Spatial and Temporal Variability of Shoreline Biota in South and Central Puget Sound; 2001 samples and analyses

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LIST OF FIGURES	ii
LIST OF TABLES	iii
LIST OF APPENDICES	iii
EXECUTIVE SUMMARY	iv
INTRODUCTION Project Objectives	1 1
METHODS Sites General SCALE Methodology Pebble Sampling Mud Sampling Analytical Methods	2 2 4 4 4 4 5
RESULTS	5 9 13 18 20 24 25 28
DISCUSSION Taxonomic Resolution: Recommendations Indices of Benthic Community Health: Recommendations Use of SCALE for Studies of Degraded Beaches	34 35 36 37
LITERATURE CITED	

TABLE OF CONTENTS

LIST OF FIGURES

Figure 1.	Map of the 2001 sample sites.
Figure 2.	Species richness at the south and east-central Sound sites over 3 years.
Figure 3.	Cumulative species-richness curves for 3 years at each of the sites sampled in 2001.
Figure 4.	Species-area curves for each area sampled in 1999 and 2001.
Figure 5.	Species richness at all sites sampled in 2001.
Figure 6.	Percentage of the number of taxa that showed significant variation at different levels of analysis.
Figure 7.	Ordinations of community similarities among years, basins, sites, and transects for the south sound and east side sites.
Figure 8.	Contour plot of sea surface salinity in June 1999.
Figure 9.	Results of community similarity ordinations for the basins, sides, and quadrants of Puget Sound.
Figure 10.	Community ordination at the high-resolution species level, showing sites by site-code.
Figure 11.	Community ordination from the pristine vs. degraded mud sites.
Figure 12.	Community-similarity ordinations for the 2001 data based on different levels of resolution.
Figure 13.	Species richness and Shannon's Diversity index for 2001 sites sampled at high and low resolution.
Figure 14.	Coefficients of variation for transects across years for 5 common taxa.
Figure 15.	Coefficients of variation for sites in 2001, based on values at 3 transects per site.
Figure 16.	Coefficients of variation for three species of capitellid polychaete, based on 21 transects with at least 3 years of data (1999-2001).
Figure 17.	Coefficients of variation for three species of capitellid polychaete, based on 13 sites with at least 3 transects in 2001.
Figure 18.	Coefficient of variation for species richness in cores.
Figure 19.	Coefficient of variation for univariate metrics of assemblage composition in cores.
Figure 20.	Coefficient of variation for species richness in quadrat cover.
Figure 21.	Coefficient of variation for univariate metrics of assemblage composition
	in quadrat cover.
Figure 22.	Coefficient of variation for species richness in quadrat counts.
Figure 23.	Coefficient of variation for univariate metrics of assemblage composition
	in quadrat counts.

LIST OF TABLES

Table 1.	Number of low-resolution taxa in each of 5 north-south distribution pattern categories, and each of 10 level-of-variation categories.
Table 2.	Species contributing more than 2% to the dissimilarity between pristine and degraded beaches.
Table 3.	Results of ordinations of 2001 data at different taxonomic levels.
Table 4.	Summary of results of principal components analysis of 13 normalized univariate metrics of community structure.

LIST OF APPENDICES (all digital only)

- Appendix A. Detailed results of nested ANOVAs using low-resolution data.
- Appendix B. Results of ordinations of variation among years and spatial scales.
- Appendix C. Results of ordinations for different regions of Puget Sound.
- Appendix D. Results from Principal Components Analysis of 13 univariate metrics of community structure.

EXECUTIVE SUMMARY

Estuarine organisms contend on a daily and seasonal basis with highly fluctuating environmental gradients, especially in salinity and temperature, and also with anthropogenic changes in water quality. Our ongoing studies in the southern and central basins of Puget Sound have shown that there is strong coupling between the estuarine waters of Puget Sound, the physical environment on the beach, and the organisms in intertidal habitats. Previous reports have quantified this linkage in various habitat types (cobble, sand, pebble, and mud beaches), and have correlated gradients in physical features throughout southern and central Puget Sound with the biota of pebble beaches. In 2001 we continued to examine spatial and temporal variability of shoreline biota in southern and central Puget Sound using the Shoreline Classification and Landscape Extrapolation (SCALE) model. We focused our 2001 fieldwork and analyses on:

- 1. measuring temporal change in biota in Carr, Case and Budd Inlets and on the eastern side of central Puget Sound;
- 2. expanding the geographic scope of our study by sampling sites on the western shore of central Puget Sound; and
- 3. evaluating the ability of our biotic sampling protocol to differentiate pristine versus degraded mud beaches in southern Puget Sound.

In addition to analyses of these sources of variation, we also tested how well differences among biotic communities can be detected using different levels of taxonomic resolution and various univariate parameters, such as species richness and measures of species dominance, that are used by monitoring programs elsewhere.

A total of 68 beaches were sampled in May-July 2001; 9 pebble beaches in south Sound, 36 in east-central Sound, 15 (new) in west-central Sound, and 8 (new) mud beaches in south Sound for the Pristine-vs-Degraded comparison.

As found in previous years, the 2001 data from pebble beaches show a distinct increase in species richness from south to north Puget Sound. We further examined spatial distribution at the taxon level along the south-north gradient. The most common distribution pattern (51 of the 135 low-resolution taxa) is to be more abundant in the north and less abundant in the south; this parallels the finding of higher species richness in the north and probably reflects the more-marine environment there. A similar number of taxa (53) show no north-sound distribution pattern; these are usually species found so rarely that no trend is discernable. Only 11 taxa were found more abundantly in the south than the north; these include one (Crepidula fornicata) found only in association with ovster culture, 5 taxa expected in muddler sediments as are characteristic of the southern sites (Edwardsia, Magelonids, Terebellids, Leptosynapta, and Neotrypaea), 3 taxa found higher on the shore (and thus not in our samples) at the more wave-exposed northern sites (Littorina, Hemigrapsus nudus and H. oregonensis), and 2 snail species whose distribution needs further study (Alia and Nassarius). There were 19 taxa that show a trend towards being less common in the north and south than in the middle of the study area; these appear to fall into 2 categories, species that are found on larger, more stable

cobbles (e.g. kelps, anemones, *Tonicella* chitons, and jingle shells) and those that are sand-loving (e.g. *Dendraster*, Chaetopterids, *Anthopleura*, *Zostera*).

Species-area curves for pebble beaches showed that 3 transects (beaches) per site are not sufficient to capture all the richness per site, i.e. the curves do not always level off after 3 transects. At larger spatial scales, 9 transects (3 sites) do appear to capture virtually all the richness in an area.

The data show distinct spatial separation of community structure at multiple scales in all years. At the basin scale, transects in the south are distinctly different in community composition and structure from the south-central transects, and the southcentral is separate from the north central At the area scale within a basin, beaches within a distance of 10s of km have more similar organisms to each other than to beaches farther apart. Variation is highest at the site-to-site scale, as physical conditions (e.g. salinity) change with increasing distance along the shoreline. Variation is moderately high at the spatial scale of transects within sites (explaining the species-area results above), especially for organisms living in the sediment. Some sites, however, show high withinsite consistency; it would be much easier to detect change of these beaches than at sites where natural variability is high. Variation is also fairly high among years, but in this case especially for organisms living on the surface (less so for infauna); variation appears to be especially high for organisms (algae and invertebrates) with short generation times. Multivariate ordinations at these different spatial and temporal scales are useful for visualizing unusual (outlier) beaches, as well as larger-than-normal shifts in biota within a beach among years. The data overall continue to suggest an important role for the effects of nearshore salinity and of sand content on the beach.

As is the case with any monitoring program, the data become increasing valuable with time, as an 'envelope of normalcy' becomes established through interannual sampling. The SCALE data for relatively pristine pebble beaches in southern and central Puget Sound are now extensive enough that we can begin to detect ecologically significant differences among beaches and changes through time; these lead to hypotheses about causes of change and suggest directions in which to focus future efforts. In addition, we are beginning to be able to make predictions about directions and types of changes in benthic biota that might follow significant environmental events, such as a localized decline in salinity.

Our pilot study comparing mud sites that were relatively pristine vs. degraded (by historic log processing and other industry) suggest that the SCALE technique can be used to help detect and quantify biotic differences between such sites. Even though the sites sampled had low-density and low-diversity communities, differences among the 'treatments' were visible. Larger sample sizes and/or working in a more diverse habitat would make detecting trends easier.

Spatial patterns in biotic communities (e.g. among groupings of beaches) were almost equally well distinguished using low-resolution biotic data as using highresolution, species-level data. Our analyses suggest that use of family-level data should be sufficient to detect both gross changes (e.g. loss of whole families), and more subtle among-site differences (e.g. within central Sound) or among-year differences within a site. We thus conclude that gathering data using low-resolution categories in the field and identifying infauna to the family level in the lab is a reasonable alternative to the more time consuming species-level monitoring. Lumping data into higher taxonomic categories (e.g. Class or Phylum) or into trophic levels made spatial patterns harder to distinguish. A reasonable long-term scenario might involve annual monitoring of most sites at this lower level of resolution, with periodic (e.g. every 3-5 years) gathering of data at the species level to allow examination of trends in species richness, which cannot be calculated from the low resolution data.

The potential indicators that we applied to the SCALE data set show some promise in summarizing spatial and temporal patterns in community structure. Three desirable traits of indicators are: consistency across sites and times that share environmental conditions; variability across sites and times that do not share environmental conditions; and ease of communication to managers and stakeholders. Of the univariate indicators examined, species richness fits these criteria most closely. Interestingly, this more formal analysis of univariate indicators converged on a reporting strategy already provided by SCALE researchers: trends in species richness through Puget Sound emerged early as a strong and easily communicated pattern of community structure. Among additional univariate indicators, evenness (J') is a likely candidate: it also helps distinguish locations, based on principal components analysis, and shows low coefficient of variation at a variety of spatial scales. Species richness and evenness do not, however, reduce the amount of data that must be collected to define beach health. Rather, they are analyses that can be carried out in addition to multivariate analyses when communities have been fully described. Thus no obvious, simple-to-measure "indicator" for the health of Puget Sound emerges from our data.

INTRODUCTION

Estuarine organisms contend on a daily and seasonal basis with highly fluctuating environmental gradients, especially in salinity and temperature. In addition, because cities are often built around estuaries, organisms in these ecosystems must survive, or may succumb to, changes in water quality, sediment quality, or habitat alteration caused by anthropogenic factors. Benthic (bottom-dwelling) species encounter natural and anthropogenic change in both sediment and water quality, and thus may serve as indicators of change (Warwick and Clarke, 1993). These organisms (invertebrates, seaweeds, and fishes) may have life spans ranging from days to seasons or years, and they frequently occur in large numbers, thus providing a useful baseline for statistical analyses. For these reasons, and because of logistical accessibility, monitoring benthic species from the intertidal zone of estuaries should provide a mechanism for detecting change in estuarine ecosystems.

Our ongoing studies in the southern and central basins of Puget Sound (Schoch and Dethier 1997, 1999, Dethier and Schoch 2000) have shown that there is strong physical and biological coupling between the nearshore waters of Puget Sound, the physical environment on the beach, and the organisms in intertidal habitats. Previous reports have quantified this linkage in various habitat types (cobble, sand, pebble, and mud beaches), and have correlated gradients in physical features throughout southern and central Puget Sound with the biota of pebble beaches. We have found great spatial and interannual variability in shoreline biota, and have shown that much of this variability follows predictable patterns along environmental gradients. Ultimately, we hope to be able to explain much of the variation seen in shoreline communities by the geophysical differences among them, allowing us to then assess the impacts of other (including anthropogenic) events.

Project Objectives

In 2001 we continued to examine spatial and temporal variability of shoreline biota in southern Puget Sound and central Puget Sound using the Shoreline Classification and Landscape Extrapolation (SCALE) model. In particular, we focused our 2001 fieldwork and analyses on:

- Measuring temporal variation in biota over multiple years in Carr, Case and Budd Inlets and in central Puget Sound
- Examining spatial variability in shoreline biota in south Sound and central Sound through comparing results within and among regions
- Expanding the geographic scope of our study in Puget Sound, and testing the role of another environmental parameter, by sampling sites on the western shore of central Puget Sound
- Evaluating the ability of biotic samples to differentiate relatively pristine versus degraded mud beaches in southern Puget Sound
- Testing the ability to detect differences among biotic communities using different levels of taxonomic resolution

METHODS

Sites

In 1999 we performed an extensive sampling of pebble beaches in numerous oceanographic cells from southern to north-central Puget Sound, and found clear north-south trends in diversity and various physical parameters. In 2000 we resampled a subset of these sites to test for interannual variation and to see if the north-south trends persisted from year to year. In 2001 we resampled all of the 2000 and some of the 1999 pebble sites (45 beach transects), and in addition added 5 sampling sites (15 beach transects) on the west side of central Puget Sound (Figure 1). Following our hierarchical sampling scheme from previous years, we sampled 3 beach transects at each site.

In addition, for the first time in 2001 we used our standard sampling scheme to examine the biota in mud beaches in South Sound for which data existed suggesting they were either relative pristine (4 beaches) or relatively degraded by pollution (4 beaches). We identified pristine and degraded sites using existing sediment chemistry data from a regional database (Washington State Department of Ecology, 1999). Degraded sites were placed in areas where concentrations of one or more harmful chemicals exceeded the state regulatory standards. Pristine sites were placed in areas where no samples exceeded the chemical concentration limits.

Degraded sites were selected in Budd Inlet, near Olympia, and in Henderson Inlet, near Shelton. Both of these embayments were historic sites for log processing and other industrial uses. In each bay, sites with two hypothesized levels of degradation were selected. Budd West and Shelton North were placed in areas where multiple chemical parameters exceeded regulatory standards. Budd East and Shelton South are adjacent to the other sites and are known to be degraded by wood waste and other stressors, but chemical samples did not exceed regulatory standards.

Relatively pristine sites were placed in areas where existing chemical samples do not exceed regulatory standards (Case Inlet, Carr Inlet, Eld Inlet and Totten Inlet). While these areas were not used extensively for industrial purposes, they have a long history of human use.

We recognize that our definition of degradation is limited. Many of the bays in Puget Sound are polluted to varying degrees, from a suite of possible sources. These sources include other industrial chemicals, sewage treatment facilities, septic systems, storm water runoff, and mariculture activities. We chose muddy habitats for the study because they represent one of the lowest levels of wave and current energy in Puget Sound and are thus more likely to accumulate toxic levels of industrial chemicals. The infauna in this habitat type were sampled in 1998 and we have a good baseline of the community structure in Budd, Case, and Carr Inlets.



Figure 1. Map of the sites sampled in 2001 in central and southern Puget Sound. Each + is a beach (transect), each set of 3 beaches is a site.

General SCALE Methodology

Our general site-selection and sampling approach involves choosing replicate sampling beaches based on the physics and physical structure of the shoreline. By decreasing the physical variability among sample sites, we increase the statistical power of comparisons among communities and populations. In order to choose replicate beaches, we segment a complex shoreline (which encompasses numerous environmental gradients) using a combination of qualitative and quantitative partitioning criteria. For example, at the spatial scales of bays and inlets in Puget Sound, geophysical parameters such as sediment grain size, wave energy, substrate dynamics, and pore water chemistry are quantified. At large spatial scales such as within the basins of Puget Sound, water chemistry attributes such as temperature and salinity are used to identify major oceanic climates. These nested segments can be used to study within-segment and amongsegment variability, which in turn will support studies of the biotic and abiotic processes that control variability. Detailed descriptions of these methods can be found in Schoch and Dethier (1997, 1999).

Pebble Sampling

Samples were collected in the lower zone only (MLLW or 0 meters elevation) in May and June. At this tidal level the biota are diverse and therefore sensitive to changes in the marine environment. In addition, this low level is subject to anthropogenic stressors from both land (when emersed) and sea (when immersed). We collected 10 random samples along a 50 m horizontal transect positioned near the center of the beach segment. Each sample consisted of quantifying surface macrofloral and faunal abundance in a 0.25 m² quadrat, and infauna in a 10-cm diameter core dug to 15 cm depth. Percent cover was estimated for all sessile taxa in the quadrats, and all mobile epifauna were counted. Surface organisms were identified to species when possible, although for some analyses this information was collapsed into "low resolution" categories (see below). Core samples were sieved through a 2 mm mesh and taxa were counted. All organisms not identifiable to the species level in the field were placed in formalin and identified in the lab. Taxonomic identifications for invertebrates were according to Kozloff (1996) and Blake et al. (1996ff), and Gabrielson et al. (2000) for macroalgae.

Mud Sampling

Mud sites were sampled during low tides in late July and early August. Mud samples were collected using the same methods as pebble samples, with exceptions noted below. At mud sites, we did not quantify surface macroflora and fauna using a quadrat. From a safety perspective, the extremely soft mud substrate made walking along the transect difficult, and anyone who stood in one place got stuck quickly. From a methodological perspective, we assumed that the infauna inhabiting polluted mud habitats are more likely to show the effects of habitat degradation. Eliminating quadrat data was acceptable because previous sampling results showed that surface biota is very sparse in muddy environments (Schoch and Dethier 1999). Finally, by eliminating surface samples, we avoided sampling some introduced epibiota that we previously found near to historic mariculture sites but that are less common at historic industrial sites.

At each transect, we described mud density using a penetrometer or soil compaction tester. The penetrometer is an approx. 2-meter long pole with a movable 1 kg weight on the top portion of the pole. We measured penetration by placing the pole on the substrate, releasing the weight from the top of the pole, and measuring the distance that the weight drove the pole into the substrate.

Analytical Methods

Community structure was analyzed using the multivariate ordination methods of Clarke and Warwick (1994) and PRIMER software (Clarke and Gorley 2001). We looked for relationships between community structure and geographic location, and the biological and physical components that drive community similarity. Nested ANOVAs were run for each organism to assess how much variability was added at each spatial increment from transects to sites to areas (Sokal and Rohlf 1995). Because withintransect variability is high and generally not of concern (see below), all analyses used mean values per transect.

RESULTS

General Patterns

As found in earlier analyses, the 2001 data show a distinct trend in species richness from south to north Puget Sound. Some year to year variation in richness per site is seen (Figure 2), but the trends among sites are very consistent. The data shown in Figure 2 represent the cumulative species richness of all three transects sampled at each site in the south and east-central regions, but do not show the west-central sites because only 2001 data were available.



Figure 2. Species richness at the south and east-central sites over 3 years (1999-2001). Sites are ordered from South to North.

When species/area curves are plotted across these sets of 3 beaches, they often do not level out, or reach an asymptote (Fig. 3). This shows that sampling more beaches per site would allow us to 'capture' more species, i.e. 3 beaches is not quite an adequate sample size to characterize a site.



Figure 3. Cumulative species -richness curves for 3 years at each of the sites sampled in 2001.



Figure 4. Species-area curves for each area (set of 9 sites) sampled in 1999 and 2001. Area 1 = Budd, Case, and Carr; Area 2 = Browns, Redondo, and Normandy; Area 3 = Seahurst, Brace, and Alki; Area 4 = West, Carkeek, and Wells; Area 5 = Edmonds, Possession, and Double.

In contrast, when species/area curves for entire areas (e.g., all of south Sound, or all of the northeast quadrant of central Sound) are examined, the 9 sites comprising each area do show curves that asymptote (Fig. 4).

The beaches sampled on the west side of Puget Sound (from Maury Island in the south to Hansville in the north) also show a trend in richness (Fig. 5), although less smoothly than sites on the east side; most of the trend is driven by very low richness at Maury and very high richness at Hansville, near the mouth of Puget Sound proper. When sites are visualized as paired across Puget Sound, Redondo and Maury are very similar in richness, Brace and Blake are somewhat higher, and the more northern areas on both sides of the Sound are clearly richer than the southern ones (Figure 5).



Figure 5. Species richness (surface biota and infauna combined) at all sites sampled in June 2001. Each datum is the cumulative richness among the 10 samples per site. Similar patterns can be seen in data from 1999 and 2000, and when the data are broken down into surface vs. infaunal species. Cooper Pt = Budd Inlet in the other figures, McMicken = Case Inlet, and Glen Cove = Carr Inlet.

Patterns across different spatial and temporal scales

We used nested ANOVAs to examine the temporal and spatial scales at which most community variability is seen; how does biotic community structure vary from year to year, and how similar are intertidal communities within and among regions? Data were examined only from south Sound and the east-side sites of central Sound, since we do not yet have inter-annual data from the west-side sites. Appendix A shows the detailed results of the nested ANOVAs and a qualitative indication of distribution of each taxon along the south-north gradient. Table 1 summarizes the overall distribution patterns and scales of variability.

Table 1. Numbers of low-resolution taxa for 1999-2001 in each of 5 north-sounddistribution pattern categories and each of 10 level-of-variation categories. Numbers in eachrow are the numbers of taxa that showed significant variation (p < .05) at those levels in the nestedANOVAs.

	North-South Distribution Pattern					
	High in	High in	High in	Low in	No N/S	Total
Levels of Variation	Ν	S	N&S	N&S	Pattern	
Just Transect	5	1	0	2	0	8
Just Site	8	4	0	1	5	18
Just Area	3	1	0	1	1	6
Transect and Year	0	0	0	0	1	1
Transect and Site	4	2	0	1	2	9
Site and Area	1	0	0	0	0	1
Year	5	1	0	4	5	15
Site and Year	5	0	0	0	0	5
Area and Year	1	0	0	0	0	1
No significant variation	19	2	1	10	39	71
Totals	51	11	1	19	53	135

The table shows, first, that the most common distribution pattern among the 135 lowresolution taxa is to be more abundant in the north and less abundant in the south; this parallels the finding, above, of higher species richness in the north and probably reflects the more-marine environment there. A similar number of taxa (53) show no north-sound distribution pattern; these are usually species that were found so rarely that no trend was discernable. Only 11 taxa were found more abundantly in the south than the north; these include one (*Crepidula fornicata*) found only in association with oyster culture, 5 taxa expected in muddier sediments as are characteristic of the southern sites (*Edwardsia*, Magelonids, Terebellids, *Leptosynapta*, and *Neotrypaea*), 3 taxa found higher on the shore (and thus not in our samples) at the more wave-exposed northern sites (*Littorina*, *Hemigrapsus nudus* and *H. oregonensis*), and 2 snail species whose distribution needs further study (*Alia* and *Nassarius*). There were 19 taxa that show a trend towards being less common in the north and south than in the middle of the study area; these appear to fall into 2 categories, species that are found on larger, more stable cobbles (e.g. kelps, anemones, *Tonicella* chitons, and jingle shells) and those that are sand-loving (e.g. *Dendraster*, Chaetopterids, *Anthopleura*, *Zostera*). These possible patterns would be interesting to pursue further, and examine with respect to the west-side sites.

Table 1 also summarizes the data on scales of variability seen in the nested ANOVAs; trends are illustrated in Figure 6.



Figure 6. Percentage of the number of taxa (at the low-resolution level) that showed significant variation (p<.05) at different levels of analysis in 1999, 2000, and 2001.

At the scale of transects within sites, 18 taxa (summing all rows in Table 1 listing Transect as a factor) showed significant variation, suggesting that for these species, the sites were less homogeneous than we expected. Figure 6 shows that almost all this variation was in infauna, not epifauna. In fact, the infauna overall are most variable at the among-transect scale; sets of beaches that appear homogeneous to us (and to the epibiota) are heterogeneous to the infauna. The greatest variability for all the biota is seen at the site-to-site scale; as we move to a new site, with slightly different salinities and other physical features, many organisms (both infauna and epibiota) show different abundances than at adjacent sites. Variation among years (1999, 2000, and 2001) is moderately high; interestingly, at this level the infauna are less likely to show variation than are the epibiota. Many of the taxa variable at this level appear to be ones with short generation times (weeks to months) such as amphipods, isopods, nudibranchs, small polychaetes, and small filamentous algae, or juveniles of longer-lived species (Clinocardium bivalves, sand dollars) that probably show high year to year variation in recruitment. These taxa would presumably make poor indicator species to monitor through time, but might be ones providing a good 'signal' to look for short-term change.

Appendix B and Figure 7 show the results of ordination analyses of the biota of these sites and illustrate these patterns of variation in a different way. Figure 7A compares community structure among years (1999, 2000, and 2001) at all sites. While the statistical test indicates that there was significant among-year variation (App. B), the very low Global R value and an examination of the ordination plot show that community structure of all sites was very similar from year to year; data from the 3 years (i.e., the 1s, 2s, and 3s) are very interwoven in the plot, indicating a lack of major shift among years. In contrast, when all years are combined, the data show very distinct separation of

community structure among basins and among sites. Figure 7B illustrates that all the transects from the south basin are distinctly different in community composition and structure from the south-central transects, and that south-central is also quite separate from north-central. Similarly, Figure 7C shows that the nine transects at each Area (e.g., the 1s in Budd Inlet) clump with each other and separately from the 9 transects at each other Area; that is, the beaches within a distance of 10s of km are more similar to each other than they are to the beaches farther apart. Some sites show much higher within-site consistency; for example, Budd Inlet (code 1 on Figure 7C) and Possession (code 7) are each tightly clumped, whereas Carr Inlet (code 3) and Brace (code 5) are much more variable. This indicates that it would be much easier to detect change on the sampled beaches in Budd Inlet than from the sampled beaches in Carr Inlet, where natural beach to beach variability is higher.

Figure 7. Ordinations of community similarities among years (1999-2001), basins, sites, and transects for the south sound and east side sites.

Stress: 0.15

Stress: 0.15

Stress: 0.15



Case: 4,5,6 Carr: 7,8,9 Redondo: 10,11,12 Brace: 13,14,15 Carkeek: 16,17,18 Possession: 19,20,21

Global R = 0.840p = 0.001



Figure 7D illustrates variation at the finest level analyzed, among-transects. There is a significant separation among transects within a site when data from all three years are analyzed together; for example, in the figure, all the "1s" (Budd transect 1 over 3 years) do not stack on top of each other. This figure thus illustrates not only how much transects differ within a site (e.g., how transects 4, 5, and 6 in Case Inlet vary), but also how much each transect changes from year to year (e.g., how transect 6 'moves' across the ordination). Again, it is possible to see not only that some sets of transects are much more similar to each other (e.g., transects 1-3 in Budd Inlet vs. transects 7-9 in Carr), but also that some transects are similar from year to year (e.g. 1, 4) while others are relatively different (e.g. 10, 15). It is notable that the latter two transects showing very high interannual variability have apparent correlates in a physical variable; at transect 10 (Redondo south) the pebble-sand transition low on the shore has shifted markedly from year to year, so that some years we have sampled in zones of much higher sand abundance than in others. At transect 15 (Brace N, which is at Lincoln Park), there may also have been a significant sediment shift; Fred Goetz (Army Corps of Engineers, Seattle) noted that a large landslide south of Fauntleroy Cove in winter 2001 may have delivered an unusual amount of sand to that site before our June 2001 sampling (the outlier "3" at the lower right of the ordination in Figure 7A is that of Brace North in 2001). Note also that while most transects shift in biotic community structure to some extent from year to year, they remain within the fairly tight cluster characteristic of their 'basin' (compare Figures 7B and 7D); if one transect were to shift into the cluster characteristic of another basin (e.g. a "3" shifting into the cluster of "1"s in Figure 7B), this would be an excellent indicator of a substantial change at that beach.

Patterns between the Eastern and Western shores of Central Sound

We previously hypothesized (Dethier and Schoch 2000) that the substantial differences in salinity between the east and west sides of central Puget Sound might result in consistently different biotic communities in similar habitats on the two sides of the Sound. Since salinities on the west shore were much higher (ca. 5 psu in our 1999 sampling: Figure 8), we predicted that the flora and fauna there would be more marine and diverse. Sea surface temperatures are patchier and more unpredictable. The patterns observed in our 2001 data comparing 15 beaches on the east and 15 beaches on the west show no significant difference in community structure (Figure 9, Appendix C); there is overlap in the ordination in Figure 9A between the two sets of sites, mostly because the 3 beaches from Maury Island (the three "2"s to the left in Figure 9A) encroach into the east side data. Figure 9B plots a comparison of the 12 northern (east and west) beaches with the 18 southern beaches and illustrates the pattern noted above, that the northern sites are significantly different (more rich, and with higher abundances of many taxa as noted in Appendix C). When the beaches are broken down further into four quadrants of central Sound (southeast, southwest, northeast, and northwest), the regions driving these patterns can be discerned; the two southern quadrants are not significantly different from each other and show highly overlapping ordinations (Fig. 9C), although abundances of some organisms, such as *Lacuna* and some macroalgae, are much higher on the west side. The two western quadrants are also not significantly different, although the ordination shows

them to be rather separate; again, the 3 Maury beaches increase the spread in the southwest samples. The northwest samples are quite similar to the northeast samples. The most different quadrants are the southeast versus the northwest; these are clearly the most different in terms of salinity, and many key organisms have very different abundances (Appendix C). Species richness also varies substantially, with 41-52 in the southeast, 56-59 in the northeast, 37 (Maury) to 57 in the southwest, and 55-58 in the northwest. Interestingly, the abundance of sand, which we believe is often critical in driving biotic community structure patterns, is similar in the various quadrants (in the 2001 data) (Appendix C).

A more detailed view of similarities among beaches in different areas can be gained from Figure 10. In this ordination, the south Sound sites (BD, CR, CS) clearly cluster separately from all the central Sound ones. The southeast-central sites (BP, RE, BR) tend to cluster together, except that the 3 beaches in Brace (BR) tend to be very different from each other (which we believe is a sand effect, discussed above). Maury Island (MA) is more similar to the southeast sites than to the more northern sites in its quadrant, suggesting that this site may be influenced by the lower salinity there. Further salinity sampling needs to be done, but it appears that Vashon and Blake receive salty, incoming surface water as it moves south along the west side of the Sound. At various points there is mixing with freshwater entering the Sound, and this mixed, lower-salinity water moves north along the east side (Fig. 8). The other sites in the quadrant with Maury, Vashon (VA) and Blake (BL), are more similar to sites (e.g. Carkeek, CK) much further to the north, perhaps because all these sites have salinities less influenced by the southern pulses of freshwater. Interestingly, at the north end Possession (PO) is more similar to the northwest sites (Jefferson and Hansville) than would be predicted by its proximity to the low-salinity plume issuing from Possession Sound.



Figure 8. Contour plot of sea surface salinity (psu) in June 1999 for the central and south basins of Puget Sound.

Figure 9. Results of community similarity ordinations for the basins, sides, and quadrants of Puget Sound.

A. East vs. West

East Shore: 1 West Shore: 2

Global R = 0.039p = 0.152



B. North vs. South Central South Central Basin: 2 North Central Basin: 3

Global R = 0.279 p = 0.001



C. Quadrants

Southeast: 1 Northeast: 2 Southwest: 3 Northwest: 4

Global R = 0.212p = 0.002



Figure 10. Community ordination at the high-resolution species level, showing sites by site-code.

BD = Budd

CR = Carr

- CS = Case
- BP = Browns
- RE = Redondo
- BR = Brace
- CK = Carkeek
- PO = Possession
- MA = Maury
- VA = Vashon
- BL = Blake
- JE = Jefferson
- HA = Hansville



Patterns in pristine versus degraded mud beaches in Southern Puget Sound

To test whether our sampling scheme could detect differences in community structure associated with sediments thought to be degraded by anthropogenic influences, we ran transects at 4 relatively pristine beaches (one each in Case, Carr, Eld, and Totten Inlets) and 4 less-pristine (two in Budd Inlet, east and west of Cascade Pole, and two near Shelton) (see Methods: Sites).

With only 4 transects sampled per treatment, our power to detect differences was low, especially because biota were sparse in most samples and thus the dataset was very zero-rich. Because of the great difficulty of working in these soft-mud sites, no data were gathered on surface flora or fauna, but epibiota are rare in this habitat type (Schoch and Dethier 1997). The infauna at all areas consisted of crustaceans (mostly amphipods), polychaete worms, and small numbers of clams. Species richness was almost identical between the two treatments, with 2-10 taxa per beach in the pristine areas, and 2-8 in the degraded (mean richness of 5.5 vs. 5.8, respectively). Figure 11 shows the ordination of the 8 beaches, and also illustrates the relative softness of the substrate at each transect. The degraded sites clearly cluster separately from the relatively pristine ones (left vs. right side of the ordination), and the degraded sites also tended to have softer sediments (larger circles) than the pristine. The ordination was not significant (p = .089) but the trend is clear, and the Global R value was fairly high as illustrated by the separation of treatments.

Figure 11. Community ordination from the pristine vs. degraded mud sites. Size of the circle indicates degree of softness of the sediment (bigger = softer).

Degraded sites: BE = Budd East (Cascade Pole east) BW = Budd West (Cascade Pole west) SN = Shelton North SS = Shelton South

Relatively pristine: CS = Case 11 CR = Carr 300 TT = Totten EL = Eld



Table 2. Species contributing more than 2% to the dissimilarity between pristine and degraded beaches.

	Degraded	Pristine		
More in Degraded	Av.Abund	Av.Abund	Contrib%	<u>Comments</u>
Monocorophium acherusicum	14	0.25	10.18	introduced suspension-feeding amphipod
Glycinde picta	6.25	1	8.29	carnivorous goniadid worm
Aphelochaeta multifilis	2.25	0	4.95	deposit-feeding cirratulid worm
Grandidierella japonica	1	0	4.41	introduced suspension-feeding amphipod
Cryptomya californica	2.25	0	3.06	small clam in ghost shrimp burrows
Mediomastus californiensis	0.75	0	2.83	deposit-feeding capitellid worm
Macoma inquinata juveniles	0.5	0	2.41	deposit-feeding clam
More in Pristine				
Spiophanes berkeleyorum	0.25	3	6.93	deposit-feeding spionid worm
Sigambra tentaculata	1.5	2.75	6.2	carnivorous pilargid worm
Macoma nasuta	1.25	3.5	5.8	deposit-feeding clam
Pseudopolydora kempi japonica	0	3	4.96	introduced deposit-feeding spionid worm
Crangon franciscorum	0.75	1.25	4.18	mud-dwelling shrimp
Amphiodia urtica	0	1	3.21	pollution-sensitive brittle star
Pinnixia schmitti/occidentalis	0	0.75	3.09	pea crab in burrows of other species
Clinocardium nuttallii juveniles	0.25	0.75	2.98	suspension-feeding cockles
Lucina tenuisculpta	0	0.75	2.25	tiny clam

Table 2 shows the average abundances of the species contributing most to community dissimilarity between the two treatments (excluding taxa whose average abundance was always <1 and that were minimally dissimilar between treatments). The most common organism in the degraded beaches was an amphipod (*Monocorophium*) thought to have been introduced with Japanese oysters; this is also true for *Grandidierella*, another corophiid amphipod present in the degraded muds. Both are suspension feeders that presumably thrive on the high organic content in these areas, as would the deposit feeding worms and clams in Table 2. The abundance of *Cryptomya* (a commensal clam found in ghost shrimp burrows) in the degraded areas suggests the presence of *Neotrypaea*; the ghost shrimp themselves are seldom captured in our relatively shallow cores. The polychaete *Mediomastus californiensis* was slightly more common in the degraded area but is found much more abundantly in our pebble-beach samples than in this mud. Interestingly, both it and *Sigambra tentaculata* (more common in pristine) are called "opportunistic and/or tolerant estuarine taxa" in the Gulf of Mexico by Rakocinski et al. (2000).

The more pristine areas also contained one introduced species, the common spionid polychaete *Pseudopolydora kempi japonica*; this and another spionid were two of the most abundant infauna in the more pristine areas, but not the degraded areas. Spionids are called "interface feeders", able to either deposit or suspension feed at the sediment-water interface, and they are broadly distributed (as a family) at our study sites; their relative lack at the degraded sites is of interest. Other species found more commonly in the pristine than the degraded sites include a variety of worms and bivalves (Table 2) and the brittle star *Amphiodia urtica*; this burrowing mud-dweller is used in southern

California as an indicator species for wastewater pollution, since its numbers are diminished near outfalls (Blake 1996). While relatively few individuals of this species were found, perhaps because it prefers slightly sandier substrates, this pattern is of interest. Both sets of sites contained organisms at a variety of trophic levels; there was no pattern, for instance, of the degraded sites being dominated by deposit feeders vs. the more pristine sites by suspension feeders.

Patterns at different levels of taxonomic detail

To test for the necessity or value of gathering field and lab-identification data at the species vs. higher taxonomic level, we used the 2001 dataset from 13 sites, analyzing them for spatial patterns at the species level versus the "low-resolution" family/functional group level (for most invertebrates and for algae, respectively). Figure 12 illustrates the ordinations at each of 3 spatial scales for these two contrasting analyses, and Table 3 gives the statistical results. At each spatial scale, it can be seen that gathering data at the species level adds a small degree of resolution of spatial patterns but no statistical improvement in our ability to distinguish patterns. For example, Figure 12A gives the ordination of community structure of the 13 sites at the species level, showing that the beaches are generally clumped with others at the same site (e.g., the three beaches at site 1, or site 8), although some are less well clumped (e.g. site 3 or 6). A comparison with Figure 12B, at the lower level of resolution, shows an almost identical pattern; sites that group well or poorly at the species level do the same at the low-resolution level. The Global R value for the species level is somewhat higher (0.73 rather than 0.65), but the significance of both ordinations is very high (Table 3). Similar differences are seen for the quadrant-level and basin-level ordinations.

		<u>Global R</u>	<u>p value</u>
Site Level	High-Res species	0.727	0.001
	Low-Res species	0.652	0.001
	Family	0.667	0.001
	Class	0.373	0.001
	Phylum	0.295	0.001
	Trophic	0.419	0.001
Quadrant Level	High-Res species	0.489	0.001
	Low-Res species	0.409	0.001
Basin Level	High-Res species	0.611	0.001
	Low-Res species	0.553	0.001

Table 3. Results of ordinations of 2001 data at different taxonomic levels.

Figure 12. Community-similarity ordinations for the 2001 data based on different levels of resolution.

- A. High-resolution Species level
- 1 = Budd
- 2 = Case
- 3 = Carr
- 4 = Brown's
- 5 = Redondo
- 6 = Brace
- 7 = Carkeek
- 8 = Possession
- 9 = Maury
- 10 = Vashon
- 11 = Blake
- 12 = Jefferson
- 13 = Hansville



B. Low-resolution Species level



C. High-resolution Family level



Figure 12, continued. D. High-resolution Class level

- 1 = Budd
- 2 = Case
- 3 = Carr
- 4 = Brown's
- 5 = Redondo
- 6 = Brace
- 7 = Carkeek
- 8 = Possession
- 9 = Maury
- 10 = Vashon
- 11 = Blake
- 12 = Jefferson
- 13 = Hansville
- E. High-resolution Phylum level





F. High-resolution Trophic level



To test whether lumping the high-resolution (species-level) data into higher taxonomic levels and into trophic groups results in a loss of critical information (i.e. ability to detect patterns in the data), we analyzed the same dataset in 5 ways (Table 3 and Figure 12). Table 3 shows that there is a steady loss in ability to detect patterns (separation of sites) at higher taxonomic levels, with lumping by trophic group falling in between family-level and class-level in degree of resolution. Collapsing the data from species to family level caused relatively minor changes in patterns, increasing the degree of separation among the 3 transects at some sites, for instance the 3 beaches in Budd (code 1 in Figure 12) and in Redondo (code 5). This implies that there may have been several species within a family that were held in common among these transects, but when that within-family information was lost, the transects no longer looked as similar. A larger-scale shift is that all the central-Sound sites, especially the most southern ones (e.g., 4, 5, 6, and 9) appear as much less different from the south-Sound sites at the family level than they do at the species level. This implies that these sites share many of the same families of organisms but have different species within these families. The trophic-level analyses look similar to the family ones in terms of degree of clumping of each set of sites, although some positions changed; e.g. at the trophic level, sites 1 and 3 are most similar to each other whereas at the family level, 2 and 3 are closer. Another interesting shift is that site 9 (Maury Island) shifted in ordination position from being close to the other beaches in south-central Sound at the species and family levels to being closer to the south Sound sites (especially Case Inlet) at the trophic level. The overall similarity of family and trophic analyses is not surprising, since species within most families of invertebrates are similar in trophic mode.

At the higher levels of resolution (Class and Phylum), similarities of sites become much less clear, and the Global R values drop substantially. Even the usually-clear separation of the South basin sites from the Central basin sites is lost. This is not surprising, since relatively few phyla are present in these samples overall, so the ability to distinguish beaches at that level is poor. The richest sites (Possession 8, Blake 11, Jefferson 12 and Hansville 13) still tend to clump in one corner of the ordination at both the Class and Phylum levels, presumably because they have some classes (and even Phyla) not represented at the other sites.

Figure 13 shows that the major disadvantage in examining biota at the lowresolution level is that overall "Richness" is much lower; when species-level information is collapsed into higher taxa, numbers of different taxa are obviously decreased. The same trend is seen in comparing Shannon's Diversity index. Thus for looking at trends such as the dramatic north-south richness patterns, the low-resolution data are clearly much less powerful.



Figure 13. Species richness and Shannon's Diversity index for 2001 sites sampled at high and low-resolution.

Univariate Indicators of Ecological Structure

A variety of univariate indicators have been proposed for benthic systems in marine environments. Some emphasize species richness, others their relative abundance (Simpson's, Swartz' Dominance, Pielou's Evenness). Special attention has also been paid to the richness and abundance of particular taxonomic groups – polychaetes, crustaceans, echinoderms and molluscs. Curves showing the relationship between abundance and species rank provide a visual representation of the distribution of individuals among species, but such curves are difficult to compare statistically. Abundance-biomass (A-B) curves, which simultaneously show cumulative biomass and abundance across species ranks, may indicate environmental stress when biomass begins lower than abundance – that is, the most abundant organisms are small and contribute relatively little biomass (PRIMER 5.0). Statistical analyses of A-B curves are possible (W statistic) but cannot be applied to SCALE data due to lack of information on biomass. In some cases, univariate indicators have been combined to produce a single benthic index. In east coast estuaries, for instance, 30 poor-quality sites (based on chemical toxicity and anoxia) were best distinguished from 30 higher-quality sites using the following formula: Benthic Index = 0.0489 G - 0.00545 T - 0.00826 S - 2.338, where G is salinity-normalized diversity, T is salinity-normalized tubificid abundance, and S is spionid abundance (Strobel et al. 1999). A benthic index below zero is considered characteristic of impacted sites.

Univariate indicators are advantageous for describing environmental quality because they tend to be easier to compare than shifts in multivariate "community space." However, they may also gloss over important aspects of community structure. For instance, species richness is a common univariate indicator, but it cannot account for wholesale replacements of species. Two communities with identical species richness might in fact have very different membership. Here we take two approaches common in the literature to analyzing the SCALE data in a univariate framework, emphasizing particular species and a suite of indicators based on species richness and organism abundance. Our major questions are:

- What is the power to detect change in particular taxa? (Coefficient of variation)
- How correlated are various indicators of community structure, in particular species richness (S), total abundance (N), molluscan, crustacean, and polychaete richness and abundance, Margalef's d, Simpson's dominance (1-λ), Pielou's J, Shannon-Wiener diversity (H), and Swartz dominance? (Principal Components Analysis)
- How well do these potential indicators distinguish among locations? (Principal Components Analysis)
- What power do these univariate indicators have to detect change, and how does this power vary with the scale of analysis? (Coefficient of variation)

Particular species:

Potential indicator taxa were chosen based on their contribution to patterns in multivariate analyses of assemblage structure. We selected species that figured prominently in a) non-metric multidimensional scaling analyses, based on their contributions to differences among samples, and b) "nested sum" analyses, based on significant variation at the site or area scale. Only a few species met both criteria: *Alia* spp., live *Balanus* spp., gammarid amphipods, lottiid limpets, ulvoid green algae, *Notomastus tenuis* and *Mediomastus californiensis*. Analyses were based on mean values for each transect, using high-resolution pebble beach data. We calculated the coefficient of variation (SD/mean) for each species within transects across years (using years as samples), and within 2001 for all transects reported that year (using three transects as samples for each site). Means and standard deviations were not calculated when a species was entirely absent from samples. All calculations were carried out on untransformed counts or cover. The coefficient of variation is simply an easy way to compare how easily change would be detected in each species.

Coefficient of variation across years



Figure 14. Coefficients of variation for 5 common taxa, based on 21 transects with 3 years of data (1999-2001).

Figure 14 shows that for these taxa, CV's are relatively high, suggesting that three years has not been sufficient time to establish "normal" levels from which departures could be easily distinguished. Ulvoids have the lowest CV, although realistically, the high seasonality and opportunistic nature of this taxon make it an unlikely candidate for detecting change.



Coefficient of variation across transects

Figure 15. Coefficients of variation for 5 common taxa for sites in 2001, based on values at three transects per site.

Figure 15 shows that in general, coefficients of variation were lower across space than across time, so "impacted" sites might be relatively straightforward to detect based on barnacles, limpets, or algae, simply by comparing their abundance to other sites in Puget Sound. *Alia* spp. and gammarids, with high CV's, would not make good indicators. Of course, it would also be good to know the range of observed values of these species, because power to detect moderate levels of change is moot if change is either always small or unpatterned.

Some of the most common polychaete species in cores were the capitellids *Mediomastus californiensis*, *Notomastus lineatus*, and *N. tenuis*. These species showed high variability among samples, whether years or transects were considered samples (Figures 16 and 17). Most CV's were above 0.5, whereas most CV's for ulvoids and barnacles in quadrats were below 0.5.



Figure 16. Coefficient of variation for three species of capitellid polychaete, based on 21 transects with at least three years of data (1999-2001).



Figure 17. Coefficient of variation for three species of capitellid polychaete, based on 13 sites with at least three transects in 2001.

Univariate metrics:

Methods

1. We separated species quantified in cores, quadrat (surface) percent covers, and quadrat (surface) counts to make it easier to compare abundances of species within samples. This also allowed us to ask whether similar patterns in univariate indicators were apparent in our three sampling methods.

2. We selected data to examine variation within transects (among three years), within sites (among three transects in 2001), and within regions (among 2-3 sites in 2001). In the future, it would be interesting to consider within-transect variation based on quadrat-level data. Seven sites have three years of data (Carr, Budd, Case, Brace, Redondo, Carkeek, Possession). Thirteen sites have data for 2001 (the seven above plus Browns Point, Maury, Vashon, Blake, Pt Jefferson, Hansville). Transects within sites were averaged to examine variation within regions: 3 sites each in the south, southeast, and southwest Sound; 2 sites each in the northeast and northwest Sound.

3. We calculated the following metrics on nine data sets (three sample methods x three scales [transect, site, region]): species richness (S), total abundance (N), Margalef's d, Pielou's evenness (J), Shannon-Wiener diversity (H), and Simpson's dominance (1- λ). All were calculated in PRIMER v. 5. In addition, we calculated the species richness and abundance of molluscs, crustaceans, and polychaetes, and Swartz dominance (the minimum number of species contributing at least 75% of total abundance in a sample).

4. We performed a principal components analysis on normalized univariate metrics. This procedure generated a graph showing the distribution of sample locations in "indicator space"; essentially, if locations cluster, then univariate metrics might help distinguish among locations. The procedure also provided information on the loadings of univariate metrics onto the principal component axes, showing which potential indicators were correlated.

5. We selected a few univariate metrics to compare among locations and assess power at different spatial scales. "Power" was determined by examining the coefficient of variation (standard deviation divided by the mean) of selected indicators. At the transect level, CV was based on three years of average transect values. At the site level, CV was based on three transects recorded for each site in 2001. At the region level, CV was based on 2-3 sites (three transects within each site were first averaged to provide single values for each sampling site). As potential indicators, we selected species richness and Pielou's evenness, as these emphasize different aspects of each sample's composition. Swartz' dominance has received substantial attention as a criterion for establishing community-level sediment quality standards in Washington Department of Ecology. We also added polychaete or mollusc abundance when considering core or quadrat data, respectively.

Results from principal components analysis:

We performed a principal components analysis of 13 normalized univariate metrics. Quantitative results appear in Appendix D and are summarized in Table 4. Nine graphs (with text results) show principal components analysis results for three sampling methods (cores, quadrat counts, quadrat cover), with results examined at three scales (transect, site, region). When clustering of locations maps roughly onto geographical space, any potential indicators that load strongly on the principal component axes are likely to be able to distinguish among locations in Puget Sound. When potential indicators load strongly on different PC axes, it indicates that they are relatively uncorrelated with each other, and therefore information-rich.

Samples	Correlates of PC 1	Correlates of PC 2	Are closer samples more similar?
Cores 1999-2001 (within transect variation)	Less diverse	More even, fewer individuals	Yes on both axes
Quadrat counts 1999- 2001	Less diverse	More even, fewer individuals	No
Quadrat cover 1999- 2001	More even	More diverse but fewer mollusc species	No
Cores 2001 (within site variation)	Less diverse	Fewer worms, more even	Yes on PC 1
Quadrat counts 2001	More even	More species	Some sites on PC 1
Quadrat cover 2001	More even and diverse	Less species rich, more molluscs	Most sites
Combined cores 2001 (within area variation)	Fewer species	More even	Yes on PC 1
Combined quadrat counts 2001	More even	Fewer species, especially molluscs	Only South Sound distinguished on PC 1
Combined quadrat cover 2001	More even	More species	South Sound distinguished on PC 2

Table 4. Summary of results from principal components analysis of 13 normalized univariate metrics of community structure. PC 1 and 2 refer to the first and second principal components of the analysis.

In general, metrics of community structure that emphasized species richness loaded strongly on one of the major axes, whereas metrics that emphasized evenness loaded strongly on another (Table 4). Overall, the first three principal components explained about three-quarters of the variation in potential univariate indicators across sample units. Indicators that are driven by species richness (S, H', taxon-specific richness) tended to be correlated with each other, and relatively uncorrelated with indicators driven by evenness (d, J', 1- λ). The third principal component often was associated with the abundance of a taxonomic group (polychaetes, molluscs, or crustaceans).

At the within-transect (among-year) level, only data from cores allowed transects to be distinguished from each other using these univariate metrics. Transects are

intermingled using quadrat cover and counts. These results are consistent with the fact that diversity in cores is responsible for the major north-south gradient in diversity observed in our data.

In contrast to the among-year comparisons, among-transect comparisons for 2001 showed reasonable separation of sites for all sampling methods. That is, the three transects in each site generally grouped in consistent areas of ordination space, with some notable exceptions (e.g. Hansville).

Finally, for sites within areas, core samples still tended to distinguish sample units: southern sites have fewer species. For quadrat counts, southern sites tended to have higher evenness than northern sites. For quadrat cover, southern sites tended to have lower richness.

Based on this principal components analysis of potential indicators, we conclude that it is reasonable to pursue only a few in more detail, because most are highly correlated. We selected two metrics of species richness (S and Swartz dominance), a metric of evenness (J'), and a metric of abundance (polychaete or mollusc, depending on sample method). We considered both S (total number of species) and Swartz dominance (the number of species making up at least 75% of total abundance) because of the importance attributed to the latter metric as a biological indicator of subtidal sediment quality (DOE documents). The next step in selecting indicators is to understand how much a given metric would have to change before a perturbation could be detected.

Results from coefficients of variation:

The ease of detecting change in a particular indicator depends in part on how variable it is among sample units (years or sites). Coefficient of variation (standard deviation divided by the mean of sample units) is a simple way of approaching the power of statistical tests: for a given shift in value, it will be easier to detect change if coefficients of variation are low. For the three sampling methods (cores, quadrat cover, and quadrat counts), we provide coefficients of variation for the four potential indicators (S, J', polychaete or mollusc abundance, and Swartz dominance). In most cases, we explore coefficients of variation at three spatial scales: within transects (each of three years is a sample unit, using average transect values), within sites (each of three transects is a sample unit in 2001), and within areas (using average site values; in 2001, 2-3 sites were examined in each of five areas). However, we performed an additional analysis for species richness, examining the coefficient of variation within transects in a single year (2001) based on 10 quadrats. The following four graphs show the coefficient of variation for selected indicators at three spatial scales in cores. A low coefficient of variation will tend to provide high power for detecting change (always contingent on a perturbation causing the same magnitude of change in all univariate measures of assemblage structure).



Figure 18. Coefficient of variation for species richness in cores. Each point represents one transect, site, or area, respectively. For within transect CV, 2001 data are based on 10 cores, whereas across-year data are based on average values in each of three years.



Figure 19. Coefficient of variation for univariate metrics of assemblage composition in cores. Each point represents one transect, site, or area, respectively. No core-level data are included: within-transect data are based on average values in each of three years.

For cores, the lowest coefficients of variation appear for species richness at the within-transect level based on several years of transect averages (Fig. 18, filled symbols). Using core-level data to define within-transect CV resulted in substantially higher values (open symbols). Other metrics, except perhaps evenness, show higher CV's at all scales. For most metrics, CV dropped or remained similar as the spatial scale under consideration increased, except for polychaete abundance, where CV actually increased with scale.



Figure 20. Coefficient of variation for species richness in quadrat cover. Each point represents one transect, site, or area, respectively. For within transect CV, 2001 data are based on 10 quadrats, whereas across-year data are based on average values in each of three years.



Figure 21. Coefficient of variation for univariate metrics of assemblage composition in quadrat cover. Each point represents one transect, site, or area, respectively. No quadrat data are included: within-transect data are based on average values in each of three years.

As in cores, CV's for quadrat cover appear lowest for species richness measured within transects (across years) (Fig. 20, filled symbols). Variability among the 10 quadrats in 2001 was much higher. CV for mollusc abundance declined with spatial scale (Fig. 21), whereas CV for Swartz dominance increased with spatial scale, and CV for species richness and evenness remained similar across scales.



Figure 22. Coefficient of variation for species richness in quadrat counts. Each point represents one transect, site, or area, respectively. For within transect CV, 2001 data are based on 10 quadrats, whereas across-year data are based on average values in each of three years.



Figure 23. Coefficient of variation for univariate metrics of assemblage composition in quadrat counts. Each point represents one transect, site, or area, respectively. No quadrat data are included: within-transect data are based on average values in each of three years.

Quadrat count data also show lowest CV's for within-transect, among-year data for species richness (Fig. 22, filled symbols). Barring consideration of quadrat-level data, the CV's of all metrics remain consistent across scales (Fig. 23), although polychaete abundance shows higher CV's than other metrics at all scales.

We conclude that it should be possible to describe ranges of natural variation for each transect based on long-term monitoring, and that variation in some metrics within transects (among years) is generally lower than within sites or within areas. Conveniently, it is also likely that many perturbations will affect the shore at the scale of transects (bulkheading, point and non-point source pollution), rather than sites or areas (global change). Within transects across years, species richness would have to vary by 20-30% from the mean before one could conclude that this metric had been affected by some environmental stress or perturbation. Of course, it is quite possible that environmental change could substantially alter assemblage composition with no concomitant change in species richness. Therefore, species richness should be considered a conservative indicator of change. Multivariate approaches are much more likely to detect changes in assemblage composition independent of a change in richness. Comparing across sampling methods, CV's are lowest for quadrat cover. Therefore, simply tracking the richness of space occupants in quadrats on pebble beaches would provide a relatively easy and powerful method for detecting change based on univariate metrics. Although Swartz dominance incorporates some aspects of species richness, it proved much more variable among sample units in this case. Possibly, this variability arises because Swartz values are often low – often just three species are sufficient to account for 75% of individuals (or cover). Because of low measures of Swartz dominance, small variation (1-2 species) around the mean can actually lead to relatively high CV's.

DISCUSSION

As is the case with any monitoring program, the data become increasing valuable with time, as an 'envelope of normalcy' becomes established through interannual sampling. The SCALE data for generally representative pebble beaches in southern and central Puget Sound are now extensive enough that we can begin to detect ecologically significant differences among beaches and changes through time; these lead to hypotheses about causes of change and suggest directions in which to focus future efforts. In addition, we are beginning to be able to make predictions about directions and types of changes in benthic biota that might follow significant environmental events. For example, most of our data on patterns of species richness and abundance suggest that nearshore salinity is a key forcing function; thus if there was a year with very high rainfall or very rapid spring snowmelt that decreased the salinity in the Sound (perhaps especially during key recruitment events), we predict that there would be a significant change in the biota in many beaches, particularly those near major river mouths. Such changes might appear as declines in local richness relative to previous years, or in a shift in community structure of those beaches out of the 'ordination space' in which they have been found for years, into a different space (perhaps more similar to that of the low-salinity, south Sound communities). Similarly, physical events such as flooding, landslides, or beach nourishment projects that increased the sediment supply to beaches over 'normal' levels (recognizing that normal levels during our years of sampling may be below those during historic periods) are predicted to cause declines in abundance of surface flora and fauna, and a shift in character of the infauna.

The combination of two large datasets now available for Puget Sound is particularly powerful in terms of our understanding of the Sound and our ability to detect change. The Shorezone dataset shows coarse-scale habitat types on the shorelines of the whole Sound; if remapped occasionally, it should allow the detection of large scale shifts in habitat types, and increases in human alterations of the shore such as bulkheading. The SCALE dataset provides much higher-resolution data about biotic communities, especially in the broadly studied, common pebble beaches but to some extent in other substrate types (especially in south Sound). The SCALE data, whether gathered at the species or family/low-resolution level, allow detection of much smaller changes, both in terms of spatial extent of a change and in terms of subtlety of community shift. The following paragraphs summarize our recommendations for future monitoring and analyses based on the research described in this report.

Taxonomic Resolution: Recommendations

Dethier and Schoch (2001) reviewed the literature on 'taxonomic sufficiency', which suggests that analyses of biota at the level of family or even order are as good at detecting trends as are species-level analyses, allowing substantial savings of time and taxonomic expertise. If the same inferences about patterns in nature can be drawn from both species- and higher-taxa information, then the latter has been termed "sufficient" (Ellis 1985), or the former even "redundant" (Ferraro and Cole 1992). In the one similar study in an undisturbed (unpolluted) system, James et al. (1995) assessed the ability of family-level analyses to detect the same spatial patterns seen at the species level. They found that differences among depth gradients in infaunal sand-habitat communities were detected just as well at the family level, using both multivariate and univariate analyses.

Analyses of our 1997 data from Carr Inlet (for mud, sand, and cobble) showed that aggregating species to the family level distinguishes among communities in different substrate types almost as well as does the species-level data; the different substrate types in Carr Inlet contain significantly different communities at the family level. Our current analyses suggest that for pebble beaches in Puget Sound, the changes in flora and fauna that accompany much more subtle shifts in salinity and wave energy among sites in central Puget Sound are almost as well distinguished at the family as at the species level. Some sites underwent a slight 'shift' or 'spreading' in ordination space between species analyses and family analyses, but these were relatively minor, and the Global R for these 2 analyses were quite similar (0.73 for species, 0.67 for family). The overall separation between south Sound and north-central Sound sites was less clear, presumably because these regions share many families but in some cases have different species within those families. Our analyses suggest that use of family-level data should be sufficient to detect both gross changes (e.g. loss of whole families), and more subtle among-site differences (e.g. within central Sound) or among-year differences within a site.

In our Puget Sound samples, much of the time and expense of processing infaunal samples has been in identifying polychaetes to species in the laboratory; identifying them to family is quite simple and rapid, and the process is relatively straightforward for a non-expert to learn. Field data collection (of surface biota) is most rapidly done not at the taxonomic/family level but instead using a modification of the 'low resolution' categories proposed in 2001 (Schoch and Dethier 2001). Our analyses of the 'low-resolution' database, which groups some species by family and others (especially epibiota) by

functional group, suggests that these are almost as powerful as the family-level groupings and in some cases result in clearer patterns (e.g. the central vs. south-Sound separation, Figure 12B). We thus conclude that gathering data using the low-resolution categories in the field and identifying infauna to the family level in the lab is a reasonable alternative to the more time consuming species-level monitoring. A reasonable long-term scenario might involve annual monitoring of most sites at this lower level of resolution, with periodic (e.g. every 3-5 years) gathering of data at the species level to allow examination of trends in species richness, which cannot be calculated from the low resolution data. In addition, if there was reason to suspect that a given beach or region was suffering from some new stressor (e.g. change in sediment supply due to bulkheading, or change in salinity due to unusual precipitation), then data could be gathered at the species level to check for more subtle biotic changes.

Indices of Benthic Community Health: Recommendations

The potential indicators that we applied to the SCALE data set show some promise in summarizing spatial and temporal patterns in community structure. Three desirable traits of indicators are: consistency across sites and times that share environmental conditions; variability across sites and times that do not share environmental conditions; and ease of communication to managers and stakeholders. When there is one major form of environmental concern (e.g. nutrient pollution) and particular taxa known to be sensitive to it, these are clear choices as indicators; unfortunately, in Puget Sound we have many possible stressors and virtually no information on local 'pollution-sensitive' species.

Of the univariate indicators examined, species richness most closely fits the "good indicator" criteria for cores, quadrat cover, and quadrat counts. Species richness varies across locations, based on high loadings in principal components analysis, but it also remains consistent within locations, based on low coefficients of variation (generally <0.4). CV is particularly low within sites across years, arguing strongly for a long-term commitment to monitoring of richness in order to detect shifts in the health of Puget Sound beaches. However, at this scale (within beaches among years), only cores effectively pick up spatial variation; quadrat richness is consistent among years (low CV) but also too consistent among transects to distinguish them well. Instead, quadrat cover works at the within-site scale: CVs are low and sites are readily distinguished.

Interestingly, this more formal analysis of univariate indicators converged on a reporting strategy already used by SCALE researchers: trends in species richness through Puget Sound emerged early as a strong and easily-communicated pattern of community structure. Among additional univariate indicators, evenness (J') is a likely candidate: it also helps distinguish locations, based on principal components analysis, and shows low CV at a variety of spatial scales. Species richness and evenness do not, however, reduce the amount of data that must be collected to define beach health. Rather, they are analyses that can be carried out in addition to multivariate analyses when communities have been fully described. A coarse taxonomic level may be sufficient, but we have not applied an indicator approach to the same data set at different resolutions. In general, the

spatial patterns revealed by species richness and evenness converged on patterns revealed by multivariate approaches: a gradient in community structure from north-central to south Sound, with some parts of the variation most evident among sites and some among areas, and usually lower temporal variation.

In contrast to univariate indicators that summarize full community structure, indicator taxa appear much less promising for defining beach health. The species that drive multivariate ordination patterns (yet still account for small total amounts of the variation) tend to be common species that vary in abundance in space and time: ulvoid green algae, barnacles, amphipods, and capitellid worms. The problem with using these as indicator taxa is that they are too variable: at several scales, coefficients of variation are generally >0.5. The life histories of many of these species, including pulsed recruitment and sensitivity to seasonal temperature change, also make them unlikely indicator taxa.

Use of SCALE for Studies of Degraded Beaches

Our pilot study using the SCALE method to compare the biota in mud beaches thought to be relatively pristine versus degraded suggest that, with the addition of some more data, we would have the power to detect biotic differences among such areas. We sampled only 4 beaches in each category, and since each beach was relatively depauperate (fewer than 10 species, versus over 50 per transect in many pebble beaches), there was relatively little data available for running statistical analyses. Several types of data could strengthen future attempts to find differences and attribute them to anthropogenic influences. First, sampling more beaches would greatly strengthen the dataset. Second, sampling more cores per transect might add more species, helping to clarify differences among sites; however, our other studies showed that richness increases more quickly among transects, so it would be better to add transects. Third, simultaneously gathering data on known or suspected degrading influences (e.g., levels of PAHs, organics, or metals in the sediment) and other sediment features such as abundance of benthic diatoms would allow additional analyses to be run, seeking linkages between the biota and quantitative factors.

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