Benthic diatoms in lakes: environmental drivers and ecological assessment

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Abstract

In order to protect or improve surface waters ecosystem response to pressures needs to be quantified. Diatoms are frequently used for assessing ecological status in streams and for reconstructing water quality of lakes. However, ecological status assessment of European lakes based on extant diatom assemblages is rare.

The overall aim of this thesis is to facilitate the application of benthic diatoms in water quality assessment of boreal lakes, using methods developed for stream assessment. Therefore, I investigated (i) the main environmental drivers of lentic diatom assemblages, (ii) the structural differences between lentic and lotic diatom assemblages (iii) the implications of differences between lake and stream diatom assemblages for assessment, (iv) the power of different aquatic organisms as indicators of nutrient and acidity status in lakes, and (v) ecological thresholds of diatom and phytoplankton assemblage structure along a nutrient gradient in boreal lakes.

The major environmental drivers of benthic diatom assemblage composition in boreal lakes were gradients in acidity and nutrient status. In contrast, the distribution of ecological diatom guilds seems to be decoupled from nutrient availability in acidic compared to high pH aquatic systems. Diatom assemblage composition can thus be recommended for ecological status assessment of boreal lakes, whereas species-specific growth morphology and the drivers affecting growth morphology need further study. Most diatom taxa occurred in Swedish lakes and streams, but differences in frequency and abundance among lakes and streams were noted. However, the diatom indices IPS and ACID, developed for streams, responded similarly to nutrient and acidity gradients, respectively, in Swedish lakes and streams and can thus also be recommended for ecological status assessment of lakes. In nutrient poor boreal lakes, both primary producers and primary consumers were correlated with nutrient concentrations. Primary producers, especially diatoms, displayed a stronger response to pH than macroinvertebrates, possibly due to shorter generation times implying faster response. Nutrient thresholds in taxonomic and guild structure of benthic diatom assemblages in boreal lakes were found at low TP concentrations (18-26 µg L^{-1}). However, caution is advised in interpreting the response of some species to nutrients, as pH dependencies might obscure the response of some nutrient sensitive diatom species to TP.

Keywords: benthic diatoms, lakes, environmental drivers, streams, indicators, phytoplankton, littoral macroinvertebrates, efficacy, nutrient thresholds

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Dedication

To my family…

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Gottschalk, S., Kahlert, M. (2012). Shifts in taxonomical and guild composition of littoral diatom assemblages along environmental gradients. *Hydrobiologia* 694:41-56.

II Kahlert, M. Gottschalk, S. (2014). Differences in benthic diatom assemblages between streams and lakes in Sweden and implications for ecological assessment. *Freshwater Sciences* DOI: 10.1086/675727

III Gottschalk, S., Kahlert, M. Response of aquatic organisms to gradients of nutrients and acidity in boreal lakes. (manuscript).

IV Gottschalk, S., Kahlert, M. Ecological thresholds of benthic diatoms and phytoplankton to nutrients in boreal lakes. (manuscript).

Papers I and II are reproduced with the permission of the publishers.

The contribution of Steffi Gottschalk (SG) to the papers included in this thesis was as follows:

I All authors planned the study. SG was responsible for collecting some diatom samples. Diatom sample processing, diatom enumeration, compilation of water chemistry data and statistical analyses were done by SG. SG wrote the paper with contributions from MK.

II All authors planned the study. Data on stream diatom assemblages and water chemistry were compiled by MK, SG was responsible for collecting some lake diatom samples and processing of all diatom samples. Diatom enumeration and compilation of water chemistry data from lakes were done by SG. Statistical analyses were done by MK, with contributions from SG. MK wrote the paper with contributions from SG.

III All authors planned the study. SG was responsible: for collecting some diatom samples, processing of all diatom samples, all diatom enumerations, compilation of phytoplankton, littoral macroinvertebrate and water chemistry data and statistical analyses. SG wrote the paper with contributions from MK.

IV All authors planned the study. SG was responsible: for collecting some diatom samples, processing of all diatom samples, all diatom enumerations, compilation of phytoplankton and water chemistry data and statistical analyses. SG wrote the paper with contributions from MK.

Abbreviations and terms

ACID	ACidity Index of Diatoms
ANOSIM	ANalysis Of SIMilarities
ANOVA	ANalysis Of VAriance
ASPT	Average Score Per Taxon index
benthic biofilm	association of algae, bacteria, fungi and small animals attached to a substrate, embedded in a matrix of mucopolysaccharides (Burkholder 1996)
benthic diatoms	diatoms living attached to or on a substrate in the littoral zone, part of the phytobenthos
C	Carbon
CCA	Canonical Correspondence Analysis
chl *a*	chlorophyll *a*
CO_2	Carbon dioxide
DCA	Detrended Correspondence Analysis
DFA	Discriminant Function Analysis
GLM	General Linear Models
H^+	Hydrogen ion
HCO_3^-	Bicarbonate
IndVal	Indicator Values according to Dufrêne and Legendre (1998)
IPS	Specific Pollution Sensitivity index
lentic	referring to standing or still water (i.e. lakes)
littoral	near shore areas in aquatic ecosystems, down to a depth were ~ 1% of the surface light levels are available
lotic	referring to flowing water (i.e. streams)
MILA	Multimetric Index for Lake Acidity
MANOVA	Non-metric Multivariate ANalysis Of Variance
MRPP	Multi-Response Permutation Procedures

N	Nitrogen
NMDS	Non-metric MultiDimensional Scaling
P	Phosphorus
pCCA	partial CCA
phytobenthos	micro- and macroalgae living attached to a substrate in the littoral zone
R^2	coefficient of determination
RDA	Redundancy Analysis
RMSE	Root Mean Square Error
SIMPER	analysis of SIMilarity PERcentage
sqrt	square root
SRP	Soluble Reactive Phosphorous (phosphate)
TEV	Total Explained Variance
TITAN	Threshold Indicator Taxa ANalysis
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorous
TPI	Trophic Plankton Index
VIF	Variable Inflation Factor

1 Introduction

Surface waters provide a number of ecosystem services to humans, such as water supply and purification, climate and flood regulation, fishery and recreation (Millennium Ecosystem Assessment 2005, Wallis *et al.* 2012). Due to population growth and economic development, water quality in surface waters is deteriorating worldwide (Schindler 2006, Smol 2008), affecting many ecosystem services (Millennium Ecosystem Assessment 2005). Blooms of nuisance algae due to excess nutrients, increased turbidity following macrophyte loss and loss of fish species due to oxygen depletion or acidification are examples of adverse human-induced effects on aquatic ecosystems and ecosystem services. Human-induced effects need to be quantified in order to protect or improve ecosystems. Emphasis has switched from simply measuring pollution (i.e. water chemistry variables) to assessing the structural and functional integrity of aquatic ecosystems using aquatic organisms. Water chemistry measurements are often restricted to providing a snap-shot of water quality, whereas bioindicators continuously record water quality (Lowe and Pan 1996). In Europe, the use of different aquatic organisms in environmental assessment of surface waters is mandatory, due to the implementation of the European Water Framework Directive (EU WFD; European Commission 2000). Ideally, different indicator organisms respond differently to the multiple pressures ecosystems are exposed to (Johnson and Hering 2009, Johnson *et al.* 2014), facilitating the integrated assessment of all aspects of ecological status.

Eutrophication, i.e. the excessive enrichment with plant nutrients phosphorus (P) and nitrogen (N), is the most widespread anthropogenic pressure on aquatic ecosystems worldwide (Schindler 2006). Primary producers are generally considered to be good indicators of eutrophication due to their reliance on inorganic nutrients (P and N) for growth (Stevenson *et al.* 2004, Hering *et al.* 2006, Johnson *et al.* 2006a, b). Among the lake (lentic) primary producers, phytoplankton and benthic diatoms (as part of the phytobenthos) are considered most efficient indicator organisms, as they have short generation times and rely on inorganic nutrients from the water column, in contrast to macrophytes which may use nutrients in the sediment when water column nutrient levels are low (Best and Mantai 1978, Barko and Smart 1981, Rattray *et al.* 1991). The response of phytoplankton assemblages to nutrient enrichment is assumed to be faster as they are directly exposed to nutrients in the water column (Cattaneo 1987) and have a higher affinity to inorganic nutrients than phytobenthos (reviewed by Vadeboncoeur and Steinman 2002). By contrast, the response of consumers is expected to be less pronounced and slower due to longer life cycles and the "indirect" relationship to nutrients. Indeed, the assemblage structure of primary producers (benthic diatoms) has been found to be stronger correlated to nutrient status than the structure of e.g. macroinvertebrates or fish assemblages in streams (Johnson and Hering 2009, Johnson *et al.* 2014).

Acidification, i.e. the release and deposition of sulphate and nitrate ions, is a large-scale environmental issue in Sweden. In acid-sensitive regions with weathering-resistant bedrock and limited amounts of base-cations, sulphate and nitrate ions will be accompanied by hydrogen ions in the runoff to the surface water system, causing a drop in pH. Primary producers are suggested to respond rapidly to changes in pH due to different ability to acquire the various forms of inorganic carbon (C) from the water column (Baekken *et al.* 2004). Either CO_2 or HCO_3^- or both can be used as carbon source for photosynthesis by different autotrophic species or taxonomic groups (Giordano *et al.* 2005, Roberts *et al.* 2007), resulting in distinct species turnover along pH gradients (Maurice *et al.* 1987). Availability of inorganic C species is determined by pH, with maximal free carbon dioxide (CO_2) levels at pH 4 and maximal bicarbonate (HCO_3^-) concentrations at pH 8.5 (Wetzel 2001). Consumers are expected to respond differently than primary producers, as they have longer generation times and life cycles (Lewis *et al.* 2007). Increased levels of hydrogen ions (H^+) impair gill function of consumers (ion regulation, osmoregulation, acid-base balance, nitrogen excretion and respiration; Brakke *et al.* 1992, Okland and Okland 1986). Both primary producers and consumers are additionally affected by toxic trace metals such as inorganic aluminium, as the bioavailability is affected by pH (Gensemer and Playle 1999). For example, in clear-water systems, metal bioavailability, and thus toxicity to organisms, increases markedly with decreasing pH (Gensemer and Playle 1999).

1.1 Benthic diatoms as part of the phytobenthos in lake food webs

In lakes, focus of research and environmental monitoring has traditionally been on the phytoplankton (Vadeboncoeur *et al.* 2002, Brucet *et al.* 2013), whilst benthic primary producers are often not considered in lake monitoring programs. Some countries, however, use macrophytes (aquatic vascular plants) or phytobenthos (Birk *et al.* 2012, U.S. EPA 2012, Brucet *et al.* 2013, Kelly 2013). Phytobenthos is an important part of lake food webs, in particular in shallow lakes (Vadeboncoeur *et al.* 2002): the phytobenthos can significantly contribute to lake primary production (0.5-92 %; Vadeboncoeur and Steinman 2002) and is an important food source to many consumers (Vadeboncoeur *et al.* 2002, Vander Zanden *et al.* 2011). Additionally, the presence of benthic primary producers (e.g. benthic diatoms) contributes to microbial nutrient cycling by stimulating bacterial processes as denitrification (Kalscheur *et al.* 2012) and leaf litter decomposition (Danger *et al.* 2013), by providing labile C sources to the bacteria.

Diatoms, characterised by a silica impregnated cell wall (frustule), are amongst the most common benthic primary producers, in addition to Cyanobacteria and Chlorophyta in lakes (Stevenson 1996). In environmental assessment, benthic diatoms are commonly used as a proxy for the entire phytobenthic assemblage for two reasons. First, inferences of lake nutrient status have been shown to be similar when based on benthic diatoms and the entire phytobenthos (including diatoms) (Kelly *et al.* 2008). Second, processing and analysis of diatoms is less problematic compared to the non-diatom “soft” algae of the phytobenthos, as diatom frustules are inert and thus easily preserved. Moreover, there is also a long tradition of diatom taxonomy; resulting in extensive floras to facilitate enumeration based on size, shape and sculpture of the empty diatom frustules (e.g. Krammer and Lange-Bertalot 1986-1991; Krammer 2000-2003; Lange-Bertalot 2001; Lange-Bertalot and Metzeltin 1996). Sensitivity and tolerance to environmental variables and optima along environmental gradients (e.g. pH, salinity, oxygen requirements, trophic state) have been empirically derived for many species (van Dam *et al.* 1994, Birks *et al.* 1990) and are frequently used in metrics for assessing stream water quality (Coste 1982, Kelly and Whitton 1995, Rott *et al.* 1999) or for reconstructing past water quality in lakes (Birks *et al.* 1990, Bennion *et al.* 2004). Diatom-based assessment often focusses on nutrient and acidity status, due to the direct relation between primary production and the inorganic macronutrients C, P and N.

In addition to taxonomic structure, diatom growth forms have been related to environmental gradients (Passy 2007, Berthon *et al.* 2011, Rimet and Bouchez 2012, Stenger-Kovács *et al.* 2013). Growth morphology, describing the position of diatom taxa in a biofilm, has been suggested to be an adaptation to resource availability (nutrients and light) and the degree of disturbance (grazing and shear stress, Passy 2007). Inside a biofilm, sharp resource gradients are established, with decreasing light levels from the top to the bottom (Meulemans 1987). Additionally, the establishment of a thick biofilm is usually suppressed under low nutrient conditions (Lange *et al.* 2011, Proia *et al.* 2012), favouring the low profile taxa, living firmly attached to the substrate (Passy 2007). Low guild diatoms are thus suggested to be adapted to low nutrient and light conditions at the bottom of the biofilm (Burkholder *et al.* 1990). Motile and high profile diatom taxa are suggested to be stronger competitors for dissolved nutrients (Passy 2007), as motile taxa are capable of moving to a suitable habitat and high profile taxa can easily access water column nutrients due to their exposed position in the upper part of the biofilm. Tychoplanktonic diatom taxa, on the other hand, are considered to be favoured in mesotrophic conditions (Ptacnik *et al.* 2008, Gottschalk 2011), as they have been suggested to be superior competitors for inorganic nutrients at moderate nutrient levels due to a high cell surface to cell volume ratio and efficient light harvesting (Reynolds *et al.* 2002). There is some uncertainty of the use of ecological guilds for predicting environmental gradient. First, observed guild affiliation of certain species may differ from that based on morphology (Passy 2007). Moreover, a guild is often assigned to all species of a genus (e.g. Berthon *et al.* 2011), while there are probably some genera comprising different guilds (e.g. Gomphonema species on short [low guild] or long stalks [high guild]; Lange *et al.* 2011). As observation of guild affiliation in live samples is difficult and time-consuming, researchers usually use literature compilations (e.g. Passy 2007; Rimet and Bouchez 2011, 2012). The benefit of using ecological traits to complement taxonomic composition is better elucidation of mechanisms underpinning ecological effects (e.g. for nutrients, DeNicola and Kelly 2014). Attempts to include trait information in diatom metrics for the assessing ecological status are few (Stevenson *et al.* 2013) and might be partly due to the above discussed uncertainties.

1.2 Assessment of ecological status based on benthic diatoms

Benthic diatom assemblage structure has been shown to be a precise indicator of changes in nutrient status in streams (Hering *et al.* 2006, Johnson *et al.* 2006a, b, Johnson *et al.* 2014). Accordingly, one might assume that benthic diatoms are likewise applicable for assessment of water quality in lakes. Indeed, diatom metrics developed for stream bioassessment have been shown to work equally well for lake bioassessment (King *et al.* 2000, Blanco *et al.* 2004, Poulíčková *et al.* 2004, Ács *et al.* 2005, Jüttner *et al.* 2010). However, the efficacy of benthic diatoms as indicators in lakes has rarely been studied and compared to other aquatic organisms, partly due to the traditional focus on phytoplankton in lake ecosystems (Vadeboncoeur and Steinman 2002, Vadeboncoeur *et al.* 2002). In streams, drivers of benthic diatom assemblage composition have been studied extensively, including water chemistry, land use and geography (e.g. Pan *et al.* 1999, Rimet *et al.* 2004, Soininen *et al.* 2004, Charles *et al.* 2006, Göthe *et al.* 2013). In lakes, only a few studies have related diatom assemblage composition to water chemistry, geography and catchment characteristics, and identified acidity, nutrient status, ionic concentrations or salinity as the main drivers of assemblage composition (King *et al.* 2000, Albert *et al.* 2009, Schönfelder *et al.* 2002, Jüttner *et al.* 2010). However, extrapolating these findings from one region to another is problematic, as lake types differ among regions. For example, lake types in the Nordic countries differ markedly from those commonly found in Central Europe, with higher variability in acidity, water colour and nutrient concentration. Thus, environmental factors and the gradients structuring littoral diatom assemblages might differ between Central and Northern Europe.

1.3 Differences in lentic and lotic benthic diatom assemblages

As discussed above, assessment of the ecological status of European lakes is dominated by phytoplankton-based tools, whereas methods based on benthic diatoms are less commonly used. For example, only seven out of 20 European countries that used phytoplankton-based methods also used phytobenthos-based methods (Brucet *et al.* 2013). Moreover, different approaches for the implementation of diatom-based assessment tools were followed in these countries: adopting metrics developed for streams (summarized in Kelly 2013), optimizing stream metrics for the application in lakes (Bennion *et al.* 2014) and development of lake-specific metrics (Schaumburg *et al.* 2004, Stenger-Kovács *et al.* 2007, Flemish Environment Agency 2009). Justification of the use of methods developed for streams in lake assessments or development of specific lake metrics are seldom given. Poulíčková *et al.* (2004) concluded that tools developed for streams provide meaningful predictions of lake nutrient status, but emphasized that indices need to be calibrated when used in other contexts, such as different geographical regions, substrates and ecosystem types, as species occurrences are driven by complex biotic interactions and abiotic gradients. Indeed, autecological parameters for widely distributed species may vary significantly among geographical regions (Charles *et al.* 2006, Alvarez-Blanco *et al.* 2011, Rott and Schneider 2014), suggesting the calculation of precise ecological data for each geographic region for successful water quality assessment. Also, the water quality assessment of a water body may vary if different substrates are sampled (Besse-Lototskaya *et al.* 2006). However, structural and functional differences between lotic and lentic benthic diatom assemblages are poorly studied.

The only direct comparison of diatom assemblages in streams and lakes to my knowledge showed significant differences in species composition, with differences attributed to unmeasured local physical factors such as current, disturbance and light regime (Soininen and Weckström 2009). Differences in lentic and lotic benthic diatom assemblage composition can be expected based on differences between these ecosystem types. Streams have unidirectional flow and often higher physical and chemical variability in streams compared to lakes (Allan 1995). For example, macroinvertebrate assemblages have been shown to differ between streams and lakes (Johnson *et al.* 2004), with water retention time argued as one of the main explanatory factors. Furthermore, the presence of a pelagic zone and phytoplankton is probably an important structuring factor for benthic diatoms in lakes, in contrast to streams. Phytoplankton can be the dominant primary producers in lakes, depending e.g. on lake morphometry (Vadeboncoeur and Steinman 2002). In particular, elevated nutrient levels can result in dense phytoplankton biomass and shading of benthic primary production (Sand-Jensen and Borum 1991, Scheffer *et al.* 2001). This effect is expected to be less in small or medium-sized streams as phytoplankton is rare (Kalff 2002).

1.4 Response of aquatic organisms to gradients of nutrients and acidity

Knowledge of taxon-specific response and uncertainty is essential for designing robust and cost-effective monitoring (Johnson *et al.* 2007). When evaluating the efficacy of bioindicators in assessment of ecological status, much focus has been on assessing the statistical power of different approaches (i.e. indices or metrics) to detect ecological change (Hering *et al.* 2006, Besse-Lototskaya *et al.* 2011, Cellamare *et al.* 2012, Dudley *et al.* 2013). By contrast, few studies have addressed the efficacy of different assemblages to detect environmental gradients and the uncertainties associated with using different taxonomic groups (Johnson *et al.* 2006b, Johnson and Hering 2009, Johnson *et al.* 2014). Response to environmental gradients can differ among different taxonomic groups, due to different constraints on life cycles (e.g. short and long; Lewis *et al.* 2007) and life history characteristics (e.g. primary producer, primary and secondary consumer; Johnson *et al.* 2006b, Johnson and Hering 2009). Moreover, biological response to human-induced and natural environmental gradients has been shown to vary across habitats within systems (e.g. between pelagic and benthic habitats of lakes; e.g. Stendera and Johnson 2008) and among systems (e.g. between lakes and streams; e.g. Johnson *et al.* 2014, or lowland and mountain streams; e.g. Johnson and Hering 2009).

1.5 Threshold responses of aquatic organisms

An ecological threshold is a point or short zone along a single or multi pressure gradient at which a sudden change in a certain parameter occurs, resulting in a shift of ecosystem condition or attributes (Groffman *et al.* 2006). Organisms are assumed to show a unimodal, symmetric response along broad environmental gradients (Potapova *et al.* 2004). However the response of aquatic organisms and organisms groups to environmental gradients is often asymmetric (Johnson *et al.* 2006b, Johnson and Hering 2009); many species exhibit TP responses following complex asymmetric patterns, for example threshold responses (e.g. diatoms in Potapova *et al.* 2004). The observed asymmetric “ecological” response to TP is attributed to the presence of additional species and biotic interactions, in contrast to the “physiological” response to TP that is assumed in the absence of species interactions (Potapova *et al.* 2004). The sudden disappearance of macrophytes with progressive eutrophication is a well-studied example of such an asymmetric or threshold community-response (Sand-Jensen and Borum 1991, Scheffer *et al.* 2001), as it is partly due to nutrient effects on other organisms, i.e. the increasing phytoplankton biomass shading macrophytes.

Thresholds in pressure-response relationships have become an important tool in environmental protection for management of eutrophication, aiming for healthy and stable ecosystems (Davies and Jackson 2006, Brucet *et al.* 2013). For example, numerical criteria based on ecological threshold response to nutrients may be used to define a border between a desired and undesired ecosystem state (Ptacnik *et al.* 2008, Stevenson *et al.* 2008, Smucker *et al.* 2013) and thus facilitate the precautionary principle. European countries are striving to achieve good ecological status of all surface waters (European Commission 2000). Quantification of the boundary between good or poor ecological status is therefore important for environmental objectives, as well as having strong economic implications. Failing to achieve at least good status may require expensive restoration procedures (European Commission 2000, Brucet *et al.* 2013).

For example, the sudden nutrient-induced loss of macrophytes, providing food sources and shelter from secondary consumers, affects all other trophic levels in lake food webs (Jeppesen *et al.* 2000, Scheffer *et al.* 1993). Similarly, abrupt shifts in benthic and planktonic algae assemblages will have consequences for consumers, for example, via shifts in edibility of algae (Johansson *et al.* 2013), algae nutrient ratios (Evans-White *et al.* 2009) or oxygen depletion as a consequence of degradation of excessive biomass (Istanovics 2009). Restoration of eutrophied lakes is often prevented for years by feedback mechanism as internal phosphorus loading (e.g. Moss 1990). Additionally, once an alternative stable state is reached, decreasing nutrient concentrations to the level at which the ecosystem shift occurred is often not sufficient to return the system to pre-disturbed conditions due to hysteresis (Scheffer *et al.* 2001).

2 Objectives

The overall objective of this work is to facilitate the application of benthic diatoms in water quality assessment of lakes, using methods developed for stream assessment. The specific objectives were to:

1. Determine the main drivers of benthic diatom assemblages in lakes (I)

2. Study structural differences between lentic and lotic diatom assemblages and important environmental drivers of both assemblages (II)

3. Study the implications of differences between lake and stream diatom assemblages for environmental assessment (III)

4. Compare the power of different aquatic organisms as indicators of nutrient and acidity status in boreal lakes (IV)

5. Search for ecological thresholds of benthic diatoms and phytoplankton assemblage structure along a nutrient gradient in boreal lakes (V)

3 Material and methods

3.1 Data collection

To assess the drivers of benthic diatom communities in lakes and implications for environmental assessment diatom assemblage structure was analysed in samples from 98 Swedish lakes (Fig. 1). Lakes were distributed over the whole country and cover broad gradients in nutrients and acidity; the main environmental gradients affected by humans in Swedish lakes. For a subset of lakes, data on phytoplankton (46 lakes) and littoral macroinvertebrate assemblage structure (35 lakes) were available from the Department of Aquatic Sciences and Assessment (Swedish University of Agricultural Sciences), Naturvatten i Roslagen AB and the Erken Laboratory at Uppsala University.

3.1.1 Benthic diatoms

Benthic diatoms were collected from the littoral habitat between 2003 and 2010 during autumn (late August to November) following the standard protocol (CEN 2003). Fourteen lakes were sampled during several seasons (spring and/or summer in addition to autumn) or several years. Eleven lakes were sampled at two to three different locations. Together, 123 diatom samples were collected. At each site diatoms were sampled from at least five cobbles (10-25 cm); if stones were not available diatoms were sampled from at least five submersed macrophyte stems. The biofilm on the upper surface of the stones was brushed off with a toothbrush and lake water, whereas the biofilm on macrophyte stems was removed by shaking the stems in lake water. The solution was then preserved with ethanol (final concentration about 70%). In the laboratory, samples were treated with hydrogen peroxide and hydrochloric acid (hot hydrogen peroxide oxidation; CEN 2003). Permanent slides were prepared using Naphrax (refractive index = 1.74, Brunel Microscopes Ltd).

Identification of benthic diatoms was done using a Nikon Eclipse 80i microscope (1000 times magnification with an oil-immersion Plan Apochromat objective), equipped with a camera. At least 400 valves were identified to the lowest taxonomical level based on the standard enumeration literature (Krammer and Lange-Bertalot 1986-1991; Krammer 2000-2003; Lange-Bertalot 2001; Lange-Bertalot and Metzeltin 1996). Taxa belonging to the *Achnanthidium minutissimum* (Kützing) Czarnecki complex (*A. minutissimum* and its varieties according to Tafel 32-34, Krammer and Lange-Bertalot 1991) were assigned to one of three groups according to the Swedish standard (Kahlert *et al.* 2007) for **paper I** and the calculation of IPS and ACID in **papers II** and **III**. The *A. minutissimum* groups were differentiated by mean cell width of 20 individuals: group 1 with a mean width <2.2 µm; group 2 with a mean width between 2.2-2.8 µm and group 3 with a mean width >2.8 µm. All diatom counting data were converted into relative abundance. Diatom taxa in the category "unidentified" were excluded from all statistical analysis. All taxa encountered were assigned to three ecological guilds (**paper I** and **IV**) according to their growth morphology according to Passy (2007) and Rimet and Bouchez (2011): low profile guild (having a short stature, belonging to the biofilm understory), high profile guild (large or colony-forming taxa belonging to the biofilm overstory) and motile guild (freely moving within the biofilm). In addition to the three ecological guilds a fourth guild (tychoplanktonic [adapted to the pelagial according to Rimet and Bouchez (2012)]) was used as category in threshold analysis (**paper IV**). Furthermore, taxa were grouped into two groups by their ability to fix nitrogen due to cyanobacterial endosymbionts (**paper I**).

3.1.2 Phytoplankton

Phytoplankton samples were collected from the epilimnion in the central part of each lake; the epilimnetic layer was determined via a temperature profile for each lake and sampling occasion. When lakes were not stratified samples were taken from 0-8 m depth. Phytoplankton samples were preserved with Lugol's iodine solution in the field and kept dark until analysed. Analysis was done with an inverted light microscope (CEN 2006), using a modified Utermöhl technique according to Olrik *et al.* (1998). Taxa were identified to the lowest taxonomical level (usually species level) and biovolume (mm^3 L^{-1}) was estimated from cell geometry and the abundance of each taxon. Small colonies were counted as one algal unit due difficulties in separating the individual cells.

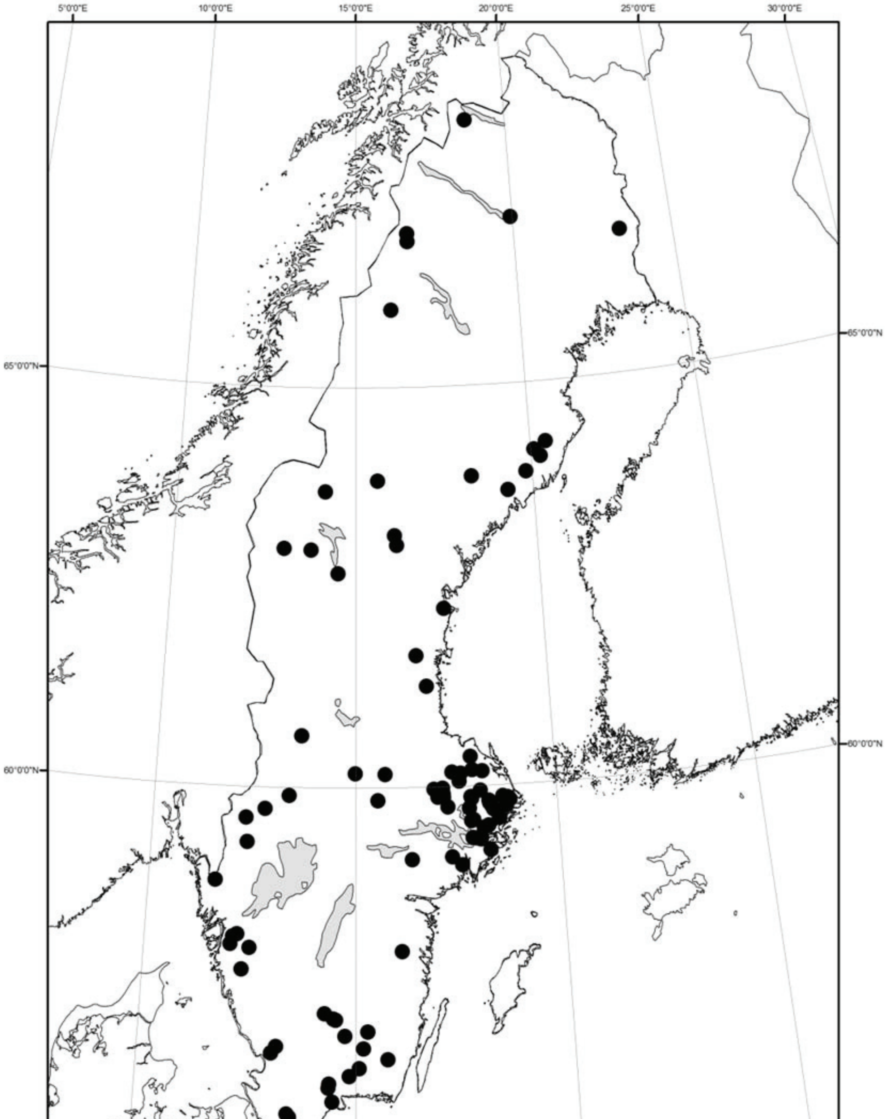

Figure 1. Location of the 98 studied Swedish lakes.

3.1.3 Littoral macroinvertebrates

Macroinvertebrate samples were collected from the littoral using standardised kick sampling with a hand net (0.5 mm mesh). A composite sample of five standardised kick samples (sampling duration per kick: 20 s, sampling distance per kick: 1 m, sampling depth: 0.5-1 m) was taken along a 10 m long, stony, vegetation-free stretch in each lake. Samples were preserved in 70% ethanol in the field. Sorting of the macroinvertebrates was done using dissecting and light microscopes. All taxa were identified to the lowest possible taxonomic resolution (usually species level), except for Oligochaeta.

3.1.4 Physicochemical data

Water chemistry data were analysed at the Department of Aquatic Sciences (**paper I-IV**); additionally data were provided by Erken Laboratory at Uppsala University (1 lake), Ekologgruppen i Landskrona AB (2 lakes), Stockholm Vatten AB (2 lakes) and Stina Drakare at the Deptartment of Aquatic Sciences and Assessment (5 lakes). Water chemistry data samples were collected from the epilimnic layer (water depth: 0.5 m) in the central part of the lakes on one or several occasions per year. Water chemistry data used for statistical analyses were annual mean values of the 12 months before the biology samples were taken. Benthic diatoms, phytoplankton and macroinvertebrates were sampled at different times of the year, resulting in individual data sets on mean annual water chemistry, i.e. one data set for each taxonomic group.

Geographical information (lake area, altitude, lake depth) was gathered from the database Svenskt Vattenarkiv (Swedish Meteorological and Hydrological Institute), catchment area and catchment land use data were determined with the database Svenska Marktäckedata, part of the CORINE Land Cover database (European Environment Agency). The category “forest” comprised coniferous, mixed and deciduous forests, including forests on wetlands. The category “anthropogenic” comprised the following land use practices: pasture, agriculture, exploited land, densely populated area, planted area, clear cut and other cultivated land. Lake depth classification of the lakes and assignment of the lakes to an ecoregion were done according to the Swedish lake classification (Swedish Agency for Marine and Water Management 2011); for some lakes the modelled lake depth class was taken from the database Vatteninformationssystem Sverige (http://www.viss.lst.se/). Data on quaternary deposits and bedrock type in the catchments were obtained from the Swedish National Atlas (Fredén 1994).

3.2 Statistical analysis

Biological indices, according to the Swedish environmental quality criteria, were calculated to evaluate the effects of eutrophication (**paper III**); the Specific Pollution Sensitivity Index (IPS, Coste 1982) based on benthic diatoms, the Average Score Per Taxon index (ASPT, Armitage *et al.* 1983) based on littoral macroinvertebrates and total phytoplankton biomass, Trophic Plankton Index (TPI) based on phytoplankton, epilimnion chlorophyll *a* concentrations and proportion of cyanobacteria (Willén 2007). Acidity was evaluated using the ACidity Index of Diatoms (ACID; Andrén and Jarlman 2008) for benthic diatoms, the Multimetric Index for Lake Acidity (MILA; Johnson and Goedkoop 2007) for macroinvertebrates and taxa richness (Willén 2007) for phytoplankton. Calculation of benthic diatom indices for both lakes and streams (**paper II** and **III**) were done using OMNIDA 5.3 (Lecointe *et al.* 1993).

For ordination analysis, discriminant function analysis and regression analysis environmental variables, with the exception of categorical variables and pH were transformed; catchment data (percent land use, quaternary deposits and bedrock type) were arcsine sqrt transformed, the remaining variables were log (x+0.1)(**paper I, III, IV**) or log (x) (**paper II**) transformed.

3.2.1 Drivers of benthic diatom assemblage composition

To explore the relationship of benthic diatom assemblage composition and 51 environmental variables (Table 1; additionally latitude and longitude and dummy variables for lake depth, sampling season, substrate and Swedish ecoregion 1-7) in 73 lakes (98 sites) we used canonical correspondence analysis (CCA) using CANOCO 4.5 (ter Braak and Šmilauer 2002). To avoid the influence of rare taxa only diatom taxa occurring in at least three samples with a maximum relative abundance higher than one percent were included in multivariate analyses. Furthermore, diatom relative abundances were square-root (sqrt) transformed to downweight the influence of dominant taxa. Detrended correspondence analysis (DCA), with detrending by segments and downweighting of rare taxa, gave a gradient length of 4.6 SD (first DCA axis) indicating unimodal responses. A series of CCA were run to test for multicollinearity in the explanatory dataset. Hill's scaling, recommended for long gradients, was chosen with focus on inter-sample distances. Variables with a high variable inflation factors (VIF; >20) were omitted from subsequent analysis (ter Braak and Šmilauer 2002). Stepwise, the variable with the highest VIF was deleted from the dataset and a new CCA was run until all variables with a VIF>20 were omitted (alkalinity, ecoregion 4, latitude, % granite, conductivity, % bare rock, TP, TN and TOC were omitted). Subsequently, a CCA was run with manual forward selection, Monte Carlo permutation tests (full model, n = 99) and Bonferroni correction of the significance levels to determine the factors significantly contributing to the model. Significance of all axes were tested using permutation tests (n = 99) in R version 2.12.2 (R Development Core Team 1999-2012), using the vegan package.

To quantify the importance of the main drivers of diatom assemblage composition partial CCA was done using the varpart function included in the vegan package for R version 2.12.2 (R Development Core Team 1999-2012). The important explanatory variables were classified into different groups: local (water chemistry), catchment (land use, lake surface area, catchment area, quaternary deposits, bedrock type) and geography (latitude, longitude, altitude). Total variance was partitioned into the unique variance for each group after removing the effects of the remaining variable groups and combined variance of the combined groups.

3.2.2 Distribution of ecological guilds and diatoms inhabited by nitrogen-fixing endosymbionts

To explore if changes in water chemistry are reflected in distinct distribution patterns of ecological guilds and diatoms with nitrogen-fixing endosymbionts, cluster analysis (Bray-Curtis similarity measure (paired-group algorithm) was applied to group lakes according to their diatom community composition using PAST 2.05 (Hammer *et al.* 2001). To determine the environmental variables which best differentiate the lake groups based on community composition discriminant function analysis (DFA) with stepwise forward selection was done in JMP 8.0.1 (SAS 2009-2012). Categorical variables, quaternary deposits and bedrock type were excluded from this analysis. Lakes were separated into three groups based on diatom assemblage structure: (1) acidic and nutrient poor, (2) circumneutral and nutrient poor and (3) alkaline and nutrient rich. Analysis of Variance (ANOVA) with a subsequent post hoc test (Tukey HSD) was performed in JMP 8.0.1 (SAS 2009-2012) to test the differences in ecological guild composition and distribution of nitrogen-fixing diatoms (sqrt transformed, mean abundances) between the lake groups.

Table 1. Minimum, mean and maximum values of the environmental variables related to benthic diatom composition in CCA. Latitude, longitude and categorical variables are not shown.

	variable	minimum	mean	maximum
water chemistry	pH	4.9	7.1	9.2
	conductivity (mS/m)	0.8	13.4	54.7
	alkalinity/ acidity (mekv/L)	-0.02	0.77	3.02
	ammonia (µg/L)	3.0	37.6	1168.0
	nitrite & nitrate (µg/L)	1.0	89.2	1247.3
	total nitrogen (µg/L)	66.0	665.7	2984.0
	phosphate (µg/L)	1.0	12.1	262.0
	total phosphorus (µg/L)	2.0	36.2	433.0
	silica (µg/L)	0.0	1.7	6.6
	absorbance	0.004	0.126	0.574
	total organic carbon (mg/L)	0.9	11.8	28.2
geography	altitude (m a.s.l.)	1	154	1155
catchment	lake area (km^2)	0.0	3.6	24.7
	catchment area (km^2)	0.4	528.5	22649.0
land use	wetland (%)	0.0	2.7	22.5
	water (%)	1.3	11.9	29.4
	forest (%)	0.0	57.6	88.5
	anthropogenic activity (%)	0.0	20.5	79.9
bedrock type	granite (%)	0.0	57.9	100.0
	gabbro (%)	0.0	1.2	35.3
	gneiss (%)	0.0	12.6	100.0
	acidic igneous rocks (%)	0.0	12.2	100.0
	basic igneous rocks (%)	0.0	0.6	20.8
	sandstone (%)	0.0	1.1	77.7
	schist/ greywacke (%)	0.0	8.3	100.0
	limestone (%)	0.0	4.8	100.0
quaternary deposits	peat (%)	0.0	4.1	48.9
	clay & silt (%)	0.0	4.6	41.8
	coarse sediment (%)	0.0	0.5	13.0
	glacial outwash (%)	0.0	7.1	100.0
	clay moraine (%)	0.0	4.5	72.1
	moraine (%)	0.0	48.0	100.0
	bare rock (%)	0.0	28.2	100.0
	moraine weathering (%)	0.0	1.7	75.3

3.2.3 Differences in lentic and lotic diatom assemblages

Data on the benthic diatom assemblage structure of Swedish streams were available from the Department of Aquatic Sciences and Assessment, lotic diatom samples were collected, processed and analysed in the same manner as the lentic diatom samples. To facilitate the comparison between lentic and lotic benthic diatoms, 179 streams were selected to be distributed over the same geographical region and displaying broad environmental gradients in nutrient concentrations and acidity as the 98 lakes. There were no significant differences between the environmental conditions observed in lakes and streams (non-metric multivariate analysis of variance [MANOVA], $p = 0.95$, Bray-Curtis distance, 9999 permutations; PAST 2.17b [Hammer *et al.* 2001]). Differences in composition between stream and lake diatom assemblages were tested at the genus and species levels. For the genus level analyses, all species within a genus were summed. All diatom data were harmonised according to conventions adopted by the NORdic-Baltic network for Benthic Algae in Freshwater (Kahlert and Albert 2005), and the species lists were checked to ensure harmonisation of species names.

To test the hypothesis of no difference in diatom composition between streams and lakes, Multi-Response Permutation Procedures (MRPP) with relative Euclidean distance implemented in the software PCOrd 6.08 (McCune and Mefford 2011) was used on genus- and species-level data (**paper II**). Rare taxa were omitted from the analysis to reduce noise (species or genera occurring only once in the data set or having a relative abundance <1% in the entire data set). For the species analysis, taxa identified only to genus were omitted from the analysis.

CCA was used to study relationships between taxa (both genus and species level) and environmental variables (**paper II**). The recommendations of McCune and Grace (2002) were followed for data inspection and multivariate analyses. Rare taxa were removed as above for the MRPP analyses, and diatom taxa data were arcsine sqrt transformed prior to analysis. The environmental matrix contained values of pH, TP, TN, absorbance, latitude, longitude, altitude, catchment size and the percentage of intensive agriculture and “forest” in the catchment. For the CCA, axis scores were centred and standardised to unit variance, and axes were scaled to optimise representation of sites. Scores for graphing were derived by linear combinations of environmental variables (LC scores). The null hypothesis for the Monte Carlo permutation test was no relationship between the taxon and the environmental variable matrices. The number of randomisations was randomly set to 563 (species analysis) and 5908 (genus analysis), but a stable solution was achieved after 47 and 18 iterations, respectively. Intraset correlation coefficients (ter Braak 1986) between environmental variables and the CCA axes >0.5 were interpreted as strong relationships. To quantify the importance of habitat as a predictor of the diatom assemblage composition, pCCA was performed in CANOCO 4.5 (ter Braak and Ŝmilauer 2002). The environmental variables were sorted into 2 groups: ecosystem type and other (all other environmental variables). Total explained variance (TEV) was partitioned into the unique variance for each group after removing the effects of the remaining variable group and the combined variance of the combined groups.

Indicator species analysis (IndVal) implemented in the software PCOrd 6.08 was used to compare the taxonomic composition of streams and lakes (**paper II**). The method gives an indicator value for each taxon that weights its relative abundance in streams and in lakes and relative frequency across all sites. Relative frequency is calculated to prevent rare taxa, which might be distributed more randomly than common taxa, from receiving a too high indicator value for either group. The statistical significance of each indicator value was tested by a Monte Carlo permutation test with 999 randomisations. A 'significant indicator' defined by this method describes a taxon (not necessarily a species) with a high and statistically significant indicator value. In our study, an indicator taxon was defined as a taxon typical of a certain ecosystem type (lake or stream) rather than an indicator used for assessment. Some taxa had high indicator values in the IndVal analysis but were not obvious indicators for one ecosystem type. To identify distinct ecosystem type indicators, the indicator values were used in combination with the sum of the differences between relative abundance and relative frequency for each taxon in lakes and streams.

3.2.4 Response of aquatic organisms to gradients of nutrients and acidity

Non-metric multidimensional scaling (NMDS) was used to condense assemblage data of 35 lakes into three multivariate metrics for further analysis: scores of the first, second and third NMDS axes (**paper III**). NMDS ordinations along three axes were based on Bray-Curtis dissimilarity matrices and were done separately for each taxonomic group. Species by site matrices for benthic diatoms, phytoplankton and macroinvertebrates were sqrt transformed to reduce the influence of abundant taxa. NMDS analysis was done using the function metaMDS (auto-transformation set to false) implemented in the vegan package for R version 3.0.1 (R Development Core Team 1999-2012).

We used regression analysis in JMP 10.0.0 (SAS 2009-2012) to compare the power of benthic diatoms, phytoplankton and macroinvertebrates from 35 lakes as indicators of nutrients and acidity (**paper III**). The NMDS scores of the first, second and third axes of each taxonomic groups were regressed against the biological available SRP (log10 SRP as a measure of nutrient status) and pH (as a measure of acidity). Additionally, NMDS scores were plotted against TP levels (log10 TP) to be able to compare results from this study with other studies. Linear and quadratic models were calculated applying the least-squares minimization approach. The statistical fit of the two models compared to a horizontal line at the mean was tested by ANOVA (null-hypothesis: slope equal to zero). Only significant models were taken into consideration. If both models were significant, the quadratic model was chosen to describe the relationship between biological and environmental variables if the addition of the quadratic term improved the fit compared to a linear model. This was tested using a t-test (null hypothesis: b2 coefficient equal to zero). When more than one multivariate metric of each biological indicator (i.e. scores of several NMDS axis) was significantly related to one environmental parameter, only the relationship with the highest precision (adjusted R^2) was displayed and discussed.

A comparison of the efficacy of diatoms, phytoplankton and macro-invertebrates was done using three metrics derived by regression analysis, according to Johnson and Hering (2009). First, the coefficient of determination (adjusted R^2) was considered as a measure of precision; i.e. the variance in the biological data explained by nutrients or acidity. Relationships were considered strong when 50% or more variation in the biological multivariate metrics or biological indices were explained by the explanatory variable (adjusted R^2 ≥ 0.5). Second, the slope (the magnitude of change) of the predicted model was interpreted as sensitivity of biological groups to environmental gradients, with high slope values indicating a high sensitivity. If a quadratic fit was chosen the slope was calculated as the sum of the absolute values of both slope coefficients. Third, the root mean square error (RMSE) was considered as a measure of the robustness of the model or prediction. The RMSE is the standard deviation of the random error of a model, i.e. an estimate of the spread of the values of the response variable around the regression line; small RMSE values thus indicate a robust model.

3.2.5 Response of indices based on different organism groups to gradients of nutrients and acidity

General Linear Models (GLM), implemented in the software STATISTICA, were used to test if the diatom indices IPS and ACID differed between lakes and streams and to show the impact of ecosystem type, i.e., whether potential differences were important for assessment (**paper II**). GLM tests whether the slope or the intercept of the relationships of ACID vs pH and IPS vs TP differed between lakes and streams. For the ACID tests, all sites with pH >8.4 were removed from the analysis because more lakes than streams were in this pH range.

Biological indices based on benthic diatoms, phytoplankton and macroinvertebrates (according to the Swedish environmental quality criteria) were plotted against SRP and TP or pH using JMP 10.0.0 (SAS 2009-2012), and the adjusted R^2 were used as a measure of precision (**paper III**).

3.2.6 Threshold response of primary producers to TP

In this study, Threshold Indicator Taxa ANalysis (TITAN; Baker and King 2010) was used to detect thresholds in the assemblage structure of benthic diatoms and phytoplankton along total phosphorus gradients in Swedish lakes (benthic diatoms: n = 107 sites [3-433 µg TP L^{-1}], benthic diatoms and phytoplankton: n = 60 sites [2-74 µg TP L^{-1}]) (**paper IV**). Taxonomic composition of both groups and ecological diatom guilds were examined for shifts in the abundance or biovolume of the single species or functional traits. Only taxa occurring at least in 5% of all samples and determined to species (benthic diatoms) or genus level (phytoplankton) were included. Taxa abundance, biovolume and guilds were log10 (x+1) transformed to down-weight dominant taxa or traits.

TITAN calculates the indicator response to an environmental gradient individually for each taxon, comprising the magnitude, direction and uncertainty of change points in the taxon abundance and frequency. The response is based on Indicator Values (IndVal; Dufrêne and Legendre 1998); the threshold of a taxon is the change point with the IndVal maximum. Bootstrapping (n = 500) is applied to test the reliability and purity of the threshold indicator taxa. A taxon is considered a pure indicator if the response direction (e.g. decreasing or increasing) is consistent with the observed response direction in at least 95% of all bootstrap samples. Decreasing taxa are negatively responding to an environmental variable and can be considered sensitive to that variable, whereas increasing taxa are positively responding and considered tolerant. A taxon is regarded a reliable indicator if the magnitude of the IndVal is equal to or larger than the observed IndVal in at least 95% of the bootstrap samples. To determine estimates of change independent of abundance or frequency, all IndVal are rescaled to z scores, which is the basis for estimating assemblage thresholds. The change point along the TP gradient having the largest cumulative positive (sum (z+)) and negative z scores (sum (z-)) correspond to the positive and negative assemblage threshold. The values of the sum (z) scores are a general estimate of the strength of response, i.e. low values indicating a weak or variable response of the single taxa. The 5% and 95% quantiles of the cumulative threshold frequency distribution in 500 bootstrap replicates provide an estimate of uncertainty around the assemblage threshold. The analyses were done in R version 3.0.1 (R Development Core Team 1999-2012), using the mvpart package and the TITAN script provided by Baker and King (2010).

4 Results and discussion

4.1 Environmental drivers of benthic diatom assemblages

Local water chemistry is the main predictor of diatom community composition in Swedish lakes according to ordination analysis (**paper I** and **II**). Species turnover was mainly driven by gradients in acidity (pH) and nutrient status (P and N levels), in agreement with studies from Northern and Central Europe (King *et al.* 2000, Schönfelder *et al.* 2002, Albert *et al.* 2009, Soininen and Weckström 2009, Jüttner *et al.* 2010). Factors such as geography and catchment variables (land use and hydromorphology) are important determinants of local water chemistry (Stevenson 1997), but were of secondary importance for benthic diatom species turnover in Swedish lakes.

Besides taxonomical structure, diatom taxa inhabited by nitrogen-fixing endosymbionts and ecological guilds based on the growth morphology were differently distributed under shifting acidity and nutrient conditions (**paper I**). Surprisingly, acidic, oligotrophic lakes were co-dominated by motile and high profile taxa. Motile and high profile taxa are suggested to become prevalent under high nutrient conditions (Passy 2007), however, light has a stronger effect on guild distribution than nutrients (Lange *et al.* 2011). Although acidic lakes were characterised by low nutrient levels, they also had low mean TOC levels. Consequently, light levels were probably higher in the studied acidic lakes as light quantity (depth of the euphotic zone) is often increasing with decreasing TOC concentrations (Steinberg 2003). Furthermore grazing pressure decreases with decreasing pH (Stokes 1986), probably benefiting the high profile taxa of the overstory of biofilms that are most vulnerable to grazing (Steinman 1996). The dominance of motile and high profile taxa in acidic, oligotrophic lakes is based to the high abundances of motile *Brachysira* Kützing and *Frustulia* Rabenhorst species, and high profile species belonging to *Eunotia* Ehrenberg. Guild assignment was based on Passy (2007), however, different growth morphologies are reported for the dominant genera in acidic, oligotrophic lakes: *Brachysira* may grow unattached or attached to the substrate by stalks (Spaulding and Edlund 2009), *Frustulia* may grow as single cells or as colonies in tubes (Spaulding and Edlund 2008) and *Eunotia* may grow singly, attached to the substrate by stalks or in long colonies (Furey 2010). There are two possible explanations for variations in growth morphology. Either the growth morphology is species- and not genera-specific, or it is driven by abiotic and biotic gradients. Hence, the application of ecological guilds for ecological status assessment should be used with caution, species-specific growth morphology and the drivers affecting growth morphology need to be studied further, especially in low pH systems.

Alkaline, nutrient rich lakes had significantly more motile taxa than the circumneutral, nutrient poor lakes, in agreement with Passy (2007), Berthon *et al.* (2011) and Lange *et al.* (2011), but we did not observe an increased abundance of high profile diatoms. The benthic diatoms *Rhopalodia* Müller and *Epithemia* Kützing, inhabited by nitrogen-fixing endosymbionts, were nearly exclusively found in alkaline and nutrient rich lakes (**paper I**). *Epithemia* is usually observed in alkaline water (Spaulding 2010). *Rhopalodia* and *Epithemia* may become dominant in nitrogen limited systems (Marks and Lowe 1993). Most Swedish lakes inhabited by these two genera had low N: P ratios, though commonly higher than the proposed threshold ratio of TN:TP =16 (Redfield 1958).

4.2 Differences in lentic and lotic diatom assemblages

Gradients in acidity and nutrients were the main drivers of benthic diatom assemblage composition in Swedish lakes and streams; however, lentic and lotic diatom assemblages differed as frequency and occurrence of diatom species and genera varied among ecosystems types (**paper II**). About 8% of the explained variance in the genus data and 10% of the explained variance in species composition was attributable to ecosystem type, whereas most variation (>85%) was explained by pH and nutrient status. In general, the differences found between lakes and streams are difficult to explain as the niches of different diatom species and genera are poorly known (McCormick 1996, Mann and Chepurnov 2004), with only a few exceptions (Jewson 1992, McDonald *et al.* 2007, Whitton *et al.* 2009). Little is known of the relative importance of biotic and abiotic drivers for occurrence and frequency of single diatom species or genera. Indicator taxa were identified for both ecosystems; most taxa occurred in both streams and lakes but were less abundant or frequent in one or the other system. The littoral zone of a lake can resemble a stream habitat in many ways (Kalff 2002), and therefore many similar diatom taxa are expected.

Ordination separated lake from stream sites along an axis correlated to ecosystem type and light absorbance of the water. Absorbance is often used as a proxy for water colour or (coloured) organic carbon levels (Wunsam *et al.* 2002); high organic carbon levels affect the aquatic biota by mitigating toxicity of heavy metals, attenuating potentially harmful ultraviolet radiation, reducing the bioavailability of nutrients (Jones 1998) and inducing light limitation (Hill 1996). In Sweden, lakes have a lower light absorbance than streams with similar water chemistry (Fölster *et al.* 2007), probably due to the higher retention time in lakes, allowing for sedimentation of humic material (Kalff 2002, Fölster *et al.* 2007).

4.3 Response of aquatic organisms to gradients of nutrients and acidity

Earlier studies have shown changes in primary producer assemblage composition at low TP levels (<50 µg L^{-1}), i.e. where nutrients are still limiting (Ptacnik *et al.* 2008, Johnson and Hering 2008, Johnson *et al.* 2014). By contrast, changes in macroinvertebrate assemblages have been shown to occur at much higher nutrient concentrations (Johnson and Hering 2009, Johnson *et al.* 2014). Comparing benthic diatoms and littoral macroinvertebrate assemblages showed similar precision to SRP concentrations (**paper III**), which contrasts with our prediction that primary producers are better related to nutrients than consumers. Also somewhat surprisingly was our finding that phytoplankton displayed a very precise response to TP, but a considerably less precise response to SRP (**paper III**).

Aquatic primary producers displayed a strong response to pH, whereas the relation between pH and littoral macroinvertebrates was very weak (**paper III**). The relationship of benthic diatoms to acidity had the highest precision and sensitivity and had low prediction error. Benthic diatom assemblages commonly display clear shifts from acid-sensitive to acid-tolerant taxa with decreasing pH (**paper I**, King *et al.* 2000, Andrén and Jarlman 2008) and are routinely applied for inference of contemporary and historical pH (Renberg *et al.* 1993, Andrén and Jarlman 2008, Battarbee *et al.* 2010). Schneider *et al.* (2013) suggested that benthic diatom assemblage composition is closely related to pH due to a greater physiological stress exerted by shifts in pH compared to shifts in nutrient levels.

4.4 Application of stream assessment tools in lakes

The diatom indices IPS and ACID, developed for streams, responded similarly to nutrient and acidity gradients in Swedish lakes, in agreement with findings from other studies (Blanco *et al.* 2004, Poulíčková *et al.* 2004, Ács *et al.* 2005, Cellamare *et al.* 2012, Novais *et al.* 2012). Thus, our findings imply that diatom stream indices can be used for ecological status assessment of lakes when excess nutrients or acidification are the main pressures (**paper I** and **II**). However, this recommendation is only true if indices are based on the environmental preferences of all species observed in a sample, as we observed relatively small quantitative differences between the stream and lake assemblages (**paper II**). Due to the observed differences in the abundance and frequency of diatom taxa in the different ecosystem types (**paper II**) ecological status assessment might yield different results when only considering the environmental preferences of certain diatom taxa, for example taxa that are abundant and or frequent in streams but not in lakes.

Most of the variation in IPS and ACID was explained by the corresponding environmental factor (IPS: 56.5% explained by TP, ACID: 72.2% explained by pH), with only minor shares of variation explained by ecosystem type, i.e. lake vs. stream (0.04% for ACID and 2.9% for IPS) (**paper II**). The slight difference in IPS as a function of TP between streams and lakes is an indication of different mechanisms underpinning responses. At low phosphorus levels, the IPS indicated more oligotrophic conditions in streams than in lakes. Streams in our study had higher concentrations of humic substances (as indicated by the light absorbance) than lakes, which can bind P and thereby reduce the amount of bioavailable P (Francko 1986, Jones 1990). In lakes, longer water retention times might lead to higher sedimentation of organic material and, therefore, less humic substances in lakes (Fölster *et al.* 2007). Thus, the IPS would correctly indicate more oligotrophic conditions in streams than in lakes, especially when P is limiting growth. At high phosphorus levels, IPS indicated a higher degree of pollution in streams compared to lakes having similar TP concentration. In the studied nutrient rich streams (>35 µg TP L^{-1}), ammonia levels were about three times higher compared to the epilimnion of the studied nutrient rich lakes (data not shown), possibly indicating a larger accumulation and subsequent decomposition of organic matter in streams than in lakes.

Correlations between IPS and TP levels were weaker at low TP levels (1-30 µg L^{-1}, **paper III**) compared to the entire gradient (1-433 µg L^{-1}; **paper II**).

Similarly, the phytoplankton-based TPI and macroinvertebrate-based ASPT responded only weakly at the low end of the TP gradient (adj. $R^2 < 0.5$, **paper III**). As benthic diatoms, phytoplankton and macroinvertebrate assemblage composition are closely related to TP levels, an adjustment of the indices to very oligotrophic conditions should result in a higher predictive power.

4.5 Threshold response of primary producers to TP

Distinct changes in benthic diatom assemblages were found at relatively low TP concentrations (~25 µg L^{-1}) in Swedish lakes, supporting the conjecture that diatoms are responding to small changes in nutrients (TP thresholds: 10-50 µg L^{-1} observed by: Ptacnik *et al.* 2008, Stevenson *et al.* 2008, Johnson and Hering 2009, Stevenson *et al.* 2012, Smucker *et al.* 2013, Taylor *et al.* 2014). The major changes in assemblage structure at relatively low TP levels indicate that nutrients are limiting the growth and production of benthic diatoms (Johnson *et al.* 2006, Johnson and Hering 2009). As nutrient levels increase, competition for light (e.g. due to increasing phytoplankton biomass) is likely to be more important than nutrient competition for benthic and planktonic primary producers in lakes (including self-shading of the phytoplankton; Vadeboncoeur and Steinman 2002, Lyche Solheim *et al.* 2008). Moreover, biofilms become thicker with nutrient enrichment (Proia *et al.* 2012) resulting in additional light attenuation (Kuehl *et al.* 1996).

Among the diatom taxa identified as TITAN threshold indicators, negatively responding taxa are generally linked to nutrient poor systems (**paper I**) and positively responding taxa to systems with higher nutrient levels (**paper I**, King *et al.* 2000). Caution should be exercised, however, in interpreting the response of some species to nutrients, as several of the nutrient-sensitive diatom species are also related to acidity (e.g. *Eunotia* spp.). Acidophilic and acidobiontic diatom species are often considered to be nutrient-sensitive (Schneider *et al.* 2013), although empirical support is often lacking due to the paucity of nutrient rich aquatic systems that are also acidic. For environmental assessment, this is not an important issue as eutrophication is mostly a problem in areas not prone to acidification (Schneider *et al.* 2013). However, the distribution of acidophilic or acidobiontic species might not be driven by nutrient concentrations (Hargreaves *et al.* 1975) and hence our findings of nutrient thresholds might indicate change points that co-vary with pH.

Low-guild diatoms exhibited a negative (~18 µg TP L^{-1}), while motile and tychoplanktonic guilds exhibited a positive threshold response to TP concentration (~19 and 13 µg TP L^{-1}, respectively), as predicted based on their position in the biofilm and subsequent adaptations to resource availability. High-guild diatoms were the only guild not displaying the expected threshold response to elevated TP concentrations. A large portion of the high-guild diatoms belong to the genus *Eunotia*; a genus considered to be almost exclusively acidophilic and acidobiontic species (Van Dam *et al.* 1994). Hence, pH might be an important driver of high-guild diatoms, lending credence to our conjecture that pH maybe obscuring response to TP.

Comparing ecological threshold of benthic diatoms and phytoplankton in a subset of lakes, phytoplankton displayed a higher TP threshold than benthic diatoms, indicating shifts in benthic diatom assemblages before planktonic communities responded to increasing nutrient levels. Hence, our findings do not support the conjecture that thresholds of phytoplankton are lower than those of benthic diatoms due to the direct exposure and higher affinity to nutrients.

Gradient length affected the ability to detect a threshold response as well as its numerical value; thresholds were more distinct and at higher TP concentrations when using the larger benthic diatom dataset covering a broader range of nutrient concentrations. Therefore, the change points observed for phytoplankton assemblages need to be validated by studying turnover along the entire TP range observed in Swedish lake; in particular, if these type of analyses are to be used to set numerical nutrient criteria or indicator species for water quality assessment.

5 Conclusions

The major environmental drivers of benthic diatom assemblages in boreal lakes are similar to those occurring in Central European lakes. Species turnover in Swedish boreal lakes is mainly driven by gradients in acidity (pH) and nutrient status (P and N), whereas the water colour gradient did not contribute significantly to species turnover. Benthic diatoms can thus be recommended as indicator organisms for acidity and nutrient status of boreal lakes.

In acidic aquatic ecosystems, the distribution of ecological diatom guilds seems to be decoupled from nutrient availability, in contrast to circumneutral or alkaline aquatic systems. Motile and high guild taxa were dominating acidic lakes, possibly due to higher light availability and lower grazing pressure in low pH lakes in my data set or problems related to guild assignment for taxa that dominate under low pH. The application of ecological guilds in ecological status assessment cannot be recommended without determination of the drivers affecting growth morphology or detailed species observations, especially in low pH systems.

Most diatom taxa occurred in both ecosystems types, i.e. Swedish lakes and streams. However, differences in frequency and abundance among lakes and streams occurred but the observed differences are difficult to explain as the niches of different diatom species and genera are poorly known.

The diatom indices IPS and ACID, developed for streams, responded similarly to nutrient and acidity gradients, respectively, in Swedish lakes and streams and can thus also be recommended for ecological status assessment of lakes. Most of the variation in both indices was explained by the corresponding environmental drivers (i.e. nutrients or acidity), with only minor shares of variation explained by ecosystem type, i.e. lake vs. streams.

In nutrient poor boreal lakes, both primary consumers and primary producers were found to be correlated with nutrient concentration. Phytoplankton displayed a very precise response to TP, but a considerably less precise response to SRP. Primary producers, especially diatoms, displayed a stronger response to pH than macroinvertebrates, possibly due to shorter generation times implying faster response.

Nutrient thresholds in taxonomic and guild structure of benthic diatom assemblages in boreal lakes were found at low TP concentrations (18-26 µg L^{-1}), similar to TP thresholds reported from other aquatic ecosystems. Phytoplankton displayed a higher TP threshold than benthic diatoms, indicating shifts in benthic diatom assemblages before planktonic communities respond to increasing nutrient levels. Caution needs to be taken in the interpretation of the response of some species to nutrients, as several of the nutrient-sensitive diatom species are also related to low pH (e.g. *Eunotia* spp.) and pH dependencies might obscure their response to TP.

Future research

Benthic diatom assemblages display distinct structural shifts along environmental gradients and can thus be recommended as indicators of water quality in monitoring. However, to better understand how changes in diatom indices are coupled to ecosystem function and finally ecosystem services it would be interesting to relate current bioindicators to changes in benthic algal biomass, primary production or the presence of other benthic non-diatom algae.

Despite a long tradition of using diatoms as indicators of water quality and extensive lists of empirically derived species-specific sensitivities and tolerances to environmental variables and optima along environmental gradients, surprisingly little is known about the ecological niches of different diatom species and genera. What drives differences in abundance, frequency or growth morphology of a diatom species among habitats, sites or ecosystem types?

Future studies should focus on the application of phytobenthos function in environmental assessment, evaluating both benthic diatoms and non-diatom algae. Experiments using algal cultures and natural biofilms will enhance our understanding of the ecological niches of diatom species and the consequences of changing environmental conditions for ecosystem functioning and services.

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