

## APPLIED ISSUES

# Using diatoms to assess the biological condition of large rivers in Idaho (U.S.A.)

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## SUMMARY

1. Diatoms were collected from 49 sites located on 23 rivers and identified to species. We defined 26 attributes of the diatom assemblage on the basis of tolerance and intolerance, autecological guild, community structure, morphological guild and individual condition.
2. We grouped sites into three geographical regions on the basis of altitude, land cover and use. Within each region, we tested for an association between diatom attributes and human disturbance.
3. We selected nine attributes for inclusion in a multimetric index, the river diatom index (RDI): percentage of valves belonging to species sensitive to disturbance, percentage of valves belonging to species very tolerant of disturbance, eutrophic species richness, percentage of valves belonging to nitrogen heterotrophic species, percentage of valves belonging to polysaprobic species, alkaliphilic species richness, percentage of valves belonging to species that require high oxygen, percentage of valves belonging to very motile genera and percentage of deformed valves.
4. The RDI was significantly correlated with measures of human disturbance made at the site (conductivity, percentage of fine sediments and number of human activities) and at the catchment level (percentage of urbanisation and agriculture in the upstream catchment).
5. The percentage of the total variance of RDI related to transect location was very small (1%) compared with among-site differences (73%) and time of sampling (26%). The RDI could reliably detect approximately three categories of biological condition based on annual monitoring and potentially more if sampling were restricted to the same month each year.

**Keywords:** assessment, biological monitoring, diatoms, multimetric index, periphyton

## Introduction

Although much is known about diatom responses to human-induced degradation, relatively less work than with fish or invertebrates has been carried out to formalise this knowledge as a monitoring tool for biological assessment of lotic waters (Rosen, 1995; Whitton & Kelly, 1995; Davis *et al.*, 1996; Hill *et al.*,

2000). This situation is changing rapidly as European countries develop indices to monitor eutrophication (Kelly & Whitton, 1998) and U.S. monitoring programmes incorporate algal sampling into their routine assessments (Rosen, 1995; Charles, 1996; Hill *et al.*, 2000; Leland & Porter, 2000).

The importance of algae to riverine ecology is easily appreciated when one considers their role as primary producers and transformers of inorganic nutrients into organic forms useable by other organisms (Lamberti, 1996; Mulholland, 1996). Structurally, algae

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stabilise the substrate and create mats that form habitat for fish and invertebrates. Some invertebrates use algae to construct cases (Bott, 1996).

Algal monitoring evolved from the early indices of saprobity (Reid *et al.*, 1995; Lowe & Pan, 1996) which were developed for European streams. Tolerance indices typically summarise the relative abundances of species weighted by their sensitivity to specific stressors (Prygiel & Coste, 1993; Kelly & Whitton, 1998; Stevenson & Pan, 1999). Many studies have linked changes in algal assemblages, particularly diatoms, to changes in water chemistry such as pH, phosphorus and nitrogen (Carrick, Lowe & Rotenberry, 1988; Pan *et al.*, 1996; Winter & Duthie, 2000). Water chemistry variables are meaningful proxies for human disturbance in some cases: for example, when nutrient enrichment results from agriculture (McCormick & O'Dell, 1996; Pan *et al.*, 1996). For other disturbances, chemistry may fail to capture changes associated with loss of instream or riparian vegetation, increased sunlight or altered flow regime (Barbour, Stribling & Karr, 1995; Karr, Allan & Benke, 2000). Consequently, other studies have taken a broader view of human influence and evaluated algal response to more direct measures of human disturbance, such as catchment land cover/use and riparian disturbance (Kutka & Richards, 1996; Chessman *et al.*, 1999; Pan *et al.*, 1999; Carpenter & Waite, 2000; Hill *et al.*, 2000; Leland & Porter, 2000).

The purpose of this study was to test a series of hypotheses relating attributes of the diatom assemblage to changes in human disturbance, at either the site or catchment scale. Our goal was to develop and test a multimetric index based on diatoms that could be used for the biological assessment of Idaho's rivers as required under the Clean Water Act (Karr *et al.*, 1986; Karr, 1991; Ransel, 1995; Stevenson & Bahls, 1999; Mebane, 2001). From the periphyton data collected, we selected diatoms for analysis because much is known about their natural history and they dominate algal assemblages in Idaho (Van Dam, Mertens & Sinkeldam, 1994; Stevenson & Pan, 1999).

## Methods

We followed four steps in developing and testing a multimetric index for Idaho rivers. First, we defined three geographical regions on the basis of physical features and types of human disturbance. Secondly,

we ranked sites according to the intensity of human disturbance (southern sites) or we grouped sites according to the type of disturbance (northern sites). Thirdly, we tested metrics in each region and selected metrics that were not redundant to include in a multimetric index. Lastly, we evaluated the index in terms of its statistical precision and association with disturbance.

### *Periphyton collection and identification*

Periphyton were collected from 49 river sites on 23 rivers from mid-August until late October (base flow period) between 1997 and 1998 (Fig. 1). In 1999, eight sites were selected for repeat sampling: twice on the same day in September and once 1 month later. For purposes of monitoring, the Idaho Department of Environmental Quality distinguishes river sites from wadeable stream sites as those >30 m wide, >0.4 m deep and fourth order or greater on a 1 : 100 000-scale map (Strahler, 1957).

Field crews collected periphyton from riffles at three transects located 200–300 m apart, depending on channel width. Three rocks were collected from each transect at the right, left and centre of the transect, for a total of nine rocks. For very deep sites, rocks were collected closer to the river bank. For same-day samples, crew members sampled from the same transect locations but selected rocks independently. Periphyton were sampled with a brush and syringe from each of the nine rocks and combined for a total sample area of approximately 28 cm<sup>2</sup> (Porter *et al.*, 1993). Samples were preserved in 2% formalin.

In the laboratory, samples were cleaned using nitric acid digestion and a microwave apparatus before slide-mounting with Naphrax™. A minimum of 800 valves was counted at 1000× magnification and identified to species where possible. 'Soft' (non-diatom) algae such as Cyanophyta and Chlorophyta were identified to genus in 1997 and 1999.

### *Geographical classification of sites*

We classified sites according to landscape features because human activities followed topography (Fig. 2; Omernik & Gallant, 1986). We grouped sites from similar ecoregions into three geographical regions: southern basins (18 sites), eastern mountains (eight

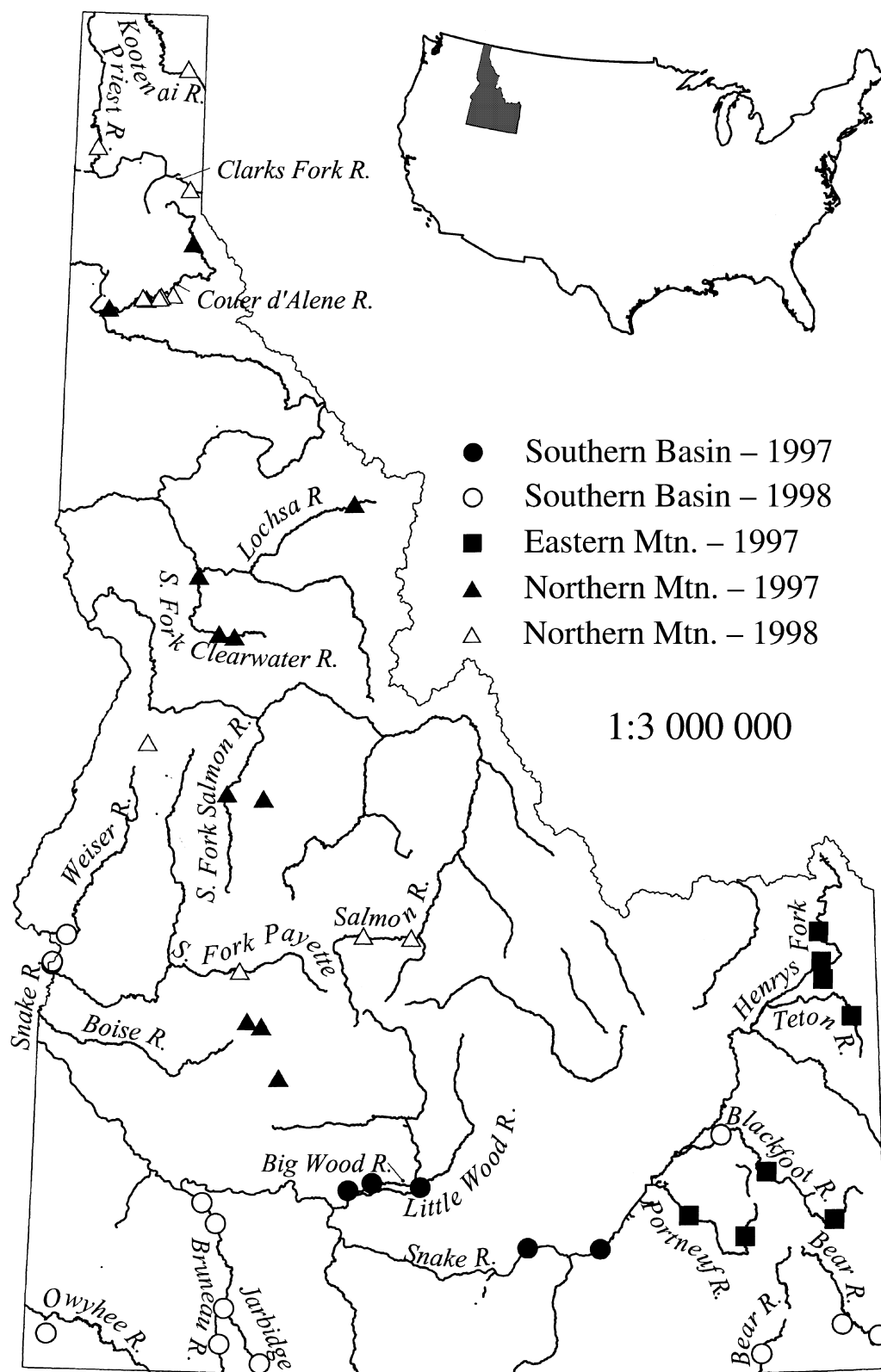


Fig. 1 River sampling sites, year sampled and geographical region.

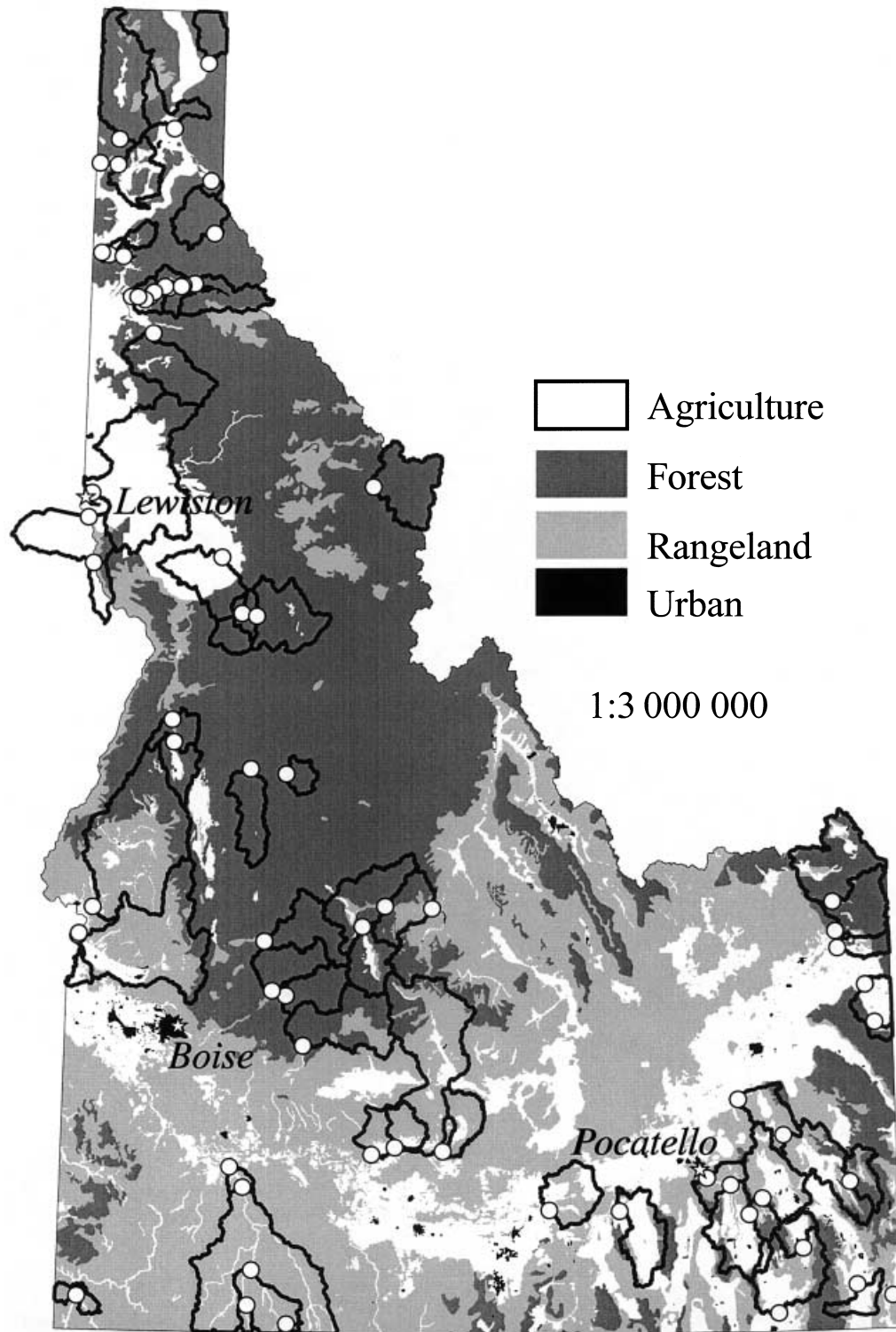


Fig. 2 Major land coverage classes for the state of Idaho. River sampling sites and their upstream catchments are shown.

sites) and northern mountains (23 sites). Southern basins (SB) included 14 sites from the Snake River Basin/High Desert, two from the Wyoming Basin and two from the Northern Basin and Range. Eastern mountain (EM) and northern mountain (NM) sites were all located in the Northern Rockies ecoregion, except for one site in the Middle Rockies (Omernik, 1995). We split this ecoregion into two groups because of the differences in latitude, land cover and land use. Compared with SB and EM sites, NM sites had less urbanisation and agriculture, higher forest cover and lower temperatures. The EM sites were more forested and at higher altitude than SB sites, but had comparable catchment areas classified as agriculture and urbanisation.

### *Quantifying human disturbance*

We quantified human disturbance at three spatial scales: the sample reach, 8 km upstream of the sample site and the upstream catchment. At the reach scale, seven measurements were made: the percentage of stream bank erosion, the percentage of fine sediments (fines) and the extensiveness, condition and predominant type of riparian vegetation on each bank. Four measurements of water quality were taken: temperature, dissolved oxygen, conductivity and pH. No data on nutrient concentrations were collected for this study. We used principal components analysis to reduce these 12 related measures to a single measure of site condition (PC1-HAB).

At a larger scale, field crews noted the types of human activities 8 km upstream of the site. Regional land managers were contacted to confirm their observations. Recorded activities included forestry, mining, agriculture, grazing, urbanisation, channel alteration and recreation and were noted separately near the site and further upstream. Evidence of logging included managed stands, clear cuts or slash piles. Evidence of mining activity included dredging equipment, mine dumps, adit tunnels, discoloured water or rocks or old mining structures. Agricultural activity was indicated by cropland or irrigation equipment. Grazing was indicated by the presence of livestock, water tanks or pastures. Urbanisation was indicated by buildings or construction; pipes or drains; roads, bridges or culverts; sewage treatment, manufacturing or power facilities; or quarries. Channel alteration included streambank stabilisation structures, bridges, dams,

diversions or channelised areas. Recreation was indicated by campgrounds, boat ramps, parking lots, hiking trails or litter. Evidence of any type of activity either near the site or upstream was counted equally and the number of activities was summed to obtain an indicator of the intensity of human disturbance. Two exceptions were recreation and mining which were counted only if they were observed near the site because of their local influence.

From satellite data the percentage of catchment area upstream of each site was classified as agricultural, urban, forested or rangeland. Our large-river sites had potentially huge land areas in their upstream catchments; therefore, rather than using the entire catchment to calculate land cover, we based calculations on the fourth-level hydrological unit as defined by the U.S. Geological Survey (Seaber, Kapinos & Knapp, 1987). The USGS has delineated all the catchments in the U.S. based on surface hydrological features. At the first level, there are 21 catchments; subsequent levels divide these catchments hierarchically. At the fourth level, there are 2262 units. For this study, if a site was near a unit boundary, we included the next upstream unit as well. The average area upstream was approximately 2300 km<sup>2</sup>.

Although grazing is associated with rangeland, the area of the catchment defined as range was not an indication of grazing intensity. Livestock grazing is an important activity in Idaho and can be very destructive to water resources but could not be quantified for this study (Fleischner, 1994). Similarly, forested area only measured vegetation cover and did not distinguish forest type based on stand age or crown cover.

### *Identifying candidate diatom metrics*

For this study, we distinguished between the terms *attribute*, *candidate metric* and *metric*. *Attribute* refers to any feature of the algal assemblage: diatoms that are tolerant of polysaprobic conditions, for example. *Candidate metric* refers to how an attribute is measured because most metrics could be expressed in more than one way. For example, candidate metrics for saprobic condition could be summarised as number of polysaprobic species or as percentage relative abundance of valves belonging to polysaprobic species. Metrics were 'promoted' from candidacy if they demonstrated a significant correlation with human disturbance. Percentages were calculated as the number of valves in the

group of interest divided by the total number of valves identified. All told, we selected 26 attributes from the literature, tested 55 candidate metrics and chose 12 metrics for possible inclusion in the final index.

We tested attributes related to tolerance and intolerance, autecological guild, community structure, morphological guild and individual condition (Table 1).

**Table 1** Diatom attributes, their predicted response to human disturbance, results of five tests for their association with disturbance and taxonomic level. For autecological guild metrics, only the general attribute is listed below because test results for measures based on either number of taxa or percentage of valves tended to be the same. Metrics considered for inclusion in RDI are underlined. We used Spearman's  $r$  to test eastern mountain (EM) and 1998 southern basin (SB) sites; for 1997 SB sites, 'agree' indicates  $r > 0.6$ ; and we used the Mann–Whitney  $U$ -test for northern mountain (NM) sites

| Diatom attribute                      | Predicted response | EM<br>1997<br>$n = 8$ | SB<br>1998<br>$n = 13$ | SB<br>1997<br>$n = 5$ | NM<br>district<br>$n = 10, 6$ | NM<br>mining<br>$n = 10, 7$ | Level<br>of ID |
|---------------------------------------|--------------------|-----------------------|------------------------|-----------------------|-------------------------------|-----------------------------|----------------|
| Tolerance and intolerance             |                    |                       |                        |                       |                               |                             |                |
| Pollution tolerance index†,‡          | Decrease           | **                    |                        |                       |                               | **                          | Species        |
| <u>Sensitive individuals (%)†,‡</u>   | Decrease           | **                    | *                      |                       |                               | **                          | Species        |
|                                       |                    | *                     | **                     |                       |                               |                             | Genus          |
| No. of sensitive species†,‡           | Decrease           | *                     |                        |                       |                               | **                          | Species        |
| <u>Tolerant individuals (%)†,‡</u>    | Increase           | **                    | *                      |                       |                               | **                          | Species        |
| No. of tolerant species†,‡            | Increase           | *                     | *                      |                       |                               |                             | Species        |
| <u>Very tolerant individuals (%)†</u> | Increase           | **                    | **                     | Agree                 |                               | **                          | Species        |
| No. of very tolerant species†         | Increase           |                       | **                     | Agree                 |                               | **                          | Species        |
| Salinity tolerance§                   | Increase           | *                     |                        |                       |                               | X                           | Species        |
| Autecological guild                   |                    |                       |                        |                       |                               |                             |                |
| <u>Eutrophic§,¶</u>                   | Increase           | *                     | **                     | Agree                 | *                             |                             | Species        |
|                                       | Increase           |                       | **                     | Agree                 |                               | X                           | Genus          |
| Oligotrophic§                         | Decrease           |                       |                        |                       |                               | X                           | Species        |
| Nitrogen fixers††                     | Decrease           |                       |                        |                       |                               | **                          | Genus          |
| <u>Nitrogen heterotrophs§</u>         | Increase           | *                     | **                     | Agree                 |                               | *                           | Species        |
|                                       |                    |                       | *                      | Agree                 |                               |                             | Genus          |
| <u>Polysaprobic§</u>                  | Increase           | **                    | **                     | Agree                 | *                             | **                          | Species        |
| <u>Oligosaprobic§</u>                 | Decrease           | *                     |                        |                       |                               | **                          | Species        |
| <u>Alkaliphilic§</u>                  | Increase           |                       | **                     | Agree                 | *                             | X                           | Species        |
|                                       |                    |                       | **                     | Agree                 |                               |                             | Genus          |
| <u>Require high oxygen§</u>           | Decrease           | *                     | **                     | Agree                 | X                             |                             | Species        |
|                                       |                    | **                    | *                      |                       |                               |                             | Genus          |
| <u>Tolerate low oxygen§</u>           | Increase           | **                    | *                      | Agree                 |                               | **                          | Species        |
| Community structure                   |                    |                       |                        |                       |                               |                             |                |
| Total taxon richness‡                 | Decrease           |                       | X                      | X                     |                               | **                          | Species        |
| Diversity index‡                      | Decrease           |                       |                        |                       |                               | **                          | Species        |
| Dominance (%) (1–5 taxa)‡,¶           | Increase           |                       | X                      |                       |                               | **                          | Species        |
| Percentage <i>A. minutissima</i> ‡    | Increase           |                       |                        |                       |                               | **                          | Species        |
| Morphological guilds                  |                    |                       |                        |                       |                               |                             |                |
| <u>Motile (%)†</u>                    | Increase           | **                    | **                     | Agree                 |                               |                             | Genus          |
| Very + moderately motile (%)‡‡        | Increase           | **                    | **                     |                       |                               |                             | Genus          |
| <u>Very motile (%)‡‡</u>              | Increase           | *                     | **                     | Agree                 | *                             |                             | Genus          |
| <u>Prostrate (%)‡‡</u>                | Increase           |                       | **                     |                       |                               |                             | Genus          |
| <u>Erect (%)‡‡</u>                    | Decrease           |                       |                        | X                     |                               | X                           | Genus          |
| <u>Stalked (%)‡‡</u>                  | Decrease           |                       | **                     |                       |                               | **                          | Genus          |
| <u>Unattached (%)‡‡</u>               | Increase           |                       |                        |                       |                               |                             | Genus          |
| Individual condition                  |                    |                       |                        |                       |                               |                             |                |
| <u>Deformed cells (%)</u>             | Increase           |                       |                        |                       |                               | **                          | None           |

†Bahls, 1993; ‡Stevenson & Bahls (1999); §Van Dam *et al.* (1994); ¶Hill *et al.* (2000); ††Cuffney *et al.* (1997); ‡‡R. J. Stevenson, personal communication.

All tests were one-sided; \* $P < 0.05$ ; \*\* $P < 0.025$ . Significant results in the *opposite* direction of prediction are marked with an X.

*Tolerance and intolerance.* We categorised taxa as sensitive, tolerant or very tolerant on the basis of their general tolerance to high nutrients (eutrophic), organics (polysaprobic), temperature (euthermal), salts (euhalobus), toxics, suspended solids or unstable substrate (Lowe, 1974; Lange-Bertalot, 1979; Bahls, 1993). We calculated taxon richness and percentage relative abundance of tolerant and sensitive species. We also calculated a pollution tolerance index as the sum over all species of the number of valves within each species multiplied by that species' tolerance value. This format is typical for many algal indices used in Europe (Whitton & Kelly, 1995).

*Autecological guilds.* Van Dam *et al.* (1994) listed attributes for diatom species related to tolerance of salt, inorganic nutrients, saprobic conditions and high or low levels of pH and oxygen concentration. We predicted that diatoms that tolerate salt and alkaliphilic diatoms would increase with agriculture and livestock grazing. Evaporation of irrigation water from agricultural fields can leave salt or alkaline residue that is washed into the river by precipitation or irrigation return; fertilisation and erosion can also increase soil alkalinity.

Trophic state refers to the presence of inorganic nutrients such as nitrogen, phosphorus, silica and carbon; in contrast, saprobity refers to the presence of biodegradable organic matter and low oxygen concentrations (Van Dam *et al.*, 1994). We expected eutrophic and polysaprobic diatoms to increase if inorganic or organic nutrients were present in large amounts. Fertiliser from irrigated fields would be one potential source of nutrient enrichment; livestock excrement and wastewater return could be sources of organic waste. In contrast, oligotrophic and oligosaprobic diatoms should decline with disturbances. Although Van Dam *et al.* (1994) originally classified species as tolerant or intolerant of high or low oxygen levels in the context of organic waste decomposition, this attribute may apply to Idaho rivers where dams create stagnant, poorly oxygenated water.

Diatoms in the genera *Epithemia* and *Rhopalodia* are nitrogen fixers because they harbour endosymbiotic cyanobacteria that allow them to convert atmospheric nitrogen into biologically useful forms such as ammonia (Mulholland, 1996). Diatoms classified as nitrogen heterotrophs can use amino acids created by other organisms as sources of carbon and nitrogen

(Tuchman, 1996). Thus, nitrogen fixers should decline and nitrogen heterotrophs should increase with disturbances that increase organic nitrogen.

Overall, Idaho river sites tended towards alkalinity: pH values ranged from 6.5 to 9.1 and only two sites were below neutral (7.0). Consequently, acidophilic taxa may not be common in these rivers. We nevertheless tested this attribute because of its potential sensitivity to acid mine waste.

*Community structure.* We calculated dominance as the percentage relative abundance of the single most abundant species in a sample and also as the percentages of the second through fifth most abundant species. An additional attribute was based on the presence of *Achnanthes minutissima* Kutz., a common diatom associated with scouring or extreme flows (Stevenson & Bahls, 1999).

*Morphological structure guilds.* Motile diatoms include species that can move across unstable substrates without being buried; thus, they are somewhat tolerant of sediment. We tested the percentage of three different categories of motile diatom genera: very motile genera (*Cymatopleura*, *Gyrosigma*, *Hantzschia*, *Nitzschia*, *Stenopterobia* and *Surirella*), very motile plus moderately motile genera (all genera with a raphe) and genera included in Bahls' siltation tolerant index (*Navicula*, *Nitzschia* and *Surirella*; Stevenson & Bahls, 1999).

Algal mats are hypothesised to follow a pattern of succession (McCormick, 1996; Peterson, 1996). The first algae to colonise scoured surfaces attach along their length (prostrate) and are followed by algae that attach apically (adnate). Next come algae that attach perpendicularly to the substrate (erect); last are the stalked and filamentous algae that are typically taller and intolerant of fast current (Kutka & Richards, 1996). Diatom genera were assigned to morphological guilds based on how cells attach to the substrate and to one another (Round, Crawford & Mann, 1990; R. J. Stevenson, personal communication). We considered only percentages, not taxon richness, as candidate metrics because the physical structure of the assemblage depends more on the percentage of valves of each type than the presence of a particular taxon.

*Individual condition.* Cell deformities have been associated with contamination by heavy metals and should increase with this type of disturbance

(McFarland, Hill & Willingham, 1997). This attribute was only noted for 1998–99 samples.

### *Identifying species-level patterns*

We evaluated species-level response to human disturbance by grouping diatom species according to their attributes and sorting them according to their relative abundance or frequency of occurrence. We compared these values for site groups defined according to region and level of human disturbance. Only species with attributes that were selected as metrics were included. In addition, we only included species that were found in 25% of the sites of at least one group.

### *Criteria for metric selection*

Metrics selected for river diatom index (RDI) satisfied three criteria: (1) they were significantly associated with disturbance in at least two geographical regions, (2) they responded to disturbance in the predicted direction and (3) they were not redundant with other metrics.

For SB and EM sites, we tested for significant correlation (Spearman's  $r$ ) of candidate metrics against a gradient of human disturbance measured as the total number of human activities (NUM\_ACT) near the site. We selected this measure for two reasons. First, it was significantly correlated (Spearman's  $r$ ,  $P < 0.05$ ) with measures of disturbance made at the reach scale (PC1-HAB) and the catchment scale (percentage of agriculture, forested and urban land cover). Secondly, NUM\_ACT represented a compromise between measures of human influence at the site and catchment scales.

For NM sites, a gradient could not be defined for testing because the range of disturbance was not as broad as it was for SB and EM sites. Instead, we defined three site groups based on type and intensity of disturbance and tested for significant differences between groups (Mann–Whitney  $U$ -test). Ten sites with low, or minimal, disturbance were influenced by timber harvest and a small amount of urbanisation in the catchment (<0.4%). The second group was moderately disturbed and included four sites with agriculture or urbanisation greater than 0.4%, one site with very high levels of timber harvest and one with a large hydropower facility that severely altered daily

peak flows. A third group included seven sites with a history of silver mining upstream, which are still contaminated by heavy metals, particularly zinc (Farag *et al.*, 1999). Six of seven mining sites were on the Coeur d'Alene River; the data were therefore not independent. Consequently, significant differences should not be generalised to other rivers without additional testing.

Multiple independent tests ensure that observed patterns are broadly applicable and not unique to the data set. We divided our data by region and year and repeated the statistical test for significance for each subset of site-visits. Because of the large number of hypotheses (candidate metrics) being tested, we expected some significant correlations by chance alone. We tested candidate metrics for their association with disturbance using five independent tests, including two tests for the SB region (for 1997 and 1998), one test for the EM region and two tests for the NM region (low versus moderate disturbance and low versus mining disturbance). The percentage of significant results expected by chance equals the alpha-level of the test. In our case, 5% of 55 tests (the number of candidate metrics tested) is approximately three, or nearly one-quarter of the 12 metrics ultimately selected. By restricting our selection to those candidate metrics that satisfied two independent tests of significance, significant results by chance alone decline to 0.25% of 55, or much less than 1.

We used one-sided tests in all cases because we were testing specific predictions about how diatom attributes should change in response to human disturbance. One-sided tests increased the power of the test to detect differences.

Some pairs of metrics were redundant, either because they measured the same attribute or because they were based on the same taxa. We evaluated the redundancy of metric pairs by first testing for statistical correlation. For metrics that were highly correlated (Spearman's  $r > 0.75$ ), we evaluated the percentage of taxa in common. If greater than half the taxa were the same, we selected the metric that was significant for the most tests and did not include the other metric in the index.

### *Constructing a multimetric index*

We combined metrics into an overall multimetric index, the RDI. We rescaled each metric because the

metrics had different ranges of possible values. We defined scoring criteria based on cumulative distribution plots of metric values. We assumed that rivers sampled for this data set were evenly spread across a gradient of human disturbance and defined scoring breaks to follow the percentiles of the distributions of metric values. This assumption may not be correct and scoring criteria should be reevaluated as more data are collected.

We scored metrics using two different sets of scoring criteria, based on three and 10 scoring categories. We then compared the two versions of RDI to determine whether scoring method affected the index's precision. For both versions of RDI, higher values indicated better biological condition.

#### *Evaluating the statistical properties of the index*

A reliable multimetric index should be influenced more by site differences than by sampling location or time of sampling at the site. We used components of variance analysis to compare the relative influence of site differences, time and sampling location on the variability of RDI using eight sites that were sampled three times in 1999 and once in a previous year. For both versions of RDI and for eight of its nine component metrics, we used an ANOVA model to estimate the proportion of the total variance associated with site differences, transect location within a site and time of sampling.

Using the estimates for mean squared error from ANOVA, we also estimated the number of categories of biological condition that RDI could reliably detect on the basis of the minimum detectable difference, or MDD (Zar, 1984). We used a simple statistical model, a two-sample *t*-test with three replicates, and commonly accepted values for alpha of 0.05 and power ( $1 - \beta$ ) of 0.80 (Peterman, 1990; Carlisle & Clements, 1999). This model answers the question, 'How large a difference between RDI values do we have an 80% chance of detecting with a *P*-value  $< 0.05$ ?' We divided the possible range of RDI by the MDD to obtain the number of distinct categories of biological condition that RDI could detect (Fore, Karr & Conquest, 1994; Fore, Paulsen & O'Laughlin, 2001).

We calculated the number of distinct categories of biological condition that RDI could reliably detect for three different sampling scenarios. Each scenario used 16 values for RDI (eight sites  $\times$  two repeat visits); but

in each case, we defined repeat visits differently using either samples made on the same day, in different months or in different years to estimate mean squared error. For the first scenario, we used same-day samples collected in September 1999 as the repeat visits. For the second scenario, we averaged RDI from the same-day samples collected in September to obtain a single RDI value for September and used that value along with the RDI from October sample as repeat visits. For the third scenario, we averaged all three samples from 1999 to obtain a single RDI value for 1999 and used the two yearly samples as repeat visits.

## Results

### *Algal sampling*

Periphyton sampling yielded 350 diatom species in 46 genera. The most abundant species, *A. minutissima*, was found at every site. Many species were rare; only a single valve was found for 11% of the species. For 'soft' (i.e. non-diatom) algae, 27 genera were identified in 1997; *Calothrix* sp. was present at most sites. We did not test attributes for soft algae because very few genera were collected at each site (2.7 on average) and many sites had none.

Averaging across sites, about 10 species per sample, or 15% of the individual valves, were not listed by Van Dam *et al.* (1994). For these species, we did not have autecological information. For SB and EM sites, neither the number of unassigned species nor the percentage of unassigned valves showed any correlation with human disturbance. In contrast, for the NM region, significantly more unidentified species were collected at less disturbed sites.

### *Diatom response to human disturbance*

*Species level.* Of the 350 diatom species identified, a subset of 105 species were assigned one or more of the attributes used to define metrics and were also present in 25% of the sites of at least one site group (Table 2). Species that prefer high oxygen were more common in NM sites which were characterised by higher altitude and less human disturbance. Two species, *A. laterostrata* Hust. and *Cymbella reichardtii* Krammer, were only present in less disturbed NM sites. Other species, such as *Gomphonema ventricosum*

**Table 2** Mean abundance (or frequency of occurrence) of diatoms for seven site groups based on geographic region and level of human disturbance. The taxa listed occurred in 25% or more of at least one site group and are ordered according to their species attributes and the frequency of occurrence across sites. When a species was present in <50% of the sites, frequency was coded as follows: \*present at less than 25% of sites; and \*\*present in >25% but <50% of sites. Mean relative abundance for species present in at least 50% of the sites was coded as follows: \*\*<1%; \*\*\*1–2%; \*\*\*\*2–10%; and \*\*\*\*\*>10%. Species that require high oxygen (ox), are motile or sediment tolerant (mo), prefer alkaline (al), eutrophic (eu) or saprobic (sp) conditions, or are nitrogen heterotrophs (nh) are indicated

| Region                                                           | NM    |       | EM    |       | SB    |       |       | Attributes |
|------------------------------------------------------------------|-------|-------|-------|-------|-------|-------|-------|------------|
| Disturbance level                                                | Low   | Mod   | Low   | Mod   | Low   | Mod   | High  |            |
| Number of sites                                                  | 10    | 6     | 5     | 5     | 5     | 7     | 6     |            |
| Taxon                                                            |       |       |       |       |       |       |       | Attributes |
| High Oxygen                                                      |       |       |       |       |       |       |       |            |
| <i>Achnanthes laterostrata</i> Hust.                             | **    |       |       |       |       |       |       | OX         |
| <i>Diatoma mesodon</i> (Ehr.) Kütz.                              | **    | *     | *     |       |       |       |       | OX         |
| <i>Tabellaria flocculosa</i> (Roth.) Kutz.                       | *     | **    |       |       |       |       |       | OX         |
| <i>Gomphonema subtile</i> Ehr.                                   | *     | **    |       |       | *     |       |       | OX         |
| <i>Eunotia incisa</i> W. Sm. ex Greg.                            |       |       | **    |       |       |       |       | OX         |
| <i>Achnanthes levanderi</i> Hust.                                |       |       | **    |       |       |       |       | OX         |
| <i>Melosira distans</i> (Ehr.) Kutz.                             | **    | **    |       |       |       | *     | *     | OX         |
| <i>Cymbella reichardtii</i> Krammer                              | ***   |       |       |       |       |       |       | OX         |
| <i>Achnanthes bioretii</i> Germain                               | ***   | *     |       |       |       |       |       | OX         |
| <i>Gomphonema clavatum</i> Ehr.                                  | **    | ****  |       |       | *     |       | ***   | OX         |
| <i>Navicula ignota</i> var. <i>acceptata</i> (Hust.) Lange-Bert. | **    |       | ***   | ***   |       |       | **    | OX         |
| <i>Cymbella cymbiformis</i> Ag.                                  | ***   | **    |       |       | ***   | *     |       | OX         |
| <i>Gomphonema ventricosum</i> Gregory                            | ***   | ***   | **    | *     | *     | **    | ***   | OX         |
| <i>Achnanthes minutissima</i> Kutz.                              | ***** | ***** | ***** | ***** | ***** | ***** | ***** | OX         |
| <i>Cymbella sinuata</i> Greg.                                    | *     | ***   | ***   | *     | ***** | ***   | *     | OX         |
| Motile (sediment tolerant)                                       |       |       |       |       |       |       |       |            |
| <i>Nitzschia siliqua</i> Archibald                               |       |       |       |       |       | **    |       | mo         |
| <i>Surirella ovata</i> Kutz.                                     |       |       |       | **    |       |       |       | mo         |
| <i>Nitzschia dissipata</i> var. <i>media</i> (Hantz.) Grun.      |       |       | **    |       |       |       |       | mo, al     |
| <i>Nitzschia sociabilis</i> Hust.                                |       |       |       |       |       | **    |       | mo, eu     |
| <i>Nitzschia apiculata</i> (Greg.) Grun.                         |       |       |       |       | *     | **    |       | mo         |
| <i>Nitzschia incognita</i> Legler & Krasske                      | *     | *     |       |       |       | **    |       | mo         |
| <i>Nitzschia liebetruthii</i> Rabh.                              | *     |       |       |       | *     |       | **    | mo         |
| <i>Surirella brebissonii</i> Krammer & Lange-Bertalot            |       |       |       |       | *     | *     | **    | mo, al     |
| <i>Nitzschia frustulum</i> v. <i>subsalina</i> Hust.             |       |       |       | **    | **    | ***** | *     | mo         |
| <i>Nitzschia gracilis</i> Hantzsch                               | *     | **    |       |       | ***   | **    | *     | mo         |
| <i>Nitzschia intermedia</i> Hantzsch ex Cl. et Grun.             |       |       | *     | *     | *     | *     | ***   | mo, eu     |
| <i>Nitzschia linearis</i> Ag. (W. Sm.)                           | ***   | ***   |       | *     | **    | **    | **    | mo, al     |
| <i>Nitzschia dissipata</i> (Kutz.) Grun.                         | ***** | ***** | ****  | ***** | ***** | ***** | ***** | mo, al     |
| <i>Nitzschia fonticola</i> Grun. in Cl. et Moll.                 | ****  | ***** | ****  | **    | ***   | ***   | ****  | mo, al     |
| Eutrophic and alkaliphilic                                       |       |       |       |       |       |       |       |            |
| <i>Stephanodiscus minutus</i> Cl. et Moll.                       |       |       |       |       |       | **    |       | eu, al     |
| <i>Cyclotella atomus</i> Hust.                                   |       |       |       |       |       | *     | **    | eu, al     |
| <i>Stephanodiscus parvus</i> Stoermer & Håkansson                |       |       | **    |       |       | *     |       | eu, al     |
| <i>Navicula menisculus</i> Schumann                              | **    |       |       | **    |       |       | **    | eu, al     |
| <i>Diatoma tenue</i> Ag.                                         |       | **    |       |       |       | *     | ***   | eu, al     |
| <i>Melosira granulata</i> (Ehr.) Ralfs                           |       | *     |       |       |       | *     | ****  | eu, al     |
| <i>Navicula viridula</i> v. <i>rostellata</i> (Kutz.) Cl.        |       | **    |       |       | *     | **    | *     | eu, al     |
| <i>Navicula elginensis</i> (Greg.) Ralfs                         |       | **    |       | *     |       | *     | *     | eu, al     |
| <i>Cocconeis placentula</i> v. <i>euglypta</i> (Ehr.) Cl.        |       | *     | ****  | *     |       |       | *     | eu, al     |

Table 2 (Continued)

| Region                                                            | NM    |      | EM    |       | SB    |       |      | Attributes     |
|-------------------------------------------------------------------|-------|------|-------|-------|-------|-------|------|----------------|
| Disturbance level                                                 | Low   | Mod  | Low   | Mod   | Low   | Mod   | High |                |
| Number of sites                                                   | 10    | 6    | 5     | 5     | 5     | 7     | 6    |                |
| Taxon                                                             |       |      |       |       |       |       |      |                |
| <i>Rhopalodia gibba</i> (Ehr.) O. Mull                            | *     | ***  |       |       |       | *     | *    | eu, al         |
| <i>Navicula gregaria</i> Donk.                                    | ***   | **   |       | ***   |       | **    | ***  | eu, al         |
| <i>Navicula lanceolata</i> (Ag.) Kutz.                            | *     |      | *     | **    | *     | **    | **   | eu, al         |
| <i>Cocconeis pediculus</i> Ehr.                                   |       | **** | ****  | ***   | ***   | ****  | ***  | eu, al         |
| <i>Gomphonema minutum</i> (Ag.) Ag.                               | *     | **** | ***   | *     | ****  | ***   | **   | eu,            |
| <i>Epithemia sorex</i> Kutz.                                      | ***   | **** | **    | *     | *     | ****  | **** | eu, al         |
| <i>Fragilaria vaucheriae</i> (Kutz.) Peters.                      | ****  | **** | ***   | *     | ***   | **    | ***  | eu, al         |
| <i>Achnanthes lanceolata</i> Breb. ex Kutz.                       | ****  | **** | ***   | ****  | ***   | ***   | ***  | eu, al         |
| <i>Amphora pediculus</i> (Kutz.) Grun.                            | **    | **   | *     | ****  | ****  | ****  | **** | eu, al         |
| <i>Cocconeis placentula</i> v.<br><i>lineata</i> (Ehr.) V. H.     | ***** | **** | ***** | ***** | ****  | ****  | **** | eu, al         |
| <i>Gomphonema olivaceum</i> (Lyngb.) Kutz.                        | *     | **   | ***   | ***   | **    | ***   | ***  | eu, al         |
| <i>Navicula tripunctata</i> (O. F. Müller)                        | *     | ***  | ****  | ****  | **    | ****  | **** | eu, al         |
| <i>Rhoicosphenia curvata</i> (Kutz.) Grun.                        | ***   | **** | **    | ****  | ***   | ***   | **** | eu, al         |
| <i>Navicula capitatoradiata</i> Germain                           | *     | ***  | *     | ***   | ****  | ****  | **** | eu, al         |
| <i>Asterionella formosa</i> Hass.                                 |       |      |       |       |       |       | ***  | al             |
| <i>Anomoeoneis vitrea</i> (Grun.) Ross                            | *     | ***  |       |       |       | **    |      | al             |
| <i>Caloneis bacillum</i> (Grun.) Cl.                              |       | *    |       | *     |       | *     | **   | al             |
| <i>Amphipleura pellucida</i> (Kutz.) Kutz.                        | *     | **   |       |       | **    | *     |      | al, sp         |
| <i>Fragilaria leptostauron</i> v.<br><i>dubia</i> (Grun.) Hust.   | ***   | *    |       | **    |       | **    |      | al             |
| <i>Navicula cryptotenella</i> Lange-Bertalot                      | *     | **   |       |       | ****  | ****  |      | al             |
| <i>Fragilaria crotonensis</i> Kitton                              |       | **   | ***   | *     |       |       | ***  | al             |
| <i>Navicula capitata</i> Ehr.                                     | *     | *    |       | *     |       | **    | *    | al             |
| <i>Navicula decussis</i> Ostr.                                    | **    |      |       | *     | **    | **    | **   | al             |
| <i>Achnanthes clevei</i> Grun.                                    | *     | **   |       | **    |       | **    | *    | al             |
| <i>Navicula pelliculosa</i> (Breb. ex Kutz.) Hilse                | **    | ***  | *     | *     |       | **    | **   | al             |
| <i>Navicula reichardtiana</i> Lange-Bertalot                      |       | ***  | *     | *     | ***   | ***   | **   | al             |
| <i>Navicula minuscula</i> Grun.                                   | *     | *    | ***   | ***   | *     |       | *    | al             |
| <i>Navicula menisculus</i> v.<br><i>upsaliensis</i> (Grun.) Grun. | *     | *    | *     |       | ***   | **    | **   | al             |
| <i>Achnanthes biasolettiana</i> (Kutz.) Grun.                     | **    | **** |       | *     | ***** | ***** | *    | al             |
| <i>Epithemia turgida</i> (Ehr.) Kutz.                             | ***   | ***  | **    | **    |       | *     | *    | al             |
| <i>Navicula costulata</i> Grun.                                   | *     | **   | *     | *     | **    | **    | **   | al             |
| <i>Diatoma vulgare</i> Bory                                       | **    | ***  | ****  | ***** | ****  | ****  | **** | al             |
| Three or more negative indicators                                 |       |      |       |       |       |       |      |                |
| <i>Amphora veneta</i> Kutz.                                       |       |      |       |       |       | **    | **   | eu, al, sp     |
| <i>Surirella minuta</i> Breb. in Kutz.                            |       | *    |       |       |       | **    |      | mo, eu, al     |
| <i>Nitzschia sigmoidea</i> (Nitzsch) W. Sm.                       |       |      |       | *     |       |       | **   | mo, eu, al     |
| <i>Navicula goeppertiana</i> (Bleich) H.L. Smith                  |       |      |       |       |       | *     | **   | eu, al, nh, sp |
| <i>Thalassosira pseudonana</i> (Hust.) H. & H.                    |       |      |       | *     |       | *     | **   | eu, al, nh     |
| <i>Gyrosigma attenuatum</i> (Kutz.) Rabh.                         |       | *    |       |       |       | **    | *    | mo, eu, al     |
| <i>Surirella angustata</i> Kutz.                                  |       |      |       | *     | *     | **    |      | mo, eu, al     |
| <i>Nitzschia filiformis</i> (W. Sm.) V.H.                         |       |      |       | *     | *     | **    | *    | mo, eu, al, nh |
| <i>Stephanodiscus hantzschii</i> Grun.                            |       |      |       | ***   | *     | **    | **** | eu, al, nh, sp |
| <i>Navicula atomus</i> (Kutz.) Grun.                              | *     | *    | ***   | *     |       |       |      | eu, al, nh, sp |
| <i>Nitzschia frustulum</i> (Kutz.) Grun.                          | *     | *    |       |       | *     | **    | *    | mo, eu, al, nh |
| <i>Nitzschia acicularis</i> (Kutz.) W. Sm.                        | **    | *    |       |       | *     | *     | *    | mo, eu, al, nh |
| <i>Navicula subminuscula</i> Manguin                              | *     | *    |       | **    | **    | **    | ***  | eu, al, nh, sp |
| <i>Nitzschia amphibia</i> Grun.                                   | *     | **   |       | **    | **    | ****  | **** | mo, eu, al, nh |
| <i>Nitzschia paleacea</i> (Grun.) Grun.                           | ****  | **** | *     | *     | *     |       | *    | mo, eu, al, nh |

Table 2 (Continued)

| Region                                           | NM    |       | EM    |       | SB    |       |       | Attributes     |
|--------------------------------------------------|-------|-------|-------|-------|-------|-------|-------|----------------|
|                                                  | Low   | Mod   | Low   | Mod   | Low   | Mod   | High  |                |
| Disturbance level                                | 10    | 6     | 5     | 5     | 5     | 7     | 6     |                |
| Number of sites                                  |       |       |       |       |       |       |       |                |
| Taxon                                            |       |       |       |       |       |       |       |                |
| <i>Melosira varians</i> Ag.                      | *     | ***   | **    | *     | ***** | *     | **    | eu, al, nh     |
| <i>Gomphonema parvulum</i> (Kutz.)               | *     | **    | *     | ****  | ***   | ***   | ***   | eu, nh, sp     |
| <i>Cyclotella meneghiniana</i> Kutz.             | *     | **    | **    | ***   | **    | **    | ***   | eu, al, nh, sp |
| <i>Nitzschia inconspicua</i> Grun.               | ***   | ***   | ****  | ****  | ***** | ***** | ***** | mo, eu, al, nh |
| <i>Navicula minima</i> Grun.                     | ****  | ***   | ***   | ****  | **    | ****  | ****  | eu, al, nh, sp |
| <i>Nitzschia palea</i> (Kutz.) W. Sm.            | ***   | ***** | *     | ****  | ***** | ***** | ***** | mo, eu, nh, sp |
| Mixed indicators: oxygen and negative indicators |       |       |       |       |       |       |       |                |
| <i>Cymbella brehmii</i> Hust.                    |       |       |       |       |       | **    |       | ox, al         |
| <i>Synedra parasitica</i> (W. Sm.) Hust.         |       |       |       |       |       |       | **    | ox, al         |
| <i>Gomphonema intricatum</i> v.                  |       | *     | ***   |       |       |       |       | ox, al         |
| <i>pumila</i> Grun. in V.H.                      |       |       |       |       |       |       |       |                |
| <i>Cymbella prostrata</i> (Berkeley) Cl.         |       | *     |       |       |       | *     | **    | ox, eu, al     |
| <i>Navicula mutica</i> Kütz.                     | *     |       |       |       | *     |       | ***   | ox, eu         |
| <i>Nitzschia lacuum</i> Lange-Bertalot           | *     | **    |       |       |       | *     |       | ox, mo, al     |
| <i>Nitzschia hantzschiana</i> Rabh.              |       | *     | ***   |       | *     |       | *     | ox, mo         |
| <i>Fragilaria construens</i> (Ehr.) Grun.        | *     | **    |       | *     |       | ***   | ***   | ox, al         |
| <i>Fragilaria leptostauron</i> (Ehr.) Hust.      | *     | **    | ***   | ***   |       |       | *     | ox, al         |
| <i>Fragilaria pinnata</i> Ehr.                   | *     | ***   | ***   | *     |       | *     | **    | ox, al         |
| <i>Fragilaria brevistriata</i> Grun.             | *     | ***   | ***** |       | *     | *     | *     | ox, al         |
| <i>Cymbella affinis</i> Kutz.                    | ***** | ***** | ***** | ***** | ***** | ***** | ***** | ox, eu, al     |
| <i>Nitzschia perminuta</i> (Grun.) M. Perag.     | ***   | ***   | *     | *     | *     | *     | *     | ox, mo, al     |
| <i>Fragilaria construens</i> v.                  | ***   | ***   | ****  | ****  | *     | ***   | ***   | ox, al         |
| <i>venter</i> (Ehr.) Grun.                       |       |       |       |       |       |       |       |                |

Gregory, *A. minutissima* and *C. sinuata* Greg. were found in all site groups.

Sediment tolerant species, many from the genus *Nitzschia*, were most common in SB sites; however, species abundance of individual taxa were not consistently associated with the intensity of human disturbance within a region. Three species, *Nitzschia linearis* Ag. (W. Sm.), *N. dissipata* (Kutz.) Grun. and *N. fonticola* Grun., were found in all site groups.

Many species that were designated as eutrophic were also alkaliphilic. Of these species, 11 were found in all site groups and were unable to discriminate between site groups. In contrast, less common species tended to increase in abundance at more disturbed sites within a region. *Diatoma tenue* Ag., *Melosira granulata* (Ehr.) Ralfs, *Navicula elginensis* (Greg.) Ralfs and *N. gregaria* Donk. followed this pattern. Many of the species that were alkaliphilic but not eutrophic were present more often in moderately and highly disturbed sites than sites with a low level of disturbance for all three regions.

Of the species with three or more negative indicators, *Amphora veneta* Kutz., *Surirella minuta* Breb. in

Kutz., *N. sigmoidea* (Nitsch) W. Sm., *N. geoppertiana* (Bleich) H.L. Smith, *Thalassosira pseudonana* (Hust.) H. & H., *Gyrosigma attenuatum* (Kutz.) Rabh., *S. angustata* Kutz., *N. filiformis* (W. Sm.) V.H. and *Stephanodiscus hantzschii* Grun. were commonly found at sites with moderate or high disturbance and rarely found at sites with low disturbance. Several other species were found in all site groups and failed to discriminate between them.

Several species were both positive and negative indicators of site condition, that is, they preferred high oxygen but also tolerate alkaline or eutrophic conditions or sediment. These species were not consistently associated with human disturbance.

**Community level.** Of the 26 attributes tested, 12 were consistently associated with human disturbance in different geographical regions (see Table 1). Eight metrics were associated with disturbance in all three regions: percentage of valves belonging to sensitive species, percentage of valves belonging to tolerant species, percentage of valves belonging to very tolerant species, number of eutrophic species, percentage

of valves belonging to nitrogen heterotrophic species, percentage of valves belonging to polysaprobic species, number of species that tolerate low oxygen and percentage of valves belonging to very motile genera. Three metrics were significant in two regions (usually SB and EM): number of alkaliphilic species, percentage of valves belonging to species that require high oxygen and percentage of valves belonging to motile genera. One metric, percentage of deformed valves, was only higher for mining sites in the NM region.

Results from EM and SB tended to agree for most candidate metrics, probably because similar types of human disturbance were common in both regions. Percentage of valves belonging to both sensitive species and species that require high oxygen declined with increasing disturbance. For higher levels of disturbance, percentages of valves belonging to very tolerant, polysaprobic and nitrogen heterotrophic species and percentage of valves belonging to very motile genera all increased. The number of alkaliphilic

and eutrophic species also increased with disturbance (Fig. 3).

In the NM region, somewhat fewer candidate metrics were significantly associated with moderate disturbance, probably because minimally and moderately disturbed sites were not very different. For moderately disturbed sites, percentages of valves belonging to polysaprobic species and very motile genera increased with disturbance. The number of eutrophic and alkaliphilic species were also significantly higher for moderately disturbed sites (Fig. 4).

For mining sites in the NM region, most candidate metrics related to tolerance and intolerance were significantly different from minimally disturbed sites. Candidate metrics related to saprobity were significantly associated with disturbance in the direction predicted; in contrast, candidate metrics related to trophic status and alkalinity were significant in the opposite direction predicted, possibly because of the presence of acid mine waste. Attributes related to

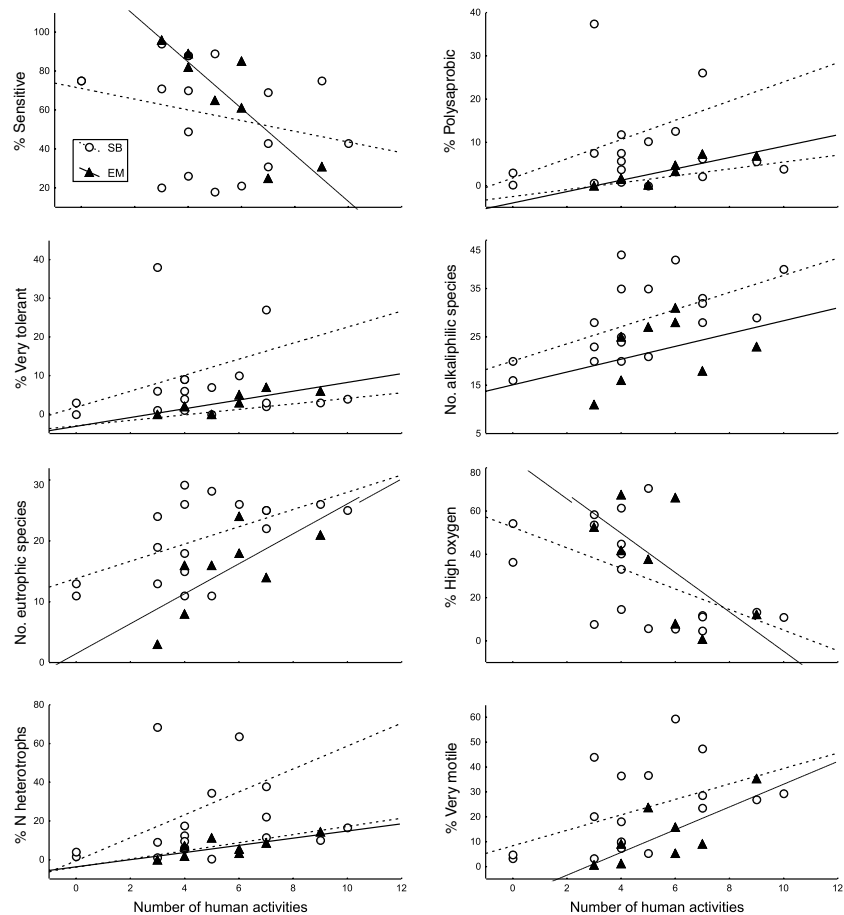


**Fig. 3** Eight diatom metrics were significantly associated with human disturbance measured as the number of human activities 8 km upstream of the site. Least-squares regression lines drawn separately for southern basins (SB) (○) and eastern mountain (EM) (▲) sites. For three metrics in the SB region, regression lines differed by year and are drawn separately for each year.

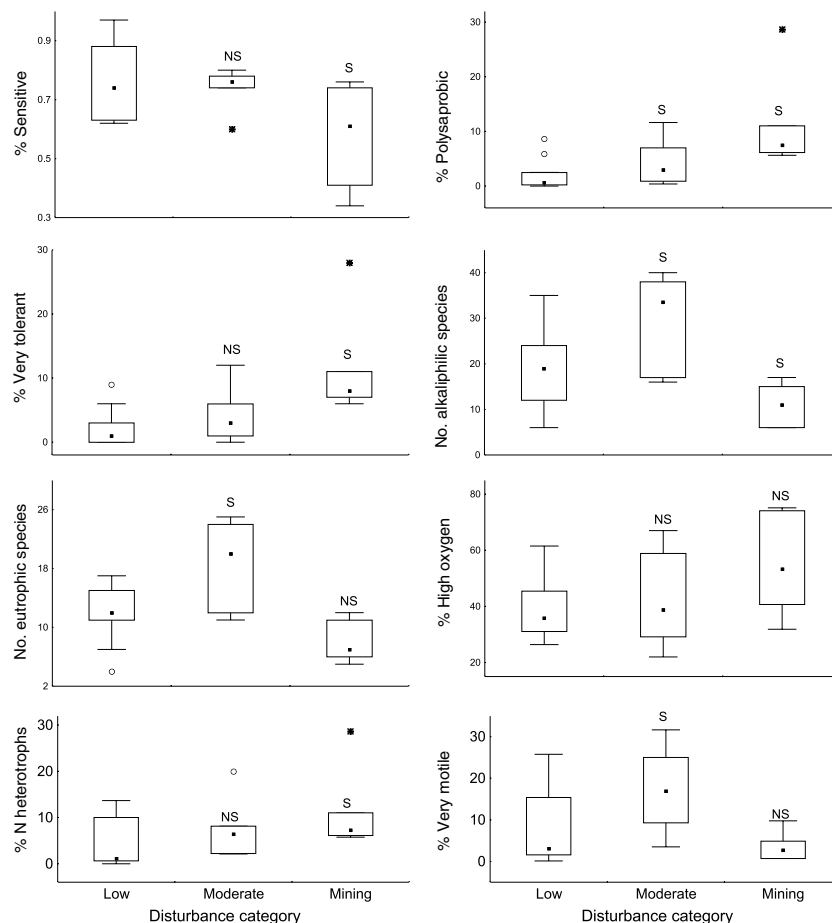


**Fig. 4** Comparison of eight metrics for groups of sites classified as low human disturbance, moderate disturbance and mining disturbance in the northern mountain region. The outlier in the mining group for percentage of valves belonging to very tolerant, nitrogen heterotrophic and polysaprobic species was a site just downstream of a wastewater treatment plant. Boxes marked with an 'S' were significantly different from the low disturbance groups; 'NS' means not significantly different (Mann–Whitney *U*-test).

**Table 3** Diatom metrics were correlated (Spearman's *r*) with measures of disturbance made at the reach (temperature, conductivity, percentage of fine sediments and PC1-HAB), 8 km upstream (number of human activities) and the catchment (percentage urban, agriculture and forested land cover) for 49 river sites. Only significant correlations are shown (\**P* < 0.05; \*\**P* < 0.01)

| Metric               | Temperature | Conductivity | pH     | Fines (%) | PC1-Hab | Num_Act | Urb (%) | Ag (%)  | For (%) |
|----------------------|-------------|--------------|--------|-----------|---------|---------|---------|---------|---------|
| Sensitive (%)        |             |              |        | −0.35*    | −0.44** | −0.40** |         |         |         |
| Very tolerant (%)    | 0.43**      |              |        | 0.39**    | 0.49**  |         |         |         |         |
| Eutrophic species    |             | 0.41**       | 0.38** | 0.33*     | 0.38**  | 0.33*   | 0.29*   | 0.56**  | −0.39** |
| N heterotrophs (%)   |             |              |        | 0.40**    | 0.40**  | 0.37**  | 0.38**  | 0.35*   |         |
| Polysaprobic (%)     | 0.40**      |              |        | 0.38**    | 0.51**  |         | 0.31*   |         |         |
| Alkaliphilic species |             | 0.29*        | 0.43** |           |         |         |         | 0.51**  | −0.37** |
| High oxygen (%)      |             |              | −0.31* |           | −0.32*  | −0.28*  |         | −0.40** |         |
| Very motile (%)      |             |              | 0.29*  | 0.33*     | 0.34*   | 0.35**  | 0.34*   | 0.57**  | −0.29*  |
| RDI                  |             | −0.38**      |        | −0.39**   | −0.50** | −0.38** | −0.38** | −0.40** |         |

community structure (total taxon richness, diversity, percentage dominance and percentage *A. minutissima*) were significantly different for mining sites only.

When sites from all regions were combined, metrics were significantly associated with measures of human disturbance made at the reach scale, 8 km upstream and at the catchment scale (Table 3). Of the site scale

measures, metrics were most frequently associated with the percentage of fines and the derived variable, PC1-HAB. At a larger scale, metrics tended to associate more closely with urbanisation and agriculture than with forested area. The number of eutrophic species and percentage of valves belonging to very motile genera were significantly correlated with the

largest number of different measures of human disturbance.

#### *Metric selection for the RDI*

Of the 12 metrics, three pairs were redundant. Most of the species that were tolerant of low oxygen were also polysaprobic. We chose the polysaprobic metric because it was significantly associated with disturbance in more regions. Two other pairs of metrics were conceptually redundant in that they measured similar attributes. The metric pairs were percentage of valves belonging to tolerant and very tolerant species and percentage of valves belonging to motile and very motile genera. For these metrics, the more specific version was chosen. We retained other metrics for the index although they were statistically correlated with each other, because they were derived from a different set of species.

We selected nine metrics for RDI representing biological information related to tolerance and intolerance, autecological guild, morphological guild and individual condition. No metrics related to community structure were selected because they were only associated with disturbance related to mining in the NM region.

#### *Index performance*

Site differences contributed the largest component to the overall variability of RDI (73%), indicating that

the index was sensitive to the differences in site condition that it was designed to measure (Fig. 5). Variability associated with sampling location within a reach was very small (1% of total variance). For same-day samples, RDI differed by one point on average, or 3% of its potential range from nine to 45. At one site, a thick algal mat was observed only at one of the transects, yet RDI changed by only two points.

Variability associated with specific months, that is, September versus October, was also very small (1%); variability associated with the interaction of month with site, however, was relatively large (25%). The small relative variance associated with month means that there was no systematic change in RDI associated with month; in other words, RDI was not consistently higher in October, for example. The larger interaction effect means that sites varied in different ways across the sampling season. For example, at five of eight sites with large agricultural areas in their upstream catchment, RDI improved for later samples. For another site downstream of a large dam, a high flow event (as a result of dam release) between sampling occasions did not seem to influence RDI values which differed by only two points.

Results were similar for the component metrics: site differences and the interaction of site and month contributed much more to overall variability than did transect location or specific month. Compared with its component metrics, measurement error was lower for RDI indicating greater precision. Error as a percentage of the total variability was 2% for RDI and ranged from

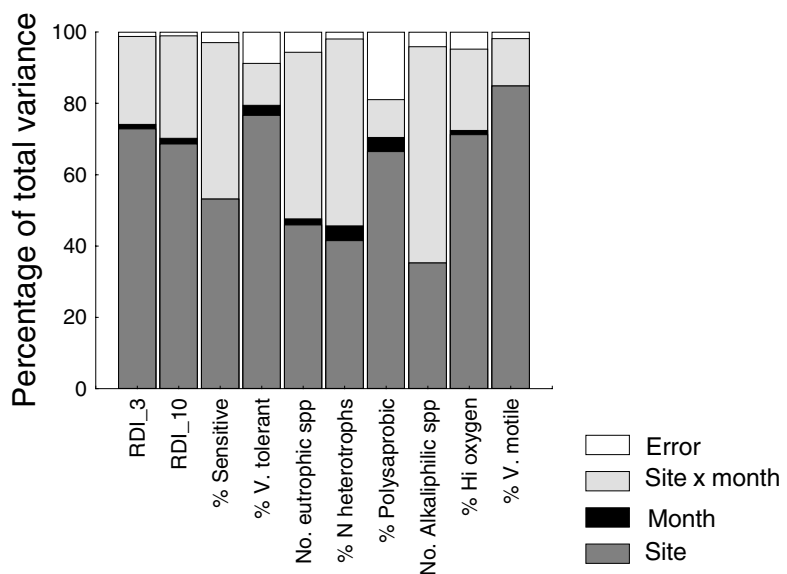


**Fig. 5** Components of variance for two versions of the river diatom index (RDI), based on three (RDI\_3) or 10 (RDI\_10) scoring categories and nine component metrics. Variability associated with site differences, e.g. human disturbance, was highest for RDI and five of the metrics. Samples taken during different months varied more by site (interaction of site and month) than according to time of year (month). Measurement error associated with samples taken on the same day was very low for all measures.

**Table 4** Biological metrics for the river diatom index, response to human disturbance and scoring criteria used to rescale metric values

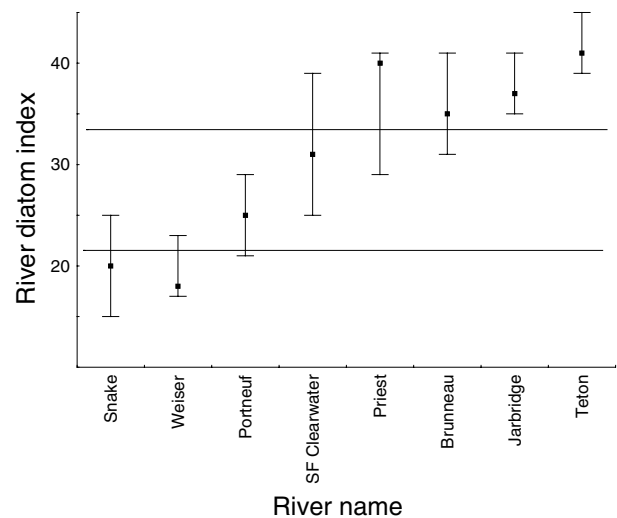
|                               |          | Scoring criteria |          |     |
|-------------------------------|----------|------------------|----------|-----|
| Metric                        | Response | 1                | 3        | 5   |
| Tolerance and intolerance     |          |                  |          |     |
| Sensitive (%)                 | Decrease | <60              | (60, 80) | >80 |
| Very tolerant (%)             | Increase | >15              | (3, 15)  | <3  |
| Autecological guild           |          |                  |          |     |
| Eutrophic species richness    | Increase | >20              | (12, 20) | <12 |
| Nitrogen heterotrophs (%)     | Increase | >20              | (7,20)   | <7  |
| Polysaprobic (%)              | Increase | >10              | (5, 10)  | <5  |
| Alkaliphilic species richness | Increase | >30              | (18, 30) | <18 |
| Requiring high oxygen (%)     | Decrease | <25              | (25, 55) | >55 |
| Morphometric guild            |          |                  |          |     |
| Very motile (%)               | Increase | >25              | (7, 25)  | <7  |
| Individual condition          |          |                  |          |     |
| Deformed cells (%)            | Increase | >1               | (0, 1)   | 0   |

3 to 18% for the component metrics. When monthly variability and site-month interaction were included as part of the error variance, two metrics were more precise than RDI; these were percentage of valves belonging to taxa that were very tolerant and very motile. Sources of variability other than site differences equalled 27% of the total variability for RDI and ranged from 16 to 64% for the component metrics.

Measurement error was very similar for the two RDI versions based on three and 10 scoring categories. Percentages related to monthly differences and same-day (error) differences were equal and percentages related to site differences and site-month interaction

**Table 5** We calculated measurement error of RDI for three types of repeat visits collected on the same day, during different months and years. We summarised index variability as mean squared error from ANOVA, percentage error relative to variability associated with site differences and in terms of the number of distinct categories the RDI could detect. For all comparisons,  $n = 16$ 

| Type of repeat samples | Mean squared error | Percentage error | Categories |
|------------------------|--------------------|------------------|------------|
| Same day               | 1.0                | 1.0              | 11.9       |
| Different months       | 22.8               | 29.2             | 2.5        |
| Different years        | 19.2               | 22.3             | 2.7        |

**Fig. 6** Range of values for the river diatom index (RDI) for eight sites sampled three times in 1999 and once in a previous year. Horizontal lines indicate categories of biological condition (good, fair and poor) that RDI can reliably detect for comparisons across years (for comparisons within years, see text).

differed by only 3%. For simplicity, we based further analyses on the more traditional three-category version (Karr, 1981; Table 4).

The RDI could reliably detect 11.9 categories of biological condition when repeat samples collected on the same day were used as replicates (Table 5). When monthly repeat visits were used as replicates (with same-day samples averaged), RDI was much less precise and could detect 2.5 categories of biological condition (Fig. 6). When annual repeat visits were used as replicates (with same-year samples averaged), the results were similar and RDI could detect 2.7 categories.

The RDI was significantly correlated with measures of natural variability only if the measure was also correlated with human disturbance (Table 6). The RDI was not correlated with latitude, altitude, channel width, temperature or pH. The RDI was significantly correlated with stream order, channel slope and channel depth; but these measures were also significantly correlated with the number of human activities.

At the catchment scale, RDI in the SB region was more closely associated with agricultural land use and in the EM region, with forested area (Fig. 7). One site on the Little Wood River had a higher RDI value than expected, based on 15% agricultural land use. This site differed from other sites in that agricultural activity was concentrated in the extreme upstream

**Table 6** Correlation (Spearman's  $r$ ) of RDI and number of human activities with geographical features (latitude and altitude), channel features (order, channel slope, depth and width) and water chemistry (temperature and pH) for 49 river sites (\* $P < 0.05$ ; \*\* $P < 0.01$ )

|                   | Latitude | Altitude | Order   | Slope   | Depth   | Width | Temperature pH |
|-------------------|----------|----------|---------|---------|---------|-------|----------------|
| RDI               |          |          | −0.53** | 0.44**  | −0.36** |       |                |
| No. of activities | −0.28*   |          | 0.34**  | −0.36** | 0.45**  |       | −0.35**        |

end of the catchment. For the NM region, catchment-scale measures of disturbance varied little and could not be used to evaluate RDI. Instead, we used the disturbance categories used initially to test metrics and found that RDI was lower for both moderately disturbed sites and sites disturbed by mining.

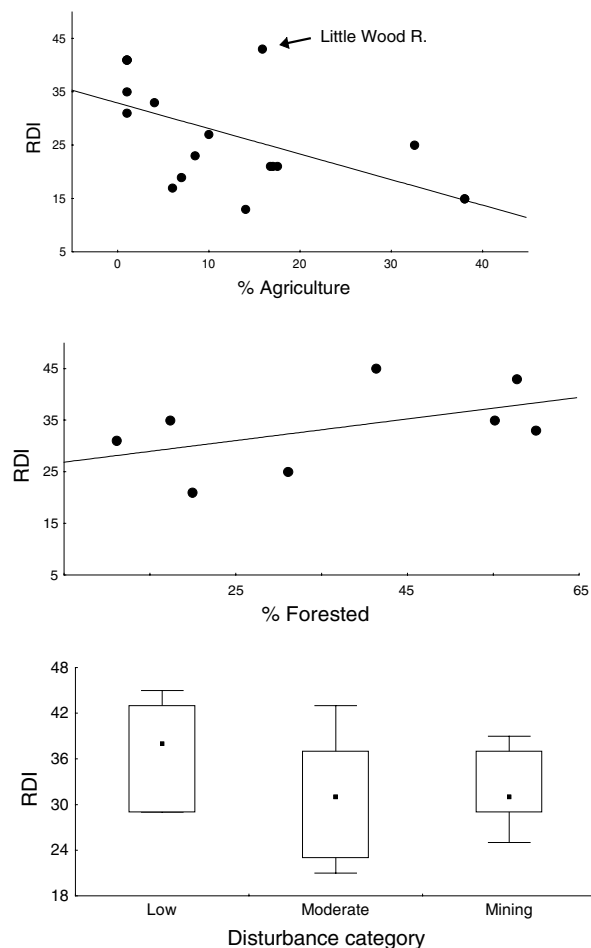


**Fig. 7** For southern basin sites, RDI values declined as agricultural area in the catchment increased (upper panel; see text for explanation of outlier). For eastern mountain sites, RDI values increased with forested area (middle panel). Least-squares regression lines are shown. For northern mountain sites, RDI was lower for both moderately disturbed and mining disturbed sites.

## Discussion

Our study differed somewhat from similar studies relating diatom assemblages to human disturbance. First, we emphasised statistical hypothesis testing rather than exploratory techniques. Secondly, for assessment purposes, we developed a multimetric index rather than a summary index based on species-specific tolerances. Finally, our data were from large rivers rather than small streams. These are only general differences and many recent studies have tested metrics similar to those presented here. Whether species-level data or metrics were used in data analysis, our results generally agreed with previous studies in terms of the consistent and predictable response of diatoms to human disturbance.

We aggregated species according to their attributes and used metrics as our response variables rather than information related to the abundance of individual species. Exploratory techniques such as canonical correspondence analysis, principal components analysis or cluster analysis have been used to link changes in the diatom assemblage to agriculture, urbanisation, alkalinity and organic pollution (Carrick *et al.*, 1988; Leland, 1995; McCormick & O'Dell, 1996; Pan & Stevenson, 1996; Pan *et al.*, 1996; Rott, Duthie & Pipp, 1998; Chessman *et al.*, 1999; Pan *et al.*, 1999; Carpenter & Waite, 2000; Leland & Porter, 2000). Because we were interested in testing specific hypotheses, we instead developed a gradient of human disturbance independent of our analysis of the diatom data. At the species level, we found that the abundance and frequency of diatom species that tolerate nutrient enrichment, sediment or alkaline conditions increased with disturbance. However, by grouping species according to their attributes, we were able to amplify the patterns observed at the species level and define metrics that can be used as biomonitoring tools.

Although often recommended, data analysis based on metrics is less common for diatoms (Pan *et al.*, 1999; Stevenson & Pan, 1999; Stevenson & Bahls,

1999). When tested, results of metric testing tend to agree with those presented here. As for this study, Cuffney *et al.* (1997) and Fore (2002) found that tolerant and sensitive species increased and decreased, respectively, with intensity of human disturbance. We found more eutrophic and alkaliphilic species and more valves belonging to nitrogen heterotrophic and polysaprobic species at disturbed sites. Studies from very different geographical regions have also noted an increase in eutrophic and nitrogen heterotrophic diatoms with disturbance (Cuffney *et al.*, 1997; Leland & Porter, 2000; Hill *et al.*, 2000; Fore, 2002). In some cases, salt tolerant diatoms were found to increase in catchments influenced by urbanisation and agriculture (Cuffney *et al.*, 1997; Leland & Porter, 2000; Fore, 2002). This metric was significantly correlated with disturbance only in the EM region and was not included as a metric in the RDI.

Lists of diatom species attributes used in the previous studies were typically developed initially for other regions or continents (e.g. Van Dam *et al.*, 1994). Cuffney *et al.* (1997) modified their taxa attributes to reflect specific tolerances noted for their study area, but found that the changes made little difference to the final assessment of biological condition. Although we were rather pleasantly surprised that the metrics derived from these lists were correlated with disturbance, we recommend that new lists be developed using quantitative methods and larger data sets as they become available for this region. A similar exercise for fish in the north-eastern U.S. found that many long-held beliefs about several species' natural history were not supported by statistical analysis (Whittier & Hughes, 1998).

As predicted from other studies, we found more diatom valves belonging to motile genera at sites with high levels of fine sediment (Kutka & Richards, 1996; Cuffney *et al.*, 1997; Detenbeck *et al.*, 2000; Fore, 2002). These taxa can move across the substrate and thus avoid being buried by shifting sand. Although decline in the total number of taxa is a strong indicator of biological condition for both fish and invertebrate assemblages (Karr & Chu, 1999), the number of taxa is not a consistent indicator for diatoms. For Idaho rivers, the total number of diatom taxa declined at mining sites but increased with other types of disturbances. Similarly, other studies have found that the number of taxa increased for moderate disturbance and declined with extreme urbanisation or metal

contamination from mining (Juttner, Rothfritz & Ormerod, 1996; Medley & Clements, 1998; Stewart, Butcher & Gerovac, 1999; Genter & Lehman, 2000; Verb & Vis, 2000; Sonneman *et al.*, 2001; review in Hill *et al.*, 2001). Our results support the idea that the total number of species only declines at intense levels of disturbance (Chessman *et al.*, 1999); therefore, we did not include it in RDI.

Algal indices developed for biomonitoring purposes are often calculated by summing the product of each species' tolerance value multiplied by its relative abundance. Tolerance is defined according to a single dimension of human disturbance, typically eutrophication or saprobity (Prygiel & Coste, 1993; Kelly, Penny & Whitton, 1995; Reid *et al.*, 1995; Kelly & Whitton, 1998). In contrast, multimetric indexes, such as RDI, summarise multiple biological attributes related to general tolerance, autecological guild, morphological guild and individual condition. Multimetric indices include measures from different levels of biological organisation so as to respond to many types of disturbance.

Component metrics can respond independently to different types of disturbance and suites of metrics may define a 'signature' for a particular disturbance (Yoder & Rankin, 1995). Other studies have distinguished between organic and inorganic effluents based on diatom samples (Kelly, 1998; Rott *et al.*, 1998; Leland & Porter, 2000) and between agricultural and urban land use (Fore, 2002). For this study, mining sites differed from sites disturbed by agriculture or urbanisation; they had more deformed valves, fewer alkaliphilic taxa and lower taxon richness. Although not included as metrics, an increase in the number of oligotrophic species at mining sites supports the idea that heavy metals associated with mining disturbance affect diatoms differently by interfering with phosphorus uptake (Kuwabara, 1985). When nutrients are unavailable, oligotrophic species that can tolerate nutrient-poor conditions replace those that cannot. Pan & Stevenson (1996) made a similar distinction between wetland sites affected by mining and agriculture.

#### *Natural variability and RDI*

Fair and enforceable regulations must distinguish human-caused changes in the biological assemblage from natural variability (Hughes *et al.*, 1998). We used

multiple, independent tests in three geographical regions to ensure that our selected metrics were robust indicators for the disturbances common in Idaho. Across regions, disturbance tended to correlate with the same diatom attributes, geography made little difference in metric response. Pan *et al.* (2000) also found that human disturbance structured diatom assemblages more than did ecoregional differences; similarly, Leland & Porter (2000) found land use to be a better predictor of algal assemblages than basin geology.

For our study, RDI did not correlate with latitude, altitude, channel width, temperature or pH. RDI was, however, significantly lower at higher-order sites that were deeper and of lower gradient, but these sites also tended to be more intensely disturbed. Thus our RDI was associated with natural features only when they, in turn, influenced patterns of human land use; otherwise, RDI was not associated with measures of natural variability.

#### *Sampling and analysis protocol for diatoms*

A robust monitoring tool should be sensitive to site differences associated with human disturbance but not much affected by small differences in location or time of sampling (Fore *et al.*, 1994; Barbour *et al.*, 1999; Kaufmann *et al.*, 1999). RDI values for same-day samples differed by only 3%, indicating that the present sampling protocol yields precise measures of the diatom assemblage and that neither the number of valves nor the area sampled needs to be increased. Variability associated with time of sampling was much higher, 22–29%. In comparison, a multimetric index for stream invertebrates showed the opposite pattern, with about 10% of the variability in index values associated with different sampling locations within the same reach and 0% associated with time of sampling (Fore *et al.*, 2001). These differences are probably because of the greater mobility and longer life cycles of invertebrates.

Our sample size was too small (eight sites) to determine whether changes in RDI through time stemmed from natural seasonal shifts in the diatom assemblage or from changes in human activity. Agriculture took place in the upstream catchment of five of the eight sites and RDI increased for all five from September to October. At this time of year, irrigation, fertilisation and herbicide application all

cease while crops are harvested; thus, diatom assemblages may well reflect real changes in human land use. It would be more accurate to use only reference sites to estimate the influence of seasonality on RDI values, but large-river sites with little or no human influence are difficult to find.

#### *Quantifying human disturbance*

The approach used to quantify human disturbance is necessarily specific to geographical regions because natural physical processes and features determine which activities are possible, e.g. farming in river valleys and timber harvest on mountain slopes (Omernik & Gallant, 1986). In southern Idaho, the number of human activities was a reasonable measure of human influence because different activities clustered together. The strong correlation between urban and agricultural land use reflected the fact that the southern economy is based largely on agriculture. In northern Idaho, human activities were not as strongly clustered geographically.

For this study, our measures of disturbance were approximate at best. We evaluated the association of diatom metrics with multiple measures of human disturbance because human activities degrade catchments and surface waters in diverse ways: by altering or destroying the natural habit, disrupting energy cycles, modifying flow regimes, releasing chemicals and propagating alien species (Karr *et al.*, 2000). In the course of relating diatom attributes to human disturbance, we could estimate the measurement error associated with the biological metrics and index; but we could neither quantify nor mitigate the error associated with measures of disturbance.

At the catchment level, livestock grazing was common and pervasive but could not be quantified for this study. Cattle grazing damages river ecosystems through erosion, loss of riparian cover, nutrient enrichment from excrement and loss of instream habitat (Armour, Duff & Elmore, 1994; Fleischner, 1994). The influence of disturbance in upstream parts of the catchment may also be important but was not considered for these rivers because of their large size. At the reach scale, information on water chemistry was not available, so we could not assess the relative influence of nitrogen, phosphorus and heavy metals. Sorting out the relative influence of different human activities may be more easily accomplished for smaller

streams where different activities are isolated within catchments (Richards, Johnson & Host, 1996; Roth, Allan & Erickson, 1996).

#### *Statistical considerations*

The diatom samples we used for statistical testing were not necessarily independent because we sampled more than one site on some rivers. Statistical testing assumes independence because correlation and significance can be inflated when values are similar as a result of physical proximity rather than the independent factors being tested (Hurlbert, 1984; Dunham & Vinyard, 1997). The average range in RDI for different sites on the same river system was 11 (out of a possible 36) points, indicating that sites located on the same river could have quite different RDI values; in one case, two sites differed by 32 points. This disparity does not prove independence, but does support the idea that biological condition was not constrained by upstream conditions and could vary in response to human activities near the site. We elected to include all the sites in the statistical tests for two reasons. First, all sites (except on the Coeur d'Alene, see below) were at least 50 km apart and often further. Secondly, a sufficiently large sample size of independent sites is difficult to obtain for rivers of this size.

Lack of independence was much more of a concern for mining sites in the NM region because six of seven sites were located along an approximately 40 km section of the Coeur d'Alene River. We reported the results for three reasons: (1) the data set was adequate to characterise the changes in the diatom assemblage, if not support a specific statistical test; (2) the differences associated with these sites were dramatic and suggest that diatoms may be very robust indicators of metal contamination; and (3) biological endpoints are in great demand for assessing the remediation of abandoned mine sites (Medley & Clements, 1998; Clements *et al.*, 2000).

#### *Diatoms as indicators*

Fish, invertebrates and diatoms represent different trophic levels and integrate environmental conditions over different temporal and spatial scales. Therefore, we expect them to be affected differently by different types of disturbance (Allen *et al.*, 1999). For example, physical barriers such as dams are probably more

disruptive to fish populations than to diatoms. On the other hand, heavy-metal contamination that eliminates many diatoms may be tolerated by fish that can travel farther to refugia. For rivers in Idaho, diatoms may represent a biological alternative to fish when sites are too deep to effectively sample fish or when endangered and protected species prohibit sampling. In contrast with longer-lived organisms, the quick response of diatoms to riverine conditions makes them an excellent tool for evaluating and comparing management practices within a year or season. Finally, in cases where chemical and biological (faunal) information disagree about site condition, diatoms may provide clues for resolving the conflict because of their sensitivity to water chemistry and their nature as living organisms.

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