Aquatic ecosystems in hot water

Effects of climate on the functioning of shallow lakes

Sarian Kosten

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1 General introduction

Lakes and reservoirs serve multiple purposes for society including supply of drinking water, power generation; fisheries and recreation (Chapman 1996). Although inland waters comprise only about 3% of the continental land surface (Downing et al. 2006) they also play a significant role in global cycles of different elements. For instance, they may retain large fractions of the nitrogen and phosphorus loads from the watershed before the rivers reach the ocean (De Klein 2008). Additionally, inland waters, turn out to be hot-spots of carbon processing in terrestrial landscapes (Cole et al. 2007). While they typically emit CO_2 they also collectively bury more organic carbon annually than the world's oceans (Duarte et al. 2008, Williamson et al. 2009).

Traditionally, the study of human impacts on lakes has concentrated largely on the effects of nutrient loading (Scheffer 1998, Wetzel 2001, Kalff 2002). However, with global climate change becoming increasingly evident, the question how climate affects the structure and functioning of lake ecosystems has become an important new focus of attention. Among the approaches taken to address this question are time series analysis (Scheffer et al. 2001, Jeppesen et al. 2003b, Van Donk et al. 2003), experiments in heated mesocosms (McKee et al. 2002, McKee et al. 2003, Christoffersen et al. 2006, Ventura et al. 2008, Feuchtmayr et al. 2009), in situ mesocosm experiments replicated in different lakes along climate gradients (Moss et al. 2004, Vakkilainen et al. 2004, Van De Bund et al. 2004, Meerhoff et al. 2007a, Bécares et al. 2008), comparisons between regions (Jackson et al. 2007), laboratory experiments (Reynolds 1997, Domis et al. 2007a) and modeling efforts (Genkai-Kato and Carpenter 2005, Mooij et al. 2007). The common pattern that starts to emerge from these studies is that nutrients play a major role in the functioning of lake ecosystems (Christoffersen et al. 2006, Ventura et al. 2008), whereas climate change may exacerbate eutrophication effects (McKee et al. 2003, Jeppesen et al. 2007a, Mooij et al. 2007, Jeppesen et al. 2009). The results, however, vary widely between studies.

Clearly each approach has its specific limitations. For instance, time series analyses may reveal effects of warmer or wetter years but such short term effects are difficult to extrapolate to long term effects of structural climate change. While experiments are a powerful tool to reveal mechanisms, they inevitably have scale limitations in both time and space that make extrapolation to predict effects of long term climatic change in lakes problematic. The limitations of modeling for predicting ecological change are well known too. Comparisons between lakes in different climate zones may in principle help to identify long term effects of climate, but comparability of lakes in other aspects than climate is often limited. Methodological differences between studies can hamper comparisons as well.

The work reported in this thesis is part of the South American Lake Gradient Analysis (SALGA) project, a project aiming at complementing the studies done so-far by systematically sampling 83 comparable lakes along a large climatic gradient in South America. Unlike Europe, the South American continent with its rather continues shoreline offers an excellent opportunity to study sets of comparable shallow lakes along a large latitudinal climatic gradient. The lake ecosystems we studied were distributed along a latitudinal gradient (5 – 55 ^{o}S) (Figure 1.1). The lakes were selected to resemble each other as closely as possible in morphology and altitude but to vary as much as possible in trophic state within each climate zone. This was done to help distinguishing between the effects of climate and the effects of nutrient level. All lakes were sampled once during summer (subtropical, temperate and tundra lakes) or the dry season (tropical lakes) between November 2004 and March 2006 by the same team. Each lake was extensively surveyed for physical, chemical and biological characteristics both in the water column and in the sediment. In all lakes we sampled nutrients (nitrogen and phosphorus), carbon, oxygen, submerged vegetation, fish, phytoplankton, zooplankton, bacteria and protozoa.

The summer or dry-season water temperature ranged from 10 ^{o}C in the South to 28 ^{o}C in the North. Annual minimum monthly air temperatures varied between -0.8 and 26.3 ^{o}C, whereas the maximum monthly air temperatures varied between 8.2 and 28.7 ^{o}C (New et al. 2002). The range in minimum and maximum temperatures illustrates that the yearly variation in temperature is much larger in the South than in the North. The same goes for the minimal daily irradiance, during the short days in winter the most Southern lakes only receive around 10 cal.cm^{-2}.day^{-1}, whereas the Northern lakes receive around 420 cal.cm^{-2}.day^{-1}. The total annual and maximum annual irradiance between the Northern and Southern lakes is, however, quite similar (Lewis 1987).

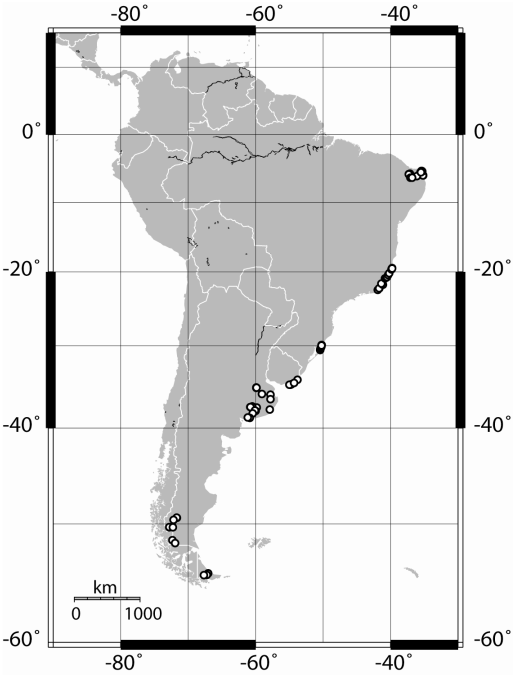

Figure 1.1 Geographical location of the 83 South American lakes studied.

Obviously, there is no silver bullet approach to predict effects of future climate change on lake ecosystems. The 'space-for-time' approach we take has important limitations as well. Certainly, interpretation of the causality behind the patterns observed in a field study such as ours remains difficult, and I discuss potential caveats and alternative explanations throughout this thesis. On the other hand our results offer a complement to what is observed so-far in many detailed but relatively short and small-scale studies of climatic effects on lakes.

Outline of the thesis

This thesis is focused on the question how climate might affect the chances of lakes to become dominated by submerged macrophytes, by algae, or by cyanobacteria, and on the question what might be the impact of climate on the carbon metabolism of lakes. This work is complemented by two companion theses within the SALGA project addressing changes in the plankton community and the food web.

In the first 3 chapters (2, 3, and 4) I address the question how climate might affect the critical nutrient level at which lakes shift from dominance by one primary producer to dominance by another group. As a first step, in chapter 2 I sketch a theoretical framework based on a simple graphical model linked to empirical data from our lakes. Chapter 3 presents the results of a study in which we combine our data with data from studies across Europe and North America to reveal systematic differences among climate regions in the relationship between submerged macrophyte abundance and environmental factors such as nutrient levels and lake depth. In chapter 4 the differences among climate regions in the effect of submerged vegetation on water clarity are discussed. This effect is one of the backbones of the theory of alternative stable states in shallow lakes represented in the minimal model presented in chapter 2. In chapter 5 I discuss the changes that occur in nitrogen or phosphorus limitation along the climate gradient. This chapter also addresses the risk of cyanobacterial dominance under different nutrient conditions. Chapter 6 further elaborates the effects of nutrients and temperature on cyanobacterial dominance combining our data with results from a large European study. In chapter 7 I show how CO_2 emissions from our lakes may change with warming. Chapter 8 provides a synthesis of the results in a broader context.

Lagoa Cutias – Rio Grande do Norte – Brazil – October 2005

2 Potential climate impacts on the stability of alternative states in shallow lakes

Abstract

We explore how climate change may affect the critical nutrient level to maintain submerged vegetation dominance in shallow lakes combining a graphical model with data from 83 lakes along a large climate gradient in South America. The data suggest that in warmer climates submerged macrophytes may tolerate more underwater shade than in cooler lakes. By contrast, the relationship between phytoplankton biomass (approximated by chlorophyll-a (chla) or biovolume) and nutrient levels did not change consistently along the climate gradient. In warmer climates the correlation between phytoplankton biomass and nutrient concentrations was overall weak. This weak correlation in warm lakes was especially clear at low TP concentrations where the chla:TP ratio could be either low or high. Although the enhanced shade tolerance of submerged plants in warmer lakes might promote the stability of their dominance, the potentially high phytoplankton biomass at low nutrient concentrations suggest an overall low predictability of climate effects. Importantly, more intense nutrient recycling and lower near-bottom oxygen concentrations in warm lakes imply that similar nutrient levels in the lake water may be realized at lower external nutrient loadings compared to cooler lakes.

Introduction

Feedback mechanisms between submerged vegetation and phytoplankton are believed to cause temperate shallow lakes to have two alternative states dominated by either one of these groups of primary producers (Jeppesen et al. 1990, Moss 1990, Scheffer 1990, Scheffer et al. 1993). A range of studies, however, suggests that mechanisms thought to play a regulating role in these ecosystems may be sensitive to climatic change (Scheffer et al. 2001, Jeppesen et al. 2003b, Van Donk et al. 2003, Genkai-Kato and Carpenter 2005, Mooij et al. 2005, Meerhoff et al. 2007a). For instance, climate may affect phenology and community composition (Nõges et al. 2003b, Winder and Schindler 2004, Adrian et al. 2006, Sommer and Lengfellner 2008, Jeppesen et al. 2009) biogeochemical cycles (Mulholland et al. 1997, Monteith et al. 2000, Nõges et al. 2007, Weyhenmeyer et al. 2007; chapter 7) and water levels (Beklioglu et al. 2007, Jeppesen et al. 2009). The numerous aspects of lake ecosystems that may be affected by climate must have consequences for the competition between submerged vegetation and phytoplankton, but, as different effects may counteract each other, the overall result is difficult to predict.

For instance, higher temperatures may cause an increase in denitrification (Golterman 2000, Piña-Ochoa and Álvarez-Cobelas 2006, Herrman et al. 2008) potentially leading to lower in-lake nitrogen concentrations. Nitrogen limitation of periphyton and phytoplankton growth may subsequently favor submerged macrophytes (e.g. chapter 3; Kosten et al. 2009b). To which extent temperature induced nitrogen limitation actually occurs, however, is not clear as a meta analysis (Elser et al. 2007) and a study of shallow lakes along a climate gradient in South America (chapter 5; Kosten et al. 2009a) did not find a particularly high incidence of nitrogen limitation in warm lakes.

Instead of favoring submerged macrophytes, warmer conditions may also promote phytoplankton dominance through an alteration of top-down regulation. Warmer waters often have high densities of small planktivorous fish (Jeppesen et al. 2007a, Meerhoff et al. 2007a, Teixeira-de Mello et al. 2009) and consequently higher predation pressure on zooplankton and lower grazing pressure on phytoplankton. The subsequent increase in phytoplankton densities may, in turn, negatively affect submerged macrophyte growth (Scheffer et al. 1993, Scheffer and Jeppesen 1998). These examples illustrate that the different climate effects may affect the competitive balance between phytoplankton and submerged macrophytes in complex ways.

In recent decades efforts have been made world-wide to reduce external nutrient loadings in order to restore macrophyte dominance and to improve water quality and the ecological status of shallow lakes (Jeppesen et al. 2007a, Søndergaard et al. 2007). It has been suggested that climate warming may counteract the effect of such measures (Mooij et al. 2005, Jeppesen et al. 2007b). However, the relationship between climate, nutrients, and the balance between competing groups of primary producers is still poorly understood.

Here we explore how changes in climate might influence the occurrence of alternative states and the critical nutrient level at which switches between states occur. We use data from 83 South American lakes to examine how the relationships assumed in the graphical model of Scheffer et al. (1993), might be altered along a climatic gradient. The model is based on three assumptions: (i) turbidity increases with nutrient level, (ii) vegetation reduces turbidity, and (iii) submerged vegetation disappears when a critical turbidity is exceeded (Scheffer et al. 1993, Figure 2.1a). Higher temperatures may affect phytoplankton and macrophyte growth in different ways, thereby influencing the critical nutrient level favoring either the dominance by phytoplankton or by macrophytes (Figure 2.1).

Which of these options will be most realistic is still unclear as there are few empirical studies available to evaluate how the competitive balance between phytoplankton and submerged macrophytes may be affected by climate change. Here we will use our lake data to obtain a first estimate of how the underlying relationships might change with climate.

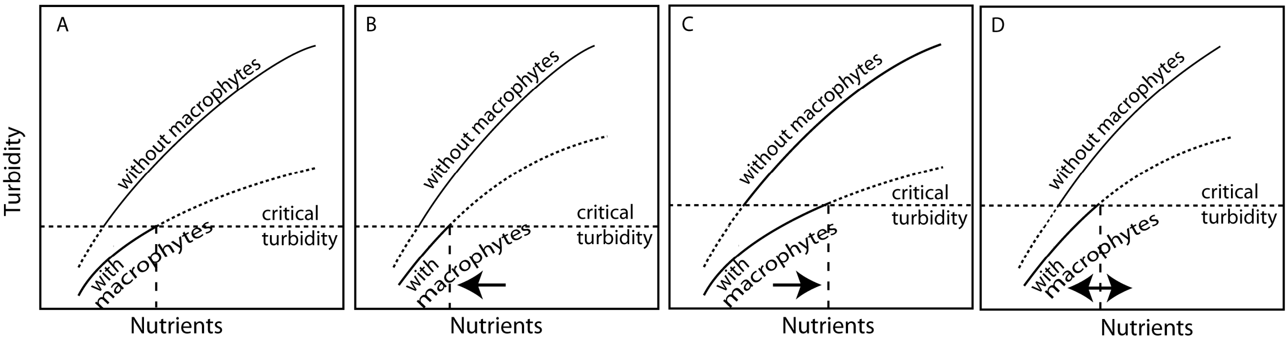

Figure 2.1 The relationship between nutrient level and turbidity in lakes when dominated by phytoplankton (upper curve) or submerged macrophytes (lower curve). B, C and D show hypothetical variations of the general model presented in A, the temperature in B, C, and D being higher than in A. A: Gradual nutrient enrichment of a macrophyte dominated lake will result in a modest turbidity increase along the lower curve until the critical turbidity is reached at which macrophytes disappear and a shift to a more turbid equilibrium (upper curve) occurs. B: Phytoplankton profits from higher temperatures and reaches higher biomass at a given nutrient level (note the increase in slope between figures A and B) *resulting in a lower critical nutrient level (vertical line indicated by the arrow). C: Macrophytes perform better at higher temperatures tolerating higher turbidities. A rise in the critical turbidity results in a higher critical nutrient level. D: Both phytoplankton and macrophytes perform better at higher temperatures resulting in an uncertain effect on the critical nutrient level.*

Methods

Study sites – We sampled 83 shallow lakes along a latitudinal gradient (5 – 55 ^{o}S) in South America. The lakes were classified into three groups based on the prevailing climate (New et al. 2002): ‘warm’ sites had an average monthly air temperature above 25^{o}C in at least one month, at ‘cold’ sites ground frost occurred for more than 120 days a year, and the other lake sites were classified as ‘intermediate’ (Figure 2.2). All lakes were shallow and had a surface area smaller than 2.53 km^2. In every group lakes were selected to vary as much as possible in vegetation coverage and trophic state (See table 2.1, Kosten et al. 2009a, and 2009b for details on the lakes).

Sampling and sample analysis – All lakes were sampled once during summer (cold and intermediate lakes) or dry season (warm lakes) between November 2004 and March 2006 by the same team. We collected depth-integrated water samples at 20 random points in each lake. Filtration for various analyses was conducted directly after collection. Water and filters were then frozen until analysis. Total phosphorus (TP), total nitrogen (TN), chlorophyll-a (chla), phytoplankton biovolume, and inorganic suspended solids (ISS) concentrations were determined using standard laboratory protocols (See Kosten et al. 2009a for details). Oxygen concentrations near the bottom of the lake were measured at the deepest point, approximately 5 cm above the sediment at noon.

Table 2.1 General features of the sampled lakes

	Range	**Mean**	**Median**
Area [ha]	9 – 253	62	46
Mean depth [m] [1]	0.5 – 4.5	1.8	1.6
Altitude [m above sea level]	1 – 500	84	20
Conductivity [µS cm^{-1}]	38 – 4930	527	167
Total nitrogen [mg N L^{-1}]	<0.1 – 25.8	1.8	0.4
Total phosphorus [mgP L^{-1}]	0.02 – 9.14	0.27	0.10
Chlorophyll-a [µg L^{-1}]	1 - 2889	80	5
Vegetation cover [%]	0-100	21	5
Vertical light attenuation [m^{-1}]	0.5 - 43.6	4.6	2.5
Surface water temperature [^{o}C]	10.4 – 30.3	24.3	25.5
Average monthly air temperature [^{o}C] [2]			
Cool	4.0 – 7.6	6.3	11.6
Intermediate	13.8 – 18.9	16.5	16.4
Warm	22.6 – 27.6	25.2	25.2
Number of frostdays [y^{-1}] [2]			
Cool	134 - 169	146	141
Intermediate	1 - 68	26	15
Warm	0 - 2	0	0

1) Based on 40 measurements in the lake
2) Average of the years 1961 – 1990 by New et al. (2002)

The maximum colonization depth of submerged vegetation (Z_{col}) was estimated based on observations of vegetation presence/absence at 20 random points in the lake combined with more detailed macrophyte observations at 13 – 47 points (average 22) equally distributed on 3 to 8 parallel transects. The number of transects varied with the shape and size of the lake. Observations were made from a boat using a grapnel when water transparency was insufficient to get a clear view of the bottom. Light measurements were conducted at different depths at noon in the center of the lake with a LICOR LI-192SA.

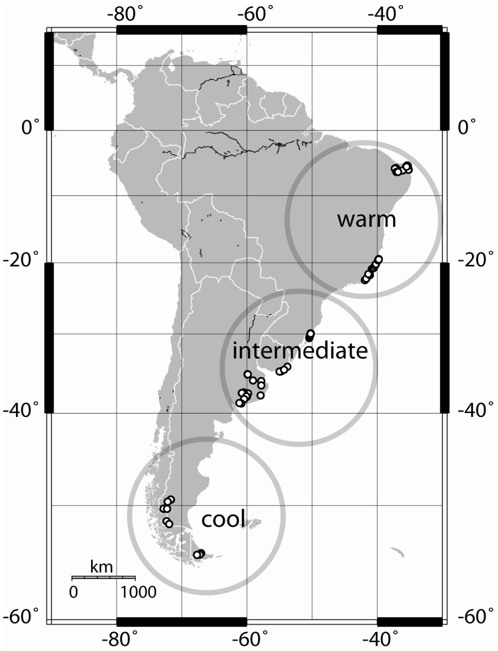

Figure 2.2 Location of the sampled lakes in climatologically different regions.

Calculations and data analysis – To explore the potential influence of average climatic conditions on the competition between phytoplankton and submerged macrophytes we analyzed the differences among the climate regions in: (1) the relationship between nutrients and phytoplankton biomass (approximated by chla and biovolume), referred to hereafter as '*phytoplankton performance'*; and (2) the relationship between light availability and submerged vegetation occurrence, referred to hereafter as '*vegetation performance'*. To examine the possible influence of warming on potential sediment phosphorus release as a result of an increase in anoxic conditions at the sediment surface, we also analyzed the relationship between temperature and sediment oxygen concentrations.

Phytoplankton performance in the different climate regions was analyzed using linear regressions on log transformed chla and biovolume concentrations versus log transformed TP and TN concentrations for each climate region. To test for significant differences between the regression lines we used an ANCOVA using climate region as a fixed factor. The TP and TN range may strongly influence the slope of the regression line especially because the chla-nutrient relationships tend to be sigmoidal when high nutrient concentrations are included in the analysis (McCauley et al. 1989). As the lakes in our dataset vary widely in nutrient concentration and as the range in nutrient concentrations differs among the climate regions we also performed linear regressions using only data of lakes with TP ranging between 0.05 and 0.35 μgL^{-1}.

The difference in vegetation performance in the 3 climate regions was assessed by comparing the maximum colonization depth with the depth at which the light compensation point was reached (Z_{comp}). We used 9 $\mu mol\ m^{-2}s^{-1}$ as an estimate of the minimum light requirement of submerged vegetation (Sand-Jensen and Madsen 1991). If vegetation performs better under warmer conditions we would expect plants to grow deeper (at lower light intensities) than in colder conditions. For each lake we calculated the light attenuation coefficient (Kd) based on the light measurements and the Lambert-Beer law. The same law was used to calculate Z_{comp} for which we used an average climate region light intensity measured just under the water surface (I_0) at noon in the different lakes in the region. Very turbid lakes (i.e. lakes with a Kd > $3m^{-1}$) were excluded from this estimation of the regional I_0.

Phosphorus release from the sediment depends strongly on oxygen levels at the sediment surface (e.g. Jensen and Andersen 1992, Barko and James 1998). As an indication for the potential climate influence on phosphorus cycling within the lake we therefore analyzed the temperature influence on near bottom oxygen by means of non-linear regression:

$$O_2(T) = \alpha * \frac{h^q}{T^q + h^q}$$

where α represents the bottom oxygen concentration at the lower temperatures in the dataset (~10 ^oC) , *h* is the temperature at which the bottom oxygen concentration is reduced by 50%, and *q* is the exponent determining the steepness of the oxygen decrease around this temperature. Climate influence on nitrogen dynamics in the 83 study lakes was analyzed elsewhere (Kosten et al. 2009a).

All statistical analyses were performed in SPSS 15.0.1.1.

Results

Phytoplankton biomass as a function of nutrient concentration

Analysis of the field data demonstrated a significant difference in the relationship between chla and TP in the cool and the intermediate region when using the complete dataset (Figure 2.3). The chla-TP relationship between the warm and the other two regions, however, was not significantly different (Figure 2.3). Using only lakes with TP between 0.05 and 0.35 μgL^{-1} indicated no significant difference between the cool and the other two regions ($p>0.05$), whereas the regression line between the intermediate and warm region significantly differed ($p=0.014$). The relationship between chla or phytoplankton biovolume and TN did not significantly differ among the climate regions ($p=0.730$ and $p=0.798$, respectively, for the parameter ‘climate region’; $p= 0.505$ and $p= 0.711$, respectively, for the interaction term ‘climate region*TN’).

Notably, the explained variance in the chla – TP relationship in the warm region was low in comparison to that in the other two regions (Figure 2.3). This did not change when the TP range was narrowed down (R^2 for the cool, intermediate and warm region: 0.98, 0,60 and 0,04 respectively, with the p-value for the cool and intermediate being <0.001 and for the warm region 0.354). A stepwise linear regression using both TP and TN indicated that whereas TP explained most of the variance in the cool region ($R^2=0.94$, $p<0.0001$), TN explained most of the variance in the intermediate ($R^2=0.84$, $p<0.0001$) and warm ($R^2=0.33$, $p=0.012$) region. Using phytoplankton biovolume as an indicator for phytoplankton biomass gave comparable results.

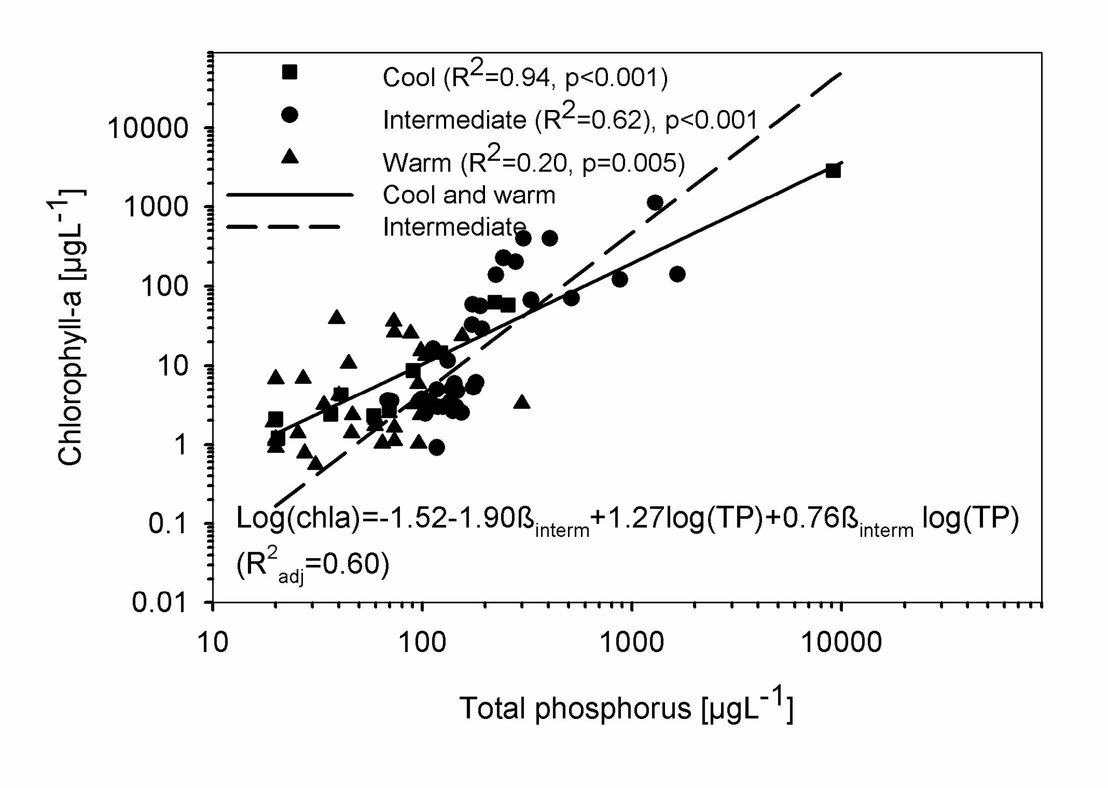

Figure 2.3 Relationship between total phosphorus (TP) and chlorophyll-a (chla) in different climate regions. The explained variance of the regional regression lines is shown in the legend. The ANCOVA model shows the relationship between chla and TP in the overall dataset, β_{interm} is 1 for the data in the intermediate zone, 0 in the others. The regression line in the warm region did not significantly differ from the regression line in the cool region (p>0.05).

Vegetation shade tolerance

In several lakes in the warm and intermediate region submerged vegetation was found till relatively greater depths than in the cool regions, taking the available light at the sediment surface into account (Figure 2.4). For a statistical analysis we removed lakes in which the vegetation colonized the maximum depth of the lake (37% of the lakes, Figure 2.4). Comparison of regression lines indicated that the relationship between the maximum colonization depth and the light attenuation in the lake differed significantly between the cool and the intermediate region on the one hand and between the warm region on the other hand (p<0.001 and p=0.002, respectively). The intermediate region did not differ significantly from the cool region (p=0.12).

Overall the colonization depth could be predicted best from the vertical attenuation coefficient if we accounted for the difference between warm lakes and other lakes in a regression model:

$$\log(Z_{col})=0.41+(-1.09+1.06\beta_{warm})*\log(Kd)$$

where β_{warm} is ‘1’ for the lakes situated in the warm climate region and ‘0’ for the other lakes (n_{cool}=6, n_{interm}=9, n_{warm}=17, R^2_{adj}=0.60).

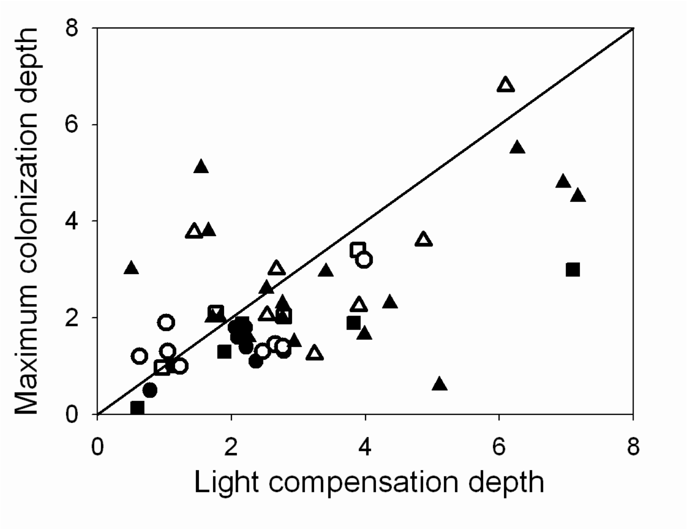

Figure 2.4 Maximum colonization depth of submerged macrophytes plotted against the estimated depth at which light intensity falls below 9 µmol m^{-2}s^{-1}. The diagonal line indicates the depth until which submerged macrophytes are expected to grow if a light intensity of 9 µmol m^{-2}s^{-1} at the bottom indeed limits their depth distribution. In lakes depicted above the diagonal line submerged macrophytes grew under darker conditions, in lakes depicted below the line they grew under lighter conditions. Symbols as in figure 2.2. Open symbols identify lakes where submerged macrophytes grew until the deepest point in the lake, and thus potentially could have grown deeper.

Bottom oxygen levels

Fitting a Hill function to the nonlinear relationship between bottom oxygen concentration and lake temperature, only 37% of the variance could be explained. A closer examination, however, revealed that a group of oligotrophic lakes with relatively sandy sediments with little organic matter deviated relatively strongly from the model. Including only eutrophic lakes (TP>200µgL^{-1}) we obtained a model with an R^2 of 0.78 (Figure 2.5).

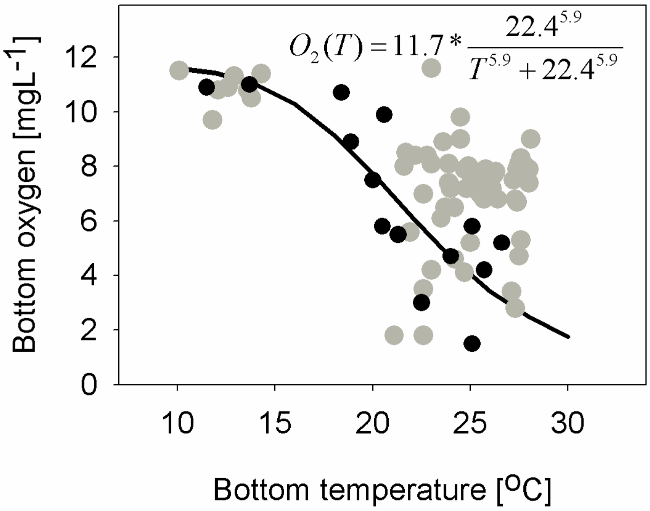

Figure 2.5 Temperature dependence of the sediment oxygen concentration. Grey dots are lakes with TP<200 µgL^{-1}. Dark dots are eutrophic lakes (TP>200 µgL^{-1}), on these lakes the regression model is based (R^2 = 0.78).

Discussion

Given the fact that we find little evidence for a climate effect on phytoplankton biomass (expressed as chlorophyll-a or biovolume) per unit of nutrients, the probable increase in shade tolerance of submerged vegetation with temperature suggests that the competitive balance might shift more easily towards macrophyte dominance in a warmer climate. On the other hand, the lower bottom oxygen concentrations we find in warmer climates imply that internal recycling of phosphorus from lake sediments may be promoted, potentially causing higher phosphorus availability for phytoplankton growth at the same external nutrient loading. Here we discuss our results in the light of earlier findings and subsequently speculate on the potential overall effect of climate on the complex web of interactions that affects the balance between submerged plants and phytoplankton in shallow lakes.

The effect of temperature on phytoplankton biomass

Warmer conditions have been argued to yield higher phytoplankton biomasses (Flanagan et al. 2003, Domis et al. 2007a, Mooij et al. 2007). This higher yield has been attributed to different phenomena: (i) higher enzyme activities (Markager et al. 1999) in combination with longer growing seasons (Flanagan et al. 2003), (ii) the dominance of cyanobacteria under warmer conditions (Paerl and Huisman 2008; chapter 6) which generally reach a higher chlorophyll-a concentration at a certain TP concentration than many other phytoplankton groups (e.g. Scheffer et al. 1997), and (iii) the absence of large zooplankton grazers in warmer lakes (Gillooly and Dodson 2000, Meerhoff et al. 2007b, Lacerot et al in prep.) reducing grazing losses of algal biomass (Mazumder 1994). A differential temperature influence on respiration and production (Rivkin and Legendre 2001, Lopez-Urrutia et al. 2006), on the other hand, could result in a lower phytoplankton biomass in warmer lakes (e.g. Huszar et al. 2006).

These counteracting temperature influences on phytoplankton yield may explain the lack of a consistent change in the relationship between nutrients (TN and TP) and phytoplankton biomass along our South American climate gradient and along a European climate gradient (Gyllström et al. 2005). A multiple regression model with data from lakes in South America and Europe combined also indicated that temperature hardly added to the variance in chlorophyll concentrations explained by (log) total-phosphorus (P) or (log) total-nitrogen (N) concentrations (Chapter 6).

The phytoplankton biomass at a given nutrient concentration was often comparable among the three climate zones (see overlapping points in Figure 2.3). This has been noted in a comparison of different climate zones in North America as well (Brown et al. 2000). When studying alternative states, the phytoplankton biomass in the lower nutrient range is especially important, as this is where macrophyte dominance occurs (Jeppesen et al. 1990, Gonzalez Sagrario et al. 2005, Kosten et al. 2009b). Notably the phytoplankton biomass in the warm region varies considerably at low TP concentrations, indicated by the low and high chla:TP ratios (Figure 2.3). This also causes the low percentage of explained variance of phytoplankton biomass by TP and TN in the warm climate region. This finding concurs with an earlier study comparing a large number of tropical and subtropical lakes (Huszar et al. 2006, Kosten et al. 2009a). It is indicative of low predictability of phytoplankton biomass in warmer climates.

The effect of temperature on performance of submerged vegetation

The climate dependence of submerged vegetation shows a different pattern as the phytoplankton performance across climate zones. The relationship between the colonization depth of submerged vegetation and vertical light attenuation in the lake revealed no difference between the cool and the intermediate climate region. Submerged vegetation in the warm region, however, reached relatively deeper zones than those in the other two regions, which may indicate lower light requirements. Earlier work has demonstrated the positive relationship between latitude and light requirements as well (Middelboe and Markager 1997). Temperature may be its most important driver (Barko et al. 1982) as the maximum daily irradiance does not vary substantially between lower and higher latitudes (Lewis 1987). This presumption concurs with findings in a Canadian lake where the colonization depth and the submerged vegetation biomass increased during a relatively warm year, in spite of increased turbidity (Rooney and Kalff 2000). Another factor that may contribute to the deeper colonization depth in warmer lakes is the increase in fish herbivory (Teixeira-de Mello et al. 2009) and the consequential higher grazing on periphyton (Meerhoff et al. 2007a) improving light conditions for host plants. Periphyton density, however, is influenced by other factors as well. Along a latitudinal gradient in Europe, for instance, relatively low periphyton biomasses in warmer lakes were attributed to an increase in shading by phytoplankton (Bécares et al. 2008).

Potential implications for critical thresholds in nutrient load across climates

A higher submerged vegetation performance in the warmer region may raise the critical nutrient concentration at which a shift to a phytoplankton dominated state occurs (Figure 2.1c). On the other hand, the low predictability of phytoplankton biomass in these warm lakes makes it more difficult to predict the competitive outcome between phytoplankton and submerged vegetation and complicates the estimation of a critical nutrient concentration in this region. Indeed, submerged vegetation cover has been found to be poorly predictable from nutrient concentrations and lake depth in warm climates (Kosten et al. 2009b).

Importantly, nutrient concentrations in the water column are not simply a function of external nutrient loading, but depend strongly on recycling within the lake ecosystem. Recycling may be affected by climatic conditions in various ways, for instance through the fish community. Warm lakes often have higher fish densities (reviewed by Jeppesen et al. 2005b). This was substantiated by the fish catches in the 83 study lakes (Kosten et al. 2009c; Mazzeo unpublished data). Higher fish densities in warmer lakes may enhance nutrient cycling in the lake water column (Hansson et al. 1987, Schindler and Eby 1997) decreasing organic matter deposition. Additionally, even though we have not found indications for systematic changes in densities of benthivorous fish along the latitudinal gradient (Kosten et al. 2009c), warming may lead to an increase in overall bioturbation activity enhancing nutrient recycling (Mooij et al. 2005).

Importantly, the metabolism of lakes may change markedly with climate. Systematically higher partial CO_2 pressures in warmer lakes (Kosten et al. in press; chapter 7), suggest that higher temperatures promote decomposition. This implies that sediments in warmer lakes are likely to be less efficient carbon sinks, but also less efficient nutrient sinks. In the case of phosphorus the change of lake metabolism with temperature may have a marked additional effect. This is because the capacity of sediments to retain phosphorus often depends strongly on oxygen concentrations (e.g. Jensen and Andersen 1992, Barko and James 1998, Genkai-Kato and Carpenter 2005, Bicudo et al. 2007). Our field data indicate a strong drop in near-bottom oxygen concentration with temperature. This pattern may be caused by an increase in bacterial activity in the sediment, lower oxygen solubility at higher temperatures, and temporarily enhanced stratification. Although it has been suggested that in contrast to phosphorus, nitrogen concentrations might be reduced in warmer climates due to higher denitrification, we found no evidence for such climate related shift along our climate gradient (Kosten et al. 2009a).

In conclusion, while enhanced shade tolerance of submerged plants in warmer climates might favor this group over phytoplankton, the potentially higher internal nutrient recycling in shallow warm lakes might imply that external nutrient loads have to be lower in warmer lakes to prevent a shift from a submerged vegetation to a phytoplankton dominated state (Jeppesen et al. 2007a, 2007b, Mooij et al. 2007).

The ambiguity of this conclusion fits with the wide variation in outcomes of earlier studies on the effect of climate on macrophytes and phytoplankton. For example, in shallow Mediterranean lakes, macrophytes in enclosures with different nutrient concentrations gave way to phytoplankton dominance at lower nutrient concentrations than in similar studies conducted in North European lakes (Moss et al. 2004, Romo et al. 2004). In contrast, it has been argued that a longer plant growing season, higher light intensities and temperatures, and strong water level fluctuations will typically lead to higher critical nutrient concentrations at warmer latitudes (Bécares et al. 2008). Similarly, conclusions from studies in temperature controlled mesocosms are not clear-cut. For instance, the effects of nutrient addition on phytoplankton abundance tended to be largely independent of temperature (McKee et al. 2002, Moss et al. 2003a), although the increase of floating plant abundance with warming caused an indirect negative influence on phytoplankton through shading (Feuchtmayr et al. 2009). Similarly, the response of submerged macrophyte volume to nutrient addition was found to be largely temperature-independent (McKee et al. 2002, Feuchtmayr et al. 2009) and already established macrophyte dominance tended to persist even with increased nutrient loading and warming (Moss et al. 2003a).

Our findings are thus in line with the emerging pattern that climate effects on the competitive balance between submerged macrophytes and phytoplankton are not straightforward.

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3 Climate-related differences in the dominance of submerged macrophytes in shallow lakes

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Abstract

It has been suggested that shallow lakes in warm climates have a higher probability of being turbid, rather than macrophyte dominated, compared to lakes in cooler climates, but little field evidence exists to evaluate this hypothesis. We analyzed data from 782 lake years in different climate zones in North America, South America, and Europe. We tested if systematic differences exist in the relationship between the abundance of submerged macrophytes and environmental factors such as lake depth and nutrient levels. In the pooled dataset the proportion of lakes with substantial submerged macrophyte coverage (>30% of the lake area) decreased in a sigmoidal way with increasing total phosphorus (TP) concentration, falling most steeply between 0.05 and 0.2 mg L^{-1}. Substantial submerged macrophyte coverage was also rare in lakes with total nitrogen (TN) concentrations above 1-2 mg L^{-1}, except for lakes with very low TP concentrations where macrophytes remain abundant until higher TN concentrations. The deviance reduction of logistic regression models predicting macrophyte coverage from nutrients and water depth was generally low, and notably lowest in tropical and subtropical regions (Brazil, Uruguay and Florida), suggesting that macrophyte coverage was strongly influenced by other factors. The maximum TP concentration allowing substantial submerged macrophyte coverage was clearly higher in cold regions with more frost days. This is in agreement with other studies which found a large influence of ice cover duration on shallow lakes' ecology through partial fish kills that may improve light conditions for submerged macrophytes by cascading effects on periphyton and phytoplankton. Our findings suggest that, in regions where climatic warming is projected to lead to fewer frost days, macrophyte cover will decrease unless the nutrient levels are lowered.

Introduction

Submerged macrophytes play an important role in the ecological functioning of shallow lakes (Jeppesen et al. 1998c). Numerous studies in European and North American lakes have shown that with increasing nutrient loading, lakes tend to shift from a clear state dominated by submerged macrophytes to a turbid state dominated by phytoplankton (Moss 1990, Scheffer 1998, Bayley and Prather 2003). The nutrient concentration above which submerged macrophytes disappear varies among lakes. So far, studies indicate that it may depend on factors such as water depth and lake size (Scheffer 1998, Jeppesen et al. 2007a). In North European lakes the conditions allowing macrophyte dominance in shallow lakes are relatively well studied (e.g. Timms and Moss 1984, Jeppesen et al. 1990, Mjelde and Faafeng 1997). Less is known about the phosphorus and nitrogen concentrations at which submerged macrophytes cease to be abundant on other continents and in different climates (Jeppesen et al. 2007b). It has been suggested that submerged macrophyte dominance gives way to phytoplankton dominance at lower phosphorus and nitrogen concentrations in warmer lakes (Beklioglu et al. 2007, Mooij et al. 2007).

The relationship between nutrients, temperature, and macrophyte coverage is particularly important in view of climatic change, yet few empirical studies are available to evaluate how the competitive balance between phytoplankton and submerged macrophytes may be affected by changing temperatures. Moreover, the results from these studies are inconclusive. For instance, experiments have shown that the effects of nutrient addition on phytoplankton abundance were independent of temperature (McKee et al. 2002, Van De Bund et al. 2004). Similarly, the response of submerged macrophyte volume to nutrient addition in those experiments was temperature-independent (McKee et al. 2002). Conversely, in shallow Mediterranean lakes, macrophytes in enclosures with different nutrient concentrations gave way to phytoplankton dominance at lower nutrient concentrations than in similar studies conducted in North European lakes (Romo et al. 2004). Lastly, in some lakes, the temperature in early spring had a positive effect on macrophyte coverage (Scheffer et al. 1992).

Clearly, observations from small scale enclosure experiments and interannual fluctuations cannot be easily extrapolated to predict effects of long-term climate change. Here we use another approach. We analyze data from shallow lakes in different climatic regions to test the hypothesis that macrophytes disappear at lower nutrient concentrations in warmer climates. Specifically, we use data from 782 lake years in temperate to tropical regions in North America, South America, and Europe to explore the relationship between macrophyte coverage and nutrient levels, lake characteristics and climate variables.

Methods

Dataset compilation

We compiled a dataset of lakes from 7 different regions on 3 continents with data on submerged macrophyte coverage, total phosphorus (TP), total nitrogen (TN), chlorophyll-a (chla), Secchi depth, lake area, and lake depth. We only included lakes with a surface area between 10 and 250 hectares and a mean depth of less than 5 meters. Data were collected over multiple years for some lakes, while for other lakes data were collected during a single year. Each lake-year combination was considered a data point, resulting in a total of 782 lake years for the analysis. All samples were taken in summer with a few exceptions in Wisconsin where some lakes were sampled for TP and TN in spring. In the datasets of the (sub)tropical regions we used a wider time window than in the colder regions (Table 3.1), because of their less pronounced seasonal variation in solar radiation, temperature (Lewis 1987), and TP and TN concentrations (Brown et al. 1998). Since we had data on only a few Uruguayan lakes, these data were clustered with data from the climatically similar Brazilian lakes.

Table 3.1 Database characteristics for the 7 regions

Region	**Data source**	**Number of lakes**	**Number of lake years**	**Timing of nutrient analysis**	**Timing of macrophyte cover assessment**	**Year in which data were collected**
Denmark	NERI	51	189	Averages for late summer[a]	Late summer[a]	1989 - 2005
Netherlands	STOWA, Waterboards & RIZA	81	151 (112 have TN and 88 mean depth data)	Averages for late summer[a]	Late summer[a]	1982 - 2005
Wisconsin	Department of Natural Resources	33	33	Single spring or summer sample[b]	Late summer[c]	2003 - 2005
Minnesota	Department of Natural Resources	133	133	Single sample in late summer[c] (no TN data)	Late summer[c]	2002 - 2006
Argentina	Universities cooperating in SALGA[d]-project	27	27	Single summer sample[e]	Summer[e]	2005 - 2006
Brazil and Uruguay[f]	Universidad de la República and universities cooperating in SALGA[d]-project	64	64	Single summer sample[g]	Summer[g]	2002 - 2005
Florida	University of Florida	132	185	Summer averages[h]	Summer[h]	1984 - 1999

[a] July and August, [b] 14 lakes were sampled in April, the rest in August and September, [c] July, August and September, [d] South American and European universities cooperating in the South American Lake Gradient Analysis project, [e] January, February and first week of March, [f] The bordering regions Brazil and Uruguay were combined due to a low number of lakes, [g] August to December, [h] May to September.

The total percent of the lake bottom covered by submerged macrophytes (hereafter referred to as macrophyte coverage) was estimated in different ways among regions. In Denmark, the lake was divided into 15-22 sub-areas in which macrophyte density was fairly constant. If the maximum depth of plants occurring in a sub-area was less than 1.5 m, total macrophyte coverage was determined at ten randomly selected locations within an area of approximately 2 * 2 m at 25 cm depth intervals reaching from the shore to the maximum depth of plant cover; otherwise a depth interval of 50 cm was used. Coverage was determined from a boat using an underwater viewer or by a diver. The following categories were used: < 1, 1-5, 5-25, 25-50, 50-75, 75-90 and 90-100%.

Data on Dutch lakes came from multiple water managers. Macrophyte coverage was estimated from a boat using a grapnel when water transparency was insufficient to get a clear view of the bottom. In some of the cases coverage for different species or groups was rated in categories of <5, 5-12, 13-25, 26-50, 51-75 and 76-100%. We transformed these classes to estimated cover percentages using mid-class values. Coverages for the individual submerged species or species groups were summed. In four of the 151 cases this resulted in a cover higher than 100%; here we set the coverage to 100%.

In Wisconsin maximum depth of plant occurrence was determined for each lake. Within this littoral zone, submerged macrophyte coverage was documented by divers at approximately 250 quadrates along 14 transects placed perpendicular to the shore. Macrophyte coverage was surveyed within 0.25 m^2 quadrates up to the maximum depth of plant cover or to a distance of 45 m from the shore. Submerged macrophyte coverage in the quadrates was averaged. This littoral zone average was then scaled to a lake-wide estimate of total percent cover by estimating the ratio of littoral zone: total lake area using bathymetric maps.

In Minnesota, the percent cover was based on point-intercept sampling in which 11 to 177 (mean 51) points in a grid pattern were sampled per lake. At each point, presence or absence was assigned based on observations and sampling using a grappling hook cast along a 2 m section of the lake bottom. The presence/absence information was used to calculate the frequency of occurrence for the entire lake. Using the frequency of occurrence as an estimate for percent cover would systematically overestimate the percent cover of the macrophytes. Therefore, we used a model derived from the logistic regression between the percent cover and the frequency of occurrence of the Danish data ($p<0.0001$, deviance reduction is 55%, average absolute difference between predicted and observed coverage is 4%) to convert the Minnesota frequency of occurrence to the percent cover.

In Florida, submerged macrophytes were sampled following the protocol established by Canfield *et al.* (1990). Between 14-16 transects were run across the lake with a Raytheon DE-719 recording fathometer, and the percent cover was determined from the fathometer charts (Maceina and Shireman 1980). The transects started as close to the shore as possible depending on water depth and the thickness of emergent or floating leafed macrophyte beds. The unnavigable littoral area might have contained submerged macrophytes as well; therefore, we removed all lakes from the dataset where the unnavigable littoral area made up more than 10% of the total area. The percent cover thus derived possibly contained patches of navigable emergent or floating macrophytes.

In Argentina, Uruguay and Brazil, coverage of submerged macrophytes was estimated based on observations of macrophyte presence/absence in 20 random points in the lake combined with coverage estimations of macrophytes in 13 – 47 points (average 22) equally distributed on 3 to 8 parallel transects. The number of transects varied with the shape and size of the lake. Observations were made from a boat using a grapnel when water transparency was insufficient to get a clear view of the bottom.

Data analysis

As macrophyte coverage was determined with different methods, our data should be interpreted with care. Therefore, we used two approaches to characterize the macrophyte coverage within study lakes. The first was a dichotomous classification of lakes as “macrophyte dominated” and “macrophyte poor”. In the second approach we used the estimated macrophyte percent cover per lake. For the dichotomous classification we classified lakes with >30% cover as macrophyte dominated as this has earlier been found to be the threshold associated with a shift from a turbid to a clear water state (Jeppesen et al. 1990). In a preliminary analysis we also used other thresholds (>25% and >50%). Although the proportion of macrophyte dominated lakes decreased when the threshold was raised, we found that different thresholds did not substantially change the pattern in the relationship between macrophyte dominance and nutrient levels.

We used classical logistic regression on the dichotomous version of our data, and logistic regression on the percent cover version of the data using quasi-likelihood (McCullagh and Nelder 1990) to explore how macrophyte dominance and macrophyte percent cover were related to environmental factors (logistic procedure, SAS Institute Inc. 2004). When using the macrophyte percent cover we used the SAS events/trials syntax. Because the conclusions of the two types of analyses did not substantially differ, the logistic regression analyses of the dichotomous dataset are presented in the supplementary material to this chapter and we focus on the results of the logistic regression on the percent cover classification throughout the rest of this manuscript. Our multiple logistic regression approach involved two steps:

First, we evaluated which variables could significantly explain variance in macrophyte coverage among lakes using single logistic models comprising all available variables. Given that at low nutrient concentrations macrophytes may be nutrient limited, we included quadratic terms for TP and TN to test for possible unimodal relationships between macrophytes and nutrients (Toivonen and Huttunen 1995, Sand-Jensen et al. 2000). We followed this procedure for each region individually and for the pooled dataset. When analyzing the pooled dataset we assigned weights in such a way that all 7 regions contributed equally to the outcome of the analysis. To account for over-dispersion in the data, which happens when the observed variance is higher than the variance of the theoretical model, we multiplied the covariance matrix by the heterogeneity factor (Deviance / degrees of freedom, SAS Institute Inc. 2004). Subsequently, we determined which combination of significant parameters could most reduce the deviance of each regional model. Deviance reduction was calculated as the percent difference in the -2*log likelihood of the full model, and the model containing an intercept only.

We used the resulting parameterized regional logistic models to calculate the TP and TN concentration at which a lake on average would have 30% submerged macrophyte coverage (TP_{30} or TN_{30}). The confidence interval of the TP_{30} and TN_{30} of each region was determined by a bootstrap procedure (Efron and Tibshirani 1986). For each region we simulated 1000 datasets by randomly taking samples from the original datasets with replacement. In each region the new dataset had the same number of observations as the original dataset. We ran a logistic regression with the data of all 1000 bootstrapped datasets and compiled a frequency distribution of the TP_{30} and TN_{30}. The confidence limits of both variables were estimated as the 5^{th} and 95^{th} percentiles of the obtained 1000 TP_{30} and TN_{30} concentrations per region.

Several climate variables are known to affect macrophyte growth. Examples are temperature, ice cover, light intensity and day length (Boylen and Sheldon 1976, Barko and Smart 1981, McCreary et al. 1983, Vadstrup and Madsen 1995). Proxies for these variables were derived from a high-resolution data set with mean monthly data on temperature, number of frost days and fraction of the day without clouds (New et al. 2002). The number of frost days was generally defined as days with a temperature below $0^{o}C$ recorded in open air with the bulb of the thermometer just in contact with the tips of the blades of grass, but see New *et al.* (2002) for regional assessment differences. Above a minimum number of frost days we assume the number of ground frost days to be positively correlated with the duration of ice cover of the lakes. We calculated the number of sun hours during the growing season by multiplying the fraction of the day without clouds (New et al. 2002) by the average day length (Meeus 1991), and considered this a proxy for solar radiation.

Results

General climatic and limnological characteristics

Climate characteristics varied substantially among regions (Table 3.2). Long term spring temperatures were highest in Florida and Brazil-Uruguay, intermediate in Argentina, and coldest in Denmark, The Netherlands, Minnesota, and Wisconsin. The average number of days with ground frost varied between less than 10 days in Brazil-Uruguay and Florida to about 180 days in Wisconsin and Minnesota (New et al. 2002). The average number of sun hours during the growing season varied between 5.6 hours in the Netherlands to 8.9 hours per day in Florida (New et al. 2002).

The ranges in nutrients, lake morphology (where we used the ratio between maximum and mean depth as an indicator of the flatness of the lake bottom), chla and macrophyte coverage were also different, but overlapped (Figure 3.1, Table 3.3). The lakes in Denmark covered the largest range in TP concentration. The dataset of Wisconsin lacked lakes with high TP concentrations. Argentina covered the largest range in TN concentration; Wisconsin the smallest.

Table 3.2 Average (years 1961 – 1990) climate characteristics of the different regions in the lake dataset

Region	**Spring[b] mean temperature [Celsius]**	**Growing season[c] mean temperature [Celsius]**	**Frost days [d]**	**Growing season[c] sun hours [e] [d^{-1}]**
Denmark	10.3	12.6	98	6.8
Netherlands	11.6	13.7	82	5.6
Wisconsin	12.5	15.3	178	8.9
Minnesota	12.3	15.2	186	8.8
Argentina [a]	15.0	16.2	90	8.3
Brazil-Uruguay [a]	22.3	23.0	5	6.3
Florida	23.9	25.5	10	8.9

[a] Climate characteristics determined in the 300 km wide coastal zone where lakes were situated, [b] On the Northern Hemisphere: April-June; on the Southern Hemisphere: October-December, [c] On the Northern Hemisphere: April-September; on the Southern Hemisphere: October-March, [d] Number of days with ground frost per year, [e] Average number of sun hours per day (day length multiplied by the fraction of the day without clouds)

Sources: climate data are average of the years 1961 – 1990 by New et al. (2002), day length algorithm by Meeus (1991) tabulated by http://www.orchidculture.com/COD/daylength.html

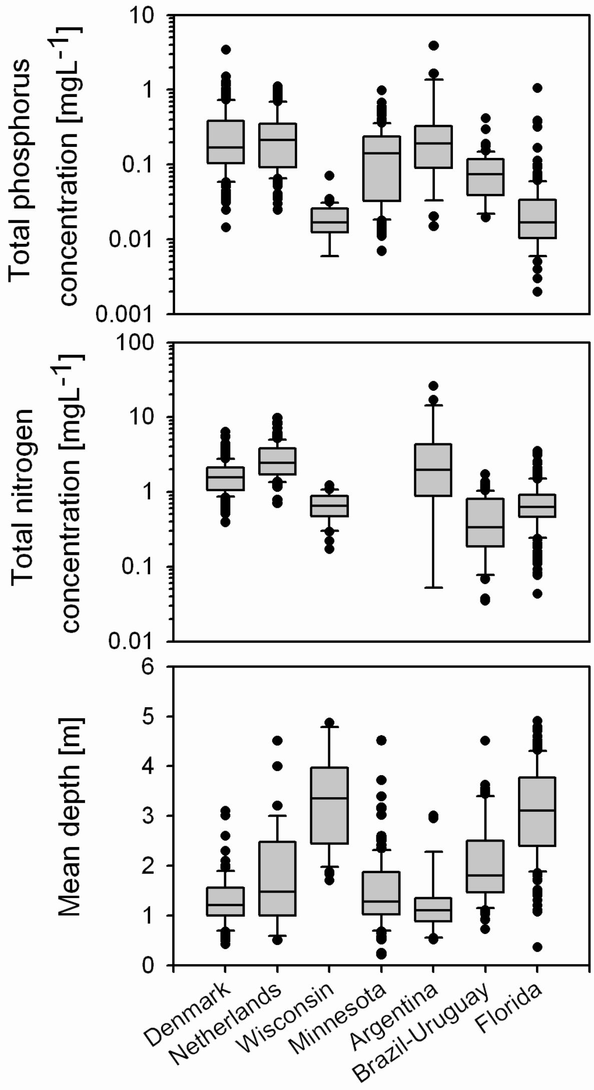

Figure 3.1 Distribution of total phosphorus concentration, total nitrogen concentration and mean depth in the different regions. Boundaries of the boxplots indicate the 25th and 75th percentile. Whiskers above and below indicate the 90th and 10th percentiles.

Table 3.3 Ranges of lake characteristics (minimum, maximum, median)

Region	**Surface area [hectares]**	**Maximum depth / mean depth [-]**	**Submerged macrophyte cover [%]**	**Chlorophyll-a [µgL^{-1}]**	**Secchi depth [m]**
Denmark	9 - 197 (32)	1.1 – 4.5 (1.8)	0 – 94 (0.03)	2 – 843 (86)	0.2 – 3.6 (0.6)
Netherlands	10 – 193 (46)	1.0 – 4.0 (1.5)	0 – 100 (0.1)	3 – 458 (80)	0.1 – 1.3 (0.3)
Wisconsin	20 - 165 (54)	1.3 – 5.0 (2.7)	8 – 76 (33)	1 – 27 (5)	0.9 – 3.5 (2.0)
Minnesota	11 – 194 (67)	1.1 – 4.2 (1.4)	0 – 83 (36)	2 – 466 (57)	0.1 – 4.6 (0.5)
Argentina	9 – 253 (40)	1.0 – 4.0 (1.4)	0 – 100 (10)	1 – 2889 (40)	0.04 – 6.8 (0.3)
Brazil-Uruguay	9 – 243 (45)	1.1 – 3.1 (1.6)	0 – 100 (5)	0 – 79 (3)	0.1 – 4.5 (0.9)
Florida	10 – 239 (62)	No data	0 – 100 (16)	1 – 241 (10)	0.3 – 6.4 (1.7)

General relationship between macrophyte occurrence and nutrients

The total proportion of lakes dominated by submerged macrophytes varied considerably among regions (Figure 3.2). In the pooled dataset, the proportion of lakes dominated by submerged macrophytes decreased in a sigmoidal way with increasing TP concentration, falling most steeply in the TP range of 0.05-0.2 mg L^{-1} (Figure 3.2a). The same trend was visible in all regional datasets except that of Wisconsin (Figure 3.2d), where lakes with high TP concentrations were not captured in the dataset. A large proportion of the lakes in Minnesota had submerged macrophyte coverages higher than 30% even at high TP concentrations (Figure 3.2e), whereas in Brazil-Uruguay, far fewer lakes were dominated by macrophytes (Figure 3.2g).

Macrophyte dominance was increasingly rare with increasing TN concentrations (Figure 3.3). Again, marked differences occurred among regions. For instance, in various lakes in The Netherlands, Argentina, and Florida, macrophyte dominance occurred at TN concentrations above ~2 mg L^{-1}, whereas in Denmark no macrophyte dominated lakes were found above this concentration. In Wisconsin and Brazil-Uruguay we did not collect data from lakes with high TN concentrations.

A 3D-mesh plot from the pooled dataset shows that macrophyte dominance is most likely in lakes that have low levels of both TP and TN (Figure 3.4). Nonetheless, at low TP concentrations up to 20% of the lakes are macrophyte-dominated even at high TN concentrations. The same holds for TP-rich lakes with low TN concentrations.

The logistic regression model using percent macrophyte coverage as the dependent variable confirmed the relationships between TP, TN, and macrophyte coverage (Table 3.4). We found no evidence for unimodal relationships of macrophyte coverage to nutrients, as quadratic terms for TP and TN did not improve the model significantly. When TP and TN were added to the model simultaneously, this only resulted in significant parameters in Danish lakes.

Effects of lake size and depth

Surface area and mean depth significantly explained macrophyte coverage in some regions (Table 3.4). Adding surface area to a logistic model including either TP or TN generally did not increase the significance of the model (not shown; note we only considered lakes between 10 and 250 ha). Mean depth improved the model fit in most regions especially in combination with either TP or TN (Table 3.4). In Wisconsin, none of these models were significant.

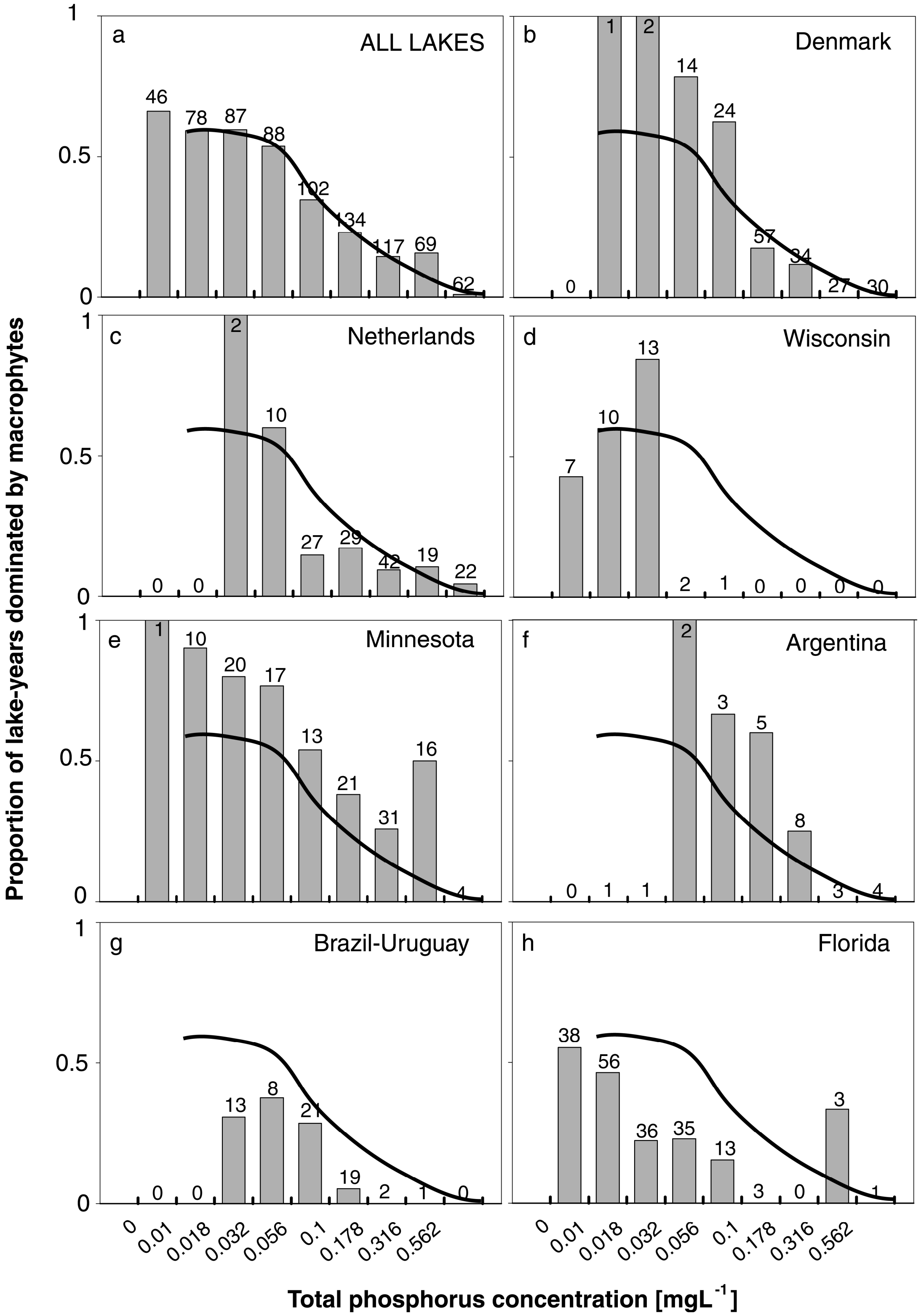

Figure 3.2 Proportion of lakes dominated by macrophytes (coverage >30%) at different total phosphorus (TP) concentrations. The curve represents the average proportion of lakes dominated by macrophytes in the different regions (panel A) and is reproduced in the other panels to facilitate comparison only. Class widths are equal on a logarithmic scale. The number of lakes in a certain TP class are represented on top of the bars.

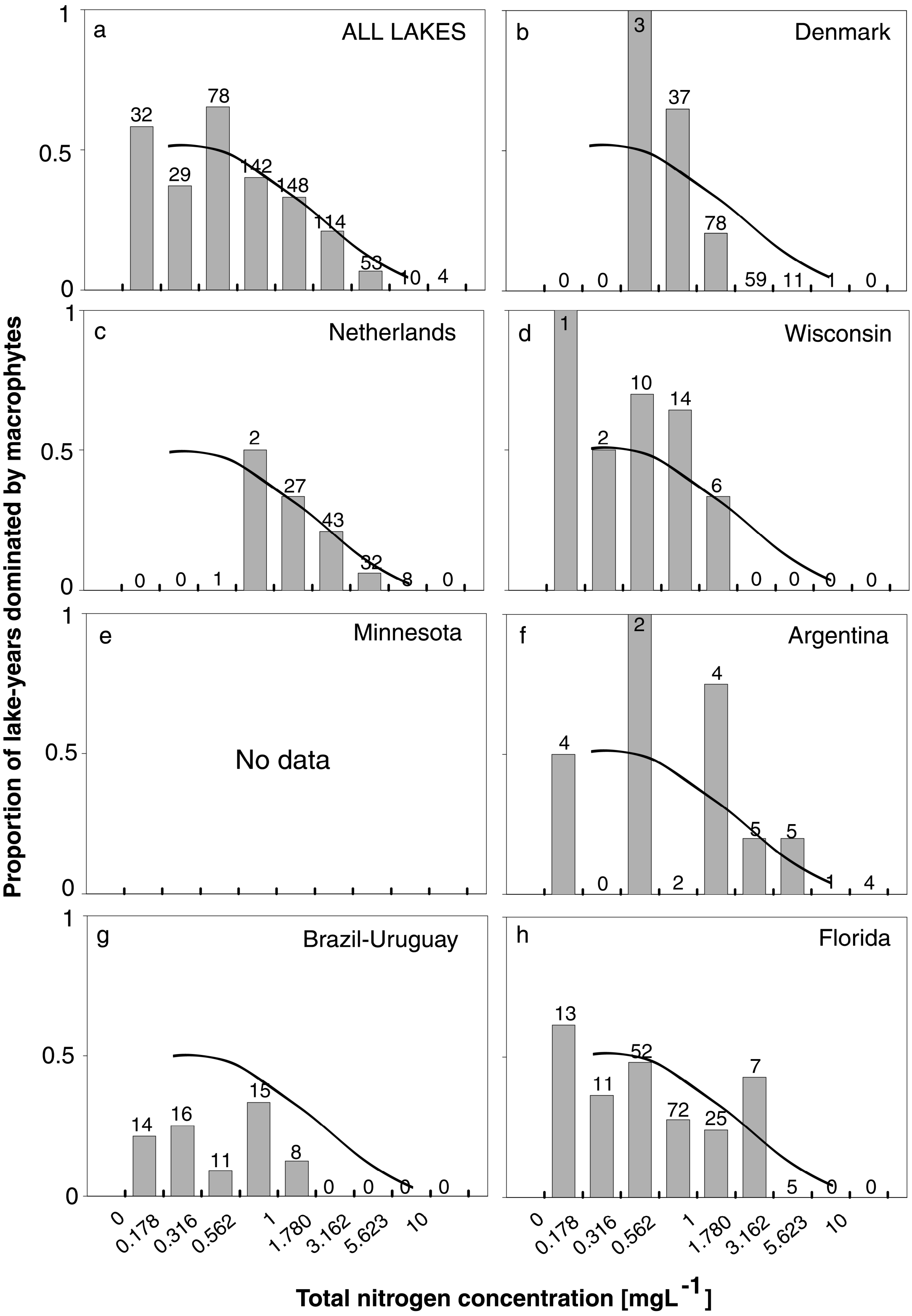

Figure 3.3 Proportion of lakes dominated by macrophytes (cover >30%) at different total nitrogen (TN) concentrations. The curve represents the average proportion of lakes dominated by macrophytes in the different regions (panel A) and is reproduced in the other panels to facilitate comparison only. Class widths are equal on a logarithmic scale. The number of lakes in a certain TN class are represented on top of the bars.

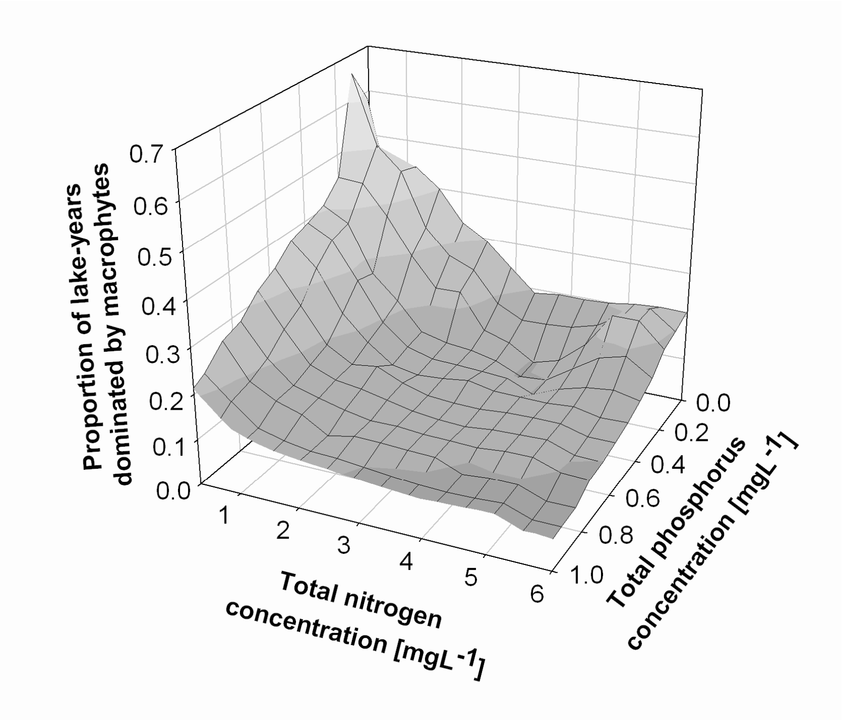

Figure 3.4 Proportion of lakes in the combined data set dominated by macrophytes at different total phosphorus and total nitrogen concentrations.

Table 3.4 Deviance reduction [%] of logistic models predicting submerged macrophyte coverage containing one (left hand side) or multiple (right hand side) explaining variables.

Region	**Area**	**Mean depth**	**TP**	**TP and TP^2**	**TN**	**TN and TN^2**	**TP and mean depth**	**TN and mean depth**
All			7***		4***		12***	4***
Denmark	1*		24***	24***	18***	18***	25***	18***
Netherlands	3*	15**	5**		10***		29***	37***
Wisconsin								
Minnesota		4**	8***		No data		15***	No data
Argentina			20**		13*		30***	24**
Brazil-Uruguay	6**		5**				5*	
Florida			1**		1*		3**	2**

Only models with a significant likelihood ratio test are shown:
*** $p<0.0001$, ** $0.0001<p<0.01$, * $0.01<p<0.05$

Regional differences in macrophyte-nutrient relationships

We used these regression models to show how the relationship between macrophyte coverage and nutrient concentration varied among regions for a standard lake with a mean depth of 1 m (Figure 3.5). Lakes with a low TP concentration in Argentina and Minnesota had on average a higher submerged macrophyte coverage than in Denmark and The Netherlands. Note that the coverage of the Brazilian-Uruguayan and Florida lakes at low TP concentrations cannot be easily interpreted as the intercept and/or parameters of the coefficient for mean depth of these models were not significant (Table 3.5).

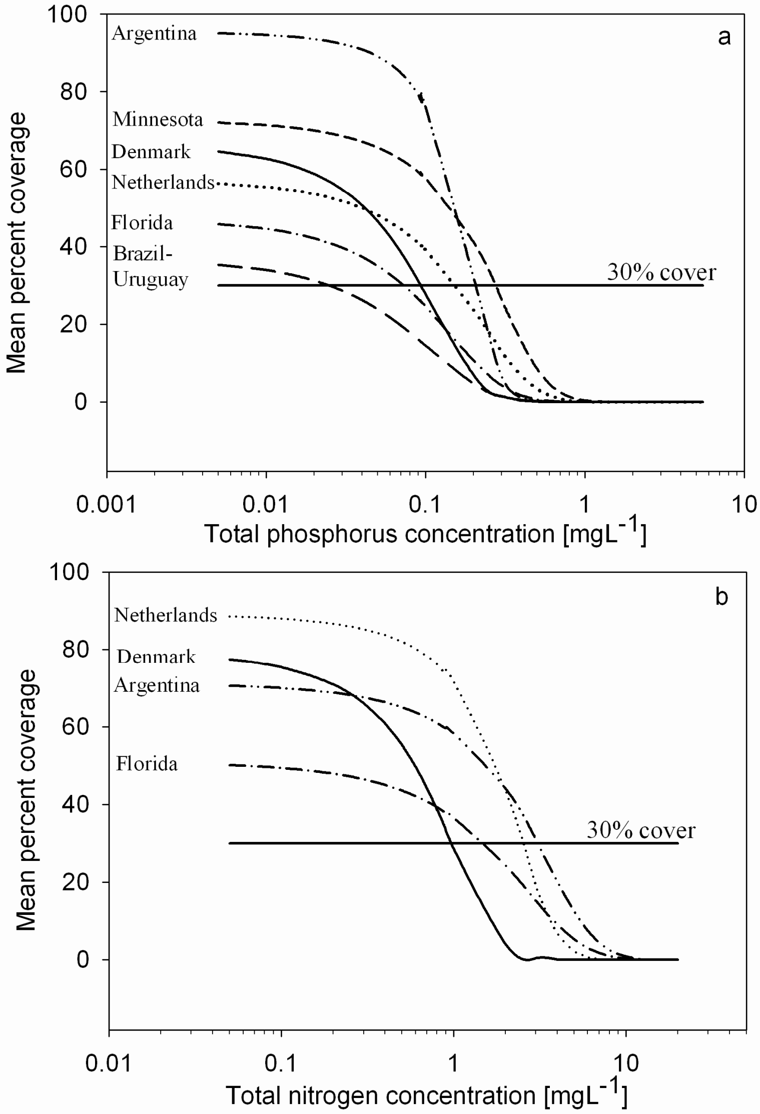

Figure 3.5 Predicted mean percent macrophyte cover in lakes with a mean depth of 1 m along a total phosphorus (TP) and total nitrogen (TN) gradient. The model parameters were: TP and mean depth (a) and TN and mean depth (b). Only regions where the logistic model was significant (likelihood ratio test p<0.05) are shown.

The TP concentrations at which 1-m deep lakes have a mean coverage of 30% (TP^{1}_{30}) varied between 0.02 and 0.3 mg L^{-1} (Figure 3.5a, Table 3.6). Minnesota lakes had the highest TP^{1}_{30} followed by the Argentinean, the Dutch, the Danish and the Florida lakes. In Brazil-Uruguay, lakes had the lowest TP^{1}_{30}. The Wisconsin model was not significant; therefore, we could not calculate the TP^{1}_{30} of this region. We found a significant correlation between the TP^{1}_{30} and the average number of frost days per year (Pearson correlation 0.88, p=0.021) (Figure 3.6). Note, however, that the deviance reduction of the regional models for Brazil-Uruguay and Florida was very low (Table 3.4). The confidence intervals of the TP^{1}_{30} concentration for the Argentinean and the Dutch lakes overlapped, as did the Dutch, the Danish and the Florida intervals (Table 3.6, Figure 3.6). The TP^{1}_{30} did not correlate with the other climate characteristics considered.

Table 3.5 Regression coefficients for logistic models predicting submerged macrophyte coverage including either ‘total phosphorus’ or ‘total nitrogen’ and ‘mean depth’.

Region	**Model including total phosphorus and mean depth**			**Model including total nitrogen and mean depth**		
	a	**b**	**c**	**a**	**b**	**c**
Denmark	1.3	-16.4	-0.6	1.9	-2.3	$-0.5^{\#}$
Netherlands	1.5	-7.3	-1.2	3.6	-1.2	-1.5
Wisconsin	$^{\$}$	$^{\$}$	$^{\$}$	$^{\$}$	$^{\$}$	$^{\$}$
Minnesota	2.1	-6.7	-1.1		No data	
Argentina	5.6	-19.0	-2.5	2.6	-0.6	-1.7
Brazil-Uruguay	$-0.4^{\#}$	-12.3	$-0.1^{\#}$	$^{\$}$	$^{\$}$	$^{\$}$
Florida	$0.2^{\#}$	-10.0	-0.3	$-0.3^{\#}$	-0.6	-0.3

$$p = \frac{e^{a+b*nutrientconcentration+c*meandepth}}{1+e^{a+b*nutrientconcentration+c*meandepth}}$$

, p is the mean macrophyte coverage

$^{\#}$ Regression coefficient not significant (p>0.05), $^{\$}$ Model not significant (likelihood ratio test p>0.05)

Table 3.6 Total phosphorus (TP) and total nitrogen (TN) concentration at which lakes with a mean depth of 1 m have an average submerged macrophyte coverage of 30% (TP^1_{30} and TN^1_{30}). The bootstrapped 5 – 95% confidence limits are presented between brackets.

Region	**TP^1_{30} [mg L^{-1}]**	**TN^1_{30} [mg L^{-1}]**
Denmark	0.09 (0.08 – 0.11)	1.0 (0.9 – 1.1)
The Netherlands	0.16 (0.09 – 0.22)	2.5 (2.0 – 2.9)
Wisconsin	$^{\$}$	$^{\$}$
Minnesota	0.27 (0.22 – 0.33)	No data
Argentina	0.21 (0.16 – 0.25)	3.1 (1.6 – 4.5)
Brazil – Uruguay	0.02 (0 - 0.07)	$^{\$}$
Florida	0.07 (0.03 – 0.15)	1.5 (0.8 – 2.5)

$^{\$}$ Model not significant (likelihood ratio test p>0.05)

The logistic model showed a sharper decrease in macrophyte coverage with increasing TN concentration in Denmark than in The Netherlands and Argentina (Figure 3.5b). The TN^1_{30}, the TN concentrations at which 1-m deep lakes have a mean coverage of 30%, varied between 1.0 and 3.1 mg TN L^{-1} (Figure 3.5b, Table 3.6). Denmark had a lower TN^1_{30} than The Netherlands and Argentina. The confidence intervals of the latter two overlapped (Table 3.6). Florida occupied an intermediate position, with a TN^1_{30} of 1.5 mg TN L^{-1}, but had a wide confidence interval which overlapped with those of the other regions (Table 3.6). The TN concentration was not related to macrophyte coverage in Wisconsin and Brazil-Uruguay. The Minnesota dataset did not contain TN data. TN^1_{30} was not significantly correlated with any of the climate characteristics.

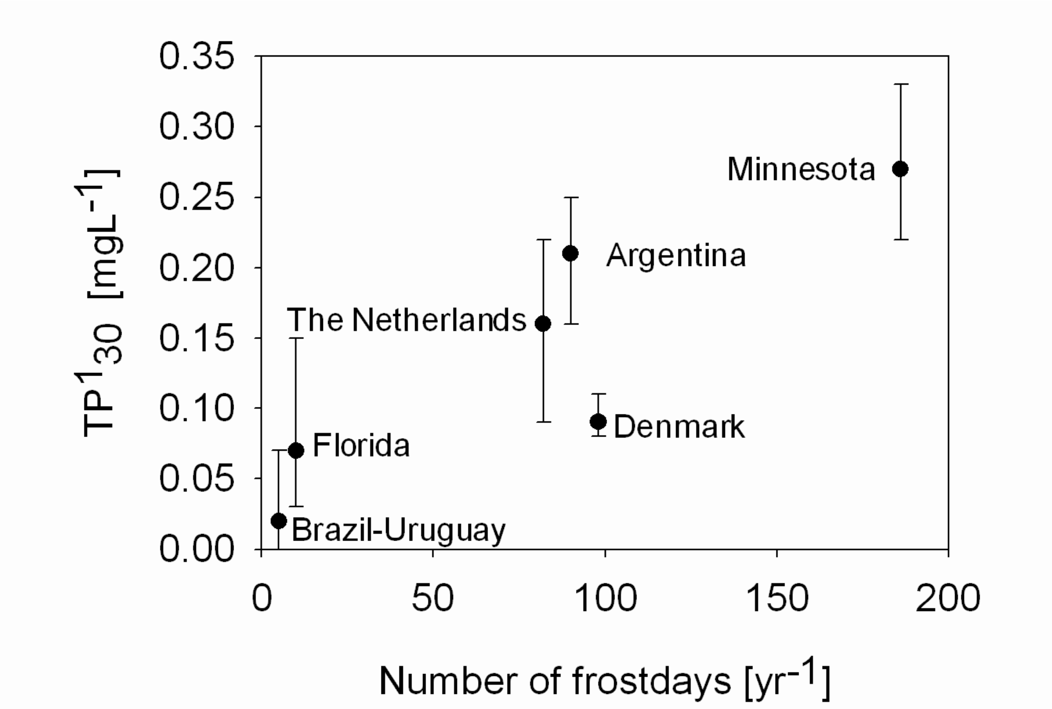

Figure 3.6 Estimated total phosphorus concentration at which lakes with a mean depth of 1 m have an average submerged macrophyte coverage of 30% (TP^{1}_{30}) in regions with a different number of days with ground frost per year. Only regions where the logistic model was significant (likelihood ratio test $p<0.05$) are shown. The whiskers show the 5 and 95% confidence limits.

We examined the influence of mean depth on the TP_{30} and TN_{30} using a pooled dataset including all lakes from those regions where the logistic model with TP (or TN) and mean depth was significant. For lakes with a mean depth of 1 m, we found a TP_{30} of 0.16 mg L^{-1}. Lakes with a depth of 2 m had a TP_{30} of 0.07 (Figure 3.7a). Similarly, we found TN_{30}'s of 1.8 and 0.9 mg TN L^{-1} for lakes with a mean depth of 1 and 2 m, respectively (Figure 3.7b).

We compared the light conditions under which macrophytes were growing in the different regions (Figure 3.8). We used a ratio between mean depth and Secchi depth as a ‘shading index’: a greater value for this ratio means that a smaller portion of the lake area receives enough light to allow macrophyte growth. Over the whole TP range, the shading index was mostly highest in Florida and Brazil-Uruguay (Figure 3.8a). Similar results are obtained when using TN instead of TP (not shown). Also, a number of Minnesota lakes had relatively high shading indices compared to the Danish, Argentinean, Wisconsin and Dutch lakes with similar TP concentrations (Figure 3.8b). Generally, we found that high macrophyte coverage ceased to occur at shading indices higher than 3. Yet, in several Minnesota lakes, macrophyte coverage remained high even when the shading index was much higher than 3.

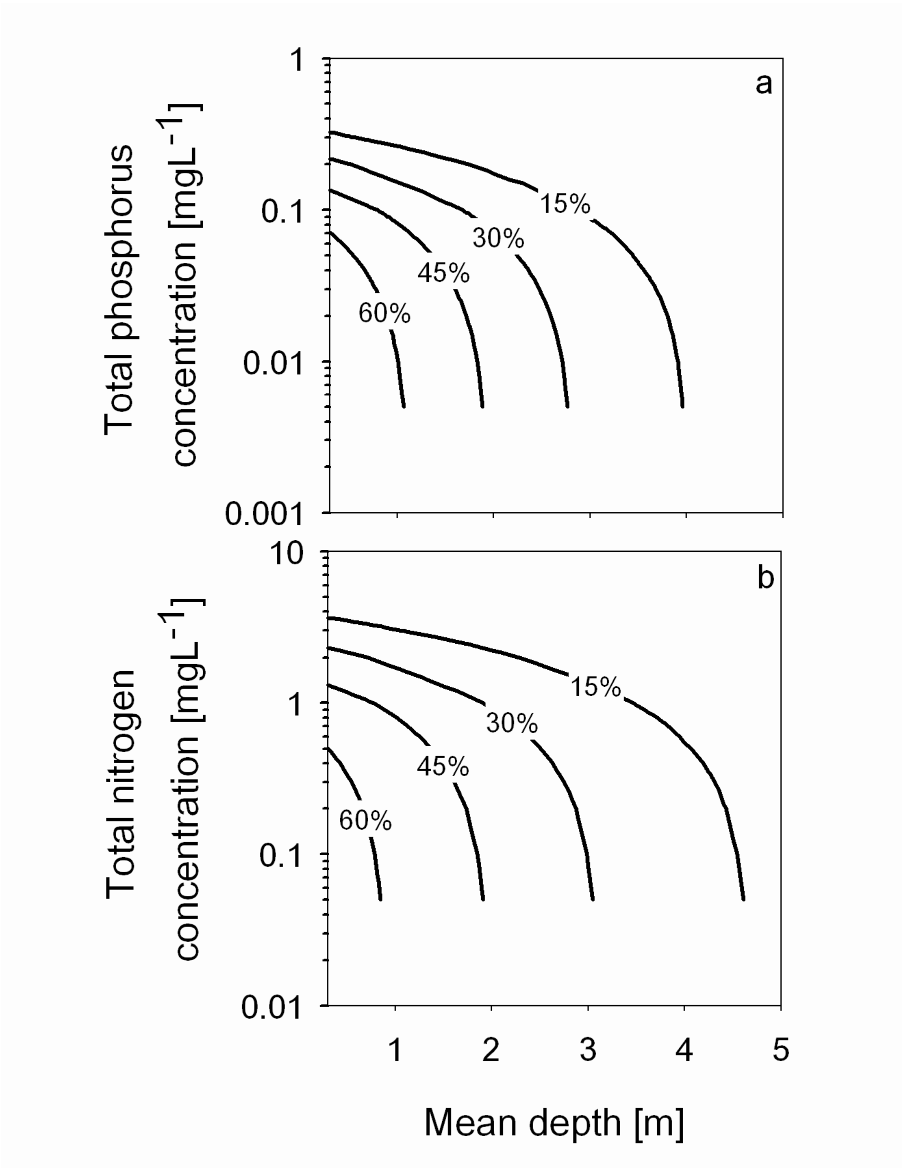

Figure 3.7 Mean percent submerged macrophyte coverage at different mean depths and total phosphorus concentration (a) or total nitrogen concentration (b). The logistic models incorporated data from Denmark, The Netherlands, Minnesota, Argentina, Brazil – Uruguay, and Florida.

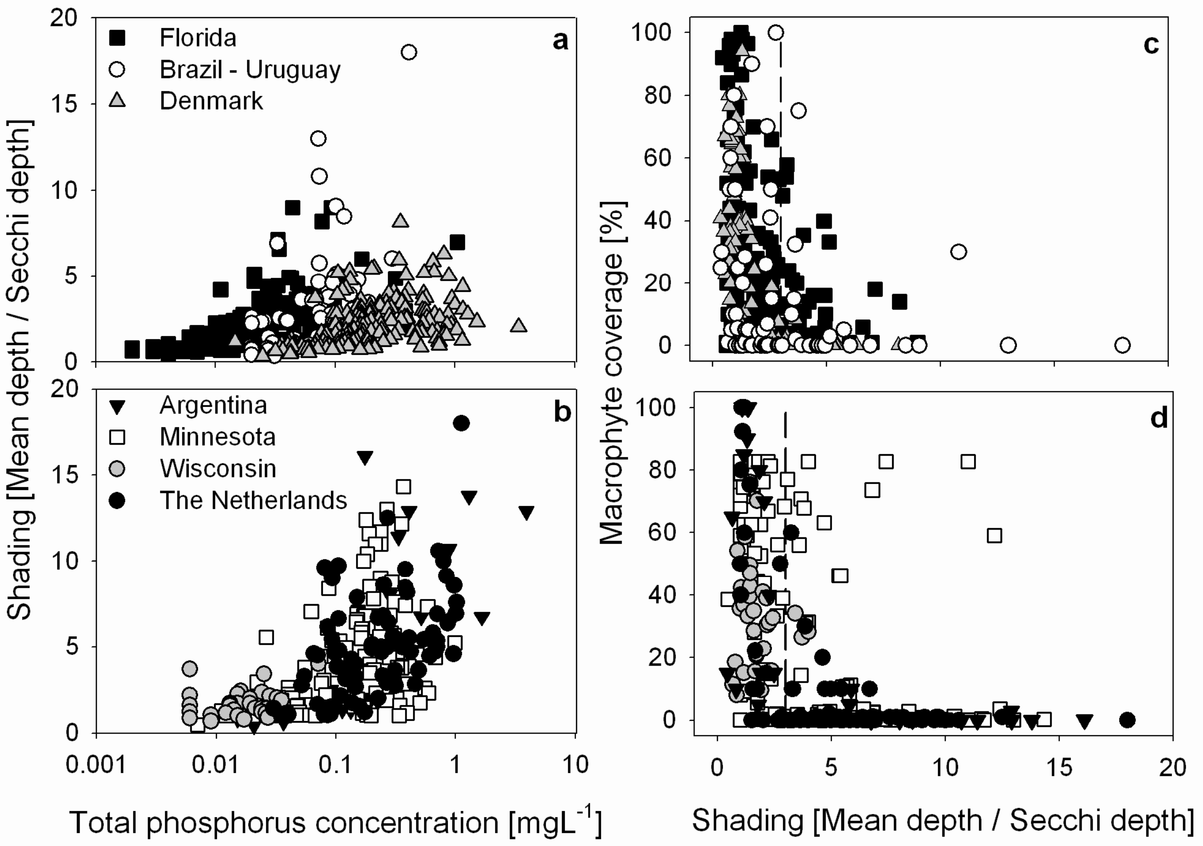

Figure 3.8 Observed relation between total phosphorus concentration and light availability (a and b), and the observed macrophyte coverage along a light availability gradient (c and d). The vertical line represents a shading index value of 3 above which few lakes have abundant macrophyte coverage.

Discussion

Clear patterns emerge from our analysis of this multi-lake, cross-continental dataset, despite inherent caveats associated with analyzing such a diverse dataset. Our results indicate that the nutrient level above which few lakes have abundant macrophyte coverage differs among climate regions. In warmer regions, the nutrient levels until which substantial macrophyte coverage occurs tend to be lower. The explained variance in the relationships between submerged macrophyte coverage, nutrients, and depth, however, remains relatively low, highlighting the importance of factors other than nutrients and lake depth in determining macrophyte dominance in shallow lakes. The explained variance is particularly low in the warmer climate regions (Brazil, Uruguay and Florida).

A caveat of our approach is that the way in which macrophyte coverage was assessed varied considerably among regions. In The Netherlands, for example, we had to derive the overall percent macrophyte coverage from the coverage of individual species and in Minnesota we had to convert presence/absence data into percent cover. Such procedures not only add uncertainty to the estimates, but may also introduce systematic differences between regions. Additionally, in some of the lakes in Florida, Wisconsin and Minnesota macrophyte coverages may have been managed. Nonetheless, the different methods do allow us to distinguish whether a lake is macrophyte dominated or macrophyte-poor. When using this dichotomous classification of the lakes in our analysis the outcome is similar to the outcome of the analysis based on the coverage percentage, which indicates that our results are robust against the detail of abundance estimates (see supplementary material).

Despite the uncertainties that come with our approach, our analyses reveal some clear relationships. For instance, the overall analysis shows that the proportion of lakes with substantial macrophyte coverage decreases in a sigmoidal way with increasing TP concentration, falling most steeply around a TP concentration of about 0.05-0.2 mg L^{-1}. These values correspond quite well with critical TP values found in detailed studies of more confined datasets which range from 0.06 to 0.25 mg L^{-1} (Jeppesen et al. 1990, Gonzalez Sagrario et al. 2005, Heinis and Evers 2007).

Gonzalez Sagrario *et al.* (2005) pointed out that not only TP, but also TN may affect macrophyte coverage. While their study was limited to Northern European lakes, our analysis suggests that a relationship between nitrogen and macrophyte abundance may be widespread. We found a significant relationship between TN and macrophyte coverage in Denmark, The Netherlands, Argentina, and Florida, which were also the regions where the highest nitrogen concentrations were found. Our pooled data suggest that substantial submerged macrophyte coverage is rare in lakes with a TN concentration above 1-2 mg L^{-1}. This is close to the TN concentration of 1.2 -2 mg L^{-1} above which macrophytes were generally absent in Denmark (Gonzalez Sagrario et al. 2005). Our data also indicate that lakes with a high TP concentration could still have substantial macrophyte coverage as long as the TN concentration is low. This fits well with results from more specific studies of this issue in Norway (Mjelde and Faafeng 1997), the UK (Moss et al. 1994) and Denmark (e.g. Michels et al. 2007). The pattern might be explained by nitrogen limitation of phytoplankton and periphyton growth, and suggests that it may be useful to focus eutrophication management of shallow lakes not only on phosphorus, but also on nitrogen load reduction (Gonzalez Sagrario et al. 2005, James et al. 2005).

In addition to nutrients, morphometric characteristics such as lake area (Van Geest et al. 2003, Jeppesen et al. 2007a) and depth (Mjelde and Faafeng 1997) are known to affect macrophyte occurrence. Lake area had little effect on macrophyte coverage in the lakes in our dataset. This is likely due to the fact that we avoided lakes smaller than 10 ha because such lakes are more likely to be macrophyte dominated (Van Geest et al. 2003). In contrast, even though we also constrained our dataset to a small range of mean depths (up to 5 m), mean depth considerably influenced macrophyte coverage.

Certainly not only mean depth, but the entire depth profile of the lake is important for macrophyte coverage. However, although the ratio between maximum and mean depth was relatively high in Wisconsin it was fairly constant among the other regions (Table 3.3), suggesting that this would not be an important factor explaining differences among the regions.

It seems likely that the depth effect is related to the light availability which is a major factor explaining macrophyte occurrence in lakes. For instance, pooling the data of all regions we found that a 2-m deep lake on average needed a two times lower TP concentration than a 1-m deep lake in order to maintain an average macrophyte coverage of 30%. The same is true for the TN concentration. The vertical light attenuation, which determines the available light at the bottom of a lake, tends to be linearly related to TP and TN levels (e.g. Scheffer 1998).

Although we generally found significant relationships between macrophyte coverage, nutrients, and mean depth, most of the variance in macrophyte abundance remains unexplained, as reflected by the low overall deviance reduction of the logistic models. Certainly, many other factors than the ones covered by our analyses may affect macrophyte abundance. Indeed, predicting submerged macrophyte coverage is notoriously difficult (Scheffer 1991), as exemplified by large interannual variation in macrophyte coverage in shallow lakes with no apparent explanation (Søndergaard et al. 1997, Bayley et al. 2007, Hargeby et al. 2007). Importantly, the deviance reduction of the models for the warmer (sub)tropical regions (Brazil, Uruguay and Florida) was far lower than for the temperate regions, which suggests that a model based on nutrients and mean depth is not sufficient to determine macrophyte coverage in warmer shallow lakes. This agrees with findings of Bachmann *et al.* (2002) and Jeppesen *et al.* (2007b) who found, respectively, no or only a weak relationship between macrophyte abundance and TP and TN in warm lakes. These results suggest that among-lake variation in macrophyte dominance might be driven by factors other than nutrients and depth, particularly in tropical and subtropical regions. Higher fish density in the (sub)tropics (Meerhoff et al. 2007a) may play a role.

Despite the considerable amount of unexplained variance in the relationship between nutrients and submerged macrophyte coverage within regions, we found substantial differences in these relationships among regions. These differences may be explained by factors that systematically vary among regions such as the functional characteristics of macrophyte and fish species, and variations in food-web structures. These regional variations may be linked to climate and potentially have strong implications for water clarity and submerged macrophyte growth (Jeppesen et al. 2007a, Meerhoff et al. 2007a).

We found that differences in the relation between nutrient concentrations and light availability among the regions may explain part of the difference in the relation between nutrient concentrations and macrophyte abundance. Along the nutrient gradient the available light, approximated by the shading index, is generally lower in lakes in Florida and Brazil-Uruguay than in lakes in the other regions. Minnesota lakes, as well, frequently had high shading indices, and seemed to be exceptional in the sense that they were macrophyte dominated at shading index values that were inhospitable to macrophytes in other regions. One possible explanation could be that the macroinvertebrate community, particularly snails (Sheldon 1987, Brönmark 1990, Sheldon 1990), controls periphyton more strongly in these lakes than in the other regions of our study, which would enable macrophytes to grow under more turbid conditions (Brönmark and Weisner 1992, Jones and Sayer 2003). The potentially strong invertebrate grazing may be triggered by partial fish kills due to long ice cover duration (Schindler and Comita 1972, Brönmark and Weisner 1992, Jackson et al. 2007) in Minnesota lakes. This hypothesis is well in line with the observed correlation between the number of frost days and the TP concentration until which abundant submerged macrophyte growth is found. Lack of comparable fish, macroinvertebrate, and periphyton data for the different regions prevents us from evaluating this hypothesis.

In conclusion, our analysis suggests that macrophytes may be abundant up to higher nutrient levels in regions with cold winters. In particular the relationship to the number of frost days is remarkable (Figure 3.6). The idea that ice cover duration is of major importance for the ecological state of shallow lakes agrees with results from long-term monitoring studies comparing lakes after severe and mild winters and cross-climate studies. Long ice cover may lead to anoxia under the ice, smaller fish communities, and stronger zooplankton control on phytoplankton and periphyton (Gyllström et al. 2005, Jackson et al. 2007). A decrease in ice cover duration may therefore cause the decline of submerged macrophyte coverage as has been found in Sweden (Hargeby et al. 2004). Taken together, this suggests that lower nutrient levels might be needed to maintain substantial submerged macrophyte coverage in regions where climatic warming is projected to lead to a reduction of frost days in winter.

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Supplementary material to chapter 3

Results from the binominal logistic regression

To examine the relationship between submerged macrophytes and total phosphorus (TP) and total nitrogen (TN) we used a binominal logistic regression with macrophyte dominance as the dependent variable. We applied an *a priori* threshold of 30% submerged macrophyte coverage to distinguish between lakes dominated by submerged macrophytes (>30%) and macrophyte-poor lakes (<30%). We found that at low TP concentrations the probability of macrophyte dominance in lakes with a mean depth of 1 m was highest in Argentina, Denmark and Minnesota, followed by The Netherlands and Brazil-Uruguay (Figure 3.Ia). The relationship between TP and submerged macrophyte dominance was not significant in Wisconsin and Florida. As a metric to compare the relationship between TP and TN and submerged macrophytes among regions we used the TP (or TN) concentration at which 50% of the lakes are still dominated by submerged macrophytes. The TP concentration at which 50% of the lakes are dominated by submerged macrophytes ranges from 0.04 to 0.26 mg TP L^{-1} (Table 3.I). The lowest concentration corresponds to Brazil-Uruguay and the highest to Argentina. These results are similar to those of the logistic regression in which we used coverage percentage as the independent variable and the TP^{1}_{30} (TP concentration at which an average coverage percentage of 30% is found) as the comparison metric (see main text).

The deviance reduction of the logistic model including TP and mean depth was highest in Argentina, Denmark, The Netherlands and Minnesota and lowest in Brazil-Uruguay (Table 3.II). This outcome as well is similar to the logistic regression results using coverage percentage, except for Florida. The Florida model was significant when using the coverage percentage, but insignificant when the classes macrophyte-dominated or macrophyte-poor were used. Note, however, that even though the Florida model is significant when using the coverage percentage the deviance reduction of the model is very low.

The binominal logistic regression resulted in significant relationships between TN and submerged macrophyte-dominance in Denmark, The Netherlands, Argentina and Florida, just as the logistic regression using cover percentage. The TN concentration at which 50% of the lakes are dominated by submerged macrophytes is higher in The Netherlands and Argentina than in Denmark and Florida (Figure 3.Ib, Table 3.I). This is similar to the TN^{1}_{30} outcome of the logistic regression using coverage percentages (see main text). The deviance reduction of the binominal model including TN and mean depth was – just as in the models with coverage percentage – highest in Denmark, The Netherlands, and Argentina and lowest in Florida (Table 3.II).

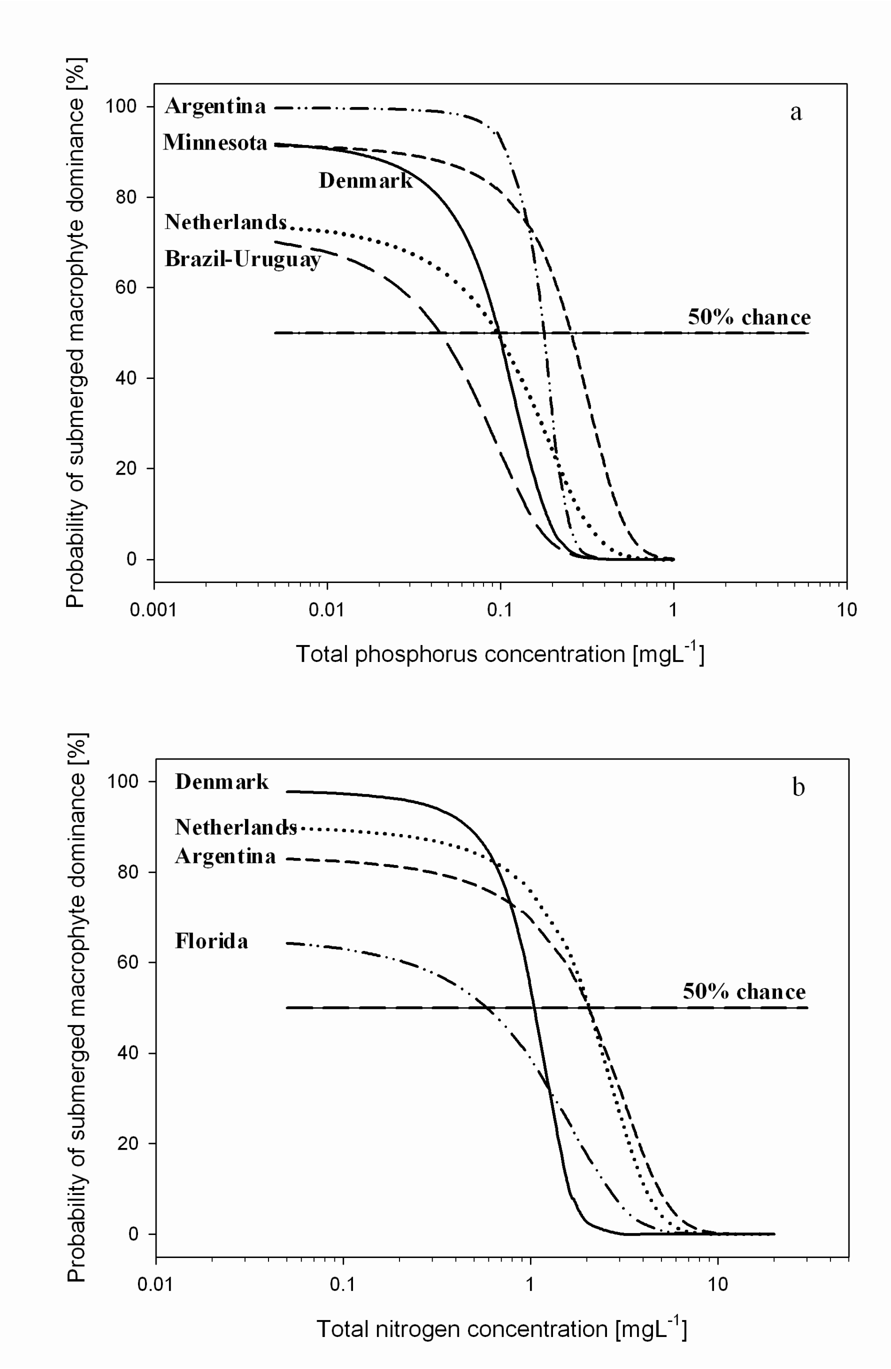

Figure 3.I Predicted probability of submerged macrophyte dominance (i.e. coverage > 30%) in lakes with a mean depth of 1 m along a total phosphorus (TP) and total nitrogen (TN) gradient, the logistic model parameters were: TP and mean depth (a) and TN and mean depth (b). Only regions were the logistic model was significant (likelihood ratio test p<0.05) are shown.

Table 3.I Total phosphorus (TP) and total nitrogen (TN) concentrations at which there is a 50% chance to find submerged macrophytes dominating (coverage > 30%) in lakes with a mean depth of 1 m. The bootstrapped 5 – 95% confidence limits are presented between parentheses.

Region	**TP [mgL^{-1}]**	**TN [mgL^{-1}]**
Denmark	0.10 (0.08 – 0.12)	1.0 (0.9 – 1.2)
The Netherlands	0.10 (0.01 – 0.14)	2.1 (1.0 – 2.5)
Wisconsin	[\$]	[\$]
Minnesota	0.26 (0.20 – 0.32)	No data
Argentina	0.18 (0.13 – 0.21)	2.0 (0.35 – 3.3)
Brazil – Uruguay	0.04 (0 – 0.09)	[\$]
Florida	[#]	0.6 (0 – 1.1)

[\$] Model not significant (likelihood ratio test p>0.05), [#] regression coefficient for TP not significant

Table 3.II Deviance reduction [%] of logistic models predicting macrophyte coverage.

Region	**TP and mean depth**	**TN and mean depth**
Denmark	40	36
The Netherlands	36	33
Wisconsin	[\$]	[\$]
Minnesota	24	No data
Argentina	56	32
Brazil – Uruguay	13	[\$]
Florida	[#]	36

[\$] Model not significant (likelihood ratio test p>0.05), [#] regression coefficient for TP not significant

Lagoa Limpa – Rio de Janeiro – Brazil – August 2005

4 Effects of submerged vegetation on water clarity across climates

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Abstract

A positive feedback between submerged vegetation and water clarity forms the backbone of the alternative state theory in shallow lakes. The water clearing effect of aquatic vegetation may be caused by different physical, chemical and biological mechanisms and has been studied mainly in temperate lakes. Recent work suggests differences in biotic interactions between (sub)tropical and cooler lakes might result in a less pronounced clearing effect in the (sub)tropics. To assess whether the effect of submerged vegetation changes with climate, we sampled 83 lakes over a gradient ranging from the tundra to the tropics in South America. Judged from a comparison of water clarity inside and outside vegetation beds, the vegetation appeared to have a similar positive effect on the water clarity across all climatic regions studied. However, the local clearing effect of vegetation decreased steeply with the contribution of humic substances to the underwater light attenuation.

Looking at turbidity on a whole-lake scale, results were more difficult to interpret. While lakes with abundant vegetation (>30%) were generally clear, sparsely vegetated lakes differed widely in clarity. Overall the effect of vegetation on water clarity in our lakes appears to be smaller than that found in various Northern hemisphere studies. This might be explained by differences in fish communities and their relation to vegetation. For instance, unlike in Northern hemisphere studies we find no clear relation between vegetation coverage and fish abundance or their diet preference. High densities of omnivorous fish and coinciding low grazing pressures on phytoplankton in the (sub)tropics may, furthermore, weaken the effect of vegetation on water clarity.

Introduction

Temperate shallow lakes tend to have two contrasting states over a range of conditions: a clear state dominated by aquatic vegetation or a turbid state (Jeppesen et al. 1990, Moss 1990, Scheffer 1990, Scheffer et al. 1993). This phenomenon has been explained by the alternative stable state theory (Scheffer et al. 1993). The backbone of this theory is the positive feedback between vegetation and water clarity: vegetation enhances water clarity and clearer water promotes vegetation growth. Several mechanisms may contribute to the impact of submerged vegetation on water clarity. Vegetation may provide a refuge for zooplankton (Timms and Moss 1984, Schriver et al. 1995); prevent resuspension and promote sedimentation of suspended matter (Barko and James 1998, Madsen et al. 2001); compete, together with periphyton, for nutrients and light with phytoplankton (Ozimek et al. 1990); or the vegetation may excrete allelopathic substances inhibiting growth of phytoplankton (Gross and Sütfeld 1994). Different combinations of these mechanisms have been held responsible for the higher water transparency in the presence of plants.

How strong the different physical, chemical and biological water clearing mechanisms are and how they interact under different conditions is still unclear. Especially differences in biological interactions in (sub)tropical and temperate lakes may result in a less pronounced clearing effect in the (sub)tropics (Jeppesen et al. 2007a, 2007b, Meerhoff et al. 2007a). Zooplankton, for instance, tends to be much smaller in warmer regions (Gillooly and Dodson 2000) which decreases their filtering capacity and likely weakens the top down control on phytoplankton. Furthermore, the high density of continuously reproducing omnivorous fish (generally small-sized species) in the (sub)tropics and their association with submerged vegetation (Sazima and Zamprogno 1985, Conrow et al. 1990, Mazzeo et al. 2003) decreases the potential control of phytoplankton by zooplankton (Jeppesen et al. 2005b, Van Leeuwen et al. 2007). These aspects could weaken or eliminate the zooplankton mediated part of the water clearing effect of aquatic vegetation (Meerhoff et al. 2007b).

Fish may also influence water clarity by resuspending sediment, by increasing nutrient cycling from the sediment to the water column (Lammens 1988), or by grazing on aquatic vegetation (Hansson et al. 1987). Differences in diet and feeding behavior among fish in cool and warm lakes may therefore also cause a difference in the water clearing effect of submerged macrophytes.

The effect of vegetation on water clarity through nutrient competition with phytoplankton may differ between climates as well. As periphyton densities tend to be lower in warmer lakes (Meerhoff et al. 2007a, Bécares et al. 2008), likely due to grazing by omnivorous fish (Meerhoff et al. 2007a), the macrophyte – periphyton ensemble might compete less for nutrients with phytoplankton in warm than in cool lakes.

Insight into the water clearing effect is important not only from a theoretical point of view, but also for understanding the probability of success of management options in different climate zones (Jeppesen et al. 2007a). For instance, effects of a temporary fish stock reduction (‘biomanipulation’) can be long lasting if a self-stabilizing vegetated state is reached. Also, the positive feedback resulting from the clearing effect is important for lake restoration measures such as inoculating lakes with vegetation (e.g. Hilt et al. 2006) or protecting plants from grazing by waterfowl (Søndergaard et al. 1996) with the aim to create favorable conditions for plants to recover.

The effect of vegetation on water clarity can be studied experimentally using enclosures with and without plants (Hasler and Jones 1949, Schriver et al. 1995). While this is a straightforward approach, results remain difficult to translate to whole-lake situations where fish and wind influences are different. Alternatively, the effect of vegetation on clarity can be evaluated by comparing individual lakes in years with vegetation dominance and in years with algal dominance (e.g. Meijer and Hosper 1997) or by comparing water clarity among lakes with different macrophyte coverages (e.g. Jeppesen et al. 1990, Bachmann et al. 2002). Obviously, there remains an issue of causality in such studies, as one can never be sure to which extent clarity is the cause or the effect of differences in vegetation abundance. Another common way to evaluate the effect of vegetation on transparency is to compare water clarity between open water and vegetation stands within a lake (e.g. Pokorny et al. 1984, Jones 1990). While this is a straightforward approach to assess local effects, it does not capture the potential effects of vegetation beds on open water characteristics, occurring, for instance, through alteration of the fish community or through the promotion of large herbivorous zooplankton species that may migrate to the open water (Timms and Moss 1984, Jeppesen et al. 1998b).

Here we explore the potential effect of climate on the capacity of submerged plants to clear the water, by comparing water clarity and seston characteristics in lakes with and without abundant macrophyte growth as well as inside and outside vegetation beds in 83 lakes across a climatic gradient ranging from tundra to tropical regions in South America.

Methods

Study sites – We sampled 83 shallow lakes along a latitudinal gradient (5 – 55 °S) in South America. The lakes were classified into three groups based on the prevailing climate (New et al. 2002): 'warm' sites had an average monthly air temperature above 25°C in at least one month, at 'cold' sites ground frost occurred for more than 120 days a year, and the other lake sites were classified as 'intermediate' (Figure 2.2). All lakes were shallow and had a surface area smaller than 2.53 km^2. In every group lakes were selected to vary as much as possible in vegetation coverage and trophic state (Table 4.1).

Table 4.1 General data on the lakes sampled.

	Range	Mean	Medium
Area [km^2]	0.09 – 2.53	0.62	0.46
Mean depth [m]	0.5 – 4.5	1.8	1.6
Average air temperature during warmest month [°C]*	8.2 – 28.7	23.2	23.5
Number of ground frost days [y^{-1}]$^{(1)}$	0 – 169	30	2
Conductivity [µScm^{-1}]	37.8 - 4930	527	167
Total nitrogen [mg N L^{-1}]	<0.1 – 25.8	1.8	0.4
Total phosphorus [mg P L^{-1}]	0.02 – 9.14	0.27	0.10
Total suspended solids [mg L^{-1}]	2 – 663	43	9
Chlorophyll-a [µg L^{-1}]	0.6 - 2889	79.6	4.7
Turbidity [NTU]	0.1-303	19	5
Fluorescence [µg L^{-1}]	$7 \cdot 10^{-2}$-204	5	1
Humic substances [absorption m^{-1} at λ=380nm]	0.3 – 54.2	5.9	4.0
Light attenuation coefficient [m^{-1}]	0.5-43.6	4.6	2.5
PVI [%]	0 – 81	10.5	0.5
Zooplankton biomass [µg Dry Weight L^{-1}]	4 - 4466	530	142
Fish [CPUE; individuals net^{-1} 12hour^{1}]	0 – 2016	218	80

* Monthly means over the period 1961-1990 (New et al. 2002)

Sampling - All lakes were sampled once during summer (cold and intermediate lakes) or dry season (warm lakes) between November 2004 and March 2006 by the same team. We collected integrated water samples at 20 random points in each lake. From each point sample two liters were used to pool into a single large bulk sample. Subsequent subsampling for different analyses was done from the bulk sample. Filtration for various analyses was conducted directly after collection. Water and filters were then frozen until analysis. Phytoplankton samples were fixed in a Lugol's solution. Another two liters were filtered on a 50µm sieve for quantitative zooplankton determination. The zooplankton sample was preserved in a 4% formaldehyde solution. Light measurements were conducted at different depths at noon in the center of the lake with a LICOR LI-192SA. Coverage of submerged vegetation (cov$_{lake}$) was estimated based on observations of vegetation presence/absence at 20 random points in the lake combined with coverage estimations of macrophytes at 13 – 47 points (average 22) equally distributed on 3 to 8 parallel transects perpendicular to the maximum length of the lake. The number of transects varied with the shape and size of the lake. Observations were made from a boat using a grapnel when water transparency was insufficient to get a clear view of the bottom. Furthermore, we measured depth and the length of the plants at each sample point along the transects.

At approximately one third of the sample points along the transects we measured water clarity using three different techniques: by measuring fluorescence, turbidity and mini-disk depth. These techniques comprise different components influencing water clarity. Fluorescence gives an indication of the phytoplankton abundance, turbidity measurements include phytoplankton, detritus, and inorganic suspended solids, and the mini-disk depth is influenced by all these components plus humic substances. We determined fluorescence and turbidity using a Turner Design Handheld Fluorometer. The fluorescence channel was calibrated daily with a solid secondary standard (PN 8000-950, Turner Designs) and the turbidity channel with a 50 NTU “non-ratio” standard (GFS chemicals). If macrophytes were present we took care not to resuspend loosely attached epiphytes. We measured the ‘mini-disk depth’ by filling a 0.5 m long metal tube of 7 cm in diameter with water in which we submersed a black and white disk until it just disappeared. The mini-disk method allows visual quantification of water transparency in places where a Secchi disk would have hit the bottom or disappeared between the plants. Fish were sampled overnight by multimesh gillnets (Appelberg 2000: Mazzeo et al., unpublished data). Data represent the average catch among nets, expressed as catch per unit effort (CPUE; individuals net^{-1} 12 hour^{1}).

Sample analysis – Total phosphorus (TP) and total nitrogen (TN) concentrations were analyzed using a continuous flow analyzer (Skalar Analytical BV) following NNI protocols (1986, 1990), with the exception of the UV/Persulfate destruction which was not executed beforehand but integrated in the system. Suspended solids were determined on pre-weighed GF/F Whatman filters after drying at 105^{o}C for one night. Loss on ignition (3 hours at 500^{o}C) was used as a proxy for organic matter (OM). As a measure for humic substances spectrophotometric light absorption at 380 nm (Buiteveld 1995) was measured in filtered (0.45 µm S&S) water against distilled water using a 5-cm cuvet. Chlorophyll-a (chla) was extracted from filters (GF/C S&S) with 96% hot ethanol and absorbance was measured at 665 and 750nm (Nusch 1980). Zooplankton taxa were counted and identified (Lacerot et al., unpublished data; Kosten et al. 2009c). Fish were classified according to their habitat preference (benthic - including benthic-pelagic species - or pelagic) and their diet (potentially piscivores, omnivores, periphyton feeders, and detritivores) based on literature on each species (Mazzeo et al., unpublished data.). We did not encounter fish with a diet of aquatic plants.

Every type of analysis was undertaken by a single person, mostly in one laboratory.

Calculations and data analysis – The percentage of the lake’s volume filled with submerged vegetation (PVI) was determined analogously to Canfield et al. (1984). The PVI of the individual sampling locations (PVI_{loc}) was calculated by multiplying the coverage percentage by the average length of the macrophytes divided by the depth. The PVI of the entire lake (PVI_{lake}) was calculated by multiplying the area of the lake covered by macrophytes (m^2) with the average height of vegetation in the vegetated locations that were sampled (m) divided by the total volume of the lake (m^3).

To evaluate local effects of vegetation we compared water clarity inside and outside the vegetation beds. In lakes where the vegetation coverage allowed us to take at least 2 samples inside and outside the vegetation beds, we calculated the average fluorescence (in 20 lakes), turbidity (in 19 lakes) and mini-disk depth (in 10 lakes) for open water (PVI_{loc}<30%) and vegetated areas (PVI_{loc}>70%). Subsequently, the difference in values in the open water and the vegetated areas was expressed as a percentage of the open water values. We defined this percentage as the 'water clearing effect' of the vegetation. A positive water clearing effect means that the water clarity inside the vegetation beds was higher than in the open water. We checked for correlations between the water clearing effect on the one hand and lake area, PVI_{lake}, concentrations of suspended solids, total phosphorus, humic substances, chlorophyll-a, and fish density on the other, using Spearman rank correlation. In bi-plots we checked for possible nonlinear relationships.

When verifying the water clearing effect of the most frequently occurring taxa of submerged species we compared the fluorescence, turbidity, and mini-disk depth measurements at the locations of homogeneous stands (PVI_{loc}>70%) with the average values of the open water. When Kruskal Wallis indicated differences ($p<0.05$) among the species then post hoc Mann Whitney was used to identify similarity of medians ($p>0.05$).

Additionally, we were interested in what the enhanced water clarity meant in terms of the increase in potential colonization depth. Submerged vegetation may colonize a lake until a depth where approximately 1% of the incoming irradiance reaches the bottom (e.g. Hudon et al. 2000): the euphotic depth. Our estimation of how much deeper the vegetation could potentially colonize the lake by its own enhancement of the water clarity had four steps. First, we calculated the vertical light attenuation of each lake (K_d) using Lambert-Beer's law and irradiation data. Second, we used different light attenuation compounds measured in the bulk sample as independent variables describing the K_d in a multiple linear regression (analogous to Buiteveld 1995, and De Lange 2000): fluorescence as a proxy for algal biomass, turbidity as a proxy for suspended solids, and humic substances as a proxy for the dissolved substances. Third, we used this model in each lake to estimate the K_d in the open water and in the vegetated area with the fluorescence and turbidity data measured in the open water and in the vegetation beds, respectively. Finally, we calculated the depth at which 1% of the surface irradiance penetrated from the calculated K_d's using Lambert-Beer's law. We did this both in the open water and in the vegetation bed. The difference between these two depths then gives the increase in potential maximum colonization depth caused by the water clearing effect of the vegetation.

Furthermore, the multiple regression model describing K_d allowed us to determine the contribution of humic substances to the K_d simply by dividing the model term for humic substances by K_d.

To evaluate the influence of submerged vegetation on whole-lake water clarity we assessed whether PVI adds to the explained variance of K_d or OM by TP. As TP and PVI influence each other we also checked for direct relationships between PVI, K_d, and OM using linear regressions. Since fish may strongly influence the water clarity (e.g. Lammens 1988, Mazumder et al. 1990) we included the benthic fish CPUE as a co-variable. Additionally, we evaluated the relationship between PVI and the potential grazing pressure to obtain insight into the possible influence of PVI on water clarity using a two-way ANOVA. As an indication for the potential grazing pressure of zooplankton on phytoplankton we used the zooplankton biomass : phytoplankton biomass ratio. We used a factor of 66 to convert chlorophyll-a concentrations to phytoplankton biomass (Jeppesen et al. 1999).

All statistical analyses were performed using SPPS for Windows v. 15.0 (SPSS Inc., Chicago, IL, U.S.A.). Data was log transformed to approach normality, to avoid zero's the lake's PVI was enlarged by 1% and the CPUE was increased by 1 individual net^{-1} 12 hour^{-1}. Differences between groups were determined using ANOVA's. When the data was not normally distributed (Shapiro-Wilk $p<0.05$) differences were assessed with Kruskal-Wallis and Mann Whitney U as a post hoc comparison. The Levene statistic was used to test for the equality of group variances.

Results

Local effects on transparency

In almost all lakes, turbidity was lower inside the vegetation beds than in the open water, indicating that vegetation has a rather consistent positive effect on local water clarity (Figure 4.1). Also, the overall photosynthetic capacity of phytoplankton (measured as fluorescence) was often lower inside the vegetation beds than in the open water (Figure 4.1).There was no significant difference in these local effects of vegetation on turbidity and fluorescence among the three climate regions (one way ANOVA, $p=0.57$ and $p=0.34$, respectively). Also, we did not find significant correlations between the water clearing effect (measured as difference in fluorescence or turbidity) and the variables lake area, PVI_{lake}, concentrations of suspended solids, total phosphorus, humic substances, chlorophyll-a, total fish density, and potential grazing pressure. However, we found a strong negative correlation between the clearing effect of vegetation determined with mini-disk readings and the concentration of humic substances (Spearman's $\rho=-0.81$, $p<0.01$). The clearing effect of vegetation was very small or even negative in lakes with a high content of humic substances.

Not surprisingly, the water clearing effects measured in different ways were significantly correlated (Spearman's ρ varied between 0.6 and 0.8, $p<0.05$). The water clearing effect of different submerged vegetation taxa varied among the sampling locations. Generally, we did not find differences in the water clearing effect estimated from turbidity and mini-disk depth among the genera (Kruskal Wallis $p=0.532$ and 0.171, respectively). However, *Egeria densa,* the only species collected for the *Egeria* genus, appeared to have a weaker effect on phytoplankton than the other taxonomic groups (Kruskal Wallis $p=0.041$, Figure 4.2).

Effect on light climate

A linear regression with the light attenuation coefficient (K_d) – measured in the center of the lake - as the dependent variable, and fluorescence (*fluo* in µgl^{-1}), turbidity (*turb* in NTU), and humic substances (*humic* in m^{-1}) –measured in the bulk water sample - as the independent variables resulted in the following model (explaining 92% of the variance, $p<0.0001$, $n=20$):

$K_d=1.05+0.051\,fluo+0.101\,turb+0.148\,humic$

Using this model, the relative contribution of humic substances to the K_d was estimated to range between 10 and 66% (Figure 4.3). Using fluorescence and turbidity data measured inside and outside the vegetation bed, the model allows us to estimate the effect of vegetation on the euphotic depth, suggesting a potential increase of approximately 60cm. The estimated effect of vegetation on euphotic depth was negatively correlated to the humic contribution to the K_d (Figure 4.3, R^2=0.38, p=0.005).

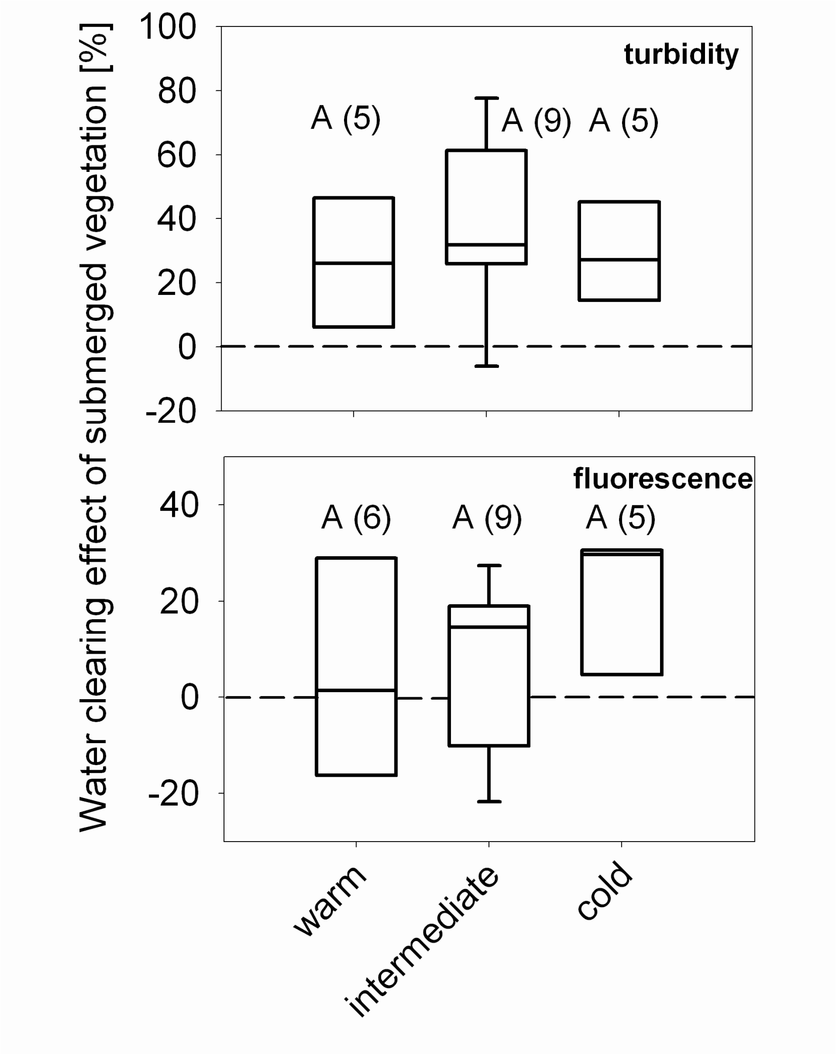

Figure 4.1 Water clearing effect of submerged vegetation in three different climate regions based on fluorescence and turbidity measurements (mini-disk measurements are not shown because of lack of data in the cold climate zone). Boundaries of the boxplots indicate the 25th and 75th percentile. Whiskers above and below indicate the 90th and 10th percentiles. Capital letters indicate groups with significantly similar medians. The number of data points is given between brackets.

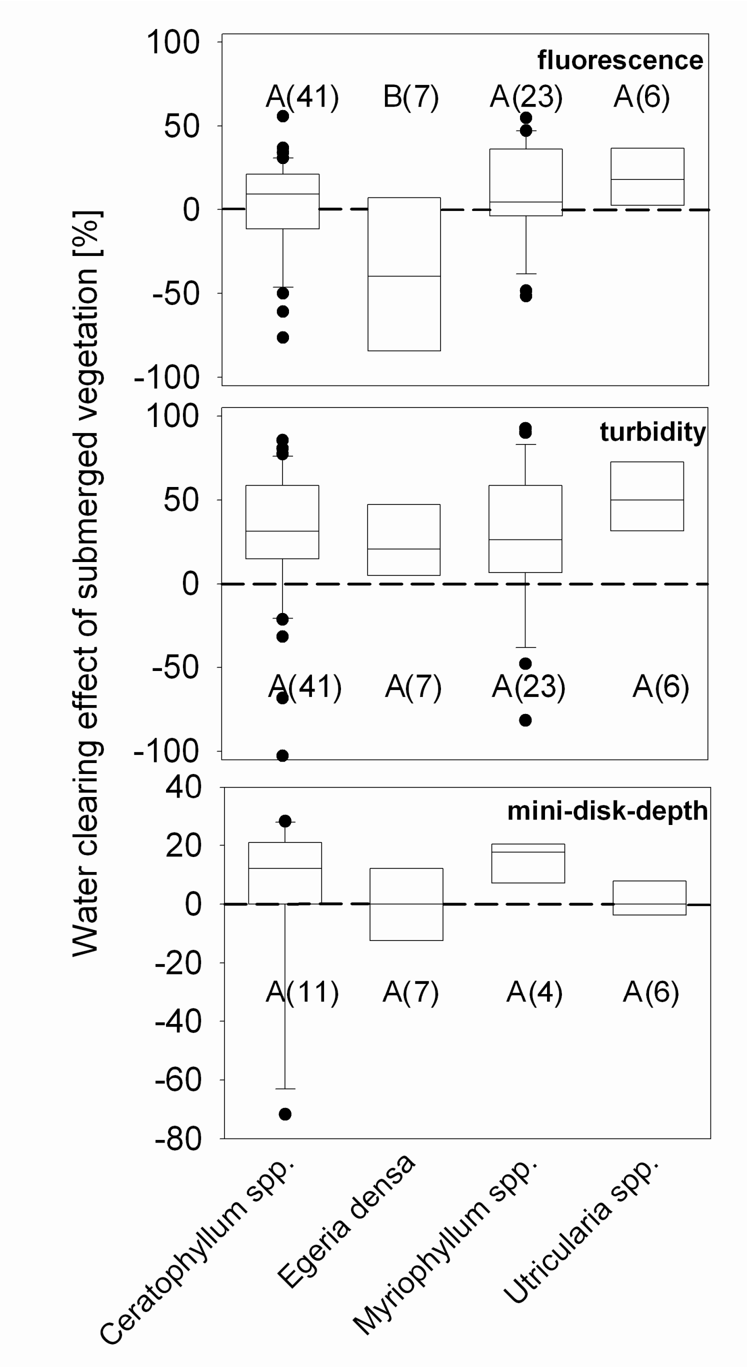

Figure 4.2 Water clearing effect of different Genera based on fluorescence, turbidity and mini-disk measurements. Dots are outlying points. See Figure 4.1 for details.

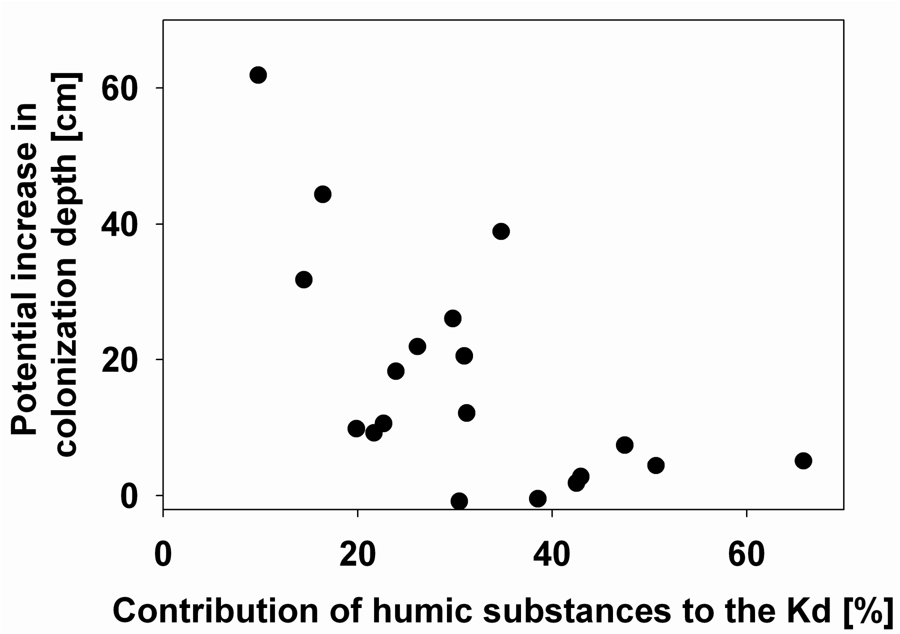

Figure 4.3 Calculated increase in maximum colonization depth due to the water clearing effect of submerged vegetation in lakes where the light attenuation is influenced by humic substances in different degrees. In humic lakes submerged vegetation has a relatively small influence on the turbidity. The vegetation, therefore, does not enhance the light availability and does hardly increase its maximum colonization depth.

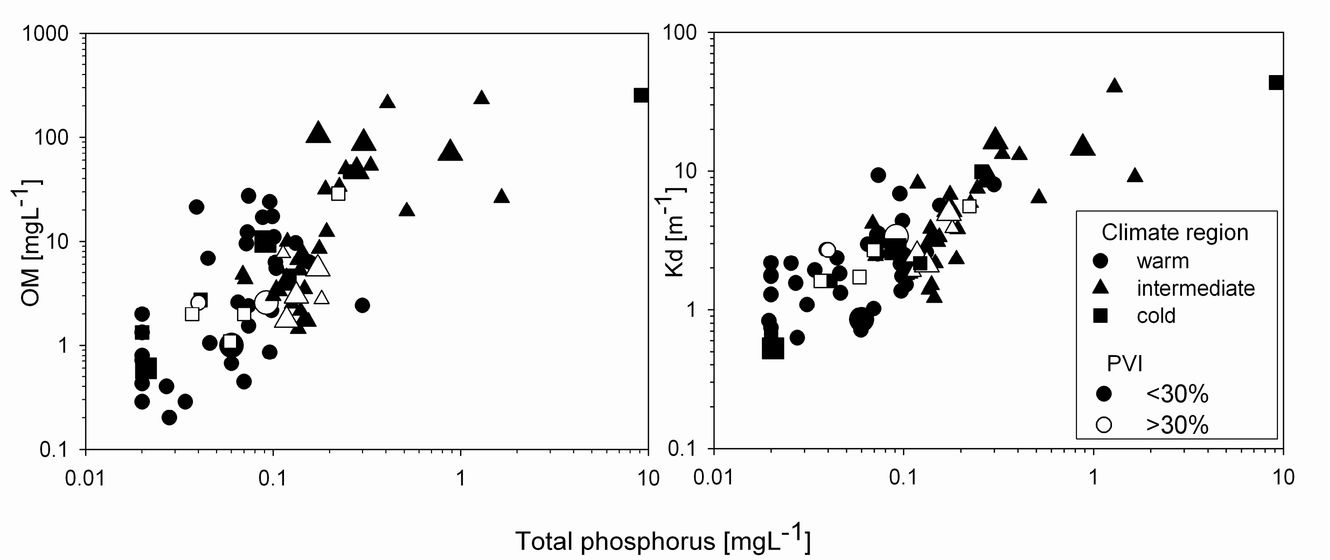

Figure 4.4 (a) Light attenuation (K_d) and (b) organic matter concentration (OM) in lakes with scarce or abundant vegetation growth along a total phosphorus concentration gradient in lakes in different climate regions. The larger symbols depict lakes > 100ha.

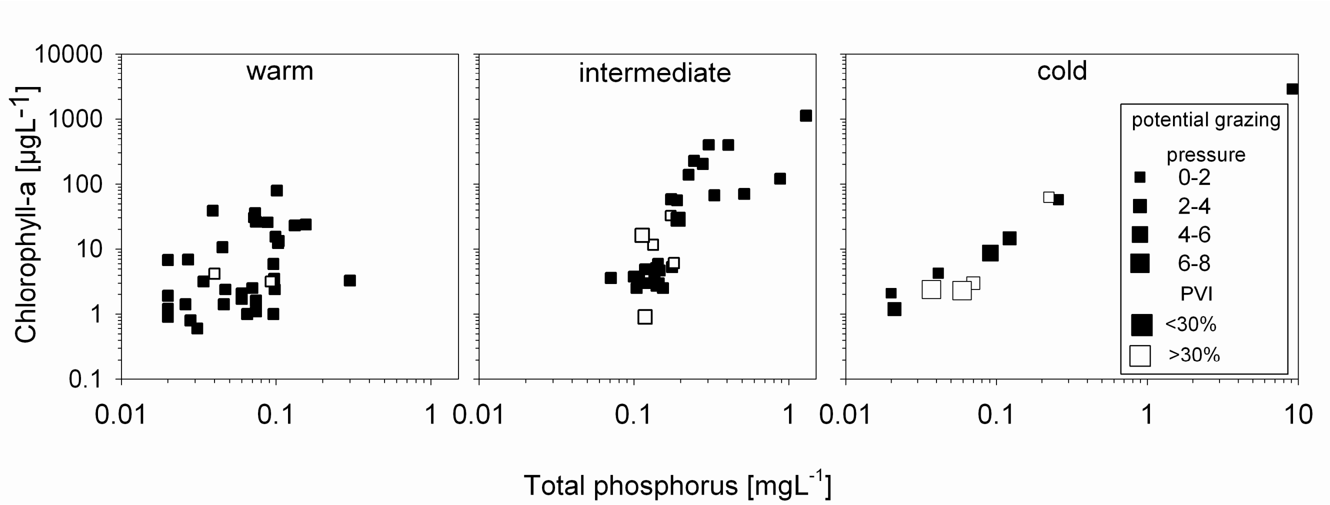

Figure 4.5 Chlorophyll-a concentration along a total phosphorus concentration gradient in lakes in with various degrees of potential grazing pressure (zooplankton : algal biomass ratio) in the warm, intermediate, and cold climate region, with scarce (PVI<30%) or abundant (PVI>30%) vegetation growth.

Whole-lake comparisons

Lakes with abundant vegetation (PVI_{lake}>30%) were generally clearer, as indicated by low K_d's (measured in the center of the lake), low OM, and low chla concentrations (both measured in the bulk water sample taken at 20 points across the lake), whereas sparsely vegetated lakes (PVI_{lake}<30%) had a larger range of turbidities (Figure 4.4 and 4.5). The variance in both K_d and OM significantly differed between lakes with abundant and sparsely vegetated lakes ($P<0.05$). In linear regressions, K_d and OM were strongly related to TP and TN (Table 4.2, Figure 4.4). PVI and the lake area did not significantly enhance this relationship, but the potential grazing pressure did (Table 4.2). PVI was not significantly related to K_d measured in the center of the lake. However, PVI alone, or in combination with TN, did significantly explain part of the variance in OM (Table 4.2). The slope of the relationship between PVI and OM was not significantly different among the climate regions (ANCOVA: PVI*climate interaction term $p>0.05$). The benthic and detrivorous fish density did not significantly increase the explained variance in OM nor in Kd.

In all climate regions, the potential grazing pressure of zooplankton was generally higher in lakes with abundant vegetation, although the difference was only significant in the intermediate region (Figure 4.6a). Comparison among the climate regions indicated that the colder lakes had the highest potential grazing pressure (Figure 4.6a). These lakes also had lower densities of omnivorous fish, i.e. potentially zooplanktivorous fish, than lakes in the other climate regions (Figure 4.5b). In the cold lakes with high potential grazing pressures and high PVI the chlorophyll-a concentration was also generally lower than expected solely from the TP concentration (Figure 4.5). Similar results are obtained when using TN concentrations (not shown). Using a different approximation of the potential grazing pressure in which only cladocerans and herbivorous copepods are taken into account, assuming that they ingest 100% and 50% of their biomass per day respectively (Jeppesen et al. 1994), did not considerably change the outcome of the analysis (not shown).

The fish community did not vary substantially between lakes with and without abundant vegetation growth. The percentage of potentially piscivorous fish was not significantly correlated to PVI in any climate region (P-values were 0.284, 0.252, and 0.905 in the warm, intermediate and cold region respectively). Furthermore, we did not find an increase in the proportion of benthic fish with a decrease in PVI in the warm and cold region ($\rho=0.04$, $P=0.41$ and $\rho=-0.57$, $P=0.07$ respectively). In the intermediate region we even found an increase in the percentage of benthic fish with an increase in PVI ($\rho=0.54$, $P<0.001$). Furthermore, we did not find a correlation between the CPUE of benthic fish and the OM concentration in any of the climate regions. Only a small percentage (warm 0.7%, intermediate 2.8% and cold 0%) of these benthic fishes is known to feed on sediment or organisms herein.

Table 4.2. Models describing the lake’s chlorophyll-a (chla) concentration, light attenuation (Kd), and organic matter concentration (OM) by total phosphorus concentration (TP), total nitrogen concentration (TN), lake’s volume filled with submerged vegetation (PVI), and potential grazing pressure (PGP) of zooplankton on phytoplankton.

Dependent	**Model**	**R_{adj}^2**
Log(Kd)	$-0.90^{(<0.001)} + 0.68\log(TP)^{(<0.001)}$	0.59
	$-0.64^{(<0.001)} + 0.65\log(TP)^{(<0.001)} - 0.09\log(PGP*10^{3})^{(0.038)}$	0.61
	$-0.93^{(<0.001)} + 0.50\log(TN)^{(<0.001)}$	0.62
Log(OM)	$-1.81^{(<0.001)} + 1.25\log(TP)^{(<0.001)}$	0.54
	$-1.16^{(0.002)} + 1.19\log(TP)^{(<0.001)} - 0.22\log(PGP*10^{3})^{(0.011)}$	0.57
	$0.86^{(<0.001)} - 0.32\log(PVI)^{(0.008)}$	0.09
	$-2.02^{(<0.001)} + 0.98\log(TN)^{(<0.001)}$	0.65
	$-1.85^{(<0.001)} + 0.96\log(TN)^{(<0.001)} - 0.23\log(PVI)^{(0.001)}$	0.70
Log(chla)	$-1.70^{(<0.001)} + 1.31\log(TP)^{(<0.001)}$	0.51
	$-0.58^{(0.123)} + 1.20\log(TP)^{(<0.001)} - 0.37\log(PGP*10^{3})^{(<0.001)}$	0.59
	$1.08^{(<0.001)} - 0.31\log(PVI)^{(0.023)}$	0.05
	$-2.11^{(<0.001)} + 1.10\log(TN)^{(<0.001)}$	0.69
	$-1.13^{(<0.001)} + 1.02\log(TN)^{(<0.001)} - 0.32\log(PGP*10^{3})^{(<0.001)}$	0.75

Only significant models are shown, the P-value of the parameters are shown in superscript between parentheses.

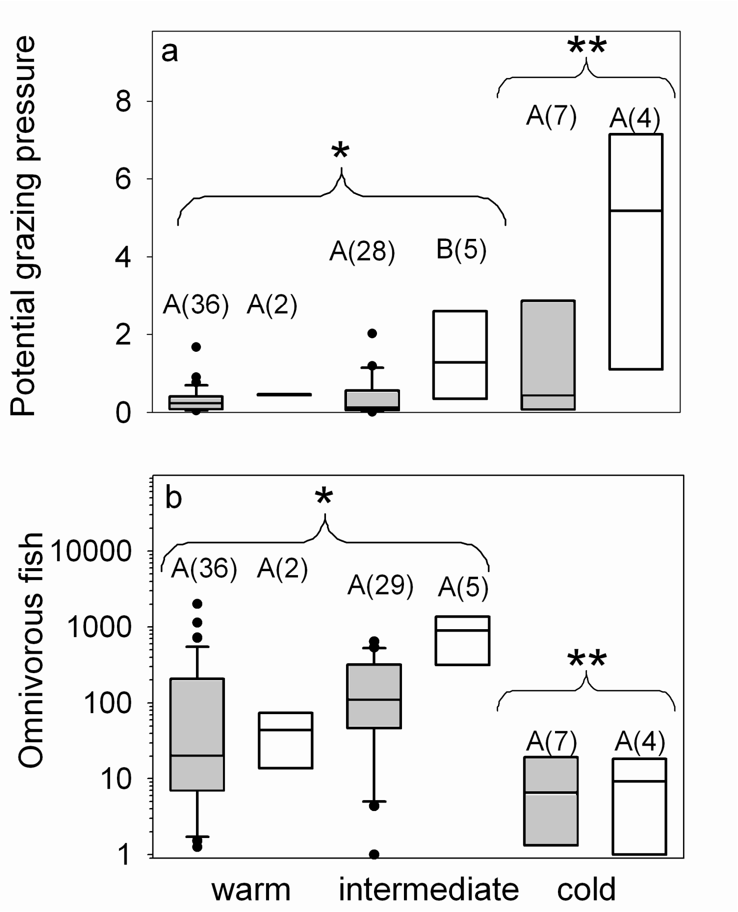

Figure 4.6 Potential grazing pressure (zooplankton : algal biomass ratio)(a) and CPUE (individuals net^{-1} 12 hours^{-1}) of omnivorous fish (b) in different climate zones in lakes with scarce vegetation (PVI<30%, grey bars) and abundant vegetation (white). The stars indicate homogenous subsets for the climate zones (post-hoc Tukey). See Figure 4.1 for more details.

Discussion

Our study shows that submerged vegetation can have a marked positive effect on local water clarity across all South American climate zones studied, with the notable exception of lakes where humic substance concentrations are high. From a comparison between entire lakes with and without abundant vegetation the water clearing effect was less obvious. In the Northern Hemisphere, lakes with abundant vegetation are often clearer also in the open water than lakes of comparable trophic state without abundant vegetation (Jeppesen et al. 1990). In the South American lakes we did not find this systematic difference.

The interpretation of the (lack of) differences in water clarity between lakes with abundant and sparse vegetation at comparable trophic state is not straightforward. Vegetation may influence water clarity, but may affect nutrient (TN and TP) concentrations as well (e.g. Carpenter 1980, Van Donk et al. 1993). Although we did not see differences in water clarity at similar nutrient concentrations, the South American lakes with abundant vegetation may very well be clearer than their counterparts without vegetation while receiving similar nutrient loads. Furthermore, the relationship between water clarity and submerged vegetation works both ways: in more turbid water it is less likely to find abundant vegetation. The critical turbidity at which submerged macrophytes disappear, however, may vary with climate; presumed low periphyton shading (e.g. Meerhoff et al. 2007a, Kosten et al. 2009b), high irradiances and strong water level fluctuations (Bécares et al. 2008) may lead to a higher persistence of submerged vegetation at higher turbidities in some climates.

Different mechanisms could account for the apparent similarity in water clarity in lakes with and without abundant vegetation found in the South American lakes. The fish community composition may play an important role. In North European lakes the percentage of piscivorous fish is generally high in oligotrophic-mesotrophic lakes, whereas with an increase in TP, catches of cyprinids increase strongly (Jeppesen et al. 2000). Bream, *Abramis brama*, has an important share in the fish community in these TP-rich and vegetation-poor lakes causing resuspension of sediment due to bioturbation (Lammens 1989). In contrast, in the lakes studied here, a decrease in PVI did not coincide with a decrease in the proportion of piscivorous fish or with an increase in the proportion of benthic fish. Furthermore, we found no correlation between the CPUE of benthic fish and suspended organic matter, which may indicate that the benthic fish do not bioturbate the sediment to the same extent as in North temperate lakes while feeding. This assumption is strengthened by the low share of benthic fish with diets based on (organisms in the) sediment, which would require bioturbating foraging activities. This may imply that the turbidity in the vegetation poor lakes studied here is not further increased through bioturbation by their fish population. Experimental research is needed to substantiate this hypothesis.

Fish may also affect water clarity through predation on zooplankton. Depending on the fish density, diet and habitat preference, vegetation may provide a refuge function for zooplankton (Schriver et al. 1995, Perrow et al. 1999, Romare and Hansson 2003, Romare et al. 2005, Meerhoff et al. 2007b). Our data confirmed this: we found that the potential grazing pressure of zooplankton on algae is generally higher in lakes with abundant submerged vegetation. Lakes with abundant vegetation in combination with low omnivorous fish densities had the highest potential grazing pressures. This combination mainly occurred in the cold climate zone (South Argentina). In the intermediate and warm lakes (Provincia de Buenos Aires in Argentina, Uruguay and Brazil) the higher densities of omnivorous fish most likely impede the establishment of larger-sized zooplankton usually responsible of high grazing pressures (see also Jeppesen et al. 2007a). A detailed analysis of the zooplankton taxa in the study lakes, furthermore, confirmed that fish is likely to be the main driver of the zooplankton community size structure, whereas the influence of vegetation seems limited (Lacerot and others, unpublished data). In the warmer lakes this is likely caused by strong fish zooplanktivory due to a combination of multiple or continuous reproductive events (Van Leeuwen et al. 2007), lower densities of large specialist piscivores (see also Quirós 1997), widespread omnivory (Branco et al. 1997, Blanco et al. 2003; Mazzeo et al. unpublished data) and high fish densities (Teixeira-de Mello et al. 2009). The lower grazing pressure in warmer lakes as compared to colder lakes found in these South American lakes concurs with findings in a European study (Gyllström et al. 2005) and comparative studies of lakes in Europe and Florida (Havens et al. 2009).

Even though the grazing pressure in lakes in the intermediate and warm climate region is lower than in the cold lakes, overall the potential grazing pressure is often higher in lakes with abundant vegetation when compared to lakes with scarce vegetation within the same climate region. The potential grazing pressure positively influences the water transparency and negatively influences the organic matter and chlorophyll-a concentration, indicating an indirect water clearing effect of the vegetation through zooplankton. The increase in water transparency due to the top-down control of grazing on phytoplankton may be noticeable in the entire lake when zooplankton grazes outside the vegetation beds or it may be more local when they mainly stay inside the beds. The estimated effect on phytoplankton biomass was highest in the coldest lakes where the potential grazing pressure was highest.

The local water clearing effect did not significantly differ among climatic regions, instead it varied strongly within each climatic zone. The effect was, however, generally lower than observed in a variety of Northern hemisphere temperate lakes where the water clearing effect ranged between 80 and 96% (Table 5.1 in Scheffer 1998). This difference may be caused either by differences in the refuge function for zooplankton (see also Meerhoff et al. 2007b) or by differences in vegetation taxa between the Northern hemisphere temperate lakes and the South American lakes studied here. Differences in morphology and biomass distribution among vegetation types may influence the water clearing effect through its effect on turbulence and sedimentation (Newall and Hughes 1995). A comparison of the water clearing effect of different vegetation taxa in the study lakes confirmed that taxa specific variations exist, especially concerning the effect on phytoplankton.

In addition to the type of vegetation, the concentration of humic substances influences the water clearing effect of the vegetation. In lakes where humic substances contributed relatively much to water transparency, vegetation hardly cleared the water. In some cases transparency was even lower inside than outside the vegetation beds. This may be explained by the fact that vegetation (together with organic soils) can be a major source of humic substances (Thurman 1985). In our lake data set, water clearing mechanisms such as the promotion of grazing and sedimentation, did not influence humic substances.

Depending on the vegetation characteristics and the water composition, vegetation may thus enhance water clarity in various degrees. By increasing the transparency, the vegetation positively influences the euphotic depth and thereby its potential colonization depth. Depending on the morphology of the lake this may lead to the colonization of a large fraction of the lake area.

Final remarks

Judged from a comparison of water clarity inside and outside vegetation beds, the vegetation appeared to have a similar positive effect on the water clarity across all coastal plain lakes in the South American climate regions studied. From a comparison between entire lakes with and without abundant vegetation the water clearing effect is less easily deduced. We found indications, however, that the key mechanism for alternative states to occur – i.e. vegetation enhancing water clarity – also works in these South American lakes, albeit it may be less pronounced than in Northern hemisphere temperate lakes as also demonstrated in comparative studies of temperate Danish lakes and subtropical Florida lakes (Jeppesen et al. 2007b). This may have implications for their management. Many restoration measures are developed in temperate lakes and aim to increase vegetation growth to restore or stabilize the clear water state. Even though success is not guaranteed, measures such as vegetation transplantation or temporary lowering of the water level may therefore also be successful in other climatic regions, though the effect on clarity is expectedly lower. In humic lakes or lakes with a high density of omnivorous fish these measures will have a small chance of success.

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5 Lake and watershed characteristics rather than climate influence nutrient limitation in shallow lakes

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Abstract

Both nitrogen (N) and phosphorus (P) can limit primary production in shallow lakes, but it is still debated how the importance of N and P varies in time and space. We sampled 83 shallow lakes along a latitudinal gradient (5 – 55 ^{o}S) in South America and assessed the potential nutrient limitation using different methods including nutrient ratios in sediment, water, and seston, dissolved nutrient concentrations, and occurrence of N-fixing cyanobacteria. We found that local characteristics such as soil type and associated land use in the catchment, hydrology, and also the presence of abundant submerged macrophyte growth influenced N- and P-limitation. We found neither a consistent variation in nutrient limitation nor indications for a steady change in denitrification along the latitudinal gradient. Contrary to findings in other regions, we did not find a relationship between the occurrence of (N-fixing and non-N-fixing) cyanobacteria and the TN:TP ratio. We found N-fixing cyanobacteria (those with heterocysts) exclusively in lakes with dissolved inorganic nitrogen (DIN) concentrations < 100µg/L, but notably they were also often absent in lakes with low DIN concentrations. We argue that local factors such as land use and hydrology have a stronger influence on which nutrient is limiting than climate. Furthermore, our data show that in a wide range of climates N-limitation does not necessarily lead to cyanobacterial dominance.

Introduction

Whether nitrogen (N) or phosphorus (P) limits primary production in shallow lakes depends on their relative availability in the lake ecosystem. This, in turn, depends on both the amount of N and P entering the system and on in-lake processes. Nutrient loads are strongly affected by hydrology and watershed characteristics such as soil type and land-use (Downing and McCauley 1992, Downing et al. 1999) which may vary with climate. Nitrogen export rates of undisturbed tropical watersheds, for instance, were found to be greater than those in temperate regions, given similar rates of runoff (Downing et al. 1999). Anthropogenic influences may, however, neutralize this background effect. Human population density, for example, strongly influences N-loads in both tropical and subtropical systems (Peierls et al. 1991).

In-lake processes such as sedimentation, flushing, mineralization and denitrification can vary on local or larger scales. The hydraulic residence time varies locally and is known to influence in-lake N (Windolf et al. 1996) as well as P (Vollenweider 1976) concentrations. Among the other factors affecting N or P concentrations in shallow lakes, temperature will change most systematically along a latitudinal gradient. An increase in temperature can strongly increase denitrification rates (Golterman 2000, Pinay et al. 2007, Herrman et al. 2008), which may lead to N-limitation of primary producers. Controversy exists among authors as to whether climate influences the frequency of N-limitation (Talling and Talling

1965, Lewis 1996, Downing et al. 1999, Lewis 2000) or not (Huszar et al. 2006, Elser et al. 2007).

The prediction of N- or P-limitation by local or regional factors could help in prioritizing measures to reduce nutrient loading. If, for instance, N-limitation is indeed more frequent in warmer climates, eutrophication amendment in the (sub)tropics could possibly benefit more from reducing external N than P loading (Jeppesen et al. 2007a). On the other hand, some research has shown that low N:P ratios or low N concentrations, independent of P concentrations, might increase the chance of cyanobacterial dominance (Bulgakov and Levich 1999, Smith and Bennett 1999, Schindler et al. 2008).

Since eutrophication is still one of the major threats to lakes in developed and developing countries (UN 2006), understanding the spatial patterns of nutrient limitation is of high relevance for lake restoration. We assessed potential nutrient limitation from 83 South American shallow lakes on a latitudinal gradient from 5 to 55 ^{o}S using dissolved nutrient concentrations and ratios of nutrients and algal biomass. Subsequently, we tested the relative importance of climate and local factors, such as lake and watershed characteristics, on the N and P-limitation. Furthermore, we evaluated its consequences for the occurrence of cyanobacteria.

Methods

Study sites – We sampled 83 lakes along a latitudinal gradient (5 – 55 ^{o}S) in South America. The lakes were selected to resemble each other as closely as possible in morphology and altitude and to vary as much as possible in trophic state within each climate zone (see supplement for lake details). All lakes were shallow, had a surface area between 0.09 and 2.53 km^2 and were situated at a maximum 500 m above sea level (Table 5.1). Most lakes had no or only a small surface water inlet. The lakes were grouped in five different categories based on the prevailing climate characteristics following the Köppen climate system (1936) digitized by Leemans and Cramer (1991): tropical*i* (19 lakes), tropical (19 lakes), subtropical (18 lakes), maritime temperate (16 lakes), and tundra (11 lakes) zone (Figure 1). The Köppen classification is based on monthly rainfall and temperature. Tropical*i* is an isothermal subzone in the tropics, which has a smaller annual temperature range than the tropical zone (Table 5.1).

Sampling – All lakes were sampled once during summer (subtropical, temperate and tundra lakes) or dry season (tropical*i* and tropical lakes) between November 2004 and March 2006 by the same team. The average depth of the lake was determined using depth measurements from 20 random points and 20 points along transects perpendicular to the longest axis of the lake. We collected integrated water samples at 20 random points in each lake. Two liters of each integrated sample were gathered in a bulk sample totaling 40 liters. Filtration for various analyses was conducted directly after collection. Water and filters were then frozen until analysis. Phytoplankton samples were fixed in Lugol's solution. Another two liters of each of the 20 integrated samples were filtered on a 50µm sieve for quantitative zooplankton quantification and determination. The zooplankton samples were preserved in a 4% formaldehyde solution. Light measurements were conducted at different depths at noon in the center of the lake with a flat underwater quantum sensor LICOR LI-192S. Sediment samples of the top 2 cm were taken in the center of the lake with a Kajak corer. We made estimations of vegetation cover for 13 – 47 points, depending on lake shape and size, and determined presence or absence of vegetation at 20 random points. Organic carbon density in soils (kg C/m^2 to 1 m depth) was taken from IGBP-DIS (1998). Soil type data were obtained from FAO (1998).

Sample analysis – Total P (TP) and total N (TN) were analyzed using a continuous flow analyzer (Skalar Analytical BV) following NNI protocols (NNI 1986, 1990), with the exception of the UV/Persulfate destruction, which was not executed beforehand but integrated in the system. For the soluble reactive phosphorus (SRP), ammonium and combined nitrate and nitrite concentrations, water was filtered over a 0.45 μm Schleicher and Schuëll filter (NC45). The filtrate was then analyzed by the same continuous flow analyzer (NNI 1986, 1990, 1997). For the N and P content of the seston, water was filtered through pre-heated (500^{o}C) GF/F Whatman filters. The seston and homogenized sediment material was digested with salicylic acid, hydrogen peroxide and selenium following Novozamsky et al. (1983, 1984) after which the same methodology was used as for the TN and TP analyses in water. For the dissolved organic carbon (DOC) analysis water was filtered over GF/F Whatman filters. DOC was determined by a Total Organic Carbon analyzer (Model 700, O.I.C International BV). Chlorophyll-a (chla) was extracted from filters (GF/C S&S) with 96% ethanol, and absorbance was measured at 665 and 750nm (Nusch 1980). The δ^2H and δ^{18}O of the bulk water sample were determined in a Multiflow system connected to an Isoprime Mass Spectrometer (Thermo Electron, Waltham, MA). We used platinum black powder (Sigma-Aldrich, St. Louis, MO, Product # 205915) to equilibrate hydrogen gas with water vapor for a 24 h period and analyzed the resulting equilibrated gas to derive the hydrogen isotope ratio of the water. Carbon dioxide was equilibrated with water for a period of 48 h and also analyzed to derive the oxygen isotope ratios of the water. All isotopic ratios are expressed in δ units:

$$\delta D \text{ or } \delta^{18}O = \left[\frac{R_{sample}}{R_{std}} - 1\right] \cdot 1000$$

in which R_{sample} and R_{std} are ^{2}H/H or ^{18}O/^{16}O ratios of the sample and standard, respectively. The standard used here is Vienna mean standard ocean water (vSMOW) and the precision of analysis is ±2.0‰ and ±0.2‰ for δ^2H and δ^{18}O values, respectively. The inlet water δ^2H and δ^{18}O were approximated by the isotopic composition of the precipitation at the lake location (data obtained from Bowen and Wilkinson 2002).

Phytoplankton populations were enumerated in random fields (Uhelinger 1964) using the settling technique (Utermöhl 1958) and inverted microscopes, Nikon Diaphot and Zeiss Oberkochen Axiovert 10. The units (cells, colonies, filaments and number of heterocysts in cyanobacteria) were enumerated for at least 100 specimens for the most frequent species ($p<0.05$, Lund et al. 1958). The organisms were identified according to the main morphological and morphometrical characteristics of the vegetative and reproductive phases. Zooplankton was counted in 1-5 mL aliquots, depending on sample concentration. Counting stopped when 100 specimens of the most abundant species of each taxonomic group (rotifers, copepods, cladocerans) were reached. If necessary, the whole sample was counted.

Every type of analysis was done by the same person mostly in one laboratory.

The percentage of the lake volume inhabited with submerged vegetation (PVI) was determined by multiplying the average length of submerged plants by the coverage percentage of the lake, consequently divided by the lake volume (Canfield et al. 1984).

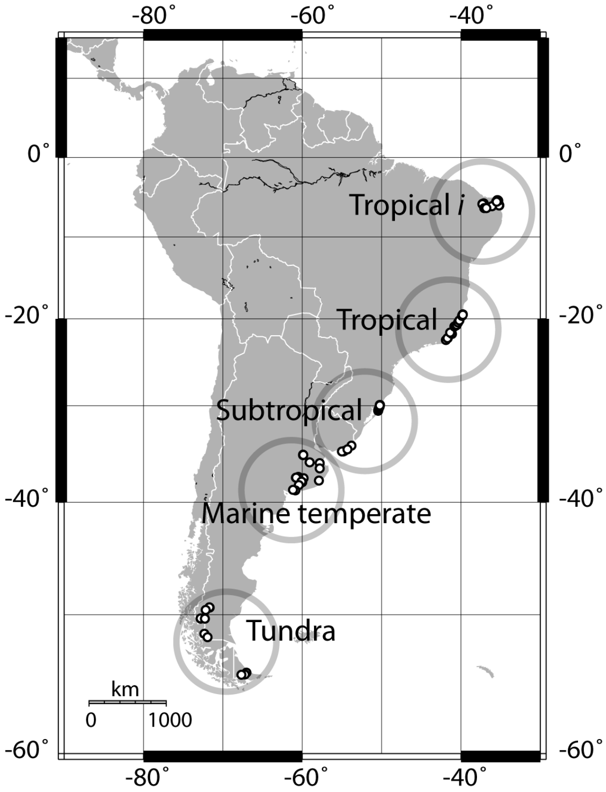

Figure 5.1 Location of the lakes sampled in South America where potential N and P-limitation was assessed with the prevailing climate following the Köppen (1936) classification. The abbreviations are as used in subsequent figures. See supplementary material for exact location and other information on each lake sampled.

Calculations and data analysis – Dissolved inorganic nitrogen (DIN) was calculated as the sum of ammonium, nitrate and nitrite. Light attenuation (Kd) was estimated by using the Lambert-Beer law and irradiation data. Besides being nutrient limited, phytoplankton may also be light limited. We used the shade index as an indication for light availability -or lack thereof- at the bottom of the lake. It was evaluated as Kd times average depth (Scheffer 1998). We derived a proxy for the relative influence of inflow from the catchment and evaporation using δ^2H and δ^{18}O. The derivation is based on the principle of light isotopes evaporating more quickly than heavy isotopes. The ‘heavier’ the lake water in comparison to the incoming water then the more the lake water has been subject to evaporation. We calculated the inlet:evaporation ratio using the Gat – Bowser model (Gat and Bowser 1991, quoted by Rozanski et al. 2001). For the stable isotope composition of the inlet water, we used the average composition of precipitation at the lake location that was extracted from a map provided by Bowen (2003) and based on data from Bowen and Revenaugh (2003). However, as the incoming water may have already been subject to evaporation in the watershed before it entered the lake, we may be overestimating the evaporation in the lake. The relative humidity input for the model was obtained from the dataset of New et al. (2002). The more the lake was subject to evaporation, for example due to a long hydraulic residence time, the smaller the inlet: evaporation ratio.

Phytoplankton biovolume (mm^3/L) was estimated by multiplying the abundance of each species by the average cell volume (Edler 1979, Hillebrand et al. 1999), based on measurements of at least 30 individuals.

Zooplankton biovolume (μm^3) was estimated by measuring a minimum of 20 individuals of each rotifer or nauplii larvae species using formulas from Ruttner-Kolisko (1977). Biomass (µgDW/L) was estimated assuming a density of 1.0 (Ruttner-Kolisko 1977). In the case of copepods and cladocerans, we measured 50 specimens of each species and estimated biomass using length/weight regressions available in the literature (Bottrell et al. 1976, McCauley 1984, Culver et al. 1985, Güntzel et al. 2003). The zooplankton biomass: algal biomass (chla multiplied by 66) ratio was used as a proxy for grazing pressure (Jeppesen et al. 1998b). It is based on the assumption that zooplankton use phytoplankton as a sole food source. It gives a rough indication of the percentage of phytoplankton standing stock that is grazed per day assuming that zooplankton consumes its biomass per day (Jeppesen et al. 1994).

To evaluate the differences in potential N-limitation to phytoplankton growth among the regions, we used the following indicators: (i) seston N:P ratios, lakes with a ratio below 9 (molar based) (Guildford et al. 2000) were considered potentially N-limited, and lakes with a ratio above 23 were considered P-limited (Hecky et al. 1993); (ii) sediment N:P ratios for which we used the same thresholds as for seston; (iii) lake water TN:TP ratios, below 20 (molar based) a lake was considered N-limited and above 38 P-limited (Sakamoto 1966); (iv) ratios between dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus concentrations (SRP), below 13 (molar based) a lake was considered N-limited and above 50 P-limited (Figure 2 in Morris and Lewis 1988); (v) DIN and SRP were compared to concentrations that have roughly been considered to limit phytoplankton growth. P was considered limiting below 10 µg P/L (~0.3 µmol P/L) (Sas 1989) and N below 100 µg N/L (~6-7 µmol N/L) (Reynolds 1999). Clearly this is only an approximation as it depends on the affinities and storage capacities of the individual species (Reynolds 1999); (vi) algal biomass:TN and algal biomass:TP ratios, where lakes with low ratios are considered to be limited by factors other than TN and TP, respectively (Hosper 1980); and (vii) when N-fixing cyanobacteria occurred we considered two (overlapping) groups: those that have the potential to fix N (potential N- fixing cyanobacteria, Order Nostocales) and those that had heterocysts (N-fixing cyanobacteria).

We first evaluated the climatic influence on the occurrence of potential N-limitation by comparing the different nutrient-limitation-indicators among various climate zones along a latitudinal gradient. To assess the influence of local factors, we then checked for Spearman correlations between the different nutrient-limitation-indicators and several local factors. The following local factors were taken into account: lake area, average depth, PVI, grazing pressure, shade, soil organic carbon, the inlet:evaporation ratio, and DOC. In a stepwise multiple regression, latitude and the before mentioned local factors were used to explain the variance in the different nutrient-limitation-indicators. The nutrient-limitation-indicators were log transformed to normalize the data (Shapiro-Wilk p>0.05).

Finally, we also verified whether there were differences in nutrient limitation in lakes with abundant and limited submerged vegetation (PVI below or above 30%, respectively; Canfield et al. 1984, Jeppesen et al. 1990) and also among lakes situated in regions with different soil types. These soil types were observed to be related to land use. Our lakes were situated in 15 different types of soil, but in this analysis we only used soil types with at least 7 lakes (Table 5.2).

Differences between more than two groups of lakes were determined by using Kruskal-Wallis tests. If differences were significant ($p<0.05$), then post hoc Mann Whitney was used to identify similarities ($p>0.05$). All statistical analyses were performed using SPPS for Windows v. 15.0 (SPSS Inc., Chicago, IL, U.S.A.).

Table 5.1 General features of the sampled lakes

	Range	Mean	Median
Area [ha]	9 - 253	62	46
Average depth [m]	0.5 – 4.5	1.8	1.6
Altitude [m above sea level]	1 - 500	84	20
Conductivity [µS/cm]	38 - 4930	527	167
Total nitrogen [mg N/L]	<0.1 – 25.8	1.8	0.4
Dissolved inorganic nitrogen [µgN/L]	<4 - 2043	90	34
Total phosphorus [mgP/L]	0.02 – 9.14	0.27	0.10
Soluble reactive phosphorus [µgP/L]	<4 - 6711	124	8
Chlorophyll-a [µg/L]	0.6 - 2889	79.6	4.7
Average air temperature during coldest month [°C] *			
Tropical*i* (AM*i*)	23.9 – 26.3	24.7	24.6
Tropical (AW)	19.9 – 22.2	21.3	21.4
Subtropical (CF)	11.2 – 14.6	13.6	14.4
Maritime temperate (CFb)	7.1 – 9.7	8.1	7.5
Tundra (ET)	-0.8 – 1.3	0.3	0.3
Average air temperature during warmest month [°C] *			
Tropical*i*	26.7 – 28.7	27.7	27.7
Tropical	25.3 – 27.6	26.7	26.9
Subtropical	21.9 – 23.6	23.0	23.2
Maritime temperate	20.2 – 23.4	22.0	21.9
Tundra	8.2 – 13.2	11.3	12.6
Average yearly rainfall [mm] *			
Tropical*i*	575 - 1573	1083	1213
Tropical	837 - 1081	983	999
Subtropical	1048 - 1450	1328	1412
Maritime temperate	676 - 1020	868	861
Tundra	251 - 1218	632	468

* Average of the years 1961 – 1990 (New et al. 2002). The five different categories, with abbreviations, are based on the prevailing climate characteristics following the Köppen climate system (1936). Tropical*i* is an isothermal subzone in the tropics, which has a smaller annual temperature range than the Tropical zone.

Results

Latitudinal gradient

The range of the sediment N:P ratio was relatively constant along the latitudinal gradient and ranged mostly between 9 and 23, indicating no clear tendency of the impact of N- or P-limitation on primary production. The tropical*i* lakes were an exception with higher N:P ratios, indicating potential P-limitation (Figure 5.2a, Table 5.2). The seston N:P ratios showed the same pattern (Figure 5.2b; note that we did not have seston N:P data for the subtropical lakes). The lake water TN:TP ratio (Figure 5.2c) varied more than the seston N:P ratio, indicating high occurrence of potential N-limitation along the entire latitudinal gradient.

The dissolved nutrient ratio (DIN:SRP) also indicated more potential N than potential P-limitation along the whole gradient. Many lakes, however, had a DIN:SRP ratio between 13 and 50, representing no clear tendency for exclusive N- or P-limitation (Figure 5.2d, Table 5.3). When assessing the dissolved nutrients separately, distinct differences in the SRP and DIN concentrations in the lakes among the climate zones occurred (Figure 5.3a and b). The SRP concentration was generally highest in the temperate lakes (Figure 5.3a). The DIN concentration in the tropical lakes tended to be higher than those in the other lakes, although the median did not differ significantly from the median DIN concentration in the tundra lakes (Figure 5.3b). Most lakes had DIN and SRP concentrations below 100 µg N/L and 10 µg P/L, respectively. Therefore, when considering the dissolved N and P concentrations, phytoplankton is likely co-limited by N and P in most lakes along the latitudinal gradient. Most of the lakes with exclusive N-limitation were found in the marine temperate and the tropical zone (Table 5.3). In the tropical*i* and tropical lakes, the percentage of DIN composed of nitrate and nitrite was significantly higher than in the other lakes (Figure 5.3c).

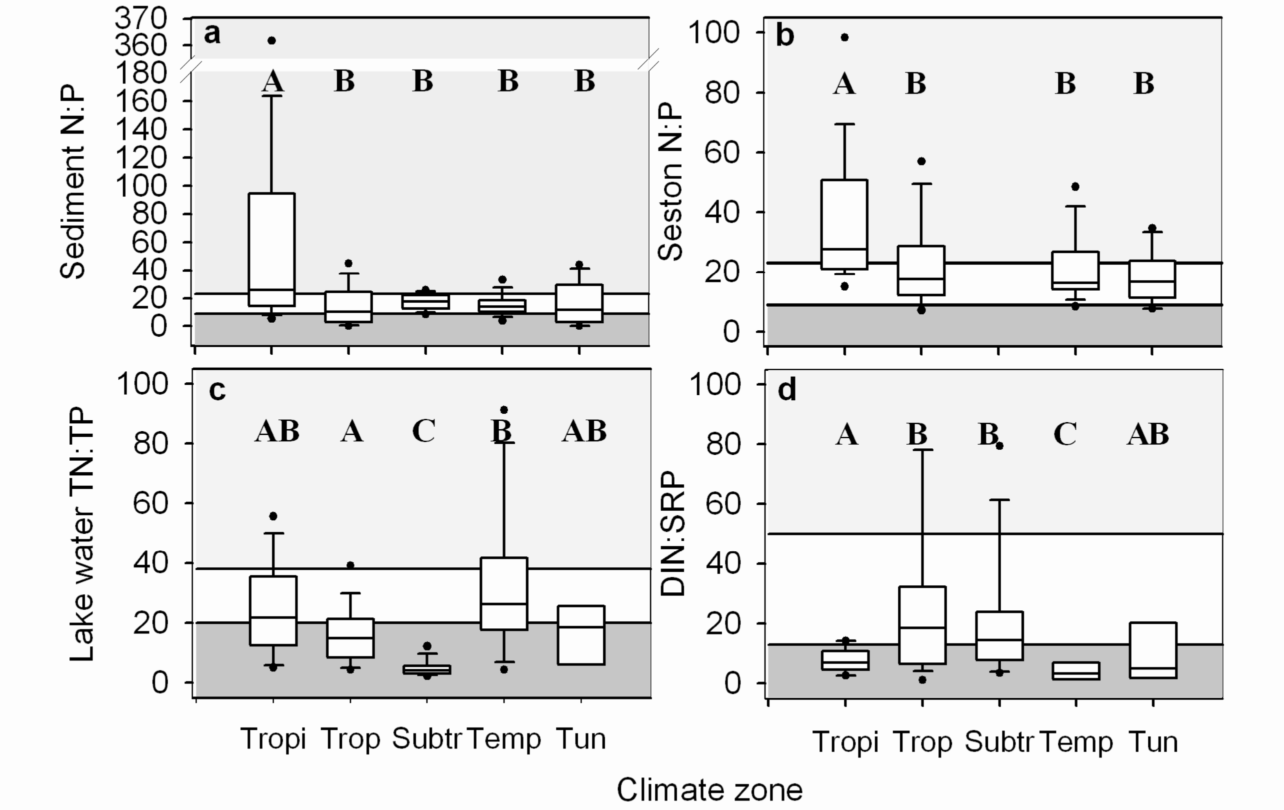

Figure 5.2 Boxplots of the sediment N:P ratio (a), seston N:P ratio (note that there is no data for the subtropical zone) (b), the lake water TN:TP ratio (c), and Dissolved Inorganic Nitrogen concentrations (DIN) and Soluble Reactive Phosphorus (SRP) ratio (d) found in the lakes in different climate zones. Boundaries of the boxplots indicate the 25th and 75th percentile. Whiskers above and below indicate the 90th and 10th percentiles. Dots are outlying points. Capital letters indicate groups with significantly similar medians. Lakes with SRP or DIN concentrations below the detection limit are not included. Light grey area represents potentially P-limited conditions and dark grey are represents potentially N-limited conditions.

The median algal biomass:TN ratio did not differ significantly among the climate zones (Figure 5.4a). The algal biomass:TP ratio was highest in the marine temperate zone (Figure 5.4b). We found a positive correlation between the percentage of the algal biovolume composed of cyanobacteria and the algal biomass:TP ratio (Spearman rank correlation ρ_{rS} =0.26, p=0.016). There was no significant correlation between algal biomass:TN and the percentage of cyanobacteria (p=0.112).

N-fixing cyanobacteria occurred in 20% and potential N-fixing in 36% of all lakes, neither of them showing a latitudinal increase nor decrease in occurrence (Table 5.3).

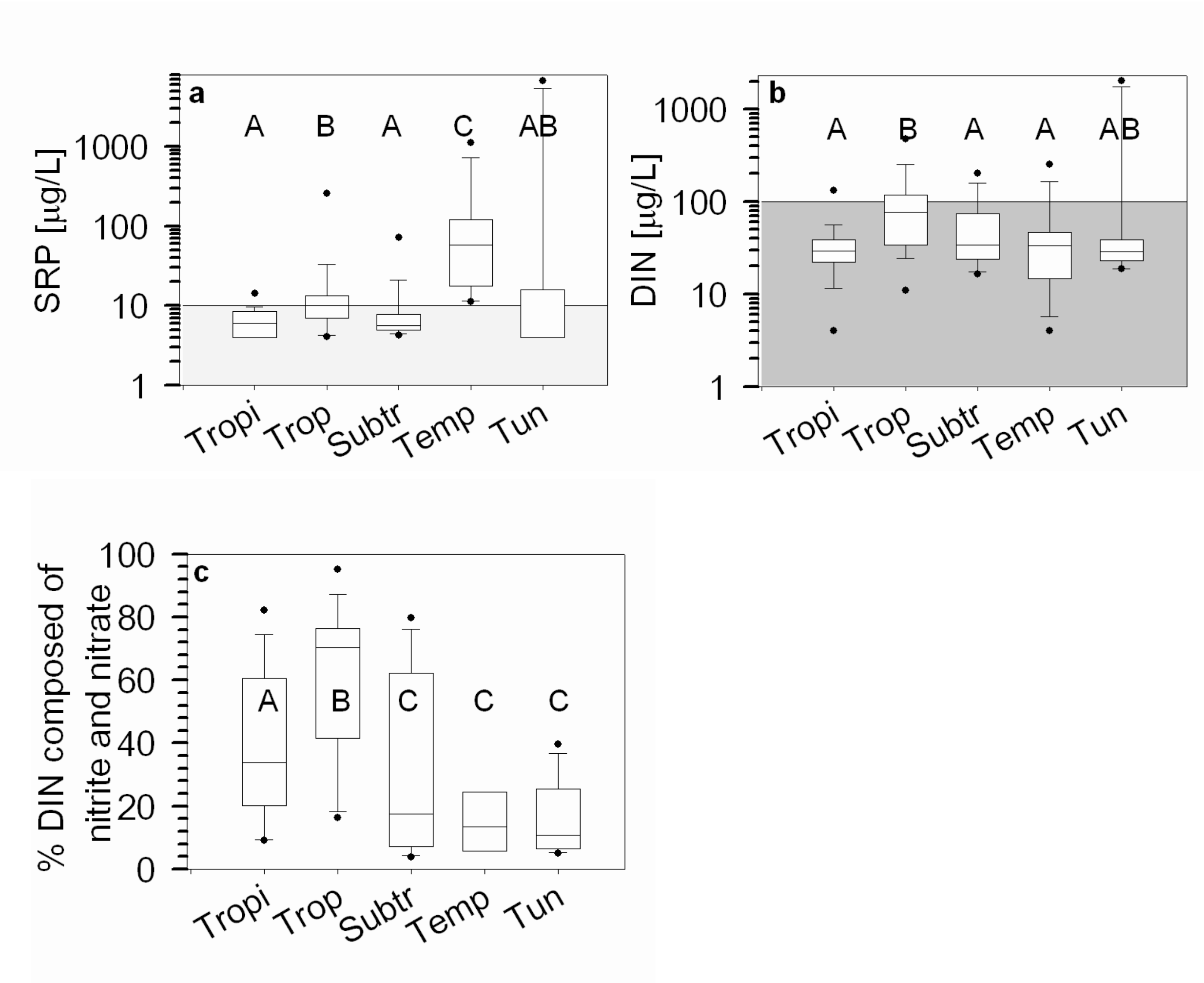

Figure 5.3 Boxplots of the Soluble Reactive Phosphorus (SRP) concentrations (a), Dissolved Inorganic Nitrogen (DIN) (b), and percentage of DIN composed of nitrate and nitrite (c) found in the lakes in the different climate zones. In lakes were SRP or DIN values were below the detection limit of respectively 4 µg P/L and 2 µg N/L the concentrations were set to the detection limit. See Figure 5.2 for further explanation.

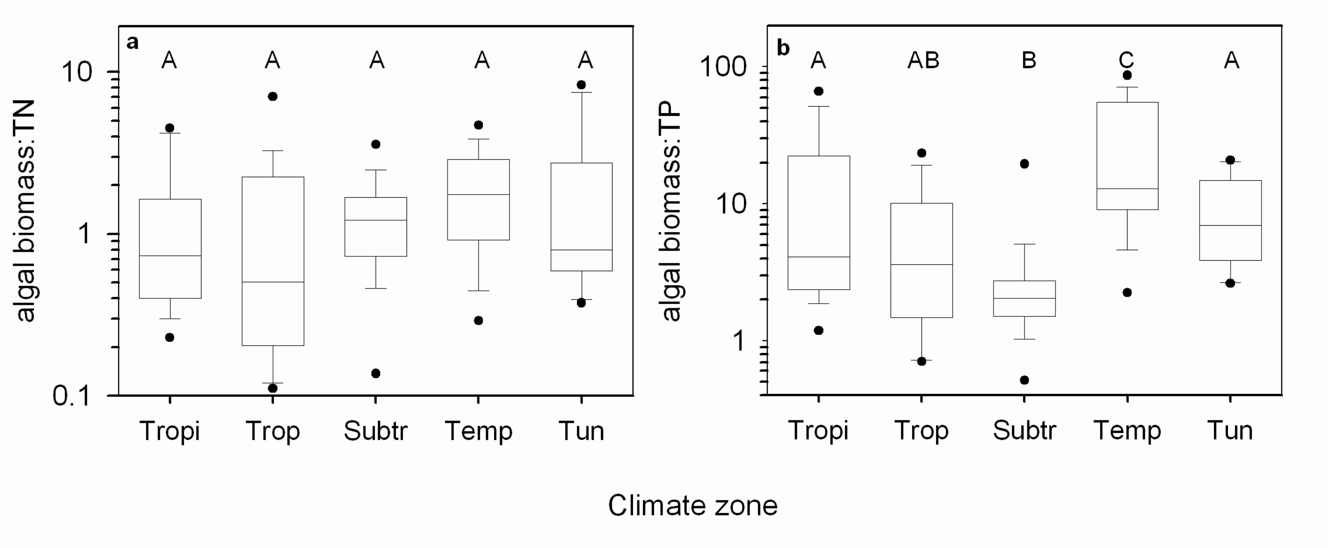

Figure 5.4 Boxplots of the algal biomass : total nitrogen ratio (a); and the algal biomass: total phosphorus (algal biomass : TP) ratio (b). See Figure 5.2 for further details.

Table 5.2 Most common occurring soil types at lake locations.

Soil type	Description	Number of lakes	Climate zones
Haplic arenosols (ARh)	Sandy-textured soils that lack any significant soil profile development.	23	Tropical*i*, Subtropical
Haplic ferralsols (FRh)	Red and yellow weathered soils whose colors result from an accumulation of metal oxides. They are formed on geologically old parent materials in humid tropical climates.	7	Tropical
Haplic phaeozems (PHh)	Soils characterized by a humus-rich surface layer.	12	Marine temperate, Tundra
Gleyic podzols (PZg)	Soils have a characteristic subsurface layer made up of accumulated humus and metal oxides. Above this layer there is often a bleached-out layer from which clay and iron oxides have been leached, leaving a layer of coarse-textured material containing primary minerals and little organic matter.	11	Subtropical

Table 5.3 Percentage of lakes potentially limited by nitrogen (N), phosphorus (P), co-limited (NP) or not limited by either N or P (no) in different climatic regions assessed using different methods

Climate zone	Nutrient ratio[1]								Total nutrient ratio lake water[3]			
	sediment[2]				seston[2]							
	N	P	no	n[7]	N	P	no	n	N	P	no	n
Tropical*i*	11	56	33	18	0	74	26	19	54	23	23	13
Tropical	42	26	32	19	11	37	53	19	78	6	17	18
Subtropical	6	22	72	18	No data			0	100	0	0	16
Maritime temperate	13	19	69	16	6	38	56	16	44	31	25	16
Tundra	45	36	18	11	18	36	45	11	50	13	38	8

Climate zone	Dissolved nutrient ratio[4]				Dissolved nutrient concentration[5]					(Potentially) N-fixing cyanobaceria[6]	
	N	P	no	n	N	P	NP	no	n	N	n
Tropical*i*	80	0	20	10	5	5	89	0	19	(53) 21	19
Tropical	33	17	50	18	32	26	26	16	19	(16) 16	19
Subtropical	46	8	46	8	6	6	83	6	18	(17) 5	18
Maritime temperate	100	0	0	8	88	0	0	13	16	(69) 50	16
Tundra	80	0	20	5	18	0	64	18	11	(27) 9	11

1) All ratios are atom based, 2) $N:P < 9$ potentially N-limited, $N:P > 23$ potentially P-limited, $9 < N:P < 23$ no limitation, 3) $TN:TP < 20$ potentially N-limited; $TN:TP > 38$ P-limited, $20 < N:P < 38$ no limitation, 4) DIN: $SRP < 13$ N-limited, $DIN:SRP > 50$ P-limited, $13 < DIN:SRP < 50$ no limitation, 5) DIN < 100µgN/L N-limited, SRP < 10µgP/L P-limited, “NP” limited by both N and P, 6) Percentage of lakes where potentially N-fixing cyanobacteria Nostocales) (between brackets) or N-fixing cyanobacteria (with heterocysts) were found, 7) The number of lakes (n) varies because in the ratios lakes with N or P concentrations below detection limit were removed from the analysis.

Local factors

Among the local factors investigated, DOC, the shade index and the inlet:evaporation ratio correlated with most nutrient limitation indicators (Table 5.4). DOC correlated negatively with the seston N:P ratio and positively with DIN, SRP, the algal biomass:TP ratio, the biovolume of N-fixing and potential N-fixing cyanobacteria. DOC correlated most with lake water TN:TP ratios. Lakes with high TN:TP ratios generally had high DOC concentrations (Figure 5.5a, Table 5.4). The inlet:evaporation ratio correlated positively with the DIN:SRP ratio and negatively with the lakewater TN:TP ratio, SRP, algal biomass:TP ratio and the biovolume of the two groups of cyanobacteria (Table 5.4). The correlation with the biovolume of N-fixing cyanobacteria was strongest with high biomass in high evaporative lakes (i.e., low inlet:evaporation ratio; Figure 5.5b). The high evaporative lakes were, however, also generally more eutrophic. The inlet:evaporation ratio was highly significantly correlated to both (log transformed) TN and TP ($p<0.0001$), and in a linear regression they explained 40 and 19 percent of the variance, respectively. Shade was negatively correlated to the seston N:P ratio and positively to DIN, SRP, algal biovolume:TN ratio, algal biovolume:TP ratio, and the biovolume of N-fixing cyanobacteria. The strongest correlation was found between the shade index and SRP (Figure 5.5c).

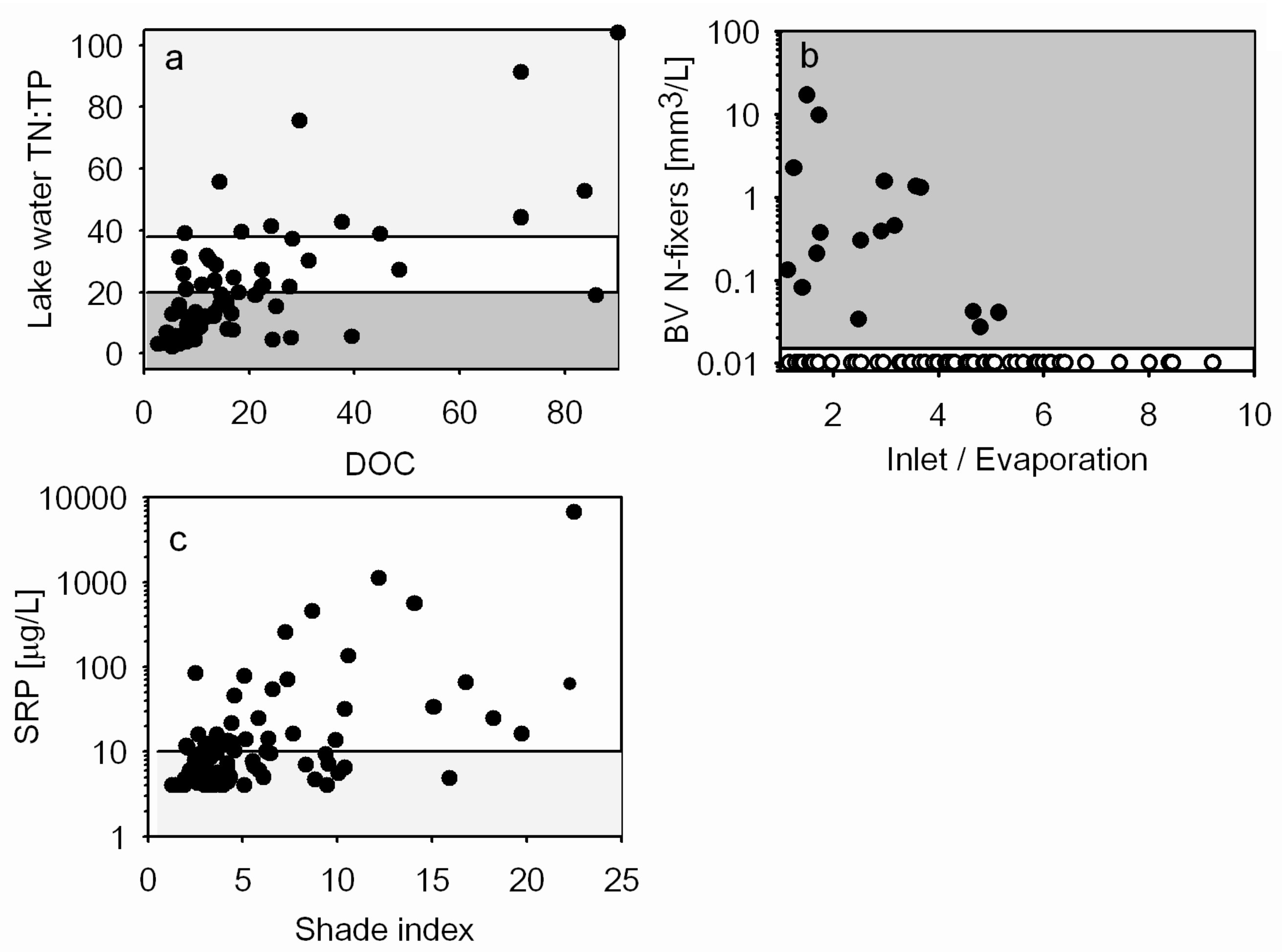

Figure 5.5 Relation between local factors and nutrient-limitation indicators: lake water TN:TP and dissolved carbon concentration (DOC) (a); Biovolume (BV) of cyanobacteria with heterocysts and the evaporation proxy “inlet : evaporation” (see “Calculations and data analysis” for derivation of this proxy), lakes where no cyanobacteria with heterocysts were found are represented with open circles (b); Soluble Reactive Phosphorus (SRP) concentration versus shade index (c). See Figure 5.2 for further details. See “Calculations and data analysis” for derivation of the evaporation proxy and the shade index.

The algal biomass:TN and seston N:P ratios significantly varied between lakes with abundant and scarce submerged vegetation (p=0.020 and p=0.010, respectively; Figure 5.6a,b). The grazing pressure proxy was significantly higher in lakes with abundant submerged vegetation (p=0.005), and both algal biomass:TN and algal biomass:TP ratios were negatively correlated with grazing pressure (Table 5.4). Lakes with abundant submerged vegetation did not have significantly lower DIN concentrations than lakes with scarce submerged vegetation (p=0.149, Figure 5.6c).

Table 5.4 Spearman correlation between nutrient limitation indicators and local factors

	N:P (sediment)	**N:P (seston)**	**TN:TP (water)**	**DIN:SRP**	**DIN**	**SRP**	**Chla:TN**	**Chla:TP**	**Biovolume heterocystous cyanobacteria**	**Biovolume Nostocales**
Lake area										
Mean depth				0.38**		-0.38***		-0.27*		
PVI							-0.39***			
Grazing pressure							-0.53***	-0.43***	-0.25**	
Shade- index		-0.36**			0.34**	0.52***	0.22*	0.25*	0.23*	
Soil carbon						0.45***				
Inlet :evaporation			-0.53***	0.35*		-0.25*		-0.49***	-0.54***	-0.34***
DOC		-0.41**	0.62***		0.23***	0.57***		0.45***	0.39***	0.31**

*** p<0.0001, **0.001<p<0.01,*0.01<p<0.05, only significant correlations are shown

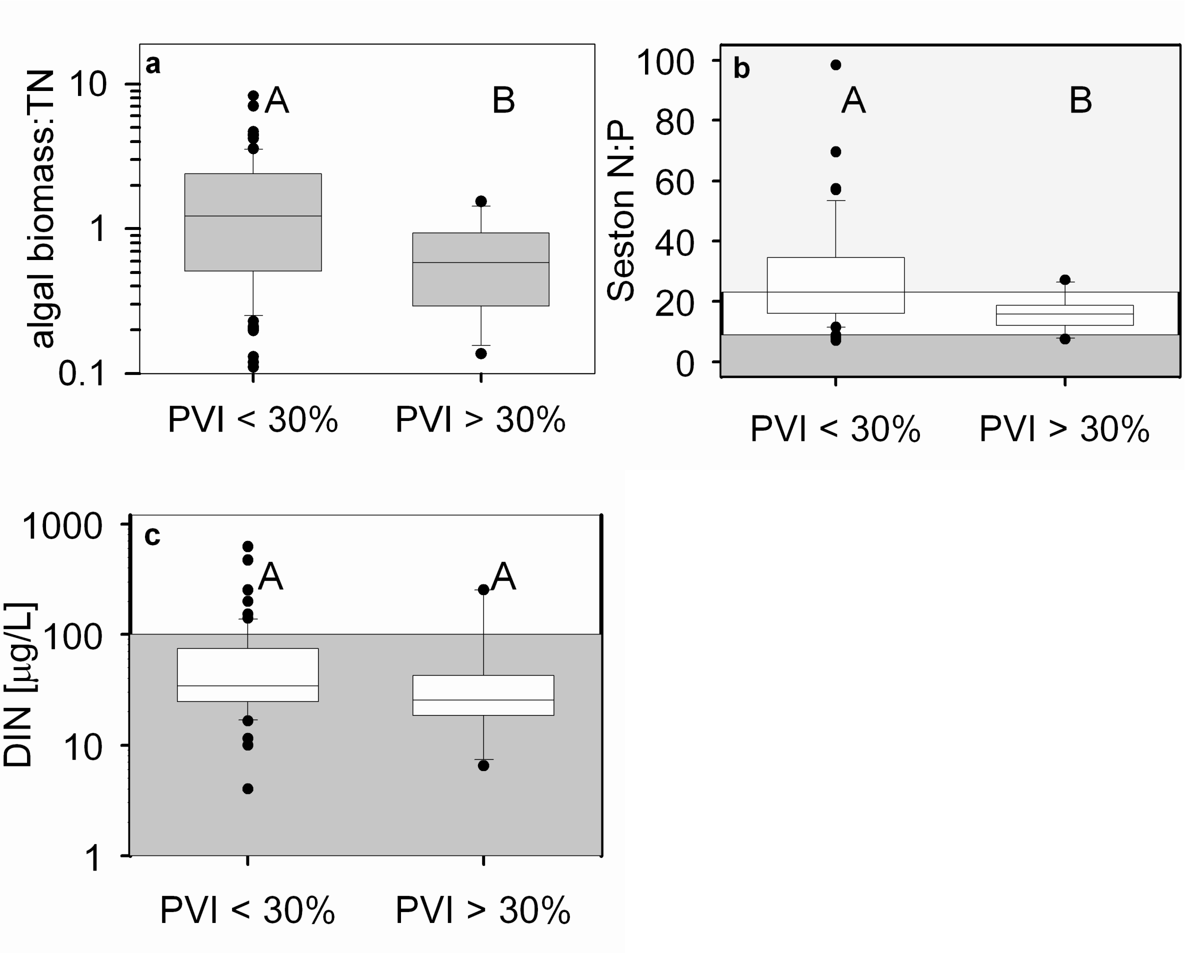

Figure 5.6 Boxplots of algal biomass:TN ratio (a), sestonic N:P ratio (b), and dissolved inorganic nitrogen (DIN) (c) in lakes with a plant infested volume (PVI) below (n=72) and above (n=11) 30%. See Figure 5.2 for further details

Nutrient-limitation-indicators were distinctly different among soil types. All indicators, except algal biomass:TN, differed significantly between the soil types ($p<0.05$). The seston N:P ratio, for example (Figure 5.7), indicated that N-limitation was rare in lakes in sandy areas (arenosols). These lakes were surrounded by bare sandy dunes, forest, and, in the tropical region, also by palm tree plantations. The seston N:P ratio in arenosols was generally higher than in the lakes in the ferralsol (sugar cane, wooded and urban areas), phaezem (pasture) and podzol (pasture and palm trees) soils.

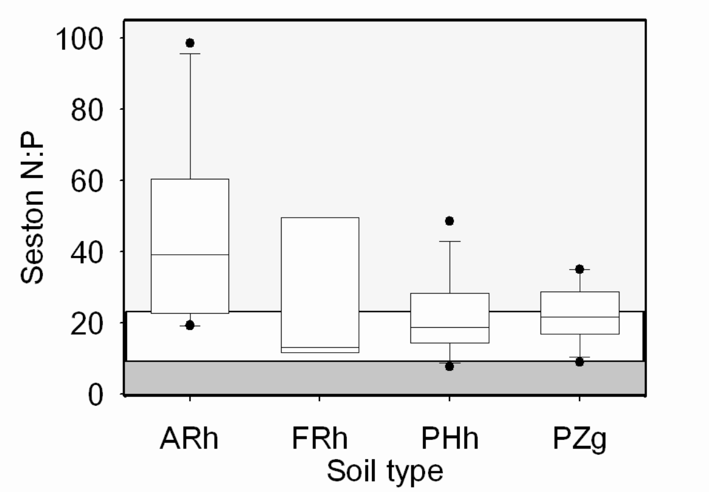

Figure 5.7 Boxplots the N:P ratio in seston found in the lakes situated at locations with different soiltypes. ARh =Haplic Arenosols , FRh = Haplic Ferralsols, PHh = Haplic Phaeozems, PZg = Gleyic Podzols. See Figure 5.2 for further details.

N-fixing cyanobacteria (i.e. cyanobacteria with heterocysts) occurred more frequently in phosphorous rich (TP>~200 µg/L) than in phosphorus poor lakes. However, also in the less enriched lakes (TP<50 µg/L) the phytoplankton biovolume often consisted (40% of the lakes) of more than 50% cyanobacteria (Figure 5.8a). We did not find N-fixing cyanobacteria when the DIN concentration was above approximately 100 µg/L, and the SRP concentration did not seem to be related to their occurrence (Figure 5.8b). The occurrence of potentially N-fixing cyanobacteria (i.e. cyanobacteria from the Order Nostocales) was not confined to lakes with low DIN concentrations. We found them in lakes with DIN concentrations up to approximately 600 µg/L (not shown).

Multiple linear regression models between log transformed nutrient-limitation-indicators, local factors and latitude often explained only a low percentage of the variance, with the exception of the models for lake water TN:TP ratios and for algal biomass:TP ratios (Table 5.5). Furthermore, the lakewater TN:TP model is the only model where both a local factor (DOC) and latitude were incorporated. The algal biomass:TP model confirms the Spearman rank correlations in that grazing pressure explains most of the variance.

Accordance between nutrient limitation indicators

Correlation between the different nutrient-limitation-indicators varied and was often absent (Table 5.6). SRP concentrations were negatively correlated with seston N:P ratios; high SRP concentrations generally co-occurred with low seston N:P ratios, both indicating potential N-limitation. On the other hand, high seston N:P ratios often occurred in lakes with DIN concentrations below 100 µg N/L, which resulted in a negative correlation between DIN and seston N:P. Notably, the seston N:P ratio and the lakewater TN:TP ratio were not significantly correlated. Also the sediment N:P ratio was not related to the nutrient ratios in water and seston. Especially lakes with high DOC tended to have low seston N:P concentrations compared to the lake water TN:TP ratio (Figure 5.9). The eutrophic, strong evaporative lakes also had high lake water TN:TP ratios compared to the seston N:P ratios. A linear regression indicated that (log transformed) DOC was highly significantly related ($p<0.0001$) to lake water TN:TP and explained 50% of the total variance, whereas it only explained 13% of the variance in the seston N:P ratio ($p=0.002$). DOC was linearly related to the algal biomass (log transformed) as well ($R^2=0.41$, $p<0.0001$). Biovolume of N-fixing cyanobacteria was positive and significantly related to lake water TN:TP ratios, but not to seston or dissolved ratios. N-fixing cyanobacteria were also positively and significantly related to algal biomass:TN and algal biomass:TP.

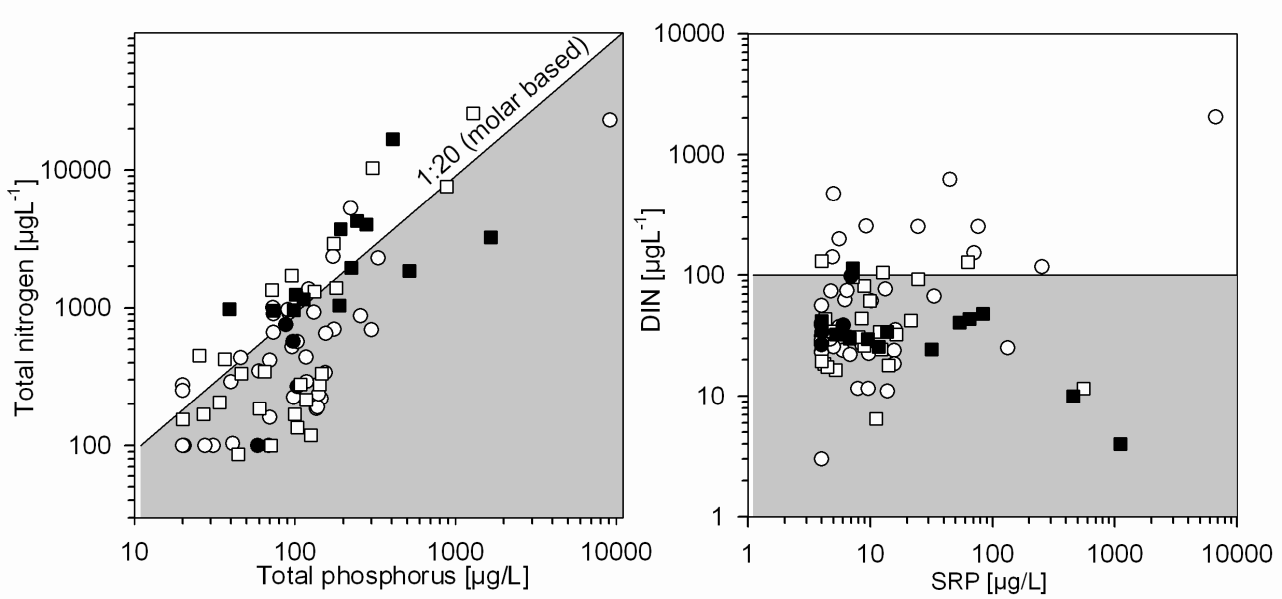

Figure 5.8 Lakes where nitrogen-fixing cyanobacteria (with heterocysts) where found (black symbols) or not found (open symbols) and where more (squares) or less (circles) than 50% of the algal biovolume consisted of cyanobacteria (N-fixing and non-N-fixing combined) with: different lake water total phosphorus and total nitrogen concentrations (a); and different dissolved nitrogen (DIN) and soluble reactive phosphorus (SRP) concentrations (b). When concentrations were below detection limit the detection limit was used in the plot (see Figure 5.2 for further details).

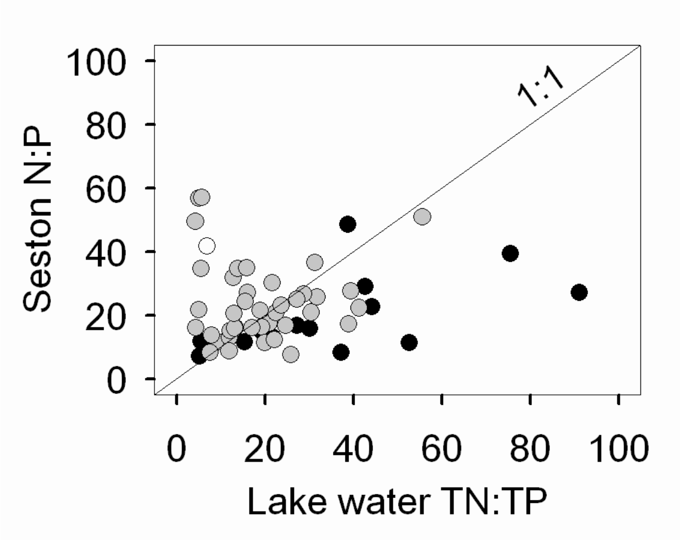

Figure 5.9 Lack of correlation between the seston N:P ratio and the lake water TN:TP ratio. White dots represent lakes with a dissolved carbon concentration (DOC) below 5 mgC L^{-1}, grey 5<DOC<25, and black DOC>25mgC L^{-1}.

Table 5.5 Models of stepwise multiple regressions. Only normally distributed (Shapiro-Wilk p>0.05) nutrient limitation indicators are shown.

Model	**$R^2_{adjusted}$**
Log(TN:TP) lake water	
0.17 + 0.85logDOC	0.50
0.29 + 0.95logDOC + 0.01LATITUDE	0.58
Log(N:P) seston	
1.67 – 0.26logDOC	0.16
1.67 – 0.34logDOC – 0.15logGRAZING	0.27
1.91 – 0.45logDOC – 0.16logGRAZING – 0.04(INL:EVAPO)	0.32
1.85 – 0.35logDOC – 0.19logGRAZING – 0.03(INL:EVAPO) – 0.01SHADE	0.36
Log(DIN:SRP)	
0.65 + 0.08(INL:EVAPO)	0.15
Log(chla:TP)	
0.14 – 0.48logGRAZING	0.25
0.64 – 0.47logGRAZING – 0.13(INL:EVAPO)	0.47
0.35 – 0.51logGRAZING – 0.13(INL:EVAPO) + 0.05SOILCARBON	0.50

grazing= potential grazing pressure; (inl:evapo)= inlet:evaporation ratio

Discussion

We found that local characteristics such as soil type and associated land use in the catchment, hydrology, and also the presence of abundant submerged macrophyte growth influence potential N and P-limitation. We found neither an increase in N-limitation nor indications of lower nitrate concentrations (which would have been expected if denitrification had increased) along the latitudinal gradient towards the equator.

Indicators

To assess potential nutrient limitation, different methods have been proposed (see Fisher et al. 1995, for an overview), all having their merits and caveats (see e.g. Beardall et al. 2001, and Kruskopf and Flynn 2006 for critical analyses of different methods). We found that the correlation between different nutrient-limitation-indicators was sometimes straightforward, sometimes poor and sometimes opposite to what we anticipated. There was an apparent lack of correlation between lakewater TN:TP and seston N:P. Lakewater TN:TP ratios tended to increase with increasing DOC, whereas this was not the case for seston N:P ratios. This difference suggests that N bound to dissolved organic matter (i.e. dissolved organic nitrogen, DON) strongly influences the lakewater TN:TP ratio. DON of fresh water often constitutes over 50% of the total soluble N (Wetzel 2001) and can even contribute up to 66% of TN (Lewis et al. 1999). When TN is largely comprised of non-bioavailable DON, high TN:TP ratios can give an inaccurate interpretation of P-limitation. The high SRP concentrations in most of the lakes with high TN:TP ratios point in this direction. Alternatively, high concentrations of bioavailable N and SRP can co-occur when another factor, for instance light, is limiting phytoplankton growth. Indeed, we found both high DIN and SRP concentrations in lakes with high shade indices (≥16). The relatively constant seston N:P ratio, on the other hand, makes it difficult to use this ratio as an indicator of nutrient limitation of a mixed species phytoplankton community (Morris and Lewis 1988, Hall et al. 2005). We, therefore, combined different approaches to strengthen the interpretation of the results.

The lakes in our study were all shallow, which effectuates a strong interaction between the sediment and the water column. Through resuspension, nutrients from the sediment may be returned to the water column. We, however, found that the sediment N:P ratio did not correlate with the nutrient ratio in seston and water. This lack of correlation may be caused by temporal changes in nutrient concentrations in the water column. It is well known that nutrient concentrations and nutrient limitation may vary in time (e.g. Setaro and Melack 1984, Philips et al. 1993). Our assessment of nutrient limitation using nutrient concentrations in the water column may, therefore, only represent the situation in a limited time frame around the moment of sampling. On the other hand, the discrepancy between seston and sediment N:P ratios may be caused by nutrient processes taking place in the sediment, such as the adsorption of phosphorus or denitrification. These processes can play a considerable role in the shallow lakes' nutrient budget (e.g. Eriksson and Weisner 1997, Jeppesen et al. 1998a).

Latitudinal effects

Denitrification is known to increase strongly with increasing temperatures (Golterman 2000, Pinay et al. 2007, Herrman et al. 2008). Therefore, it has often been hypothesized that N may be more often limiting in (sub)tropical lakes than in temperate lakes (Talling and Talling 1965, Lewis 1996, Downing et al. 1999, Lewis 2000). Whether an increase in denitrification actually leads to N-limitation of primary producers also depends on the N- and P-load to the lake. Recent studies did not find N-limitation to be more frequent than P-limitation in (sub)tropical lakes (Huszar et al. 2006) or found only a very weak correlation (R^2= -0.17, p=0.001) between latitude and the relative response of freshwater autotrophs to enrichment with N (Elser et al. 2007). Our analyses not only confirm that both N and P limitation may occur in a wide range of climates, we furthermore did not find indications for an increase in denitrification towards the equator. Denitrifying bacteria use nitrate and the identified DIN concentrations in the tropical*i* and tropical lakes were not lower than in the other lakes. The percentage of DIN composed of nitrate and nitrite was even higher than at higher latitudes.

Denitrification is also influenced by the concentration and quality of organic matter (Golterman 2004). The organic carbon content of the sediment did not significantly differ among the climate zones ($p=0.339$). However, quality of the organic matter may vary, and we found a positive relationship between the C/N ratio of the sediment and the percentage of DIN composed of nitrate and nitrite ($R^2=0.27$, $p<0.001$) with high C/N ratios (often an indication of low quality organic matter) combined with the high nitrate and nitrite percentages occurring in tropical lakes.

Local factors

Land use and catchment characteristics can strongly influence N- or P-limitation (e.g. Downing et al. 1999, Maberly et al. 2003). In U.K. lakes with N-limited phytoplankton, the phytoplankton growth was negatively linked to the proportion of bare ground, and P-limitation was negatively linked to the presence of pastures (Maberly et al. 2003). We found similar results: N-limitation was likely of little importance in lakes surrounded by a relatively large area of bare ground in the arenosol areas. These lakes were generally oligotrophic (mean chla concentration of 3.0 µg/L and SD=1.7 µg/L) and largely situated in areas with high N-deposition (1-2 g N m^{-2} yr^{-1}; Galloway et al. 2004). P-limitation was not frequent in the phaeozem and potzol areas with a high proportion of pastures in the catchments.

Besides land use, the hydrological characteristics of a lake and its catchment were found to affect nutrient limitation. When the influx of water from the watershed and the amount of direct precipitation were relatively high compared to the amount of water evaporating from the lake, potential N-limitation apparently occurred to a lesser extent than when the total inlet:evaporation ratio was low. This may reflect variations in residence time that affect net loss of nitrogen (e.g. Windolf et al. 1996). The SRP concentration, the DIN/SRP ratio, the chla/TP ratio and the biovolume of N-fixing cyanobacteria all decreased with a stronger influence of inlet water on the hydrological budget of the lakes. In contrast, the lake water TN:TP ratio increased in eutrophic, high evaporative lakes. This increase is likely caused by the high DOC concentrations in these lakes, possibly due to evapoconcentration or DOC derived from phytoplankton.

Two other factors that apparently limited phytoplankton biomass in some lakes were submerged vegetation and light. Seston N:P ratios were lower in lakes with abundant vegetation, although severe N-limitation did not appear to occur. Additionally, the algal biomass:TN ratio was significantly lower in lakes with abundant submerged vegetation. This observation could not be attributed to a low TN concentration in these lakes as the TN concentration and the algal biomass:TN ratio were not significantly correlated. The low algal biomass:TN ratio in lakes with abundant submerged vegetation might, however, reflect a higher grazing pressure in the lakes with abundant submerged vegetation. This suggestion is substantiated by the significant negative correlation between algal biomass:TN and algal biomass:TP ratios versus the grazing pressure proxy. It furthermore suggests that in several shallow lakes the algal biomass cannot reach the values expected from the resources available because of the biomass removal process by zooplankton, as demonstrated elsewhere (Mazumder and Havens 1998, Jeppesen et al. 2003a, Jackson et al. 2007). The positive correlation between SRP and DIN concentrations and shading in the water column might indicate that, besides nutrient limitation and grazing, also light limited algal biomass in some lakes.

Cyanobacteria occurrence

The occurrence of N-fixing cyanobacteria was not correlated with the TN:TP ratio. We did not encounter N-fixing cyanobacteria in most of the lakes with low TN:TP ratios whereas they were often present in lakes with high TN:TP ratios. Notably, N-fixing cyanobacteria, but also non-N-fixing cyanobacteria, were absent in many lakes with low DIN concentrations, which agrees with earlier findings (Jensen et al. 1994, Diaz et al. 2007). The concern that N-loading reduction and the consequent lowering of the TN:TP ratio will increase the risk of cyanobacterial dominance (Bulgakov and Levich 1999, Smith and Bennett 1999, Schindler et al. 2008) was, thus, not substantiated by our data from 83 South American shallow lakes. Studies in Denmark (Jensen et al. 1994) and Argentina (Diaz et al. 2007) confirm that neither low N nor low N:P ratios necessarily lead to cyanobacterial blooms.

Final remarks

Our finding that local factors more than climate influence which nutrient limits phytoplankton growth is in agreement with a European cross-climate study where human influences were found to be the most important factor affecting trophic state variables in shallow lakes (Nõges et al. 2003a). It implies that one cannot *a priori* prioritize N- or P-loading reduction depending on the climatic region, and emphasis should, therefore, remain on local factors. Contrary to findings in other regions, our analysis furthermore indicated that, in a wide range of climates, lower N-concentrations or lower N:P ratios do not necessarily lead to cyanobacteria (N-fixing or not) dominance.

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Supplementary material to chapter 5

Lake details

X	Y	Name	Climate zone	T Warm (^{o}C)	T Cold (^{o}C)	Soil type	Lake area (km^{2})	Average depth (m)	Altitude (masl)	Conduct. (µS/cm)	TN (mg/l)	TP (mg/l)	Chla (µg/l)
-50.4388	-30.5597	unknown	subtropical	23.5	14.2	ARh	0.46	3.01	20	115.5	0.338	0.154	2.5
-50.4260	-30.5483	unknown	subtropical	23.5	14.2	ARh	0.61	2.98	20	105.3	0.186	0.136	3.5
-50.4295	-30.5292	unknown	subtropical	23.5	14.2	ARh	0.16	1.58	21	101.9	0.220	0.145	3.0
-50.3611	-30.3580	Lagoa da Lavagem	subtropical	23.6	14.3	ARh	0.77	1.52	29	200	0.190	0.139	5.1
-50.2595	-30.1470	Lagoa Suzana	subtropical	23.5	14.6	ARh	1.16	1.71	18	137.4	0.279	0.143	5.9
-50.2572	-30.1378	Lagoa Lessa	subtropical	23.5	14.6	ARh	0.75	1.71	20	133.1	0.334	0.147	4.7
-50.2407	-30.0566	Lagoa da Prainha	subtropical	23.5	14.6	ARh	0.40	1.62	23	74.8	0.135	0.104	2.5
-50.2248	-30.0562	Lagoa da Tapera	subtropical	23.5	14.6	ARh	0.86	1.46	12	72.9	0.119	0.126	3.0
-50.2330	-29.9109	Lagoa Horácio	subtropical	23.2	14.4	ARh	0.69	1.46	31	52.9	<0.1	0.069	3.6
-50.2200	-29.9102	Lagoa Inácio	subtropical	23.2	14.4	ARh	0.51	1.34	15	55.2	<0.1	0.071	3.6
-50.2053	-29.9074	Lagoa do Rincão	subtropical	23.2	14.4	ARh	0.83	1.57	6	54.1	0.279	0.108	3.6
-50.2365	-29.9682	unknown	subtropical	23.2	14.4	ARh	0.40	1.46	19	62.3	0.169	0.100	3.8
-50.2236	-29.9697	Lagoa Emboaba	subtropical	23.2	14.4	ARh	1.47	1.84	9	68.3	0.215	0.118	4.9
-54.8333	-34.8967	Laguna Blanca	subtropical	21.9	11.4	PHg	0.46	1.74	5	325	1.039	0.190	56.2
-54.7097	-34.8507	Laguna del Barro	subtropical	21.9	11.4	PHg	0.22	1.27	12	270	0.292	0.119	3.0
-55.0072	-34.9079	Laguna del Diario	subtropical	22.2	11.4	PHl	2.43	1.46	13	397	0.439	0.118	0.9
-53.8053	-34.2954	Laguna Clotilde / Laguna Briosso	subtropical	22.2	11.5	PHg	0.17	2.45	2	168.6	0.237	0.140	2.7
-54.2958	-34.6786	Laguna de las Nutrias	subtropical	22	11.2	PHg	0.65	1.09	1	87	0.697	0.176	5.3
-59.9066	-35.2844	Las Encadenadas / San Carlos	maritime temperate	23.4	9.7	PHh	0.38	0.66	42	1023	3.712	0.193	28.7
-59.8856	-35.2295	Estancia Bellavista	maritime temperate	23.4	9.7	PHh	0.33	0.88	42	1095	4.277	0.244	226.1
-59.8565	-35.2451	Honda / Noria (on Estancia Bellavista)	maritime temperate	23.4	9.7	PHh	0.31	1.14	40	785	1.389	0.181	6.1
-59.0812	-36.0084	Difunto Manuel	maritime temperate	22.9	9.1	PHh	0.57	1.08	28	584	1.148	0.113	16.2

-59.8478	-37.6245	Los Abuelos	maritime temperate	21	7.1	PHl	0.39	0.55	217	3160	25.769	1.290	1123.9
-60.5022	-37.4802	Quillalauquen	maritime temperate	21.7	7.2	SNm	1.16	1.45	220	833	2.935	0.174	58.5
-60.7928	-37.5674	El Paraíso	maritime temperate	21.9	7.2	SNm	1.14	1.11	213	2130	10.383	0.304	398.7
-60.7585	-37.5619	Piorno	maritime temperate	21.9	7.2	SNm	0.78	1.29	215	3250	16.759	0.407	397.8
-60.0159	-38.0290	El Coraje	maritime temperate	21.2	7.2	PHl	1.14	0.96	190	4930	7.567	0.879	120.6
-60.0887	-37.9687	El Bagual	maritime temperate	21.1	7.1	PHl	0.84	0.80	200	1607	2.302	0.331	66.9
-60.4401	-38.3102	del Holandés	maritime temperate	21.5	7.3	PHl	0.33	1.35	151	1141	3.226	1.653	140.9
-60.8617	-38.8379	Santa Barbara	maritime temperate	21.9	7.6	PHh	0.78	1.35	41	964	1.858	0.515	70.5
-61.1747	-38.7692	de Oviedo	maritime temperate	22	7.4	PHh	0.25	1.68	81	651	1.955	0.225	138.8
-57.8948	-37.8667	Peregrina	maritime temperate	20.2	7.8	PHh	1.14	1.20	54	811	4.007	0.279	202.1
-41.9088	-22.5033	Lagoa Iriry / Coca Cola / Iodada	tropical	25.3	19.9	ACf	0.11	0.90	1	191.7	0.693	0.299	3.3
-41.6557	-22.2740	Lagoa Comprida	tropical	25.9	20.4	PZg	0.24	1.62	1	109.2	0.904	0.074	1.6
-41.6838	-22.2840	Jurubatiba / Carapebus	tropical	25.6	20	PZg	0.81	3.22	1	383	0.347	0.065	1.0
-41.1263	-21.8007	Lagoa Toca do Fereti / Lagoa Tai da Praia / Lagoa Pipeiras	tropical	26.3	20.8	PZg	0.76	1.70	3	712	0.754	0.088	25.7
-41.1357	-21.7738	Lagoa Palacete	tropical	26.3	20.8	PZg	0.76	1.38	2	838	0.956	0.074	26.2
-41.3635	-21.6195	Lagoa Limpa	tropical	26.4	20.2	FRh	1.27	2.79	36	1021	0.917	0.092	3.2
-40.8182	-20.9276	Lagoa Sete Puntas	tropical	27	21.4	FRh	0.60	4.50	2	129.2	0.662	0.074	1.1
-40.5858	-20.7856	Lagoa 'Es lo que hay'	tropical	26.9	21.4	FRh	0.24	2.49	12	162.8	0.225	0.098	2.4
-40.5243	-20.7135	Lagoa do Gaucho	tropical	26.9	21.4	FRh	0.36	1.11	1	110.9	<0.1	0.045	10.7
-40.5289	-20.7215	unknown	tropical	26.9	21.4	FRh	0.34	1.52	1	220	0.268	0.104	13.3
-40.3788	-20.4409	Lagoa Jabaete	tropical	27.4	22	FRh	0.34	2.14	27	167.1	0.520	0.096	1.0

-40.2013	-20.1892	unknown	tropical	27.6	22.2	FRh	0.13	1.03	2	159.2	0.653	0.155	23.7
-39.8749	-19.6472	Lagoa Cacimbas	tropical	27.1	21.7	PZg	0.34	2.30	1	71.9	0.333	0.047	2.4
-39.8423	-19.6150	Lagoa Parda	tropical	27.1	21.7	PZg	0.67	2.31	1	99.8	0.206	0.034	3.2
-39.8454	-19.6345	Lagoa Nova	tropical	27.1	21.7	PZg	0.18	1.61	1	73	0.169	0.027	6.9
-39.8400	-19.5694	Lagoa Boa Vista	tropical	27.1	21.7	PZg	0.48	2.32	1	68.7	0.436	0.046	1.4
-39.7633	-19.5122	Lagoa da Viuva	tropical	27	21.7	PZg	0.50	2.21	1	75.9	0.155	<0.02	1.2
-39.7772	-19.4803	Lagoa do Doutor	tropical	26.9	21.7	PZg	0.33	1.92	1	75.6	0.291	0.040	4.2
-39.7826	-19.5148	Lagoa Nova	tropical	27	21.7	PZg	0.19	2.89	1	70.5	0.450	0.026	1.4
-35.1797	-6.0468	Lagoa Boa Agua	tropical*i*	27.1	23.9	ARh	0.62	3.62	17	94.4	0.347	0.060	1.7
-35.1619	-6.0602	Lagoa do Carcara	tropical*i*	27.1	23.9	ARh	0.43	3.44	12	57.2	0.277	0.020	1.9
-35.1644	-6.0449	Lagoa do Ferreira Grande	tropical*i*	27.1	23.9	ARh	1.24	3.50	11	75.7	0.186	0.060	2.1
-35.1751	-6.0257	Lagoa do Urubu	tropical*i*	27.1	23.9	ARh	0.96	3.35	13	46.2	<0.1	<0.02	0.9
-35.1848	-6.0370	Lagoa Redonda	tropical*i*	27.1	23.9	ARh	0.91	3.55	18	162.5	0.160	0.070	2.5
-35.3219	-5.4243	Lagoa Baiaozinho	tropical*i*	27.7	24.7	ARh	0.43	1.35	3	76	0.251	<0.02	1.1
-35.3539	-5.3952	Mutucas	tropical*i*	27.7	24.7	ARh	0.91	1.53	7	77	<0.1	0.031	0.6
-35.3781	-5.3726	Lagoa Cutias	tropical*i*	27.7	24.7	ARh	0.61	2.36	7	71	<0.1	0.028	0.8
-35.4342	-5.3880	Lagoa Catole	tropical*i*	27.7	24.7	ARh	0.75	2.70	35	97	<0.1	<0.02	6.8
-35.2856	-5.4669	Lagoa da Mata	tropical*i*	27.6	24.5	ARh	0.41	0.73	1	92	<0.1	0.020	1.1
-35.5530	-5.5695	Lagoa do Cujueiro	tropical*i*	27.6	24.6	PLe	0.27	1.61	67	2100	0.571	0.097	3.5
-35.5193	-5.5754	do Mato (?)	tropical*i*	27.6	24.6	PLe	0.31	3.50	56	1180	1.004	0.073	35.9
-37.2918	-5.8622	Açude Recreio	tropical*i*	28.6	26.1	LVx	0.20	1.47	121	154	0.982	0.039	39.1
-37.1294	-5.7712	Açude do fazenda Bola	tropical*i*	28.6	26.3	LVx	0.40	1.48	89	384	0.965	0.099	15.4
-37.3133	-5.8773	Açude Belo Monte	tropical*i*	28.6	26.1	LVx	0.09	0.92	122	186	1.705	0.096	5.9
-36.1145	-6.1382	Açude das Lajes Pintadas	tropical*i*	26.7	23.9	LPe	0.24	2.27	318	1395	1.246	0.101	79.3
-37.0607	-6.3944	Açude Mondo Novo	tropical*i*	28.7	25.7	LVx	0.87	2.73	189	531	1.344	0.072	30.6
-36.7846	-6.3890	Açude Cruzetas	tropical*i*	28.3	25.3	LVx	0.27	2.57	209	564	0.567	0.103	12.3
-35.4856	-5.5781	Lago do Mineiro	tropical*i*	27.8	24.6	PLe	0.24	1.96	50	464	0.926	0.132	22.9
-67.0256	-54.4912	Lago del Pescado	tundra	8.4	-0.8	CMu	0.55	0.93	473	120.8	<0.1	0.059	2.3
-67.2428	-54.6253	Aguas Blancas	tundra	8.2	-0.8	CMu	0.28	2.04	500	37.8	0.104	0.041	4.3
-67.6628	-54.6335	Laguna Santa Laura	tundra	9	-0.1	LPq	1.05	3.00	166	84.6	<0.1	0.021	1.2
-72.3402	-51.5369	Dique San Jose	tundra	10.6	0.6	PHh	0.37	2.12	408	162.9	1.363	0.122	14.6

-71.9384	-51.7984	Laguna Larga	tundra	11.7	1.3	PHh	2.53	1.27	137	254	0.969	0.091	8.7
-72.8023	-50.3188	Laguna del Cisne	tundra	12.6	1.2	PHh	0.40	0.55	225	660	5.290	0.222	62.8
-72.7875	-50.3020	Laguna Don Leonardo	tundra	12.6	1.2	PHh	0.35	2.94	225	60.9	<0.1	0.015	2.1
-72.2641	-50.3211	Laguna Nimez	tundra	13.2	0.8	ANz	0.09	0.52	105	540	22.993	9.141	2889.0
-71.6731	-49.4174	Laguna del Barron	tundra	12.9	0.2	LVh	0.39	0.58	429	982	0.871	0.257	57.7
-71.7016	-49.4077	Laguna Sarian	tundra	12.9	0.2	LVh	0.09	0.99	429	414	0.415	0.070	3.0
-72.1759	-49.6153	Laguna Estancia San Agustino	tundra	12.6	0.3	PHh	0.18	1.10	400	265	0.427	0.037	2.4
-57.7874	-36.0922	Laguna Rosita	maritime temperate	22.1	9.3	SNm	2.42	1.00	2	1610	1.307	0.133	11.6
-57.7950	-36.6357	Laguna Mirador	maritime temperate	21.6	9	SNm	1.43	1.02	9	590	2.362	0.173	32.7

‘X’ and ‘Y’: coordinates in decimal degrees, ‘name’: some lakes have multiple names, ‘climate zone’: categories based on the prevailing climate characteristics following the Köppen (1936) climate system digitized by Leemans and Cramer (1991), ‘T warm’ and ‘T cold’: average air temperature during the warmest and coldest month (New et al. 2002), ‘soil type’: according to the FAO (1998), ‘lake area’: determined using landsat Orthorectified Landsat Thematic Mapper Mosaics of the year 2000. If image was cloudy, images of 1990 were used in stead. In rare cases when image deviated much from area observed in the field, all waypoints measured in the field were plotted on top of the image and the area of the polygon around the lake was calculated, ‘average depth’: determined using 40 depth measurements per lake, ‘altitude’: meters above sea level according to the dem from gtopo30, converted to an Arcview grid using the procedure published on www.herpnet.org, ‘conduct.’: conductivity, ‘TN’: total-nitrogen concentration, ‘TP’: total-phosphorus concentration, ‘chla’: chlorophyll-a concentration.

Açude das Lajes Pintadas – Rio Grande do Norte – Brazil – November 2005

6 Warmer climate boosts cyanobacterial dominance in lakes

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Abstract

Dominance by cyanobacteria hampers human use of lakes and reservoirs worldwide. Excessive nutrient loading and warmer conditions are often suggested to promote cyanobacteria, but evidence for both aspects has remained circumstantial. Here we show that while warmer climates do not result in higher overall phytoplankton biomass, the proportion of cyanobacterial biovolume in phytoplankton communities increases steeply with temperature. Our analysis, based on a study of 143 lakes along climatic gradients across Europe and South America, also reveals that cyanobacteria are more prominent in lakes with low underwater light levels. Such situations are promoted by high phytoplankton biomass and therefore by nutrient loading. Our results imply that nutrient levels required to prevent cyanobacterial dominance need to be substantially lower in a warmer climate.

Introduction

Cyanobacteria rank high when it comes to problems with water quality of lakes and reservoirs. Although cyanobacterial blooms predate human settlement (McGowan et al. 1999), the frequency and extent of cyanobacterial problems is strongly expanding (Paerl and Huisman 2008). Cyanobacteria are an ancient and diverse group of organisms with a wide range of growth forms (Reynolds 2006, Haselkorn 2009). Being prokaryotes they are quite distinct from other phytoplankton in various aspects. Importantly, the range of notorious toxins they can produce implies a major threat to the human use of freshwater resources (Huisman et al. 2005).

In view of the magnitude of the problems cyanobacteria cause for society, it is remarkable how poor our understanding is of the conditions that favor them. For some cyanobacterial sub-groups an understanding of their ecology has allowed successful prediction and management. For instance, species that can regulate their buoyancy to optimize their position in the water column need stable hydraulic conditions to do so. Such species can sometimes be combated by actively mixing the water using air bubbles (Visser et al. 1996). Also, particularly slow growing species in some situations may be eliminated from lakes by flushing (Scheffer et al. 1997). However, overall, it has remained difficult to predict under which conditions cyanobacteria will dominate lakes.

It is often asserted that cyanobacteria are promoted by high nutrient levels and a warmer climate (McGowan et al. 1999, Romo et al. 2004). However, evidence for these assertions is in fact ambiguous. For instance, cyanobacteria are found across all climate zones, including the cold ones, and in temperate shallow lakes extreme nutrient enrichment typically results in dominance by green algae rather than by cyanobacteria (Jensen et al. 1994). Systematic studies to evaluate the interactive effects of nutrients and temperature at realistic scales are scarce (Moss et al. 2004). Laboratory experiments are available for only a few species (Trainor 1998), and while they are indispensible to reveal mechanisms, they remain a poor basis to predict what drives dynamics in most complex natural communities. Studies of interannual variation hint at a positive temperature effect on cyanobacteria. For instance, blooms of particular buoyant cyanobacteria have been convincingly linked to warm summers in some cases (Huisman et al. 2005, Reynolds 2006), and also a study of Danish lakes indicates a higher likelihood of cyanobacterial dominance in warmer year (Jeppesen et al. 2009). However, in the absence of wider scale field studies it has remained unclear whether across climate zones there is in fact a systematic relationship between cyanobacterial dominance, nutrient levels and temperature.

Methods

Sampling - We sampled 83 lakes (5-55 ^{0}S) along the east coast of South America (2004-2006) and 60 lakes (38-68 ^{0}N) in Europe (2000-2001) (Figure 6.1). Some of these lakes were sampled in multiple years, resulting in a total of 188 lake-years. The lakes were explicitly selected to cover a wide climatic range and to vary markedly in nutrient levels, independently of climate (Figure 6.1, Table 6.1, and see Moss et al. 2003b, Kosten et al. 2009a for details). All lakes were shallow (average of mean depth=1.9m) and sampled during dry season in tropical lakes in South America and during summer in all other lakes in South America and Europe. In South America we collected depth-integrated water samples at 20 random points in each lake. Two litres of each integrated sample were gathered in a bulk sample. Water was frozen until analysis. In Europe, depth-integrated water samples were taken from the central part of the lake.

Sample analysis - Phytoplankton was counted in water samples preserved in Lugol's iodine using an inverted microscope. Biovolume was estimated according to standard geometric formulae (Hillebrand et al. 1999). In South American lakes total-phosphorus (TP) and total-nitrogen (TN) in the lake water were analyzed using a continuous flow analyzer (Skalar Analytical BV, Breda, The Netherlands) following NNI protocols (NNI 1986, 1990). In Europe TP was measured by the molybdenum blue reaction (Murphy and Riley 1962) after potassium persulfate digestion. TN was analyzed with the Kjeldahl flow or analyzer method. Chlorophyll-a was extracted from filters (GF/C S&S, Whatman's) with 96% ethanol and absorbance was measured at 665 and 750 nm (Nusch 1980, Arvola 1981). Further details on the methodology are reported elsewhere (Moss et al. 2003b, Nõges et al. 2003a, Kosten et al. 2009a, Peeters et al. 2009).

Data analysis - Shade was characterized as the ratio of average lake depth to the Secchi depth transparency (Scheffer 1998). Simple and multiple linear regressions were used to test the relationships between chlorophyll-a, total phytoplankton biovolume, and cyanobacteria biovolume versus TN, TP and water temperature. All variables were log transformed. To prevent zeros in lakes where cyanobacteria were not detected we used log cya BV + 0.001 mm^3L^{-1}. In order to explore relationships between relative share of cyanobacteria (% to total biovolume) with TN, TP, shade and water temperature, we performed a logistic regression using quasi-likelihood (McCullagh and Nelder 1990) with the Nagelkerke R^2 as coefficient of determination (Nagelkerke 1991).

Although nutrient levels varied largely independently from climate, the four coldest (Swedish) lakes had the lowest nutrient concentrations (Supplementary Figure 6.1). Removing those oligotrophic lakes from the dataset did not significantly affect the results of the logistic analysis. All statistical analyses were performed using SPSS for Windows version 15.0 (SPSS, Chicago, Illinois, USA). The response surface shown in panel (d) of Figure 6.2 in the main text was produced by interpolating the raw data using inverse distance weighting in Sigmaplot 10.0.

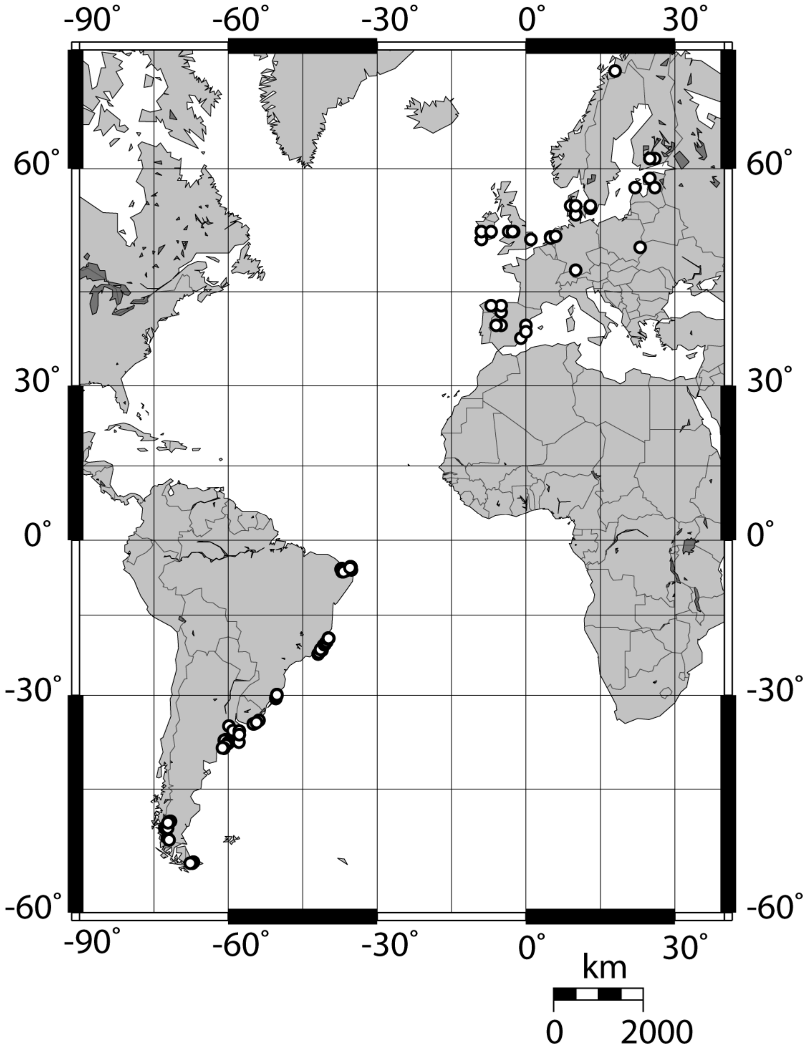

Figure 6.1 Geographical location of the studied lakes.

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Table 6.1 General characteristics of the lakes used in the analysis (n=188, except for chlorophyll a where n=184).

	min	**max**	**mean**	**median**
Area (ha)	0.6	27000	502	39
Mean depth (m)	0.5	5.6	1.9	1.6
Conductivity (µS cm^{-1})	10	6400	439	212
Temperature (^{o}C)	6.7	28.8	20.5	20.0
Chlorophyll-a (µg L^{-1})	0.4	2889.0	52.7	10.1
Total nitrogen (µg L^{-1})	35	25769	1552	888
Total phosphorus (µg L^{-1})	5	9141	170	70
Total phytoplankton biovolume (mm^3 L^{-1})	0.02	916.82	24.14	4.68
Cyanobacteria biovolume (mm^3 L^{-1})	0.00	511.17	13.62	0.75
% of cyanobacteria total phytoplankton	0	100	36	27
Shade	0.31	19.99	2.73	1.77
Secchi depth (m)	0.04	6.90	1.38	0.90

Results and discussion

Despite the large climatic gradient, temperature hardly added to the variance in chlorophyll concentrations explained by (log) total-phosphorus (P) or (log) total-nitrogen (N) concentrations (Table 6.2, multiple regression models 1-4). However, temperature adds to the nutrient-explained variance in the total biovolume of cyanobacteria (Table 6.2, models 9-12). Also, regression models for (log) total phytoplankton biovolume explain slightly more variance if temperature is included as an independent variable (Table 6.2, models 5-6). Thus warmer lakes appear to have the same chlorophyll-a concentration, but a somewhat higher biovolume, especially of cyanobacteria, for given nutrient levels.

Table 6.2 Linear regression models for chlorophyll-a (log Chla), total phytoplankton biovolume (log TotBV), and cyanobacterial biovolume (log CyaBV + 0.001) explained from the independent variables total nitrogen (log TN, μgL^{-1}), total phosphorus (log TP, μgL^{-1}), and water temperature (Temp, ^{o}C). (Regressions with at least one non significant p value are in italic).

	Intercept	**p**	**Temp**	**p**	**log TN**	**p**	**log TP**	**p**	**R^2_{adj}**	**F**	**n**
					Log(Chla)						
1	-2.15	<0.0001			1.11	<0.0001			0.60	277.16	184
2	*2.423*	*<0.0001*	*0.01*	*0.0905*	*1.12*	*<0.0001*			*0.61*	*141.47*	*184*
3	-0.65	<0.0001					0.91	<0.0001	0.39	119.92	184
4	*-0.296*	*0.1603*	*-0.02*	*0.0118*			*0.96*	*<0.0001*	*0.41*	*64.99*	*184*
					Log(totBV)						
5	-2.28	<0.0001			1.01	<0.0001			0.37	110.17	188
6	-2.99	<0.0001	0.03	0.0022	1.05	<0.0001			0.40	62.46	188
7	-1.00	<0.0001					0.89	<0.0001	0.27	70.53	188
8	*-0.99*	*<0.0003*	*0.00*	*0.9599*			*0.89*	*<0.0001*	*0.27*	*35.08*	*188*
					Log(Cya BV)						
9	-3.341	<0.0001			1.06	<0.0001			0.13	29.51	188
10	-5.756	<0.0001	0.10	<0.0001	1.18	<0.0001			0.26	32.03	188
11	-2.658	<0.0001					1.28	<0.0001	0.18	41.03	188
12	-3.738	<0.0001	0.07	0.0011			1.11	<0.0001	0.22	27.42	188

Interpreting causality in relationships between total nutrient concentrations and algal biomass is always problematic, as much of the total amount of nutrients is typically locked up in algal cells, and relationships could thus be consequential or causative. Especially in shallow lakes where sediment-water exchange is intense, nutrient levels in the water column may reflect algal biomass to a large extent (Scheffer 1998, Søndergaard et al. 2003). This problem is circumvented if we focus on explaining the relative share of cyanobacteria (expressed in biovolume) instead of absolute biomass (Table 6.3 and Figure 6.2). Regression models reveal a highly significant increase in the percentage cyanobacterial biovolume with temperature (Table 6.3, model 4). The share of cyanobacteria also increases with total-N and total-P concentrations, but in single regressions these nutrients explain less of the variation in cyanobacterial share than temperature (Table 6.3, models 4, 6, and 7). The temperature effect is unlikely to be explained by a systematic difference in nutrient levels over the climatic gradients, as the lakes have been selected in such a way that climate and nutrient levels vary largely independently (See Supplementary Information, Figure 6.1). Indeed, multiple logistic regression models confirm the independent effects of temperature and nutrients on the share of cyanobacteria in the phytoplankton community (Table 6.3, models 2 and 3). Our indicator of shade experienced by the community explained more of the variance in the relative abundance of cyanobacteria than nutrient concentrations (Table 6.3 compare model 1 with 2 and 3, and model 5 with 6 and 7). This fits well with results from controlled experiments and field data (Smith 1986, Scheffer et al. 1997, Jeppesen et al. 2009) suggesting that light availability rather than nutrient concentrations might affect the competitive balance between a large group of shade-tolerant cyanobacteria –mainly Oscillatoriacea - and other phytoplankton groups (Scheffer et al. 1997).

Table 6.3 Logistic regression models for the relative share of cyanobacteria (expressed as biovolume) as a function of water temperature (Temp, ^{o}C), total nitrogen (log TN, µgL^{-1}), total phosphorus (log TP, µgL^{-1}), and shade (n=188).

	Inter-cept	**p**	**Temp**	**p**	**log TN**	**p**	**log TP**	**p**	**log Shade**	**p**	**R^2 (Nagelkerke)**
1	-3.12	<0.0001	0.11	<0.0001					1.13	0.0003	0.14
2	-5.75	<0.0001	0.14	<0.0001	0.76	0.0010					0.13
3	-4.19	<0.0001	0.11	<0.0001			0.63	0.0074			0.12
4	-3.15	<0.0001	0.12	<0.0001							0.10
5	-0.99	<0.0001							1.39	<0.0001	0.08
6	-2.05	<0.0001					0.78	0.0013			0.04
7	-2.1	0.0025			0.52	0.0268					0.02

In conclusion, our results suggest that the probability that cyanobacteria dominate a phytoplankton community is affected directly by temperature, and indirectly (through shading) by nutrient loading to a lake (Figure 6.3). Obviously, nutrient load is also important in determining the total biomass of cyanobacteria in phytoplankton communities where they dominate, and therefore the impact these organisms may have on ecosystem functioning and human use.

Importantly, there are indications that warming may increase nutrient levels as well. Sediments of warmer lakes appear to be less efficient carbon and nutrient sinks (Kosten et al. in press), implying that, external nutrient loadings being equal, higher temperatures might lead to higher nutrient concentrations in the water column (Figure 6.3). In the case of phosphorus, sediment release may further be boosted by reduced oxygen concentrations mediated by enhanced metabolic rates of bacteria decomposing organic matter at the water-sediment interface (Søndergaard et al. 2003). On top of this climate change may enhance phosphorus loading from the catchment to the lakes, especially through modifications in precipitation regimes (Jeppesen et al. 2009). Also, warmer conditions could enhance phytoplankton biomass through an alteration of top-down regulation. Warmer waters often contain high densities of small planktivorous fish (Jeppesen et al. 2007a, Teixeira-de Mello et al. 2009) and the resulting high predation pressure on zooplankton may reduce the grazing pressure on phytoplankton (Gyllström et al. 2005, Jeppesen et al. 2007a, Jeppesen et al. 2009) (Figure 6.3). Thus, the direct temperature effect on the competitive advantage for cyanobacteria suggested by our analysis may well work in synergy with a set of mechanisms leading to lower underwater light levels under warmer conditions.

Our findings have straightforward implications for lake management in a warming climate. Nutrient control may often be difficult and expensive to achieve. However, our results demonstrate that in a warmer climate, nutrient loading will have to be reduced substantially more if we wish to prevent the risk of cyanobacterial dominance to rise.

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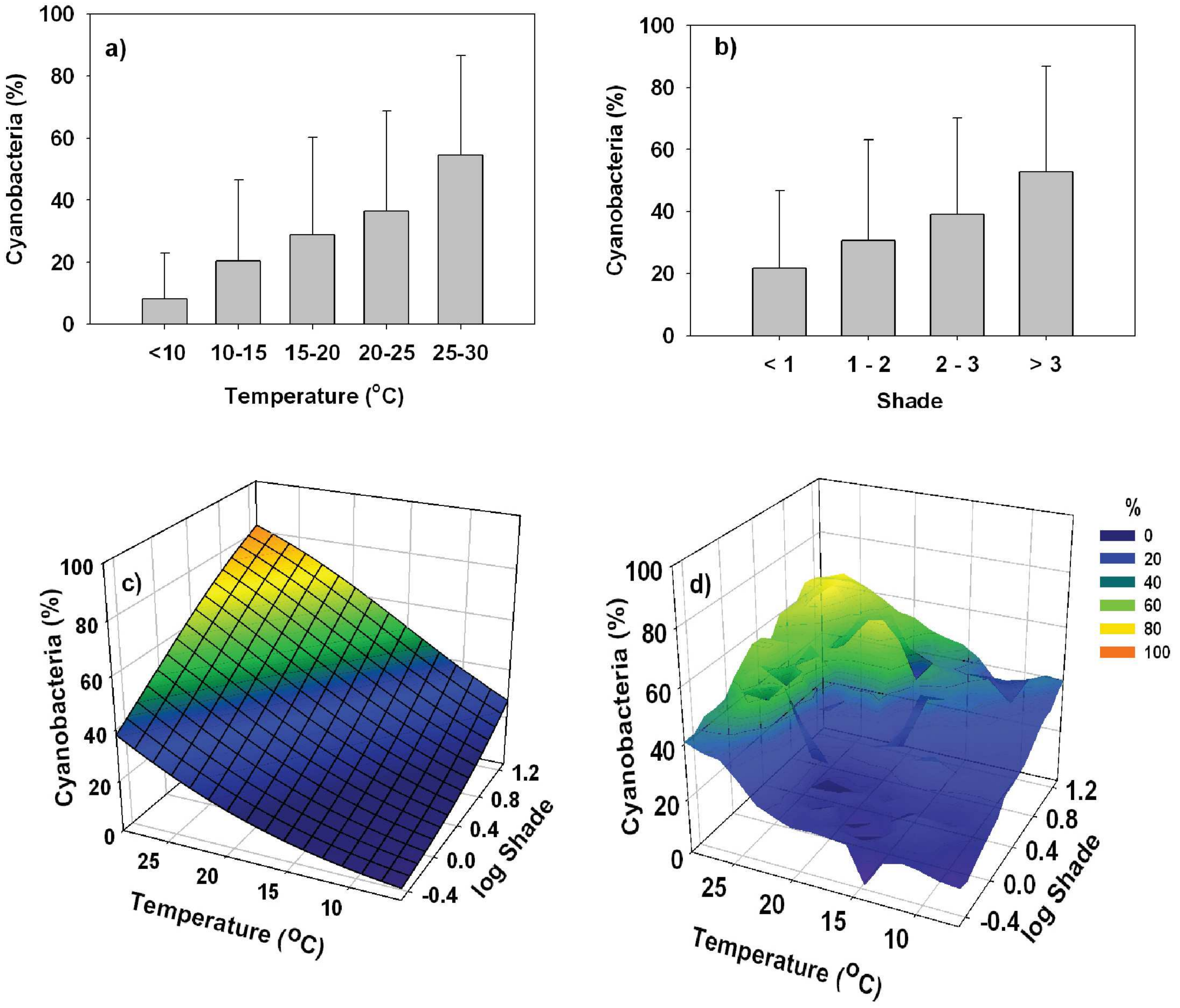

Figure 6.2 Percentage of cyanobacterial biovolume in phytoplankton communities as a function of water temperature and underwater shade level (indicated by the ratio of average lake depth to Secchi depth) in 143 lakes along climatic gradients in Europe and South America. Single relationships are illustrated in panels (a) and (b) where error bars represent one standard deviation. The lower panels illustrate the combined effects of temperature and shade as captured by a Logistic regression model (panel c, corresponding to Table 6.3 model 1), and a response surface obtained from interpolation of the raw data using inverse distance weighting (panel d).

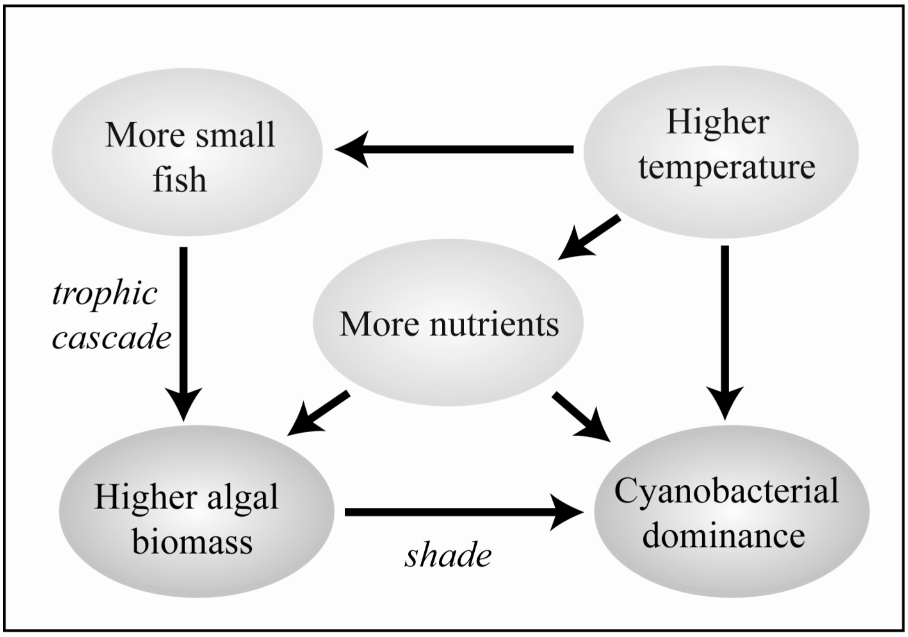

Figure 6.3 Hypothesized causal structure of the effects of climatic warming and nutrient load on the probability that a phytoplankton community will be dominated by cyanobacteria.

Acknowledgements

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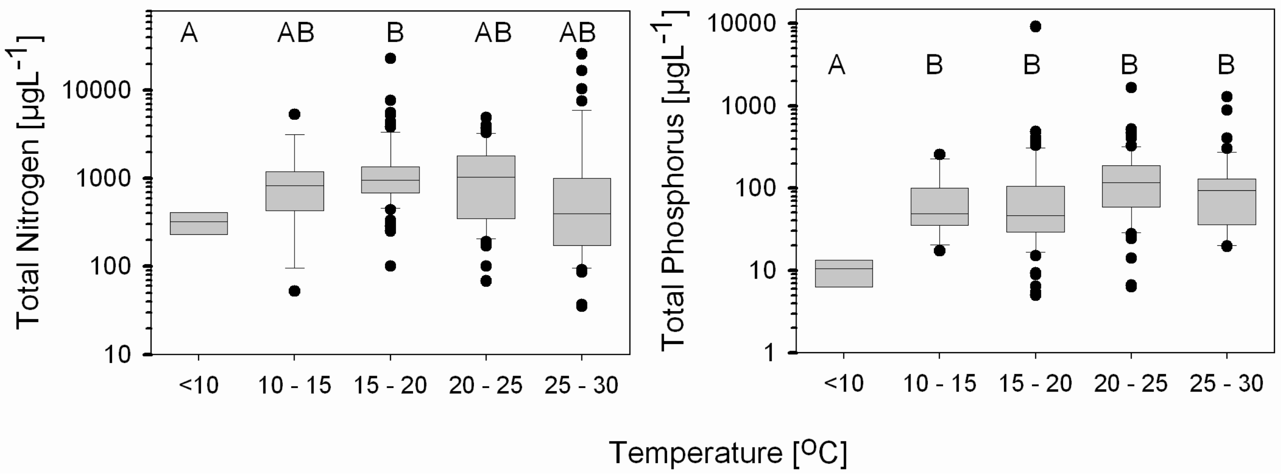
Supplementary material to chapter 6



Supplementary Figure 6.I Nutrient concentrations varied largely independently of temperature. Capital letters indicate homogeneous subsets (post-hoc Tukey). Only in the coldest lakes the nutrient concentrations were significantly lower than in the other regions. Removing these lakes from the dataset does not change the outcome of our analysis. Box plots delimit the 25th and 75th percentile, whiskers indicate the 10th and 90th percentile. Median is plotted within the boxes.

Bacupari – Rio Grande do Sul – Brazil – November 2004

7 Climate-dependent CO_2 emissions from lakes

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Abstract

Inland waters, just as the world's oceans, play an important role in the global carbon cycle. While lakes and reservoirs typically emit CO_2, they also bury carbon in their sediment. The net CO_2 emission is largely the result of the decomposition or preservation, of terrestrially-supplied carbon. What regulates the balance between CO_2 emission and carbon burial is not known, but climate change and temperature have been hypothesized to influence both processes. We analyzed patterns in carbon dioxide partial pressure (pCO_2) in 83 shallow lakes over a large climatic gradient in South America, and found a strong, positive correlation with temperature. The higher pCO_2 in warmer lakes may be caused by a higher, temperature-dependent mineralization of organic carbon. This pattern suggests that cool lakes may start to emit more CO_2 when they warm up due to climate change.

Introduction

The importance of the world's oceans in global carbon cycling is well known and their influence on atmospheric CO_2 concentrations is explicitly incorporated in climate change models (IPCC 2007). So far, however, the role of inland waters has received less attention even though recent studies indicate that they play an important role in regulating carbon fluxes as well (Cole et al. 2007, Downing et al. 2008, Duarte et al. 2008). A significant part of the organic carbon initially sequestered as CO_2 by terrestrial ecosystems ends up in rivers and lakes. Only about half of this carbon is transported to the oceans (Cole et al. 2007). Much of the terrestrially produced carbon entering inland waters is buried in sediments or emitted as CO_2 to the atmosphere (Cole et al. 2007). In addition, primary production within inland waters represents a substantial carbon flux, especially in lakes with high concentrations of nutrients allowing high productivity (Williamson et al. 2009). This turns inland waters into carbon processing hot spots in terrestrial landscapes and despite the fact that inland waters occupy a relatively small fraction of the Earth's surface, they play an important role in the global carbon cycle by processing large amounts of terrestrially derived carbon (Battin et al. 2009). Depending on the balance between processes such as respiration, primary production, groundwater carbon inflow and calcite precipitation, these systems may be carbon sinks, or become supersaturated with CO_2 and act as CO_2 sources to the atmosphere (Cole et al. 1994, Cole et al. 2000, Duarte and Prairie 2005, Sobek et al. 2005). All these processes are likely sensitive to changes in temperature and hydrology.

Very little is known about the overall effects of climatic change on the carbon cycling in inland waters. Temperature, for example, may affect carbon cycles in a direct way through its influence on aquatic respiration (Sand-Jensen et al. 2007) and primary production (Flanagan et al. 2003), which may be most evident when it coincides with an increase in nutrient loading (Christoffersen et al. 2006). A differential temperature-dependence of respiration and photosynthesis may lead to a decrease in carbon fixation and an increase in carbon emission (Lopez-Urrutia et al. 2006). Altered precipitation regimes may influence lakes' metabolism as well. For instance through its effect on the hydraulic residence time, which can have several effects including: altering carbon sedimentation and mineralization (Curtis 1998, Algesten et al. 2003); changing terrestrial inputs of nutrients and organic matter, and possibly primary production as well (Reynolds 1994, Schallenberg and Burns 1997). Temperature and precipitation also have an indirect effect on lake's carbon cycle through their influence on terrestrial carbon fixation and the subsequent carbon leaching to the lake (Sobek et al. 2005).

To explore the potential net effects of climate on carbon emissions from lakes, we sampled 82 comparable shallow lakes along a latitudinal gradient (5 – 55 °S) in South America (Figure 1.1) ranging in annual mean temperature from 4.0 to 27.6 °C.

Methods

Site description. We sampled 82 lakes in the East of South America (Figure 1.1). Lakes were selected to resemble each other as much as possible morphologically, but to vary as much as possible in climate and – within climate regions – in trophic state. The lakes in our data set ranged from being oligotrophic to hypertrophic (Table 7.1). All lakes were shallow (maximum mean depth 4.5m) and relatively small (surface area ranged between 0.09 and 2.53 km^2) (Table 7.1). The climate conditions at the sampling sites varied considerably; the most northern lake sites had maximum monthly air temperature up to 28.7 °C whereas at the most southern lake locations the maximum was only 8.2 °C (New et al. 2002). At the time of sampling the lake water temperature ranged from approximately 10 to 30 °C. In each climate region, lakes were selected to vary as much as possible in trophic state (Table 7.1, and Kosten et al. 2009a).

Sampling. Lakes South of 25°S were sampled once during summer, the lakes nearer to the equator were sampled during dry season between November 2004 and March 2006.
We collected water samples along the whole water column with a 1.5 m long tube at 20 random points in each lake between 9:30 and 12.00 am. Two litres of each of these depth-integrated samples were gathered in a 40 litres bucket resulting in a depth and area integrated bulk sample. Filtration for various analyses in the laboratory was conducted directly after collection. Water and filters were then frozen until analysis. Sediment samples of the top 2 cm were taken in the centre of the lake with a Kajak corer.

Annual mean precipitation data was obtained from a high-resolution data set of surface climate over global land areas (New et al. 2002).

Table 7.1 General data on the lakes sampled.

	Range	**Mean**	**Median**
Area [km^2]	0.09 – 2.53	0.62	0.46
Mean depth [m]	0.50 – 4.50	1.80	1.60
Conductivity [μScm^{-1}]	38 – 4930	527	167
Total nitrogen [mg N L^{-1}]	<0.10 – 25.80	1.80	0.40
Total phosphorus [mg P L^{-1}]	0.02 – 9.14	0.27	0.10
PVI [%] *	0 – 81	11	1
Chlorophyll-a [µg L^{-1}]	0.6 – 2889.0	79.6	4.7
The extinction of light at λ= 380nm - as a proxy for humic substances – [m^{-1}]	0.3 – 54.2	5.9	4.0
Total suspended solids [mg L^{-1}]	2 – 663	43	9
Light attenuation coefficient [m^{-1}]	0.50 – 43.60	4.60	2.50

* The percentage of the lake's volume filled with submerged vegetation

Sample analysis. We determined the acid neutralizing capacity (ANC) titrimetrically using 0.05N HCl on unfiltered samples in the field directly after sampling. pH was determined also on unfiltered samples using a probe. Chlorophyll-a (chla) was extracted from filters (GF/C S&S) with 96% ethanol and absorbance was measured at 665 and 750nm (Nusch 1980). For dissolved organic carbon, (DOC) analysis water was filtered through GF/F Whatman filters. DOC was determined by a Total Organic Carbon analyzer (Model 700, O.I.C International BV). As a measure for humic substances spectrophotometric light absorption at 380 nm was measured (Buiteveld 1995) in filtered (0.45 µm S&S) water. The concentration of organic N and C in sediment was determined, after carbonate removal, using a CNS analyzer (NA-1500).

The δ^2H and $\delta^{18}O$ of the bulk lake water sample were determined in a Multiflow system connected to an Isoprime Mass Spectrometer (Thermo Electron, Waltham, MA). All isotopic ratios are expressed in δ units relative to the Vienna mean standard ocean water, here as:

$$\delta D \text{ or } \delta^{18}O = \left[\frac{R_{sample}}{R_{std}} - 1\right] \cdot 1000$$

in which R_{sample} and R_{std} are $^2H/H$ or $^{18}O/^{16}O$ ratios of the sample and standard, respectively. The precision of analysis is ±2.0‰ and ±0.1‰ for δ^2H and $\delta^{18}O$ values, respectively.

Calculations and data analysis

pCO_2 was calculated from the pH and the ANC, adjusting for temperature, ionic strength and air pressure (Cole et al. 1994). Subsequently, the results were expressed as under- or supersaturated with CO_2 relative to the atmosphere (Relative Saturation, RS). We used the global average atmospheric CO_2 pressure for the year 2005 as a reference (Tans 2009). Lakes were classified as strongly CO_2 supersatured (RS> 1.2); strongly undersaturated (RS< -1.2); or near saturation (-1.2<RS<1.2). The percentage of the lake's volume filled with submerged vegetation (PVI) was determined analogously to Canfield et al. (1984, for details see Kosten et al. 2009b).

As a proxy for the hydrological character of the lake we derived an inflow: evaporation ratio using δ^2H and $\delta^{18}O$ of the lake water. The derivation is based on the principle of light isotopes evaporating more quickly than heavy isotopes. The ‘heavier’ the lake water in comparison to the incoming water the more the lake water has been subject to evaporation. We calculated the inflow: evaporation ratio using the Gat – Bowser model (Gat and Bowser 1991, Rozanski et al. 2001). The relative humidity input for the model was obtained from a global dataset (Bowen and Revenaugh 2003). For the stable isotope composition of the inlet water we used the average composition of precipitation at the lake location as a proxy (extracted from map provided by Bowen (2003) based on data from Bowen and Revenaugh (2003)). However, as the incoming water may have already been subject to evaporation in the watershed before it enters the lake, we may be overestimating the evaporation in the lake. The more the lake was subject to evaporation, for example due to a long hydraulic residence time, the smaller the inflow:evaporation ratio.

Relations of pCO_2 versus climatological variables and local variables were first explored using simple linear regressions. The climatological variables included temperature, mean annual precipitation and the inlet:evaporation ratio. The local variables included PVI, chlorophyll-a (chla), the extinction of light at λ=380nm used as a proxy for humic substances (humic), dissolved organic carbon concentration (DOC), and two indicators for the relative influence of aquatic primary production and terrestrial carbon input: (1) the ratio between chlorophyll-a and the extinction at λ= 380nm (chla:humic); and (2) the ratio between organic carbon and nitrogen in the sediment (C:N). A high chla:humic ratio indicates that the primary production is relatively large compared to influence of terrestrial organic matter on the lake’s metabolism. To the contrary, a high C:N ratio indicates a relatively strong terrestrial influence on the lake’s metabolism, as the C:N ratio of terrestrial organic matter is generally higher than that of aquatic material (Elser et al. 2000). Next, we applied multiple linear regressions. The multiple linear regressions modeled pCO_2 using temperature and the variables explaining most of the variance in pCO_2 in the simple linear regressions. To enhance normality both the dependent and the independent – except for temperature - variables were log transformed before analysis.

All statistical analyses were performed using SPSS for Windows version 15.0 (SPSS, Chicago, Illinois, USA).

Results

Only in a small fraction (6 %) of the lakes, the carbon dioxide partial pressure (pCO_2) was within a range of ±20% from the equilibrium with the atmosphere (near saturation group). Most lakes (80%) were supersaturated (RS> 1.2, Figure 7.1). The pCO_2 saturation increased with increasing temperature (Table 7.2). Temperature alone explained 13% (R^2) of the variance in pCO_2 (Table 7.1). Lakes with high abundances of primary producers (either phytoplankton or submerged macrophytes) generally have lower pCO_2’s than expected based on temperature alone (Figure 7.2, see also negative parameters in the models 4 and 5 in Table 7.2), whereas lakes with low abundances of primary producers generally fall above the pCO_2-temperature regression line.

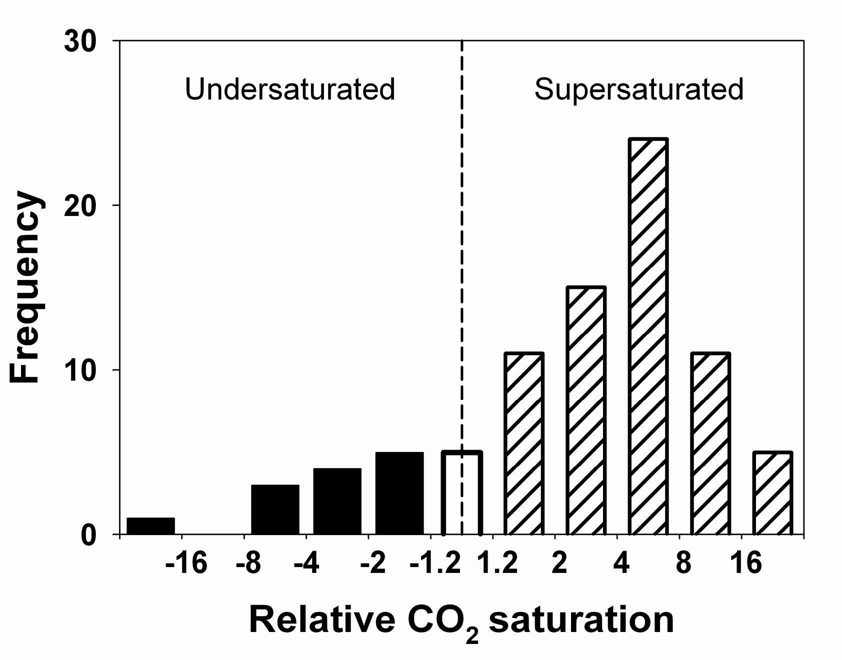

Figure 7.1 Frequency distribution of lakes undersaturated and supersaturated with CO2 relative to the atmosphere. For undersaturation: RS= - pCO_2(air)/pCO_2(water), for supersaturation the relative saturation (RS): RS= pCO_2(water)/pCO_2(air).

Table 7.2 Simple linear regression models describing the relationship of lakes' partial CO_2 pressure and different climatological and local variables. The p-value of the parameters are presented between parentheses.

		Regression statistics		
	Log(pCO_2) =	**F_1**	**R^2**	**n**
1	$2.08^{(<0.001)} + 0.04^{(0.001)}$ temperature	12.13	0.13	82
2	$-0.17^{(0.877)} + 1.08^{(0.005)}$ log(total annual precipitation)	8.37	0.08	82
3	$2.55^{(<0.001)} + 0.98^{(<0.001)}$ log(inflow:evapo) [a]	21.87	0.24	70
4	$3.33^{(<0.001)} - 0.30^{(<0.001)}$ log(chla)	18.14	0.19	82
5	$3.15^{(<0.001)} - 0.18^{(0.047)}$ log(PVI) [b]	4.06	0.05	82
6	$3.10^{(<0.001)} - 0.08^{(0.548)}$ log(humic) [c]	0.36	0.01	82
7	$3.50^{(<0.001)} - 0.42^{(0.010)}$ log(DOC)	6.96	0.08	82
8	$3.20^{(<0.001)} - 0.36^{(0.001)}$ log(chla:humic) [d]	21.63	0.21	82
9	$0.70^{(0.103)} + 2.27^{(0.001)}$ log(C:N) [e]	32.45	0.30	79

a) Inflow : evaporation ratio, b) The percentage of the lake's volume filled with submerged vegetation (PVI) was augmented with 1% to avoid zero's, c) The extinction of light at λ= 380nm is used as a proxy for humic substances, d) Chlorophyll-a concentration : extinction at λ= 380nm ratio, e) Organic carbon : total nitrogen ratio in the top-sediment

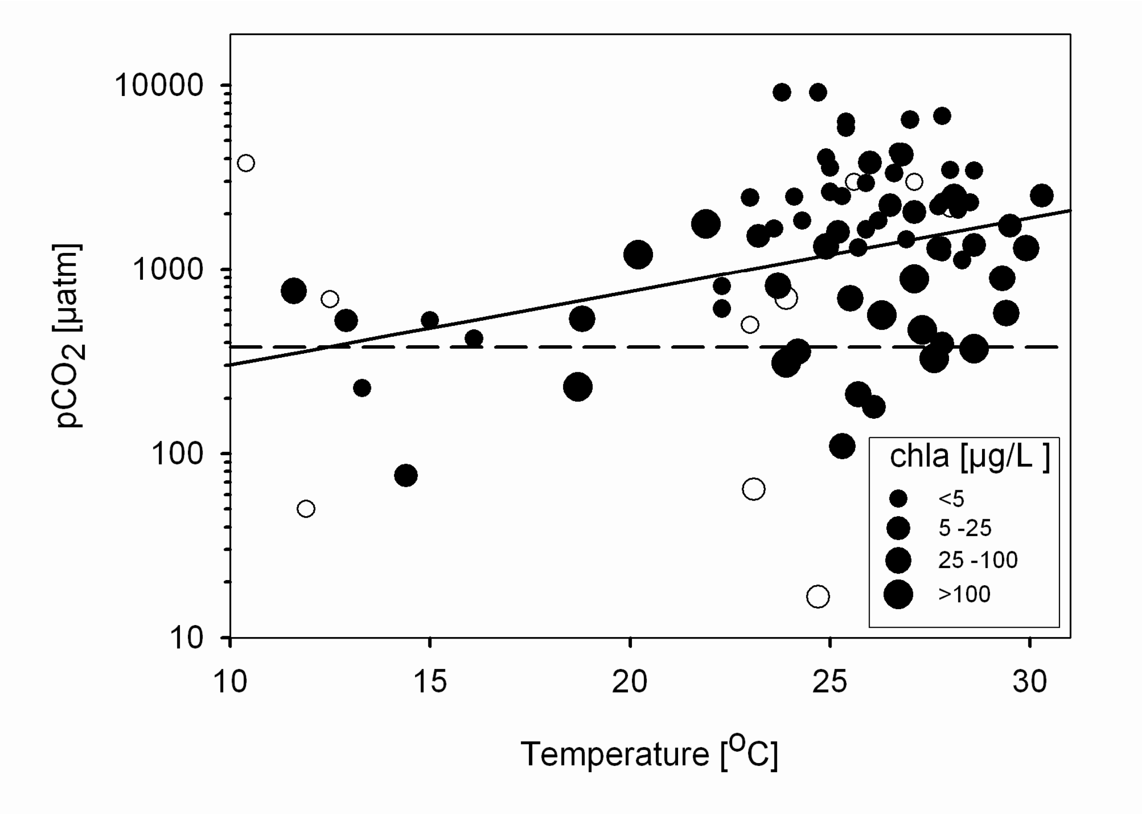

Figure 7.2 Partial CO_2 pressure (pCO_2) in lakes along a water temperature gradient in South America. Lakes differed in trophic status (size of symbols indicate chlorophyll-a concentrations, open symbols represent lakes with substantial growth of submerged vegetation (>25% of the lake volume is filled with vegetation)). Continous line represents the regression line between pCO_2 and the water temperature measured in the lakes. Lakes depicted below the dashed line are undersaturated in pCO_2 relative to the atmosphere.

In the simple linear regressions pCO_2 was best explained by the inflow:evaporation ratio (R^2=0.24, model 3 in Table 7.2), the ratio between algal biomass (expressed as chla) and the light extinction λ= 380nm (used as a proxy for humic substances) (R^2=0.21, model 8 in Table 7.2), and the C:N ratio in the sediment (R^2=0.30, model 9 in Table 7.2). Lakes with a high inflow:evaporation ratio had relatively high pCO_2's compared to lakes with a low inflow:evaporation ratio. Furthermore, in lakes with a relatively strong terrestrial influence (i.e. a low chla:humic ratio or a high C:N ratio), the pCO_2 tended to be high as well.

The pCO_2 could significantly be explained by a combination of temperature and the before mentioned variables, increasing the degree of explanation (adjusted R^2) of pCO_2 (Table 7.3). At similar inflow: evaporation ratios, the cooler lakes had a lower pCO_2 than the warmer lakes (Table 7.3, Figure 7.3a). In the same way, the warm lakes generally had a higher pCO_2 than the cool lakes at similar chla:humic ratios (Table 7.3, Figure 7.3b).

Table 7.3 Multiple linear regression models describing the relationship of lakes' partial CO_2 pressure, temperature and local environmental variables. In the lower part we used the ratio between organic carbon and nitrogen in the sediment (C/N) instead of the chla/humic ratio as an indicator for the relative influence of terrestrial and aquatic organic matter. Only significant models are shown. The p-value of the parameters are presented between parentheses. For explanation of the variables see Table 7.2.

		Coefficients[a]			**Regression statistics**		
Intercept	**Temperature**	**Inflow:evapo**[b]	**Chla:humic**[c]	**C:N**[d]		**R^2_{adj}**	**N**
$1.93^{(<0.001)}$	$0.03^{(0.035)}$	$0.99^{(<0.001)}$			F_2=13.84	0.27	70
$2.20^{(<0.001)}$	$0.04^{(<0.001)}$		$-0.37^{(<0.001)}$		F_2=21.48	0.34	82
$2.17^{(<0.0001)}$	$0.03^{(0.023)}$	$0.67^{(0.005)}$	$-0.23^{(0.011)}$		F_3=12.29	0.33	70
$-0.03^{(0.94)}$	$0.04^{(<0.001)}$			$2.15^{(<0.001)}$	F_2=25.93	0.39	79
$0.48^{(0.36)}$	$0.03^{(0.020)}$	$0.54^{(0.015)}$		$1.62^{(0.001)}$	F_3=12.6	0.35	67

a) All variables (except of temperature) were log transformed before analysis, b) Inflow:evaporation ratio, c) Chlorophyll-a concentration:concentration of humic substances ratio, d) Organic carbon:total nitrogen ratio in the top-sediment

Discussion

The small fraction of lakes that is in equilibrium with the atmosphere indicates that most lakes were either sinks or sources to the atmosphere (Figure 7.1). As in surveys in other parts of the world (Cole et al. 1994, Sobek et al. 2003), most lakes were supersaturated, indicating that these lakes are net sources of CO_2 to the atmosphere. In fact, pCO2 tends to be lowest in summer (Kelly et al. 2001) or dry season (Richey et al. 2002) precisely when our samples were taken. Therefore the annual average relative saturation per lake is likely even higher than we report here.

The ratio between phytoplankton biomass and the light extinction at λ= 380nm explained 21% (R^2) of the variation in pCO_2 (Table 7.2). Most likely this is because the light extinction at this wavelength, which we used as a proxy for the level of humic substances can be used as an indicator for terrestrial carbon input. When the terrestrial carbon is mineralized this may lead to a net CO_2 production. Phytoplankton, on the other hand, takes up CO_2 during photosynthesis, reducing CO_2 concentrations in the water. The higher the density of phytoplankton compared to the level of humic substances, the lower the pCO_2 tends to be. Besides phytoplankton, submerged vegetation may contribute substantially to the primary production in shallow lakes thereby lowering the pCO_2. Indeed, the pCO_2 is systematically lower in lakes with high abundances of submerged vegetation (Table 7.2 and Figure 7.2).

In other lake data sets pCO_2 was found to be positively correlated to DOC concentrations (Prairie et al. 2002, Sobek et al. 2003, Sobek et al. 2005). Although our lakes had a wide range in extinction at λ=380nm - used as a proxy for humic substances - and dissolved organic carbon (respectively 0.3 – 54.2 m^{-1} and 1.7 -86 mg C L^{-1}) we did not find a significant relation between pCO_2 and the light extinction and the explained variance in pCO_2 by DOC was low (R^2=0.08, Table 7.2). Our lakes, however, also covered a wide range in productivity and as pCO_2 is influenced by primary production and respiration simultaneously, the chlorophyll-a:humic substances ratio may therefore be most informative. A relatively constant primary production in comparison to the variation in DOC in the other data sets may explain the overruling influence of DOC on the pCO_2 in those earlier studies. The strong correlation between DOC and chlorophyll-a in our dataset (R^2=0.48, p<0.001) might indicate that in eutrophic lakes, much of the DOC is algal derived which may explain the negative correlation between pCO_2 and DOC (Table 7.2).

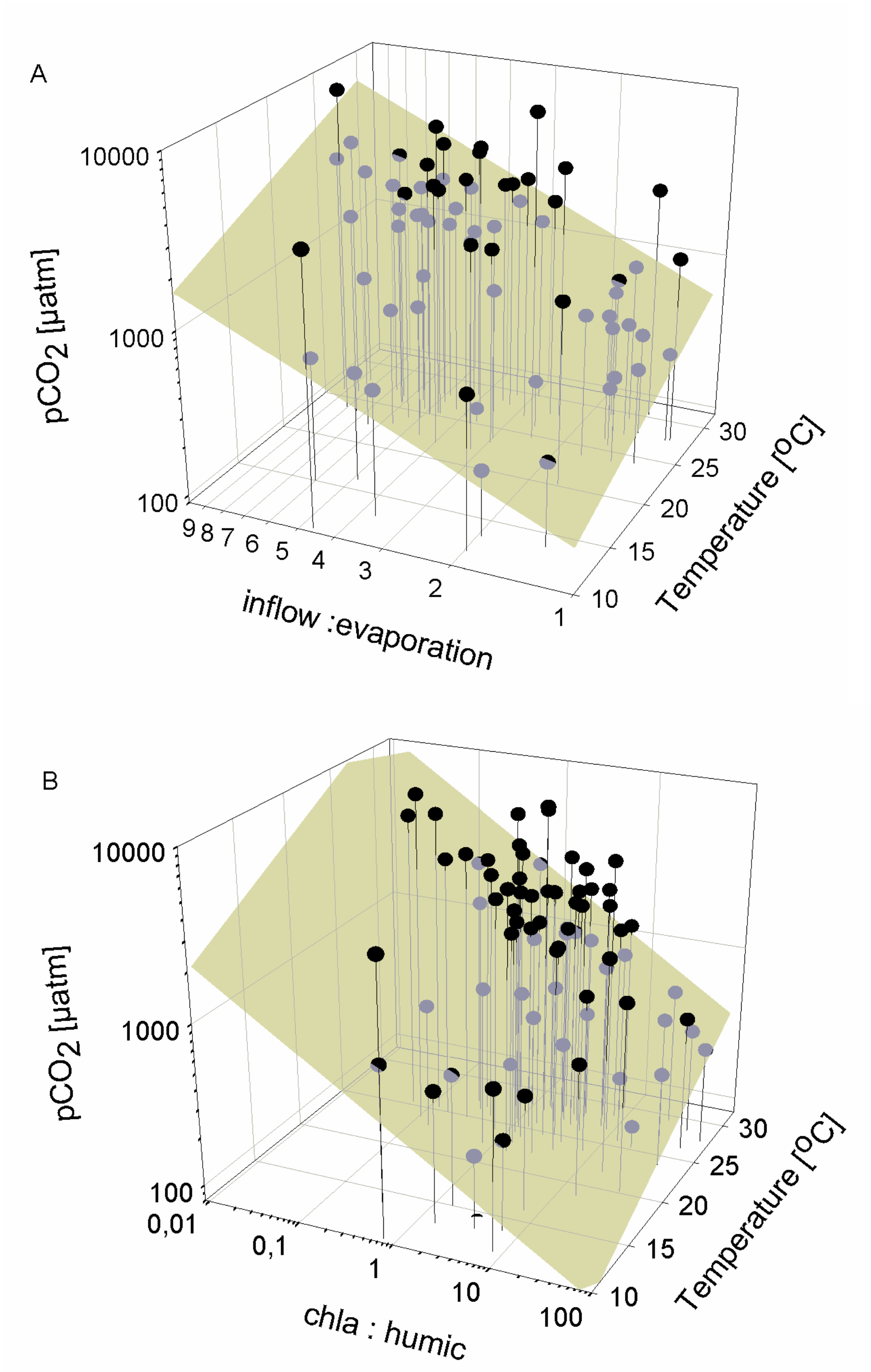

Figure 7.3 Partial CO_2 pressure (pCO_2) in South American lakes over a temperature gradient and a gradient of (a) a ratio between inflow and evaporation and (b) a ratio between algal biomass (expressed as chlorophyll-a) and the extinction of light at λ= 380nm - as a proxy for humic substances. The transparent planes indicate the multiple linear regression models. Grey points have a lower and black points have a higher pCO_2 than expected based on the models.

Our data furthermore suggests that hydrological characteristics of the lake, i.e. the ratio between inflow and evaporation, strongly affects the pCO_2 (Table 7.2). In part this may reflect a temperature effect. Evaporation plays an important role in the energy budget of the lake and strong evaporation cools down the lake (Lenters et al. 2005). Hydrology, however, also affects the input of terrestrial carbon to the lake. Indeed, pCO_2 was generally high in lakes where the volume of incoming water from the watershed was large in comparison to the volume that had evaporated. The importance of lakes' hydrology is in line with results from studies in other parts of the world (Algesten et al. 2003). Although the pCO_2 may be influenced by the inflow of CO_2 rich groundwater as well (Striegl and Michmerhuizen 1998), a dominant role of the terrestrial input in our lakes is suggested by the fact that 21% of the variance in pCO_2 could be explained by the chlorophyll-a:humic substances ratio.

As an alternative indicator of the relative importance of terrestrial input, we analyzed the ratio between organic carbon and nitrogen in the sediment, a high C:N ratio indicating a relatively strong terrestrial influence on the lake's metabolism. Indeed we found this indicator to explain a comparable part (30%) of the variance in pCO_2 levels as the inflow: evaporation ratio (Table 7.2). Adding the ratios chlorophyll-a:humic substances or C:N to regression models explaining pCO_2 levels from the hydrology indicator did not add to the explanatory power (Table 7.3), suggesting that the hydrology might affect the carbon balance largely through its relative effects on allochthonous carbon input and in-lake primary productivity.

For the lakes in our data set cooler lakes generally had a lower pCO_2 than warmer lakes (Table 7.3); the coldest lakes in our data set generally being carbon sinks and the warmer lakes in our data set being carbon sources (Figigure 7.2). To filter out the effects of idiosyncratic differences in terrestrial inputs and productivity among lakes we looked at relationships between pCO_2 and the main indicators of these processes along the temperature gradient (Table 7.3, Figure 7.3). This again suggested a significant influence of temperature on the pCO_2. At similar inflow:evaporation ratios, or similar chlorophyll-a: humic ratios the warmer lakes had a higher pCO_2 than the cooler lakes (Table 7.3, Figure 7.3). The logarithm of the pCO_2 in lakes with a water temperature of 30℃ was 0.3 to 0.4 units higher than in lakes with a water temperature of 20 ℃ (see temperature regression coefficients Table 7.3), suggesting an approximate 2-2.5 times increase in pCO_2 per 10 degrees Celcius in comparable lakes.

Although the pCO_2 is not a direct measure of the carbon flux in lakes, it is the most important factor influencing this flux (Sobek et al. 2005). Therefore, our findings indicate a substantial influence of climate on CO_2 efflux from lakes. The apparent effect of hydrology on the pCO_2 in lakes suggested by our results implies that future changes in evaporation (Roderick and Farquhar 2002) and precipitation regimes (IPCC 2007) may have a strong impact on lake carbon emissions. In addition there is a clear relationship between pCO_2 and temperature. Although correlations do not give insight in causal relationships, various mechanisms could contribute to such a temperature effect. In addition to the direct effect of temperature on the CO_2 flux –gasses dissolve better in colder than in warmer water – there is possibly an increase in net-heterotrophy with temperature. Rates of respiration tend to increase stronger than production with temperature (Rivkin and Legendre 2001, Biddanda and Cotner 2002, Lopez-Urrutia et al. 2006, Sand-Jensen et al. 2007). Importantly, the relatively strong increase of respiration with temperature (Acuña et al. 2008) implies that warm lakes might metabolize a substantially larger portion of the terrestrial organic matter influx than cooler ones (Biddanda and Cotner 2002, Jansson et al. 2008). Primary production, on the other hand, may be limited by other factors than temperature (e.g. nutrients or light) this may prevent primary production from ‘keeping up’ with respiration when temperature increases.

Final remarks

Most likely, rising global temperatures will promote increased atmospheric greenhouse gas concentrations (Cox and Jones 2008). In fact, estimates based on ancient climate variation suggest that this effect may be quite large (Scheffer et al. 2006a). Future climate prognosis often neglect the feedback effect of temperature on greenhouse gas fluxes as our knowledge of the processes involved is insufficient leading to large uncertainties (Jones et al. 2006). Our results suggest that warm inland waters emit more CO_2 than comparable cooler lakes. Carbon emission from cool lakes may therefore increase with climate warming. While this positive feedback mechanism has negative consequences from a climate change perspective, the sensitivity of the carbon balance of lakes to in-lake productivity and hydrology also suggests that a better understanding of the regulating mechanisms might give opportunities for managers to design climate friendlier management strategies of these hotspots of carbon channeling.

Acknowledgments

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Lagoa Nova – Espírito Santo – Brazil – September 2009

8 Synthesis: effects of climate and climate change on shallow lakes

"Observational records and climate projections provide abundant evidence that freshwater resources are vulnerable and have the potential to be strongly impacted by climate change, with wide-ranging consequences for human societies and ecosystems." (IPCC 2008)

8.1 Climate and shallow lakes

Climate may influence the functioning of shallow lake ecosystems in various ways (Figure 8.1). Temperature, wind, precipitation, and cloud cover can all affect important ecosystem processes. In addition to the direct effects of climate on lakes, climate change may also have an indirect effect through the changes it causes in the terrestrial realm. In some situations, changes in terrestrial ecosystems such as altered primary production and the subsequent export of organic material to the lake may well have a stronger effect on in-lake processes than direct climate effects on the lake itself (e.g. Jansson et al. 2008). Many climate change effects may exacerbate current anthropogenic stresses on freshwater systems, including increasing demands for water, increasing waste heat loadings and land use changes that alter the quantity and quality of runoff to inland waters (Mulholland et al. 1997).

The many facets of climate change and the multitude of processes that they influence render it difficult to unravel causal relationships between observed ecological changes and climate change. Linking ecosystem change to climatic variation becomes even more challenging as many non-climate related changes, such as eutrophication or re-oligotrophication (Kangur et al., 2002; Carvalho and Kirika, 2003; Jeppesen et al., 2005a), occur simultaneously. Nevertheless, the physical aspects of lakes in different parts of the world have clearly changed under influence of climate change and many ecological changes have been attributed to climate change as well.

The most conspicuous effects of climate change on lakes are likely those on water temperature, ice cover period, and water level. For all three there is ample evidence of changes over the last decades. Confirmation of water temperature rises comes from a wide range of lakes varying from relatively shallow lakes in the United Kingdom (Carvalho and Kirika 2003) and the Netherlands (Scheffer et al. 2001) to the deepest and most voluminous of the world's great lakes, Lake Baikal (Hampton et al. 2008). In most cases the annual mean water temperature has risen approximately 1^{o}C within the last decades. Even greater temperature increases are observed for winter and spring (Carvalho and Kirika 2003). Ice cover duration on lakes in the Northern Hemisphere has considerably shortened over the last century. During the last 15 years lakes gradually froze later (5.8 days per 100 years) and ice breakup dates advanced (6.5 days per 100 years) (Magnuson et al. 2000). Variation in lake water levels is another highly visible signal of climate change. Climate oscillations have been identified as the main drivers of water level variation in both small and large North American lakes (Williamson et al. 2009).

Various, complementary, methods exist to study the effects of these physical changes on the aquatic ecology and chemistry in lakes. They include long term monitoring, lake comparisons between climatically different regions, temperature regulated enclosure experiments, mesocosm experiments, laboratory experiments, and modeling. These approaches all have contributed to a broader understanding of the impact of climate change on aquatic ecosystems. (For reviews on various effects of climate forcing on lakes see Carpenter et al. 1992, Schindler 1997, Gerten and Adrian 2002, and Straile et al. 2003).

In this synthesis, I will focus on the interrelated temperature impacts on biochemical cycles, community composition, and shifts in alternative states in shallow lakes, using not only the results presented in this thesis, but also other key results from the ‘South American Lake Gradient Analysis’ (SALGA) project.

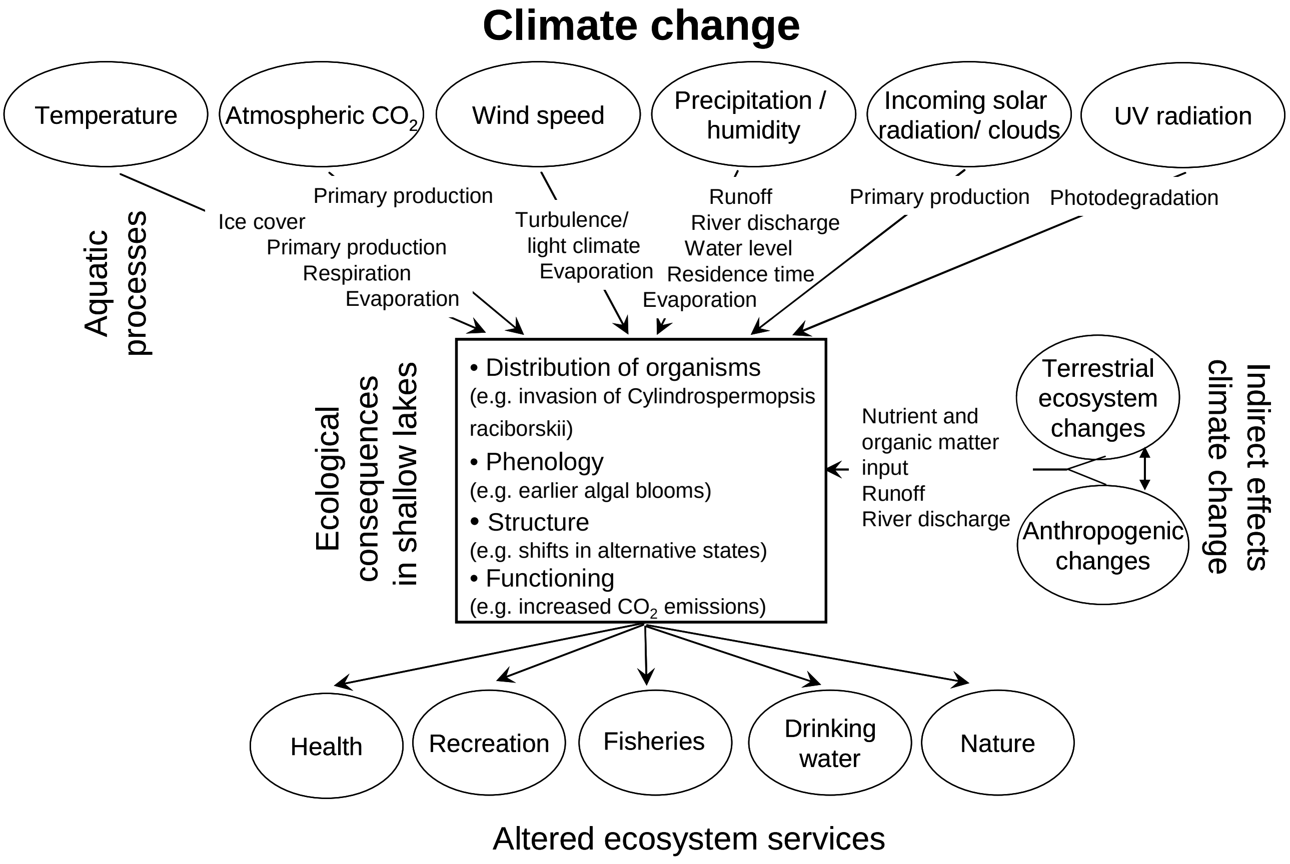

Figure 8.1 Climate change impacts on shallow lakes.

8.2 Biochemical cycles

Oxygen (O), carbon (C), nitrogen (N), and phosphorus (P) are key elements in numerous shallow lake processes. The biochemical cycles of all four are closely interrelated and can be strongly influenced by climate variables. Basic effects of warming are the decrease in solubility of gasses in water, the increase in metabolic rates, and alterations in mixing patterns.

Oxygen and phosphorus

Due to the decrease in gas solubility oxygen concentrations will tend to drop when temperatures rise (Figure 8.2). As respiration rates typically rise more than production rates with temperature (Rivkin and Legendre, 2001; Lopez-Urrutia et al., 2006; Sand-Jensen et al., 2007), oxygen concentrations will be reduced further. The increase in metabolic rates of bacteria, decomposers and bioturbators enhances phosphorus release from the sediment (Jensen et al. 1992). When the oxygen decrease leads to sediment anoxia sediment P-release will be boosted further (e.g. De Groot 1981, Jensen and Andersen 1992, Barko and James 1998). Therefore, a temperature rise will tend to lead to an increase in phosphorus concentrations in the water column (Figure 8.2).

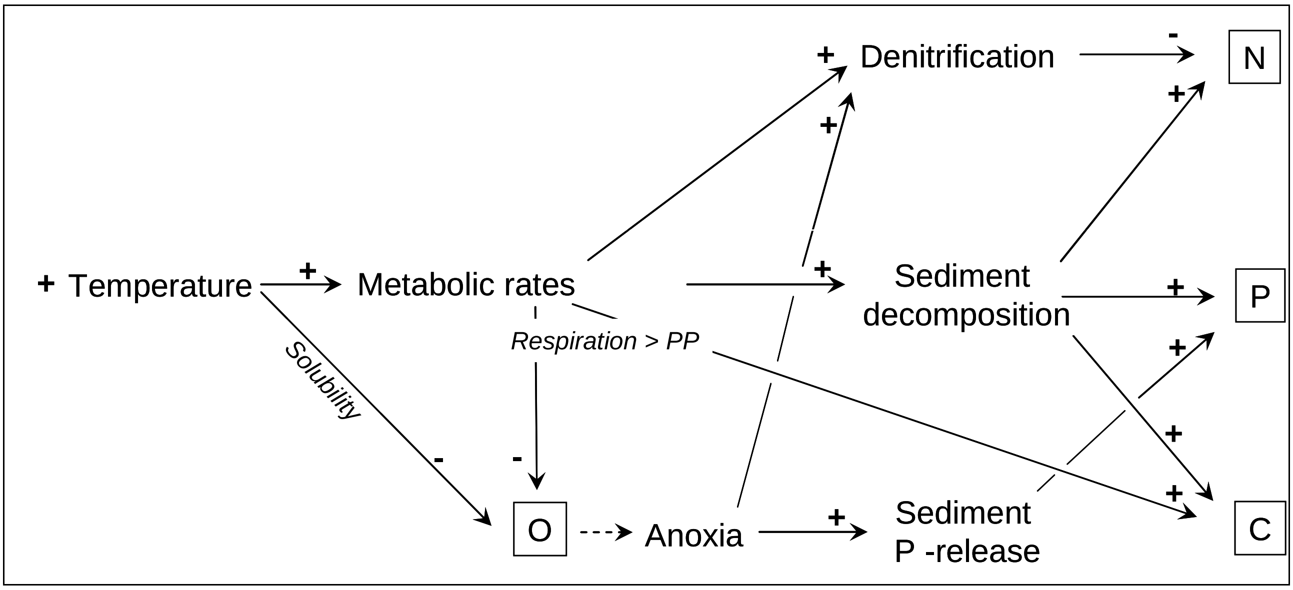

Figure 8.2 Simplified scheme of the temperature influence on oxygen (O), inorganic carbon (C), inorganic nitrogen (N), and inorganic phosphorus (P) in the water column. Lake specific circumstances may lead to different temperature effects than the ones shown here (see main text). PP refers to primary production.

Another way in which temperature may influence the elemental cycles in lakes is through its effect on stratification. While the consequences of warming on stratification and the subsequent effects on aquatic processes are relatively well known for deep lakes (Mulholland et al., 1997; Winder and Schindler, 2004; Berger et al., 2007) and the world oceans (Behrenfeld et al. 2006), less studies have been conducted into the effect of climate change on the stratification of polymictic shallow lakes (Gerten and Adrian, 2001). Nonetheless, especially warm and turbid shallow lakes, have a tendency to develop diurnal stratification. Warm lakes stratify more easily than cool lakes as they require a lower temperature difference between the upper and lower water layers to stratify; this is due to the temperature dependency of water density (Lewis 1987). Turbidity contributes to stratification of lakes as it causes the upper water layers to absorb relatively more heat, resulting in a larger temperature difference between the upper and lower water layers (Figure 8.3A).

Increased stratification in combination with high sediment oxygen demands in warm eutrophic waters may cause a steep oxygen gradient (Figure 8.3B). While diurnal stratification is common in shallow low latitude lakes, warm summers may lead to stratification at higher latitudes as well (Riley and Prepas, 1984; Kallio, 1994) and with climate warming the incidence of such stratification periods seems likely to increase. During recent heat waves, for instance, pronounced stratification events have already been observed in the shallow German lake Müggelsee (Wilhelm and Adrian 2008). These events were followed by strong phosphorus pulses into the euphotic zone and subsequent intense phytoplankton growth (Wilhelm and Adrian 2008). Such increased internal phosphorus cycling with warming has been identified as an important factor complicating re-oligotrophication of warm lakes (Genkai-Kato and Carpenter 2005, Bicudo et al. 2007, Jeppesen et al. 2009; chapter 2).

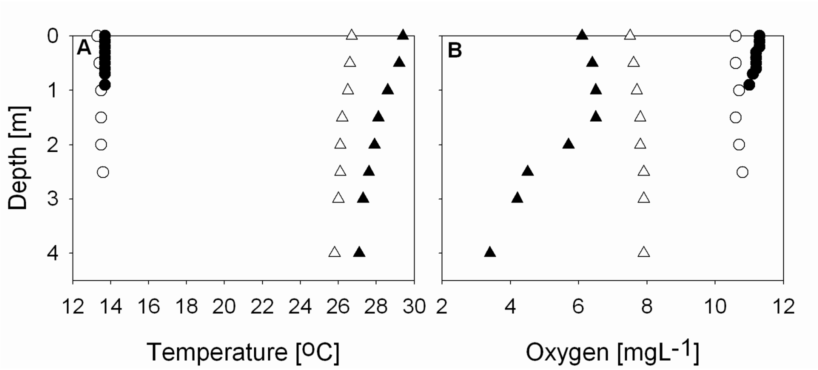

Figure 8.3 Temperature (A) and oxygen (B) profiles of 2 South Argentinean (circles) and 2 Brazilian (triangles) shallow lakes differing in trophic state (black lakes are eutrophic and white are oligotrophic lakes). Note the strong gradient in the oxygen profile in the eutrophic warm Brazilian lake in combination with only minor vertical temperature differences (Kosten and Lacerot, unpublished data).

Carbon

While a high mineralization rate in warm lakes will typically reduce oxygen concentrations, it may at the same time lead to raised dissolved inorganic carbon concentrations. Indeed, in our SALGA lakes we found strong indications for a climate effect on the carbon dynamics (chapter 7). Warm lakes tended to have higher partial CO_2 pressures (pCO_2) than cool lakes of comparable trophic state and concentrations of humic substances. The higher pCO_2 in warm lakes may be caused by a higher, temperature-dependent mineralization of organic carbon. This pattern suggests that cool lakes may start to emit more CO_2 when they warm up (chapter 7). As the released CO_2 contributes to global warming, this implies a positive feedback between climate warming and inland water CO_2 emissions. We found an approximately 3-5 times higher pCO_2 in the warm Brazilian lakes than in comparable cool Argentinean lakes. Clearly, these findings cannot directly be extrapolated to a global increase in C-emissions from inland waters due to climate change as not all lakes will undergo climatic changes, and if they do their increase in temperature will likely not be as large as the temperature difference between the cool and warm lakes in our study. Nonetheless, even a more modest increase in the percentage of carbon emissions from inland waters may have noticeable consequences. Inland waters are estimated to emit approximately 0.28-0.32 gigatons C per year (Duarte et al. 2008). To counterbalance a doubling of inland water emissions a 5% reduction in global fossil fuel burning is required, equivalent to 2.1 times the total emission of the Netherlands.

Nitrogen

Just as oxygen, phosphorus, and carbon cycles, the nitrogen cycle may be affected by climatic variation in many ways. There are reasons to expect that nitrogen concentrations in the water column will decrease with warming (Figure 8.2). One mechanism for this would be an increase in denitrification rates with temperature (Ventullo and Rowe, 1982; Christensen et al., 1990; Golterman, 2000; De Klein, 2008). Indeed, observed temperature effects on denitrification are remarkably strong. Denitrification rates, for instance, have been found to double between 8 °C and 25 °C in river sediments (Herrman et al. 2008) and increased with one order of magnitude from high to mid latitudes in alluvial soils (Pinay et al. 2007). Recent work suggests that this strong effect is due to a combination of increased metabolic rates of denitrifying bacteria and decreasing oxygen concentrations caused by warming (Veraart et al., in prep.). The latter has a large effect as oxygen availability strongly reduces denitrification. Thus, just as in the case of the phosphorus cycle, the full effect of temperature on the nitrogen cycle can only be understood by taking the reduction of oxygen levels in warmer waters into account (Kaushik et al. 1981). Additionally, denitrification is influenced by the concentration and quality of organic matter (Golterman 2004). Submerged macrophytes provide organic matter (Golterman 2004) and also ample oxic – anoxic interfaces as well as substrate for epiphytic microbial communities (Reddy et al. 1989, Weisner et al. 1994, Eriksson and Weisner 1997). As a result, submerged macrophytes might have a large effect on overall dentrification. Indeed, variations in organic matter quality and macrophyte cover might explain the lack of indications for an increase in denitrification towards the equator in the SALGA lakes (chapter 5).

Limiting nutrient

In view of the contrasting influence of temperature on the basic N and P processes (Figure 8.2), it has often been suggested that, while P is frequently limiting in temperate waters, N might be more often limiting in (sub)tropical freshwaters (Talling and Talling, 1965; Lewis, 1996; Downing et al., 1999). In a recent meta-analysis, however, Elser and others (2007) only found a very weak correlation between N-limitation and latitude, and Huszar and others (2006) did not find any indications of N being more frequently limiting than P in 192 (sub)tropical lakes. Our study of potential N and P limitation in the SALGA lakes suggests that temperature effects on aquatic processes are generally overruled by the relative influx of these elements to the lake (chapter 5). We found that local characteristics such as soil type and associated land use in the catchment, hydrology, and also the presence of abundant submerged macrophyte growth correlated stronger to N- and P-limitation than lake water temperature or the climate zone in which the lake was located.

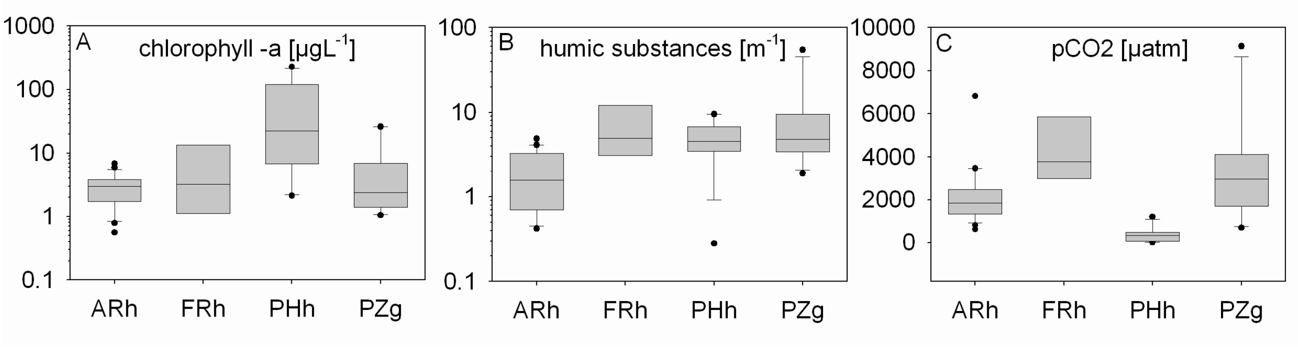

Figure 8.4 Boxplots of (A) the chlorophyll-a concentration, (B) the concentration of humic substances – approximated by the light absorption at 380 nm - and (C) the partial CO_2 pressure in the lakes situated at locations with different soil types. ARh =Haplic Arenosols , FRh = Haplic Ferralsols, PHh = Haplic Phaeozems, PZg = Gleyic Podzols. (For details on the soil types see Table 8.2 chapter 5) Boundaries of the boxplots indicate the 25th and 75th percentile. Whiskers above and below indicate the 90th and 10th percentiles. Dots are outlying points.

Soil type and associated land use not only correlated to the relative concentrations of N and P but also to the absolute concentrations (chapter 5), which, in turn, are closely correlated to chlorophyll levels (Figure 8.4A). The concentrations of terrestrial dissolved organic carbon – approximated by the light absorption at 380 nm - were strongly linked to watershed characteristics as well (Figure 8.4B and chapter 7), which concords with evidence from a wide range of landscapes on other continents (Magnuson et al., 1997; Schindler et al., 1997; Nõges et al., 2007). As the pCO_2 is largely influenced by primary production and ecosystem respiration (chapter 7), it is evident that watershed characteristics also influence the carbon balance in lakes (Figure 8.4C).

In conclusion, while temperature clearly influences in-lake elemental processes, the concentrations of N, P and C are also strongly influenced by watershed characteristics and consequent terrestrial loadings to the lake.

8.3 Community composition

Each species has a thermal range in which its fitness is highest and therefore temperature has a direct effect on interspecific competition and community composition (e.g. Magnuson et al. 1979). Temperature, furthermore, influences community composition through its effect on oxygen and nutrient availability and on stratification patterns (paragraph 8.2). The overall temperature effect on organisms is therefore complex. Much work on temperature effects is focused on changes in phenology (Carvalho and Kirika 2003, Winder and Schindler 2004, Domis et al. 2007b). Also, considerable attention has been given to gradient of increasing biodiversity from the poles to the equator (e.g. Allen et al. 2002).

Changes in phenology may be important for ecosystem functioning because the level of response to climate change may vary across functional groups and multiple trophic levels (Edwards and Richardson 2004). Ample evidence exists for the impact of climatic conditions on the seasonal succession of plankton (Straile 2000, Carvalho and Kirika 2003, Winder and Schindler 2004, Berger et al. 2007, Sommer and Lengfellner 2008), but also on seasonal dynamics of macrophytes (Barko and Smart 1981, Scheffer et al. 1992, Rooney and Kalff 2000) and fish (Hall and Rudstam, 1999; Nyberg et al., 2001; Ahas and Aasa, 2006). The potential alteration of trophic relationships with phenological shifts has important ramifications for food-web structures and may eventually lead to ecosystem-level changes (Edwards and Richardson 2004). What follows is an overview of the emergent patterns for different groups of organisms and biodiversity.

Plankton

Much research on the impact of climate on plankton phenology has been conducted in deep lakes where the on- and off-set of stratification plays an important role in the consecutive appearance of different species. Lake Constance and lake Washington are two lakes with well studied climate induced shifts in community composition and dynamics of zooplankton and phytoplankton (Straile and Adrian 2000, Winder and Schindler 2004). There are, however, also indications for climate impact on plankton phenology in shallower lakes. In Loch Leven in Scotland with a mean depth of 3.9 m, winter mean values of chlorophyll-a are positively related to water temperature and spring *Daphnia* densities are closely related to spring water temperatures (Carvalho and Kirika 2003). The high density of grazers in early spring may change the timing of the phytoplankton bloom. Both earlier spring blooms (Scheffer et al. 2001) and later blooms (Sommer and Lengfellner 2008) have been observed under warmer circumstances. These contrasting findings suggest that direct temperature effects on zoo- and phytoplankton growth are not the only factors influencing the timing of the bloom. Whether and when zooplankton can exert sufficient grazing pressure on phytoplankton to limit its biomass depends to a large extent on the fish community –in turn also influenced by temperature (Jeppesen et al. 2003b). In warmer lakes zooplankton species tend to be smaller (Gillooly and Dodson 2000), which has been attributed to intense predation by fish. The reduced size of zooplankton lowers grazing pressures on phytoplankton (Gyllström et al. 2005; and chapter 4). However, nutrient availability is still a major force in driving on plankton dynamics. A detailed mesocosm study of the temperature influence on plankton indicated that direct effects of warming on freshwater pico- and nanoplankton populations are far less important than the effects of nutrients (Christoffersen et al. 2006). Their results furthermore revealed that the combination of warming and nutrients sets off complex interactions.

The interactive effects of nutrients and temperature also appear to drive the occurrence of - potentially toxic - cyanobacterial blooms. Nutrient enrichment has led to a world-wide increase in cyanobacterial blooms (Paerl and Huisman 2008). It has long been recognized that higher temperatures may also favor cyanobacteria. A literature review of growth rates for different groups of phytoplankton back in 1974 (Canale and Vogel 1974) already suggested that at lower temperatures diatoms have the highest growth rate, whereas at higher temperatures cyanobacteria grow better, and green algae take an intermediate position. Later work, however, has shown that species specific and even strain specific responses are highly variable and that a large overlap in growth rates among the broad phytoplankton groups exists (Reynolds 1984, Teffera 2009). This makes it difficult to predict which group will win the competition when temperatures rise on the basis of laboratory experiments with isolated species. Also, in addition to the direct temperature effect on growth rate, temperature may influence cyanobacterial dynamics in other, more indirect ways. For instance, it is well know that warm conditions can favor dominance by some groups of cyanobacteria through enhanced stratification (Robarts and Zohary 1987). Climate change induced early summer stratification has been held responsible for earlier cyanobacterial growth in a large range of European lakes (Weyhenmeyer 2001, Blenckner et al. 2007). In addition, ice-free winters have been related to higher cyanobacterial abundances compared to years with prolonged ice cover in shallow temperate lakes (Mooij et al. 2005).

Despite the extensive work on cyanobacterial dynamics, the interactive effect of nutrients and temperature on cyanobacterial blooms has received relatively little attention. Our analysis of 143 lakes in South America and Europe (chapter 6) confirms the image that nutrient concentrations are the most important factor influencing total phytoplankton biomass, temperature having only a minor influence. However, our analysis also shows that temperature strongly affects the share of cyanobacteria in the phytoplankton community. At higher temperatures, the proportion of phytoplankton composed of cyanobacteria increases. Also, lakes with a higher phytoplankton biomass have a higher probability of being dominated by cyanobacteria. The latter might be related to the overall higher shade tolerance of cyanobacteria as compared to other groups of phytoplankton (Smith 1986, Jensen et al. 1994, Scheffer et al. 1997). Thus, while nutrients mostly determine algal biomass, the combination of this biomass and temperature may largely determine the probability of cyanobacterial dominance. These results imply that nutrient levels required to prevent cyanobacterial blooms may need to be substantially lower in a warmer climate (chapter 6).

Fish

Temperature influences various life history traits of fish. Overall, data from long-term surveys and experimental data suggest that higher temperatures tend to lead to an increase in the proportion of small-sized species and young age classes and to a decrease in size-at-age (Daufresne et al. 2009). In Danish lakes, for instance, warm early summers often lead to higher abundances of planktivorous young-of-the-year fish (Jeppesen et al. 2003b). In specific cases higher temperatures have also been related to increased fish growth (Lake Oneida, NY, USA; Hall and Rudstam 1999). Also, warming has been related to advanced migration and spawning of different North European fresh water fish species (Ahas and Aasa 2006). While in cold and temperate lakes spawning has been found to shift to an earlier moment (Ahas and Aasa 2006) in warmer lakes multiple spawning events per year may occur (Fernando 1994).

Ice cover may have strong effects on fish communities. Oxygen depletion induced by extended periods of ice cover can cause total or partial fish kills (Schindler and Comita 1972, Brönmark and Weisner 1992, Jackson et al. 2007). Climate warming shortens total ice cover periods, thereby potentially reducing fish kill events. Although this may be regarded as a positive effect of climate warming from a fisheries point of view (Fang and Stefan 2000), an increased survivorship of planktivores can lead to a stronger control of zooplankton. This, in turn, might decrease zooplankton control on phytoplankton, leading to ‘greener’ lakes (Jackson et al. 2007). An increased survival of benthivores may favor phytoplankton growth further through enhanced internal nutrient recycling (Hargeby et al. 2004, Hargeby et al. 2007).

While warming may prevent winter kills, fish mortality in summer may increase if higher temperatures lead to lower oxygen concentrations. In the north-central and north-eastern shallow lakes of the USA, for instance, large negative impacts on coolwater fish as a result of climate warming have been predicted (Stefan et al. 2001). Decaying fish during summer may lead to further oxygen depletion and potentially to botulism outbreaks (Dodd 1992). On the other hand, summer kills of planktivorous fish can have a cascading effect leading to a decrease of the phytoplankton biomass (Temte et al., 1988).

The difference in fish community composition between warm and cooler lakes has been reviewed by Jeppesen and others (2005b). In short, warmer lakes tend to have higher abundances of small fish, more omnivorous fish, and less large piscivores than cooler lakes. Many of the small zooplanktivorous fish in warm lakes inhabit the macrophyte beds (Meerhoff et al. 2007a). These differences in fish community composition may have far reaching consequences for the functioning of shallow lakes (Jeppesen et al. 2005b, Meerhoff et al. 2007a, Teixeira-de Mello et al. 2009). In particular the differences may lead to either more (Mooij et al. 2005) or less (chapter 4) bioturbation, enhancing phytoplankton growth and potentially limiting macrophyte growth, and to a higher predation pressure on zooplankton in warmer lakes (see ‘plankton’, paragraph 8.4, and chapter 4).

Macrophytes

Experimental work has shown that an increase in temperature may positively influence macrophyte growth, although it may compress the growth cycle (Barko and Smart 1981). Comparisons of macrophyte cover in Dutch lakes between years with warm and cold springs confirm the positive influence of temperature (Scheffer et al. 1992). Earlier season warming has furthermore been related to deeper macrophyte growth and higher biomasses (Rooney and Kalff 2000).

Macrophytes have a strong ability to adapt to changes in temperature (Hargeby et al. 2004), but how temperature will influence macrophyte growth on a longer time scale is less clear. Winter temperatures seem to play a crucial role (see also chapter 3); however, the result of warm winters is not unambiguous. Severe winters delayed macrophyte growth in spring and lowered the maximum biomass yield in a Dutch lake (Best and Visser 1987), whereas they triggered abundant macrophyte growth in a Swedish lake (Hargeby et al. 2004). Clearly, factors other than temperature play a role as well. The decrease in macrophyte growth in The Netherlands was contributed to low temperatures slowing down plant development, so that full maturity was attained only after the diurnal insolation had already decreased (Best and Visser, 1987). In Sweden, on the other hand, severe winters with long ice cover cause fish kills resulting in low biomasses of benthivorous and planktivorous fish, which may set off a complex set of community interactions - including low bioturbation and low internal nutrient recycling - favoring macrophyte growth (Hargeby et al. 2004).

As different macrophyte species respond differently to changes in temperature (Barko and Smart 1981, Scheffer et al. 1992, McKee et al. 2002) warming may also directly influence macrophyte community composition. More importantly from the point of view of ecosystem functioning, it may affect their competitive strength relative to phytoplankton (paragraph 8.4).

Biodiversity

Climate impacts on phenology and interactions inevitably affect biodiversity. The connection may, however, not be straightforward. Many latitudinal gradients of increasing biodiversity from the poles to the equator are observed. While this is one of the most prominent features of life on Earth, its causes remain poorly understood (Allen et al. 2002). Different explanations have been proposed based on such as gradients in historical perturbation, environmental stability, habitat heterogeneity, productivity and interspecific interactions (Gaston 2000). Many of these mechanisms are not mutually exclusive and merely offer different levels of explanation.

In the SALGA lakes we found, contrary to expectations, that the average species richness per lake did not increase towards the equator, but was highest in the subtropical region. We found this pattern for zooplankton (Lacerot et al., unpublished data), fish (Mazzeo et al., unpublished data) and macrophytes (Kosten et al. unpublished data). Average species richness of phytoplankton per lake increased from the tundra region to the subtropics and then stayed constant towards the tropics (Costa and others, unpublished data). Other studies have shown deviations from the pattern of increasing richness towards the equator as well (Roy et al., 1998; Lyons and Willig, 1999; Dolan et al., 2006; Fuhrman et al., 2008; Huston and Wolverton, 2009). Gaston (2000) hypothesized that for marine and terrestrial communities these latitudinal asymmetries might be explained by asymmetries in contemporary climate, historical climatic events, and latitudinal complexities of the geometry and area of land and ocean. In lakes local factors have been shown to affect biodiversity of various groups profoundly. For instance, plankton biodiversity may vary with trophic state (Leibold 1999, Jeppesen et al. 2000, Declerck et al. 2007, Louette et al. 2008, Vanormelingen et al. 2008), presence of macrophytes (Declerck et al., 2007; Kruk et al., 2009), turbidity (Costa et al., unpublished data), lake size (Søndergaard et al. 2005), and isolation (Scheffer et al. 2006b); the number of fish species depends upon trophic state (Jeppesen et al. 2000), macrophyte cover (submerged and/or floating) (Suarez et al. 2001, Agostinho et al. 2007), lake size (Søndergaard et al. 2005, Kruk et al. 2009), piscivore abundance and depth (Suarez et al. 2001), and the diversity of macrophytes has been shown to vary with trophic state (Jeppesen et al. 2000, James et al. 2005), alkalinity (Søndergaard et al. 2005), lake area, depth, and pH (Jeffries 1998). Systematic variation in such lake characteristics along the latitudinal gradient may cause deviations from the typical increase of biodiversity towards the tropics, such as the subtropical peak in species richness that we found in the SALGA lakes.

8.4 Shifts in alternative states

The multifaceted effects of climate on biochemical cycles and species dynamics affect species interactions and may therefore affect the outcome of the competition between different groups of primary producers. Once a specific group of primary producers, submerged macrophytes, floating macrophytes, algae, or cyanobacteria, dominates a shallow lake positive feedback mechanisms often reinforce the dominant position of this primary producer (reviewed by Scheffer and van Nes 2007). A long list of examples exists of lakes that show switches between such alternative states. Most come from temperate regions in Europe (e.g. Timms and Moss 1984, Jeppesen et al. 1990, Moss et al. 1990, Scheffer et al. 1993, Hargeby et al. 1994, Van Donk 1998, Jeppesen et al. 1999, Hargeby et al. 2005, Rip et al. 2006, Sayer et al. 2006), but there are also examples from colder regions in Canada and the USA (Engel and Nichols, 1994; Jackson, 2003; Rosenthal et al., 2006). Cases from other regions are scarce, but studies suggesting alternative stable states exist for lakes in high Andean Bolivia (Cadima 1997), temperate New Zealand (Dugdale et al. 2006), mediterranean Turkey (Beklioglu 2006), subtropical Florida (Schelske et al. 2005), China (Yang et al. 2006), and tropical Brazil (Jeppesen et al. 2005a).

The influence of warming on the competition between different primary producers is difficult to predict. Our analyses (chapter 2) and the outcome of various experiments indicate that critical nutrient levels for the switch between submerged macrophytes and phytoplankton dominance are not very clear, especially in warm lakes. Studies comparing experimental results in lakes in different climate regions are largely inconclusive. For example, in shallow Mediterranean lakes macrophytes in enclosures with different nutrient concentrations gave way to phytoplankton dominance at lower nutrient concentrations than in similar studies conducted in North European lakes (Moss et al. 2004, Romo et al. 2004). In contrast, it has been argued that a longer plant growing season, higher light intensities and temperatures, and strong water level fluctuations lead to higher critical nutrient concentrations at warmer latitudes (Bécares et al. 2008). While temperature controlled mesocosm experiments suggest that the effects of increased nutrient loads on phytoplankton and submerged macrophyte abundance are largely independent of temperature (McKee et al. 2002, Moss et al. 2003a, Feuchtmayr et al. 2009).

Winter temperatures may play an important role in the competition between different primary producers. It has been hypothesized that absence of a cold winter generally favors phytoplankton dominance (Sand-Jensen and Borum 1991, Hargeby et al. 2004, Hargeby et al. 2007, Jackson et al. 2007). Long ice covers may cause fish kills reducing bioturbation by benthivorous fish (Hargeby et al., 2004; 2007) and release predation pressure on zooplankton by planktivorous fish (Hargeby et al. 2007, Jackson et al. 2007). These fish kills may therefore improve growth conditions for macrophytes (see also paragraph 8.3). On the other hand, at low temperatures submerged macrophyte re-growth usually occurs much later and at much lower rates than the phytoplankton populations, and the success of phytoplankton versus macrophyte development during spring may be the key to the shifting dominance of submerged macrophytes to phytoplankton between years (Sand-Jensen and Borum 1991). In a cross-continental study, we found evidence for the idea that ice cover indeed plays an important role in the competition between primary producers: the maximum TP concentration allowing substantial submerged macrophyte coverage was higher in cold regions with more frost days than in colder regions. Although winter and spring dynamics may play an underestimated role in determining which primary producers will dominate, the effect of temperature is obviously complex (Figure 8.5). Therefore, the net effect of warming on the competition between the different groups of primary producers may well be less predictable than generally thought (chapters 2, 3, and 4). Nonetheless, there is some evidence that higher temperatures promote the chances for dominance by floating plants (Scheffer et al. 2003, Scheffer and van Nes 2007, Feuchtmayr et al. 2009; Netten et al. submitted) or cyanobacteria (see chapter 6).

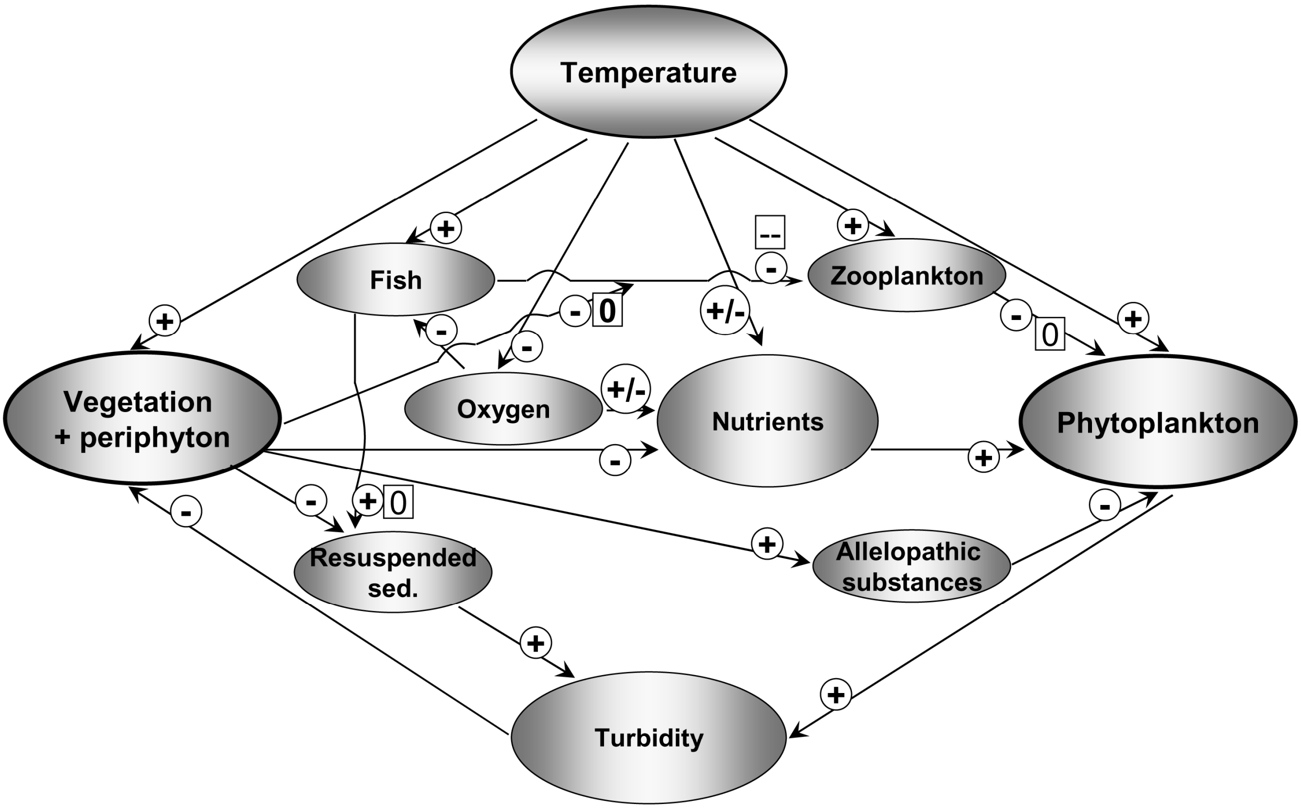

Figure 8.5 Effects of temperature on feedback mechanisms between aquatic vegetation, phytoplankton and other ecosystem variables. The feedback effects (-- very negative, - negative, 0 neutral, + positive) are indicated in circles. Effects that are expected to be fundamentally different in warmer climates are indicated in squares. The complexity of the web of direct and indirect temperature effects implies that the net effect on the competition between different groups of primary producers may be hard to predict.

In conclusion, the effect of increasing temperature on aquatic ecosystems is highly complex and may interact with effects of other aspects of climatic change. Nonetheless, our findings, in combination with the outcome of an increasing number of studies (Moss et al. 2004, Jeppesen et al. 2005b, Mooij et al. 2005, Jeppesen et al. 2007a, Jeppesen et al. 2007b), suggest that the effect of climate warming in many aspects mimics anthropogenic eutrophication, likely lower nutrient levels are required to prevent cyanobacterial blooms and to preserve or establish high coverages of submerged macrophytes.

Lagoa Catole – Rio Grande do Norte – Brazil – November 2005

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Aguas Blancas – Tierra del Fuego – Argentina – January 2006

Summary

There is concern that a warmer climate may boost carbon emissions from lakes and promote the chance that they lose their vegetation and become dominated by phytoplankton or cyanobacteria. However, these hypotheses have been difficult to evaluate due to the scarcity of relevant field data. To explore potential climate effects we sampled 83 lakes along a latitudinal gradient of more than 6000 km ranging from Rio Grande do Norte in Brazil to the South of Argentina (5-55 ^{o}S). The lakes were selected so as to be as similar as possible in morphology and altitude while varying as much as possible in trophic state within regions. All lakes were sampled once during summer (subtropical, temperate and tundra lakes) or during the dry season (tropical lakes) between November 2004 and March 2006 by the same team.

In the first chapters I address the question how climate might affect the chances for shallow lakes to be dominated by submerged plants. It has been shown that temperate lakes tend to have two contrasting states over a range of conditions: a clear state dominated by aquatic vegetation or a turbid state. The turbid state is typically dominated by phytoplankton and often characterized by poorer water quality than the clear state. The backbone of the theory explaining this pattern is a supposed positive feedback of submerged vegetation on water clarity: vegetation enhances water clarity and clearer water, in turn, promotes vegetation growth. The theory furthermore asserts that submerged vegetation coverage diminishes when nutrient concentrations increase until a critical point at which the entire vegetation disappears due to light limitation. Both aspects of the alternative state theory have been well studied in temperate shallow lakes, but the validity of the theory for warmer lakes has been questioned. In chapter 2 a graphical model is used to show how climate effects on different mechanisms assumed in the theory may affect the general predictions. An analysis of our data presented in chapter 4 reveals that submerged vegetation has similar overall effects on water clarity across our climatic gradient. Nonetheless, the results hint at differences in the underlying mechanisms between climate zones. For example, the data suggest that the positive effect of vegetation on top-down control of phytoplankton by zooplankton is lost at high densities of fish that are often found in warmer regions. The main factor explaining differences in the water clearing effect of vegetation among lakes in our data-set was the concentration of humic substances. In lakes with a high concentration of humic substances vegetation did not enhance the water clarity.

Combining our data with results from North America and Europe (chapter 3) we found that the critical nutrient level to maintain a substantial submerged vegetation coverage is much less predictable in warm lakes than in cold lakes. This might be related to a poor correlation between phytoplankton biomass and nutrients in warm areas (chapter 2). Despite the large unexplained variability in plant coverage in warm lakes our data suggest that the phosphorus concentration allowing substantial submerged macrophyte coverage increases steeply with the average number of frost days in a region. This implies that a reduction of phosphorus levels may be needed in regions where climate warming reduces the number of frost days if we aim at maintaining an equal submerged vegetation coverage.

In chapter 5 I show that nitrogen limitation may play an important role in shallow lakes as well. Our results indicate that submerged vegetation may tolerate higher phosphorus concentrations when nitrogen concentrations are low. Contrary to our expectations, evidence for nitrogen limitation was not systematically related to climate in our lakes. Also, unlike studies in other regions I did not find a relationship between the occurrence of cyanobacteria and the ratio between total nitrogen and phosphorus.

To explore the potential effect of climate on cyanobacterial dominance we combined our data with a data-set obtained in a large European study (chapter 6). The results indicate that warmer lakes have substantially higher probabilities of being dominated by cyanobacteria than colder lakes of the same nutrient levels.

In chapter 7 I address the question how climate affects the carbon metabolism of lakes. Whether an aquatic ecosystem acts as a net source or sink of atmospheric carbon depends on the balance between organic carbon burial and carbon dioxide (CO_2) evasion, both of which are influenced by gross primary production, carbon fluxes from terrestrial ecosystems and ecosystem respiration. These processes and many other aspects of lake functioning and community composition are affected by temperature, it is therefore unclear a priori how a warm lake’s metabolism may differ from that of a colder lake. Our measurements revealed that the warm lakes were generally more supersaturated with CO_2 than the cool ones, while there was no underlying latitudinal gradient in dissolved organic carbon (DOC) or in the trophic state of the lakes. At similar algal biomass:DOC ratios, the cool (South Argentinean) lakes were undersaturated, whereas the warm (Mid and North Brazilian) lakes were oversaturated. Correcting for the phenomenon that gasses dissolve better in colder water, our results indicate that going from cool to warm lakes the CO_2 releasing processes accelerated more than CO_2 absorbing processes did, making warm lakes a more intense source of atmospheric CO_2 than comparable cooler ones. Since aquatic ecosystems are important components of the global carbon circle these differences can have notable implications for regional carbon balances in a warming world: cool lakes may start to emit more CO_2 when they warm up.

Samenvatting

Met het warmer worden van de aarde zullen meren mogelijk meer koolstof uit gaan stoten. Bovendien zou de kans dat ze hun vegetatie kwijtraken en gedomineerd zullen worden door algen of cyanobacteriën toe kunnen nemen. Hoewel dit zorgelijke veronderstellingen zijn, waren deze hypothesen tot nu toe lastig te evalueren door een tekort aan relevante gegevens uit het veld. Om potentiële klimaatseffecten in kaart te brengen hebben we 83 meren bemonsterd langs een 6000 kilometer lange latitudinale gradiënt tussen Rio Grande do Norte in Brazilië en het zuiden van Argentinië (5-55^{o} S). Er zijn meren geselecteerd die qua morfologie en hoogteligging zoveel mogelijk op elkaar leken en binnen elke klimaatregio qua trofiegraad zoveel mogelijk van elkaar verschilden. Alle meren zijn eenmalig bemonsterd gedurende de zomer (subtropische, gematigde en tundra meren) of gedurende het droge seizoen (tropische meren) tussen november 2004 en maart 2006. De bemonstering is uitgevoerd door één team.

In de eerste hoofdstukken bespreek ik hoe de kans dat een meer gedomineerd wordt door ondergedoken waterplanten wordt beïnvloed door het klimaat. Eerdere studies wijzen erop dat meren zich onder bepaalde omstandigheden in alternatieve toestanden kunnen bevinden: een heldere toestand waarbij aquatische vegetatie domineert en een troebele toestand. In de troebele toestand domineert phytoplankton vaak. Bovendien is, vergeleken met de heldere toestand, de waterkwaliteit vaak slecht. De basis van de theorie die dit patroon verklaart, is de veronderstelde positieve terugkoppeling tussen ondergedoken waterplanten en de helderheid van het water: vegetatie maakt het water helder en helder water is goed voor de plantengroei. De theorie stelt verder dat de vegetatiebedekking minder wordt wanneer de nutriëntenconcentraties toenemen. Dit gebeurt tot een kritisch punt waarop de gehele vegetatie verdwijnt door lichtlimitatie. Beide aspecten van de theorie over alternatieve toestanden zijn goed bestudeerd in ondiepe meren in gebieden met een gematigd klimaat. Er zijn echter twijfels over de geldigheid van de theorie in warmere meren. In hoofdstuk 2 gebruik ik een grafisch model om te laten zien hoe klimaatverandering de mechanismen die aan de theorie ten grondslag liggen zou kunnen beïnvloeden. De analyse van onze data in hoofdstuk 4 geeft aan dat ondergedoken vegetatie een vergelijkbaar effect heeft op de helderheid van het water in verschillende klimaatzones. De resultaten duiden er echter op dat de onderliggende mechanismen mogelijk wel verschillen tussen de verschillende klimaatzones. Zo is het positieve effect van vegetatie op de ‘top-down’ regulering van fytoplankton door zoöplankton vaak nihil in meren met hoge dichtheden aan vis. Deze hoge dichtheden komen juist vaak voor in warme meren. Het verschil in water-verhelderendeffect van vegetatie tussen de meren wordt in onze dataset het best verklaard door de concentratie van humeuse stoffen. In humusrijke meren hebben de meren geen positief effect op de helderheid van het water.

De analyse van onze data samen met Noord-Amerikaanse en Europese data (hoofdstuk 3) geeft aan dat het in warme meren moeilijker te voorspellen is bij welke kritische nutriëntenconcentratie ondergedoken waterplanten verdwijnen dan in koude. Mogelijk wordt dit veroorzaakt door de zwakke correlatie tussen fytoplankton biomassa en nutriënten in warme gebieden (hoofdstuk 2). Ondanks de grote fractie onverklaarde variabiliteit in vegetatiebedekking in de warme meren lijkt onze data-analyse erop te wijzen dat de fosfaatconcentratie waarbij nog een substantiële vegetatiebedekking optreedt, toeneemt met het aantal vorstdagen in een regio. Dit geeft aan dat, wanneer we de ondergedoken vegetatie willen behouden, de fosfaatconcentraties mogelijk verlaagd zullen moeten worden in regio’s waar klimaatverandering zal leiden tot minder vorstdagen.

In hoofdstuk 5 laat ik zien dat stikstoflimitatie ook een belangrijke rol kan spelen in ondiepe meren. Onze resultaten laten zien dat ondergedoken waterplanten mogelijk hogere fosforconcentraties tolereren als de stikstofconcentratie laag is. Tegen de verwachting in vind ik in onze meren geen aanwijzingen voor een samenhang tussen stikstoflimitatie en klimaat. Bovendien vind ik geen relatie tussen het voorkomen van cyanobacteriën en de verhouding tussen totaal stikstof en fosfor, wat in andere studies wel is gevonden.

Om de potentiële effecten van het klimaat op de dominantie van cyanobacteriën te onderzoeken, hebben we onze dataset gecombineerd met een Europese dataset (hoofdstuk 6). Uit de analyses blijkt dat warmere meren een grotere kans hebben om gedomineerd te worden door cyanobacteriën dan koude meren met hetzelfde nutriënten gehalte.

In hoofdstuk 7 ga ik in op de vraag hoe klimaat het koolstofmetabolisme in meren kan beïnvloeden. Of een aquatisch ecosysteem netto koolstof opneemt of uitstoot hangt af van de balans tussen de opslag van koolstof in het sediment en de ‘uitademing’ van koolstofdioxide. Beide processen worden beïnvloed door de bruto primaire productie in het meer, koolstofstromen vanuit terrestrische ecosystemen en de respiratie in het meer. Omdat deze en andere processen en ook de samenstelling van de levensgemeenschappen in meren worden beïnvloed door temperatuur is het a priori niet duidelijk hoe het metabolisme van een warm meer verschilt van dat van een koud meer. Onze metingen laten zien dat warmere meren over het algemeen een hogere oververzadiging met CO_2 hadden dan koudere meren, dit terwijl er geen sprake was van een onderliggende gradiënt in opgelost organisch koolstof (DOC) of in trofiegraad van de meren. Bij vergelijkbare verhoudingen tussen algen-biomassa en DOC waren de koudere (Zuid-Argentijnse) meren onderverzadigd met CO_2 en de warmere (Midden en Noord-Braziliaanse) meren oververzadigd. Onze resultaten suggereren dat wanneer het warmer wordt, CO_2 genererende processen sterker toenemen dan processen die CO_2 omzetten. Hierdoor kunnen warmere meren intensere koolstofbronnen zijn dan vergelijkbare koudere meren. Omdat aquatische ecosystemen een belangrijk onderdeel uitmaken van de wereld-koolstofcyclus kunnen deze verschillen merkbare implicaties hebben voor regionale koolstofbalansen in een warmer wordende wereld: koude meren zullen mogelijk meer koolstof uit gaan stoten als ze opwarmen.

Laguna Clotilde – Uruguay – December 2004

It takes a village

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Sarian Kosten, Gissell Lacerot

A couple of years ago, we set out along with a team of ecologists to sample 100 lakes along the east coast of South America. We discovered that the task was easier said than done. Our four-wheel-drive pickup was stolen in Brazil and had to be restored by airplane. In the far south of Argentina, our boat sank with all of our equipment —and us— on it. But perhaps the most memorable adventure was the quest for our missing samples and the villagers in a small town in Argentina who came to our aid.

credit: Peter Hoey www.PeterHoey.com

We had been in the field, traveling from lake to lake, for almost 2 months straight. Our precious samples were stored in coolers in the back of our pickup truck. At night, we would park the pickup in guarded car-parks. One early morning, we took the truck to lake El Paraiso. After a long day of fieldwork, we went to store the “catch of the day” in the cooler, only to discover that the cooler was no longer there. We drove back the 30 km to the previous day’s worksite in the hope that we had left the cooler there. Upon realizing that we did not have the key to the gate of the lake, we decided to climb the fence and walk the last 2 km through a mosquito-infested swamp. But it was for naught; the cooler was not there.

Back in the village, at the suggestion of a local, we sent a plea for help through the local radio station. Even before we arrived back at our hotel, three people had called with information. Apparently, someone had stolen the cooler during the night. Disappointed with its contents, the thief had thrown the cooler with samples in a garden adjacent to the hotel. The owner of the garden found them and, thinking they were veterinary samples, informed the police. The police, in turn, contacted all vets in the area. The neighbor, the police, and a veterinarian all contacted the radio show as soon as they heard our story. What a relief: We recovered almost all of our samples and became local celebrities in the process!

I see trees of green, red roses too
I see them bloom for me and you
And I think to myself what a wonderful world.

I see skies of blue and clouds of white
The bright blessed day, the dark sacred night
And I think to myself what a wonderful world.

The colors of the rainbow so pretty in the sky
Are also on the faces of people going by
I see friends shaking hands saying how do you do
They're really saying I love you.

I hear babies crying, I watch them grow
They'll learn much more than I'll never know
And I think to myself what a wonderful world
Yes I think to myself what a wonderful world.

Louis Armstrong - What A Wonderful World

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My PhD adventure started somewhere in 2003. I was working and living in Ecuador when Marten Scheffer asked if I was interested in a PhD thesis with field work in Brazil, Uruguay, and Argentina. An offer I could not resist. Thanks Marten, for your scientific supervision, the 'parties for no particular reason' in your garden, and your friendship.

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Curriculum vitae

Sarian Kosten (1975) holds a BSc in Aquatic Ecotechnology from the Hogeschool Zeeland. In 1999 she graduated *cum laude* for her MSc at the Wageningen University. She specialized in Water Quality Management and Communication. From 1999 she worked successively for the IVN (Association of Nature and Environmental Education in Utrecht, The Netherlands), the Communication and Innovation Group and the Aquatic Ecology and Water Quality Management Group at the Wageningen University (The Netherlands) focusing on various aspects of water management.

In the year 2000 she obtained a VSB-scholarship to do research into the effects of pesticides on macroinvertebrates in streams in Ecuador. This research was conducted in coordination with the International Potato Centre (Quito, Ecuador). Afterwards she shortly worked as a volunteer for The Nature Conservancy in Ecuador. There she focused on the effects of water extraction in páramo areas (high altitude peat ecosystems) on water quality and wetland vegetation. Upon her return to The Netherlands she worked on anti-desiccation projects and eco-hydraulics at the Water Board De Stichtse Rijnlanden (Houten, The Netherlands).

In 2003 she obtained a Junior Professional Officer position at the South American office of the IUCN-World Conservation Union (Quito, Ecuador) through the Directorate General for International Cooperation (DGIS) of the Netherlands Ministry of Foreign Affairs. Here, she coordinated wetland conservation projects. She anticipated to stay in Ecuador for the years to come, but the possibility of participating in the South American Lake Gradient Analysis (SALGA) project and to sample 100 lakes between the equator and Tierra del Fuego lured her into her PhD project in April 2004. The project focused on the influence of climate on lake metabolism and the competition between submerged macrophytes and phytoplankton. In 2006 she won the Storm-van der Chijs stipendium for most promising female promovendi of the Wageningen University.

Currently, she works as a researcher in the Aquatic Ecology and Water Quality Management group in Wageningen. She is involved in the Dutch ‘Knowledge for Climate Programme’. Her work focuses on the effects of climate change on the carbon balance of inland waters, aquatic ecology, and water quality.

List of publications

Peer reviewed journals

Sarian Kosten, Amy Kamarainen, Erik Jeppesen, Egbert H. van Nes, Edwin T.H.M. Peeters, Néstor Mazzeo, Laura Sass, Jennifer Hauxwell, Nicole Hansel-Welch, Torben L. Lauridsen, Martin Søndergaard, Roger W. Bachmann, Gissell Lacerot, Marten Scheffer, 2009, *Climate-related differences in the dominance of submerged macrophytes in shallow lakes*, Global Change Biology 15:2503–2517.

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