes	L– to H[f]	L

[a] Codes used by panel members to rate benthic community measurements:

L - low
M - medium
H - high.

[b] Range of individual ratings provided by experts panel in their written comments (see Appendix B).

[c] Overall rating reflects the consensus reached by the experts panel at the workshop.

[d] Includes both pollution-tolerant and pollution-sensitive species.

[e] No range; all experts agreed on the same rating.

[f] High rating assigned to species dominance; no high ratings assigned to species diversity.

is prevented or is not possible in Puget Sound because of the limited database on which of the indigenous species are sensitive to pollution and the absence of widely supported groups of species considered to be typical of Puget Sound habitats. Currently, there is no list of indicator species for Puget Sound, unlike other areas of the United States (e.g., southern California). Development of a list for Puget Sound will require the identification of species that are indicators for organic enrichment, contamination from metals and organic compounds, and physical disturbances. Additional work remains to be done before candidate indicator species can be associated with specific environmental stresses in Puget Sound. Analysis of existing data could provide a substantial first step in this process. The national benthic experts rated indicator species abundance data high for every evaluation criteria except cost effectiveness.

Individual Species Abundance

Enumeration of all taxa to the species level (or lowest possible taxonomic level) provides considerably more information than total or major taxa abundance, particularly if the sensitivity of species to environmental stress or its association with a habitat is known. While data on the abundance of all species can be used directly for analysis and interpretation, several endpoint indices are calculated using the abundance of some or all species occurring within a sample. The experts panel strongly recommended enumerating benthic samples to the species level. Some experts suggested analytical methods that would directly use the data for all species (e.g., classification, ordination), while other experts would base analyses on endpoints derived using species data (e.g., species richness indices, dominance, infaunal index). Information on the derived indices is provided below. Because of the large amount of data obtained to assess the species-level abundance endpoint, the experts panel considered this endpoint to be highly sensitive and objective. Because species-specific knowledge is required in interpreting the data, the endpoint received a moderate rating for ease of interpretation from the panel. The additional cost of identifying samples to the species level resulted in a moderate score for cost effectiveness.

Total Abundance

The total abundance of benthic invertebrates is easily assessed. All of the members of the experts panel agreed that total abundance should be one of the primary parameters assessed during studies of benthic effects. However, total abundance should be used in conjunction with at least one other index. Important changes and adverse effects could go undetected if total abundance were used alone. For example, two stations may have identical total abundances, but the species composition of the two locations may be dramatically different (as illustrated by the case history data for Everett Harbor; see Appendix A). Nevertheless, total abundance remains a relatively quick and easy indicator of differences among benthic invertebrate communities. The national benthic experts generally rated total abundance as moderate in terms of sensitivity, objectivity, and ease of interpretation. Cost effectiveness was rated high for this endpoint because identification and enumeration of individual species is not required.

Major Taxa Abundance

Quantitative assessments of the abundance of major taxonomic groups (e.g., molluscs, crustaceans, amphipods, polychaetes) can provide more information than total abundance alone. Some major taxonomic groups such as crustaceans or amphipods are generally considered to be more sensitive to organic enrichment or toxic contaminants than other major taxa. Yet even within major taxonomic groups, some species may be tolerant of stress while other species are sensitive. Compensatory changes in the abundance of individual species within a major taxon can therefore yield the same overall abundance and obscure important shifts in community composition. The experts panel recommended that if major taxa abundance is used by regulatory agencies, additional endpoints should also be measured to increase overall sensitivity. As with total abundance, experts generally rated major taxa abundance as moderate in terms of sensitivity, objectivity, and ease of interpretation and high relative to cost effectiveness.

Biomass

Biomass is typically represented as the wet weight of organisms living within a unit area. Some assessment techniques measure biomass as a function of depth within the sediment. The sampling techniques required for vertically partitioned samples are relatively expensive. Another complication is the occasional presence of a large organism (e.g., a bivalve) in a sample. Because insufficient biomass data were available when the case studies were prepared, biomass was not considered as an endpoint in Weston (1993). Some panel members considered biomass to be an important benthic endpoint, while others did not believe biomass should be considered as a relevant endpoint in the determination of adverse benthic effects. The panel members had widely varying opinions (i.e., low to high; see Table 2) on the sensitivity of this endpoint. Biomass elicited the widest range of opinions from the experts.

Infaunal Index

The infaunal index (Word 1979, 1980) is based on an algorithm which mathematically combines the abundance of selected organisms that belong to predefined groups (based on reported feeding strategies) into a single value ranging from 0 to 100. Originally developed for southern California fauna, the infaunal index has been modified for Puget Sound species. The expert panel's assessments of the infaunal index show the index to be highly sensitive, objective, and cost effective. A weakness of the index is that best professional judgment must be used to assign organisms into species groupings. With the continued validation of taxa group assignments for Puget Sound, this index could be highly useful to assess adverse effects. The experts panel rated the ease of interpretation for this index as moderate, based on the apparent subjective nature of determining a critical value between 0 and 100 to delineate adverse effects.

Species Richness

Species richness (i.e., the number of species occurring in a unit area) was considered to be one of the more important endpoints. In the case of stations located along a gradient of organic enrichment, models have been developed that are based on species richness to describe the relative level of environmental effects. The experts panel considered the sensitivity of species richness to be high. However, because of the need for expert knowledge relative to applicable models relating richness and pollutant stress, the experts panel rated objectivity and ease of interpretation as moderate. The consensus among the experts was that species richness is a primary endpoint for the consideration of benthic effects. The case studies provided in Appendix A show that species richness is a powerful indicator of adverse effects to benthic communities.

Species Diversity and Dominance

Diversity indices such as H′ provide a single value that reflects both the number of species present in a sample (species richness) and the distribution of individuals among those species (evenness or dominance). The experts panel agreed that diversity indices are often not useful because the resulting values can be misleading. For example, two stations can have the same or similar diversity, but in fact have very different benthic communities. There may be no direct relationship between diversity values and degree of environmental stress experienced by benthic communities. Dominance indices measure the relative numerical contribution of the most abundant species to the overall abundance of benthic invertebrates. Several panel members indicated that dominance was an important secondary measure in assessing benthic effects. Limitations of dominance are linked to interpretation of the endpoint in areas of moderate stress. Diversity and dominance scored low relative to all evaluation criteria.

ANALYTICAL TECHNIQUES

The selection of analytical techniques appropriate for the detection of adverse benthic effects is dependant on the specific hypothesis being tested, the sampling design, and the endpoints being measured. Two general groups of analytical techniques exist. Univariate tests encompass *t*-tests and ANOVA, as well as the nonparametric equivalents of these tests. Multivariate tests include numerical classification (e.g., cluster analysis) and ordination (e.g., principal components analysis). The primary distinction between these two basic groups is the number of dependant variables that are simultaneously being considered. Univariate tests are performed on a single variable (e.g., total polychaete abundance or the abundance of a single polychaete species), and multivariate tests are performed using two or more variables (e.g., the individual abundance of each polychaete species). These analytical techniques are not mutually exclusive. The experts panel generally recommended that simple univariate tests be used as a primary screening tool followed by the multivariate tests when exploratory analysis is desired to determine the causes of observed patterns in endpoint values.

One benthic expert (Dr. Bruce Thompson; see Appendix B) discussed the value of performing multivariate analyses first. By performing an ordination analysis and then testing for significant differences among the ordination scores using an ANOVA, significant differences among station groupings can be detected. This approach has been used to analyze benthic invertebrate data from the Southern California Bight. In these studies, only duplicate samples at a station were required to elucidate changes in benthic infaunal patterns. However, this method is very dependent on establishing reference community characteristics.

SUMMARY OF EXPERTS PANEL FINDINGS

The experts panel resolved that total abundance and species richness were the most commonly used benthic indices. The experts also expressed that it is important to compare the affected area to a suitable reference area (see Dr. Robert Diaz, Appendix B, for possible exception). There was no clear consensus reached by the experts relative to all methods and the above evaluation criteria with the exception that species diversity was not considered a useful endpoint. Variability was evident in the expert panel's ratings of the benthic community measurements (i.e., low to high; see Table 2). In general, most of the experts recommended the use of multiple methods to determine effects, and all agreed that identification to the lowest taxonomic level (preferably species level) was necessary. Most of the experts also stated that statistical hypothesis testing using *t*-tests and ANOVA should be the first step in data analyses. Subsequent analyses may be more exploratory and use multivariate statistical techniques. A summary of the individual experts' recommendations is shown in Table 3 and described below:

- **Dr. Chapman**—Dr. Chapman recommended that species richness, total abundance, species-level abundance, numerical dominance (e.g., top few dominant taxa by station grouping), and biomass be used in regulatory management decisions. Dr. Chapman recommended that the following data analysis approaches be used: regression of richness on abundance, ANOVA followed by linear orthogonal contrasts,[1] and various other multivariate approaches (e.g., cluster techniques, ANOVA analyzing principal components). Dr. Chapman said that an integrative assessment approach should be used for benthic studies (e.g., the sediment quality triad, which combines sediment chemistry and toxicity testing with benthic community structure data). Dr. Chapman stated that the use of appropriate reference data is key to regulatory interpretation of benthic community data at suspected impacted sites. He suggested that it would be useful to define reference conditions in Puget Sound for benthic data. Dr. Chapman suggested that it may be possible to do this by examining existing benthic community data sets. Dr. Chapman supported the expert panel's decision that species-level taxonomy should be used.

- **Dr. Diaz**—Dr. Diaz reiterated that there is a need to study both the biological and physical components of an ecological system prior to the selection of the appropriate field or statistical method. It is essential

[1] Linear orthogonal contrasts are *a priori* tests performed after an ANOVA to test for specific differences among groups (stations).

TABLE 3. RECOMMENDATIONS OF BENTHIC EXPERTS

Expert	Recommended Endpoints	Analytical Approach	Comments
Dr. Chapman	Species richness Total abundance Species abundance Dominance Biomass	ANOVA with linear orthogonal contrasts, regression of species richness on abundance.	Use benthos in conjunction with sediment chemistry and bioassays.
Dr. Diaz	Depends on questions being asked. Biomass and species richness should be considered.	Depends on questions being asked. Consider methods that do not use mean values and that do not rely on a normal distribution.	Important that critical scales of temporal and spatial variability are considered in approach. Design program based on questions that need to be answered
Dr. Hyland	Species richness Infaunal index	Univariate tests (*t*-test, ANOVA) followed by multivariate tests (e.g., ordination)	Approach should focus on relationship between biological and other environmental variables. Assessing adverse effects should control for both spatial and temporal variability.
Ms Musgrove	Species richness Total abundance Dominance Infaunal index	Combination of univariate and multivariate tests.	Need to identify Puget Sound reference conditions.
Dr. Swartz	Species richness Infaunal index Indicator species Species abundance Dominance	Univariate tests followed by multivariate tests.	Additional data sets from Puget Sound should be analyzed to assist in selecting appropriate endpoints.
Dr. Thompson	Species richness Total abundance Biomass Indicator species	Classification and ordination with hypothesis testing of ordination scores.	Identification of Puget Sound reference condition should be a priority.

Note: ANOVA - analysis of variance

that the scales of variation (spatial and temporal) for a given system are clearly understood. Specific hypotheses should be developed (i.e., what questions are being answered) prior to the selection of endpoints and analytical techniques. Dr. Diaz stated that no single sampling design or list of indices or analyses will answer all possible questions. Much of the problem in assessing benthic effects is related to sampling designs that are not appropriate for the questions being asked. Dr. Diaz concluded that what is most crucial in statistical testing is to control for those factors that influence benthic organisms but are not of interest in detecting adverse effects (e.g., water depth and grain size). Use of traditional endpoints and analyses will optimize comparability to historical data but may not be as efficient as alternative analytical methods. Effort should be expended exploring analytical approaches such as non-parametric statistics, logistic regression, odds ratio, and loglinear modeling. Dr. Diaz supported the expert panel's decision that species-level taxonomy should be used.

- **Dr. Hyland**—Dr. Hyland stated that the criteria for biological change should be based on indices other than just abundances of major taxonomic groups. Decisions as to whether or not a biological effect has occurred should be based on a combination of sensitive and meaningful indices, including those based on numbers of species, abundances of individual taxa, and the distribution of individuals among important functional groups (e.g., the infaunal index). Dr. Hyland suggested incorporating approaches that look at relationships between biological and other environmental variables, so that biological patterns reflecting possible among-site or among-time differences can be examined. He also stated that the decision-making framework should stress, wherever possible, the need to examine change at a suspected impacted site in comparison with both spatial and temporal controls. Dr. Hyland commented that pollution tolerance should not be confused with opportunism. Dr. Hyland supported the expert panel's decision that species-level taxonomy should be used.

- **Ms. Musgrove**—Ms. Musgrove recommended that multiple benthic endpoints be used in assessing impacts on the aquatic environment. Ms. Musgrove endorsed the use of the following endpoints: species richness, community composition, dominance, and the infaunal index. She also stated that measures of abundance (especially total abundance) are useful screening tools. Benthic communities in Puget Sound display a large natural variability in space and time and are subjected to a wide range of physical and chemical factors (e.g., currents, salinity regimes, sediment deposition, erosion patterns, wave energy). Ms. Musgrove suggested that resources be allocated to further characterize reference community assemblages and habitat characteristics so that the southern California interpretive techniques could be applied to Puget Sound. Ms. Musgrove also recommended that the application of multivariate techniques (including hypothesis testing using ordination scores) be evaluated using Puget Sound data. In addition, she stated that if indices were based on higher levels of taxonomic organization, then family or order groupings should be used instead of class or phylum. Ms. Musgrove supported the expert panel's decision that species-level taxonomy should be used.

- **Dr. Swartz**—Dr. Swartz stated that no single index allows a thorough understanding of benthic alterations. A combination of richness, indicator species, infaunal index, species-level abundance, dominance, and numerical classification provides a comprehensive benthic assessment. Dr. Swartz suggested that the efficacy of the indices proposed at the workshop should be tested on 4–6 existing, representative data sets from Puget Sound. He contended that the example of comparative analyses that was provided in Weston (1993) could be applied to the other data sets. Dr. Swartz supported the expert panel's decision that species-level taxonomy should be used.

- **Dr. Thompson**—Dr. Thompson stated that the best measurements for use in benthic monitoring are species richness, total abundance, biomass, abundances of key indicator species, and the results of classification and ordination analyses. These parameters could be tested statistically against appropriate reference conditions for compliance purposes. Dr. Thompson recommended that multivariate ordination be used instead of the infaunal trophic index. He felt that multivariate ordination is a well-founded, published methodology and does not require that a subjectively chosen species be included *a priori*. Dr. Thompson suggested that the Ecology Ambient Monitoring Program database should be analyzed to determine if reference conditions and indicator species can be defined for various habitats in Puget Sound. Dr. Thompson supported the expert panel's decision that species-level taxonomy should be used.

RECOMMENDATIONS

Although a wide range of opinions were expressed by the experts panel, several specific recommendations for the improvement of benthic effects assessments emerged. These recommendations include the following:

1. Benthic invertebrate samples should be identified and enumerated to the lowest possible taxonomic level. Although this is more costly than simply performing major taxa identifications, the level of increased information from the more detailed analysis justifies the increased expense.

2. More than one benthic endpoint should be used to assess adverse benthic effects. Species richness and total abundance should be considered for inclusion with major taxa abundance as primary benthic endpoints. These endpoints could be used immediately without the need for further study.

3. Although other secondary endpoints (i.e., indicator species abundance and individual species abundance) were rated very high, these endpoints cannot be adopted immediately by Puget Sound programs because organisms whose presence or absence is indicative of anthropogenic stresses need to be identified *a priori*. Additional studies should therefore be implemented to expand on the case studies presented in Appendix A. Central to these studies should be the refinement of indicator species lists and the infaunal index for Puget Sound. Data collected by the Ecology Ambient Monitoring Program provide an excellent base for performing these analyses.

4. Identification of reference conditions for benthic invertebrates in Puget Sound is desirable. Benthic data from noncontaminated sites can be valuable in identifying areas where benthic invertebrates are adversely affected by anthropogenic effects. Examination of existing data sets could yield sufficient information to define reference conditions.

5. Univariate statistical tests (*t*-tests and ANOVA) should be performed to compare the study area and reference conditions. If an ANOVA is performed, *a posteriori* contrasts should be used do determine significant differences from reference conditions.

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APPENDIX A

Evaluation of Techniques for Assessing Benthic Endpoints for Use in Puget Sound Sediment Management Programs

EVALUATION OF TECHNIQUES FOR ASSESSING BENTHIC ENDPOINTS FOR USE IN PUGET SOUND SEDIMENT MANAGEMENT PROGRAMS

Prepared for:

Washington Department of Ecology

U.S. Environmental Protection Agency
Region X

Puget Sound Water Quality Authority

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EVALUATION OF TECHNIQUES FOR ASSESSING BENTHIC ENDPOINTS FOR USE IN THE PUGET SOUND SEDIMENT MANAGEMENT PROGRAM

1.0 INTRODUCTION

The Washington State Department of Ecology (Ecology) is responsible for the identification and management of contaminated sediments in Puget Sound. Ecology was mandated by various state regulations to develop marine, estuarine, and freshwater sediment quality criteria to ensure the protection of environmental and human health. Sediment quality standards were promulgated in April 1991 under the Washington State Sediment Management Standards (SMS; Chapter 173-204 WAC) and currently include biological and chemical criteria.

Biological effects endpoints, including those based on benthic community indices, are used by a number of regulatory or resource management programs in Puget Sound including the multi-agency Puget Sound Dredged Disposal Analysis (PSDDA) program, Ecology's Ambient Monitoring Program, and the federal Superfund program. In response to the availability of the sediment management standards, these programs have incorporated, to varying degrees, the SMS biological and chemical criteria into their interpretation of environmental data or as decision criteria.

1.1 SMS Benthic Criteria

Under the sediment management standards, biological criteria were developed for acute and chronic sediment toxicity tests including amphipod mortality, oyster or echinoderm larvae abnormality and mortality, juvenile polychaete growth, and benthic community alterations. Criteria for evaluating changes in benthic community structure are based on changes in major taxa (polychaetes, molluscs, crustaceans) abundance relative to abundance in reference areas. If any one taxa group has fewer than 50 percent of the mean number of individuals found in a reference area **and** is statistically different from reference, then this result is considered evidence of an adverse biological impact and the test sediment fails the criteria.

1.2 SMS Benthic Sampling Design

Under the sediment management standards, collection and analysis of benthic community samples must follow the guidance provided in the Puget Sound protocols (Tetra Tech, 1987a). Limited guidance regarding sampling design is provided in these protocols; very specific guidance is provided for sample collection, handling, and quality control/quality assurance criteria. It was recognized that the number of locations sampled and level of sample replication would vary according to the objectives of each investigation, but it was recommended that replicate samples (minimum of three) be collected at each sampling location if statistical comparisons among sites were to be conducted. A number of sampling devices are appropriate to use in benthic community studies, however the van Veen grab (0.06 or 0.10 m^2 surface area) is the most commonly used device in Puget Sound. Typically, organisms are separated from the sediment using a 0.5 mm or 1.0 mm mesh screen. Taxonomic resolution may vary by study objective, but most studies in Puget Sound have based analyses on the distribution and abundance of either major taxa or species.

1.3 Task Goals and Objectives

Ecology is currently evaluating selected methods for interpreting adverse effects on benthic infaunal communities. The overall goal of the evaluation process is to identify potential improvements to the use and interpretation of benthic data within the Sediment Management Program. The objectives of this report are to:

- Identify and summarize the technical and regulatory methods used to assess adverse benthic community effects in marine sediments in Puget Sound and coastal areas of the United States.
- Evaluate the adequacy of analytical methods with respect to identifying impacts.
- Provide recommendations regarding improvements to interpretation criteria and experimental design used in the assessment and management of Puget Sound sediments.

To meet these objectives, regulatory and resource management agencies throughout the United States were contacted and the typical sampling design and analytical methods for identifying impacts were described. Information on the specific criteria used to identify adverse impacts was also sought from these agencies. Based on the results of the agency survey, selected statistical methods were applied to a data set from Puget Sound to test the effectiveness of each method or index to identify adverse impacts. The results of the Puget Sound case study will provide the basis of the discussions among experts at the Ecology-sponsored workshop to be held in February. Final recommendations will be made following input from the workshop participants.

This report presents a brief background on the use of benthic communities in environmental monitoring, the history of the current effects criteria, the results of the agency surveys, case study analyses, and preliminary recommendations for topics of discussion among the experts.

2.0 USE OF BENTHIC COMMUNITY INDICES IN ENVIRONMENTAL MONITORING

Benthic community composition and abundance have been widely used in pollution impact studies. Long-term environmental monitoring programs in freshwater, estuarine, and marine environments have often relied upon indices of community structure to demonstrate the health of these ecosystems. Benthic communities are often ideal indicators of ecosystem health because they tend to integrate effects of both long-term and recent exposure to environmental stresses (e.g., contaminant discharge, dredging). In addition, many benthic organisms are sessile (nonmotile) and represent conditions at a specific location. Decreases in the number of taxa, shifts in community composition, and changes in abundance have all been documented responses to physical and chemical stresses in aquatic environments. The approach to sample collection, treatment, and interpretation of benthic community data has ranged from simple qualitative measures of presence/absence of indicator species to quantitative measures of community composition and abundance. Comparisons of potentially impacted communities with communities from reference areas or along physical/chemical gradients are often used to quantify the magnitude of change associated with the stresses the benthic community may have experienced.

2.1 Historical Use of Benthic Indices in Puget Sound

Benthic communities have been sampled as part of a major environmental investigations throughout Puget Sound over the last 20 years. A summary of the sampling design elements and geographic distribution of these studies is presented in Table 1 and Figure 1. The majority of these studies were based on a single sampling event and examined changes in benthic community composition as a function of contaminant concentrations in the sediments, habitat characteristics (e.g., sediment grain size or depth), or in comparison to reference communities. Several studies were of longer duration and provided seasonal baseline information prior to major construction projects in the marine environment (i.e., Seahurst and Duwamish Head Baseline Studies). The Puget Sound Ambient Monitoring Program is the most geographically widespread, sampling approximately 50 locations throughout northern, central and southern Puget Sound on an annual basis over the last four years. Several researchers (Lie 1968, 1974; Lie and Evans 1973; Nichols 1985, 1988) have sampled locations in the deep central basin on a regular basis over the last 30 years to look at long-term changes in community composition. Although sampling methods varied, most of these investigations collected replicate samples at each location to allow for statistical treatment of the data. Taxonomic resolution for the samples has been mixed, ranging from class to species level identification.

Analysis of benthic community data in these historical studies relied on commonly used statistical methods to identify trends or impacts including analysis of variance among stations or over time using community indices such as richness, species abundance, total infaunal abundance, diversity, dominance, and the infaunal trophic index (ITI; Word 1979). Many studies also relied on comparison of potentially impacted stations with reference areas to identify adverse impacts. Multivariate techniques such as cluster analyses were also used to identify similarities among benthic assemblages. In those studies concerned with adverse impacts, communities were generally considered impacted if abundance, richness, or other community indices were significantly different (usually less than) reference conditions or other sites that were sampled. Over the last 10 years, the use of a 50 percent reduction in major taxa abundance in comparison to reference abundance has been incorporated as a criterion for identifying adverse benthic effects.

Table 1—Summary of subtidal benthic data in Puget Sound

Survey/Source[a]	Location[b]	#Reps/Station	Taxa Identification	Sampling Dates	Relative Level of Contamination	PSEP Protocols[c]	Notes
Puget Sound Ambient Monitoring Program/Ecology (Tetra Tech 1990)	Sound-wide	5	Species	1989-1992	Low-high	Y	Sampled yearly at fixed and rotating stations; about 50 stations; 1mm sieve
Bellingham Bay/EPA (Broad et al. 1984)	A	2	Species	5/83 and 10/83	Low-moderate	C	Sampled once; 20 stations in bay and 2 reference stations in Samish Bay; 1.0mm sieve
Bellingham Bay/CH2M Hill (CH2M Hill 1984)	A	5	Species	10/83	Low	C	Sampled once; 14 stations; 1.0mm sieve
PSDDA Phase II Evaluation/ACOE (PTI 1989)	A,F	5	Major taxa	6/88	Low	Y	10 stations; 1.0mm sieve
PSDDA Phase I Evaluation/ACOE (PTI 1988)	B,D,E	5	Species	6/87	Low-moderate	Y	9 stations; 1.0mm sieve
Everett Harbor/U.S. Navy (Parametrix 1985)	B	5	Species	7/84	Low-high	Y	Sampled once; 9 stations
Everett Harbor/ACOE (U.S. ACOE 1985)	B	5	Species	2/85	Low-high	C	Sampled once; 11 stations
Everett Harbor Action Program/EPA (PTI and Tetra Tech 1988a)	B	5-10	Species	9/86	Low-high	Y	Sampled once; 20 stations; 0.5 and 1.0mm sieves
Annual outfall survey/Metro (Romberg 1992, pers. comm.)	C	5	Major taxa	1988, 1990, 1992	Low	Y	Sampled alternate years; 2 stations; 5 outfalls; 1.0mm sieve
Central Basin/USGS (Nichols 1988)	C	3	Species	1963-1988	Low	U	Sampled 1-2 times per year; 3 stations; 1.0mm sieve
Eagle Harbor Preliminary Investigation/Ecology (Tetra Tech 1986)	C	5	Species	1985	Low-high	C	Sampled once; 8 stations; 2 reference stations in Blakely Harbor
Eagle Harbor RI/EPA (CH2M Hill 1989)	C	5	Major taxa	3/88	Low-high	Y	Sampled once; 4 stations; 2 reference stations in Port Madison; additional observations using benthic camera sled
Puget Sound Benthic Infauna Study/USPHS (Lie and Evans 1973)	C	3-10	Species	1963, 1964, 1967, 1969	Low	U	4 stations

Survey/Source[a]	Location[b]	#Reps/Station	Taxa Identification	Sampling Dates	Relative Level of Contamination	PSEP Protocols[c]	Notes
Denny Way Cap/Metro (Romberg 1992 pers. comm.)	D	5	Species	1990-1992	Low	Y	Sampled annually in August; 2 stations; 1.0mm sieve
Duwamish Head Baseline/Metro (Stober and Chew 1984)	D	4	Species	7/84-10/84	Low	C	Sampled 3 times; 83 stations
Elliott Bay Action Program/EPA (PTI and Tetra Tech 1988b)	D	5	Major taxa and species	3/83	Low-high	Y	Sampled once; about 74 station and 4 reference stations; 1.0mm sieve
Seahurst Baseline Survey/Metro (Word et al. 1984)	D	2-5	Major taxa	4/82-12/83	Low-moderate	C	Sampled 7 times; 133 stations; 1.0mm sieve
Toxicant Pretreatment Planning Study/Metro (Comisky et al. 1984)	D	1-4	Species	9/81-8/82	Low-high	C	Sampled 3 times; about 70 stations; 1.0mm sieve
Asarco RI/FS/Asarco (Parametrix 1989a,b;1990)	E	5	Major taxa and species	1988-1990	Low-high	Y	Sampled once per year; 100 stations first year; selected stations in subsequent years; 1.0mm sieve
Commencement Bay RI/FS/Ecology and EPA (Tetra Tech 1985)	E	4	Species	3/84 and 8/84	Low-high	C	Sampled once; 48 stations; 6 reference stations in Blair Waterway; 2 reference stations in Carr Inlet; 0.5 and 1.0mm sieves
Simpson Cap/Simpson (Parametrix 1991)	E	5	Species	1989-1993	Low	Y	Annual sampling at 6 stations; reference station location varies; 1.0mm sieve
Sitcum Waterway/Port of Tacoma (Hart Crowser 1992)	E	4	Major taxa and species	1991	Low-high	Y	Sampled once; 18 stations; 0.5 and 1.0mm sieve

a PSEP = Puget Sound Estuary Program
Ecology = Washington State Department of Ecology
Metro = Municipality of Metropolitan Seattle
PSDDA = Puget Sound Dredged Disposal Analysis
USGS = U.S. Geological Survey
USPHS = U.S. Public Health Service
ACOE = U.S. Army Corps of Engineers
EPA = U.S. Environmental Protection Agency
RI/FS = remedial investigation/feasibility study

b See Figure 1 for regional locations

c Y = Sampling performed according to PSEP protocols
C = Sampling methods comparable to PSEP protocols
U = Sampling methods unknown

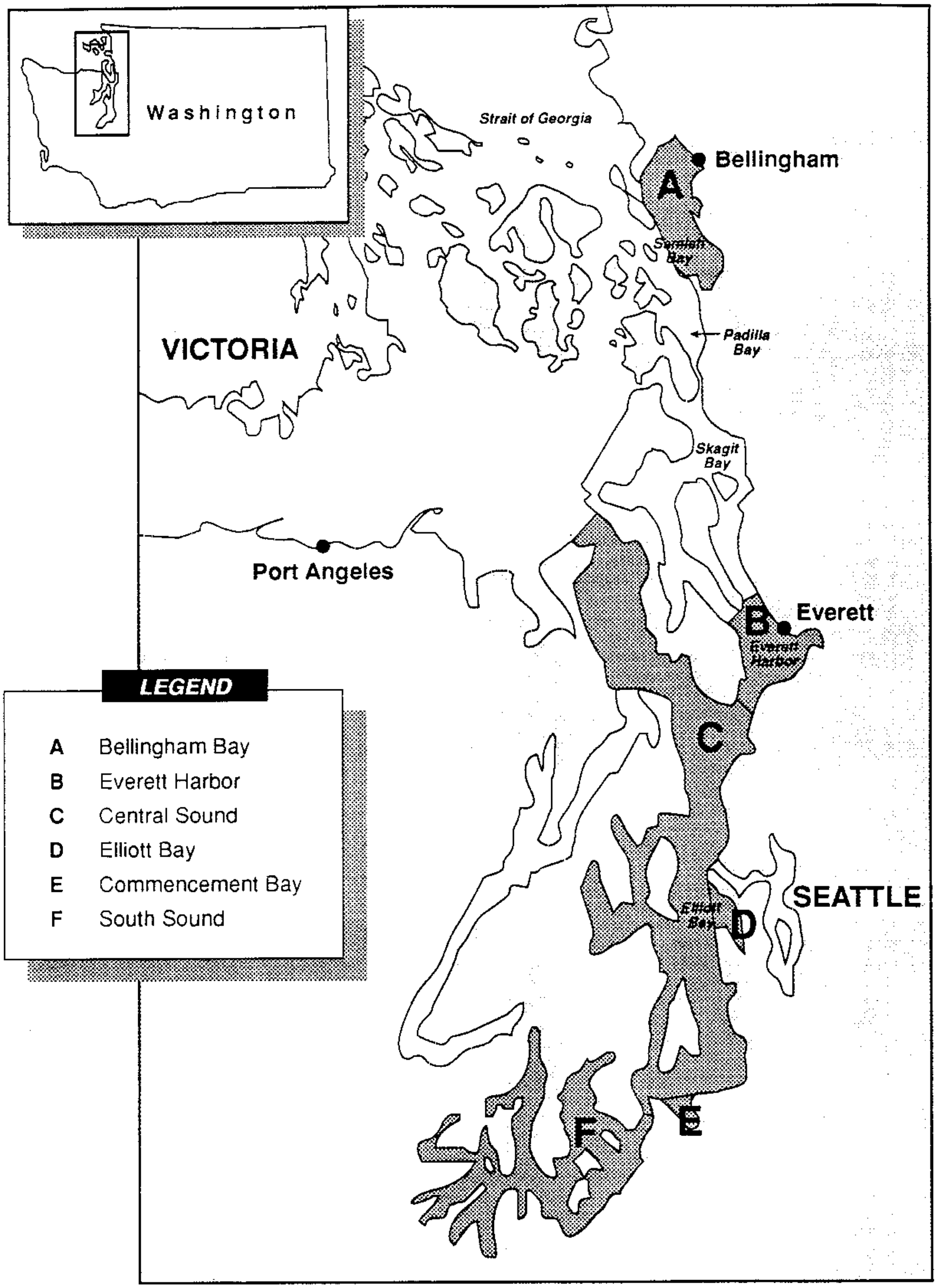

Figure 1 Generalized locations of benthic data in Puget Sound.

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2.2 Background for Selection of Benthic Effects Criteria

Selection of 50 percent reduction as the trigger for potential impacts was based on analysis of numerous benthic data sets by Tetra Tech and PTI Environmental Services in support of various federal and state programs in Puget Sound (e.g., Commencement Bay Nearshore/Tideflats Remedial Investigation, Puget Sound Estuaries Program). This guideline was derived partly from consideration of the natural variability of benthic infaunal communities. Based on a summary of seasonal data from Lie (1968), Nichols (1985), and Word et al. (1984), the abundance of major taxa may seasonally vary by a factor of 2 (i.e., the lowest mean abundance is roughly 50 percent of the highest mean abundance). Statistical analyses of major taxa abundance using five replicate samples generally can only accurately detect a change equivalent to 50 percent or more of the reference abundance. Using historical Puget Sound data, it was found that changes in abundance greater than 50 percent of the mean abundance were generally statistically significant, whereas smaller changes were not consistently detected as significant. Finally, the guideline of a 50 percent reduction was thought to provide a reasonable, cost-effective level of environmental protection for regulatory applications. However, it was recognized that lower criteria would provide greater protectiveness.

3.0 SUMMARY OF REGULATORY AND RESOURCE AGENCIES APPROACHES

Many historical Puget Sound programs used different analytical approaches and measurement endpoints to interpret benthic community alterations and decide whether or not changes were based on anthropogenic effects or natural causes. Regulatory and resource management agencies in the Puget Sound region have expended tremendous resources over the last 10 years to standardize the collection, analysis, and interpretation of environmental monitoring data. However, differences in analytical and interpretive approaches still exist among agency programs, reflecting different mandates or objectives.

Regional and national programs that measure benthic community indices as part of their environmental monitoring programs were contacted to gain information about how benthic communities were evaluated within each program and what decision criteria were used. The following section presents the results of telephone interviews and review of agency documents regarding use of benthic communities in environmental monitoring. An attempt was made to contact as many agencies as possible, but the list is not to be considered all inclusive. The general sampling design, community indices, and decision criteria are described for each program, when known. Puget Sound programs are described first, followed by other regional or national programs.

3.1 Puget Sound Programs

Washington State Department of Ecology
Sediment Management Program
Olympia, Washington
Mr. Brett Betts
(206) 459-6824

The Sediment Management Program was responsible for the development and implementation of narrative standards to protect or clean up marine sediments in Puget Sound. These sediment quality standards are authorized under both state and federal laws and are used in regulating wastewater discharges, controlling contaminant sources to aquatic environments, and remediating contaminant sediments. The standards define biological and chemical criteria for which an exceedance is considered indicative of a potential deleterious impact to environmental health. Additional criteria are also provided in the sediment standards for defining cleanup screening or minimum cleanup levels for which exceedances would require remedial actions.

Biological criteria were developed for acute and chronic sediment toxicity tests including amphipod mortality, oyster or echinoderm larvae abnormality and mortality, juvenile polychaete growth, and benthic community alterations. Criteria for evaluating changes in benthic community structure are based on changes in major taxa (polychaetes, molluscs, crustaceans) abundance relative to abundance in reference areas. If any one taxa group has fewer than 50 percent of the mean number of individuals found in a reference area **and** is statistically different from reference, then this result is considered evidence of an adverse biological impact and the test sediment fails the criteria. To further define sediment requiring remedial actions, reductions in two major taxa groups are required for a test sediment to exceed cleanup screening levels.

Washington State Department of Ecology Ambient Monitoring Program Olympia, Washington Mr. Pete Striplin (206) 586-5995
The Ambient Monitoring Program was initiated in 1989 and is in its fifth year of annual sampling. The program is based on the collection and analysis of surface sediment samples in March of each year from fifty sites throughout Puget Sound. Contaminant and indicator (e.g., caffeine, ß-coprostanol) chemical concentrations, conventional sediment parameters, amphipod toxicity, and benthic community structure are measured for each sediment sample. Field and laboratory protocols follow those provided in the Puget Sound Protocols. Five replicate benthic community samples are collected from each locations with a van Veen grab and are field-sieved using a 1.0 mm mesh screen. Taxonomic resolution is to species. Benthic community data analyses are based on richness, species abundance, total and major taxa group abundance, relative abundance of pollution-tolerant and pollution-sensitive taxa and indices of diversity, evenness, dominance, and trophic structure (i.e., Infaunal Trophic Index). Analytical methods include graphical comparisons of summary statistics (e.g., mean abundance), analysis of variance, and cluster techniques. Interpretation is augmented by correlation and regression analyses among biological, chemical, and physical variables. Prior to 1991, the identification of adversely impacted communities relied on a moderate degree of correspondence among benthic, toxicity, and chemical results and best professional judgement. Since the promulgation of the sediment management standards, use of the 50 percent reduction in major taxa abundance in comparison to reference conditions was included as part of the interpretive approach. Candidate reference sites were identified during analysis of the first years data. Reference sites were resampled in subsequent years and additional candidate locations were sampled to refine the representation of reference conditions.

Puget Sound Dredged Disposal Analysis Program
Washington State Department of Natural Resources - Division of Aquatic Lands
Olympia, WA
Mr. Gene Revelas
(206) 902-1086

The U.S. Army Corps of Engineers (Corps) and Environmental Protection Agency (EPA), and the Washington State Departments of Ecology (Ecology) and Natural Resources (DNR) are jointly responsible for the management and regulation of open-water dredge disposal sites in Central Puget Sound. Responsibilities include designation of disposal sites, permitting, compliance inspections, environmental monitoring, and data management. Under the Puget Sound Dredged Disposal Analysis study, this interagency group is involved in the development of siting criteria, evaluation procedures, and management plans for disposal sites. Environmental monitoring occurs as part of the site selection process, management of an active site, and assessment following closure of a site.

Monitoring typically includes physical, chemical and biological variables. The monitoring program design is outlined in the Evaluation Procedures Technical Appendix (Phillips et al. 1988). The Corps is responsible for physical monitoring and DNR conducts the chemical and biological monitoring. Biological sampling (i.e., benthos and toxicity) at PSDDA disposal sites is conducted prior to disposal and after disposal in offsite areas including the periphery of the disposal site, in areas immediately downcurrent of the site, and at a benchmark location. Five replicate benthic samples are collected at the benchmark site and in downcurrent locations during one season (typically spring) each monitoring year (selection of which years to monitor is based on disposal activities). Benthic community response is based on changes in abundance of major taxa groups (i.e., polychaetes, molluscs, crustaceans, and miscellaneous taxa). Post-disposal abundances are statistically compared to baseline conditions. If results indicate that benthic abundance is significantly lower than baseline abundance, benchmark data are used to interpret whether or not changes in abundance were due to disposal activities or some other factor. If offsite mean abundance is statistically different from the benchmark but the **difference** is less than 50 percent of the benchmark mean abundance, then changes are considered to be due to disposed materials. Larger differences are considered indicative of impacts from other factors. For the purpose of comparing means, α was set at either 0.20 or 0.05, depending on the sampling phase.

A detailed evaluation of the benthic sampling program design at Port Gardner was provided by SAIC (1990). They recommended modifications to the monitoring program including collection of a single sample at each station with a concomitant increase in the number of stations along the downcurrent transects and in the benchmark area. SAIC suggested that with these changes, differences between baseline and post-disposal data or differences between sites adjacent to the disposal area and the benchmark area could be efficiently analyzed using the ordination techniques developed by EcoAnalysis.

University of Washington
Fisheries Research Institute
Seattle, WA
Mr. Charles Simenstad
(206) 543-7185

Researchers at the Fisheries Research Institute have developed estuarine wetland restoration monitoring protocols in support of the U.S. EPA Puget Sound Estuary Program. The protocols were designed as a supplement to procedures for assessing overall wetland quality (e.g., Wetland Evaluation Technique or Habitat Evaluation Procedure). Nearshore subtidal habitats were considered in the development of the protocol because of their integral association with wetlands.

Recommended parameters for measuring benthic communities included abundance and wet-weight biomass per unit area. For the purpose of evaluating wetland functions, it was preferred that abundance and biomass be analyzed relative to species and lifestage. Although benthic samples are often not replicated, Simenstad et al. (1990) recommended five samples per location to support reasonable statistical analyses. For the purpose of monitoring restored or created wetlands, changes in benthic infaunal density and standing stock are generally evaluated over time and in comparison to a reference or control site.

Statistical treatment of the data depends ultimately on the sampling design and whether or not the underlying assumptions of various statistical tests (e.g., normal distribution, equal variances) are met. Parametric tests are applicable if the data are normally distributed and the variances among treatments are approximately equal. Analysis of variance (ANOVA) techniques tend to be robust with respect to data distributions and can still be applied if the data begin to depart from a normal distribution. Transformations (e.g., log-transformation) of the data may also allow assumptions of parametric tests to be met. If data do not meet the underlying assumptions, data transformations do not improve the distribution of the data, and there is some question regarding the robustness of the test to be used, nonparametric procedures using ranks can be applied with similar reliability to parametric tests.

National Oceanic and Atmospheric Administration Hazardous Materials Response and Assessment Division Seattle, WA Mr. Don MacDonald (206) 526-6271
The Hazardous Materials Response and Assessment Division provides technical support to the U.S. EPA at Superfund sites to ensure the protection of trust resources (i.e., migratory, marine, and estuarine species, the habitats used by all life stages, and prey species) during remedial activities. NOAA may recommend collection of aquatic biological data as part of a remedial investigation or feasibility study to fill data gaps necessary to complete the selection and design of cleanup actions. Recommendations for use of benthic community indices tends to be site-specific, but generally includes total abundance, taxa richness, percent contribution of dominant taxa, community similarity, and species composition (MacDonald et al. 1992). Diversity indices are specifically excluded from the recommended metrics. The recommended sampling design, data collection and handling, and sample analysis for estuarine or marine sediments typically follows the guidance provided in the Puget Sound protocols. Generally, three to five replicate samples at each station are recommended with taxonomic resolution occurring at the lowest practical taxonomic level. The number and location of stations is site-specific and reflects the pathways of contaminant migration at a given site. In most cases, benthic indices represent only one of several biological endpoints that are being measured as part of a site investigation. Sediment bioassays, bioaccumulation in resident species, and sediment chemistry are often analyzed in conjunction with benthic community indices. In order to determine what impacts are occurring or are likely to occur because of uncontrolled releases of hazardous substances to the aquatic environment, biological data are typically compared to reference conditions established during the investigation. Chemical data are compared to regulatory criteria or effects-based screening levels reported in Long and Morgan (1990).

3.2 Other Regional Programs

Southern California Coastal Water Research Project
Long Beach, CA
Dr. Bruce Thompson (now with Aquatic Habitat Institute, San Francisco)
(510) 231-9539

SCCWRP provides research and technical support to municipal dischargers and state agencies to examine the effects of anthropogenic activities on the ecological health of the Southern California Bight. The primary focus of their work has been identifying and evaluating the effects associated with municipal wastewater discharges on water and sediment quality, and biota in the bight. Sampling benthic communities has been an integral part of the monitoring programs conducted by the SCCWRP.

Much of the benthic sampling has relied on single samples collected at a large number of locations along depth contours on the coastal shelf (e.g., Word and Mearns, 1979; Thompson et al., 1987). Lack of replication precludes the use of parametric statistics for among-location comparisons. Multivariate techniques, including cluster and discriminant analysis, were used to examine patterns in species composition and abundance. In addition, researchers at the SCCWRP developed the infaunal trophic index to quantify the distribution of individuals and species among four feeding guilds in response to organic enrichment and toxic contaminant concentrations. Dominance of specific guilds was used as evidence of unimpacted, moderately organically enriched, or heavily impacted conditions. Biological patterns were also compared to the distribution and magnitude of contaminants in sediments.

U.S. Environmental Protection Agency
Region IX, San Francisco, California
Ms. Janet Hashimoto
(415) 744-1981

This regional office of the U.S. EPA has extensive involvement in the design and interpretation of individual environmental monitoring program data as part of their responsibilities under Section 301(h) of the Clean Water Act. Under current regulations, a balanced indigenous population (BIP) must exist beyond the zone of initial dilution for a given discharge. A BIP can be defined as the type of community that may exist at a particular site in the absence of an outfall. There have been several approaches used to define a BIP, including comparison to reference areas. However, criteria used to define reference conditions and statistical techniques used to test for differences between benthic communities near a discharge and reference areas has varied from discharger to discharger. In addition, comparability among programs with respect to analytical variables and procedures has been a stumbling block for any regional assessment of environmental quality.

To address this issue in part, EPA sponsored an evaluation of historical benthic monitoring data from the Southern California Bight. Comparability among data sets was evaluated, characteristic reference communities were identified, and statistical approaches for data analysis were recommended as the product of this study (Tetra Tech, 1992). Final recommendations regarding statistical approaches included use of multivariate techniques such as the ordination techniques developed by EcoAnalysis and comparison of test sites with reference conditions. Benthic community indices used in this analysis included total abundance, abundance of indicator taxa (e.g., *Parvilucina tenuisculpta, Amphiodia urtica*) and multi-species assemblages, richness, diversity, and biomass. Identification of impacted sites was partially based on community composition and degree of dissimilarity with reference station groups formed using cluster and ordination analyses. Tests of hypotheses that potentially impacted communities indices were similar to reference indices was based on a calculation of confidence bounds or tolerance intervals for reference conditions. Parameters used in the calculation include the standard deviation for reference conditions, and the number of reference stations used to calculate the standard deviation, and the desired probability for making a Type I error (α). If the mean value of a given index from a potentially impacted site falls outside of the tolerance interval (or reference envelope), then the site is considered impacted.

The current approaches proposed in this document are under review by the agency. Because of the differences of opinion among scientists regarding statistical treatment of biological data, EPA intends to hold a meeting to in the first quarter of 1993 to ask scientists to reach a consensus for a regional monitoring program in the bight.

California Water Resources Control Board
San Francisco, CA
Ms. Karen Taberski
(509) 286-1346

The Water Resources Control Board is involved in several regional monitoring programs including San Francisco Bay, Santa Monica Bay, and San Diego. The San Francisco Bay program currently does not use benthic community indices. Communities in the bay tend to be highly variable, in part because of salinity gradients and differences in sediment grain size, so discrimination between habitat characteristics and contaminant effects is difficult. In addition, much of the bay is contaminated, making selection of comparable reference areas very difficult. Their program relies on sediment toxicity bioassay results, however, their application is currently being modified.

The monitoring program conducted in San Diego includes collection of benthic infaunal samples. However, the data are currently not used in any regulatory decisions.

Florida Department of Environmental Regulations
Doug Farrell
Tampa, FL
(813) 744-6100

The Florida Department of Environmental Regulations (FDER) is currently developing a monitoring program approach for their estuarine and marine surface waters. Program development will include selection of biological indicators to be used and analytical methodologies.

Historically, the FDER used species diversity (i.e., Shannon-Weiner index) as an indicator of environmental health. Because of the tremendous variability in habitat characteristics, this index tended to be fairly insensitive.

Virginia Water Control Board
Chesapeake Bay Office
Richmond, VA
Mr. Fredrick Hoffman

The Virginia Water Quality Control Board funds a benthic monitoring program in the estuarine portions of the York, Rappahannock, and James rivers and in Chesapeake Bay within the state of Virginia. The main objective of the program is to characterize changes in estuarine infaunal communities on a regional basis. Four replicate benthic box cores are collected at 19 stations on a quarterly basis since 1985. Only three of the four samples are processed. Taxonomic resolution is to species. Analyses are based on abundance, richness, and biomass of the top 20 numerically dominant taxa. Distribution of biomass by depth within the sediment and among indicator groups (i.e., equilibrium versus opportunistic species) is also examined. Further presentation of analytical approaches in discussed in the next entry.

Old Dominion University
College of Sciences
Norfolk, VA
Dr. Daniel Dauer
(804) 683-3595

Dr. Dan Dauer is the principal investigator for the Virginia Chesapeake Bay Benthic Monitoring Program. Using the monitoring data collected from 1985 to 1989, he analyzed spatial and temporal trends in benthic community structure at 19 stations. Statistical procedures were fairly complex and used only replicated data (i.e., abundance, richness, and biomass of dominant taxa). Temporal trends were examined by a series of nonparametric trend tests including the seasonal intra-block sign test based on the Kendall Tau statistic and the aligned rank test. Unique seasonal trends or interactions between specific stations and seasons were examined using a chi-square protocol. The slope of significant trends were estimated using the Seasonal Kendall slope estimator. These tests tend to be quite robust and withstand severe departures from the assumptions of parametric statistics. The spatial distribution of benthic species were examined using multivariate techniques including cluster analysis, multivariate analysis of variance, and discriminant analyses. From these analyses, Dauer was able to identify beneficial or detrimental trends in benthic community structure in the region.

College of William and Mary Virginia Institute of Marine Sciences Gloucester Point, VA Dr. Robert Diaz (804) 642-7364
Dr. Diaz has been the principal investigator for numerous studies of benthic community structure and function in the tidal portions of the York River and adjacent areas of the Chesapeake Bay (e.g., Diaz 1989). Generally, two or three benthic samples were collected at a station. Stations were located at regular intervals along the river and were placed on either bank and in the center of the channel in most sampling reaches. Benthic community patterns and responses to point-source discharges were analyzed using descriptive indices (e.g., abundance, richness, diversity, and evenness) and multivariate techniques including cluster and principal components (ordination) analyses. Station groups were defined by physical, chemical and biological characteristics. Results were discussed in light of concordance between physical/chemical characteristics (e.g., salinity, pollutant loadings) and benthic community structure rather than statistical hypothesis testing.

4.0 METHODS FOR EVALUATING BENTHIC COMMUNITY INDICES

Analytical methods for evaluating benthic community indices in the monitoring programs described in the previous section included comparison of simple descriptive statistics, analysis of variance, and clustering and ordination techniques. Community indices included richness, species abundance, total and major taxa abundance, diversity, dominance or evenness, trophic function, and distribution of individuals among indicator taxa or assemblages. These methods are described in more detail in the following sections and a discussion of the issues affecting their use is provided.

4.1 Descriptive Community Indices

Use of descriptive community indices is fairly entrenched in environmental monitoring programs. These indices provide a simple screening of community characteristics and can be used to identify impacts to benthic communities. In addition, these indices allow a simpler, numerical representation of complex data sets which supports the comparison with numeric triggers or criteria.

Descriptive indices commonly used in benthic community studies include number of taxa (i.e., richness), number of individuals (i.e., abundance) distributed among species, major taxa groups, or indicator taxa assemblages, diversity, and dominance. These indices are often represented as the mean value for each sampling location or site. Replicate values for each index often form the basis for subsequent hypothesis testing. In addition, regulatory criteria have been developed for some of these indices (e.g., richness, diversity, total abundance, and major taxa abundance).

4.1.1 Richness

Richness is defined as the total number of species per sample area and is the most direct measure of diversity. Richness data tend to be normally distributed and the least problematic in terms of underlying assumptions for hypothesis testing. In addition, the natural variability tends to be low, resulting in accurate detection of small changes in the number of taxa using few replicate samples.

This index is best represented by species-level data, but some scientists have included taxa at higher classifications (e.g., genus) in the counts, when species-level identification is impractical. Richness is affected by the size of the sampling device and comparisons of data collected using different techniques cannot be solved by extrapolating richness based on sample area. This issue may affect inter-program comparisons and is best addressed by standardization of protocols used in regional monitoring programs.

4.1.2 Abundance

Abundance is generally represented as a measure of density and is equal to the total number of individuals per sample area. Abundance measures are also used to represent the distribution of individuals among major taxonomic groups (e.g., polychaetes, molluscs, and crustaceans) or indicator assemblages (e.g., pollution-sensitive taxa, suspended detrital feeders).

Reductions in major taxa abundance (i.e., less than 50 percent of mean reference abundance for any one group) are part of the biological criteria in the sediment management standards and are used as an evaluation criteria in the PSDDA program. The abundance of major taxonomic groups has been used in a number of monitoring programs as a reflection of gross changes in community structure in response to an environmental stress. Also, this level of taxonomic resolution has been chosen because it reduces the level of effort and concomitant costs associated with a monitoring program.

The relative abundance of major taxa groups has not been established for pristine or unimpacted aquatic environments in Puget Sound and the ecological relevance of shifts in major taxa contribution to community composition is not well established. However, the absence or reduced abundance of crustaceans has been used to indicate that the benthic community has been impacted and high abundance of polychaetes has been used to indicate an organically enriched environment. Use of major taxa abundance alone does not allow interpretation of shifts in species composition that may occur as an initial community response to contaminant exposure or organic enrichment.

Abundance tends to be naturally highly variable, both spatially and temporally. High natural variability can obscure detection of contaminant or physical disturbance effects. In addition, abundance data tend to not be normally distributed, which affects the selection of statistical methods for hypothesis testing or requires transformation of the data to meet the underlying assumptions of many of the tests.

4.1.3 Abundance of Indicator Taxa or Groups

Indicator taxa or assemblages have been used to define benthic community health in environmental monitoring programs. For example, the abundance of opportunistic or pollution-tolerant taxa has been used to illustrate gradients in organic enrichment (Pearson and Rosenberg, 1978). The abundance of species sensitive or tolerant to "pollution" has been used in Puget Sound programs to add insight to station-specific conditions and compare between the station locations. In the Southern California Bight, the relative abundance of *Amphiodia urtica* and *Parvilucina tenuisculpta* has been used to discriminate between reference assemblages and stressed benthic assemblages.

Use of opportunistic or pollution-tolerant taxa as indicators has lessened in recent years (Burd et al. 1990). These taxa tend to be cosmopolitan in their distribution and can be found in abundance in areas considered to be pristine or minimally impacted. Some researchers (e.g., Washington 1984) have advocated the use of sensitive taxa that would typically be absent in areas stressed by chemical pollutants or organic enrichment.

4.1.4 Infaunal Trophic Index

The infaunal trophic index (ITI) measures the relative abundance of organisms with specific feeding behaviors, which are used to identify the trophic function of the community (Word 1979, 1980). The ITI is based on the relative abundance of organisms classified into four categories of feeding strategies:

- suspended detrital feeders--dominant in relatively pristine areas.

- surface detrital feeders--may be abundant but not dominant in reference areas; increase in areas slightly organically enriched.

- surface deposit feeders--present but not abundant in reference areas; most abundant in areas moderately enriched with organic material.

- subsurface deposit feeders--rare at reference sites; most abundant in areas heavily affected by organic enrichment.

The species composition of each group in Puget Sound is presented in Appendix A: Table 1. This version of the ITI was developed in Southern California as an indication of benthic community response to changes in organic content of the substrate (e.g., 0 to greater than 10 percent total organic carbon). In general, as the sediments become organically enriched, deposit-feeding organisms become more abundant. Any geographic adaptations to this index requires extensive knowledge about the feeding behaviors and ecology of infaunal species. Modifications to the ITI groups and assignment of Puget Sound species to groups have been proposed by Word, but have not been widely applied. Ferraro et al. (1989) evaluated methods for comparing an impacted site in Port Gardner and a reference site using the six-group version of the ITI and found it to be a powerful index for detecting differences in community structure.

4.1.5 Diversity Indices

Diversity is a measure of community structure or complexity and has a long history of use in ecological research. There are numerous diversity indices and algorithms, but most indices represent the distribution of individuals among species. The Shannon-Weiner diversity index (H′; Pielou, 1966), used in many environmental monitoring programs, considers the proportional abundance of individuals in the determination of diversity. Pielou's evenness (J; Pielou, 1966) relates the observed diversity in benthic communities as a proportion of the maximum possible diversity for that data set (Zar, 1984). Evenness values approaching 1.0 indicate an evenly distributed population. The reciprocal value, 1-J, is termed dominance (Pielou, 1966) and high dominance values (i.e., those that approach 1.0) indicate that most of the individuals in a community are represented by only a few taxa. Swartz's Dominance Index (SDI; Swartz et al. 1980, 1985) is a representation of the number of taxa that account for 75 percent of the total abundance. Values less than 5.0 have been used to indicate stressed communities.

All of these indices tend to be normally distributed or do not assume an underlying distribution of the data, allowing their use in hypothesis testing. Difficulties arise in interpretation of index values because few indices have absolute values to define moderately stressed conditions (extreme conditions can be easily established), leading to ambiguous results. Of the above indices, the Shannon-Weiner index is most likely to give a "false positive" because of its dependence on the equitable distribution of individuals among species. Under conditions of slight to moderate stress, H′ may actually increase if the equitably increases while the number of species decreases. An example of this effect, along with the other diversity indices is provided in Appendix B.

4.1.6 Biomass

Benthic community structure has also been represented in some studies by the weight or biomass of individual species or higher taxonomic groupings. Several researchers (Dauer 1992, Weston 1990) have determined the health of benthic communities by analyzing the vertical distribution of biomass in the sediments. There are problems inherent in measuring biomass because of the association of inert material (e.g., shell, carapace, constructed tube structures) with living tissue. The occurrence of a single, large organism can bias comparisons among samples. In addition, the techniques used (e.g., use of preservatives, length of drying time) can account for variations in biomass. Warwick (Warwick 1986, Warwick et al. 1987) developed a method to examine changes in abundance and biomass in response to pollution gradients. Species level abundance and biomass data are used to plot cumulative frequency curves for a given sampling location. The relationship of the abundance curve with the biomass curve determines whether a location is highly stressed, moderately stressed, or not stressed. The abundance-biomass (ABC) method is based on the assumption that as pollution increases the larger, stable-assemblage species decline in number and biomass while the smaller, opportunistic species increase. This method tends to provide clear results for either normal communities or extremely stressed communities, but under conditions of moderate stress, results are harder to interpret. This method was tested by Weston in Sinclair Inlet (Weston, 1990) but results suggested that further testing was necessary. In addition, the method relies upon measurements of biomass for each species, which can be quite costly to include in an environmental monitoring program.

4.2 Analysis of Variance Techniques

Analysis of variance techniques are used in hypothesis testing and include ANOVA, t-tests, regression, and correlation. Use of these statistics was rare in historical ecological studies (Burd et al. 1990) but has become more prevalent in regulatory and resource management programs because of the "yes-no" nature of the results which facilitate management or compliance decisions. The effectiveness of the tests to return meaningful results is dependent upon the sampling design (e.g., number of locations and level of replication at a location) and the specificity of the hypothesis or question to be answered. In addition, these statistical methods assume an underlying normal or Gaussian distribution of the data and homogeneity of variances.

Benthic community indices tend to be log-normally distributed, especially for measures based on abundance. Transformations of the data using log (x + 1), where x = abundance, can be used to improve the distribution of the data and allow use of the statistics described above. However, benthic samples representing different environmental conditions may not follow a classic log-normal distribution. Rather, benthic communities in polluted sediments may have a binomial (Burd et al. 1990) distribution. Samples collected from geographically disparate sites or under various environmental conditions may represent more than one type of distribution which cannot be easily accounted for in most typical data transformations. In addition, the choice of the transformation to apply to the data relies upon the judgement of the scientist analyzing the data.

The methods used to test hypothesis are also affected by two types of errors. The first type of error is when the null hypothesis is mistakenly rejected. Usually the null hypothesis is stated as the assumption that two sample means are the same. The error is made when the statistical test results suggest that the sample means are significantly different, when in fact they are not. The probability (α) of making the error is typically set between 1 and 10 percent. The second type of error (β) is when the null hypothesis is accepted when the sample means are actually significantly different. The complement of β ($1-\beta$) is an estimate of the power of the test to reliably detect a significant difference when it exists. This statistic is affected by the alpha level, sample size, and the magnitude of the response being measured relative to the inherent variability of the response.

4.3 Multivariate Techniques

Classification and ordination analyses represent two basic types of multivariate statistical techniques. Both methods are used in benthic community studies to identify groups of sampling locations with similar community composition, resulting in a visual representation of the relationships or associations. These methods do not identify impacts *per se* but when the data include samples representing reference or unimpacted communities, the degree a test community is similar to or associated with reference communities can be discerned. The statistical significance of the differences among resulting is not easily tested. Best professional judgement is usually required to interpret the results and thus is difficult to use within a regulatory framework.

4.3.1 Ordination

The purpose of ordination techniques is to simplify and condense a large data set to determine the factors or components important to the ecological relationships within a study area. For example, using species abundance data, ordination techniques can be used to arrange sampling locations along coordinate axes such that their relative positions provide information regarding the degree of association among the locations sampled based on community composition.

Two major ordination techniques include principal components analysis and factor analysis. Both techniques rely upon measures of correlation or covariance among the variables (e.g., species abundance) being used to identify the associations among cases (e.g., station locations). These methods provide a unique tool in that they can incorporate the effects of different types of variables (e.g., depth, grain size, chemical concentrations) measured at each location within a single analysis.

4.3.2 Cluster

Cluster analyses identify groupings in a data set. Using species abundance data, a cluster analysis can identify "homogeneous groups" (clusters) of sampling locations based on similar species composition and abundance. The most common clustering technique used with benthic community data is a hierarchical cluster analysis of an average species abundance matrix, although other taxonomic groupings can be used. For example, cluster analyses were conducted using species abundance, and the major taxa (molluscs, crustaceans, and polychaetes) abundance in a number of the historical Puget Sound studies.

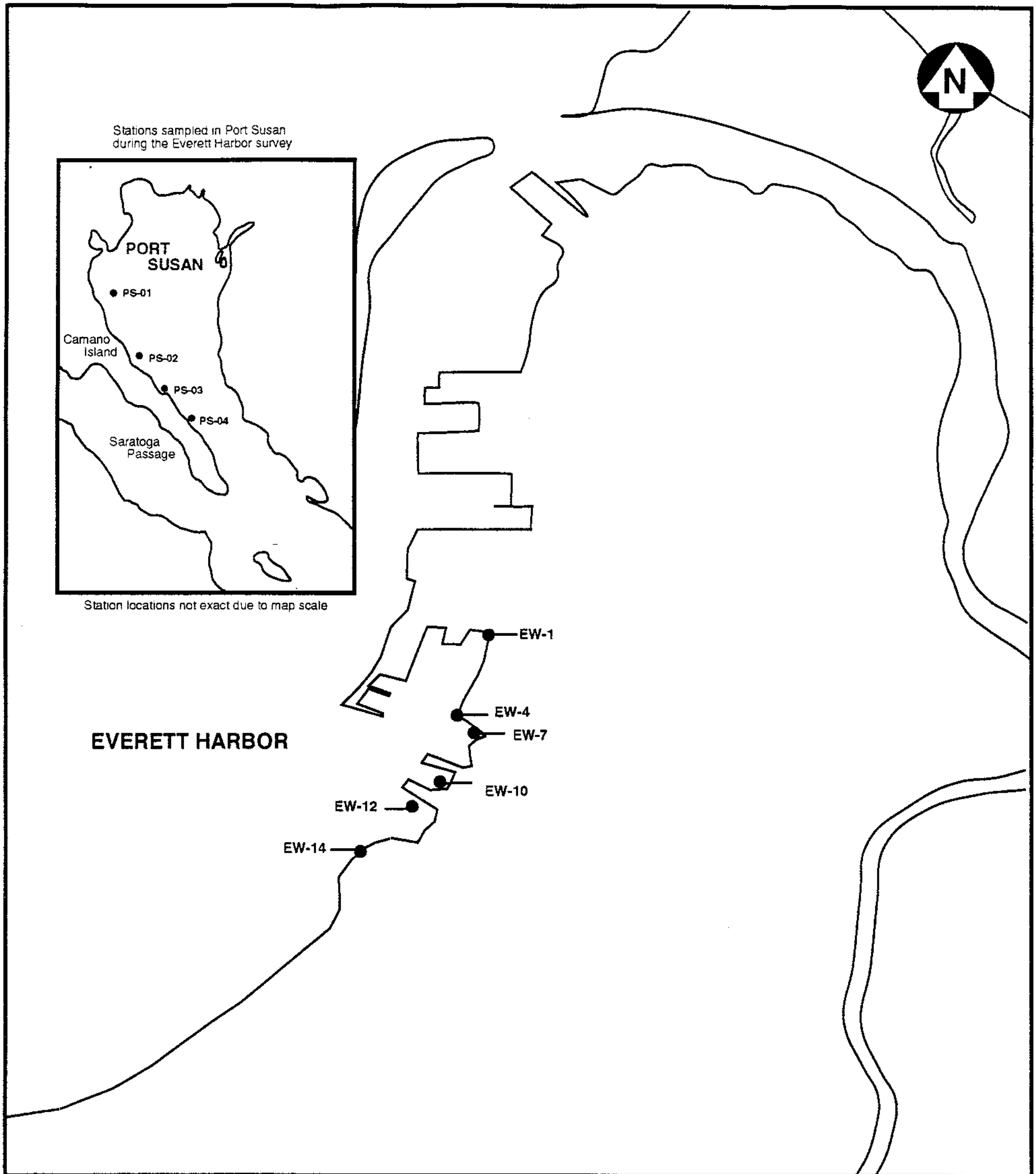
The degree of similarity between sampling locations can be affected by the degree of taxonomic resolution and the number and relative abundance of taxa present within each sample. It is often necessary to conduct cluster analyses several ways to resolve the effects of taxonomy and the distribution of taxa among sampling locations. Some strategies for calculating the similarity between locations are strongly influenced by dominant taxa. For this reason, data transformations [e.g., log (x+1)] are often performed when the analyst wants less abundant taxa to have some role in defining groups.

5.0 CASE STUDIES

Analytical methods were selected from those described above to apply to data from Puget Sound to test the effectiveness of each method or index to identify adverse impacts. Numerous historical data sets were evaluated for use as a case study. Criteria used in the selection included:

- Documentation available for sampling and QA/QC procedures
- Experienced regional taxonomists used
- Taxonomy to species or lowest practical taxonomic level
- Sample replication adequate to support statistical testing
- Samples collected from both contaminated and background areas
- Available in electronic format

The data set chosen for the case study was from the 1986 Everett Harbor Action Program (EHAP) investigation (PTI and Tetra Tech, 1988a). Benthic infaunal data from six stations located along a transect in the East Waterway, encompassing inner and outer harbor locations were selected for use in the case study (Figure 2). Reference area data from Port Susan (i.e.,



Locations of Sampling Stations for Everett Harbor Benthic Macroinvertebrates

FIGURE

2

WESTON

PROJECT # 10070-001-001-0100 DATE: January 1993

stations PS-02, PS-03, and PS-04) collected during the EHAP study were used for comparison with the Everett Harbor stations. Because these reference stations were primarily coarse-grained, a fourth reference station (PS-01) sampled during the 1985 Elliott Bay Action Program (PTI and Tetra Tech, 1988b) was selected for inclusion in the case study to represent reference communities at primarily fine-grained habitats. Habitat characteristics for all stations used in this case study are summarized in Table 2.

Table 2. Habitat characteristics of stations selected for the case study.

Station	Depth (m)	Sediment Fines (% Silt+Clay)	Total Organic Carbon (%)
EW-01	5.1	79	11
EW-04	8.7	59	29
EW-07	3.7	57	6.0
EW-10	9.1	77	12
EW-12	4.7	8	2.2
EW-14	9.8	32	4.7
PS-01	9.6	88	1.5
PS-02	7.9	12	0.4
PS-03	9.1	8	0.4
PS-04	8.7	7	0.3

As illustrated in Table 2, habitat characteristics within the study area were not homogeneous. Because habitat attributes can affect benthic community structure, stations were grouped by their relative similarity in percent sediment fines for some of the subsequent analyses to help detect potential contaminant effects on the benthos. Stations EW-01, EW-04, EW-07 and EW-10 were classified as fine-grained along with the reference station PS-01. Coarse-grained sediments included the outer harbor stations EW-12 and EW-14 and the reference stations PS-02, PS-03, and PS-04.

5.1 Descriptive Biological Indices

Richness, total abundance, major taxa abundance, relative abundance of pollution-tolerant and sensitive taxa, community composition based on numerically dominant taxa, the infaunal trophic index, and several measures of diversity were applied to the case study.

5.1.1 Richness

Mean richness (number of taxa per 0.1 m^2) among East Waterway stations ranged from 3 taxa (Station EW-01) to 59 taxa (Station EW-14)(see Appendix A: Table 2). The mean number of taxa appeared to increase along the sampling transect from the inner to the outer harbor. Total pooled richness (i.e., the number of unique taxa across all replicate samples collection from a station) among these stations also indicated an increasing gradient from the inner to outer harbor, with values ranging from 9 to 130 taxa. Total pooled richness for stations EW-12 and EW-14 fell within the range reported for the Port Susan reference stations, while the numbers of taxa at the remaining East Waterway stations were as much as 15 times lower than reference. The greatest pooled number of taxa was reported at Reference Station PS-04 (132 taxa).

5.1.2 Abundance

Abundance (number of individuals) and richness (number of taxa) data for each sample collected from six sampling stations in the Everett Harbor East Waterway and four Port Susan reference stations are presented in Appendix A: Table 2. The mean abundance, expressed as the number of individuals per 0.1 m^2, among stations located in the East Waterway ranged from 54 individuals (stations EW-01 and EW-07) to 1,610 individuals (Station EW-04). Stations EW-04, EW-12, and EW-10 had the greatest total abundance of benthic organisms, ranging from 6,036 to 8,049 individuals. The total abundances at these stations exceeded the total abundances measured at the four Port Susan reference area stations. The abundances of benthic infauna at stations EW-01 and EW-07 were an order of magnitude lower than the total abundances at the reference stations. There were no apparent gradients in total abundance of benthic organisms along the sampling transect.

5.1.3 Major Taxa Abundance

Abundance data for major taxonomic groups (crustaceans, molluscs, and polychaetes) are summarized in Appendix A: Table 3. Within the East Waterway, polychaetes were the most abundant taxonomic group at stations EW-01, EW-04, and EW-10, representing 68 to 90 percent of the total abundance at each of these stations. The remaining three stations in the waterway (EW-07, EW-12, and EW-14) were dominated by crustaceans, which accounted for 47 to 82 percent of the total abundance. At the reference stations, polychaetes were the most abundant taxonomic group at stations PS-03 and PS-04, crustaceans were the most abundant group at Station PS-01, and molluscs accounted for over 60 percent of the total abundance at Station PS-02. Overall, polychaetes had the highest relative abundance at Station EW-04 and crustaceans had the highest relative abundance at Station EW-12. Molluscs accounted for less than 15 percent of the total abundance among all East Waterway stations, which was below the range of relative abundance reported for the reference stations. Echinoderms were the least abundant and were absent from several stations. Miscellaneous taxa represented not more than two percent of the total abundance among all stations.

5.1.4 Pollution-Sensitive and Pollution-Tolerant Taxa

The abundance of pollution-sensitive and pollution-tolerant taxa was examined at each station. Taxa that have demonstrated sensitivity to toxicants in laboratory studies or are typically associated with unimpacted areas were defined as pollution-sensitive. On the basis of information from the Puget Sound Ambient Monitoring Program (PSAMP; Tetra Tech, 1990) and Word (1980), 14 taxa were identified as pollution-sensitive in the data from Everett Harbor and Port Susan. Opportunistic taxa and those tolerant of stressed conditions were defined as pollution-tolerant and were represented by 61 taxa in case study data. Pooled abundance data for these taxa are presented in Appendix A: Tables 4 and 5.

At the East Waterway stations, pollution-sensitive taxa represented 0 to 5 percent of the total pooled abundance at a station (see Appendix A: Table 4). Pollution-sensitive taxa were not present at the inner harbor transect stations (EW-01, EW04, and EW-07). The highest relative abundance (18 percent) of pollution-sensitive taxa occurred at Reference Station PS-01; pollution-sensitive taxa at the other 3 reference stations represented 1 percent of the total abundance at each station. The highest number of sensitive taxa (6 of 14) was measured at Reference Station PS-04. *Terebellides stroemi* was the most abundant of the pollution-sensitive benthic organisms identified, occurring at each of the reference stations but not at any of the East Waterway stations.

Pollution-tolerant taxa were identified at all Everett Harbor and Port Susan reference stations. These taxa represented 8 percent (Station EW-12) to 90 percent (Station EW-04) of the total abundance at the East Waterway stations, and 20 to 40 percent of the total abundance at the reference stations. Except at stations EW-12 and EW-14, the abundance of pollution-tolerant taxa was higher at the East Waterway stations than at the reference area stations. The greatest number of pollution-tolerant taxa (32 of 61) was measured at Station EW-14. *Capitella capitata* was the most abundant of the pollution-tolerant benthic organisms identified, but this species occurred only once at one reference station. Station EW-12 and Reference Station PS-01, which exhibited the highest relative abundances of pollution-sensitive taxa, had the lowest relative abundances of pollution-tolerant benthic organisms.

5.1.5 Community Composition Based on Numerically Abundant Taxa

Among all stations, the top ten numerically abundant taxa were represented by a total of 53 species (see Appendix A: Table 6). *Pectinaria granulata* was the only dominant taxon found at an East Waterway station that also occurred in the top ten dominant taxa at a reference station. This species was the 10th most abundant taxon at Station EW-12 and the second most abundant species at reference stations PS-03 and PS-04. *Nebalia* spp., *Capitella capitata*, *Platynereis bicaniculata*, *Aoroides spinosus*, *Aoroides* spp., and *Armandia brevis* were the most frequently occurring dominant taxa within the East Waterway, with each species occurring at a minimum of 4 of the 6 harbor stations. Of these species, *Capitella capitata* was the most abundant taxon at stations EW-01, EW-04, EW-07, and EW-10. At the reference stations, *Psephidia lordi* and *Axinopsida serricata* were among the most abundant species at each station. *Euphilomedes* *carcharodonta* was the most abundant taxon at stations PS-03 and PS-04, while *Psephidia lordi* and *Protomedia prudens* were the most abundant taxa at stations PS-02 and PS-01, respectively.

5.1.6 Infaunal Trophic Index

Infaunal trophic index (ITI) values are presented in Appendix A: Table 7. The ITI values calculated for the Everett Harbor stations ranged from 0.1 (Station EW-01) to 69 (Station EW-12). The ITI values generally increased from the inner to outer harbor locations along the sampling transect, indicating a shift in community composition from deposit-feeding infauna to suspended and surface detrital feeding organisms. At the reference stations, ITI values ranged from 67 (Station PS-02) to 76 (Station PS-04). These values were greater than those measured at all but one East Waterway location (Station EW-12). In general, ITI values less than 65 percent are indicative of benthic communities composed of transitional or pollution-tolerant taxa.

5.1.7 Diversity

Various indices of diversity are presented in Appendix A: Table 8. Shannon-Weiner diversity index (H′) values among the East Waterway stations ranged from 0.24 (Station EW-04) to 1.49 (Station EW-14). Except for stations EW-07 and EW-14, the values of H′ calculated for the Everett Harbor stations were lower than the range of values (1.13 to 1.42) reported for the reference stations. A similar pattern was observed for the Swartz's Dominance Index (SDI) values. The SDI values reported for stations EW-07 (7.83) and EW-14 (14.4) were within the range of SDI values calculated for the reference stations (7.41 to 11.8), but the four remaining East Waterway stations had SDI values less than the reference station SDI values. In addition, three of the four stations in Everett Harbor had SDI values less than 5.0, suggesting that the communities at these stations were stressed. Among these three stations, *Capitella capitata* accounted for 63 to 88 percent of the total abundance.

At the East Waterway stations in Everett Harbor, evenness (J) values ranged from 0.16 (Station EW-04) to 0.75 (Station EW-07). Higher evenness values indicate homogeneously distributed communities. As the complement of evenness, dominance (D) values among these stations ranged from 0.25 (Station EW-07) to 0.84 (Station EW-04). Dominance values for all but two stations in the East Waterway (EW-07 and EW-14) were higher than those reported for the Port Susan reference stations, which ranged from 0.31 to 0.43. Communities dominated by one or two relatively abundant taxa are reflected in dominance values approaching 1.0.

5.2 Differences Among Stations

Relationships among stations based on benthic data were examined using analysis of variance techniques. Independent t-tests were conducted for two-sample comparisons (i.e., East Waterway stations versus reference stations) to determine whether statistically significant differences existed between site and reference stations. The independent t-test procedure is based upon the assumption that the data are approximately normally distributed, but does not assume that the samples have equal variances. To satisfy the normality assumption, log-transformed data were used. A experiment-wise error rate of 0.01 was used to account for the repetitive testing of the stations and to reduce the probability of making Type I errors. Probabilities less than or equal to 0.01 were considered significant, indicating the benthic communities at the stations were unlikely to be the same.

ANOVA tests were also conducted to determine whether significant differences existed among stations. Tests were run using three groups of data: (1) only East Waterway stations, (2) all fine-grained stations including reference stations, and (3) all coarse-grained stations including reference stations. Log-transformed data were used in the ANOVA to satisfy the normality assumptions required by this method. As part of the ANOVA procedure, Tukey's honestly significant difference (HSD) *a posteriori* pair-wise contrasts (as applied in SYSTAT; described in Sokal and Rohlf, 1981) were calculated for all possible station pairs to identify significant differences. This test adjusts for the experiment-wise error rate.

There has been some discussion among statisticians and researchers regarding the need to adjust α for the experiment-wise error rate, particularly in a regulatory setting. It is felt that the number of stations sampled and hence, the number of comparisons made should not affect compliance decisions, therefore use of an error rate of 0.05 for all comparisons would be appropriate. For the purpose of the case study, an adjustment was made to maintain compatibility with historical approaches used in Puget Sound programs.

The ANOVA and t-tests using abundance and richness data indicated that there were significant differences among stations in richness, total abundance, crustacean abundance, mollusc abundance, and polychaete abundance. ANOVA results are summarized in Appendix A: Tables 9 through 13. All four fine-grained harbor stations had significantly fewer taxa and molluscs compared to Reference Station PS-01. Crustacean abundances were significantly lower at all inner harbor stations. Total taxa abundance and polychaete abundance was also significantly depressed a two of these stations (EW-01 and EW-07). Based on the ANOVA results, the coarse-grained harbor stations were not distinguished from the pooled, coarse-grained reference stations with the exception of total taxa abundance at Station EW-12, and crustacean abundance at stations EW-12 and EW-14, all of which were significantly greater than the mean reference values.

Generally similar results were reported for the t-tests, with few exceptions (see Appendix A: Tables 14 through 18). The lack of agreement between the ANOVA and t-test results was observed for all indices tested with the exception of total abundance. In all but one case, the t-test failed to detect a difference declared as significant by the ANOVA. Unlike the ANOVA results, the t-test indicated that the mean abundance of polychaetes at Station EW-12 was significantly *lower* than the mean pooled reference polychaete abundance.

Results of the ANOVA of among station comparisons using only the East Waterway stations also indicated that there were significant differences in abundance and richness among stations (see Appendix A: Tables 9 through 13). The mean total abundance and crustacean abundance at stations EW-01 and EW-07 were significantly lower than the mean total abundance and crustacean abundance at the other stations in the waterway. Mean richness at the three outer harbor transect stations (EW-10, EW-12, and EW-14) was significantly higher than at the three inner harbor transect stations (EW-01, EW-04, and EW-07). Stations EW-12 and EW-14 had significantly higher mean abundances of molluscs than stations located farther north along the transect. The mean polychaete abundance at Station EW-04 was significantly higher than all other East Waterway stations except Station EW-10.

5.3 Sampling Efficiency and Power Analyses

An index of sampling efficiency based on the standard error as a percent of the mean was calculated for each station using abundance and richness data, and the results are presented in Appendix A: Table 19. According to Dauer et al. (1979) and Elliott (1977), values less than 20 to 30 percent indicate that the number of samples collected at each station are adequate to estimate benthic population parameters. With few exceptions, the case study results indicated that the study design used was efficient for estimating population parameters.

Efficiency index values based on total abundance data for all stations ranged from 4 to 26 percent. Values based on richness ranged from 5 to 18 percent. For the major taxa groups, index values based on crustacean abundance ranged from 4 to 76 percent, with the index of sampling efficiency exceeding 30 percent at the inner harbor transect stations. Index values based on mollusc abundance ranged from 8 to 100 percent, with the only exceedance of the 30 percent sampling efficiency value occurring at Station EW-01. Values based on polychaete abundance ranged from 8 to 34 percent. The index values at stations EW-01 and PS-01 exceeded the sampling efficiency index of 30 percent.

Power analyses were conducted based on the approach developed by Scheffe (1959) and Cohn (1977) and found in the Ocean Data Evaluation System (Tetra Tech 1987b). Analyses were conducted using both transformed and original abundance data, in addition to original richness data, and the results are presented in Appendix A: Table 20. The estimate of the variance was derived from the mean square error term in the ANOVA results. The purpose of the analysis was to evaluate the relative power of the sampling design (i.e., 10 stations with 5 replicates per station) to detect real differences among stations. For 5 replicates, the minimal detectable difference (MDD) as a percent of the mean, using richness data, was 52 percent of the mean. The MDDs, expressed as percent of the mean for transformed data, ranged from 18 percent (total abundance) to 42 percent (crustacean abundance). Non-transformed abundance MDDs ranged from 111 percent (mollusca abundance) to 202 percent (crustacean abundance). The power of the sampling design to detect real differences increased with an increase in the number of replicate samples collected and transformation of the abundance data increased the power to detect a significant difference.

5.4 Multivariate Analyses

Overall, the cluster and principal components analyses resulted in similar associations among stations based on species abundance data. In both analyses, reference stations were not grouped or associated with Everett Harbor stations, suggesting that the benthic communities in Everett Harbor are different than in Port Susan and may have been impacted.

5.4.1 Classification Analyses

Classification analyses were conducted using the Bray-Curtis similarity index with a group-averaging clustering algorithm. Before the classification analysis was conducted, the data matrix was reduced because its size exceeded software capabilities. The matrix was reduced to 211 taxa by dropping any taxa with only one or two individuals in the entire data set. Data were log-transformed to minimize the effect of numerically dominant taxa. Results of the classification analyses are presented in Appendix A: Tables 21 through 24 and Appendix A: Figures 1 through 4.

The degree of similarity among stations tended to be low; few stations or station groups were linked at greater than 60 percent similarity. Most importantly, all reference stations formed a cluster, while all Everett Harbor stations formed a separate cluster. This result reflects the differences in community composition between reference and potentially impacted stations in Everett Harbor (only a single numerically dominant taxa was shared between these two station groups). Using all taxa with more than two individuals in the entire data set, reference stations PS-03 and PS-04 formed the first cluster, at 68 percent similarity. This group clustered with Reference Station PS-02 at 59 percent similarity and PS-01 at 30 percent similarity. Linkages based on all taxa among Everett Harbor stations ranged from 19 to 56 percent, with Station EW-01 being the least like all other sampling locations.

The mean total abundances for the two stations that clustered with highest similarity based on total abundance (PS-03 and PS-04) were approximately 400 individuals per 0.1 m^2. Sediments at these stations were composed primarily of sands (92 percent) and less than 0.5 percent total organic carbon (TOC). The benthic communities at these stations were dominated by the crustacean ostracod *Euphilomedes carcharodonta* and the polychaete *Pectinaria granulata*. Other dominant taxa shared among these stations included the bivalve *Psephidia lordi* and the ostracod *Euphilomedes producta*. Station PS-02, which clustered with these two stations at nearly 60 percent similarity, had a slightly higher mean total abundance, approaching 450 individuals per 0.1 m^2. Substrate composition at this station was similar to the first cluster, composed of approximately 88 percent fines and less than 0.5 percent TOC. *Pectinaria granulata* was absent from this station, but *Psephidia lordi* and *Euphilomedes carcharodonta* were also among the dominant taxa. These dominant taxa were absent from the East Waterway stations, which contributed to the low percent similarity between the reference stations and East Waterway stations.

Classification analyses based on species abundances within major taxa groups showed a similar pattern illustrated by all taxa, except that linkages (percent similarity) varied. For the classification analysis using crustacean abundance, reference stations PS-02 and PS-03 formed the first cluster at 66 percent similarity. The second cluster was formed by stations EW-10 and EW-14 at a similarity of 65 percent. The remaining stations were the less similar to all other the EHAP investigation for these stations.

Sediment bioassays using the amphipod *Rhepoxynius abronius* were conducted for the East Waterway and Port Susan stations sampled during the Everett Harbor investigation. Mean amphipod mortality for the East Waterway stations ranged from 13 percent (Station EW-12) to 100 percent (Station EW-01). At the reference stations, mean mortality ranged from 20 percent (Station PS-04) to 29 percent (Station PS-02). Results of the ANOVA conducted during the Everett Harbor investigation indicated that the mean mortalities at stations EW-01, EW-04, and EW-07 were significantly ($P<0.001$) higher than reference (using pooled data from PS-03 and PS-04 only). These results provided additional evidence that the benthic communities at the inner harbor stations were highly stressed relative to the outer harbor and reference areas.

The identification of the benthic community at Station EW-01 as highly stressed relative to other harbor stations and reference areas was apparent based on all statistical methods used to evaluate the data. The community at this station was characterized by extremely low abundance, which was significantly ($P<0.00001$) lower than mean reference abundance based on the results of the ANOVA and t-tests. The ANOVA results also indicated that the mean total abundance at this station was significantly lower than all other East Waterway stations except Station EW-04, which had a total abundance nearly identical to Station EW-01 (discussed below). In addition, abundances of major taxonomic groups were depressed, with abundances less than 50 percent of reference area abundances for all major taxa groups. ANOVA results provided further evidence of adverse affects, indicating that the mean abundances for all major taxa groups were significantly lower than reference. In accordance with the SMS approach, the benthic community at this station would have been identified as being adversely affected based on all major taxa group abundances.

Measures of diversity at Station EW-01 also indicated the benthic community was highly stressed. The mean richness at this station was significantly lower than reference based on both ANOVA and t-test results. ANOVA results also indicated that the mean richness at Station EW-01 was significantly lower than mean richness at outer harbor stations. Values of Shannon-Weiner diversity, Swartz's Dominance Index, and evenness were substantially lower than those measured at the reference area, providing further evidence of adverse impacts to the benthic community at this station.

The community at Station EW-01 was dominated by the pollution-tolerant species *Capitella capitata*, which was reflected in the high relative abundance of polychaetes, a dominance value approaching 1.0, and an extremely low infaunal trophic index value (less than 1) indicative of a community dominated by subsurface deposit feeders. Pollution-sensitive species were absent from this station. In addition, results of the cluster analyses indicated that Station EW-01 was dissimilar from all reference stations based on species and major taxa group abundances. Station EW-01 was identified as similar to Station EW-04 based on polychaete abundance, but Station EW-04 was also dominated by the pollution-tolerant species *C. capitata*. The identification of stations EW-04 and EW-07 as having highly stressed benthic communities was not evidenced by all statistical methods used, thus indicating the need for comparing several different measures of

Table 3—Comparison of test results in identifying adverse benthic impacts

	Conclusion Based on Numeric Criteria				Conclusion Based on Best Professional Judgement						
Station	Total	Crustacean Abundance	Mollusc Abundance	Polychaete Abundance	Richness	H′	J and D	SDI	ITI	Pollution-sensitive taxa (%)	Pollution-tolerant taxa (%)
EW-01	*	<50%*	<50%*	<50%*	*	stressed	stressed	stressed	stressed	stressed	stressed
EW-04	*	*	<50%*	*	*	stressed	stressed	stressed	stressed	stressed	stressed
EW-07	*	<50%*	<50%*	<50%*	*	unstressed	moderately stressed	moderately stressed	stressed	stressed	moderately stressed
EW-10	NS	*	<50%*	*	*	stressed	stressed	stressed	stressed	stressed	stressed
EW-12	*	*	NS	NS	NS	unstressed	moderately stressed	moderately stressed	unstressed	unstressed	unstressed
EW-14	NS	*	<50%*	NS	NS	unstressed	unstressed	unstressed	unstressed	stressed	unstressed

* Significantly different compared to reference.
NS Not significantly different compared to reference.
<50% Less than 50% of the reference abundance.

community structure to evaluate the relative health of a community. Adverse effects to benthic organisms would have been identified for both stations based on significantly ($P \leq 0.05$) reduced abundances of major taxa groups (reduced mollusc abundances at both stations and reduced crustacean and polychaete abundance at Station EW-07). However, the extremely high dominance of an opportunistic species at Station EW-04 was not reflected using the SMS biological criteria, limiting the effective interpretation of the relative degree of impact between these two stations.

Unlike stations EW-01 and EW-07, which were characterized by extremely low abundances, Station EW-04 had the highest total abundance of organisms among all stations sampled. However, review of the species-level data indicated that this station was dominated by the opportunistic species *Capitella capitata*, which can reach very high abundances in areas of organic enrichment. The TOC content measured in sediment collected from Station EW-04 was nearly 30 percent. The dominance of the community at this station by one species was also indicated by the high relative abundance of polychaetes (90 percent), a dominance value approaching 1.0, low Shannon-Weiner diversity (less than 1), and an SDI value less than 5.0. In addition, pollution-sensitive taxa were absent from this station and the low ITI value for Station EW-04 was indicative of a community dominated by subsurface deposit feeding organisms.

At Station EW-07, the evenness and Swartz's Dominance Index values were indicative of homogeneously distributed communities, and none of the major taxonomic groups appeared to be overly dominant. Based on these results alone, it would appear that the benthic community at this station was relatively unstressed compared to stations EW-01, EW-04, and the reference stations. However, Station EW-07 was characterized by an extremely low abundance (less than 300 individuals per 0.1 m^2) in addition to low richness (15 taxa). Review of species-level data indicated that the most abundant taxon at this station was *Capitella capitata*, and that other opportunistic subsurface deposit feeding organisms were also present, contributing to a low ITI value. In addition, pollution-sensitive species were absent from this station.

The need for comparing several different measures of benthic community structure to evaluate the relative health of a community was most apparent in evaluating the data from stations EW-10 and EW-12. The abundances of organisms collected from these stations were similar, at approximately 6,000 individuals per 0.1 m^2, and not statistically different from each other based on ANOVA results. Evenness, dominance, and Shannon-Weiner diversity were also relatively similar between these two stations. Based on these results alone, it would appear that the benthic communities at these two stations were both relatively unstressed. However, further review of richness and species-level data for stations EW-10 and EW-12 indicated the benthic communities at these stations were substantially different, and that the community at Station EW-10 is moderately stressed whereas EW-12 is not. Comparisons with SMS biological criteria would have indicated possible adverse affects to the benthic community at Station EW-10 (based on significantly reduced mollusc abundance) and no adverse effects at Station EW-12.

Stations EW-10 and EW-12 were statistically different from each other based on mean richness.

Mean richness at Station EW-10 (30 taxa per 0.1 m^2) was significantly lower than Station EW-12 (52 taxa per 0.1 m^2) and the pooled reference mean richness (55 taxa per 0.1 m^2). The low richness at Station EW-10 was indicative of a moderately stressed community, while richness at Station EW-12 fell within the range of values reported for the reference area and was not significantly different from reference, indicating a relatively unstressed community at this station. A similar pattern occurred using Swartz's Dominance Index and the Infaunal Trophic Index. The SDI value at Station EW-10 was substantially less than 2.0 and the ITI value was 5.0, indicating a community dominated by subsurface deposit feeding organisms. Unlike Station EW-10, the benthic community at Station EW-12 was characterized by surface detrital and suspension feeding organisms, reflected in an ITI value for this station within the range of values reported for the reference stations. The SDI for Station EW-12 exceeded 5.0, also indicating a relatively unstressed community.

Review of species-level data for these two stations also showed that pollution-sensitive species (primarily *Corophium acherusicum* and *Corophium* spp.) were present at Station EW-12 at a relative abundance of 5 percent, while these species accounted for less than 1 percent of the total abundance at Station EW-10. Conversely, pollution-tolerant species (primarily *Capitella capitata*) accounted for 69 percent of the total abundance at Station EW-10, but represented less than 10 percent of the total abundance at Station EW-12. In addition, the distribution of major taxonomic groups between these stations illustrated the differences in community structure: polychaetes accounted for 68 percent of the total abundance at Station EW-10, while crustaceans represented 82 percent of the total abundance at Station EW-12. These differences may be partially attributed to the differences in sediment composition between these stations. Station EW-10 was characterized by fine sediments and greater than 11 percent TOC. Substrate composition at Station EW-12 was dominated by sands and lower TOC content (approximately 2 percent TOC).

The distribution of major taxa groups at Station EW-12 also provided further evidence that the community at this station was relatively unstressed. The mean abundance of molluscs was not significantly ($P>0.05$) different from reference based on ANOVA and t-test results. Although the mean abundance of polychaetes was significantly ($P\leq0.01$) lower than reference based on t-test results, the ANOVA results indicated that the mean polychaete abundance at this station was not significantly ($P>0.05$) different from reference. In addition, the mean abundance of crustaceans was significantly higher than reference.

The benthic community at Station EW-14 was identified as unstressed based on all statistical results. Unlike Station EW-01, mean total abundance, major taxa abundance, and richness at Station EW-14 were not significantly difference from reference based on ANOVA and t-test results. The richness, Shannon-Weiner diversity, and ITI values at this station were within the range of values reported for the reference stations, indicating a relatively diverse benthic community dominated by suspension and surface detrital feeding organisms. Although pollution-sensitive taxa were rare at Station EW-14, the relative abundance of pollution-tolerant taxa at this station was lower than other East Waterway stations located in the inner harbor and was within the range of relative abundances reported for the reference stations.

The reference area stations were relatively similar based on abundance and richness data. Among all stations, the reference area stations tended to cluster with the highest percent similarities. These stations had significantly lower mean abundances than three of the East Waterway stations (EW-04, EW-10, and EW-12), but the mean richness values at the reference stations were significantly higher than all but two of the East Waterway stations (EW-12 and EW-14, which were identified as unstressed communities). Evenness, Shannon-Weiner diversity, and Swartz's Dominance Index indicated homogeneously distributed communities at the reference stations. The ITI values were indicative of communities dominated by surface detrital and suspension feeding organisms. Review of species-level data also indicated that pollution-sensitive species were relatively abundant at Reference Station PS-01, accounting for approximately 18 percent of the total abundance, and lower relative abundances of pollution-tolerant taxa than at the inner harbor stations.

7.0 WORKSHOP TOPICS

The information contained in this document is intended to form the basis of discussions among benthic experts at the upcoming workshop regarding the applicability of analytical methods and benthic community indices within regulatory or resource management programs. The relative advantages and disadvantages of each of the methods and indices presented above will be evaluated during the workshop using the following criteria:

- Sensitivity to detecting deleterious impacts versus natural change
- Power of the test
    - Minimization of false positives
- Robustness across different habitat types
- Objectivity of the criteria
- Ease of use and interpretation for non-statisticians
- Cost effectiveness

A preliminary assessment of the analytical methods was made based on the results of the case study and is summarized in Table 4. Based on this assessment, it is recommended that the following community indices be considered candidates for use in regulatory or management decisions and be evaluated by the experts:

Table 4—Comparison of analytical methods with criteria for use in a regulatory or management framework

	Criteria for Use in a Regulatory or Management Decision Process				
Analytical Method	Ability to Discern Impacts[a]	Robustness	Objectivity	Ease of Interpretation	Cost Effectiveness[a]
Simple comparisons					
Indicator taxa abundance	Variable	Robust	Some judgement required	Easy	Moderate
Diversity	Low	Robust	Objective	Easy	Moderate
Evenness and dominance	Variable-high for extreme impacts	Robust	Objective	Easy	Moderate
SDI	High for extreme impacts	Robust	Objective	Easy	Moderate
ITI	Moderate	Applicable on a regional basis	Some judgement required	Moderately easy	High
Analysis of variance					
Richness	High	Robust	Objective	Easy	Moderate
Total abundance	Variable	Robust	Objective	Easy	High
Major taxa abundance	Variable	Robust	Objective	Easy	High
Multivariate techniques					
Cluster	Low	Robust	Some judgement required	Complex	Moderate
Ordination	Low	Robust	Some judgement required	Complex	High
a = dependent upon sampling design					

- Species richness
- Species composition and abundance
- Total abundance
- Major taxa abundance
- Abundance of pollution-sensitive taxa
- Swartz's Dominance Index
- Infaunal Trophic Index

Other issues that are recommended for inclusion in the workshop agenda are:

- Use of ANOVA *a posteriori* contrasts versus t-tests for identifying difference between reference and test site conditions.
- Adjustment for pair-wise comparison error rates within a regulatory framework.
- Numeric criteria versus professional judgement in identifying adverse benthic effects.
- Sampling design (e.g., level of replication, scale of replication, taxonomic resolution).
- Application of multivariate techniques in regulatory programs.

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APPENDIX A

Table 1—Categories of infaunal trophic index (ITI) feeding strategies

Group I - Suspended Detrital Feeders		
Passive	Normal	Active
Ampharetidae	*Ampelisca* sp. I	*Ampelisca* sp. II
Maldanidae	*Amphiodia* spp	*Byblis* spp
Onuphidae	*Amphipholis* spp	*Crenella* spp
Phyllochaetopterus spp	Caprellida	*Cucumaria* spp
Terebellidae	*Owenia* spp	*Nemocardium* spp
	Phoronis spp	Phoxocephalidae
	Sabellidae	*Sthenelanella* spp
	Serpulidae	
Group II - Surface Detrital Feeders		
Stationary	Mobile	Specialized
Axinopsida spp	Cumacea	*Pectinaria californiensis*
Calyptogena spp	*Decamastus* spp	
Cirratulidae	*Euphilomedes* spp	
Magelonidae	*Glycera* spp	
Myriochele spp	*Goniada* spp	
Mysella spp	Lumbrineridae	
Photis spp	*Mediomastus* spp	
Psephidia spp	*Nephtys* spp	
Spionidae	Orbiniidae	
	Tanaid	
Group III - Surface Deposit Feeders		
Stationary	Mobile	
Macoma carlottensis	*Bittium* spp	
Nucula spp	*Mitrella permodesta*	
Nuculana spp	*Nassarius* spp	
Parvilucina tenuisculpta	*Nereis* spp	
Yoldia spp	*Travisia* spp	
Group IV - Subsurface Deposit Feeders		
Armandia bioculata	Oligochaeta	Stenothoidae
Capitella capitata	*Ophelina acuminata*	
Dorvilleidae	*Solemya* spp	

Table 2—Richness and abundance of benthic infaunal organisms at 6 stations in the East Waterway of Everett Harbor and at 4 reference area stations in Port Susan.

	RICHNESS (# taxa/0.1 m^2)							ABUNDANCE (# individuals/0.1 m^2)						
Station	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Mean	Total	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Mean	Total
EW-01	3	4	2	2	5	3	9	38	56	26	107	44	54	271
EW-04	14	19	13	12	17	15	31	1,241	2,410	1,698	942	1,758	1,610	8,049
EW-07	9	21	16	17	12	15	37	32	91	68	38	43	54	272
EW-10	26	35	27	27	35	30	69	1,858	1,688	585	635	1,270	1,207	6,036
EW-12	62	42	59	50	46	52	97	1,872	756	1,690	1,399	764	1,296	6,481
EW-14	72	42	65	68	47	59	130	625	181	629	716	289	488	2,440
PS-01	55	48	34	41	43	44	85	770	581	342	350	536	516	2,579
PS-02	51	47	37	45	52	46	94	322	508	441	486	451	442	2,208
PS-03	63	67	53	51	52	57	107	416	415	334	398	412	395	1,975
PS-04	56	69	62	55	72	63	132	374	529	437	373	476	438	2,189

Table 3—Average total abundance (# individuals/m^2) and relative total abundance of major taxonomic groups at 6 stations in the East Waterway of Everett Harbor and at 4 reference stations in Port Susan.

Station	Average Total Abundance (# individuals/0.1 m^2)					Relative Total Abundance (%)				
	Crustaceans	Molluscs	Polychaetes	Echinoderms	Misc. Taxa	Crustaceans	Molluscs	Polychaetes	Echinoderms	Misc. Taxa
EW-01	6	<1	48	0	0	11	<1	88	0	0
EW-04	156	4	1,446	<1	3	10	<1	90	<1	<1
EW-07	26	6	22	0	<1	47	11	40	0	1
EW-10	345	38	823	0	1	29	3	68	0	<1
EW-12	1,064	140	88	<1	4	82	11	7	<1	<1
EW-14	250	70	158	<1	10	51	14	32	<1	2
PS-01	184	154	177	0	1	36	30	34	0	<1
PS-02	65	273	100	<1	4	15	62	23	<1	1
PS-03	101	129	156	0	10	26	33	39	0	2
PS-04	134	82	215	<1	6	31	19	49	<1	1

Table 4—Pooled abundance (# individuals/0.1 m^2) and relative abundance (percent) of pollution-sensitive taxa at 6 stations in the East Waterway of Everett Harbor and at 4 references area stations in Port Susan.

Species	Station									
	EW01	EW04	EW07	EW10	EW12	EW14	PS01	PS02	PS03	PS04
Ampelisca agassizi										1
Ampelisca brevisimulata									1	
Ampelisca careyi								5	9	2
Ampelisca hancocki							5			2
Ampelisca spp										1
Amphiodia digitata								1		
Amphioda psara										1
Amphioda urtica					1					
Corophium acherusicum				1	170	1				
Corophium spp				1	138	2				
Heterophoxus oculatus							68			
Rhepoxynius abronius				1						
Rhepoxynius variatus								22	1	
Terebellides stroemi							402	1	9	14
TOTAL ABUNDANCE	0	0	0	3	309	3	475	29	20	21
RELATIVE ABUNDANCE (%)	0	0	0	<1	5	<1	18	1	1	1

Blank indicates species was absent.

Table 5—Pooled abundance (# individuals/0.1 m^2) and relative abundance (percent) of pollution-tolerant taxa at 6 stations in the East Waterway of Everett Harbor and at 4 reference area stations in Port Susan

	Station									
Species	EW01	EW04	EW07	EW10	EW12	EW14	PS01	PS02	PS03	PS04
Armandia brevis		32	12	39	68	73				
Capitella capitata	231	7,076	71	3,773	9	7	1			
Eteone spp		26	2	8				2		4
Eteone longa		57	2	6			5	2	1	3
Euphilomedes producta					26		149	28		229
Euphilomedes carcharodonta			1	5	71	9		184	347	366
Glycinde picta		2		1	52	12	2		3	
Glycinde armigera					6	2			3	
Goniada brunnea/maculata								7	11	16
Heteromastus spp									1	
Heteromastus filobranchus						2	30			
Heteromastus filiformis						1				
Leitoscoloplos panamensis								3	1	
Leitoscoloplos spp									1	
Leitoscoloplos pugettensis				1	7	1		118	68	19
Lumbrineris spp						2	103		2	4
Lumbrineris sp GR1						15		57	61	74
Lumbrineris sp GR3				1	1	14			1	1
Lumbrineris sp GR4						26				
Lumbrineris luti						7	88	78	46	47
Lumbrineris cruzensis						2				
Macoma moesta alaskana										5
Macoma carlottensis			2	18		4	63	6		2
Macoma elimata			7				4	4	10	12
Macoma spp	1	8		39						17
Macoma balthica					8	14		36	118	
Macoma inquinata						25				
Macoma nasuta			16	29	76	23				21
Macoma obliqua				4	75	7		5	4	

Table 5—Pooled abundance (# individuals/0.1 m^2) and relative abundance (percent) of pollution-tolerant taxa at 6 stations in the East Waterway of Everett Harbor and at 4 reference area stations in Port Susan (*continued*)

Species	Station									
	EW01	EW04	EW07	EW10	EW12	EW14	PS01	PS02	PS03	PS04
Mediomastus californiensis							2			
Mediomastus spp					13	7		11	1	i
Mya arenaria		2			12		1	3	1	2
Nephtys spp							13			
Nephtys punctata							5			
Nephtys ciliata						2				
Nephtys caeca			1		55				1	
Nephtys assignis										1
Nephtys cornuta										1
Nephtys ferruginea					1		14		18	10
Nereis procera		1	2		1	2		1	2	
Nereis (Neanthes) virens							1			
Nereis spp						3				
Nereis brandti				2						
Nereis ferruginea								49		
Oligochaeta		16	4	1	1	4				
Ophelina acuminata		1					12	30	8	4
Ophryotrocha spp				4						
Paraprionospio pinnata							1			
Parvilucina tenuisculpta					2	4			2	10
Prionospio multibranchiata			2	10	24	13		5	2	2
Prionospio cirrifera		3		206	25	234	1	3		1
Prionospio spp			1	11	3	17				3
Prionospio steenstrupi				5	9	58	6	6	10	15
Schistomeringos rudolphi					1	9	2			
Schistomeringos japonica						1				
Scoloplos armiger					2		1	10	3	i
Scoloplos spp					1					
Scoloplos ameceps					3				1	2

Table 5—Pooled abundance (# individuals/0.1 m²) and relative abundance (percent) of pollution-tolerant taxa at 6 stations in the East Waterway of Everett Harbor and at 4 reference area stations in Port Susan *(continued)*

Species	Station									
	EW01	EW04	EW07	EW10	EW12	EW14	PS01	PS02	PS03	PS04
Stenothoidae							2			
Tharyx spp		1			1	9		3	1	
Tharyx multifilis							18			
TOTAL ABUNDANCE	232	7,225	123	4,163	553	609	524	651	728	873
RELATIVE ABUNDANCE (%)	86	90	45	69	8	25	20	29	37	40

Blank indicates species was absent

Table 6—Top 10 taxa ranked by total pooled abundance (# individuals/0.5 m^2) at 6 stations in the East Waterway of Everett Harbor and at 4 reference area stations in Port Susan

Species	Station									
	EW01	EW04	EW07	EW10	EW12	EW14	PS01	PS02	PS03	PS04
Alvania spp				5	4	4				
Ampharete acutifrons										8
Aoroides spp		5	6	7		6				
Aoroides spinosus		3	2	4		2				
Armandia brevis		6	8	9		9				
Axinopsida serricata							10	5	6	6
Balanus spp			9							
Balanus crenatus	4		3							
Cancer oregonensis						7				
Capitella capitata	1	1	1	1						
Clinocardium nuttali								3	7	
Corophium acherusicum					6					
Corophium spp					7					
Eobrolgus spinosus					9					
Eteone spp		8								
Eteone longa		4								
Euphilomedes producta							4		4	4
Euphilomedes carcharodonta								2	1	1
Gastropteron pacificum	8									
Glycera capitata										10
Gnorimosphaeroma oregonensis	6									
Heterophoxus oculatus							8			
Leitoscoloplos pugettensis								4	8	
Leptochelia spp	7				3					
Leptochelia dubia					1					
Limnoria algarum	5									
Lumbrineris luti							6	6		9
Lumbrineris spp							5			
Lumbrineris sp GR1								7	10	7

Table 6—Top 10 taxa ranked by total pooled abundance (# individuals/0.5 m^2) at 6 stations in the East Waterway of Everett Harbor and at 4 reference area stations in Port Susan (*continued*)

Species	Station									
	EW01	EW04	EW07	EW10	EW12	EW14	PS01	PS02	PS03	PS04
Macoma nasuta			4							
Macoma balthica								10	5	
Macoma eliminata			10							
Macoma carlottensis							9			
Macoma spp	9	10		8						
Melita dentata						8				
Melita desoichada				10						
Melita californica				6						
Mysella tumida						10				
Nebalia spp	2	2	5	2		1				
Nephtys ferruginea								8		
Odostomia spp							7			
Oligochaeta		9								
Orchomene pinquis					8					
Pectinaria granulata					10				2	2
Photis brevipes					2					
Photis spp					5					
Pinnixia spp								9		
Pista spp									9	3
Platynereis bicaniculata	3	7	7			5				
Prionospio cirripera				3		3				
Protomedia prudens							1			
Psephidia lordi							2	1	3	5
Terebellides stroemi							3			

Of the top ten taxa, 1 is most abundant; 10 is least abundant.

Table 7—Abundance (# individuals/0.1m²) of benthic infauna within each feeding strategy group and associated Infaunal Trophic Index (ITI) values.

Station	Feeding Strategy Groups (# individuals/0.1m²)				ITI
	Group I	Group II	Group III	Group IV	
EW-01	0	0	0	231	0.1
EW-04	1	10	1	7,096	0.2
EW-07	1	13	4	75	12
EW-10	4	257	20	3,774	5
EW-12	114	1,178	4	11	69
EW-14	11	557	14	21	64
PS-01	208	954	66	17	70
PS-02	61	1,632	8	30	67
PS-03	218	1,056	16	8	72
PS-04	463	1,104	26	4	76

Table 8—Benthic community diversity indices at 6 stations in the East Waterway of Everett Harbor and at 4 reference area stations in Port Susan.

Station	Diversity Indices			
	H'	J	D	SDI
EW-01	0.26	0.28	0.72	1.14
EW-04	0.24	0.16	0.84	1.17
EW-07	1.17	0.75	0.25	7.83
EW-10	0.62	0.34	0.66	1.63
EW-12	1.02	0.51	0.49	5.86
EW-14	1.49	0.70	0.30	14.4
PS-01	1.20	0.62	0.38	7.52
PS-02	1.13	0.57	0.43	7.41
PS-03	1.40	0.69	0.31	11.2
PS-04	1.42	0 67	0 33	11 8

Table 9—Probability of significant differences among station pairs based on mean total abundance at 6 stations in the East Waterway of Everett Harbor and at 4 reference stations in Port Susan.

ANOVA Using Mean Total Abundance						
	Among Station Comparisons Using Only East Waterway Stations					Comparisons Between East Waterway and Reference Stations
Station	EW-01	EW-04	EW-07	EW-10	EW-12	Pooled Reference
EW-01						<0.00001
EW-04	<0.00001					<0.005
EW-07	NS	<0.00001				<0.00001
EW-10	<0.00001	NS	<0.00001			NS
EW-12	<0.00001	NS	<0.00001	NS		<0.00001
EW-14	<0.00001	<0.005	<0.00001	NS	<0.028	NS

NS: Differences between stations not significant at P≤0.05.
Probabilities based on Tukeys *a posteriori* contrasts.

Table 10—Probability of significant differences among station pairs based on mean richness at 6 stations in the East Waterway of Everett Harbor and at 4 reference stations in Port Susan

ANOVA Using Mean Richness						
	Among Station Comparisons Using Only East Waterway Stations					Comparisons Between East Waterway and Reference Stations
Station	EW-01	EW-04	EW-07	EW-10	EW-12	Pooled Reference
EW-01						<0.00001
EW-04	NS					<0.00001
EW-07	NS	NS				<0.00001
EW-10	<0.00001	<0.03	<0.03			<0.001
EW-12	<0.00001	<0.00001	<0.00001	<0.001		NS
EW-14	<0.00001	<0.00001	<0.00001	<0.00001	NS	NS

NS: Differences between stations not significant at P≤0.05.
Probabilities Based on Tukeys *a posteriori* contrasts

Table 11—Probability of significant differences among station pairs based on mean crustacean abundance at 6 stations in the East Waterway of Everett Harbor and at 4 reference stations in Port Susan.

ANOVA Using Mean Crustacean Abundance						
	Among Station Comparisons Using Only East Waterway Stations					Comparisons Between East Waterway and Reference Stations
Station	EW-01	EW-04	EW-07	EW-10	EW-12	Reference
EW-01						<0.00001
EW-04	<0.00001					<0.00001
EW-07	NS	NS				<0.00001
EW-10	<0.00001	NS	<0.001			<0.001
EW-12	<0.00001	<0.005	<0.00001	NS		<0.00001
EW-14	<0.00001	NS	<0.003	NS	NS	<0.006

NS: Differences between stations not significant at P≤0.05.
Probabilities based on Tukeys *a posteriori* contrasts

Table 12—Probability of significant differences among station pairs based on mean mollusc abundance at 6 stations in the East Waterway of Everett Harbor and at 4 reference stations in Port Susan.

ANOVA Using Mean Mollusc Abundance						
	Among Station Comparisons Using Only East Waterway Stations					Comparisons Between East Waterway and Reference Stations
Station	EW-01	EW-04	EW-07	EW-10	EW-12	Pooled Reference
EW-01						<0.00001
EW-04	<0.026					<0.00001
EW-07	<0.004	NS				<0.00001
EW-10	<0.00001	<0.00001	<0.002			<0.002
EW-12	<0.00001	<0.00001	<0.00001	<0.017		NS
EW-14	<0.00001	<0.00001	<0.00001	NS	NS	NS

NS: Differences between stations not significant at P≤0.05.
Probabilities based on Tukeys *a posteriori* contrasts

Table 13—Probability of significant differences among station pairs based on mean polychaete abundance at 6 stations in the East Waterway of Everett Harbor and at 4 reference stations in Port Susan.

ANOVA Using Mean Polychaete Abundance						
	Among Station Comparisons Using Only East Waterway Stations					Comparisons Between East Waterway and Reference Stations
Station	EW-01	EW-04	EW-07	EW-10	EW-12	Pooled Reference
EW-01						<0.009
EW-04	<0.00001					<0.00001
EW-07	NS	<0.00001				<0.00001
EW-10	<0.00001	NS	<0.00001			<0.00001
EW-12	NS	<0.00001	<0.001	<0.00001		NS
EW-14	<0.004	<0.00001	<0.00001	<0.00001	NS	NS

NS: Differences between stations not significant at P≤0 05.
Probabilities based on Tukeys *a posteriori* contrasts

Table 14—Probability of Significant Differences Between Station Pairs Based on Mean Total Abundance at 6 Stations in the East Waterway of Everett Harbor and at 4 Stations in Port Susan

T-test Using Mean Total Abundance					
Station	PS-01	PS-02	PS-03	PS-04	Pooled Reference
EW-01	<0.00001	<0.00001	<0.001	<0.00001	<0.00001
EW-04	<0.001	<0.00001	<0.001	<0.001	<0.001
EW-07	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
EW-10	NS	NS	NS	NS	NS
EW-12	<0.007	<0.004	<0.004	<0.004	<0.005
EW-14	NS	NS	NS	NS	NS
NS: Differences between stations not significant at P≤0.01.					

Table 15—Probability of Significant Differences Between Station Pairs Based on Mean Richness at 6 stations in the East Waterway of Everett Harbor and at 4 Stations in Port Susan

T-test Using Mean Richness					
Station	PS-01	PS-02	PS-03	PS-04	Pooled Reference
EW-01	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
EW-04	<0.001	<0.00001	<0.00001	<0.00001	<0.00001
EW-07	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
EW-10	NS	<0.002	<0.00001	<0.00001	<0.00001
EW-12	NS	NS	NS	NS	NS
EW-14	NS	NS	NS	NS	NS
NS: Differences between stations not significant at P≤0.01					

Table 16—Probability of Significant Differences Between Station Pairs Based on Mean Crustacean Abundance at 6 stations in the East Waterway of Everett Harbor and at 4 Stations in Port Susan

T-test Using Mean Crustacean Abundance					
Station	PS-01	PS-02	PS-03	PS-04	Pooled Reference
EW-01	<0.001	<0.004	<0.003	<0.002	<0.002
EW-04	NS	NS	NS	NS	NS
EW-07	<0.005	NS	NS	<0.009	NS
EW-10	NS	<0.01	NS	NS	NS
EW-12	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
EW-14	NS	NS	NS	NS	NS
NS: Differences between stations not significant at P≤0.01					

Table 17—Probability of Significant Differences Between Station Pairs Based on Mean Mollusc Abundance at 6 Stations in the East Waterway of Everett Harbor and at 4 Stations in Port Susan

T-test Using Mean Mollusc Abundance					
Station	PS-01	PS-02	PS-03	PS-04	Pooled Reference
EW-01	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
EW-04	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
EW-07	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
EW-10	<0.009	<0.002	NS	NS	<0.01
EW-12	NS	NS	NS	NS	NS
EW-14	NS	<0.002	NS	NS	0.024
NS: Differences between stations not significant at P≤0.01.					

Table 18—Probability of Significant Differences Between Station Pairs Based on Mean Polychaete Abundance at 6 Stations in the East Waterway of Everett Harbor and At 4 Stations in Port Susan

T-test Using Mean Polychaete Abundance					
Station	PS-01	PS-02	PS-03	PS-04	Pooled Reference
EW-01	NS	NS	<0.004	<0.001	<0.004
EW-04	<0.001	<0.00001	<0.00001	<0.00001	<0.00001
EW-07	<0.005	<0.00001	<0.00001	<0.00001	<0.00001
EW-10	<0.005	<0.00001	<0.001	<0.003	<0.001
EW-12	NS	NS	<0.005	<0.001	<0.006
EW-14	NS	NS	NS	NS	NS
NS: Differences between stations not significant at P≤0.01.					

Table 19—Sampling Efficiency (SE/mean) Based On Richness, Total Abundance, and Major Taxonomic Group Abundance For 6 Stations in the East Waterway of Everett Harbor and 4 reference stations in Port Susan

Station	Sampling Efficiency (%)				
	Total Abundance	Richness	Crustacean Abundance	Mollusc Abundance	Polychaete Abundance
EW-01	26	18	76	100	31
EW-04	16	9	47	29	12
EW-07	20	14	43	23	8
EW-10	22	7	30	25	22
EW-12	18	7	18	25	14
EW-14	22	10	24	23	23
PS-01	15	8	11	8	34
PS-02	7	6	8	10	10
PS-03	4	6	4	8	8
PS-04	7	5	6	8	10
Values < 20 to 30 percent indicate sufficient power to compare and contrast data					

Table 20—Power Analysis Minimum Detectable Differences (MDD) Using Abundance and Richness Data from 10 Sampling Stations

No. of Sample Replicates	Raw MDD (% of mean)					Log(x+1) Transformed Data MDD (% of mean)			
	Total Abundance	Richness	Crustacean Abundance	Mollusc Abundance	Polychaete Abundance	Total Abundance	Crustacean Abundance	Mollusc Abundance	Polychaete Abundance
3	193	74	285	157	232	26	60	51	34
4	157	60	233	128	189	21	49	42	28
5	136	52	202	111	164	18	42	36	24
6	122	47	180	99	147	17	38	33	22
7	111	42	165	91	134	15	35	30	20
8	103	39	153	84	124	14	32	28	18
9	97	37	143	79	116	13	30	26	17
10	91	35	135	74	110	12	28	24	16
11	87	33	128	70	104	12	27	23	15
12	83	32	122	67	100	11	26	22	15
13	79	30	117	64	95	11	25	21	14
14	76	29	112	62	92	10	24	20	14
15	73	28	108	60	88	10	23	20	13

Table 21—Percent Similarities Among Benthic Communities From Cluster Analysis Based on Total Taxa Abundance (n>2) at 6 Stations in the East Waterway of Everett Harbor and at 4 Reference Stations in Port Susan

Clusters Linked (Stations)		Percent Similarity
PS-03	PS-04	68
PS-02	PS-03	59
EW-04	EW-10	56
EW-04	EW-07	42
EW-12	EW-14	41
PS-01	PS-02	30
EW-04	EW-12	28
EW-01	EW-04	19
EW-01	PS-01	10

Table 22—Percent Similarities Among Benthic Communities From Cluster Analysis Based on Crustacean Abundance at 6 Stations in the East Waterway of Everett Harbor and at 4 Reference Stations in Port Susan

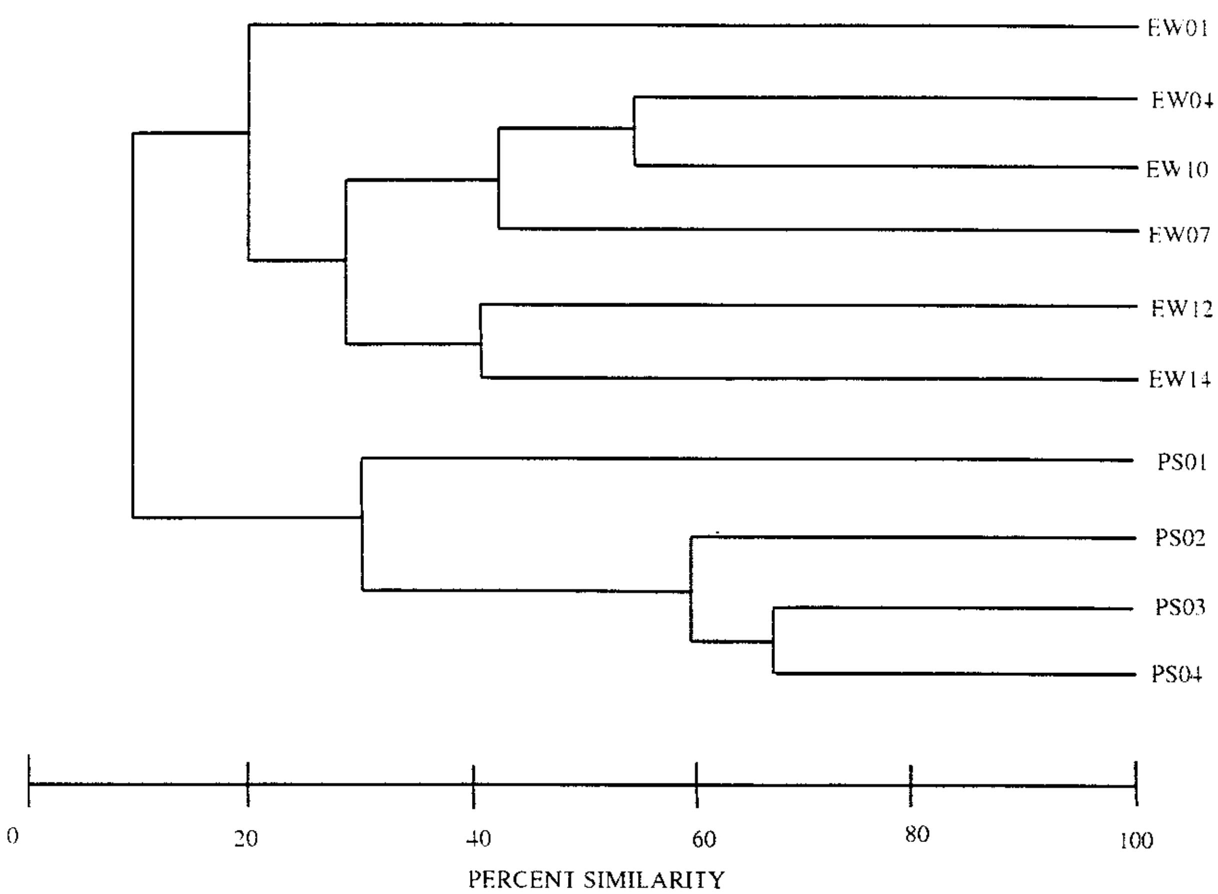
Clusters Linked (Stations)		Percent Similarity
PS-02	PS-03	66
EW-10	EW-14	65
PS-02	PS-04	56
EW-04	EW-07	49
EW-04	EW-10	40
PS-01	PS-02	19
EW-04	EW-12	17
EW-01	EW-04	12
EW-01	PS-01	6

Table 23—Percent Similarities Among Benthic Communities From Cluster Analysis Based on Mollusc Abundance at 6 stations in the East Waterway of Everett Harbor and at 4 Reference Stations in Port Susan

Clusters Linked (Stations)		Percent Similarity
PS-02	PS-03	70
EW-12	EW-14	62
PS-02	PS-04	54
EW-10	EW-12	42
PS-01	PS-02	39
EW-07	EW-10	23
EW-04	EW-07	18
EW-04	PS-01	15
EW-01	EW-04	2

Table 24—Percent Similarities Among Benthic Communities From Cluster Analysis Based on Polychaete Abundance at 6 Stations in the East Waterway of Everett Harbor and at 4 Reference Stations in Port Susan

Clusters Linked (Stations)		Percent Similarity
PS-03	PS-04	70
EW-04	EW-10	66
EW-01	EW-07	60
PS-02	PS-03	57
EW-12	EW-14	51
EW-01	EW-04	41
PS-01	PS-02	29
EW-01	EW-12	23
EW-01	PS-01	10



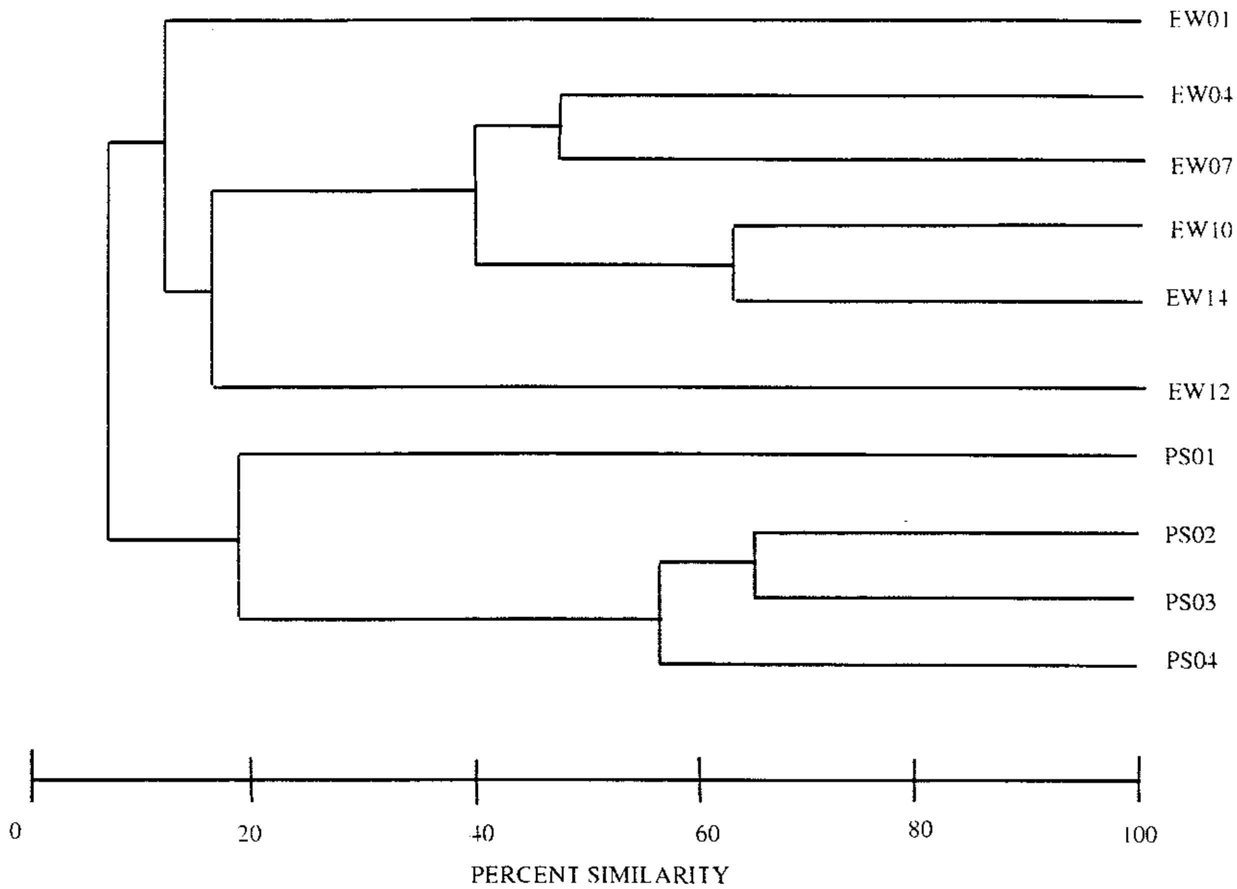
Dendrogram Resulting From a Bray-Curtis Classification Analysis Using Total Taxa Abundance (n>2) at East Waterway and Port Susan Stations

FIGURE

1

WESTON

PROJECT # 10070-001-001-100-0100 DATE January 1993



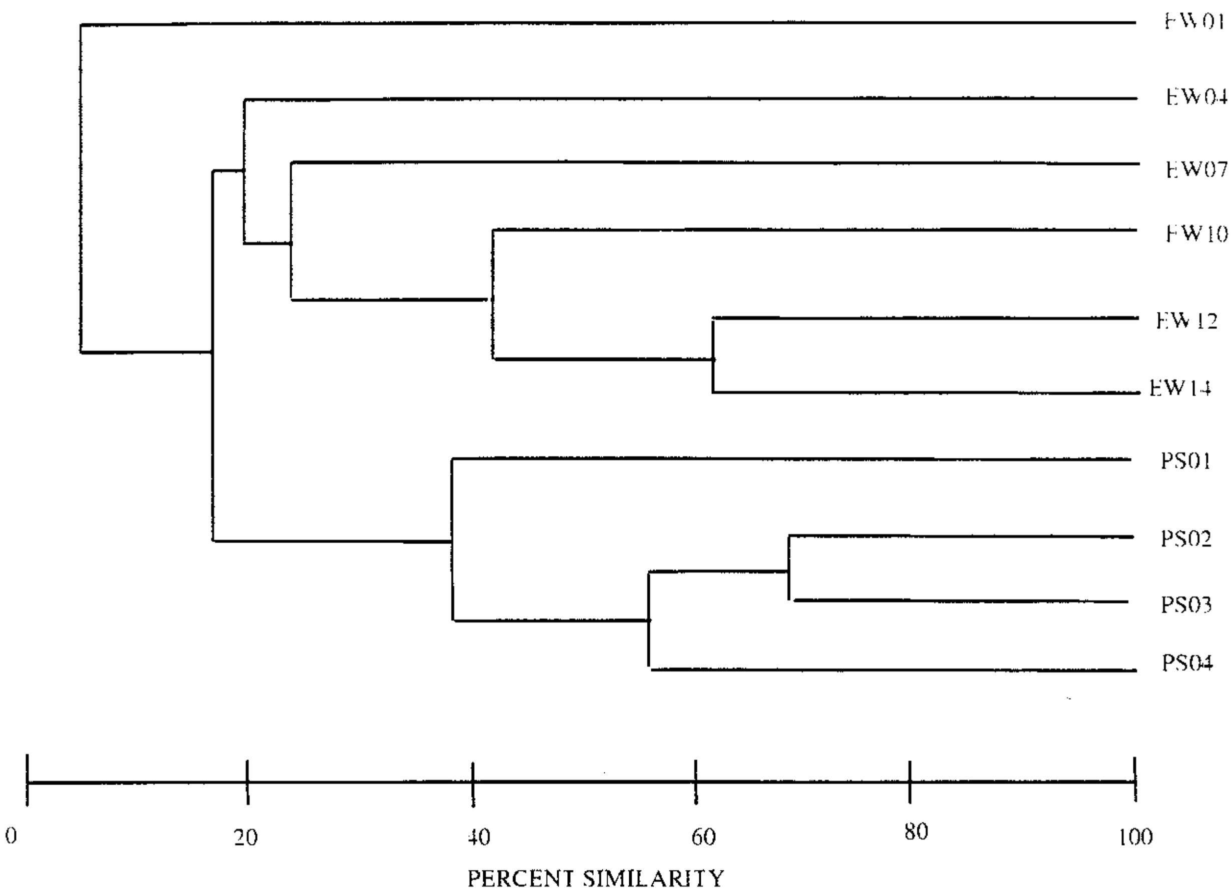
Dendrogram Resulting From a Bray-Curtis Classification Analysis Using Crustacean Abundance at East Waterway and Port Susan Stations

FIGURE

2

WESTON

PROJECT # 10070-001-001-0100 DATE January 1993

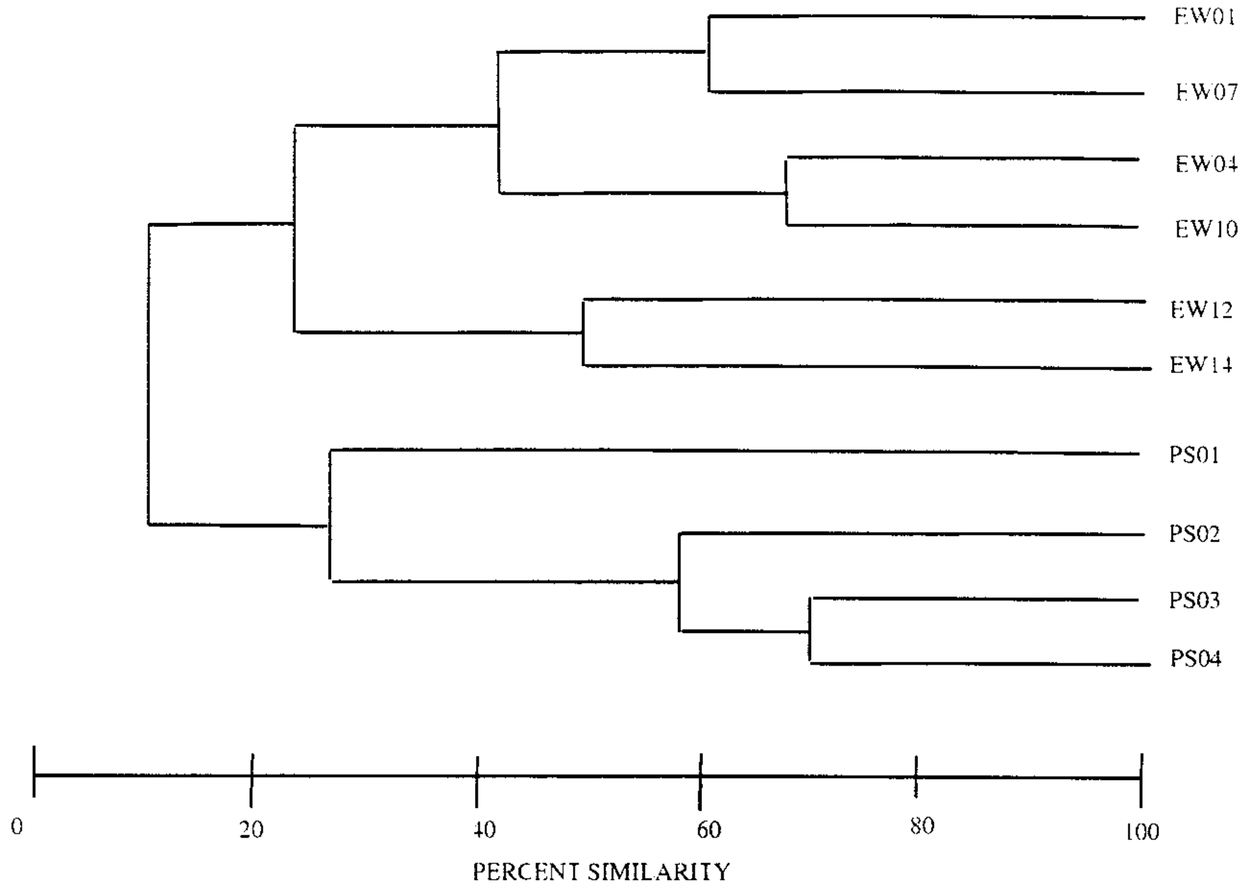


Dendrogram Resulting From a Bray-Curtis Classification Analysis Using Mollusc Abundance at East Waterway and Port Susan Stations

FIGURE **3**

WESTON

PROJECT # 10070-001-001-0100 DATE: January 1993

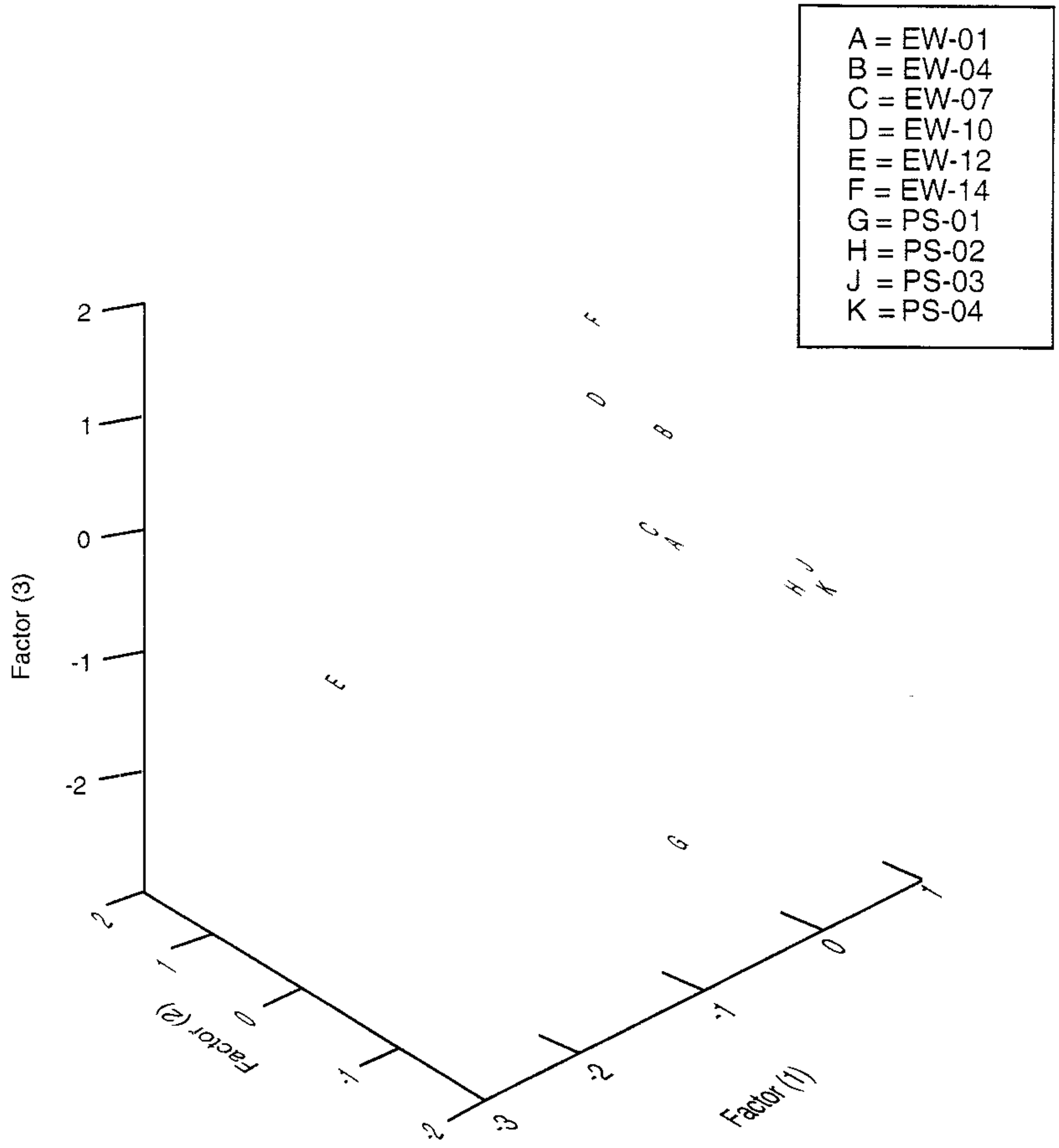


Dendrogram Resulting From a Bray-Curtis Classification Analysis Using Polychaete Abundance at East Waterway and Port Susan Stations

FIGURE
4

WESTON

PROJECT # 10070-001-001-0100 DATE: January 1993



Plot of Principal Components Analysis Results Using the Dominant Species at East Waterway and Port Susan Stations

FIGURE

5

WESTON

PROJECT # 10070-001-001-100-0100 DATE January 1993

APPENDIX B

DEMONSTRATION OF BENTHIC INDICES CALCULATIONS USING HYPOTHETICAL DATA

Species	Data Set				
	A	B	C	D	E
n_1	10	29	91	100	20
n_2	10	19	1	100	20
n_3	10	14	1	100	20
n_4	10	11	1	100	20
n_5	10	9	1	100	20
n_6	10	7	1	100	
n_7	10	5	1	100	
n_8	10	3	1	100	
n_9	10	2	1	100	
n_{10}	10	1	1	100	

Richness	10	10	10	10	5
Abundance	100	100	100	1000	100
H', Shannon diversity	1.00	0.86	0.22	1.00	0.70
H_{max}, maximum H	0.92	0.92	0.92	0.99	0.66
J, evenness	1.00	0.86	0.20	1.00	1.00
1-J, dominance	0	0.14	0.80	0	0
Swartz's dominance	7.5	4.2	0.82	7.5	3.75

Specific calculations for each of these indices are illustrated below:

- Shannon-Weiner diversity (H′; Pielou 1966)

  $H' = -\Sigma p_i(\log_{10} p_i)$ where

  p_i = the proportion of the total abundance that a single species represents

  Using data set B, the following steps are used to calculate diversity:

Species Abundance	Proportion	Log Proportion	Product	Cumulative Sum
29	0.29	-0.538	-0.156	0.156
19	0.19	-0.721	-0.137	0.293
14	0.14	-0.854	-0.119	0.412
11	0.11	-0.959	-0.105	0.517
9	0.09	-1.046	-0.094	0.611
7	0.07	-1.155	-0.081	0.692
5	0.05	-1.301	-0.065	0.757
3	0.03	-1.523	-0.046	0.803
2	0.02	-1.699	-0.034	0.837
1	0.01	-2.000	-0.020	0.857

- Pielou's evenness or equitability (J; Pielou, 1966)

  $J = H'/\log S$ where

  S = total number of species

  Using the above example, evenness is calculated as follows:

  $J = 0.86/\log_{10}(10)$ or $0.86/1 = 0.86$

- Numerical dominance (D; complement of evenness; Pielou, 1966)

  $D = 1 - J$

  Using data set B and the above evenness calculation, dominance is calculated as follows:

  $1 - 0.86 = 0.14$

- Swartz's dominance index (SDI; Swartz et al.. 1985)

  SDI = the minimum number (or fraction) of taxa whose combined abundance equals 75 percent of the total abundance

Using the above example, this index is calculated as follows:

Ranked Abundance	Cumulative Abundance	Number of Taxa	Cumulative Sum
29	29	1	1
19	48	1	2
14	62	1	3
11	73	1	4
9	82	2/9 = 0.22	4.22
7			
5			
3			
2			
1			

APPENDIX B

Recommendations from Experts Panel

Environmental Services for
Industry and Government

Seattle U S A
Vancouver Canada

EVS ENVIRONMENT CONSULTANTS

Our File: 2/368-27

February 26, 1993

Sandra Manning
Benthic Task Manager
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[FAX 206-493-2961]

Dear Sandra:

Re: National Benthic Experts Workshop

Thank you for inviting me to participate in the above, held yesterday in Seattle. I enjoyed the discussions and interactions.

In your letter of January 14, 1993 you asked that I prepare a written summary of my recommendations after the workshop. I have done so and attach same. I have not provided a separate evaluation of the different indices and analytical methods because I do not believe this to be useful without extensive caveating and explanation of what I mean (it was clear at the Workshop that each "expert" had different opinions of each category meant, thus this comparison is "apples and oranges").

Thanks again for inviting me; don't hesitate to call if you have any questions.

Sincerely,

EVS CONSULTANTS

Peter M. Chapman, Ph.D.
Partner

PMC/ubl
attach.

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MAR 8 1993

February 27, 1993

Dr Chip Hogue
PTI Environmental Services
15375 SE 30th Place, Suite 250
Bellevue, WA 98007

Dear Chip;

It was a pleasure to meet you after all those phone conversations. I feel that the workshop went well. I know that I learned a lot on how to approach similar questions of impact assessment in Chesapeake Bay. I have enclosed a summary of my workshop comments. I have also sent my review of the case study directly to Nancy Musgrove. I sent my expenses to Jane Sexton.

In response to questions from the audience for references on various topics we discussed, I have enclosed several reprints that deal with application of community structure metrics to impact assessment.

Let me know if you need any further information.

Sincerely,

Robert J. Díaz
Associate Professor
Biological Sciences

cc: J. Sexton
N. Musgrove

VIMS

NATIONAL BENTHIC EXPERTS WORKSHOP - February 25, 1993
Comments of Robert J. Diaz

The workshop had three objectives, as presented in the meeting agenda, and I perceived our job as experts was to:

1 - Review the selection of indices for use in a regulatory program detailing strong and weak points.

2 - Consider the selection of analytical techniques and propose additional approaches.

3 - Evaluate the dilemma of statistical significance vs. professional judgement

I will start with my introductory comments on the four lessons that need to be learned prior to proceeding with a impact assessment plan:

1 - Know what you want. You must come to an agreement on what is wanted, both sides of the environmental issue. The question of simplest is best may not work.

2 - Don't do more then you need too. Also, do not do anything till you know what you are going to do with it.

3 - One approach will not fit al cases. Too many variables influence benthic communities to allow for one approach to fit all cases.

4 - There is nothing new under the sun (at least not much). The approaches to assessing impacts exist. One needs to develop a subset of suitable approaches to met objectives. Spend time on data analysis, most cost effective thing you can do. Replicate at the level that is most important to what you want. Control for what is not important.

The initial list of indices (which I will call metrics since some are typically not thought of a indices) all revolve around species identity and numeric abundance. No way around one fact, → species level identification is expensive and time consuming. What you need to decide is:

1 - Do you use all that information?

2 - Is the information content in the dominant species, or some other subset of species, sufficient?

My experience indicates that identification of dominant and common species takes only a small fraction of the total identification time. Up to 80% of the time can be spent in identification of rare species. These are the ones that get cut from most multivariate analyses.

Workshop agreed upon levels are listed below with my revisions in parentheses.

Index	Sensitiv.	Objectiv.	Ease of Interpret.	Reference Comparison	Cost Effectiv.
Diversity	L	L	L	YES	L
Dominance*	(H)	(M)	(H)	(YES)	(H)
Infaunal Index	H	H(M)	M	YES	H
Total Abundance	M	M	M+	YES	H
Major Taxa Abundance	M	M	M+	YES	H
Species Level Abund	H	H	M+	YES	M
Indicator Species**	H	H	H	YES	M
Biomass***	M(H)	M	M(H)	YES	L/M(H/L)
Richness	H	M	M+	YES	L

* We talked about treating dominance separately, but I do not think we got around to doing a ranking.
** Only for indicators that are present, and not absence of species. Absence of species will work but it requires **very** careful control of non-impact variables that are likely regulating the distribution of species.
*** Measurement of biomass can range from gross total to species level, and from wet weight to AFDW. So the cost effectiveness ranges from low to high depending on what you pick. In any case biomass is the one metric in the list that integrates the affect of all physical and biological processes acting on a habitat. The only other metric on the list that integrates benthic functional level response signals is the Infaunal Index.

The assumption made in ranking the analytical methods is that proper attention was given to the sampling design so that the correct type and amount of data were collected. With **poorly** designed field studies **any** analysis approach would have low scores in all of the categories

Analytical Methods	Sensitiv.	Objectiv.	Ease of Interpret.	Reference Comparison	Cost Effectiv.
Analysis of variance	H	H	H	NO	H
Pair-wise Comparisons	M	M	H	NO	H
Cluster Techniques	H	L/M	M	NO	H
Ordination Techniques	H	L/H	M	NO	H
Graphical Methods	H	H	H	NO	H
Contrasts	H	H	H	NO	H
Regression	H	M	M	NO	H

Comparison to a reference area is not essential for ANOV and pair-testing. As long as the factor (i.e., impact gradient) is measured at an appropriate number of points from bad to better or good the strength of the impact is measurable with ANOV. Inclusion of reference areas is a good idea and may help better define the impact. But, if suitable reference areas are not available these analysis techniques will still work. The biggest problem is failing to control for sources of variation that are not related to the factor selected for the ANOV Also if regression and correlation techniques are used then a reference is also not needed. Actually none of the statistical techniques need a reference area to work They could all be effectively used without reference areas. **What is most crucial in statistical testing is to control for what is not of interest but influences the variables of interest.**

The selected metrics and analyses indicate a strong reliance on tradition methods. This will optimize the connection to historical data but may hamper your quest for efficient and effective methods. Explore other metrics like the median as a measure of central tendency. Other statistical methods that do not use the mean and the normal probability distribution should be evaluated. Consider the usefulness of logistic regression, odds ration, loglinear modeling, G-Maximum likelihood X^2.

There are as many schools of thought on analysis strategies as Carter has little liver pills. The Smith-Berstein Ecological Data Analysis (EDA, at least I think their package is called EDA) school could be seen as one extreme. EDA relies heavily on the normal probability distribution and multivariate techniques. The other extreme is straight descriptive statistics and graphical methods followed by hypothesis testing that use other probability distributions (nonparametrics, logistic regression, odds ration, loglinear modeling, G-Maximum likelihood X^2).

Process integrating metrics are a possible source of sensitive and robust variables that may have promise. The list we discussed contained two integrating metrics, Infaunal Index and Biomass. The Infaunal Index needs to be further investigated. Biomass needs to be considered for inclusion. Benthic community biomass represents an integration of factors that effect the most basic and important ecosystem functional process, that of growth and secondary productivity. Biomass changes can result form:

1- Species turnover
2- Changing numerical abundance
3- Body size changes
4- Shallowing of biomass vertical distribution
5- Changes in trophic groups

All of the metrics we considered are involved in one or more of these five changes.

In hindsight the one variable that I wish I had collected as consistently as species level abundance all throughout my carrier is biomass. Data on any form of this variable opens up another dimension in assessing impacts. For impact assessment the best form of biomass measurement is vertical distribution. Vertical distribution profiles of biomass integrate many physical and biological processes. I have found this variable to be consistently sensitive to toxic as well as organic enriched sediments. In Puget Sound Weston (1990) found vertical biomass distribution to be a very sensitive measure in assessing impacts from pen aquaculture.

Much of the improvement to selected analytical techniques will have to wait till the Lesson-1 what you want question is answered. There is no way around this. How can you select the proper technique without the proper question **Still much of the problem, if not all the problem, in analysis is related to field design and data collection methods.** Once you are out of the field and finished sample processing your options are fixed. Even voodoo statistics can not recover the answer when the data are confounded by nonrandomness or correlated effects.

Concluding Comments

Need to know your system from a biological and physical point of view prior to selection of a subset of field and statistical methods. Nothing can be done until you have an understanding of the scales of variation (spatial and temporal) you are dealing with.

Benthic organisms are just like other life forms we know, their comings and goings are erratic Many times they just seem to do what they darn well please. This means that no one design or list of metrics and analyzes will do all that we want.

You must learn to express your data Do not suppress the information contained in your numbers You spend most of your time and money getting these numbers so coddle and fondle them. Love your numbers. Before you are able to make any informed decision on environmental matters you have to have a clear understanding of simple trends in your data. By this, I mean what you are able to extract for the data when you look at them graphed and in simple summary tables, not the output of some complicated computer technique.

Most of the metrics that you put fort require species level taxonomy. This results in two things at which the managers that have their hands on the pure strings tend to balk; 1- lots of time (and remember that time is money) is needed to get an answer, 2- only a few highly trained individuals can produce an answer (an answer that you trust anyway) leaving most people as outsiders to getting an answer You end up with an evaluation approach that is not possible to implement on all your management problems. When it come down to the bottom line, if it cost to much to implement an assessment plan then it will not be used.

March 2, 1993

MAR 5 1993

PTI

Dr. Chip Hogue
PTI Environmental Services
15375 SE 30th Place, Suite 250
Bellevue, Washington 98007

Re: Written Summary of Comments and Recommendations for the National Benthic Experts Workshop, February 25, 1993

Dear Dr. Hogue:

The following are my comments and recommendations pertaining to the evaluation of techniques for assessing benthic endpoints for use in Puget Sound sediment management programs. This is a written summary of points that I presented as a panelist at the recent National Benthic Experts Workshop, February 25, 1993. As requested, I also have filled out the table of evaluation criteria for each of the various biological indices and analytical methods (see attached table filled out by hand).

Comments and Recommendations

1. Criteria for biological change should be based on indices other than just abundances of major taxonomic groups. Decisions as to whether a biological impact has occurred should be based on a **combination of sensitive and meaningful indices**, including those based on numbers of species, abundances of individual taxa, and the distribution of individuals among important functional groups (e.g., the infaunal index). The following indices are highly recommended, based on their sensitivity, objectivity, and ease of interpretation, as summarized in the table: species richness, total faunal abundance, abundances of individual species including indicator species, and the Infaunal Index. Diversity measurements such as H´ (i.e., measures of species heterogeneity) are not recommended because values can be misleading due to the dual influence of both of the components species richness and evenness (e.g., two assemblages might have the same value of H´, with one having high richness and low evenness, and the other having low richness and high evenness). Measures of dominance (e.g., Swartz's Dominance Index and Gleason's Index) and evenness are also recommended when used by themselves. Biomass measurements can also be useful, and have implications with respect to production and trophic value of the system, but must be used with some caution to avoid misleading interpretations when a few individuals of large-sized species (which may be rare numerically) swamp the biomass.

For most of these indices (probably all but the infaunal index), decisions as to whether changes have occurred between different times or sites can be based on ANOVA, or other appropriate hypothesis testing procedures, at a specified statistical confidence level (e.g., alpha of 0.05). Infauanl Index values should be evaluated qualitatively; in general, values less than 65% are indicative of benthic communitiies composed of transitional or pollution-tolerant taxa, as identified in the report prepared by Weston and PTI for the benthic workshop.

I suggest that the biological criteria component of the decision-making framework for evaluating sediment quality include the requirement to measure a suite of benthic community indices (i.e., all of those in our table, except H′ diversity). The trigger for deciding whether a pollution related impact has occurred could then be based on whether a required number (say two or more) of these indices have shown a statistically significant or other obvious change, **and** are correlated with a concomitant change in the concentration of a chemical contaminant or other physical/chemical change linked to a suspected anthropogenic activity.

2. Specimens should be identified to the species level to accommodate measurements of species richness and species-level abundances.

3. I suggest incorporating approaches that look at relationships between biological and other environmental variables, so that biological patterns reflecting possible among-site or among-time differences can be examined in light of controlling factors and possible linkages to pollution-induced impacts. Sediment quality triad studies, which combine measurements of sediment chemistry, sediment toxicity, and in-situ benthic community conditions, have demonstrated the strength of using multiple indicators as a basis for drawing conclusions about the status of pollution impacts in important coastal ecosystems.

4. The decision-making framework should stress wherever possible the need to examine change at a suspected impact site in comparison to both spatial and temporal controls. Such an approach is consistent with the highly powerful "optimal-impact study design" of Green (1979).

5. If the objective is to detect a pollution-induced change in the biological community, it is important that the sampling design controls for other natural environmental factors that may influence benthic species distributions. For example, if examining benthic distributions in relation to depth and substrate types is not among the objectives of the study, then the study design can be optimized by sampling consistently within similar depth and sediment strata for both reference and suspected impact sites.

6. Pollution tolerance should not be confused with opportunism, which often has been the case in attempts to evaluate pollution impacts on the benthos by the presence of indicator species. The presence of opportunistic species, for example, is often used to characterize polluted conditions. Some species, in fact, are opportunistic (i.e., can reproduce, grow, and colonize empty substrates rapidly) but have moderate to low tolerances to physiological stresses (e.g., high sensitivity to chemical toxicity). Ampeliscid amphipods and the small capetellid polychaete *Mediomastus ambiseta* are examples (see Hyland et al. 1985). Conversely, some species with more conservative reproductive strategies may have high physiological tolerance limits or have behavioral mechanisms that help avoid the stress. Some species of molluscs (e.g., *Mercenaria* and *Nucula*) are examples here. Determining the relative proportions of pollution-tolerant vs. pollution-sensitive species can give valuable results, but the distinction between reproductive strategies and physiological/behavioral mechanisms for dealing with stress should be kept in mind.

Some Suggested Analytical Methods

Between-site or -time differences in univariate indices (e.g., numbers of species, numbers of individuals) can be tested as a two-stage procedure consisting of a one-way ANOVA *F* test, or the distribution-free Kruskal-Wallis test in the nonparametric case, followed by *a-posteriori* comparison of all means in pairs using unplanned multiple-comparison procedures (see review by Day and Quinn 1989). ANOVA coupled to appropriately selected multiple-comparison tests are recommended for several reasons: 1) ability to examine mean differences in response variables in relation to two or more main effects; 2) greater control over the "Experimentwise" Type I error rate; 3) maximization of power to detect real differences; and 4) there is a variety of different tests, from which to choose, which are robust to violations of assumptions (e.g., unequal variations and nonnormality).

As discussed by Day and Quinn (1989), if sample variances are equal, the parametric Ryan's Q test (Einot and Gabriel 1975), or the nonparametric Joint-Rank Ryan's test (Campbell and Skillings 1985), can be used for multiple comparisons following the ANOVA in cases of normal and non-normal distributions, respectively. For unequal variances, the parametric Games-Howell test (Games and Howell 1976), or the nonparametric Fligner-Policello test (Fligner and Policello 1981), can be used in cases of normal and non-normal distributions, respectively. Tests for normality and homogeneity of variances can be performed using the procedures available in SAS (1985).

Two additional methods have been found to be very useful in determining patterns in infaunal community structure and composition: 1) graphical comparison of distributional patterns in the species-level data among the different study sites or times, using k-dominance plots (Lambshead et al. 1983); and 2) the multivariate method of nonmetric Multi-Dimensional Scaling Ordination (MDS) (Kruskal and Wish 1978) applied by site or time on a Bray-Curtis similarity matrix of appropriately transformed species data. As with the univariate methods discussed above, each of these methods can be used in conjunction with an appropriate multivariate statistical test to determine the significance of differences between replicated community samples from different study sites or times. For example, Warwick and Clarke (1991) applied the simulation/permutation test ANOSIM (Clarke and Green 1988, Clarke 1990) as a test statistic for each of these methods. ANOSIM does not assume multivariate normality in the data.

K-dominance curves rank species in order of importance on the x-axis (logarithmic scale) with percentage dominance on the y-axis (cumulative scale). It has been suggested that the distributions of numbers of individuals (or biomass) among species in marine benthic communities, as depicted in the k-dominance curves, can be used to reflect levels of response to pollution-induced disturbance. The shape of the k-dominance curve, for example, is usually steeper in polluted communities due to irruptions of dominant opportunistic and stress-tolerant species, and more sigmoid-shaped in unpolluted communities, due to a more even distribution of individuals among a greater number of species including "equilibrium species." Thus the status of pollution effects on benthic communities in contaminated versus uncontaminated sites can be evaluated by comparison of the shapes of these curves.

Both the univariate and distributional/*k*-dominance methods offer straightforward results (e.g., a reduction in diversity or elevation of *k*-dominance curves) from which value judgments regarding amounts of disturbance can be attached. However, Warwick and Clarke (1991) suggest that the multivariate MDS method offers the additional advantages of greater sensitivity in detecting change and generality of response (i.e., not sensitive to just a few faunal groups). The authors demonstrate these attributes with a wide range of pollution effects cases.

Numerical classification, or "cluster analysis," is also a useful method for exploratory data analysis and can be a powerful tool for identifying patterns of faunal similarity among sites or times from complex multivariate data sets. A number of clustering rules and resemblance measures are available (see Boesch 1977), but group-average sorting (= unweighted pair-group method; Sneath & Sokal, 1973) as a clustering method and Bray-Curtis similarity (Bray & Curtis, 1957) as a resemblance measure are commonly used in marine benthic studies. Results are usually expressed in the form of dendrograms in which samples are ordered into groups of increasingly greater similarity based on resemblances of component-species abundances. Thus samples clustered closely together display greater similarities than samples spaced further apart and the degree of separation can be used to depict spatial or temporal differences due to some environmental factor or combination of factors.

The above methods are ones that can be used for depicting differences in benthic communities among various study sites or times. Additional multivariate techniques and correlation analyses should be used to help identify what environmental variables, measured synoptically, are most responsible for the observed faunal differences. Ideally, the other environmental variables should include those that would allow testing for relationships between biological, chemical/physical, and toxicological conditions of the sediments. Correlation analysis (e.g., Pearson's product-moment correlation coefficient) can be used to determine the direction and strength of association between benthic indices and other environmental variables. The significance of the correlations, i.e., the null hypothesis that any two variables are not correlated (H_0: $p = 0$), can be tested as a t-test, with n-2 *df*.

Two additional multivariate methods have been shown to be useful as a means of exploring relationships between biological and other measured environmental variables. The first method, recommended by Green and Vascotto (1978) is a numerical classification (or cluster) analysis of the biological data followed by multiple discriminant analysis of the species-assemblage groups on the environmental variables. The discriminant (= canonical) analysis is used to derive a reduced set of discriminant functions that best describe the separation of the pre-declared station groups based on data represented by the different environmental variables. Total structure coefficients, which are the correlations between the original variables and the discriminant scores on each function, serve as a measure of the relative contribution of each variable to the group separation derived by numerical classification of the species data. Station groups are then plotted in the reduced discriminant space and interpreted visually in light of those variables that account for the majority of the separation. Hyland et al. (1991) applied this procedure to the analysis of macroinfauna distributions in relation to environmental factors on the outer continental shelf and slope off Point Conception, California.

The second multivariate method, suggested in a recent review of methods for analyzing benthic community structure by Warwick and Clarke (1991), consists of matching multivariate ordinations from subsets of environmental data to an ordination of faunistic data, with the idea of establishing a relationship between the faunal ordination and pattern in the environmental variables. Experimenting with the environmental variables in different combinations allows one to choose which of the combinations matches the faunistic ordination most closely and thus to identify which variables are the most responsible for the biological differences. The ordination procedure used in the examples given in Warwick and Clarke (1991) consisted of Multidimensional Scaling Ordination (MDS) by stations of appropriately transformed infaunal data, together with correlation-based Principal Components Analysis (PCA) on the measured environmental variables.

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End of Comments and Recommendations.

Thank you for the opportunity to participate in this important workshop. The State of Washington has taken a leading role in our nation in efforts to establish sediment quality standards for marine ecosystems and should be commended for their current progress as well as continuing interest in searching for improvements.

Sincerely,

Jeffrey L. Hyland

Jeffrey L. Hyland, Ph.D.

Telephone: 508/263-2509 (home); 617/498-5373 (office)

COMPARISON OF BENTHIC COMMUNITY INDICES WITH EVALUATION CRITERIA

	Evaluation Criteria				
	Sensitivity	Objectivity	Ease of Interpretation	Reference Area Comparison	Cost Effectiveness
Index					
Diversity = H′ (or other measures of heterogeneity)	L	L	L	Required	L
Dominance vs. evenness	M	M	M/H	Required	L
Infaunal index	H[a]	M[b]	M[b]	Required	M
Total abundance	M[c]	M	H	Required	H
Major taxa abundance	M	M	M/H	Required	H
Species level abundance	H	H	M/H	Required	M
Indicator species	H	H	H	Required	M
Biomass	M	M	M	Required	L/M
Richness	H	H	H	Required	M/H[d]

Analytical Methods

Analysis of variance (coupled to appropriately selected "unplanned" multiple-comparison tests)	Strongly recommended; approach offers 1) ability to examine mean differences in response variables in relation to two or more main effects, 2) greater control over the "experiment-wise" Type I error rate, 3) maximization of power to detect real differences, and 4) various tests from which to choose that are robust to violations of assumptions (e.g., unequal variances and non-normality).
Pair-wise comparisons (e.g., *t*-tests)	Not recommended if the objective is to determine whether more than two means are significant. Usually must decrease the error rate per individual comparison to a greater extent than with unplanned multiple comparison procedures in order to reduce the experiment-wise error rate to an equivalent amount (50 percent). Lower power will result when the Type I error per each comparison is low. A *t*-test is fine, however, when a comparison of two means was planned as part of the experimental design.
Cluster and ordination techniques	Highly recommended for exploratory data analysis as a powerful tool for identifying trends among complex multivariate data sets. Also, ordination on environmental variables, coupled to cluster analysis of environmental samples based on similarities of species, can be a very effective method of identifying what environmental factors (natural vs. anthropogenic) are accounting for the among-site differences.
Graphical methods	Highly recommended as a straightforward method of illustrating relationships between biological and other chemical and physical environmental variables.

COMPARISON OF BENTHIC COMMUNITY INDICES (cont.)

[a] The sensitivity or the infaunal index is apparently high in Puget Sound.

[b] Some subjectivity and error in interpretation can result from 1) categorizing species into the various feeding strategies, and 2) a trend may apply to organic enrichment, but chemical contaminants may be the controlling factors (i.e., physiological sensitivity to toxicity).

[c] The sensitivity does not reflect distribution of abundance among species.

[d] The resulting information is of high value, but the process is expensive relative to total abundance measures.

NATIONAL BENTHIC EXPERTS WORKSHOP
FEBRUARY 25, 1993

SUMMARY OF RECOMMENDATIONS - Nancy Musgrove

Selection of benthic endpoints for use in environmental assessment seemed to be the least controversial topic within the workshop. Most of the discussion involved refinement of the use of traditional or widely accepted endpoints rather than discussion of new endpoints. Based on my experience in evaluating benthic data including the Everett Harbor case study, I would like to make the following recommendations for selection of benthic effects endpoints:

- Multiple benthic community metrics or effects endpoints should be used in assessing impacts to aquatic environments.

- The mostly highly recommended metrics or indices are those that rely on species level information including:

    - Species or taxa richness
    - Community composition
    - Swartz's dominance
    - Pielou's dominance
    - Infaunal (trophic) index

- Measures of abundance also provide useful screening tools, with the most powerful index being total abundance.

- If indices based on higher levels of taxonomic organization are to be applied, I would suggest use of family or order groupings rather than class or phylum.

Selection and use of analytical techniques will be dependent upon programmatic goals and objectives, the "question" to be answered, and ultimately the sampling design. Most environmental monitoring programs in Puget Sound have relied upon comparison of potentially impacted sites with reference or background conditions using analysis of variance techniques. However, these comparisons continue to be a problem in most monitoring programs because of the lack of physically comparable reference sites and information about reference community assemblages. Benthic communities in Puget Sound display a large natural variability in space and time reflecting the diversity of habitat types created by myriad physical and chemical factors (e.g., currents, salinity regimes, sediment deposition and erosion patterns, wave energy, etc). Because this issue affects the efficacy of the traditional monitoring program sampling designs and hypothesis testing, the following recommendations are offered:

- Allocate resources to further characterize reference community assemblages and the characteristics of the habitats they reside in. There are potentially several sources of historical data that can be used to start this process including the Ambient Monitoring Program, work by Nichols and Lie, as well as unpublished data collected by Bob Harmon at Shoreline Community College over the last 20 years.

- Evaluate application of multivariate techniques (including hypothesis testing using ordination scores) in regulatory programs using Puget Sound data. If reference community assemblages were better characterized, interpretive techniques used by Thompson et al. in the Southern California Bight would be more suitable and potentially provide a highly cost-effective approach in Puget Sound.

Comparison of benthic community indices with evaluation criteria

	Criteria for Use in a Regulatory or Management Decision Process			
Index	Ability to Discern Impacts[a]	Objectivity	Ease of Interpretation	Cost Effectiveness[a]
Indicator taxa abundance	Variable, tends to be high if based on presence	Some judgement required in selection of taxa	Easy	Moderate, requires species-level ids
Diversity	Low	No clear cut-off values for impacted sites	Easy for extreme impacts	Moderate, requires species-level ids
Evenness and dominance	Variable-high for extreme impacts	No clear cut-off values for impacted sites	Easy for extreme impacts	Moderate, requires species-level ids
Swartz's dominance index	High for extreme impacts	Objective	Easy	Moderate, requires species-level ids for numerically dominant taxa
ITI	Moderate to high	Judgement required for initial species classifications	Moderately easy	High (based on Ferraro's analysis)
Richness	High	Objective	Easy	Moderate, requires species-level ids
Species abundance, community composition	High	Requires knowledge of species ecology	Moderate	High, based on degree of information per cost
Total abundance	Variable, moderate to high	Objective	Easy	High
Major taxa abundance	Variable, can detect extreme impacts fairly easily	Objective	Easy	High
Biomass	Variable, low to moderate	No clear cutoff values for significant changes	Low to moderate	Low

Analytical Techniques				
Analysis of variance	Tends to have high power to detect impacts	Objective	Easy	High
Cluster	Low	Judgment required in interpreting patterns	Complex	Moderate
Ordination	Low, but may increase with some refinements	Some judgement required in interpreting relationships	Complex	High, if used with hypothesis testing and well-characterized reference assemblages
Graphical methods	Moderate	Fairly objective	Easy to moderate	Moderate
a = dependent upon sampling design				

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT

ENVIRONMENTAL RESEARCH LABORATORY
HATFIELD MARINE SCIENCE CENTER
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MAR 8 1993

March 5, 1993

Dr. Jane Sexton
PTI Environmental Services
15375 Southeast 30th Place
Bellevue, WA 98007

Dear Jane:

Enclosed is a summary of my recommendations from the Benthic Endpoints workshop.

I enjoyed the discussions at the workshop. Thank you for arranging for my participation.

Sincerely,

Rick

Richard C. Swartz, Ph.D., Leader
Benthic Effects Team

National Benthic Experts Workshop
Seattle, Washington
February 25, 1993

Summary Report - Richard C. Swartz

My summary evaluation of the benthic endpoints discussed at the benthic workshop is shown in the attached table. I have followed the format given at the workshop, except for the addition of a column for the total "score" of each index. The score is quite useful. It shows that I, along with other Panel members, consider the H and J type diversity indices to be virtually useless (score = 4). I gave three indices very high scores (28-29): Richness, Infaunal Index, and Indicator species. All three of these indices require species level identifications. They are information rich, show relatively little variance, and are easily interpretable. They also may not require reference area comparisons, if adequate historic information is available. I gave a low score (12) to both biomass and total abundance because these indices cannot reflect species replacements along stress gradients, and have relatively high variance. I gave only a slightly higher score (16) to major taxa abundance primarily because stress tolerant and intolerant species can replace one another within each major taxon. I consider dominance a useful second order index (score = 18) that can quantify conditions where one or a few species are very abundant. However, the identity of the species must be known before the dominance index can be correctly interpreted. Finally, abundance at the species level received a high score (23) because knowledge of population dynamics is essential before community changes can be understood.

My major conclusions are:

- Specimens should be identified to the species level. Otherwise, the most powerful indices and multivariate analyses cannot be used.

- No single index allows a thorough understanding of benthic alterations. A combination of Richness, Indicator Species, Infaunal Index, Species Level Abundance, Dominance, and Numerical Classification provides a comprehensive benthic assessment.

- The efficacy of the indices proposed at the workshop should be tested on 4-6 existing, representative data sets from Puget Sound. The example of comparative analyses prepared by Nancy Musgrove for the workshop is a good model that could be applied to the other data sets at a reasonable cost.

Endpoint	Evaluation: Sensitivity	Objectivity	Ease of Interpretation	Reference Area Comparison	Cost Efficiency	Score*
H, J	L	L+	L-	Yes	L-	4
Dominance	M+	H	M	Yes	M	18
Infaunal Index	H+	H	M+	No	H+	28
Total Abundance	M-	M-	M-	Yes	M-	12
Major Taxa Abundance	M	M	M	Yes	M	16
Species Abundance	H+	M+	M+	Yes	M+	23
Indicator Species	H+	H	H-	No	H	28
Biomass	M	M-	M	Yes	L	12
Richness	H	H+	H	Probably	H-	29

*L- 0 M- 3 H- 6
L 1 M 4 H 7
L+ 2 M+ 5 H+ 8

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March 8, 1993

Dr. Chip Hogue
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15375 SE 30th Place, Suite 250
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Dear Chip:

I would like to thank you and the other organizers of the Benthic Workshop for including me on the panel. It was very informative for me as we will be considering similar matters for San Francisco Bay in the near future. I hope that you and the other organizers also found the discussions helpful.

As requested, this letter is to summarize my opinions about the results of the workshop. I have filled out the matrix from my personal point of view, with the following comments.

1. I added ordination scores as one of the indices that should be considered. Although their statistical use in pollution work is not well established, I believe that with a little more refinement they will find widespread application. Our report for EPA Region IX is a good start (Tetra-Tech, 1992). Ordination uses all species collected at a site, thus the scores have very high statistical power. Our work has shown full power with only 2 replicates because there is so much information in species lists. They are easy to interpret since the species themselves provide the evaluation of how sites fall along contaminant gradients. It is not the score itself that is valuable, but the ability to statistically compare sites to a reference benthic community. Thus, a reference standard for benthic assemblages could be established that is based on community composition and abundances.

I would suggest that Pete Striplin's data base needs to be analyzed to see if reference conditions and

COMPARISON OF BENTHIC COMMUNITY INDICES WITH EVALUATION CRITERIA

	Evaluation Criteria				
	Sensitivity	Objectivity	Ease of Interpretation	Reference Area Comparison	Cost Effectiveness
Index					
Ordination scores	H	M	H	Yes	M
Diversity, evenness, dominance	L	M	M	Yes	M
Infaunal index	M	M--	L	Yes	M
Total abundance	M	H	H	Yes	H
Major taxa abundance	L	L	L	Yes	H
Individual species level abundance	M	H	M	Yes	M
Indicator species	H	H	H	Yes	H
Biomass	L	M	M	Yes	M
Richness	M	H	M	Yes	M
Analytical Methods					
Analysis of variance (e.g., *t*-tests)	H	H	H	Yes	H
Pair-wise comparisions (e.g., *t*-tests)	--[a]	--	--	--	--
Cluster techniques					
Ordination techniques					
Graphical methods					

[a] Do not use except along gradients, transition vs. reference site, etc.

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