Species and genetic diversity of benthic diatom communities along a salinity gradient

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Project Summary

Benthic diatoms are important primary producers in shallow aquatic environments. Serving as a food source for heterotrophic protozoans and larger invertebrate grazers and important players in carbon cycling, benthic diatoms are integral to the health of the shallow benthic layer. Species diversity of benthic diatom communities has been investigated in varying environments. Several variables, including sediment type and salinity, can affect the distribution and diversity of benthic diatom communities. Salinity has been observed to have an inverse relationship with benthic diatom community diversity. In this study the effects of salinity on species diversity will be examined in Big Beef Creek, an estuary in Hood Canal, Puget Sound, Washington. An important component of diversity that has not been investigated with benthic diatom communities in combination with physical parameters is genetic diversity. Three sets of samples will be taken from various locations within the Big Beef Creek estuary: one set of sediment samples for pore water salinity analysis, one for microscopic species counts, and one for molecular analysis of the 18S ribosomal ribonucleic acid, rRNA, gene. Anticipated findings will confirm prior observations of the relationship between salinity and benthic diatom community diversity. These results will add to the relatively small amount known about benthic diatoms in estuaries and what influences their distributions.

Introduction

Benthic diatoms are main primary producers that live on the surface and in the upper layer of the sediments in aquatic environments. Microscopic in size, they are ubiquitous in bodies of water in which the euphotic zone reaches the sediments. Benthic diatom communities tend to be very diverse, containing many of the more than 12,000 described diatom species (Graham and Wilcox 2000). Along with cyanobacteria, benthic diatoms comprise the primary producer niche of benthic environments. They are a key food source for other benthic organisms. Each species of benthic diatom has a specific distribution range and tolerance for environmental variables.

Within an estuary, a variety of physical and chemical factors can influence the range of specific benthic diatom species. Certain sediment grain sizes, such as sands and muds, effect where particular species are found (Amspoker and McIntire 1978, 1986). Nutrient availability, for example nitrogen concentrations along an eutrophication gradient, can enable some species of benthic diatoms, such as *Nitzschia sigma*, to flourish where others may be absent (Underwood et al 1998, Agatz et al 1999). The occurrence of inorganic compounds like sulphides can also influence benthic diatom species distribution. *Nitzschia* sp. has been experimentally shown to have a low tolerance for sulphides in their environment, whereas *Navicula* sp. has a relatively high tolerance (Admiraal and Peletier 1979). Another chemical variable that can affect benthic diatom community structure is the salinity of pore water and the overlaying water column (Amspoker and McIntire 1978, McIntire 1978, Oppenheim 1988, Underwood et al 1998). Some benthic diatoms are predominantly marine or freshwater species, but others are halotolerant, *Navicula* sp. and *Nitzschia* sp., and can thrive in oligohaline environments like an estuary (Amspoker and MacIntire 1986). Many species can be expected to be observed in similar habitats in temperate estuaries, so several of the species previously observed in estuaries may be found in this study as well (Whiting and McIntire 1985).

Increasing salinity has been observed to have an inverse relationship with benthic diatom diversity (figure 1, Herbst and Blinn 1998). An inverse relationship between the number of benthic diatom taxa and salinity has also been observed (figure 2). Studies on the effects of salinity on benthic diatoms have been conducted primarily in fresh, saline, and alkaline lakes, as well as in a laboratory; marine or estuarine studies are rarer. Blinn conducted a significant study on salinity and benthic diatoms in saline lakes in western North America. Many of these lakes had ionic compositions similar to seawater. The most prominent diatoms found in his study were from the families Naviculaceae, Nitzsciaceae, Fragilariaceae, and Cymbellaceae, many of which are likely to be found in varying environments. In this study, the effects of salinity on benthic diatom community diversity in an estuary, rather than a saline lake, will be investigated in Big Beef Creek, Hood Canal, Washington, in an attempt to confirm the observed inverse relationship in another aquatic environment (figure 3). An important component of diversity that has not been readily studied is the genetic diversity of a benthic diatom community. While studies on pelagic diatoms have encompassed many applications of molecular biology, previous molecular work on benthic diatoms has been primarily for the purpose of reclassification (Rynearson, and Armbrust 2000, Beszteri et al 2001). The ecology of benthic diatoms is less well understood than their pelagic counterparts, and this study will add to the knowledge of benthic diatoms and their interactions with the chemical variables in their environment.

Proposed Research

Field component

Presampling- Sampling at Big Beef Creek, 47°38'27"N 122 °47'02"W, Hood Canal, Washington, will be done 18-19 March 2004 (fig. 2). In the event of adverse weather conditions, sampling dates may be adjusted accordingly. PAR, photosynthetically active radiation, levels will be detected from a canoe at five points in the estuary using a submergible PAR sensor on 18 March 2004 within two hours of low-high tide, 02:37PM local standard time, LST (table 1). These measurements will confirm sampling areas in which PAR level is constant, ensuring the elimination of available light as a contributing variable in benthic diatom distribution. The longitude and latitude of areas of constant PAR level will be recorded for sampling the following day.

Sampling- Sampling will occur at the previously recorded locations within a four hour window containing the high-low tide, 10:18AM LST, on 19 March 2004 (table 1). Samples will be taken in order from the creek, the areas which have been exposed for the longest time, moving progressively toward Hood Canal where the mudflat hasn't been exposed as long. Samples will be taken on foot at stations within the estuary using 10 cubic centimeter plastic syringes to obtain the top 1 cm of the sediment. Triplicate samples will be taken at each location for microscopy analysis and preserved immediately with 2% formaldehyde to preserve cell morphology and cease predation by other benthic microorganisms within the sample. A second set of triplicate samples of sediment will be taken in 15 ml centrifuge tubes to be used later for pore water salinity analysis. Additional triplicate samples will be taken from each location in 20 ml syringes to be reserved for molecular analysis in the lab. All samples will be frozen in the dark until analysis at the University of Washington.

Laboratory component

Pore water salinity analysis. Samples reserved for pore water salinity analysis will be centrifuged for 3 minutes to separate sediment and interstitial fluid in a procedure described by Underwood et al. The resulting supernate will be tested for salinity using a hand spectral refractometer.

Species identification. Analyses of samples for species diversity will be analyzed within a few days of collection in the Marine Molecular Biotechnology Laboratory (MMBL), University of Washington. Samples preserved in formaldehyde will be centrifuged for 3 minutes to resuspend benthic diatoms from the sediment into the fluid. Samples may also be treated with the addition of hydrogen peroxide to release diatom frustules from their attachment to sediment particles (Facca et al 2002). Microscopic diatom species counts will then be done using a compound light microscope. These counts will further be analyzed to yield Shannon-Wiener diversity indexes for each site sampled (Siqueiros-Beltrones 1990).

Molecular techniques. Molecular techniques will be applied to samples from one location to gain insight into the diversity of the community by examining the 18S rRNA genes. Analyses will occur in the MMBL. These analyses do not need to be completed shortly after collection as samples will be frozen and can remain viable for several months. All nucleic acids will be extracted from the frozen samples using the Qiagen DNEasy plant kit. Diatom-specific primers will then be employed in a PCR reaction to amplify only diatom 18S rRNA genes. The resulting DNA will be cloned and sequenced. The goal of ten sequences of a portion of the 18S rRNA gene has been set to determine the level of diversity within the community. Time and budget allowing, samples from a second site will be analyzed via the same methods for a comparison of benthic diatom species diversity from locations of different salinities.

Proposed budget

Equipment/Supplies	Origin	Total Cost	Effective Cost
Platform			
No platform	n/a	$0.00	$0.00
Sampling Equipment/Supplies			
Ice chest	Personal	$0.00	$0.00
Handheld GPS	Miles Logsdon	?	$0.00
PAR Sensor	Miles Logsdon	?	$0.00
Plastic 10 cc syringes, 100/box	UW Stores	$10.91	$10.91
Plastic 15 cc Falcon centrifuge tubes	Kathy Newell	?	$0.00
Formaldehyde, 500 ml	Biochemistry Stores	$7.49	$7.49
Laboratory Equipment/Supplies			
Hand spectral refractometer	Marine Chemistry Lab	?	$0.00
Centrifuge	MMBL	?	$0.00
Bench Fee, MMBL	n/a	$218.85	$0.00
DNEasy plant kit, extraction	Qiagen	$2.92	$2.92
Promega Taq	MMBL	$107.70	$107.70
Custom primer, $0.35/bp		$14.00	$14.00
dNTPs	MMBL	$92.00	$0.00
TOPO TA Cloning Kit, 20 reactions		$374.08	$18.70
QIAprep Spin Miniprep, 50 reactions		$65.00	$19.50
Sequencing mix, 20 reactions	MMBL	$125.00	$10.00
Loading solution	MMBL	$3.40	$0.00
Megabace fee, per sample		$29.20	$29.20
Slides, box	UW Stores	$8.07	$8.07
Cover slips, 100	UW Stores	$7.67	$7.67
P1000 Disposable pipette tips, box	MMBL	$8.00	$8.00
P20 Disposable pipette tips, box	MMBL	$6.31	$6.31
Total		**$1080.60**	**$250.47**

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Table 1. Tidal heights at Big Beef Creek, Hood Canal, Washington, 18-19 March 2004.

Tides	18 Mar 2004	19 Mar 2004
Higher high	03:51AM LST 12.0 H	04:23AM LST 12.1 H
Higher low	09:39AM LST 5.9 L	10:18AM LST 4.8 L
Lower high	02:37PM LST 9.8 H	03:39PM LST 10.1 H
Lower low	09:24PM LST -0.1 L	10:08PM LST 0.5 L

Figure Captions

Figure 1. Observed relationship between salinity and benthic diatom species diversity in saline lakes in western United States and southwestern Canada (Blinn 1993).

Figure 2. Observed relationship between number of benthic diatom taxa present in saline lakes in western United States and southwestern Canada and salinity (Blinn 1993).

Figure 3. Graphical representation of Big Beef Creek, Hood Canal, Washington, 47°38'27"N 122 °47'02"W.

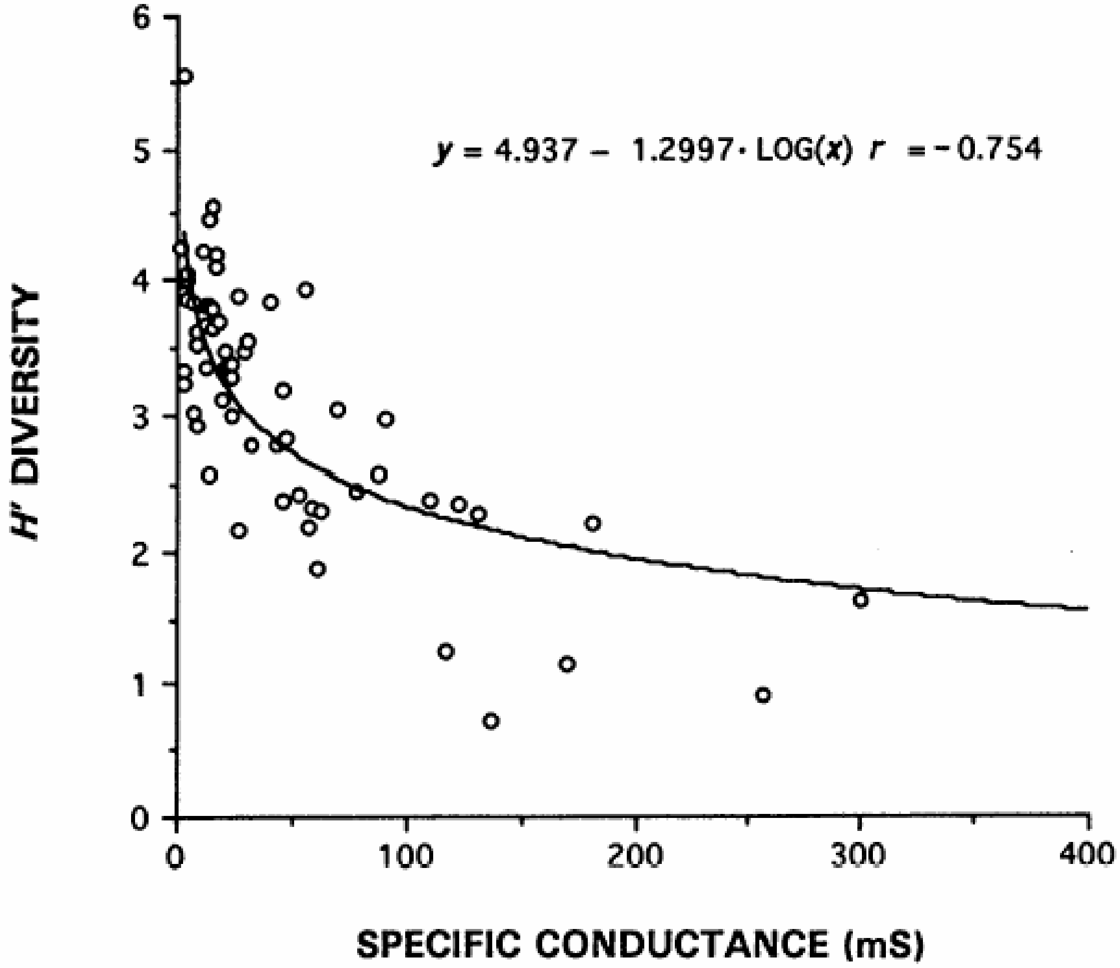

FIG. 4. Relationship between specific conductance and diatom H' diversity for 63 saline lakes in western North America.

Figure 1.

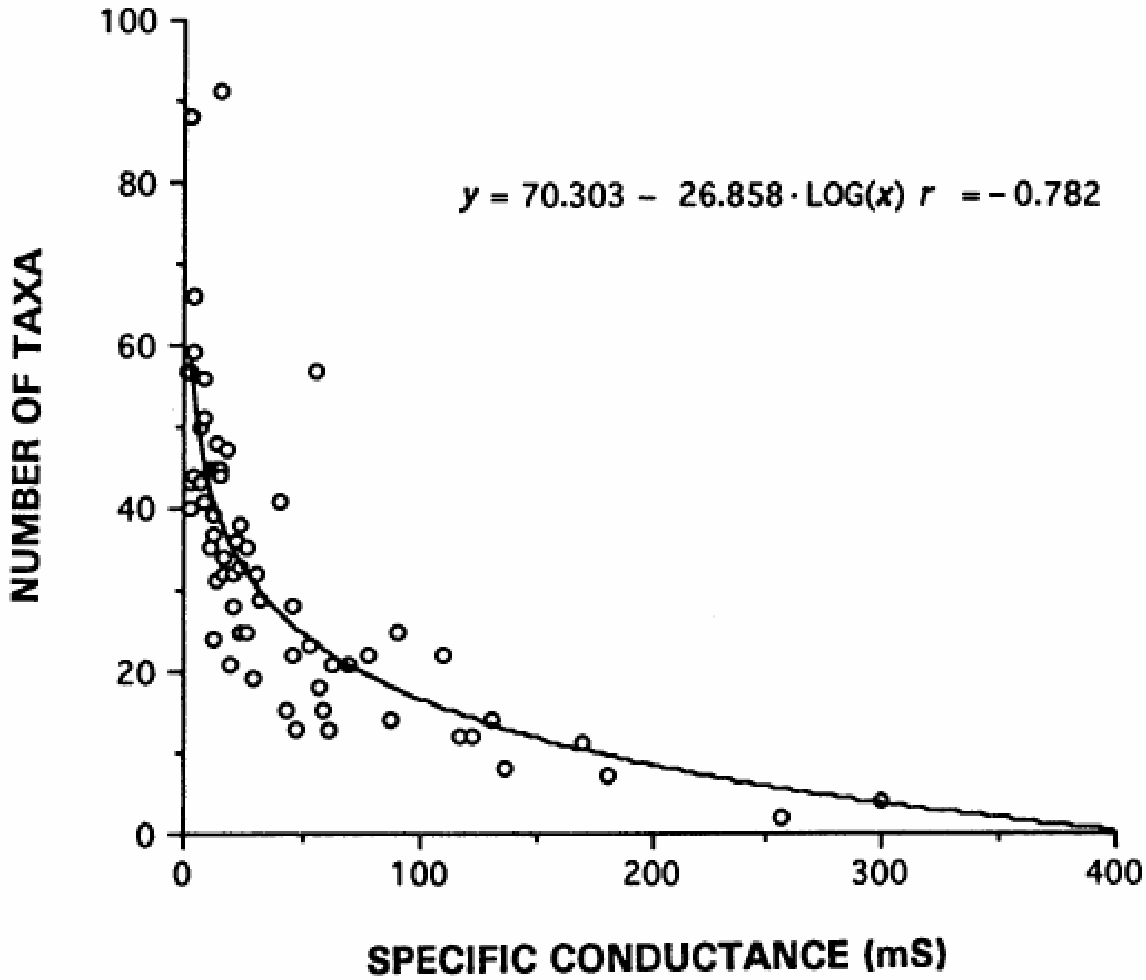

FIG. 5. Relationship between specific conductance and number of diatom taxa for 63 saline lakes in western North America.

Figure 2.

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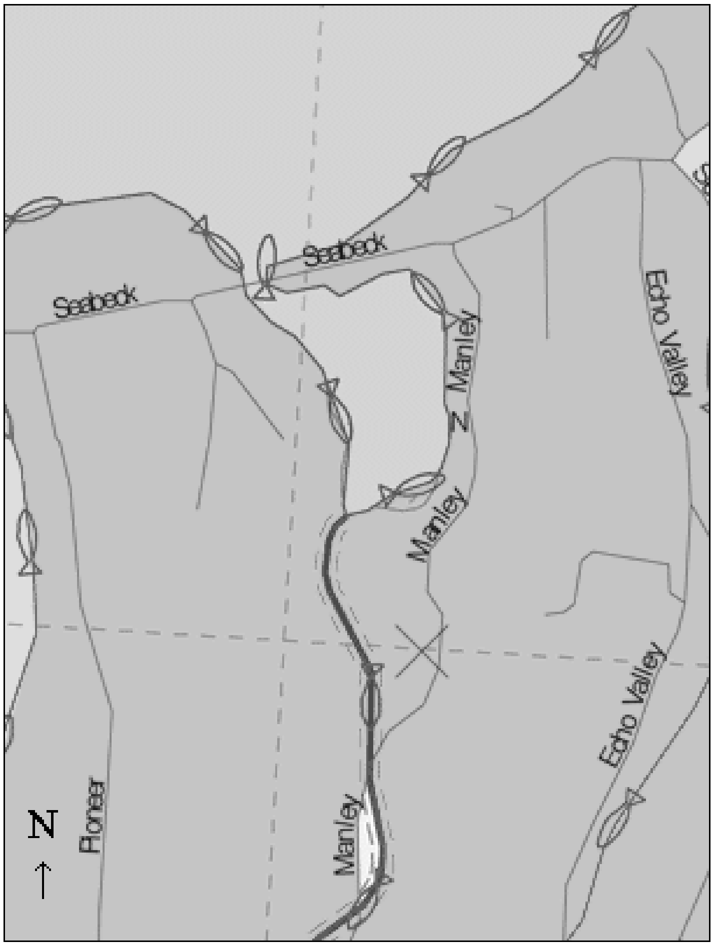
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Figure 3.