Using diatom indices for water quality assessment in a subtropical river, China

Xiang Tan[a,b,c], Fran Sheldon[b], Stuart E. Bunn[b], Quanfa Zhang[a]*

a. Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, the Chinese Academy of Sciences, Wuhan 430074, P. R. China

b. Australian Rivers Institute, Griffith University, Nathan, QLD 4111, Australia

c. Graduate School of the Chinese Academy of Sciences, Beijing 100049, P. R. China

Corresponding author:

Quanfa Zhang

Key Laboratory of Aquatic Botany and Watershed Ecology

Wuhan Botanical Garden

Chinese Academy of Sciences

Wuhan 430074, P. R. China

Tel: +86 27 87510702

Fax: +86 27 87510251

Email: qzhang@wbgcas.cn

Abstract

Diatoms have been regularly used as bioindicators to assess water quality of surface waters. However, diatom-based indices developed for a specific geographic region may not be appropriate for evaluating water quality elsewhere. We sampled benthic diatom assemblages during the dry season and wet season in the upper Han River, a subtropical river in China, to evaluate the applicability of 14 diatom-based indices routinely used worldwide for water quality assessment. A total of 194 taxa from 31 genera were identified in the dry season and 139 taxa from 23 genera in the wet season. During the dry season, significant relationships were found for all but one of the diatom-based indices (Index Diatom Artois-Picardie - IDAP) with one or more physical and chemical variables, including nutrients and ion concentrations in river waters. Among these indices, the Biological Diatom Index (IBD) and Diatom-based eutrophication/pollution index (EPI-D) were strongly related to trophic status and ionic content, while Watanabe’s Index (WAT) was related to organic pollution and conductivity. In contrast to the dry season data, the diatom indices showed weak relationships with physical and chemical variables during the wet season. These data suggest that some diatom-based indices developed in Europe, can be applied with confidence as bioindicators of water quality in subtropical rivers of China, at least during base flow conditions.

Key Words: Bioindicator, Diatom-based index, Bioassessment, Water quality,

OMNIDIA, Subtropic river

1. Introduction

Rivers, lakes and wetlands are threatened by a wide range of human activities and there is growing concern about the global impacts to freshwater biodiversity and ecosystem health (Dudgeon et al., 2006; Vörösmarty et al., 2010). This has led to the development and refinement of methods and tools for water quality monitoring, moving beyond those based solely on physical and chemical properties to include biological indicators (Kelly and Whitton, 1998; Karr 1999; Bunn et al., 2010). These include measures of benthic algae (Hill et al., 2000; Coste et al., 2009), phytoplankton (Sagert et al., 2008), macroinvertebrates (Rosenberg and Resh, 1993; Bonada et al., 2006), and fish (Karr, 1981; Kennard et al., 2006) as well as direct measures of ecosystem processes (Bunn and Davies, 2000; Feio et al., 2010).

Diatoms have been used extensively for biomonitoring of aquatic ecosystems due to their ubiquitous presence, ease of collection and preservation, and also their rapid response to environmental changes and deterioration of water quality, especially from impacts such as nutrient enrichment, acidification, and metal contamination (Descy, 1979; Kelly and Whitton, 1995; Stoermer and Smol, 1999; Hirst et al., 2002). Strong relationships between diatom assemblage and water chemistry have been established and many diatom indices for water quality assessment of rivers and lakes have been developed (Kelly and Whitton, 1995; King et al., 2000; Schönfelder et al., 2002; Kelly et al., 2008; Beltrami et al., 2012). However, some studies have shown that diatom-based indices vary in their capacity to predict trophic status, ionic composition, and organic pollution in rivers (Gómez and Licursi, 2001; Taylor et al., 2007).

Many of the widely used diatom indices have been developed from studies of European rivers and it is unclear as to whether they are as effective to evaluate water conditions in other geographic regions. For example, the Biological Diatom Index (IBD, Prygiel and Coste, 2000), widely used in France, showed no significant correlation with any water quality variables in the upper Segre basin of Oriental Pyrenees (Gomà et al., 2005) or in Austrian rivers (Rott et al., 2003). The Watanabe Index (WAT, Watanabe et al., 1986), developed in Japan, is not applicable in European waters (Zgrundo and Bogaczewicz-Adamczak, 2004). It is also known that diatom community composition is correlated with major ion gradients in saline lakes and estuaries (Blinn, 1993; Potapova and Charles, 2003), and yet the relative importance of these natural factors has rarely been studied at large scale. Furthermore, there is little information about the correlations between diatom indices in the assessment of water quality during the wet season, when high flows may also influence community composition (Riseng et al., 2004).

Previously we have used physical and-chemical variables to assess the water quality and to investigate their relationship with land use and land cover in the upper Han River basin (Li et al., 2008, 2009a, 2009b, 2009c). In this study, we conducted research on this same river to evaluate the relationships between diatom based indices and water quality variables in the dry season and wet season. Our aim was to investigate the suitability of benthic diatom assemblages to inform watershed management objectives for the river (Zhang, 2009; Zhang et al., 2009), and to validate the applicability of diatom-based indices for water quality assessment in the subtropical rivers of China.

2. Materials and Methods

2.1 Study region

The upper Han River in the Yangtze River basin is the water source area of China’s South-to-North Water Transfer Project which will transfer water to the North China Plain including Beijing and Tianjin for domestic, industrial and irrigational use (Zhang, 2009; Zhang et al., 2009) (Fig. 1). It has a drainage area of 95,200 km^2 with a length of 925 km and drains a region of north subtropical monsoon climate, with an annual mean temperature range between 12 and 16 ºC (Li et al., 2009a). The principal vegetation in the basin includes coniferous, deciduous forest, mixed coniferous and broad-leaved forest, shrubs and herbs (Li et al., 2008). Basin soils are characterized by yellow brown soil and cinnamon soil (Li et al., 2008), and the surficial lithology is dominated by carbonates (Li and Zhang, 2008). The benthic substrate of rivers is mostly composed of cobbles with low proportions of gravel and sand, and boulder substrate is found in streams and uplands.

There are small towns and villages with small populations in the uplands and there are several larger cities such as Hanzhong, Ankang, Shiyan along the Han River corridor (Li et al., 2009a). Water quality is affected by the activities of industry and the by-products of agriculture, such as pesticides and herbicides, as there are large areas of cultivation including maize, wheat, rice, cassava, vegetables and citrus in the basin (Li et al., 2008, 2009a).

2.2 Diatom sampling and identification

Sampling was undertaken in the Han River basin in November 2007 (dry season) and August 2008 (wet season). Epilithic diatoms were collected from 29 sites in November and 24 sites in August (sites 2,10,11,18 and 19 could not be sampled because of the high depth of the river) (Fig. 1). Some sites (e.g. 17, 21 and 22) were in a catchment of the Hanyin-Ankang corridor with a relatively high percentage (>20%) of agricultural land use (Fig. 1; Li et al., 2008). Other sites (e.g., 25, 26, 27, 28, 29 in Yunxi basin) were in a catchment with ~10% bareland (Fig. 1; Li et al., 2008). Although urban land use was only a small percentage (~1%) of the total catchment area above study sites, some sites (notably 10 and 29) were downstream from major urban centres or had riparian zones disturbed by human activities such as construction.

Three cobbles (with the diameter < 25cm) were collected randomly from the left, centre and right along a transect across the river. At very deep sites, cobbles were collected closer to the river bank. A circular area (diameter 7 cm) was brushed from each rock and periphyton from all nine rocks were rinsed several times with distilled water into one container. A 100ml sample was then preserved in plastic bottles in 4% formaldehyde.

In the laboratory, permanent diatom slides were mounted with Naphrax^{TM} after oxidizing organic material in samples with HNO_3 and H_2SO_4. Species were identified and a minimum of 400 valves were counted per slide at 1000× magnification in Microscope (Olympus BX51). Algal identification was undertaken using Prygiel and Coste (2000), Krammer and Lange-Bertalot (1986, 1991a, 1991b), and Krammer (2002).

2.3 Physical and chemical analyses

Water samples were taken from the same 29 sites in the river network (Fig. 1) on both sampling occasions. A 500ml sample was collected and treated with 10% (v/v) sulphuric acid (final pH <2) for analysis of total phosphorus (TP), total nitrogen (TN), orthophosphate (PO_4-P), and dissolved organic carbon (DOC); prior to analysis the sample for DOC was filtered using a cellulose nitrate membrane filters (Whatman, aperture 0.45 μm). For analysis of the major ions a separate 300ml sample was collected and filtered through cellulose nitrate membrane filters (Whatman, aperture 0.45 μm). From this sample 100ml was acidified with ultra-pure HNO_3 to pH < 2 for the determination of Na^+, K^+, Ca^{2+} and Mg^{2+}, and Si, while another 100ml was left unacidified for the determination of Cl^- and SO_4^{2-} (Li and Zhang 2008). All samples were stored at 4 ºC in acid-washed bottles.

Water temperature (Temp), pH, electrical conductivity (EC), dissolved oxygen (DO), ammonium nitrogen (NH_4-N), and nitrate nitrogen (NO_3-N) were measured *in situ* using a YSI 6920. Major cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) and Si were determined using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (IRIS Intrepid II XSP DUO, USA). Anions (Cl^- and SO_4^{2-}) were measured using a Dionex Ion Chromatograph (IC) (Dionex Corporation, Sunnyvale, USA). Reagent and procedural blanks were determined in parallel to the sample treatment using identical procedures (Li and Zhang 2008). DOC was determined TOC analyzer (TOC-V CPH, Shimadzu Corporation., Japan), TP and PO_4-P were measured by the Ammonium molybdate spectrophotometric method, TN was determined by the alkaline potassium persulfate digestion-UV spectrophotometric method (CSEPB, 2002).

2.4. Data analysis

Patterns between sites based on both diatom species abundance and environmental parameters were initially explored using Canonical correspondence Analysis (CCA) (CANOCO Version 4.53); CCA was run using 4999 iterations and the final solution in two dimensions presented. This initial analysis showed that sites 10 and 29 were markedly different to all others and were subsequently removed from further analysis. To explore the influence of the measured environmental variables on species composition, patterns between species based on both their overall abundance across the sites and the environmental variables were also explored using CCA with inter-species distances.

Fourteen diatom indices (Table 1), which have been widely employed for river monitoring, were calculated using OMNIDIA 7 software V 4.2 (Lecointe et al., 1993, 2003). Most of them (DESCY, DI-CH, EPI-D, IBD, IPS, SLAD, TDI, and TID) are based on the same equation, $ID = \sum A_j v_j i_j / \sum A_j v_j$ (Zelinka and Marvan, 1961), where A_j is the relative abundance of the species j, v_j is its indicator value ($1 \leq v \leq 3$), and i_j its sensitivity (Descy, 1979; Prygiel and Coste, 1993). Further details can be found in OMNIDIA 7 (Lecointe et al., 1993, 2003).

Initially, simple relationships between the calculated diatom indices and measured water quality variables were explored using Pearson's correlations. As there were multiple comparisons, the significance was Bonferroni corrected and only assumed where $p<0.01$. For each separate diatom index, stepwise multiple regression was then used to explore which combination of physical and chemical variables best explained the observed variation in the diatom index. All statistical analyses apart from the CCA were performed using SPSS 16.0 for windows.

3. Results

The physical and chemical characteristics of the rivers

There was considerable variability in water quality across the gradient of sites and between the dry and wet seasons (Table 2). Temperature ranged from 8.5 to 16.0 ºC in November and from 22.8 to 31.9 ºC in August. Sites were slight acidic during the dry season (November) with pH ranging from 5.57 to 6.88 but were more alkaline during the wet season (pH 6.54 to 8.04). There was considerable spatial variation in TP and PO_4-P (0.00 to 5.17 mg/L and 0.00 to 4.70 mg/L, respectively) and Cl^- (1.17 to 19.60 mg/L) in November (Table 2). Sites 10 and 29 showed marked differences in water quality compared with the other sites, especially for pH, nutrients and DOC (Table 2). Site 10 reached concentrations of 2.45 mg/L for TN, 5.17 mg/L for TP and 4.70 mg/L for PO_4-P during the dry season, and 6.41mg/L, 6.06 mg/L and 5.89 mg /L, respectively, during the wet season. At site 29, NH_4-N, NO_3-N, and DOC concentrations were 3.17 mg/L, 6.51 mg/L, 4.76 mg/L, respectively; much higher than those observed at other sites during the dry season. During the wet season at site 29, TN reached a concentration of 11.75 mg /L and DOC was 6.93 mg/L. NH_4-N and NO_3-N were 6.34 mg/L, 4.77mg/L, respectively, at this time. The highest Cl^- concentration was also recorded at site 29 (34.58 mg/L) in the wet season.

Diatom species richness and assemblages

A total of 194 diatom taxa from 32 genera were identified from the dry season, and 139 taxa from 27 genera were found during the wet season (Appendix 1). In the dry season, the most species rich genera (number given in parentheses) included *Navicula* (54), *Cymbella* (26), *Nitzschia* (25), *Achnanthidium* (19), *Gomphonema* (17), and *Fragilaria* (12). Five species accounted for more than 5% of the diatom composition at this time: *Achnanthidium pyrenaicum* (17.9%), *A. subatomus* (11.1%), *Eolimna minima* (8.7%), *A. saprophilum* (8.1%), and *A. minutissimum* (7.4%). During the wet season, *Navicula* (36), *Nitzschia* (20), *Achnanthidium* (15), *Cymbella* (11), *Gomphonema* (8), and *Fragilaria* (8) were the most species rich genera. Four species accounted for more than 5% of the diatom composition at this time: *A. subatomus* (14.6%), *A. pyrenaicum* (9.0%), *A. saprophilum* (7.8%), and *Eolimna minima* (5.3%).

The species composition at sites 10 and 29 were markedly different from those at the other sites. In the dry season, *Sellaphora seminulum* and *Surirella angusta* accounted for 58.5% and 7.9% of the diatoms at site 10, respectively, while *S. seminulum* represented 42.6% of the total at site 29. During the wet season, *S. seminulum* (39.8%), *Pinnularia subcapitata* var. *elongate* (17.1%), and *Navicula submolesta* (15.1%) dominated the diatoms at site 29, and there was no occurrence of *A.subatomus* and *A. saprophilum* which were dominant at other sites.

The CCA clearly separated sites 10 and 29 from others based on diatom composition and water quality (Fig. 2). These two sites were excluded from further analysis to explore relationships between diatom composition and water quality at the other sites (Fig. 3). During the dry season, the first three axes of CCA explained 28.5% of the total variance (Fig. 3a). The first axis was positively correlated with pH, DO, and negatively with chloride concentration. Nutrient-sensitive species such as *A. pyrenaicum*, *A. subatomus* were associated with positive values on axis 1 together with high DO, whereas nutrient tolerant species such as *Navicula reichardtiana* and *Nitzschia dissipata* were associated with negative values on axis 1 and high DOC and TN and other pollutants (Fig. 3a). As for the effects of ions, *Melosira varians and E. minima* were distributed almost along the gradients of Na^+, Cl^- and K^+ indicated by their position along the arrow of those three ions (Fig. 3a).

In the wet season, the first three axes of CCA explained 27.9% of the total variance in species composition. As in the dry season, nutrient sensitive species such as *A. pyrenaicum*, *A. subatomus* were associated with high DO and low nutrient concentrations, whereas nutrient tolerance species such as *Mayamaea.atomus*, *N. reichardtiana* and *Nitzschia palea* were associated with high values of TP and cations (Fig. 3b). Furthermore, the *M. atomus* and *Eolimna subminuscula* propagated much affected by the concentration of Na^+, Cl^- and K^+ (Fig. 3b).

Overall, the relationships between diatom composition and water quality variables were stronger during the dry season than in the wet season, as indicated by the length of arrows defining the disturbance gradients (Fig. 3).

Relationship between diatom indices and water quality

Of the 14 diatom indices, only IDAP was not correlated with any physical or chemical variables in the dry season (Table 3). Most water quality variables had strong relationships with at least one diatom index at this time, except for pH, Ca^{2+}, and Si. Among 14 diatom index predictor models (stepwise regression models), Si concentration was not a significant predictor variable during the dry season (Table 4). Phosphorus concentration was a good predictor of DESCY, IBD, IPS, and TID, while indicators of organic pollution (DOC) were good predictors of SLAD and TDI. DI-CH, IDP and SHE appeared to be responsive to measures of salinity (Cl^-, Na^+, and EC), while CEE and SID responded to phosphorus and measures of organic pollution (*i.e.*, DOC or NO_3-N). Significant spatial variation in WAT, IBD, and EPI-D indices during the dry season was explained by parameters such as DO, TP, NH_4-N, NO_3-N and ion concentrations (Table 4). In contrast, only three diatom indices showed significant correlations with water quality variables during the wet season: CEE, SHE and TDI were significantly correlated with TP ($r = -0.47$), Temp ($r = -0.45$) and NH_4-N concentration ($r = 0.42$), respectively.

Stepwise multiple regression showed only two significant models but both explained low levels of variation. The model for the index CEE included total phosphorous CEE = 12.64 – 26.476 x [TP] ($R^2 = 0.20$, $p = 0.035$); while the model for the index SHE Included Temperature SHE = 28.87 – 0.704 x Temp

(R^2 = 0.20, p = 0.038).

4. Discussion

Benthic diatoms are routinely used as bioindicators of water quality or ecosystem health and to assess the impacts of various human activities of freshwater ecosystems (Kelly and Whitton, 1995; King et al., 2000; Schönfelder et al., 2002). For instance, CEE and IBD are widely used in France (Prygiel and Coste, 2000; Gomà et al., 2005). Yet, there are varying characteristics in terms of diatom composition and physicochemical variables with seasonality among rivers in different regions (Descy, 1979; Descy and Coste, 1990; Prygiel and Coste, 1993; Kelly and Whitton, 1995; Kelly et al., 1995) which raises questions about the broader applicability of these indices.

The applicability of diatom based indices may largely depend on the similarity in species composition in the study area and the taxa used in each index. In this study, IBD performed well in the dry season in our study because 137 taxa (71% of the total 194 identified species) were included in the taxa list used in the IBD calculation. WAT was calculated based on 548 diatom taxa (Watanabe et al., 1986) and included almost all of the diatom taxa in the dry season in this study. Similarly, the IPS index performed well (R^2 = 0.49) in the upper Han River basin showing strong correlations with Cl^-, TP and Ca^{2+} probably because it includes a list of 1300 species (Descy and Coste 1991) which almost cover all the taxa we found this study. In contrast, DESCY is based on 106 species (Descy and Coste, 1990) and covered only a few diatom species in the upper Han River in the dry season (126 common species see Appendix 1). Similarly, IDP was developed with 210 species of which the most frequently occurring species included only 26 of the common species in this study. The performance of each index therefore seemed to reflect the degree of overlap between the taxa lists provided with the index and those present in our rivers, which is not surprising, but provides some direction in the selection of appropriate indices.

Other issues associated with transferring diatom indices different geographic regions from which they were developed include the natural differences in water quality. For example, the indices DI-CH respond to spatial patterns in Na^+ or Cl^- which overwhelms predicted patterns to some other variable (e.g. nutrients). Whereas, they were originally developed to evaluate and predict the nutrient from anthropogenic activities (BUWAL 2002). That may be because in the area it was developed, the water quality naturally had low concentration of Na^+ or Cl^-. In my setting, this would not be a good indicator because it is being influenced by natural factors (geology) rather than nutrient level from human impacts. Likewise, it was reported that the DESCY index does not differentiate anthropogenic pollution from natural eutrophication (Descy and Coste, 1990) suggesting it would not be applicable for detecting human related eutrophication where natural nutrients or ions levels are high.

Some of the indicators tested, however, performed well in the Han River, despite being developed for different regions; and their responses to human impacts in China were the same as shown in the original studies. For example, the indices EPI-D and IBD showed a strong relationship with Cl^- in the Han River (Tables 3, 4) which is in accordance with their described original response to Cl^- and other nutrients in rivers in Italy and France (Dell'Uomo 1996; Prygiel and Coste 2000). Likewise, the SLAD index showed a correlation with DOC in the Han River basin (Table 3), which agrees with the original studies that demonstrated a relationship with organic pollution (Sládeček 1986; Prygiel and Coste 1993). This suggests that where both the gradient to the pollution and the diatom assemblage is similar to that in the original study, the index does indeed perform well at detecting human impacts.

Another issue that can make interpretation of the response difficult relates to the range of the pollution or disturbance gradient that is sampled. If the water quality gradient in the region of interest has a much lower range than where the indicator was developed, there would be a weak (or no) response to the parameters predicted merely because we are sampling across a much narrower disturbance gradient. For example, IDP responds to the spatial gradients in EC found in the upper Han River, and this overwhelms the predicted relationship to organic pollution indicated by NH_4-N, NO_3-N, COD_{Mn} and eutrophication indicated by PO_4-P found in rivers of the Pampean plain in Argentina (Gómez and Licursi 2001). The lithology of the upper Han River basin is composed of granites, sandstones, shales, schist and limestones with natural weathering contributing to the concentration of dissolved ions (Li and Zhang 2008) and consequently the gradients of EC that appeared to influence the diatom assemblages. Comparatively, there is a high range of enrichment of organic pollution and eutrophication (NH_4-N, from <0.01 to 45.9 mg/L; NO_3-N, from <0.01 to15.63 mg/L) across sites within rivers of the Pampean plain where the IDP index was originally developed (Gómez and Licursi 2001), however, there was a relatively small variation in NH_4-N (0.12-1.14 mg/L) andNO_3-N (0.32-6.73 mg/L) in the upper Han River basin. Therefore, this suggests that were a natural gradient dominates the response of an index it may be hard to detect impacts from human disturbance.

Na^+, Cl^-, K^+ showed some strong effects on maintaining the structure of diatom community since these three ions probably is good for the propagation and growth of the species such as *M. varians*, *E. minima*, *M. atomus* and *E. subminuscula*. Due to Na^+, Cl^-, K^+ contribute to the alkalinity of surface water while the Ca^{2+}, Mg^{2+} contribute to the hardness of the water (Patrick, 1977). Moreover, the affinity of Na^+, Cl^-, K^+ with those four species has already been confirmed by the autecology that those four species that has been categorized as alkaliphilous and fresh brackish species in autecology (van Dam et al., 1994)

Silica is often a major limiting nutrient for diatom growth because it is necessary for the siliceous structures in diatom valves (Martin-Jézéquel et al., 2000). However, Si showed no significant correlation with any index and did not make any significant contribution to the indices in this study (Tables 3, 4). In the upper Han River basin, the granitic geology is rich in $Si(OH)_4$. The average Si concentration in the upper Han River is 5.52mg/L, higher than in many other rivers, e.g., 2.89 mg /L in the Yangtze River (Chen et al., 2002), 3.36 to 5.18 mg /Lin the Amazon (Stallard and Edmond, 1983), 4.43 mg /Lin the Ganga–Brahmaputra (Sarin et al., 1989), 1.87 mg /Lin the Lena (Huh et al., 1998), and 1.59 mg /Lin the Mackenzie (Chen et al., 2002). This suggests that Si is not a key factor limiting the growth of diatoms due to its higher natural concentration in the Han River.

One index that has been found to work across a number of areas is the IDAP index. It was developed in the Artois-Picardie basin where the main pollutants were NH_4-N, TN, and chemical oxygen demand (COD_{Mn}) (Prygiel et al., 1996), and studies also demonstrate its utility for water quality assessment in coastal streams (Zgrundo and Bogaczewicz-Adamczak, 2004) and highly polluted rivers (> 100 mg /L NH_4-N) (Prygiel and Coste, 1993). Therefore, it is inferred that IDAP can probably perform well in assessing organic pollution over a large range from 0 mg /L to100 mg /L of NH_4-N. The range of NH_4-N in the upper Han River in the dry season was only 0.12 to 1.14 mg/L, so IDAP it was not surprising it was the least sensitive index to the measured water variables (Table 3). The high reliability of WAT might be attributed to the similarity of the upper Han River area to the region in Japan where it was developed, particularly in relation to river habitat and organic pollution from agriculture and industrial activities (Watanabe et al., 1986).

It was apparent that the performance of diatom indices differed between dry and wet seasons. One reason for the weaker relationships between diatom indices and water quality in the wet season is that high flows or periodic spates may scour the stream bed and lead to high variability in diatom assesmblages (Stevenson, 1990; Bergey and Resh, 2006). There is also a higher likelihood that algal composition will be out of phase with water quality in the wet season; that is, the algal composition may reflect the water quality from days earlier than the sampling time. Additionally, as a result of high flow in the wet season, the species number of diatoms is much less than that in the dry season, which leads to the decrease of taxa involved in calculation of diatom indices. Another contributing factor is that average nutrient concentrations across the sites (excluding sites10 & 29) were reasonably similar between seasons (Table 2) but the ranges such as NO_3-N, Cl^-, and SO_4^{2-} were larger in the dry season, implying there was a larger spatial gradient, implying larger spatial variability during the dry season (Sheldon and Fellows 2010). This also may explain why the indicators showed stronger responses to water quality in the dry season than in the wet season.

Conclusion

A diatom index that performs well in one geographic region is not necessarily suitable to assess water quality in another region due to the regional differences in diatom composition and pollutant characteristics. The same diatom index may also not perform equally well in different seasons even in the same region. In this study, this was primarily a consequence of the degree of overlap in diatom species composition in samples compared with those considered in the index calculation. It is concluded that EPI-D, IBD, and WAT have strong correlations with some water quality variables in subtropical rivers in China, especially in the dry season, despite differences in taxonomy, geographic setting (e.g., climate, geology) and the nature of the pollutant gradient.

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Figure Legends

Fig. 1: The position of sampling sites in the Upper Han River, China, with major sub-basins and catchment features highlighted.

Fig. 2: Ordination plots of diatom assemblages at all sites with respect to environmental variables based on Canonical Correspondence Analysis (CCA) in the dry (a) and wet (b) seasons. Vectors represent the strength and direction of relationships between diatom community composition and water quality parameters.

Fig.3: Ordination plots of common diatom species at all sites (excluding 10 and 29) with respect to environmental variables based on CCA in the dry (a) and wet (b) seasons. Only those species representing more than 10% of the total abundance were included (14 of 194 species). (***ADPY***, *Achnanthidium pyrenaicum*, ***ADSU***, *Achnanthidium subatomus*, ***ADMI***, *Achnanthidium minutissimum*, ***ADSA***, *Achnanthidium saprophilum*, ***ACTT***, *Achnanthidium catenatum*, ***CAFF***, *Cymbella affinis*, ***CPEA***, *Cocconeis placentula* var.*euglypta*, ***CPED***, *Cocconeis pediculus*, ***GMIN***, *Gomphonema minutum*, ***MVAR***, *Melosira varians*, ***MAAT***, *Mayamaea atomus*, ***ESBM***, *Eolimna subminuscula,* ***NCPR***, *Navicula capitatoradiata*, ***NRCH***, *Navicula reichardtiana*, ***EOMI***, *Eolimna minima*, ***NDPA***, *Nitzschia dissipata*, ***NPAL***, *Nitzschia palea*)

Table 1.Diatom based indices and the acronym used in this study.

Abbreviation	Diatom indices
CEE	Commission for Economical Community metric (Descy and Coste1991)
DESCY(or ID)	Descy's pollution metric (Descy1979)
DI-CH	Swiss Diatom Index (BUWAL 2002; Lecointe et al. 2003)
EPID	Diatom-based eutrophication/pollution index (Dell'Uomo1996)
IBD	Biological Diatom Index (Prygiel and Coste 2000)
IDAP	Index Diatom Artois-Picardie (Prygiel et al.1996; Lecointe et al. 2003)
IDP	Pampean Diatom Index (Gómez and Licursi2001)
IPS	Specific pollution sensitivity Index (Cemagref 1982)
SHE	Schiefele and Schreiner's Index (Schiefele and Schreiner 1991)
SID	ROTT saprobic index (Rott et al. 1997)
SLAD	Sládeček's Index (Sládeček1986)
TDI	Trophic Diatom Index (Kelly and Whitton 1995)
TID	ROTT trophic index (Rott1999)
WAT	Watanabe's Index (Watanabe et al.1986; Lecointe et al. 2003)

Table 2.Water quality characteristics in the upper Han River, China in the dry and wet seasons, with and without data for sites 10 and 29 (See text for details).

Parameter	Unit	All sites				Excluding sites 10 and 29			
		Range		Mean±SD		Range*		Mean±SD*	
		Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Temp	ºC	8.50 - 16.0	22.83 - 31.90	12.40±1.68	24.14±2.09	8.5 - 15.0	22.83 - 31.90	12.28±1.51	26.01±2.11
EC	μS/cm	159.9 - 495.9	143.4 - 442.9	287.0±67.3	263.8±63.7	159.9 - 495.9	143.4 - 442.9	282.9±65.6	263.8±66.1
DO	mg/L	3.92 - 12.11	4.47 - 11.13	10.44±1.43	7.62±1.34	8.16 - 12.11	5.69 - 11.13	10.70±0.72	7.79±1.19
pH		5.57 - 6.88	6.54 - 8.04	6.37±0.32	7.42±0.27	6.00 - 6.88	7.04 - 8.04	6.51±0.27	7.44±0.26
NH_4-N	mg/L	0.12 - 3.17	0.06 - 6.43	0.39±0.58	0.48±1.17	0.12 - 1.14	0.06 - 1.11	0.28±0.22	0.23±0.25
NO_3-N	mg/L	0.32 - 6.73	0.86 - 4.92	1.70±1.57	2.47±1.11	0.32 - 6.73	0.86 - 4.92	1.51±1.31	2.32±1.00
TN	mg/L	0.62 - 6.79	0.27 - 11.75	1.93±1.17	2.17±2.18	0.62 - 6.79	0.27 - 3.28	1.86±1.18	1.66±0.80
TP	mg/L	0.00 - 5.17	0.02 - 5.89	0.24±0.96	0.35±1.07	0.002 - 0.25	0.02 - 0.34	0.04±0.05	0.14±0.09
PO_4-P	mg/L	0.00 - 4.70	0.00 - 6.06	0.21±0.87	0.26±1.12	0.00 - 0.23	0.00 - 0.19	0.03±0.05	0.03±0.04
DOC	mg/L	0.51 - 4.76	0.76 - 6.93	1.43±0.74	2.12±1.17	0.51 - 2.06	0.76 - 3.94	1.29±0.37	1.93±0.73
Cl^-	mg/L	1.17 - 19.60	2.81 - 34.58	5.96±4.72	6.23±6.02	1.17 - 19.60	2.81 - 15.78	5.54±4.48	5.18±2.63
SO_4^{2-}	mg/L	12.91 - 64.43	12.32 - 66.24	29.50±12.19	28.14±13.11	12.91 - 64.43	12.32 - 59.17	28.51±12.05	26.04±10.55
Ca^{2+}	mg/L	25.43 - 68.15	20.08 - 58.16	41.07±8.89	36.97±8.58	25.43 - 68.15	20.08 - 52.31	41.12±9.08	36.49±7.69
K^+	mg/L	0.41 - 2.40	0.13 - 10.41	1.09±0.40	1.23±1.88	0.41 - 1.62	0.13 - 2.62	1.02±0.31	0.87±0.55
Mg^{2+}	mg/L	3.55 - 20.52	2.46 - 16.01	8.97±3.90	7.35±2.89	3.55 - 20.52	2.46 - 16.01	9.08±4.00	7.17±2.88
Na^+	mg/L	1.63 - 8.42	1.32 - 18.38	3.32±1.62	3.39±3.18	1.63 - 8.42	1.32 - 6.68	3.11±1.42	2.79±1.34
Si	mg/L	2.65 - 14.08	5.37 - 11.74	5.96±2.42	7.81±1.72	2.65 - 12.13	5.37 - 10.63	5.52±1.74	7.57±1.52

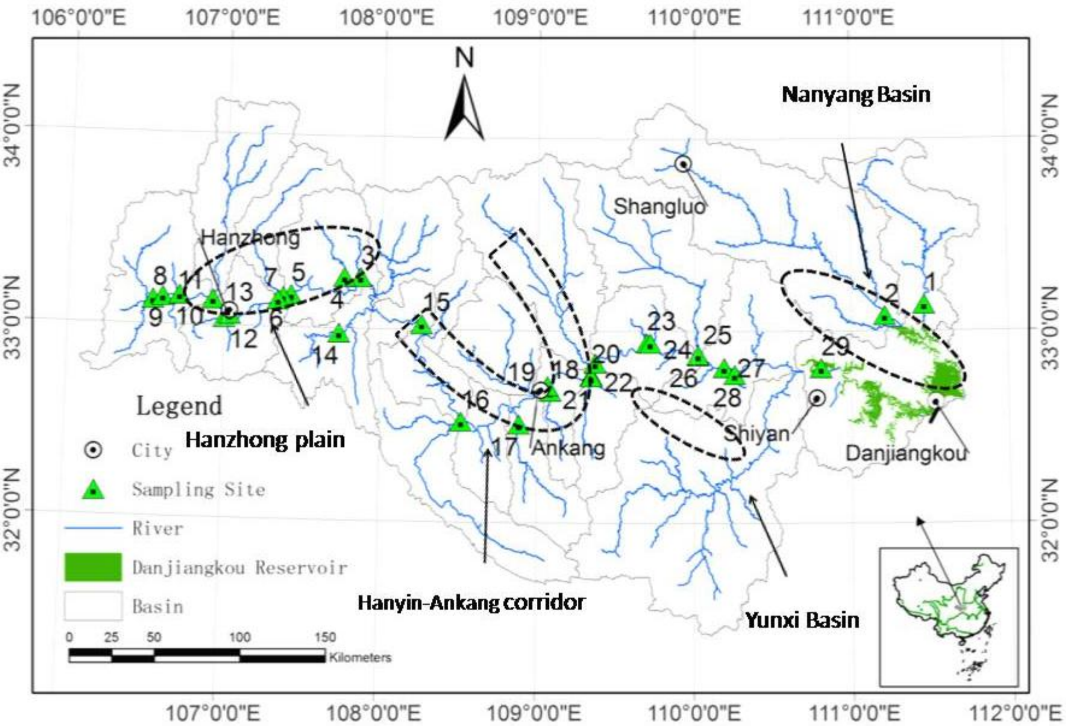
Table 3.Pearson correlation coefficients between measured water quality variables and diatom indices(n=27) in the dry season.

	Temp	EC	DO	pH	NH_4-N	NO_3-N	TN	TP	PO_4P	DOC	Cl^-	SO_4^{2-}	Ca^{2+}	K^+	Mg^{2+}	Na^+	Si
CEE			.425					-.471	-.466	**-.611****						-.420	
DESCY								-.394	-.447								
DI-CH	-.440					**-.530****	**-.516****			-.419	**-.588****			-.446		**-.525****	
EPID			**.511****							-.454	-.410					-.438	
IBD	-.386									-.485	**-.549****			-.445		**-.498****	
IDAP																	
IDP		**-.495****										-.403				-.388	
IPS										-.396	-.467					-.397	
SHE					-.406	**-.521****	**-.525****				**-.572****	-.402		**-.495****		**-.615****	
SID						**-.580****	**-.567****	-.411				-.398		-.396	-.420		
SLAD			.454					-.437	-.428	**-.609****						-.445	
TDI			.383					-.405	-.399	**-.595****	-.439					-.404	
TID								-.383			-.404						
WAT						-.433	-.437			**-.488****	**-.516****			-.384		**-.584****	

**significance at the 0.01 probability level marked in bold and assumed significant after Bonferroni correction.

Table 4. Prediction models with diatom indices as dependent variables using stepwise multiple regression in the dry season.

Dependant Variable	Formula	R square	Sig.(constant)	Sig.(variable 1)	Sig.(variable 2)	Sig.(variable 3)	Sig.(variable 4)
CEE	20.677-4.593DOC -22.450TP	0.490	0.000	0.002	0.028		
DESCY	15.314-14.122 PO_4-P	0.200	0.000	0.019			
DI-CH	13.367-0.452 Cl^-	0.346	0.000	0.001			
EPI-D	-15.699+2.796DO-0.386 Cl^-	*0.528*	0.033	0.000	0.001		
IBD	14.343-0.535 Cl^--29.821TP+0.131Ca^{2+}	*0.533*	0.000	0.000	0.008	0.038	
IDAP							
IDP	1.14+0.003EC	0.247	0.001	0.008			
IPS	12.506-0.463 Cl^--31.868TP+0.135Ca^{2+}	0.494	0.000	0.001	0.005	0.034	
SHE	16.113-1.264 Na^+	0.378	0.000	0.001			
SID	15.091-0.946 NO_3-N +6.603TP	0.477	0.000	0.001	0.018		
SLAD	19.858-3.384DOC	0.371	0.000	0.001			
TDI	17.039-4.780DOC	0.354	0.000	0.001			
TID	10.523-0.223 Cl^--20.726 PO_4-P	0.329	0.000	0.016	0.023		
WAT	16.030-3.595 Na^++17.440 NH_4-N-1.528 NO_3-N+0.022EC	*0.713*	0.000	0.000	0.000	0.001	0.009



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Hanzhong
Hanzhong plain
Hanyin-Ankang corridor
Yunxi Basin
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Fig. 1

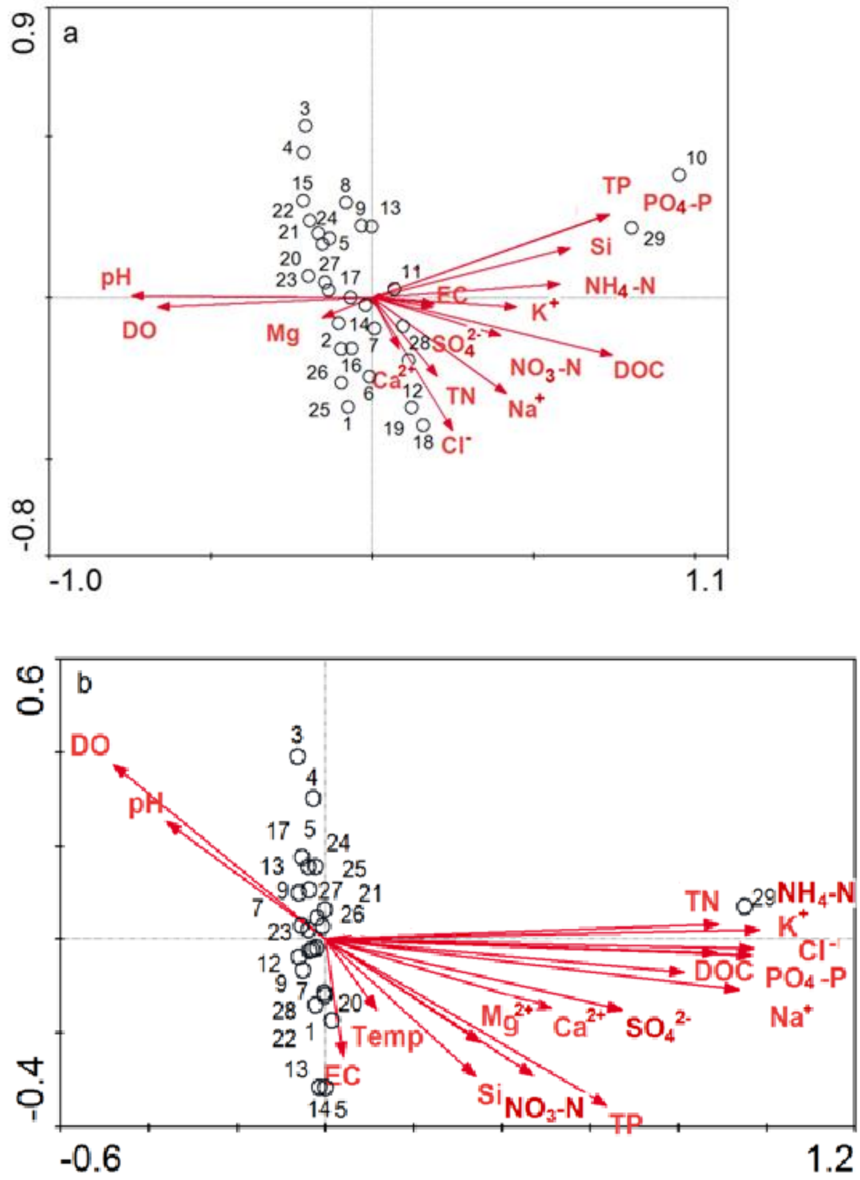

Fig. 2

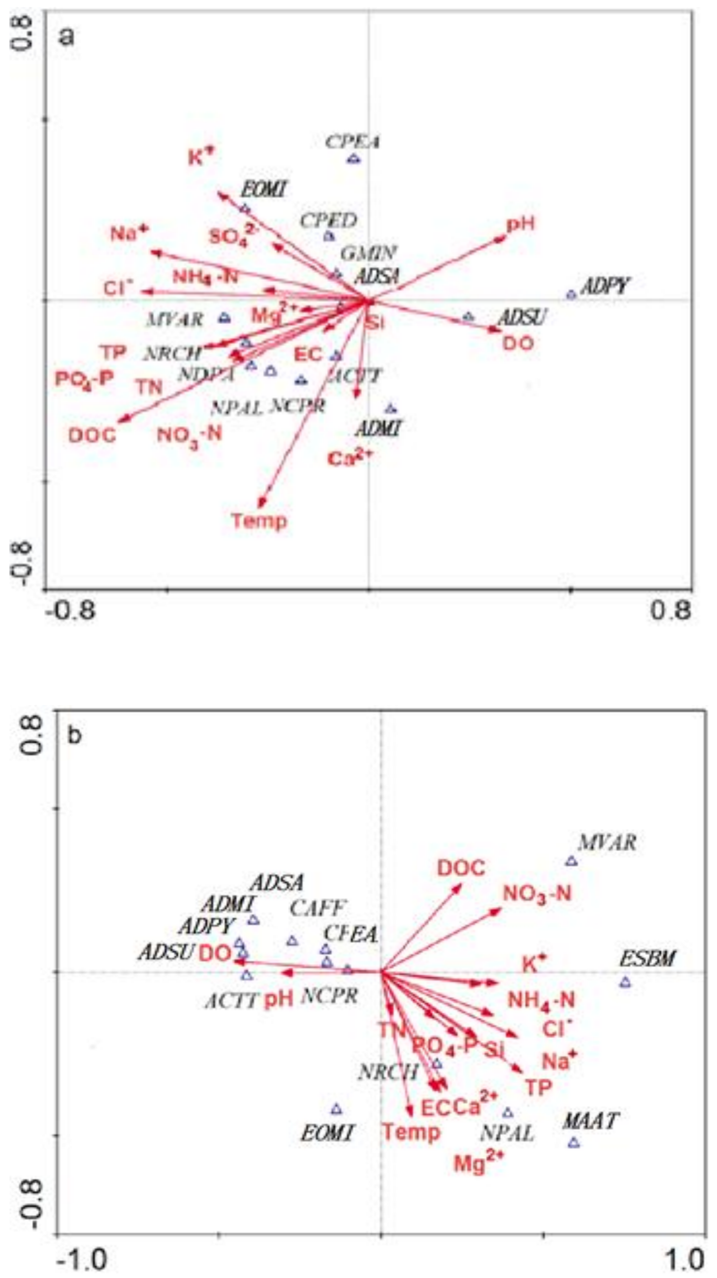

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Fig. 3

Appendix 1. List of common diatom taxa and abundances of them found in the studied rivers (common taxa here means some species appear in above two sampling sites including two sites)

Dry seaon	Wet season
Achnanthidium atomus	*Achnanthidium atomus*
Achnanthidium biasolettiana var. *biasolettiana*	*Achnanthidium biasolettiana* var. *biasolettiana*
Achnanthidium biasolettiana var. *subatomus*	*Achnanthidium biasolettiana* var. *subatomus*
Achnanthidium catenata	*Achnanthidium catenata*
Achnanthidium conspicua	*Achnanthidium conspicua*
Achnanthidium minutissima var.*minutissima*	*Achnanthidium minutissima* var.*minutissima*
Achnanthidium minutissima var. *jackii*	*Achnanthidium minutissima* var. *jackii*
Achnanthidium minutissima var. *saprophila*	*Achnanthidium minutissima* var. *saprophila*
Achnanthidium subatomoides	*Achnanthidium subatomoides*
Achnanthidium subhudsonis	*Achnanthidium subhudsonis*
Amphora inariensis	*Amphora inariensis*
Amphora montana	*Amphora montana*
Amphora oligotraphenta	*Amphora oligotraphenta*
Amphora pediculus	*Amphora pediculus*
Brachysira vitrea	*Amphora libyca*
Bacillaria paradoxa	*Caloneis molaris*
Caloneis bacillum	*Cocconeis pediculus*
Caloneis molaris	*Cocconeis placentula* var. *placentula*
Cocconeis pediculus	*Cocconeis placentula* var. *euglypta*
Cocconeis placentula var. *placentula*	*Cyclostephanos dubius*
Cocconeis placentula var. *euglypta*	*Cyclotella meneghiniana*
Cocconeis placentula var. *lineata*	*Cyclotella pseudostelligera*
Cyclostephanos invisitatus	*Cyclotella kuetzingiana* Thwaites
Cyclotella meneghiniana	*Cyclotella ocellata*
Cyclotella pseudostelligera	*Cymbella affinis*
Cyclotella kuetzingiana	*Cymbella neoleptoceros*
Cyclotella ocellata	*Cymbella tumida*
Cymbella affinis	*Cymbella turgidula*
Cymbella cymbiformis var. *nonpunctata*	*Cymbopleura kuelbsii*
Cymbella laevis	*Delicata delicatula*
Cymbella leptoceros	*Denticula tenuis*
Cymbella neoleptoceros	*Diatoma vulgaris*
Cymbella tumida	*Encyonema minutum*
Cymatopleura solea	*Encyonema perpusillum*
Delicata delicatula	*Encyonema reichardtii*
Delicata sinensis	*Encyonopsis leei* var. *sinensis*
Denticula tenuis	*Encyonopsis leei*
Diatoma vulgaris	*Eolimna minima*
Diploneis elliptica	*Fragilaria capucina* var. *capucina*
Encyonema caespitosum	*Fragilaria capucina* var. vaucheriae
Encyonema minutum	*Fragilaria* ulna
Encyonema perpusillum	*Fragilaria ulna* var. *acus*
Encyonema reichardtii	*Gomphonema clevei*
Encyonopsis microcephala	*Gomphonema minutum*
Encyonopsis leei	*Gomphonema parvulum*
Encyonopsis leei var. *sinensis*	*Gomphonema pseudoaugur*
Eolimna minima	*Gyrosigma scalproides*
Fragilaria biden	*Karayevia clevei*
Fragilaria capucina var. *capucina*	*Luticola mutica*
Fragilaria capucina var. vaucheriae	*Mayamaea atomus*

Fragilaria pinnata var. *pinnata*	*Melosira varians*
Fragilaria ulna (Nitzsch.)	*Navicula capitatoradiata*
Fragilaria ulna sippen angustissima	*Navicula caterva*
Fragilaria ulna var. *acus*	*Navicula cryptocephala*
Frustulum vulgaris	*Navicula cryptotenella*
Gomphonema clevei	*Navicula decussis*
Gomphonema helveticum	*Navicula gregaria*
Gomphonema minutum	*Navicula halophila*
Gomphonema parvulum	*Navicula lanceolata*
Gomphonema parvulum var. exilissimum	*Navicula menisculus* var. *menisculus*
Gomphonema pseudoaugur	*Navicula minuscula*
Gyrosigma acuminatum	*Navicula menisculus* var. *grunowii*
Gyrosigma scalproides	*Navicula molestiformis*
Karayevia clevei	*Navicula novaesiberica* Lange-Bertalot
Lemnicola hungarica	*Navicula oligotraphenta*
Mayamaea atomus	*Navicula pellliculosa*
Melosira varians	*Navicula pupula*
Melosira granulata	*Navicula radiosa*
Navicula atomus var. *permitis*	*Navicula radiosafallax*
Navicula capitatoradiata	*Navicula recens*
Navicula caterva	*Navicula reichardtiana*
Navicula cincta	*Navicula schroeteri* var. *symmetrica*
Navicula clementis	*Navicula subminuscula*
Navicula confervacea	*Navicula trivialis*
Navicula cryptocephala	*Navicula vandamii*
Navicula cryptotenella	*Navicula veneta*
Navicula decussis	*Navicula viridula* var. *germainii*
Navicula digitoradiata var.*rostrata*	*Navicula viridula* var. *rostellata*
Navicula gregaria	*Navicula contenta* var. *biceps*
Navicula ignota	*Navicula submolesta*
Navicula menisculus var. *menisculus*	*Navicula trophicatrix*
Navicula minuscula	*Nitzschia amphibia*
Navicula novaesiberica	*Nitzschia angustatula*
Navicula oligotraphenta	*Nitzschia capitellata*
Navicula pellliculosa	*Nitzschia dissipata*
Navicula phyllepta	*Nitzschia fonticola*
Navicula pupula	*Nitzschia frustulum*
Navicula radiosa	*Nitzschia linearis* var.
Navicula radiosafallax	*Nitzschia palea*
Navicula reichardtiana	*Nitzschia sigma*
Navicula reichardtiana var. crassa	*Nitzschia sinuata* (Thwaites) var. *delognei*
Navicula salinarum	*Nitzschia sinuata* (Thwaites) var. *tabellaria*
Navicula schroeteri var. *symmetrica*	*Nitzschia communis*
Navicula subminuscula	*Nitzschia liebetruthii*
Navicula subrotundata	*Nitzschia pumila*
Navicula viridula var. *germainii*	*Pinnularia subcapitata*
Navicula viridula var. *rostellata*	*Pinnularia subcapitata var. elongata*
Nitzschia amphibia	*Planothidium frequentissimum*
Nitzschia amphibioides	*Planothidium lanceolatum*
Nitzschia capitellata	*Planothidium rastratum*
Nitzschia denticula	*Reimeria uniseriata*
Nitzschia dissipata	*Sellaphora bacillum*
Nitzschia frustulum var. *minutu*	*Sellaphora seminulum*
Nitzschia gracilis	*Stephanodiscus neoastraea*
Nitzschia intermedia	

Nitzschia palea
Nitzschia paleaeformis
Nitzschia recta
Nitzschia sinuata (Thwaites) var. *delognei*
Nitzschia sinuata (Thwaites) var. *tabellaria*
Planothidium frequentissimum
Planothidium lanceolatum
Planothidium rastratum
Pinnularia subcapitata
Reimeria uniseriata
Sellaphora bacillum
Sellaphora seminulum
Stephanodiscus neoastraea
Surirella angusta
Surirella linearis
Surirella robusta var.robusta
Surirella suecica
Surirella terricola