

## Diatom assemblages and their associations with environmental variables in Oregon Coast Range streams, USA

Christine L. Weilhoefer\* & Yangdong Pan

*Environmental Sciences and Resources, Portland State University, Portland, Oregon, USA*

(\*Author for correspondence: E-mail: clw@pdx.edu)

**Key words:** periphyton, nonmetric multidimensional scaling (NMDS), cluster analysis, TWINSpan, bioassessment, Oregon Coast Range

### Abstract

The Oregon Coast Range, rich in natural resources, is under increasing pressure from rapid development. The purpose of this study was to examine diatom species patterns in relation to environmental variables in streams of this region. Diatoms, water quality, physical habitat and watershed characteristics were assessed for 33 randomly selected stream sites. Watershed size, elevation, geology, vegetation and stream morphology varied substantially among sites. Streams were characterized by dilute water chemistry and a low percent of fine substrate. A total of 80 diatom taxa were identified. Taxa richness was low throughout the region (median 15, range 10–26). Assemblages were dominated by two adnate species, *Achnantheidium minutissimum* and *Achnanthes pyrenaicum*. Diatoms sensitive to organic pollution dominated the assemblages at all sites (median 85%). Non-metric multidimensional scaling (NMDS) and correlational analysis showed quantitative relationships between diatom assemblages and environmental variables. NMDS axes were significantly correlated with watershed area, watershed geology, conductivity, total nitrogen, total solids and stream width. Diatom-based site classification (Two-way Indicators Species Analysis, (TWINSpan)) yielded 4 discrete groups that displayed weak correlations with environmental variables. When stream sites were classified by dominant watershed geology, overall diatom assemblages between groups were significantly different (Analysis of Similarity (ANOSIM) global  $R = 0.19$ ,  $p < 0.05$ ). Our results suggest that streams in the coastal region are in relatively good condition. High natural variability in stream conditions in the Oregon Coast Range ecoregion may obscure quantitative relationships between environmental variables and diatom assemblages. A bioassessment protocol that classifies sites by major landscape variables and selects streams along the major human disturbance gradient might allow for detection of early signs of human disturbance in environmentally heterogeneous regions, such as the Pacific Northwest.

### Introduction

Streams are a distinctive feature in the Oregon Coast Range ecoregion, with a typical density of 1–2 km of perennial streams per square km in the mountains (Omernik & Gallant, 1986). The declining status of stream biota, particularly salmonid fish, has been of interest in recent years,

resulting in the implementation of several federal, state and local programs to assess the status of these streams (Hansmann & Phinney, 1973; Fore et al., 1996; Ford & Rose, 2000). One aspect of many stream assessment plans is the use of aquatic biota as indicators of stream conditions. In Oregon, stream bioassessment is commonly performed using macroinvertebrate assemblages and fish, but

the utility of stream periphyton assemblages is only beginning to be explored (e.g., Carpenter & Waite, 2000).

Periphyton assemblages have frequently been used in stream bioassessment because they respond rapidly to changes in stream conditions (Leland, 1995; Pan et al., 1996; 2000). Streams in the Coast Range ecoregion have naturally dilute water chemistry, with low alkalinity and nutrient levels, due to regional bedrock (Welch et al., 1998). Consequently, their periphyton may be highly sensitive to anthropogenic disturbances and serve as a good bioindicator of stream conditions. For example, periphyton respond positively to nutrient enrichment in mountain streams of British Columbia (Perrin et al., 1987).

The major anthropogenic disturbances in the Coast Range are activities associated with forest management practices (McClain et al., 1998). However, the watersheds vary widely in elevation, size, geology and vegetation cover, leading to a high natural variability in water chemistry and physical habitat conditions. This high natural variability in stream conditions may interfere with the use of diatoms in bioassessment by obscuring relationships between pollution or habitat alterations and species assemblages. The main objective of this study was to characterize diatom species composition in streams of the Oregon Coast Range ecoregion. The diatom assemblages of these streams were then related to environmental variables, including water chemistry, physical habitat, watershed vegetation and watershed geology, in an attempt to explain the observed patterns. A better understanding of the role of natural landscape variability in influencing diatom assemblages may improve the utility of diatom-based bioassessment in the region.

## Methods

### *Study area and site selection*

The Oregon Coast Range ecoregion (Level III Ecoregion, Omernik & Gallant, 1986) extends along the coast from the Pacific Ocean to the Willamette Valley on the east side of the Coast Range and is characterized by rugged mountains inland and flat plains along the coast. Average

annual precipitation ranges between 140 and 320 cm (Wigington et al., 1998), the heaviest typically occurring from October through March. The natural vegetation of the area is a mixture of Sitka spruce (*Picea sitchensis* Carr.), occurring below 150 m in elevation, and western hemlock (*Tsuga heterophylla* Sargent), occurring at higher elevations (Frenkel, 1993). The bedrock geology of the study area is a mixture of marine tuffaceous sandstones and shales and basaltic volcanic rocks with unrelated intrusives (Rosenfeld, 1993). Land use/land cover in the area is predominately forested in the uplands, much of which is managed commercially for timber harvest, and a mixture of dairy farmland, cropland and orchards in the lowlands. Much of the population residing in the Coast Range is confined to small towns and a few larger urban areas on the coast.

Data were collected as part of Oregon DEQ's Oregon Plan for Salmon and Watersheds. A stratified, random probability design was used to select 1st–3rd order, wadeable streams throughout Oregon (Herlihy et al., 2000). For this study, the 35 sites that fell within the range of the Coastal Landscape Analysis and Modeling Study (CLAMS; Oregon State University College of Forestry, U.S. Forest Service and Oregon Department of Forestry joint study) were utilized. These sites were selected so that watershed geology and land use coverages for a geographic information system (GIS) could be generated. After preliminary analysis of diatom species, two sites were excluded from the study because their assemblages were dominated by one species, *Achnanthes oblongella* Oestrup, which was not present at any of the other sites ( $n = 33$ ; Figure 1). Nine sites were designated as “reference sites” a priori by Oregon DEQ. Reference sites were defined as streams with minimally impacted watersheds (Mrazik, 1999). Criteria used to select reference sites included professional opinion and the percentage of roads in the stream's watershed.

### *Field sampling*

All sites were sampled once for biological, chemical and physical variables during June through September of 1999. The sampling unit was the stream reach, defined as 40 times the average wetted width measured at 3 locations within 10 m



Figure 1. Sampling locations and streams (5th order or higher) in the Oregon Coast Range ecoregion.

of the stream coordinates selected by the random probability design. The reach was divided into 11 equally spaced transects parallel to the ends of the reach, including 1 at either end of the reach. Stream physical habitat characterization included channel morphology, substrate composition, riparian area condition and discharge. Thalweg depth and wetted width were measured at each transect and the mean of these values was used to represent the depth and width of the reach. Instantaneous stream discharge was measured using a flow meter (Marsh-McBirney “Flo-mate 2000”). Fine substrate (<2 mm) at each site was assessed by placing a grid on the streambed at 2

random locations and counting the number of intersections covered by fine sediment particles. Qualitative assessment of riparian and stream conditions were made according to the EPA’s Rapid Bioassessment Protocol (Barbour et al., 1999).

Within each reach, conductivity, dissolved oxygen and stream temperature were measured with a YSI Model 85 m. Turbidity was measured using a HACH Model 2100P Turbidimeter and pH was measured using an Orion Model 210A meter. Water samples were collected in the middle of the stream for nutrient analyses. Two water samples were taken at each site, 1 filtered on site

(47 mm Millipore<sup>®</sup> type HA filters, 0.45  $\mu\text{m}$  pore size), and the other left unfiltered and stored on ice until returned to the laboratory for nutrient analysis. Water samples were analyzed for concentrations of total and dissolved nutrients according to standard EPA methods (Clesceri et al., 1998).

Periphyton were sampled by randomly selecting 10 cobbles within riffle habitats of the stream sampling reach. A known area (7.1  $\text{cm}^2$ ) of each rock was scraped using a toothbrush and delimiter and combined into 1 composite sample per site. The composite sample was homogenized and split into 3 subsamples for identification and enumeration of diatom species and other assays. Samples for diatom identification and enumeration were preserved with formalin for a final formalin concentration of 4%.

#### *Sample and data analysis*

For diatom slide preparation, samples were rinsed repeatedly with deionized water to remove excess formalin. Samples were homogenized and digested using concentrated sulfuric acid and potassium dichromate for 12 h. Samples were rinsed repeatedly with deionized water until the pH was approximately neutral and then mounted on slides with Naphrax<sup>®</sup>, a high resolution mounting medium. Slides were scanned using transects until at least 500 diatom valves were identified and enumerated to the species level using a Nikon Eclipse E600 microscope at 1000 $\times$  magnification. The primary references for diatom taxonomy were Krammer & Lange-Bertalot (1986; 1988; 1991a; 1991b; 2000) and Patrick & Reimer (1966, 1975). Diatom autecological indices were calculated for each site based on the pollution tolerance values of Lange-Bertalot (1979) and Bahls (1993).

Geologic categories and vegetative cover within the watershed upstream of each sample reach were quantified using a GIS with ArcInfo version 8 and ArcView version 3.2 software. The spatial analyst extension of ArcInfo was used to delineate the watershed upstream from each sampling reach. Ten-meter digital elevation models (10 m resolution USGS; CLAMS, 1996) were used and sampling site coordinates were used as the outlet point for each watershed. Vegetation within each watershed was quantified from the 1996 Gradient

Nearest Neighbor Vegetation Class developed by CLAMS (25 m grid, TM satellite imagery and plot data; CLAMS, 1996). Vegetation data were grouped into three major forest classes: broadleaf, conifer forest and mixed forest (broadleaf and conifer). Major lithologic categories within each watershed were quantified from the 1991 Geologic Map of Oregon (1:500,000 coverage; Walker & MacLeod, 1991) using overlay analysis in ArcInfo. Geologic types were grouped into major lithologic categories (i.e., volcanics, sedimentary, alluvium) using guidelines in Johnson & Raines (1995).

Diatom assemblages were represented as the proportion of total species at each site (relative abundances). In an effort to reduce the influence of "rare" species in the data set on the data analyses, only species occurring with greater than 1% relative abundance at three or more sites were included in data analyses ( $n = 28$ ). Stream sites were ordered based on diatom species composition using non-metric multidimensional scaling techniques (NMDS). NMDS was performed using PC-ORD v.4 (Bray-Curtis distance measure, 40 real runs and 400 maximum iterations). The Monte-Carlo permutation procedure was used to determine if the axes extracted by NMDS explained more variation than by chance alone (PC-ORD; 50 randomized runs). An Analysis of Similarity (ANOSIM, PRIMER-4, Bray-Curtis distance measure, 999 permutations; Clarke & Green, 1988) was used to test differences in diatom species composition between reference and randomly selected sites.

Measured environmental variables were correlated to the axes of the NMDS to identify a subset of environmental variables that co-vary with changes in diatom species composition among streams. Environmental variables that were not normally distributed were  $\log_{10}$  or arcsine-square root transformed (proportional data) to produce near-normal distributions (Zar, 1999). Relations of environmental variables to diatom species composition were examined by correlating variables with NMDS axes scores. To examine the effects of classifying sites based on watershed geology on the relationships between diatom assemblages and environmental variables, sites were divided into 2 groups based on their dominant watershed geology. The watersheds of 21 sites had greater than 50% sedimentary rocks and these

sites were considered to have sedimentary rock dominated watersheds for analysis while the remaining 12 sites were considered to have non-sedimentary dominated watersheds. Rock types other than sedimentary and volcanic (primarily alluvial/unconsolidated material) were found at 6 sites, with the relative abundance ranging from <1 to 9%. To simplify data analysis, these rock types were grouped with volcanic-dominated sites. Average % sedimentary rock in the sedimentary-dominated group was 91% while average % volcanic rock in the volcanic-dominated group was 78%. The % sedimentary rock and % volcanic/other rock varied significantly between the two groups (ANOVA  $p < 0.001$ , in both cases). An ANOSIM was used to detect difference in diatom assemblages between the 2 geology-based groups (ANOSIM, PRIMER-4, Bray–Curtis distance measure, 999 permutations). Differences in environmental variables and relative abundances of dominant diatom species between geology-based site groups were examined with  $t$ -tests.

Two-way indicator species analysis (TWINSPAN, PC-ORD), a divisive cluster method using a top-down approach, was used to classify stream sites into several groups based on diatom relative abundance data (Hill et al., 1975). To determine the number of TWINSPAN groups to retain, differences in species composition between the groups were tested using ANOSIM (PRIMER-4; Bray–Curtis similarity measure, 999 permutations). A separate ANOSIM was performed on the groups created by the first 4 TWINSPAN breaks. Groups were retained if the probability of sites being members of a TWINSPAN specified group was greater than the probability of sites being members of randomly created groups. A set of indicator species for each of the retained TWINSPAN groups was characterized using Indicator Species Analysis using PC-ORD (Dufrene & Legendre, 1997). Statistical significance of each species indicator value was tested using a Monte-Carlo permutation test (999 permutations,  $p < 0.05$ ). Differences in environmental variables and diatom indices between TWINSPAN groups were detected using an Analysis of Variance (ANOVA, SigmaStat v. 1.00, Jandel Scientific). Multiple comparisons between TWINSPAN groups were performed with a Student–Newman–Keuls test using a Bonferroni-corrected alpha.

## Results

### *Environmental factors*

Watershed area and sampling point elevation varied widely among sites. Watershed area ranged between 1 and 169 km<sup>2</sup> (median = 15 km<sup>2</sup>; Table 1). Elevation ranged between 3 and 707 m (median = 171 m). Sedimentary rocks were present in the watersheds of all but 3 sites and 13 watersheds were composed entirely of sedimentary rock. Rock of volcanic origin was present in the watersheds of 18 sites and only 3 watersheds were composed entirely of volcanic rocks. Sites with volcanic-dominated watersheds tended to be in the northern part of the Coast Range ecoregion. Conifer forest was the most common vegetation type within the watersheds (median coverage = 63%; Table 1). Mixed forest covered between 1 and 36% of the watersheds, while coverage by broadleaf vegetation ranged between 0 and 25%. Spatial vegetation coverages were similar between the sedimentary and volcanic-dominated watersheds, however, the age structure for conifer forest is unknown. Correlations between watershed vegetation type (conifer, mixed and broadleaf forest) and water quality, physical habitat and watershed geology variables were weak overall ( $|r| < 0.5$ ).

Stream morphology varied substantially among sites. Mean bankful width varied between 3 and 52 m, with a median of 9 m. Mean thalweg depth ranged between 7 and 37 cm. Channel slope was low overall all, but varied between 0 and 29% of mean reach length (Table 1). Riparian canopy cover ranged between 13 and 88%. In general, the percent of fine substrate was low at most sites (median 8%); only 2 sites had greater than 50% percent fine substrate. Percent of fine substrate was the only environmental variable that was significantly different between sedimentary- and volcanic-dominated watersheds (median = 11 c.f. 2%;  $p = 0.003$ ).

The streams in the study had low stream ionic strength and nutrient levels (Table 1). Median total nitrogen (TN) concentration was 0.4 mg l<sup>-1</sup> and median dissolved inorganic nitrogen (DIN) was 0.2 mg l<sup>-1</sup>. Both total phosphorus (TP) and soluble reactive phosphorus (SRP) concentrations ranged from below detection limits (0.01 mg l<sup>-1</sup>)

Table 1. Minimum, median and maximum values for landscape, water chemistry and physical habitat variables for Oregon Coast Range streams ( $n=33$ )

	Minimum	Median	Maximum
Watershed area (km <sup>2</sup> )	1	15	169
Elevation (m)	3	171	707
Sedimentary rock in watershed (%)	0	75	100
Volcanic rock in watershed (%)	0	25	100
Conifer forest in watershed (%)	23	63	98
Broadleaf forest in watershed (%)	0	9	25
Mixed forest in watershed (%)	1	12	36
Alkalinity (mg CaCO <sub>3</sub> /l)	6	17	45
Conductivity ( $\mu$ S/cm)	38	66	146
Total Nitrogen (mg/l)	0.2	0.4	0.8
Nitrate + Nitrite (mg/l)	0.0	0.2	0.6
Total Phosphorus (mg/l)	0.01	0.02	0.10
Soluble Reactive Phosphorus (mg/l)	0.01	0.01	0.02
Total Solids (mg/l)	41	56	93
Bankful width (m)	3	9	52
Thalweg depth (cm)	7	15	37
Slope (% of mean reach)	0	2	29
Fine substrate (%)	0	8	69
Riparian canopy coverage (%)	13	51	88

to 0.1 mg l<sup>-1</sup> (TP) and 0.02 mg l<sup>-1</sup> (SRP). Alkalinity, conductivity and total solids varied throughout the study area, but were low overall. Alkalinity ranged between 6 and 45 mg CaCO<sub>3</sub> l<sup>-1</sup>, conductivity ranged between 38 and 146  $\mu$ S cm<sup>-1</sup> and total solids ranged between 41 and 93 mg l<sup>-1</sup>. Conductivity, alkalinity and total solids were positively correlated for all sites ( $r > 0.6$  for all).

#### Diatom assemblages

A total of 80 diatom species, from 22 different genera, were identified from the 33 sites. *Nitzschia*, *Navicula* and *Achnanthes/Achnantheidium* were the most common genera with 13, 10 and 9 species, respectively. Overall species richness was low, ranging between 10 and 26 species per site (median = 15; Table 2). Shannon diversity index values ranged from 0.9–2.4 (median = 1.8; Table 2). Over 50% of the total diatoms counted were from the genus *Achnanthes/Achnantheidium*. *Achnantheidium minutissimum* (Kütz.) Czarnecki was present at all sites and was the most abundant species throughout the study area (median relative abun-

dance = 19%, range = 3–64%; Table 2). *Achnanthes pyrenaicum* (Hustedt) Kobayasi was also common. Diatoms sensitive to organic pollution dominated the assemblages at all sites (median = 85%). Overall siltation index was low; however, there were a few sites with high siltation index values (median = 8%, range = 0–82).

A three-dimensional solution was obtained for the NMDS ordination that explained 87% of the variance in the diatom distance matrix (Figure 2). Seventy percent of the variance was partitioned between the second ( $r^2 = 0.28$ ) and third ( $r^2 = 0.42$ ) axes. Axis 1 explained 17% of the variance in the diatom data. The axes explained significantly more variance than would be expected by chance based on Monte-Carlo permutation tests ( $p = 0.03$ ). Axis 3 was primarily driven by relative abundance of *A. pyrenaicum* ( $r = -0.69$ ; Table 3) and secondarily by *N. inconspicua* Grunow ( $r = 0.55$ ) and *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot ( $r = 0.61$ ). Axis 2 was driven primarily by the relative abundances of *A. minutissimum* ( $r = 0.79$ ) and *Cocconeis placentula* Ehrenberg ( $r = -0.56$ ). Axis 1 was driven by the relative abundance of *Cocconeis*

Table 2. Relative abundance of the most common diatom species and diatom autecological indices values (median and ranges) for all sites and sites grouped by dominant watershed geology. Bold values indicate significant differences between the two watershed geology groupings. Pollution sensitivity values are from Lange-Bertalot (1979). Siltation index values are from Bahls (1993)

	All Sites ( $n=33$ )	Sedimentary-dominated ( $n=21$ )	Volcanic-dominated ( $n=12$ )
<i>Achnanthydium minutissimum</i>	19 (3–64)	<b>15</b> (4–64)	<b>38</b> (3–64)
<i>Achnanthydium pyrenaicum</i>	13 (0–64)	<b>21</b> (0–64)	<b>5</b> (0–47)
<i>Cocconeis placentula</i>	6 (0–52)	11 (0–46)	1 (0–52)
<i>Nitzschia inconspicua</i>	3 (0–34)	2 (0–19)	10 (0–34)
<i>Rhoicosphenia abbreviata</i>	2 (0–32)	2 (0–32)	3 (0–32)
Shannon diversity	1.8 (0.9–2.4)	<b>1.8</b> (0.9–2.4)	<b>1.5</b> (1.2–2.0)
Species richness	15 (10–26)	17 (10–25)	14 (10–26)
% Pollution sensitive taxa	85 (24–99)	85 (28–99)	84 (24–97)
Siltation Index	8 (0–82)	4 (0–37)	15 (0–82)

*placentula* ( $r = 0.59$ ) and *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot ( $r = -0.60$ ). Diatom assemblages in reference and randomly selected sites did not separate along the NMDS axis (Figure 2a). In addition, diatom assemblages were not significantly different between reference and randomly selected sites (ANOSIM global  $R = 0.13$ ,  $p = 0.09$ ).

Correlations of environmental variables with site locations along the ordination axes revealed gradients associated with changes in species composition. Stream watershed area and bankful

width positively correlated to axis 1 ( $r = 0.52$  and  $0.42$ , respectively;  $p < 0.05$  in both cases; Table 3). Conductivity and total solids positively correlated to the 3rd axis (conductivity,  $r = 0.52$ ; total solids,  $r = 0.50$ ). Total nitrogen was correlated to axes 1 and 2 ( $r = -0.39$  and  $0.41$ , respectively). Watershed vegetation was not significantly correlated to any of the ordination axes. Watershed geology correlated to the 2nd ordination axis, with % volcanic rock correlating positively ( $r = 0.58$ ; Figure 2b) and % sedimentary rock correlating negatively ( $r = -0.57$ ). The ANOSIM performed

Table 3. Pearson's correlation coefficients ( $r$ ) for environmental variables and common diatom species with significant correlations to NMDS axes. Bold values indicate significant correlations ( $\alpha = 0.05$ )

	Axis 1	Axis 2	Axis 3
Watershed area (km <sup>2</sup> )	<b>0.52</b>	0.08	-0.31
Elevation (m)	0.00	<b>-0.35</b>	0.24
Sedimentary rock in watershed (%)	-0.07	-0.57	0.00
Volcanic rock in watershed (%)	0.05	<b>0.58</b>	0.03
Conductivity ( $\mu\text{S}/\text{cm}$ )	-0.26	-0.15	<b>0.52</b>
Total Nitrogen (mg/l)	-0.39	<b>0.41</b>	0.17
Nitrate + Nitrite (mg/l)	-0.34	<b>0.52</b>	0.08
Total Solids (mg/l)	-0.24	-0.21	<b>0.50</b>
Bankful width (m)	<b>0.42</b>	0.11	-0.30
Slope (% of mean reach)	-0.38	-0.04	<b>0.44</b>
<i>Achnanthydium pyrenaicum</i>	<b>0.37</b>	<b>-0.48</b>	-0.69
<i>Achnanthydium minutissimum</i>	-0.12	<b>0.79</b>	<b>-0.40</b>
<i>Cocconeis placentula</i>	<b>0.59</b>	<b>-0.56</b>	0.25
<i>Nitzschia inconspicua</i>	-0.22	<b>0.46</b>	<b>0.55</b>
<i>Planothidium lanceolata</i>	-0.60	-0.35	<b>0.37</b>
<i>Rhoicosphenia abbreviata</i>	-0.44	-0.02	<b>0.61</b>

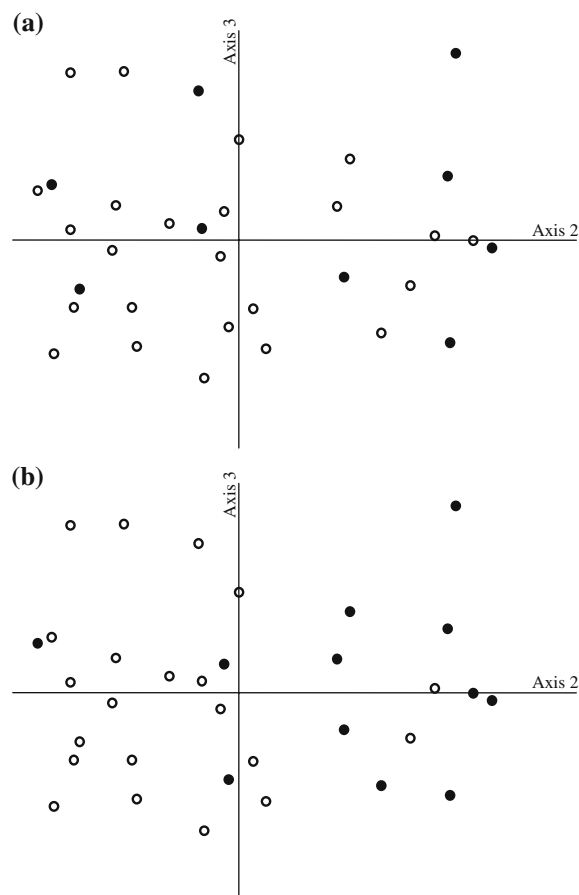


Figure 2. Non-metric multidimensional scaling ordination plot of sites based on diatom species assemblages (sites in species space). (a). Open circles are randomly selected sites and closed circles are reference sites. (b) Open circles are sedimentary-dominated watersheds and closed circles are volcanic-dominated watersheds.

on diatom assemblages between the 2 geologic groups was significant (global  $R = 0.19$ ,  $p < 0.05$ ). The relative abundance of *A. minutissimum* was significantly greater at sites with volcanic-dominated watersheds ( $p = 0.01$ ), while that of *A. pyrenaicum* was significantly lower ( $p = 0.05$ ).

TWINSPAN produced four groups with statistically significant site membership based on ANOSIM results (Figure 3). Indicator species analysis showed that Group I was characterized by *Amphora pediculus* (Kützing) Grunow and *R. abbreviata*. Group II was characterized by *A. minutissimum*, *Nitzschia fonticola* Grunow and

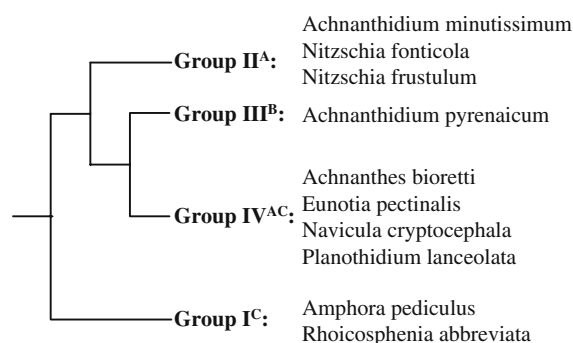


Figure 3. Dendrogram illustrating results for TWINSPAN classification of sites based on diatom species assemblages. Groups were retained if ANOSIM results were significant. Superscripts indicate significant differences in species assemblages among TWINSPAN groups. Indicator species for each TWINSPAN group are the result of the Indicator Species Analysis.

*N. frustulum* (Kützing) Grunow. Group III was characterized by a single species, *A. pyrenaicum*. Group IV was characterized by *Achnanthes bioretii* Germain, *Planothidium lanceolata*, *Eunotia pectinalis* (Dillwyn) Rabenhorst, and *Navicula cryptocephala* Kützing. Relationships between landscape level environmental variables and TWINSPAN groups were not clear (Table 4). Group III sites had significantly lower total nitrogen levels ( $p = 0.005$ ). Groups IV sites had the highest percent of fine substrate ( $p = 0.004$ ). There were no significant differences in diatom metrics among groups.

## Discussion

The strength of utilizing a random probability sampling design is that it allows for extrapolation to regional patterns of stream condition. Diatom species assemblages and autecological metrics suggest overall the Coast Range streams have relatively high water quality. The median percent of pollution sensitive species was 85%. Assemblages were not significantly distinct between reference sites and randomly selected sites (ANOSIM,  $p = 0.09$ ). Diatom richness was low throughout the region, similar to findings in other low-impacted basins of the Coast Range (Naymik et al., 2005). The dominance of stream assemblages by *Achnantheidium minutissimum* and

Table 4. Landscape, water quality, physical habitat variables and diatom metrics for diatom-based TWINSpan groups for Oregon Coast Range streams. Superscripts indicate significant differences among groups (ANOVA, alpha = 0.05). Pollution sensitivity values are from Lange-Bertalot (1979)

Variables	TWINSpan Group			
	I (n = 6)	II (n = 6)	III (n = 18)	IV (n = 3)
Watershed area (km <sup>2</sup> )	11	6	17	33
Elevation (m)	27 <sup>a</sup>	320 <sup>b</sup>	175 <sup>b</sup>	207 <sup>b</sup>
Watershed sedimentary rock (%)	100	28	83	100
Watershed volcanic rock (%)	0	72	15	0
Alkalinity (mg CaCO <sub>3</sub> /l)	28 <sup>a</sup>	14 <sup>ab</sup>	17 <sup>b</sup>	14 <sup>ab</sup>
Conductivity (μS/cm)	82	66	63	64
Nitrate + Nitrite (mg/l)	0.3	0.2	0.1	0.5
Total Nitrogen (mg/l)	0.5 <sup>ab</sup>	0.4 <sup>ab</sup>	0.3 <sup>a</sup>	0.7 <sup>b</sup>
Total Solids (mg/l)	59	55	53	62
Bankful width (m)	7 <sup>ab</sup>	15 <sup>a</sup>	10 <sup>a</sup>	4 <sup>b</sup>
Thalweg depth (cm)	9 <sup>ab</sup>	23 <sup>a</sup>	16 <sup>ab</sup>	10 <sup>b</sup>
Slope (% of mean reach)	4 <sup>a</sup>	1 <sup>b</sup>	1 <sup>b</sup>	1 <sup>ab</sup>
Fine substrate (%)	5 <sup>a</sup>	2 <sup>a</sup>	10 <sup>a</sup>	45 <sup>b</sup>
Riparian canopy coverage (%)	49	50	56	18
Species richness	18	15	14	20
Pollution sensitive taxa (%)	84	84	87	76

*A. pyrenaicum* may indicate waters with dilute water chemistry (Van Dam et al., 1994; Potapova & Charles, 2003). In addition, the widespread distribution of these adnate species may indicate the importance of the physical environment in controlling biotic assemblages. *Achnanthes/Achnantheidium* species are often small in size and associated with shaded headwater streams (Steinman & McIntire, 1986).

Although diatoms have been shown to respond to environmental factors over several scales, quantitative relationships between diatom assemblages and environmental variables were relatively weak in this study. Random probability sampling reflects regional complexity, however it may not capture the variance of human disturbance adequately. Diatom-based assessment has proven successful in areas with high variance of human disturbance. Strong relationships between environmental variables and diatom assemblages were found in streams covering a gradient of acid mine drainage impacts (Verb & Vis, 2000). Diatom assemblages were different between reference streams, streams in golf courses under construction and streams in operational golf courses

spanning a nutrient enrichment gradient (Winter et al., 2003). Diatom-based bioassessment in minimally impacted areas, such as the Oregon Coast Range, may be improved with careful site selection that ensures the coverage of the entire human disturbance gradient of interest. The impacts of logging activities on stream periphyton were detected in Oregon Coast Range streams selected to cover the entire gradient of logging activities (Naymik et al., 2005).

The effectiveness of a bioindicator is based on its ability to separate the human disturbance signal from natural environmental variability. In regions where high natural variability may obscure the human disturbance signal, diatom-based bioassessment may require a more sophisticated approach. One possible approach is to first classify sites into relatively homogeneous groups to minimize confounding factors not of interest in the assessment. There are two main classification methods employed in bioassessment: (1) landscape classification (e.g., ecoregions) and (2) biota-based classification (e.g., River Invertebrate Prediction and Classification System (RIVPACS); Wright et al., 1993). The ecoregion approach defines areas

of relatively homogeneous ecosystems based on landscape factors (soils, vegetation, climate, geology and physiography; Omernik & Gallant, 1986). The use of ecoregions to classify sites has produced mixed results. Invertebrate communities of New Zealand streams differed significantly among ecoregions (Harding et al., 1997). In Oregon, fish and macroinvertebrates differed significantly only between mountain and valley ecoregions (Whittier et al., 1988). Pan et al. (2000) also found significant differences in diatom assemblages only between montane and valley ecoregions. The weak relationships between diatom assemblages and environmental variables found in this study point to the fact that Level III ecoregion classification might be too coarse to detect the human disturbance signal in this moderately impacted area. The Oregon Coast Range ecoregion encompasses both the coastal mountain range and the flat, coastal lowlands with a mixture of geology, soil, vegetation and landuse/land cover (Clarke et al., 1991). In this study, when sites were stratified by dominant watershed geology, relationships between diatom assemblages and environmental variables became stronger. Diatom assemblages were significantly different between sedimentary and volcanic-dominated watersheds. In addition, the relative abundance of dominant species, *A. minutissimum* and *A. pyrenaicum* were significantly different between the two groups.

A major criticism of the ecoregion classification approach is that it places too much emphasis on landscape features. In summarizing several studies on the relationship between landscape classifications and stream biota, Hawkins et al. (2000a) found that the amount of variance explained by landscape variables was low. The ecoregion approach is non-hierarchical and therefore smaller-scale variation (e.g., reach-level) is overlooked. In a study of stream macroinvertebrates, reach-scale physical properties were more predictive of species assemblages than were landscape-scale properties (Richards et al., 1997). In regions with high environmental heterogeneity, biota-based stream classification may delineate discrete stream types with more clear relationships between biota and environmental conditions. The RIVPACS assessment approach first classifies sites based on biota and then uses a predictive model based on environmental variables to provide a list of taxa to

be expected in the absence of human disturbance (Wright et al., 1993). The ratio of observed taxa to expected is an estimate of the stream condition. The RIVPACS bioassessment approach using macroinvertebrates has been successful in montane streams in California (Hawkins et al., 2000b). A RIVPACS model using diatoms in Australian streams detected differences in predicted values at test sites (Chessman et al., 1999). The RIVPACS approach is data intensive and the sample size in our study was too small to employ this approach. In an attempt to explore the utility of a biota-based classification in the Oregon Coast Range ecoregion, we used TWINSpan to classify sites into 4 discrete groups. Although diatom assemblages were significantly different between groups, relationships with landscape level variables were not apparent. It may be that land cover and geology characterized at the watershed level are at a coarser scale than stream biota respond.

To improve diatom-based bioassessment in environmental heterogeneous regions more sophisticated in-stream sampling and analytical approaches may be required. The sampling design used in this study randomly selects and composites periphyton samples from the stream reach. Several studies have demonstrated that benthic algal assemblages are spatially structured, varying with habitat features, such as substrate and current velocity (Stevenson & Hashim, 1989; Sabater et al., 1998; Passy 2001). A sampling design that targets dominant stream habitats might maximize the signal to noise ratio in the data. The statistical treatment of cosmopolitan and rare taxa might also improve the detection of patterns. Hawkins & Vinson (2000) partially attributed the weak relationships between invertebrates and environmental variables to cosmopolitan taxa masking real differences among sites and rare taxa contributing little ecological information. Four of the five most common taxa in this study were among the most commonly occurring taxa in U.S. rivers (Potapova & Charles, 2002). The dominance of Coast Range diatom assemblages by cosmopolitan taxa might mask disturbance patterns. The treatment of rare taxa also has implications for bioassessment. In reviewing the effects of rare taxa in bioassessment, Cao et al. (2001) concluded that the inclusion/exclusion of rare taxa appeared to relate to the sensitivity of the bioassessment, particularly in

studies covering small spatial extents. Hawkins et al. (2000b) found that rare taxa responded positively to impacts and their inclusion in data analysis improved precision and sensitivity of the bioassessment. The Bray–Curtis distance measure used in our study is insensitive to rare taxa. However, we did not include rare taxa in our analysis due to taxonomic uncertainty of taxa encountered only a few times. This resulted in the exclusion of 35% of the taxa encountered in the study. Conventional fixed-count methods (counting 500–600 diatom valves per sample) may not be adequate to characterize rare species. Employment of a stratified counting method that stops counting dominant taxa after their abundance stabilizes but continues to count rarer taxa until taxonomic precision is established is one possible approach to adequately identify and enumerate rare species.

In summary, diatom-based assessment suggests that Coast Range ecoregion streams are in good condition overall. The high natural variability in stream condition and short human disturbance gradients in this region might obscure the patterns between diatoms and environmental variables. In minimally impacted, complex landscapes, diatom-based bioassessment might benefit from: (1) classifying sites by either major landscape variables (i.e., geology) or stream biota, (2) selecting sites that cover the entire human disturbance gradient of interest, (3) sampling dominant in-stream habitat and (4) careful statistical treatment of rare and cosmopolitan taxa.

### Acknowledgements

Stream sampling was conducted by the Oregon Department of Environmental Quality as part of the Salmon Plan for Oregon Watersheds. We thank Rick Hafele, Mike Mulvey, Doug Drake, Paul Gill and Shannon Hubler for their assistance. Watershed geology and landscape calculations would not have been possible without the CLAMS dataset. Jesse Naymik and Brian Bowder provided considerable assistance with GIS calculations. An EPA STAR Graduate Fellowship provided funding for C. L. Weillhoefer during the writing of this manuscript. The comments of Jan Stevenson and an anonymous reviewer greatly improved the quality of the manuscript.

### References

- Bahls, L. L., 1993. Periphyton bioassessment methods for Montana streams. Water Quality Bureau, Department of Health and Environmental Sciences, Helena, Montana., 23 pp.
- Barbour, M. T., J. Gerritsen, B. D. Snyder & J. B. Stribling, 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, 2nd edn. Report EPA 841-B-99-002. U. S. Environmental Protection Agency, Office of Water, Washington, DC.
- Cao, Y., D. P. Larsen & R. St-J. Thorne, 2001. Rare species in multivariate analysis for bioassessment: some considerations. *Journal of the North American Benthological Society* 20: 144–153.
- Carpenter, K. D. & I. R. Waite, 2000. Relations of habitat-specific algal assemblages to land use and water chemistry in the Willamette Basin, Oregon. *Environmental Monitoring and Assessment* 64: 247–257.
- Chessman, B., I. Grown, J. Currey & N. Plunkett-Cole, 1999. Predicting diatom communities at the genus level for the rapid biological assessment of rivers. *Freshwater Biology* 41: 317–331.
- CLAMS, 1996. Coastal Landscape Analysis and Modeling Study. [www.fsl.orst.edu/clams](http://www.fsl.orst.edu/clams).
- Clarke, K. R. & R. H. Greene, 1988. Statistical design and analysis for a 'biological effects' study. *Marine Ecology Progress Series* 46: 213–222.
- Clarke, S. E., D. White & A. L. Schaedel, 1991. Oregon, USA, ecological regions and subregions for water quality management. *Environmental Management* 15: 847–856.
- Clesceri, L. S., A. E. Greenberg & A. D. Eaton, 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC 1325 pp.
- Dufrene, M. P. & P. Legendre, 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs* 67: 345–366.
- Ford, J. & C. E. Rose, 2000. Characterizing small subbasins: a case study from coastal Oregon. *Environmental Monitoring and Assessment* 64: 359–377.
- Fore, L. S., J. R. Karr & R. W. Wisseman, 1996. Assessing invertebrate responses to human activities: evaluating alternative approaches. *Journal of the North American Benthological Society* 15: 212–231.
- Frenkel, R. E., 1993. Vegetation. In Jackson, P. L. & A. J. Kimmerling (eds), *Atlas of the Pacific Northwest*. Oregon State University Press: 58–65.
- Hansmann, E. W. & H. K. Phinney, 1973. Effects of logging on periphyton in coastal streams of Oregon. *Ecology* 54: 194–199.
- Harding, J. S., M. J. Winterbourn & W. F. McDiffett, 1997. Stream faunas and ecoregions in South Island, New Zealand: do they correspond?. *Archiv für Hydrobiologie* 140: 289–307.
- Hawkins, C. P. & M. R. Vinson, 2000. Weak correspondence between landscape classifications and stream invertebrate assemblages: Implications for bioassessment. *Journal of the North American Benthological Society* 19: 501–517.

- Hawkins, C. P., R. H. Norris, J. Gerritsen, R. M. Hughes, S. K. Jackson, R. K. Johnson & R. J. Stevenson, 2000a. Evaluation of the use of landscape classifications for the prediction of freshwater biota: Synthesis and recommendations. *Journal of the North American Benthological Society* 19: 518–540.
- Hawkins, C. P., R. H. Norris, J. H. Hogue & J. W. Feminella, 2000b. Development and evaluation of predictive models for measuring the biological integrity of streams. *Ecological Applications* 10: 1456–1477.
- Herlihy, A. T., D. P. Larsen, S. G. Paulsen, N. S. Urquhart & B. J. Rosenbaum, 2000. Designing a spatially balanced, randomized site selection process for regional stream surveys: The EMAP Mid-Atlantic pilot study. *Environmental Monitoring and Assessment* 63: 95–113.
- Hill, M. O., R. G. H. Bunce & M. W. Shaw, 1975. Indicator species analysis, a divisive polythetic method of classification and its application to a survey of native pinewoods in Scotland. *Journal of Ecology* 63: 597–613.
- Johnson, B. R. & G. L. Raines, 1995. Digital map of major bedrock lithologic units for the Pacific Northwest: A Contribution to the Interior Columbia River Basin Ecosystem Management Project. U. S. Geological Survey, Open File Report 95–680.
- Krammer, K. & H. Lange-Bertalot, 1986. *Bacillariophyceae, Teil 1. Naviculaceae*. VEB Gustav Fisher Verlag, Jena, 876 pp.
- Krammer, K. & H. Lange-Bertalot, 1988. *Bacillariophyceae, Teil 2. Epithemiaceae, Bacillariophyceae, Surirellaceae*. VEB Gustav Fisher Verlag, Jena 596 pp.
- Krammer, K. & H. Lange-Bertalot, 1991a. *Bacillariophyceae, Teil 3. Centrales, Fragilariaceae, Eunotiaceae, Achnantheaceae*. VEB Gustav Fisher Verlag, Jena, 576 pp.
- Krammer, K. & H. Lange-Bertalot, 1991b. *Bacillariophyceae, Teil 4. Achnantheaceae, Kritische Ergänzungen zu Navicula (Lineolate) und Gomphonema*. VEB Gustav Fisher Verlag, Jena, 437 pp.
- Krammer, K. & H. Lange-Bertalot, 2000. *Bacillariophyceae, Part 5. English and French Translation of the Keys*. VEB Gustav Fisher Verlag, Jena, 311 pp.
- Lange-Bertalot, H., 1979. Pollution tolerance of diatoms as a criterion for water quality estimation. *Nova Hedwigia* 64: 285–305.
- Leland, H. V., 1995. Distribution of phytobenthos in the Yakima River Basin, Washington, in relation to geology, land use, and other environmental factors. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 1108–1129.
- McClain, M. E., R. E. Bilby & F. J. Triska, 1998. Nutrient cycles and responses to disturbance. In Naiman, R. J. & R. E. Bilby (eds), *River Ecology and Management: Lessons from the Pacific Coastal Ecoregion*. Springer-Verlag, New York: 347–372.
- Mrazik, S., 1999. Reference Site Selection: A Six-Step Approach for Selecting Reference Sites for Biomonitoring and Stream Evaluation Studies. Oregon Department of Environmental Quality Technical Report BIO99-03. 14 pp.
- Naymik, J., Y. Pan & J. Ford, 2005. Diatom assemblages as indicators of timber harvest effects in coastal Oregon streams. *Journal of North American Benthological Society*: accepted.
- Omernik, J. M. & A. L. Gallant, 1986. *Ecoregions of the Pacific Northwest*. Map scale 1:2,500,000. Report EPA/600/3–86/033. U. S. Environmental Protection Agency, Corvallis, Oregon, 39 pp.
- Pan, Y., R. J. Stevenson, B. H. Hill, A. T. Herlihy & G. B. Collins, 1996. Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment. *Journal of the North American Benthological Society* 15: 481–495.
- Pan, Y., R. J. Stevenson, B. H. Hill & A. T. Herlihy, 2000. Ecoregions and benthic diatom assemblages in Mid-Atlantic Highlands streams, USA. *Journal of the North American Benthological Society* 19: 518–540.
- Passy, S. I., 2001. Spatial paradigms of lotic diatom distribution: a landscape ecology perspective. *Journal of Phycology*: 370–378.
- Patrick, R. & C. W. Reimer, 1966. *The Diatoms of the United States. Vol. 1: Monographs of the Academy of Natural Sciences of Philadelphia No. 13*, 688 pp.
- Patrick, R. & C. W. Reimer, 1975. *The Diatoms of the United States. Vol. 2: Part 1. Monographs of the Academy of Natural Sciences of Philadelphia No. 13*, 213 pp.
- Perrin, C. J., M. L. Bothwell & P. A. Slaney, 1987. Experimental enrichment of a coast stream in British Columbia: effects of organic and inorganic additions on autotrophic periphyton production. *Canadian Journal of Fisheries and Aquatic Sciences* 44: 1247–1256.
- Potapova, M. G. & D. F. Charles, 2002. Benthic diatoms in USA rivers: distributions along spatial and environmental gradients. *Journal of Biogeography* 29: 167–187.
- Potapova, M. & D. F. Charles, 2003. Distribution of benthic diatoms in U.S. rivers in relation to conductivity and ionic composition. *Freshwater Biology* 48: 1311–1328.
- Richards, C., R. J. Haro, L. B. Johnson & G. E. Host, 1997. Catchment and reach-scale properties as indicators of macroinvertebrate species traits. *Freshwater Biology* 37: 219–230.
- Rosenfeld, C. L., 1993. Landforms and geology. In Jackson, P. L. & A. J. Kimerling (eds), *Atlas of the Pacific Northwest*. Oregon State University Press, Corvallis, OR: 40–47.
- Sabater, S., S. V. Gregory & J. R. Sedell, 1998. Community dynamics and metabolism of benthic algae colonizing wood and rock substrate in a forest stream. *Journal of Phycology* 34: 561–567.
- Steinman, A. D. & C. D. McIntire, 1986. Effects of current velocity and light energy on the structure of periphyton assemblages in laboratory streams. *Journal of Phycology* 22: 352–361.
- Stevenson, R. J. & S. Hashim, 1989. Variation in diatom community structure among habitats in sandy stream. *Journal of Phycology* 25: 678–686.
- Van Dam, H., A. Mertens & J. Sinkeldam, 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. *Netherlands Journal of Aquatic Ecology* 28: 117–133.
- Verb, R. G. & M. L. Vis, 2000. Comparison of benthic diatom assemblages from streams draining abandoned and reclaimed coal mines and nonimpacted sites. *Journal of the North American Benthological Society* 19: 274–288.
- Walker, G. W. & N. S. Macleod, 1991. *Geologic Map of Oregon*, scale 1:500,000. United States Geologic Survey.

- Welch, E. B., J. M. Jacoby & C. W. May, 1998. Stream quality. In Naiman, R. & R. E. Bilby (eds), *River Ecology and Management: Lessons from the Pacific Coastal Ecoregion*. Springer-Verlag, New York, NY: 69–85.
- Whittier, T. R., R. M. Hughes & D. P. Larsen, 1988. Correspondence between ecoregions and spatial patterns in stream ecosystems of Oregon. *Canadian Journal of Fisheries and Aquatic Sciences* 45: 1264–1278.
- Wigington, P. J. Jr., M. R. Church, T. C. Strickland, K. N. Eshleman & J. Van Sickle, 1998. Autumn chemistry of Oregon Coast Range Streams. *Journal of the American Water Resources Association* 34: 1035–1049.
- Winter, J. G., P. J. Dillon, C. Paterson, R. A. Reid & K. M. Somers, 2003. Impacts of golf course construction and operation on headwater streams: bioassessment using diatoms. *Canadian Journal of Botany* 81: 848–858.
- Wright, J. F., M. T. Furse & P. D. Armitage, 1993. RIVPACS—a technique for evaluating the biological quality of rivers in the UK. *European Water Quality Control* 3: 15–25.
- Zar, J. H., 1999. *Biostatistical Analysis*. Prentice-Hall, Upper Saddle River, New Jersey, 663 pp.