

## **LINKING NEARSHORE PROCESSES WITH INTERTIDAL DIVERSITY IN PUGET SOUND**

### **Principal Investigators**

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### **Objectives**

We will investigate the ecological processes that generate conspicuous patterns of diversity and species composition along the estuarine gradient in south and central Puget Sound. Although a gradient in salinity occurs in this region, oceanographic processes also alter temperature, recruitment, productivity, and sediment type/stability of nearshore sites. We seek to understand how modifications of these processes could lead to changes in intertidal community structure. We predict that diversity should be positively related to recruitment and productivity; that sediment instability will reduce the diversity of epibiota; and that fine sediments will increase the diversity of infauna. We will draw on an extensive, multi-year database of biota and physical characteristics of 64 sites in Puget Sound to choose appropriate study sites and generalize our results.

### **Problem or Opportunity Addressed**

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 changes occurring in the environment (Warwick and Clarke 1993, Rakocinski et al. 2000, Warwick et al. 2002). These organisms (invertebrates, seaweeds, and fishes) 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, studying benthic species from the nearshore region of estuaries can provide a mechanism for detecting change in estuarine ecosystems.

Our previous studies in the southern and central basins of Puget Sound (Schoch and Dethier 1997, 1999, 2001, Dethier and Schoch 2000) have shown that there is strong coupling between the nearshore waters of the sound, the physical environment on the beach, and the organisms in intertidal habitats. We have quantified this linkage in various habitat types (cobble, sand, pebble, and mud beaches), and have examined gradients in

physical features throughout southern and central Puget Sound in the biota of pebble beaches (the most common beach type in the sound). Our 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 experimental studies.

Intertidal benthic communities in Puget Sound show a striking, temporally consistent trend in species richness from low in the south to high in the north in both epibiota and infauna of pebble-sand beaches (Fig. 1); virtually identical physical habitats are almost twice as species-rich in the north as in the south. We have documented this biotic pattern and various physical correlates in collaboration with the WA Dept. of Natural Resources, as part of a long-term monitoring program (see Dethier & Schoch 2000). The existence of this diversity gradient in one habitat type in Puget Sound provides us with a unique opportunity to test ideas about the maintenance of diversity in estuaries: what processes are important? Are there detectable general rules that could apply to other estuarine situations?

Our large dataset generates testable hypotheses about estuarine processes that maintain the health and diversity of local benthic communities, such as nearshore water flow and water characteristics, sediment characteristics (supply, character, and stability), rates of recruitment, local primary and secondary productivity, and biological interactions. To test such hypotheses, we must move beyond descriptive work and conduct experiments. DNR funding constraints do not permit such hypothesis testing. We propose a series of experiments to explore such key nearshore processes at a variety of spatial scales, thus allowing us to test hypotheses about the interplay of processes on local (small) and larger scales (Thrush et al. 1997).

## **Rationale**

Delineation of processes key to the maintenance of healthy Puget Sound intertidal habitats is critical for several efforts: 1) Monitoring programs seek to detect changes and ascribe them to an anthropogenic or natural cause. Our research will help identify key links between physical processes and biological outcomes. For example, a climatic shift that increases precipitation may drive down the salinity in south Sound, thus preventing larval clam recruitment or increasing the north-south diversity gradient. 2) Many areas of Puget Sound are prized for commercial and recreational shellfish use. Our sites overlap south Sound areas of commercial aquaculture, particularly in Carr Inlet, as well as areas throughout Puget Sound that are harvested recreationally for clams. Do sites with abundant bivalves have higher recruitment or faster growth rates? Do growth rates relate more to local temperatures, food supply, or other factors? 3) The maintenance of diversity and productivity are key issues in choosing sites for marine protected areas (MPAs). MPAs are proposed or established for a variety of reasons, including conservation of biodiversity, reference areas for scientific studies, and tools for fisheries management. For intertidal areas, fisheries management is less of an issue than it is in other habitats and regions, except for the harvest of bivalves (and a few other species).

There is a burgeoning literature on requirements for effective networks of marine preserves in terms of linking populations of threatened species, but less research on requirements for preserves focused on overall biodiversity. Effective preservation of biodiversity requires that the ecological and oceanographic conditions necessary to maintain this diversity also be encompassed (Zacharias and Roff 2000, Salomon et al. 2001); because these are open systems, local diversity will be a balance between what arrives and grows (bottom-up processes), and what survives by not getting eaten (top-down processes). Our research should provide guidance on what regions of Puget Sound would be most effective for establishing MPAs that could maintain their 'value' over time. To date, there has been little progress on choosing or establishing MPA sites in Puget Sound, and the few chosen have been selected based on opportunism rather than scientific criteria.

We propose to study the processes behind the well-documented patterns in benthic diversity in Puget Sound by examining the linkages between intertidal primary and secondary productivity, recruitment, and local diversity. We suggest that sites with high benthic diversity should make the most ecologically effective sites for preserves, and that effective sites should be supported by high and predictable productivity and recruitment. We will draw on the sites studied by DNR and on the expertise of several PIs plus the DNR's Nearshore Habitat Program to test a set of hypotheses over a variety of spatial and temporal scales. We will use the extensive datasets gathered by the Washington Department of Ecology (DOE) on offshore temperature, nutrients, and chlorophyll concentrations throughout the Sound, merge these with physical data that we will gather nearshore, and link both with process-oriented research on the shoreline.

### **Present Status**

Estuarine diversity gradients have been documented elsewhere, but seldom while controlling for substrate type and almost never with sufficient replication (Constable 1999). The sampling design used in our studies to date controls for substrate type and is hierarchical, with enough replication at each spatial scale to allow us to document and compare variability at scales ranging from meters to tens of kilometers. We have found that benthic communities in Puget Sound are very consistent among beaches that share a set of geophysical characteristics and among beaches within several km of each other, but show gradual differentiation at increasing spatial scales, and especially from south to north.

The differences in species richness between similar beaches in the south versus central basin are striking; there is a clear, almost unidirectional trend from south to north in the numbers of species found per beach (Fig. 1). The trend is visible both in the epibiota and in the infauna, although more strikingly in the latter. When the taxa are broken down by trophic mode, the south-north trend is still visible in virtually all the groups (Fig. 2). This differs from results in other, perhaps more contaminated estuaries, where trophic diversity declines with species richness (e.g., Rakocinski et al. 2000).

Higher richness in our northern samples (Fig. 1) parallels many other estuarine studies that find the greatest benthic diversity in areas near the mouth of estuaries, where salinities and temperatures tend to be the least variable (and most marine), wave action highest, turbidity and sedimentation lowest, and pollution the least. Any or all of these factors may be affecting the trends in our Puget Sound samples, although this region of Puget Sound is oceanographically well mixed compared with many estuaries. Several physical features statistically correlate with local and larger-scale patterns of differences in biota, providing clues about the key driving forces affecting algal and invertebrate populations at different spatial scales. Within a transect (i.e., among replicate samples), small-scale variation in grain sizes and pore-water characteristics, as well as local biological interactions, are probably important. At larger spatial scales, the patterns suggest that wave energy and nearshore salinity are key differences among basins (south Sound vs. central Sound), and that even within central Sound, salinity and energy differences correlate with differences in biota (Fig. 3). This agrees with previous estuarine studies emphasizing the role of salinity. Low-salinity stress may exclude many species from the south sound, although we could find no literature on experimental salinity studies for the local flora and fauna. The amount of sand found among the pebbles on these mixed-substrate beaches also correlates with differences in community structure (Fig. 2). Higher sedimentation of fines in the south may exclude some taxa, for instance suspension feeding phoronid worms, and these tube-builders in turn provide habitat for a variety of associated infauna (Rader 1980).

Our data thus provide evidence that pebble beach communities have a high degree of fidelity to specific physical habitat types. These habitats result from a combination of small and large spatial scale physical forcings. By matching the spatial scales of these forcing functions to the scale of the biological response, we improve our understanding of the significance of any observed environmental change. For example, local physical events such as flooding, landslides, or beach nourishment projects that increase the sediment supply to beaches over 'normal' levels (recognizing that normal levels now may be below those during historic periods) may cause local declines in abundance of surface flora and fauna, and a shift in character of the infauna. In addition, by clarifying the timing of recruitment and maximal growth with frequent sampling, we can characterize the physical (or biological, e.g. food supply) conditions under which these key biological processes occur. By linking DOE data on temperature and chlorophyll for the middle of the Sound with the nearshore proxy of temperature as recorded by dataloggers at each of our sites, we can use these physical data as the background against which to study ecological processes.

In summary, the gradient in diversity we have observed in south and central Puget Sound could be related to: 1) Organism physiology, if fewer species tolerate low salinity and high temperatures at the head of the estuary. We will measure temperature directly. 2) Gradients in recruitment, because surface waters move predominantly out of Puget Sound. We will measure recruitment directly. 3) Gradients in resource supply, because, in theory, enhanced productivity first increases and then reduces diversity. We will measure both primary and secondary productivity on shore, as well as examining DOE data on fluorescence from offshore stations. 4) Gradients in post-recruitment mortality. Mortality

may be due to either biotic (predation, herbivory) or abiotic factors (wave force, substrate stability), and we will focus on the role of sediment type and stability in influencing infauna and epibiota.

## **Approach**

Our experimental approach begins, critically, with 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 (the **Shoreline Classification And Landscape Extrapolation** model: Schoch and Dethier 1997). In order to choose replicate beaches, we segment a complex shoreline (which encompasses numerous environmental gradients) using a combination of qualitative and quantitative 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 regions. These nested segments can be used to study within-segment and among-segment variability, which in turn support studies of the biotic and abiotic processes that control variability (Dethier and Schoch, in review).

Our monitoring work to date has primarily involved samples collected in the lower intertidal zone (MLLW or 0 meters elevation). At this 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 collect 10 random samples along a 50 m horizontal transect positioned near the center of the beach segment. Each sample consists of quantifying surface macroflora and fauna abundance in a 0.25 m<sup>2</sup> quadrat, and infauna in a 10-cm diameter core dug to 15 cm depth. Percent cover is estimated for all sessile taxa in the quadrats, and all mobile epifauna are counted. Core samples are sieved through a 2 mm mesh and taxa counted. Organisms not identifiable to the species level in the field are placed in formalin and identified in the lab.

All experiments discussed below will be conducted at 9 sites, chosen from those sampled during our long-term monitoring program (Dethier and Schoch 2000). Following our hierarchical sampling design, we will work at 3 replicate beaches (within 10 km of each other) with shared geophysical characteristics. All experiments will be replicated within each beach (=site) as well as among beaches. To examine patterns at larger spatial scales, we will compare 3 broadly separated regions in the Sound. Thus we will sample at 3 sites in north-central Puget Sound (labeled Carkeek in Figure 1), 3 in south-central (labeled Brown, Figure 1), and 3 in south Sound (Budd, Figure 1). All are sites which we have been sampling since 1999 on a regular basis and thus for which we have excellent data on community structure and interannual variability. All have similar substrate types, although the more southern sites have lower wave exposure and a correspondingly higher proportion of sand. We will choose beaches that have low public access to minimize disturbance to experiments; if possible, we will obtain access from private landowners.

This will be important for winter sampling when sites must be reached from the land, during nighttime low tides. Washington Department of Ecology (DOE, 2003) water-quality sampling data are available from offshore waters near all three sets of sites. In addition, we will examine records for more northern sites (north Admiralty Inlet) to assess physical and biological conditions of water entering into Puget Sound.

We propose to study the nearshore processes linking physical features with the biota found in Puget Sound by testing four hypotheses. We request 3 years of funding to accomplish these studies; all observations and manipulations will be repeated over at least 2 years. This will allow us to gather data on interannual variability (e.g. in growth rates) and greatly strengthen our conclusions about relationships between diversity, recruitment, growth rates, and conditions of the nearshore environment (temperature, plankton concentrations). We have already shown that interannual variability in biota in these habitats is substantial (Dethier and Schoch 2000); thus short-term experiments (not repeated among years) would run considerable risk of having low generality.

**H1:** *Local intertidal productivity is closely linked in space and time with food supply rather than temperature.*

Background: Estuarine waters are very heterogeneous in terms of temperature, nutrients, plankton production, and other factors that may affect growth rates of both producers and consumers in the nearshore. The “textbook” estuary is highly productive because food webs are supported by nutrients entering from watersheds. While nutrients delivered to estuaries in freshwater (e.g., into south Sound) can stimulate phytoplankton population growth, freshwater can introduce osmotic stress and, at high flow, flush phytoplankton out of an estuary (Muylaert et al. 2001). Rivers can also introduce sediment that interferes with light required for phytoplankton growth (Pennock and Sharp 1986). Maurer et al. (1992) suggest that P/B (production per biomass) of secondary consumers is higher inside Delaware Bay than at coastal stations, but this may primarily reflect the dominance of deposit feeders inside the bay. Thus, although resource patterns vary consistently along exposure gradients within estuaries, the predominance of terrestrial inputs in freshwater at the head and of marine inputs at the mouth may not have consistent consequences for the production of benthic suspension feeders that consume pelagic resources. In addition, measuring food supply for benthic species is problematic; phytoplankton biomass generally declines from inner to outer portions of estuaries, but estimates of annual production do not match this pattern (Heip et al. 1995).

Shellfish harvest (wild and aquaculture) is a key industry on Washington state’s shorelines, with millions of dollars in revenues annually. Factors that improve oyster growth may also increase productivity of hardshell clams, geoducks, and other important suspension feeders that are abundant in Puget Sound. Our preliminary data on growth rates of juvenile oysters (*Crassostrea gigas*, Fig. 4) suggest that south and central Sound is a weak “textbook” estuary. Secondary production is higher at the south (head) end of Puget Sound. Oysters grow significantly faster in Budd Inlet than at other sites, and stable isotope ratios of oyster tissue are enriched in  $\delta^{15}\text{N}$  there (Fig. 5). Stable isotope ratios indicate the structure of food webs and the energetic subsidies entering an area. Many

elements on earth include several naturally occurring forms that differ in molecular weight. Carbon-12, for instance, is very common on the earth's surface, whereas Carbon-13 is rare. For stable isotope analysis, the ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  is measured as  $\delta^{13}\text{C}$ , the relative amount of  $^{13}\text{C}$  in a sample relative to a standard. Other elements commonly considered in stable isotope analyses are  $^{15}\text{N}/^{14}\text{N}$  (nitrogen) and  $^{35}\text{S}/^{34}\text{S}$  (sulfur). Two key points allow stable isotopes to be used to explore feeding relationships. First, stable isotope ratios vary among primary producers, because inorganic carbon and organic nitrogen sources occur in different ratios in different habitats. Atmospheric  $\text{CO}_2$ , for instance, contains relatively little  $^{13}\text{C}$  compared to carbon dissolved in seawater ( $\text{HCO}_3^-$ ). Second, you are what you eat. To a large extent, the stable isotope ratios of consumers reflect the stable isotope ratios of what they have been eating. If a consumer eats two things with different stable isotope ratios, the consumer will have an isotopic signature in between the two resources. Some elements, particularly nitrogen, change directionally from one trophic level to the next. So,  $^{15}\text{N}$  becomes slightly more common through the food chain, providing an indication of trophic level, not just energy source (Dawson and Brooks 2001). These isotopic differences seen in our preliminary oyster studies (Fig. 5) suggest that there is spatial variation in food sources among our study sites (Peterson and Fry 1987, Stribling and Cornwell 1997).

Bioenergetic models of *Crassostrea gigas* emphasize total suspended solids, chlorophyll, and temperature as factors most responsible for variation in growth (Ren and Ross 2001). Growth generally improves with chlorophyll, whereas suspended solids can actually interfere with suspension feeding. A peak in growth generally occurs at temperatures around  $19^\circ\text{C}$ . The temperature differences we have observed among our study sites, roughly  $1\text{-}2^\circ\text{C}$  higher in the south than north, may be sufficient to account for observed differences in growth, but we suspect resource variation is more likely.

Methods: In years 1 and 2, we will put out TidbiT temperature dataloggers and transplant juvenile oysters across the north-south estuarine gradient at the 9 sites discussed above. Temperature data from the TidbiTs will serve as local proxy allowing us to tie nearshore seasonal and interannual trends into those at the 40 offshore stations where DOE (2003) gathers monthly chlorophyll data. At each site, we will place one Tidbit in the shallow subtidal zone to record nearshore temperatures, one on the surface of the beach to record beach-surface (including groundwater runoff) temperatures, and one buried in the sediment. Monthly growth rates of oysters will then be correlated with beach and water temperatures and with offshore productivity. Oysters (*Crassostrea gigas*) will be settled on individually-labeled ceramic tiles (11 x 11 cm) in a commercial hatchery. After two weeks, when individuals are about 2 mm in shell length, they will be thinned to 5-20 per tile and outplanted to study sites. Tiles will be attached through pre-drilled holes to PVC stakes, and pushed into the substrate until the tile is just above the surface at mean lower low water. Five tiles will be outplanted to each beach. Photographs will be taken of each tile prior to outplanting and at 1-2 month intervals in the field to determine growth and survival. Transplants will be carried out in June-September (summer growth) and September-June (winter growth) over two years.

Stable isotope analyses of outplanted juvenile oyster tissue can provide an integrated picture of estuarine food webs, translated through the feeding habits of an economically and ecologically important species. We will focus on  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  because these inform us about sewage inputs and terrestrial-marine inputs, respectively (McKinney et al. 2001).  $\delta^{35}\text{S}$  will be measured in selected samples, because this element best distinguishes food derived from marine macrophytes such as eelgrass. Isotopes will be analyzed from homogenized individuals from each of the 5 tiles per site, as well as from water samples at the southern and northern ends of the estuarine gradient. We will also test potential benthic sources of organic C, especially ulvoid algae and benthic diatoms.

**H2:** *Sites with higher benthic productivity have higher species diversity and biomass.*

Background: Theory suggests a nonlinear relationship between diversity and productivity (Huston 1994), such that an increased resource base first promotes coexistence, but, at very high resource levels, monodominant stands develop and diversity declines. Proulx and Mazumder (1998), summarizing studies of consumer impacts across a range of resource levels, found that results differed consistently between natural gradients in productivity (where consumers tended to reduce diversity) and anthropogenic gradients (where consumers tended to increase diversity). Interestingly, our preliminary data with juvenile oysters suggest a negative correlation between productivity (somewhat higher in the south, Fig. 4) and overall benthic diversity (clearly higher in the north, Fig. 1).

Methods: At each of the 9 sites we will measure both primary productivity, using growth of algae, and secondary productivity, using growth of suspension feeders. We will transplant rocks with small *Fucus gardneri* (brown algae) and tiles with juvenile oysters and barnacles to each site and quantify growth through the spring and summer of years 1 and 2. We will gather rocks with young *Fucus* (less than 5 cm length) from other sites, thin the plants to leave approx. 10 per rock, and place at least 3 marked rocks per site. *Fucus* growth can easily be measured non-destructively in the field by quantifying the maximum length and total number of tips, which correlate closely with biomass (Dethier et al. in review). Barnacles (*Balanus glandula*) will be allowed to recruit onto small ceramic tiles hung from the breakwater at the Friday Harbor Labs; juveniles will be thinned and the tiles attached to stakes (5 per site) hammered into the substrate. Barnacle tiles will be photographed monthly in the field, and survival and growth measured digitally. Simultaneous oyster growth rates will be obtained from the tiles described in H1.

Overall community diversity will be measured in surface quadrats and sediment cores using our standard long-term monitoring techniques (see Approach). Species-specific biomasses will be measured by randomly collecting samples of epibiota and large infauna (clams) and returning them to the lab to get wet and dry masses; small infauna will have biomasses measured following laboratory identification. To link biomass data to our broader database on abundance, biomasses of sessile species (whose abundances are quantified by percent cover) will be assessed on a per-area, not per-individual basis. If growth of our target species (*Fucus*, oysters, barnacles) is positively



correlated with their biomass across sites, then productivity is likely to influence community structure; otherwise, recruitment or post-recruitment survival rates more strongly influence community structure. Post-recruitment survival (an indicator of top-down processes, such as herbivory and predation) can be assessed in part by measuring survival of our transplanted target species over each monthly interval.

**H3:** *Sites with higher rates of recruitment have higher diversity.*

Background: In areas where recruitment of organisms is high because supply and survival of propagules (larvae and spores or zygotes) is high and regular, we might expect overall diversity on the beach to be high. This prediction would only hold, however, if recruitment (supply-side) rather than biological interactions (top-down) limits a variety of species in this system; while this dichotomy has been debated extensively for rocky shore communities, no such literature exists for estuaries. A meta-analysis of marine studies suggests that consumers reduce diversity when overall recruitment rates are low and increase diversity when overall recruitment rates are high (Moore et al. in revision). Patterns may depend in part on whether or not species have planktonic propagules, and in estuaries, on whether species are primarily of marine or brackish origin. We can test these relationships in Puget Sound. In addition, by clarifying the timing of recruitment with frequent sampling, we can characterize the physical (or biological, e.g. food supply) conditions under which recruitment occurs.

Methods: Beginning in late winter of years 2 and 3, we will embed small flower pots of sterile sediment at each sampling site. Pots will be approx. 1.5L and will be filled with a natural size mix of sand, gravel, and cobbles; these will be embedded flush with the natural sediment. Pots will be collected and replaced to quantify colonization by infauna on a monthly basis, corresponding to physical measurements in H1. Samples will be sieved with 2 and 0.5 mm sieves to capture young recruits; our normal monitoring procedures only use 2 mm sieves.

At the same sites and sampling periods we will also measure monthly rates of colonization by epibiota (algae, barnacles, etc) on settling tiles; ceramic tiles (11 x 11 cm, 5 per site) will be attached to PVC poles embedded in the beach. Tiles will be collected and replaced at each sampling interval, and species will be identified and counted in the laboratory.

Rates and timing of recruitment of both infauna and epibiota will then be related to monthly local physical conditions (as quantified above), and to local diversity as quantified in H2.

**H4:** *The quantity of fine sediments increases recruitment of infauna but reduces epibiota due to scour.*

Background: The high diversity of these mixed-sediment beaches is due in large part to there being essentially 3 habitats: relatively stable surface cobbles, on which algae and sessile invertebrates settle; spaces under cobbles and pebbles where mobile invertebrates

and small fishes hide; and sediments below, containing enough fines to support a rich infauna of polychaetes, clams, and other invertebrates. The amount of fine sediment subsurface probably contributes positively to infaunal recruitment and diversity, but our monitoring data suggest that too much sand on the surface decreases epibiota, due to scour or smothering. Sediment supply and transport are key and controversial topics in the nearshore of Puget Sound, because watershed changes and shoreline hardening (with seawalls) have clearly altered these processes. Our research will investigate how changes in nearshore sediment processes can affect communities on this common beach type.

Methods: To quantify the effect of higher proportions of sand on infaunal recruitment, pots of sterile sediment (as in H3) will be prepared with 3 differing proportions of fines (sand) characteristic of each of the 3 sampling regions, and set out at the three northern sites in years 2 and 3. Pots will be collected and replaced at each sampling interval. In addition to quantifying recruitment, we will do final grain size analyses to determine how much the pots changed between sample periods. To quantify surface scour, painted rocks will be established at all sites each spring; at each sampling interval we will measure the amount of paint removed from each (N=5 rocks per beach). We will correlate the amount and seasonality of scour with abundance and seasonality of epibiota, both on these beaches and on our settling tiles (H3).

### Analyses

Our major question concerns the relative contributions of temperature, productivity, recruitment, post-recruitment survival, sediment grain size, and sand scour to gradients in species diversity observed in south and central Puget Sound. For each of our nine study sites, we will have information on beach temperature (average and maximum), oyster growth and survival, barnacle growth and survival, *Fucus* growth and survival, recruitment of epibiota, recruitment of infauna, sediment grain size, and sediment scour. Many of these measurements broadly reflect similar factors and are likely to be correlated. We will look for uncorrelated structure in these potential predictor variables, using principal components analysis, and then relate richness across sites to these major principal component axes, using regression (linear or polynomial).

To test hypothesis 1, two analyses will be performed. First, we will examine whether oyster growth rates are best predicted by stable isotopes (reflecting energy source) or temperature, using a multiple regression analysis with sites as samples. We will also parameterize a bioenergetic model (under development in a separate Sea Grant project: Material processing and ocean-estuarine coupling in oysters) to determine whether growth rates are more sensitive to observed differences among sites in temperature or chlorophyll (based on DOE data).

To test hypothesis 2, we will relate the biomass of each of our target species to its growth and survival across sites, using multiple regression. This analysis will emphasize barnacles and *Fucus*, as oysters occur infrequently in quadrats. If growth is an effective predictor of biomass, then productivity is likely to drive community structure. If survival

is an effective predictor of biomass, then post-recruitment sensitivity to disturbance or predation is likely to drive community structure.

To test hypothesis 3, we will relate the biomass of each of our target species to its recruitment. If recruitment is an effective predictor of biomass, then propagule delivery is likely to drive community structure. More generally, however, we will relate diversity to recruitment rates, distinguishing epibiota from infauna. If recruitment is an effective predictor of diversity, then the estuarine gradient may be set up due to propagule delivery being higher in the north.

Hypothesis 4 will involve separate tests for epibiota and infauna. For epibiota, we will relate diversity to sediment scour, using regression with sites as samples. For infauna, we will test whether our different grain size treatments affect recruitment, using analysis of variance with site as a random factor.

### **Expected Results**

Our results will improve our understanding of changes occurring in the benthic habitats of this complex estuary by getting at *mechanisms* of change. Previous work has only been able to point to correlations (e.g. with changes in sand on the beach); real understanding requires experimental work. By examining this system at a variety of scales (from quadrats on one beach to along the whole gradient of Puget Sound) we can match the spatial scale of physical forcing functions (gradients in salinity, temperature, sand supply) to the scale of biological response. By matching the temporal scale of these forcing functions with short-term events such as recruitment or growth spurts, we can test what processes critically affect these key biological parameters. By manipulating variables such as amount of sand in the sediment, we can specifically test the importance of this controversial topic. We anticipate that our results will allow improvement of the monitoring efforts of various agencies by pinpointing processes critical to benthic communities in Puget Sound. Results may affect decisions made about adding fines to beaches (beach nourishment), and building seawalls, which result in the removal of fines, because we will have data on how these human activities affect beach biota, including 'desirable' species such as clams and oysters. Our extensive data on oyster growth rates in different regions and at different temperatures, and on the source of oyster food (as seen in stable isotope analyses) may affect decisions about regions for effective aquaculture (in terms of productivity). The isotope data may also indicate how much Puget Sound productivity is altered by leaky septic systems, as this 'signal' should appear as enriched  $\delta^{15}\text{N}$  in the oysters (Lake et al. 2001). Our data will also inform decisions about siting of marine protected areas, in terms of both identifying locations with high biodiversity and understanding processes that maintain this diversity.

### **Approximate Time Table**

Note: data entry, analyses of growth data, and identification of recruits will be done on an ongoing, month-by-month basis. Data will be downloaded from the temperature

dataloggers at each sampling interval to reduce the probability of losses of large datasets if the TidbiTs are lost.

## 2004

**Feb.-March:** Gather/purchase supplies, set up field logistics, contact landowners at the 9 chosen sites, prepare substrates with *Fucus* and barnacles.

**April:** Begin fieldwork at all 9 sites: Outplant *Fucus* and barnacles. Establish Tidbits.

**May:** Measure *Fucus* and barnacles. Set out painted rocks to measure scour. Settle oyster spat on tiles.

**June:** Set out first sets of oyster tiles at all 9 sites. Measure and photograph *Fucus* and barnacles. Full SCALE sampling (surface and infauna) at all 9 sites. Collect material at each site to measure biomasses; dry and weigh in lab.

**July:** Measure oysters on all tiles. Measure and photograph *Fucus* and barnacles. Settle oyster spat on second set of tiles.

**August:** Measure and photograph *Fucus* and barnacles. Measure oysters and collect tiles: replace with new set. Harvest first set of oysters for isotope analyses.

**September:** Measure and photograph *Fucus*, barnacles, and oysters.

**October:** Measure oysters.

**Nov-December:** Measure oysters, *Fucus*, barnacles. Prepare recruitment pots and settling tiles. Begin writing paper(s) using first year's data.

## 2005

**Jan.-Feb.:** Measure oysters. Set out first set of recruitment pots, settling tiles, and extra pots at north sites with sand manipulation.

**March-April:** Replace TidbiTs if necessary. Measure oysters. Outplant new *Fucus* and barnacle tiles. Replace settling tiles and recruitment pots.

**May:** Replace pots and tiles. Set out new painted rocks. Harvest overwintered oysters for isotope analyses. Settle oyster spat on new tiles.

**June:** Set out third sets of oyster tiles at all 9 sites. Measure and photograph *Fucus* and barnacles. Full SCALE sampling (surface and infauna) at all 9 sites. Collect material at each site to measure biomasses; dry and weigh in lab. Replace settling tiles and recruitment pots.

**July:** Measure oysters, *Fucus*, and barnacles. Replace settling tiles and recruitment pots.

**August:** Measure and photograph *Fucus* and barnacles. Measure oysters and collect tiles: replace with 4th set. Harvest third set of oysters for isotope analyses. Replace settling tiles and recruitment pots.

**September:** Measure oysters, *Fucus*, and barnacles.

**October:** Measure oysters. Replace settling tiles and recruitment pots.

**Nov.-Dec.:** Measure oysters. Replace settling tiles and recruitment pots. Write paper(s) summarizing growth data (primary and secondary productivity) and their relationships to temperature data and offshore DOE data including chlorophyll concentrations.

## 2006

**Jan.-Feb.:** Measure oysters. Replace settling tiles and recruitment pots. Set out extra pots (varying sand concentrations) at all sites.

**March-April:** Measure oysters, replace pots and tiles.

**May:** Measure oysters, replace pots and tiles.

**June:** Collect 4<sup>th</sup> set of oysters for isotope analyses. Replace pots and tiles.

**July:** Replace pots and tiles.

**August:** Replace pots and tiles.

**September:** Remove/'harvest' all experimental setups from beaches.

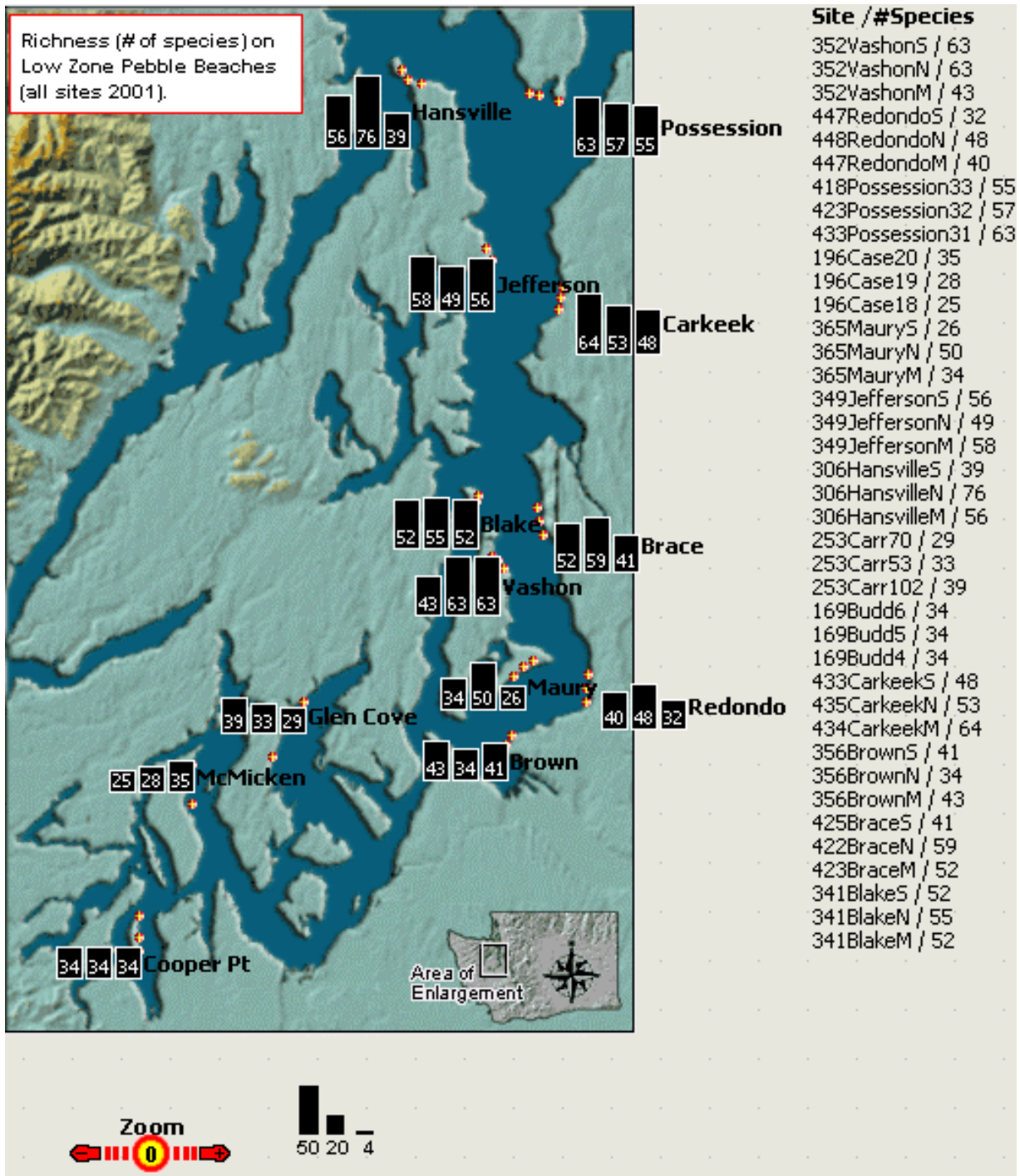
**Fall 2006:** Final analyses and writing. We anticipate writing at least 3 papers that will be submitted to peer-reviewed journals, and in addition will present results at local and national meetings beginning after year 1. Data will also be linked to the PIs' websites.

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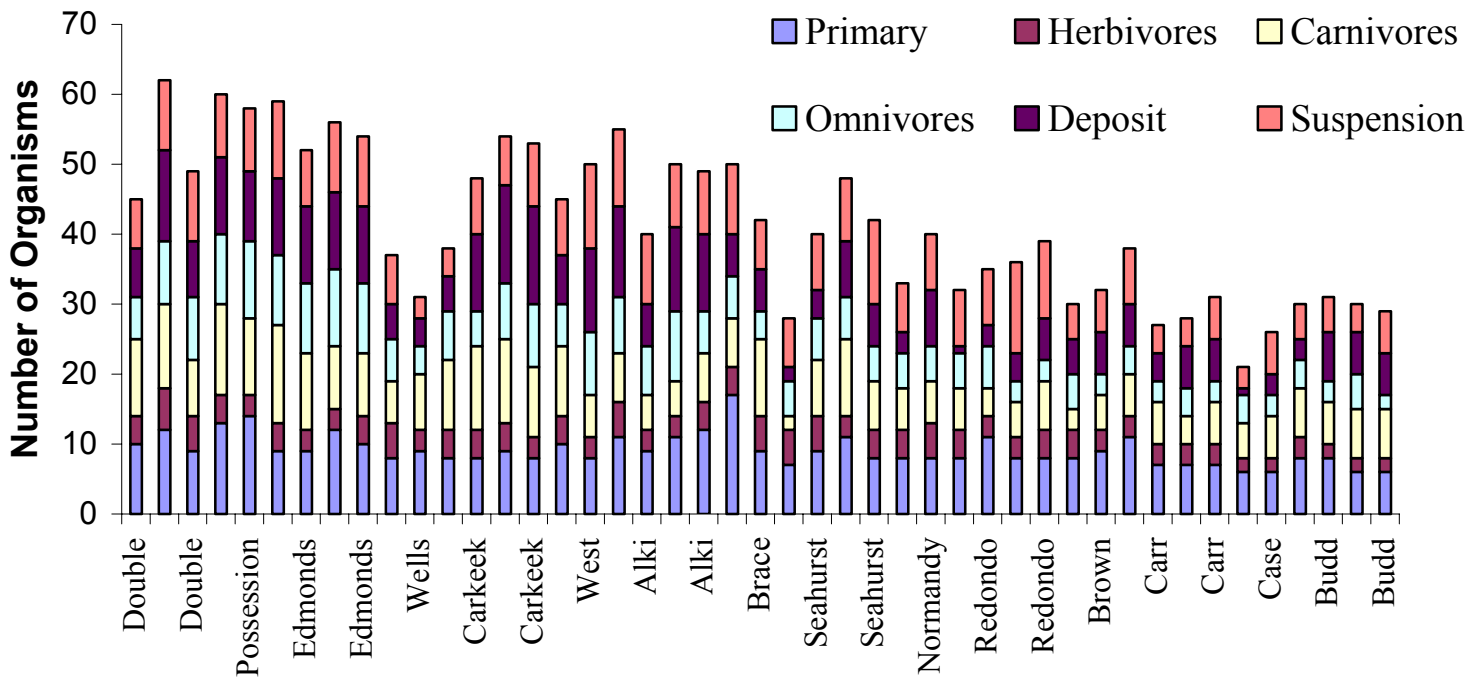
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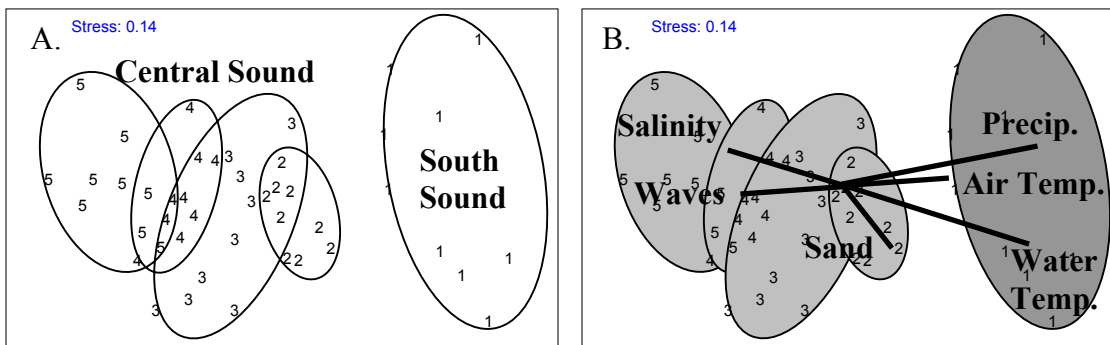


**Figure 1.** Species richness (surface biota and infauna combined) at all sites sampled in June 2001. Each datum is the cumulative richness among the 10 samples per site. Similar patterns can be seen in data from 1999 and 2000, and when the data are broken down into surface vs. infaunal species. Other data from this long-term database can be viewed at: [www2.wadnr.gov/nearshore/scale/index.asp](http://www2.wadnr.gov/nearshore/scale/index.asp).

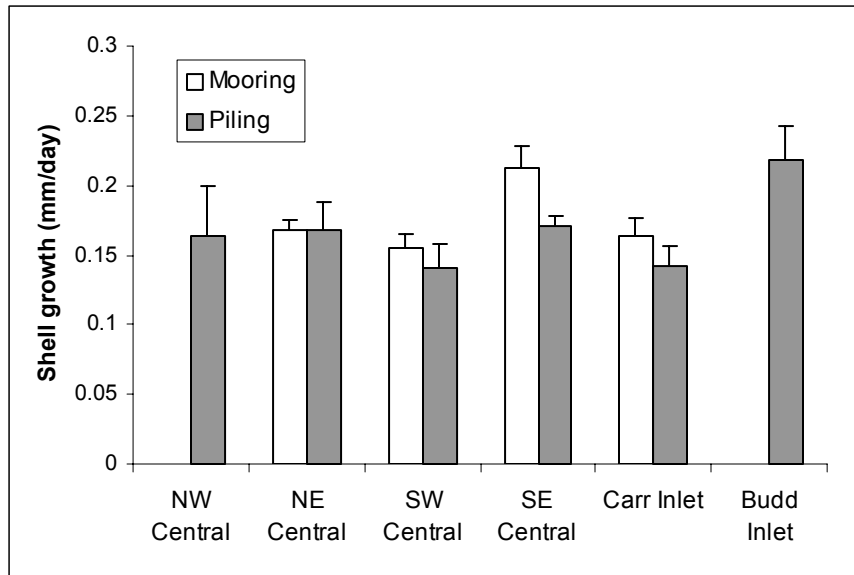




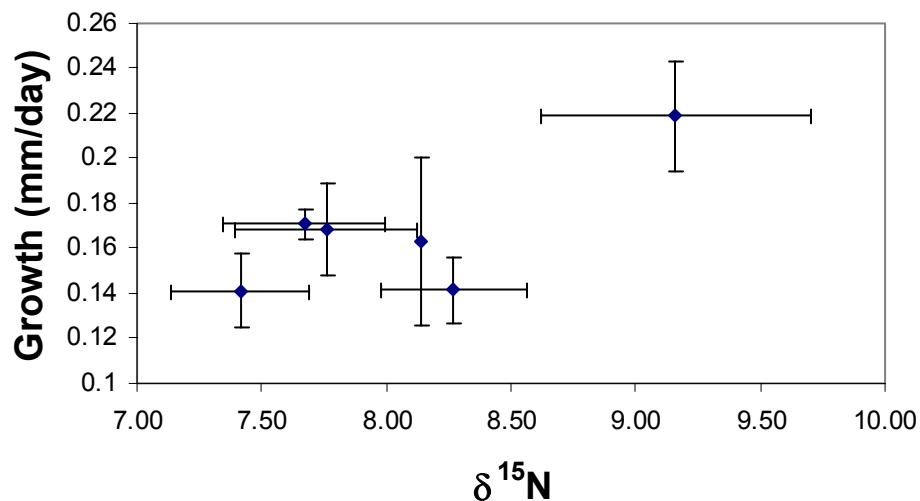
**Figure 2.** North-south gradient in species richness broken down by trophic level.



**Figure 3.** Results of a non-metric multidimensional scaling ordination (final stress = 0.14) of the communities in pebble beach sites. Each point represents the community at one beach; closer points have more similar communities (species and abundances). (A) shows the clear separation of central vs. south-sound sites; the ovals around the central sites represent different quadrants (northeast, northwest, etc.). (B) shows the vectors best representing scaled Spearman-rank correlations to the community patterns.



**Figure 4.** Daily growth (mm shell length/day) of juvenile oysters (*Crassostrea gigas*) transplanted to six sites in Puget Sound, July-September 2002. Bars are means ( $\pm$  SE) of three tiles permanently submerged (moorings) or placed at mean lower low water (pilings). Moorings at two sites disappeared.



**Figure 5.** Daily growth (mm shell length/day) and stable isotope ratios of juvenile oysters (*Crassostrea gigas*) transplanted to six sites in Puget Sound, July-September 2002. Oysters on ceramic tiles were attached to pilings at mean lower low water. Bars are means ( $\pm$ SE) of  $\leq 3$  tiles. Budd Inlet is the site in the upper right hand corner, enriched in  $^{15}\text{N}$  and with relatively high secondary productivity.