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PhD thesis, 2006

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Mariana Meerhoff

Department of Freshwater Ecology
National Environmental Research Institute

Department of Biological Sciences
University of Aarhus

Faculty of Science
University of Aarhus

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Abstract:	The effects of climate warming on the role of free-floating and submerged plants on trophic dynamics were studied by means of controlled laboratory and outdoor experiments and comparative field studies in a series of subtropical and temperate shallow lakes. In the subtropics, the typical warm-climate large free-floating plants, and submerged plants as well, do not seem to promote cascading effects generating increased water transparency, which contrasts with observations made in temperate shallow lakes. Our results suggest that a warming-related higher impact of fish may strongly affect the resilience of shallow lakes.
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A veces uno es
manantial entre rocas
Otras veces un árbol
con las últimas hojas
Pero hoy me siento apenas
como laguna insomne
con un embarcadero
ya sin embarcaciones

Una laguna verde,
inmóvil y paciente
Conforme con sus algas,
sus musgos y sus peces
Serena en mi confianza,
confiada en que una tarde
Te acerques y te mires,
te mires al mirarme

Rumbo 1980's

Sometimes one feels
like spring water within rocks
Sometimes like a tree
with the last leaves
But today I feel just
like a sleepless lake
with a wharf
and no more boats

A green lake,
immobile, and patient
Satisfied with my algae,
my mosses and fishes
Serene in my confidence,
confident that one day
You will approach and look at you,
look at you when you look at me

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I) **Meerhoff M.**, Fosalba C., Bruzzone C., Mazzeo N., Noordoven W. & E. Jeppesen. 2006.
"An experimental study of habitat choice by *Daphnia*: plants signal danger more than refuge in subtropical lakes".
Freshwater Biology 51: 1320-1330

II) **Meerhoff M**. & N. Mazzeo. 2004.
"Importancia de las plantas flotantes libres de gran porte en la conservación y rehabilitación de lagos someros de Sudamérica".
Ecosistemas. http://www.aeet.org/ecosistemas/042/revision1.htm

III) **Meerhoff M.**, Teixeira de Mello F., Clemente J. M., Iglesias C., Jensen E., Lauridsen, T.L. & E. Jeppesen.
"Effects of climate and habitat complexity on community structure and predator avoidance behaviour of zooplankton in the shallow lake littoral".
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IV) **Meerhoff M.**, Clemente J.M., Teixeira de Mello F., Iglesias C., Pedersen A.R. & E. Jeppesen.
"Can warm climate-related structure of littoral predator assemblies weaken clear water state in shallow lakes?"
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V) Liboriussen L., Landkildehus F., **Meerhoff M.**, Bramm M.E., Søndergaard Mo., Christoffersen K., Richardson K., Søndergaard Ma., Lauridsen T.L. & E. Jeppesen. 2005.
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VI) Iglesias C., Goyenola G., Mazzeo N., **Meerhoff M.**, Rodó E. & E. Jeppesen.
"Horizontal dynamics of zooplankton in subtropical Lake Blanca (Uruguay) hosting multiple zooplankton predators and aquatic plant refuges".
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VII) Jeppesen E., Søndergaard Ma., Mazzeo N., **Meerhoff M.**, Branco C., Huszar V. & F. Scasso. 2005.
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In: Reddy M.V. (ed.) *Restoration and Management of Tropical Eutrophic Lakes*.
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IX) Jeppesen E., Søndergaard Ma., **Meerhoff M.**, Lauridsen T.L. & J.P. Jensen.
"Shallow lake restoration by nutrient loading reduction- some recent findings and challenges ahead".
In press *Hydrobiologia, Special Volume Shallow Lakes*

Articles in preparation

A. **Meerhoff M.**, Bramm M.E., Pedersen A.R., Liboriussen L., Landkildehus F., Lauridsen T.L., Søndergaard Ma. & E. Jeppesen.
"Response of zooplankton communities to 1-year experimental warming in temperate freshwater ponds".

B. Clemente J. M., **Meerhoff M.** & E. Jeppesen.
"Contrasting fish-macroivertebrates-periphyton interactions in temperate and subtropical shallow lakes".

C. Teixeira de Mello F., **Meerhoff M.** & E. Jeppesen.
"Substantial differences in littoral fish community structure and dynamics in shallow lakes under contrasting climates".

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Summary

Shallow lakes, the most abundant lake type in the global landscape (Moss 1998, Wetzel 2001), are particularly sensitive to climatic changes (Scheffer 1998). Global warming expectantly impacts the length and timing of seasonal processes, the hydrological and thermal regimes (Beklioglu et al. in press), as well as the biological interactions in lakes (Schindler 1997). The key role of submerged plants for biodiversity and water transparency in shallow temperate lakes (Canfield et al. 1984, Declerck et al. 2005) may also be affected by climate warming. Thus, an earlier start of growing seasons, resulting in greater biomass and distribution of submerged plants (Asaeda et al. 2001, Scheffer et al. 2001) might be expected with warming. In contrast, eutrophication effects may worsen with this climatic change (Mulholland et al. 1997, Søndergaard et al. 2001, Jeppesen et al. 2003b), as the higher temperatures and lower water level will likely enhance the sediment resuspension and release of nutrients, especially of phosphorus. A broader shift in the geographical range of tropical and subtropical free-floating plants represents another likely consequence of increasing winter minimum air temperatures.

The general objective of this thesis was to study the effects of warming on the role of aquatic plants and trophic dynamics and, consequently, on the functioning of shallow lakes, with emphasis on submerged and free-floating plants. The approach followed consisted of the combination of both controlled experiments at different scales (laboratory and outdoor field experiments) and comparative field studies under different climates: subtropical (Uruguay: 30°-35° S) *versus* temperate (Denmark: 55°-57° N). Following a space-for-time substitution approach, the knowledge about trophic dynamics and lake functioning gained from the subtropics may be useful when evaluating the effects of climate warming on temperate lakes. The project aimed to address the following main questions:

- Do free-floating plants affect trophic interactions in shallow lakes in a manner similar to submerged plants?
- Can warming weaken the mechanisms associated with the submerged plant-dominated clear water state, in particular the refuge effect for zooplankton?
- How does the trophic structure in the littoral zone function under warmer climates?
- How does the architecture of aquatic plants impact littoral trophic interactions?
- Are the restoration strategies developed for temperate eutrophic shallow lakes applicable to warm(ing) lakes?

Our results indicate that large free-floating plants do not act as refuge for large-bodied cladocerans, and therefore do not promote cascading effects leading to increased water transparency in the subtropics (papers I & III). An expansion of the free-floating plants, especially considering their capacity to constitute an alternative stable state to submerged plants (Meerhoff 2002, Scheffer et al. 2003), would entail negative impacts on trophic dynamics, apart from the already known negative effects on general diversity and water qualtiy (paper II).

In experimentally warmed ponds (paper V) we observed some minor effects of increased temperatures on the macrophyte and zooplankton communities. Overall these communities proved quite resilient to warming, as have been reported in similar studies (McKee et al. 2003). However, these results cannot be applied directly to real lakes, as the fish structure and density were kept constant due to the size limitation of the experimental units. Therefore, warming-induced effects on fish structure and the consequent cascading effects occurring in nature could not be traced. The results from a 1-year field study in a subtropical lake (paper VI) and comparative studies conducted in subtropical and temperate lakes (paper III) contrast the observations in temperate systems, as these show strong refuge-mediated cascading effects of submerged plants leading to increased water clarity (Jeppesen et al. 2002b). Submerged plants had only a very weak refuge effect for cladocerans in the subtropics. In the subtropical lakes diel vertical migration of cladocerans was the most frequent anti-predator behaviour displayed, in contrast with the temperate lakes where diel horizontal migration seemed more common. However, the densities and composition of the cladoceran community indicate that no anti-predator behaviour of zooplankton was enough to counteract the high predation pressure in warm lakes.

In the comparative study (paper IV), we found that more fish species and densities co-occurred with less cladoceran and less macroinvertebrate genera and densities, and lower periphyton biomass, in the subtropical lakes, than in similar temperate lakes. The lower abundance of zooplankton (5.5-fold) and macroinvertebrates (8-fold), the dominance of zooplankton by small-bodied taxa, the more frequent occurrence of large-bodied taxa within the macroinvertebrates, and the contrasting behaviour of cladocerans, indicate that predation pressure is clearly stronger among the plants in the subtropical than in the temperate lakes, as an obvious consequence of the 11-fold higher fish (and likely also shrimps) densities. Furthermore, the potential for

trophic cascades seemed more truncated in the subtropical lakes, as the impacts of fish were observed at all trophic levels, from cladocerans and macroinvertebrates to periphyton.

This study suggests that, more than other environmental factors, the structure of the typical predator assembly, namely fish, embodies the main reason for the observed different structure of littoral communities in temperate and subtropical shallow lakes (paper IV). Our results also confirmed the importance of plant architecture for littoral trophic interactions.

These results suggest that, even if climate warming does not directly affect significantly submerged plants and zooplankton, warming may impact other components of shallow lakes, such as the community structure or activity level of fish, as has been reported in some experimental studies (Mehner 2000). A warming-related higher impact of fish may strongly affect the resilience of shallow lakes, pushing the lakes in the direction of a higher predation on large-bodied zooplankton and lower grazer control of phytoplankton, and of a decreased capacity of the submerged plants to create and maintain clear-water conditions. The observed 4-fold lower periphyton biomass, and consequent reduced shading to the host plants, could help explain the development of aquatic plants despite such a minimized cladoceran community and consequent low grazing pressure on phytoplankton. Lower nitrogen availability is another likely factor contributing to plant development in warm lakes.

The current process of warming in temperate lakes may lower their resilience and impose an increased sensitivity to eutrophication or changes in water level, and a threat to the high diversity, clear water state with consequent impoverished ecosystemic and social values.

Conservation and restoration strategies should incorporate the substantial differences in lake functioning under different climate regimes (papers VII, VIII & IX), and the likely future increased sensitivity of shallow lakes to several external stressors, into today's planning.

Dansk resumé

Lavvandede søer, den mest udbredte søtype i det globale landskab (Moss 1998, Wetzel, 2001), er særdeles følsomme over for klimatiske ændringer (Scheffer 1998). Den globale opvarmning forventes at påvirke varigheden af vækstsæsonen, den sæsonmæssige rytme, det hydrologiske og termiske kredsløb samt det biologiske samspil i søer (Schindler 1997). Også undervandsplanternes afgørende betydning for biodiversiteten og sigtbarheden i lavvandede, tempererede søer (Canfield et al. 1984; Declerck et al. 2005) kan påvirkes af den globale opvarmning. Således kan man forvente, at en tidligere start på vækstsæsonen vil resultere i en større biomasse og udbredelse af undervandsplanter (Asaeda et al. 2001, Scheffer et al. 2001). Eutrofieringseffekter ventes at forstærkes i takt med de klimatiske ændringer (Mulholland et al. 1997, Søndergaard et al. 2001, Jeppesen et al. 2003b), eftersom højere temperaturer og lavere vandstand sandsynligvis vil forøge sedimentets resuspension og frigivelse af næringsstoffer, især fosfor. Voldsomme ændringer i den geografiske udbredelse af tropiske og subtropiske fritflydende undervandsplanter er en anden sandsynlig konsekvens af stigende minimumstemperaturer i luften i vinterhalvåret.

Det overordnede mål med denne PhD-afhandling var at belyse effekten af opvarmning på de akvatiske planters rolle, struktur og dynamik - og dermed lavvandede søers funktion - med hovedvægten lagt på undervandsplanter og fritflydende planter. Til dette formål anvendtes en kombination af kontrollerede eksperimenter på forskellige niveauer (laboratorie- og udendørs felteksperimenter) og sammenlignende feltundersøgelser under forskellige klimatiske forhold: subtropisk (Uruguay: 30°-35° S) *versus* tempereret (Danmark: 55°-57° N). Hvis man laver en space-for-time betragtning, kan viden om den trofiske dynamik og søernes funktion i subtroperne være nyttig i forudsigelsen af, hvordan den globale opvarmning vil påvirke tempererede søer. Projektets hovedsigte var at besvare følgende spørgsmål:

- Påvirker fritflydende planter de trofiske interaktioner i lavvandede søer på samme måde som undervandsplanter?
- Vil opvarmning svække de mekanismer, der karakteriserer klarvandede søer med dominans af undervandsplanter, især zooplanktonets brug af planterne som skjulested?
- Hvordan fungerer den trofiske struktur i littoralzonen i et varmere klima?
- Hvordan påvirker de akvatiske planters struktur littorale trofiske interaktioner?
- Kan restaureringsmetoder udviklet til tempererede, eutrofierede, lavvandede søer anvendes i varme søer?

Vores resultater indikerer, at i subtroperne fungerer store fritflydende planter ikke som skjul for store cladoceer, og de fremmer derfor ikke de kaskadeeffekter, der kan føre til stigende sigtbarhed (artikel I & III). Ud over deres i forvejen kendte negative indflydelse på den overordnede diversitet og vandkvalitet vil en voksende udbredelse af fritflydende planter, især med hensyn til deres mulighed for at udgøre en alternativ stabil tilstand til undervandsplanter (Meerhoff 2002, Scheffer et al. 2003), have en negativ virkning på den trofiske dynamik.

I eksperimentelt opvarmede damme (artikel V) observerede vi en svag effekt af øgede temperaturer på udbredelsen af undervandsplanter og zooplankton, men generelt viste disse samfund sig at være modstandskraftig over for opvarmning, hvilket også har været påvist i sammenlignelige undersøgelser (McKee et al. 2003). Disse resultater kan dog ikke overføres direkte til rigtige søer, fordi fiskestrukturen og fisketætheden i forsøget var konstant på grund af de eksperimentelle enheders begrænsede størrelse. Vi kunne derfor ikke spore nogen påvirkning af fiskestrukturen og deraf følgende kaskadeeffekter forårsaget af opvarmning, som de kan tænkes at forekomme i naturen. Resultaterne af et etårigt feltstudium i en subtropisk sø (artikel VI og sammenlignende undersøgelser i subtropiske og tempererede søer (artikel III) modsiger iagttagelser fra tempererede systemer af, at undervandsplanternes funktion som skjul og de deraf følgende kaskadevirkninger fører til øget sigtbarhed (Jeppesen et al. 2002b). I subtroperne fungerer undervandsplanterne kun i ringe grad som skjul for cladoceer. I de subtropiske søer foretog cladoceerne vertikal døgnvandring som forsvar mod prædation, hvorimod horisontal døgnvandring synes mere hyppig i tempererede søer. Tætheden og sammensætningen af cladocé-samfundet indikerer imidlertid, at ingen forsvarsmekanisme kunne hindre det høje prædationstryk på zooplanktonet i varme søer.

I den sammenlignende undersøgelse (artikel IV) fandt vi i de subtropiske søer flere arter og større tætheder af fisk, færre cladoceer og færre arter og tætheder af makroinvertebrater og en lavere biomasse af periphyton end i sammenlignelige tempererede søer. Den lavere forekomst af zooplankton (5,5 gange lavere) og makroinvertebrater (8 gange lavere), dominans af små zooplanktonarter og større hyppighed i forekomsten af store arter af makroinvertebrater tyder på et betydeligt større prædationstryk mellem planterne i de subtropiske søer end i de tempererede søer, hvilket er en indlysende effekt af den 11 gange højere tæthed af fisk (og sandsynligvis også rejer). Ydermere er potentialet for trofiske kaskader mindre i de subtropiske søer, eftersom fiskenes indvirkning kunne spores på alle trofiske niveauer lige fra cladoceer over makroinvertebrater til periphyton.

Undersøgelsesresultaterne tyder på, at fiskebestandens struktur er den afgørende miljømæssige faktor for de observerede forskelle i de littorale samfund mellem tempererede og subtropiske søer (artikel IV). Vores resultater bekræfter også betydningen af plantestrukturen for de trofiske interaktioner i littoralen.

Resultaterne antyder, at selv om den klimatiske opvarmning ikke har nogen direkte afgørende indflydelse på undervandsplanter og zooplankton, påvirker opvarmningen andre elementer i de lavvandede søer, såsom fiskenes sammensætning og aktivitetsniveau, hvilket også er påvist i andre eksperimentelle undersøgelser (Mehner 2000). En opvarmningsrelateret påvirkning af fisk kan have en markant indvirkning på de lavvandede søers robusthed, stabilitet og evne til regeneration og kan føre til større prædation på det store zooplankton og dermed lavere græsningskontrol af fytoplanktonet samt en nedsat evne hos undervandsplanterne til at skabe og fastholde en klarvandet tilstand. Den konstaterede 4 gange lavere periphytonbiomasse og deraf følgende mindsket skygning af værtsplanten kan være en af forklaringerne på de akvatiske planters vækst på trods af et minimeret cladocé-samfund og det dermed lavere græsningstryk på fytoplanktonet. Lavere kvælstofkoncentrationer er en anden mulig faktor, der spiller ind på udviklingen af planter i varme søer.

Den nuværende opvarmning af tempererede søer kan skade deres robusthed og øge deres følsomhed over for eutrofiering og ændringer i vandstanden og udgøre en trussel mod den klarvandede tilstand med høj diversitet, hvilket kan føre til en forarmning af søernes økosystem og en forringelse af de deres sociale værdier. I udarbejdelsen af strategier for bevaring og restaurering bør man tage højde for de væsentlige forskelle i søernes funktion mellem klimatiske regioner (artikel VII, VIII & IX) samt for de lavvandede søers sandsynlige øgede følsomhed over for udefrakommende stresspåvirkninger.

Introduction

Climate warming

Our planet is warming unnaturally. The dynamic changes occurring in the structure and function of many of our world's ecosystems challenge the very sense of current debates about climate warming. Natural climate variability (i.e. year-to-year oscillations) depends on various factors, such as orbital variations of the Earth (Milankovitch cycles), changes in the incoming solar radiation, and changes in the concentrations of aerosols from volcanic eruptions. Natural climate variations can also occur as a result of complex interactions between components of the climate system, such as the coupling between the atmosphere and the oceans. The El Niño-Southern Oscillation (ENSO), the North Atlantic Oscillation (NAO), and the Artic Oscillation (AO) phenomena are examples of such natural variability on interannual time-scales. However, several global environmental changes that are apparent nowadays are certainly anthropogenic (IPCC 2001), and all have tremendous ecological consequences.

The best-documented global changes occur in land use and land cover, in alterations in the biogeochemistry of the global nitrogen cycle, and the increasing concentrations of greenhouse gases in the atmosphere, particularly of CO_2 (Vitousek 1994). This increase in CO_2 concentrations has been pointed out as the main responsible factor for the increase of ca. 0.6 °C (±0.2°C) in the global average surface air temperature during the 20th century (IPCC 2001). Climate models predict that the mean annual global surface temperature will further increase 1-3.5°C by the year 2100. General circulation models predict that warming is not, and will not be, uniform across the world. Warming is more pronounced at higher latitudes (Rouse et al. 1997, Phoenix and Lee 2004, Smol et al. 2005), but also of significance in subtropical regions at least in the northern hemisphere (Mulholland et al. 1997). The increased temperatures can affect ecosystems at different levels, from organism physiology and phenology, to geographical distribution of species and community structure (Hughes 2000). Clear evidence exists that climate change is affecting the seasons, more strongly in places experiencing more warming. For example, spring starts 6-8 days earlier, while autumn is delayed ca. 3 days over the last 30 years in Europe (Menzel et al. 2006). Climate warming is considered one of the most important threats to ecosystems already today (Parmesan and Yohe 2003), particularly to biodiversity (Thomas et al. 2004).

At the same time, the demand for high-quality freshwater increases steadily with human population and technological growth, and changes in climate seriously threaten its accessibility in many places (de Wit and Stankiewicz 2006). Freshwater systems are already subject to increasing deterioration processes, including wetland area loss, local extinction of native species and introduction of exotic species, water level changes due to water extraction or canalisation, and especially, eutrophication (Moss 1998). Climate warming expectantly brings a significant shortening of the duration of ice cover in temperate and cold areas (Williams et al. 2004), substantial changes in hydrological and thermal regimes in lakes, especially in arid or semiarid areas (Beklioglu et al. in press), as well as local extinction of some species due to physiological stress or via interactions with other species (Schindler 1997). Shallow lakes, the most abundant type of lake in the global landscape (Moss 1998, Wetzel 2001), seem particularly sensitive to climatic changes among freshwater systems (Scheffer 1998).

The structuring role of aquatic plants in temperate shallow lakes

Shallow lakes possess the ability to support the development of higher aquatic plants over the entire basin, or at least in large sections of their littoral area. Plants affect the biological structure and physico-chemical processes of the littoral zone of lakes, with influences in the entire lake ecosystem (Carpenter and Lodge 1986, Jeppesen et al. 1997c). Many aspects of the functioning and structure in shallow lakes depend on the presence of aquatic plants, particularly in lakes of the temperate zone (Moss 1990, Jeppesen et al. 1997c), but also in the tropics (Thomaz and Bini 2003).

In shallow temperate lakes, submerged plants can positively influence biodiversity and water transparency (Canfield et al. 1984, Declerck et al. 2005) (Fig. 1). The effects of submerged plants on trophic dynamics and water clarity have given support to the alternative states hypothesis for shallow temperate lakes (Balls et al. 1989, Scheffer et al. 1993). In a nutshell, this hypothesis states that, over a wide range of nutrient concentrations typically considered to be between 25 to 100 μg TP L^{-1} (Jeppesen et al. 1990), shallow temperate lakes can be clear with abundant submerged plants, or turbid, often with excess phytoplankton. Biological and physico-chemical mechanisms related to the presence or absence of the submerged macrophytes maintain either state. Submerged plants promote their own development by several mechanisms, that include the reduction of available light and nutrients for phytoplankton and periphyton competitors, either by direct luxurious uptake or enhanced denitrification (Stephen et al. 1998); the reduction of sediment resuspension and therefore of nonbiotic turbidity (Madsen et al. 2001); the stabilization of the interactions between carnivores

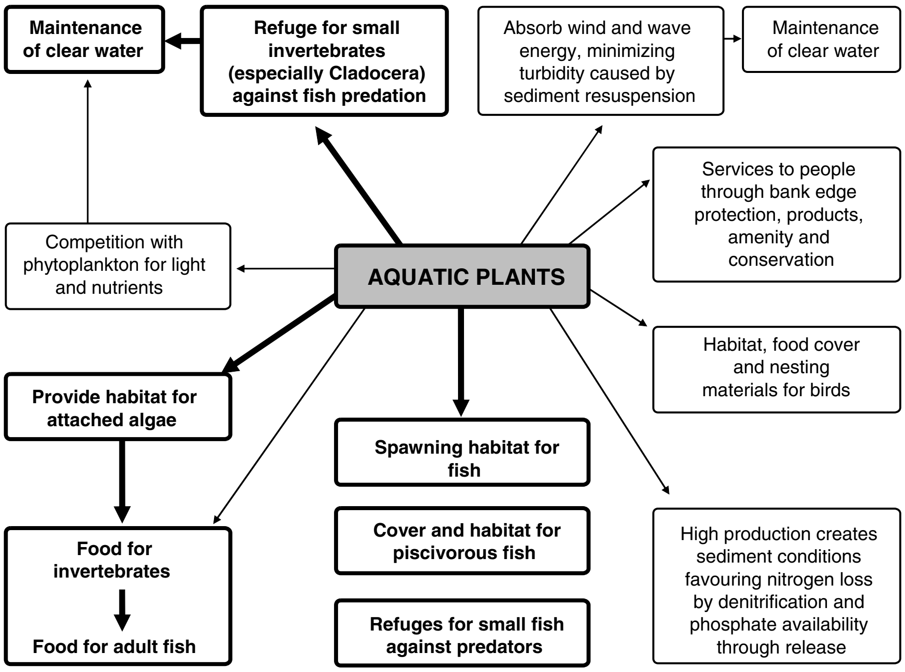

Fig. 1. Effects of aquatic plants on lake functioning and water transparency (modified from Moss et al. 1996). This thesis analysed the highlighted processes for submerged and free-floating plants.

(fish), herbivores (zooplankton and several macroinvertebrates), and primary producers (phytoplankton and periphyton) (Timms and Moss 1984, Brönmark and Weisner 1992); and although far less documented in the field, the excretion of allelopathic substances that can inhibit algal growth (Wium-Andersen et al. 1982, Gross et al. 2003, Burks et al. 2006).

The provision of refuge for large-bodied cladoceran grazers from diurnal visual predation by fish is a key mechanism by which submerged plants can lead to a decrease in phytoplankton biomass and higher water transparency (Timms and Moss 1984, Lauridsen and Buenk 1996, Burks et al. 2002). The refuge effect for cladocerans, particularly for *Daphnia*, varies with the composition of the potential predators (Jeppesen et al. 1997a), as the plants can also host juvenile fish (Persson and Eklöv 1995), and multiple predatory invertebrates (Burks et al. 2001a). The refuge effect also seems to depend on the plant architecture (Nurminen and Horppila 2002), plant bed size or density (Lauridsen et al. 1996, Burks et al. 2001b), and percent of the lake volume inhabited by the plants (Schriver et al. 1995), as well as the trophic state of the lake (Lauridsen et al. 1999). At both extremes of a nutrient (and consequent turbidity) gradient, submerged plants would offer little refuge for zooplankton, whereas the refuge effect would be stronger under meso-eutrophic conditions with high plant cover (Jeppesen et al. 1997b).

The plants can also provide such a refuge for juvenile fish against piscivores (Persson and Eklöv 1995), and support a high density of grazing invertebrates that may, in turn, maintain a low periphyton biomass for the benefit of the plants (Jones and Sayer 2003).

The role of plants under a climate warming scenario

Besides being indirectly affected by the effects that climate change will impose on the catchments (e.g. increased run-off of nutrients due to higher precipitation), aquatic plants can be directly affected by the process of warming suffered by the lakes. Temperature influences the growth and production of aquatic plants (Madsen and Brix 1997, Santamaría and van Vierssen 1997). According to field studies in north temperate lakes, earlier commencement of growing seasons, as predicted by climate change models, would result in greater biomass and distribution of submerged macrophyte communities (Rooney and Kalff 2000). This effect would be a function of lake morphometry and be most pronounced in the shallower systems (Rooney & Kalff 2000). Some models also describe a warming-related faster development of macrophytes (Asaeda et al. 2001). When also considering increased CO_2 concentrations, both phytoplankton (Schippers et al. 2004a) and macrophyte production (Schippers et al. 2004b) are expected to increase in the coming decades. Such production will occur at different rates according to the variation in external conditions, such as nutrient status and alkalinity.

A model built on the correlation between the winter NAO index and a defined clear water state has predicted a shift in the timing and a higher probability of the occurrence of clear water phases in shallow temperate lakes (Scheffer et al. 2001). These authors argue that increased temperature can generate a window of opportunity for the recolonization of submerged macrophytes. Moreover, a strong decrease in water level caused by higher evaporation can favour submerged plant development (Romo et al. 2004), or lead to fish mortality and a shift to a clear water state, due to a change in the reinforcing mechanisms mentioned above (Scheffer 1998). In contrast, eutrophication effects can worsen with climate warming (Mulholland et al. 1997, Søndergaard et al. 2001, Jeppesen et al. 2003b). While some shallow lakes and ponds might dry out, the higher temperatures and lower water level will likely enhance the sediment release of nutrients, especially phosphorus, and resuspension. Few studies have tested the effects of increased temperature using an ecosystemic approach in controlled experiments (McKee et al. 2003). In these experiments, warming did not promote algal blooms but instead increased phosphorus levels and deoxygenation (McKee et al. 2003). Contrary to expectations, these researchers did not find significant interaction effects of nutrients and warming on major freshwater communities, such as invertebrates, zooplankton, and macrophytes (McKee et al. 2002a, McKee et al. 2002b). However, the activity of small fish increases with warming, which may lead to a stronger predation pressure on zooplankton (Mehner 2000), and, with it, a weakening of the macrophyte-dominated state. Furthermore, both fish predation pressure and the importance of nutrient loading increased in warm southern lakes compared to similar temperate lakes in an experimental study along a latitudinal gradient in Europe (Moss et al. 2004).

Some macrophyte species of north temperate systems seem to be resilient to the predicted increases in temperature, although climate warming might change the proportion of species within macrophyte communities favouring exotic ones (McKee et al. 2002b). Most research into the response of shallow lakes to warming has so far focused on the performance of submerged macrophytes and phytoplankton. However, a broader shift in the geographical range of tropical and subtropical free-floating plants represents another likely consequence of increasing winter minimum air temperatures in freshwater systems. Although far less studied than the submerged plant communities, large free-floating plants play key roles in warm climates as they are not temperature-limited. Free-floating plants may constitute an alternative stable state to submerged plant (Meerhoff 2002, Scheffer et al. 2003), and likely to phytoplankton dominance (Roijackers et al. 2004), mainly due to different competition abilities involving carbon, light, and nutrients (Fig. 2). Large free-floating plants commonly get identified as some of the most important nuisance weeds in many warm-climate parts of the world (Talling & Lemoalle 1998). The effects of a dominance of free-floating plants on the functioning of lake ecosystems are undesirable according to accepted water quality parameters because of the usual strong decrease in oxygen concentrations under floating mats, with the consequent impoverishment of biodiversity.

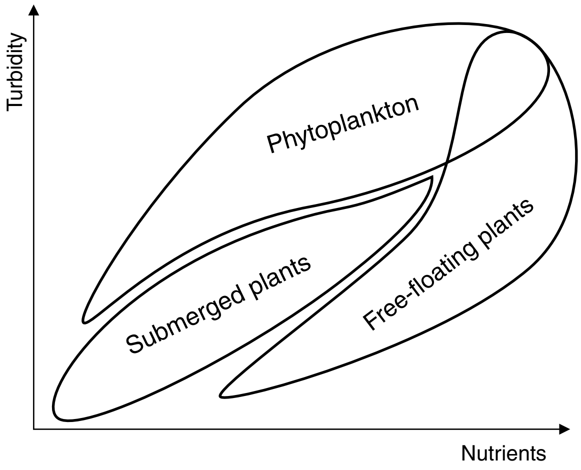

Fig. 2. Turbidity and nutrient conditions along which phytoplankton, free-floating plants and submerged plants are likely to occur in shallow lakes. Each vegetation type can, in turn, modify these conditions. (Modified from paper II).

As described above, the role of submerged plants in temperate lakes is quite well understood. However, the role of other aquatic plant life forms (such as the free-floating), and even of the submerged plants, under other climate regimes or under a climate change scenario, is far from clear. There are much fewer studies of the role of macrophytes, of any architecture, in the tropics and subtropics (Thomaz and Bini 2003). It seems quite clear that plants have an extraordinary importance for the fish assemblage in these systems, particularly affecting the number of species (Agostinho et al. 2003) and size distribution in lakes. In particular, the smallest fish species and individuals aggregate in very high numbers in the vegetation (Conrow et al. 1990, Meschiatti et al. 2000), both in submerged and free-floating plants (Meerhoff et al. 2003). Furthermore, fish communities in warmer climates are characterised by a higher share of omnivorous species (Winemiller 1990, Branco et al. 1997, Yafe et al. 2002) and multiple or frequent reproduction (Paugy and Lévêque 1999). Consequently, aquatic plants likely offer a poor refuge for large-bodied zooplankton in warm/warming lakes (Meerhoff et al. 2003), and this may potentially have consequences at the ecosystem level.

Objectives and Approach

The general objective of this thesis focused on studying the effects of warming on the role of aquatic plants on trophic dynamics and, consequently, on the functioning of shallow lakes, with focus on submerged and free-floating plants. The project aimed to address the following main questions and hypotheses:

Q1 Do free-floating plants affect trophic interactions in shallow lakes in a similar manner than submerged plants?

H1 Aquatic plants impact trophic interactions differently according to their architecture. In contrast to submerged plants, free-floating plants do not stabilize the predator-prey interactions between fish and large-bodied zooplankton by offering refuge for the latter.
(papers I, II & III)

Q2 Are the effects of submerged macrophytes on trophic dynamics affected by climate warming? Can warming weaken the mechanisms associated with the submerged plants-dominated clear water state, in particular, the refuge effect for zooplankton?

H2 Warming negatively impacts the structuring role of submerged plants in temperate shallow lakes. A warmer climate can lead to a weakening of the macrophyte-dominated clear water state.
(papers III, V, VI, & in prep.)

Q3 How does the trophic structure in the littoral zone differ between temperate and subtropical climates? What are the key drivers in community structure and function? How does the architecture of aquatic plants impact littoral trophic interactions?

H3 The trophic structure in the littoral zone in warm (subtropical) lakes is much more impacted by fish than in similar temperate lakes. The potential for trophic cascades is thus more truncated in the subtropics. The effects of aquatic plants differ greatly according to their architecture, and consequently will lead to different cascading effects.
(paper IV)

Q4 Are the restoration strategies developed for temperate eutrophic shallow lakes applicable to lakes under warmer climates?

H4 The climate warming scenario imposes serious limitations to the current strategies for the restoration of eutrophic temperate shallow lakes. The success of the application of those strategies in (sub)tropical lakes is also questionable.
(papers VII, VIII & IX)

The impacts of climate warming on lakes are currently investigated using different approaches such as long-term historical data, paleolimnological data, model simulations, experiments, and application of the space-for-time substitution approach in field studies (Battarbee 2000, Scheffer et al. 2001, Jeppesen et al. 2003a, McKee et al. 2003, Moss et al. 2004). No single approach can provide all the answers, and the interpretation of particular results must be complemented with those coming from other investigations and approaches.

The approach followed in this thesis consisted of the combination of both controlled experiments at different scales (laboratory and out-door field experiments) and comparative field studies under different climates: subtropical (Uruguay: 30°-35° S) *versus* temperate (Denmark: 55°-57° N). Following a space-for-time substitution approach, the knowledge about trophic dynamics and lake functioning gained from the subtropics might be useful when evaluating the effects of climate warming on temperate lakes. The papers presented in this thesis, together with some unpublished results discussed here, aim at providing empirical insight to the above questions.

1. Effects of free-floating plants on trophic dynamics in the subtropics

Plant architecture may determine differential impacts on lake functioning (Burks et al. 2006). Comparative studies on the effects of emergent and floating-leaved plants (Nurminen and Horppila 2002, Horppila and Nurminen 2005) and submerged and free-floating plants (Meerhoff et al. 2003) have highlighted the importance of plant architecture for several trophic interactions.

Although the necessity of a daytime refuge for zooplankton appears strong in subtropical and tropical lakes, the diel use of submerged plants is not so obviously advantageous for the zooplankton due to the usually high fish densities (Conrow et al. 1990, Mazzeo et al. 2003). However, all macrophyte growth forms (emergent, submerged, floating-leaved and large free-floating) can be extremely dense in subtropical and tropical lakes (Talling and Lemoalle 1998) and might thus provide refuge for the zooplankton against predation. Large free-floating plants attract interest as potential refuges because of their conspicuous nature in the (sub)tropics, and their dense and long root network, which enhanced by the shade produced by their shoots and leaves, could act as refuge.

Applying different research approaches, I aimed to analyse the role of free-floating plants in some key processes in lake ecosystems. Laboratory experimental studies took place to study the effects of free-floating plants on the interactions between fish and zooplankton (paper I). In a separate publication (paper II), we summarized the scarce literature on the ecology and trophic effects of free-floating plants in South America.

Free-floating plants comprise both very small taxa, such as the genera *Azolla* and *Lemna*, medium-sized taxa, such as *Salvinia* spp (water fern), and large-sized taxa, such as *Eichhornia crassipes* (water hyacinth), and *Pistia stratiotes* (water lettuce). The free-floating plants can be found over a wide latitudinal gradient, except for the large species, which are typical of (sub)tropical systems due to their high sensitivity to low temperatures and freezing events (Sculthorpe 1967). Most studies on the large free-floating plants have focused directly or indirectly on their status as dangerous weeds. In this regard, an ample literature exists on their growth and nutrient assimilation capacity, dispersion ways and control methods (Mitchell 1973). Free-floating plants are considered the main aquatic weeds in many subtropical and tropical water bodies where they are exotic, such as in Africa (Cilliers et al. 1996), Asia (Mansor 1996), North America (Gutiérrez et al. 1996), and Southern Europe (Moreira et al. 1999), but also in some parts of their native South America (Bini et al. 1999). Besides the negative impact of a massive development of these plants on several uses of the systems, including navigation, irrigation, recreation, and drinking water supply, they are believed to enhance the development of mosquitoes (Savage et al. 1990) and of other intermediate hosts of several tropical diseases (Rumi et al. 2002). The first field study into the role of free-floating plants (water hyacinth) on trophic dynamics took place in Lake Rodó, Uruguay, a hypertrophic lake containing submerged plant beds, extremely high fish densities, and a taxon-poor zooplankton community (Meerhoff et al. 2003). The results were striking. We found that despite hosting lower fish densities than the submerged plants (although still high compared to densities in temperate lakes), water hyacinth beds also had lower densities of zooplankton, particularly of the larger-bodied species. Due to the specific characteristics of Lake Rodó, the questions remained whether these results could be generalised to larger zooplankton grazers (such as *Daphnia*), and to other species of free-floating plants.

In laboratory studies (paper I) we analysed the refuge capacity of common (sub)tropical free-floating plants and a cosmopolitan submerged plant for *Daphnia*. Using tube experiments (Fig 3a), we conducted simple behaviour experiments to test the effects of three free-floating plants, *E. crassipes*, *P. stratiotes* and *Salvinia auriculata*, and the submerged plant *Ceratophyllum demersum* (coontail or common hornwort), on the horizontal movement behaviour of the water flea *Daphnia obtusa*. We performed these experiments in the presence and absence of alarm signals from crushed conspecifics (Pijanowska 1997) and chemical stimuli from a potential predator, the omnivorous-planktivorous fish *Cnesterodon decemmaculatus* (ten-spotted livebearer, local name: madrecita). The free-floating plant species are native to (sub)tropical South America, while *C. demersum* is cosmopolitan but widely distributed in subtropical lakes. The fish *C. decemmaculatus*, present in Argentina, Uruguay and the south of Brazil (Rosa and Costa 1993), is very common over a wide range of nutrient and turbidity conditions, but it is particularly dominant in eutrophic and hypertrophic lakes (Scasso et al. 2001).

The behaviour of *Daphnia* in these experiments followed our expectations when faced with the plants alone and with crushed conspecifics. In the absence of other stimuli, *Daphnia* significantly avoided both free-floating and the submerged plants. Furthermore, the response to the free-floating plants was faster and stronger than to the submerged plants (Fig. 3b). To determine whether the behaviour of the daphniids towards the free-floating plants was either chemically or mechanically induced, we used, respectively, suspended filter-paper bags with roots of *E. crassipes*

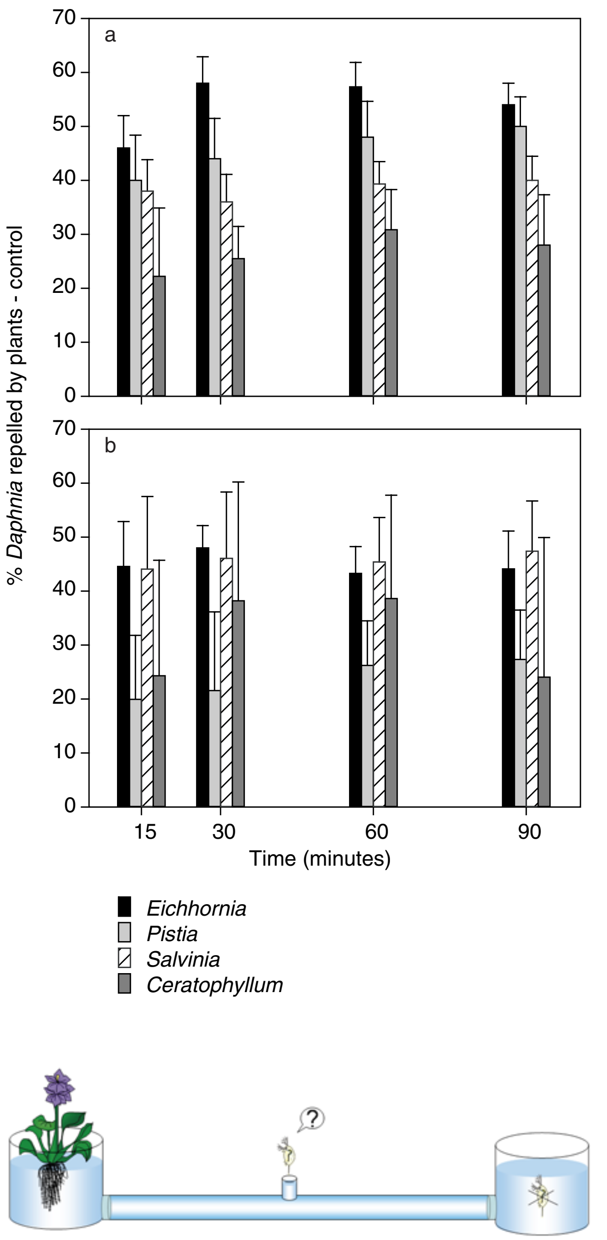

Fig. 3. Refuge potential of aquatic plants for *Daphnia* in the subtropics. Data represent the difference between the proportion of *Daphnia* repelled by the plants and the proportion in the respective side of the control unit (mean ± 1 SE). Above: Effect of the free-floating plants *E. crassipes*, *P. stratiotes*, *S. auriculata*, and the submerged *C. demersum*, on the horizontal movement of *Daphnia* in the absence of other stimuli. The weaker the repellent effect of the plant, the closer to 0% is the difference. Below: Choice of *Daphnia* when exposed simultaneously to the same plants and crushed conspecifics (schematized below). If plants were attracting the daphniids ("refuge effect"), the % differences would be negative. (From paper I).

(thus removing the shade effect of an intact plant) and plastic structures mimicking the architecture of the root system and the shade produced by the leaves of *E. crassipes*. Both the chemical cues from the roots and the plastic structure promoted *Daphnia* avoidance. The response to the roots was significantly weaker than the response to the complete plants, whereas the response to the plastic structure was intermediate between both (Fig. 2 in paper I).

Contrary to our expectations based on research on submerged plants in temperate lakes (Lauridsen and Lodge 1996, Burks et al. 2001b), *Daphnia* clearly avoided the selected set of plants, despite the risk of actual predation (cues from the fish) and implied threats (alarm signals from crushed conspecifics) (Fig. 3). Even though the repellence by plants weakened in the presence of alarm signals, our results suggest that the costs of exposure to the plants, and particularly to the free-floating species, do not outweigh the benefits of swimming away from predators or alarm signals. Thus, plants of both life- forms seemed to signal danger rather than potential refuge for daphniids in the subtropics, but remarkably more in the case of free-floating than submerged plant species.

Ecological phenomena like diel migration have proximate and ultimate causes (Ringelberg and Van Gool 2003). The proximate causes refer to the environmental factors that lead to the occurrence of the phenomenon, i.e. the signal that *Daphnia* perceives to start moving horizontally (such as changes in light environment, plant metabolites, etc). However, such behaviour must have an adaptive meaning and been subject to selection processes, which represent the ultimate causality. The ultimate cause of the strong aversion to the plants shown in our experiments may not be elicited by the plants themselves, but rather by the expectedly high densities of zooplanktivorous fish seeking refuge or feeding within all vegetation types in subtropical lakes. However, other mechanisms must also be at play to explain the stronger aversion behaviour of cladocerans towards the free-floating plants. Lower food quality (due to out-shading of phytoplankton) and particularly, detrimental physico-chemical conditions under dense floating mats (such as constant and extremely low oxygen concentrations), may be the complementary ultimate causes of such phenomenon.

2. Effects of submerged plants on fish-zooplankton interactions in warm(ing) shallow lakes

The role of submerged plants as refuge providers for large-bodied zooplankton is increasingly understood in temperate shallow lakes (Burks et al. 2002), but far from understood in lakes located in warmer regions (or in currently warming temperate lakes) (Burks et al. 2006). Shallow lakes typically lack a hypolimnetic refuge which would favour diel vertical migration (DVM) by zooplankton. In deep lakes vulnerable zooplankton migrate to the bottom layers during the day, thus minimizing the predation risk by fish, and migrate upwards during the night to graze with lower chances of being eaten (Lampert 1993, Ringelberg and Van Gool 2003). In shallow temperate lakes, chemical cues from potential predators may lead the large-bodied zooplankton to perform diel horizontal migration (DHM) into the littoral zone, particularly into the submerged plants (Timms and Moss 1984, Burks et al. 2002). The consequent plant-mediated maintenance of a herbivorous zooplankton population to graze algae has individual, population and even ecosystem consequences (Brönmark and Hansson 2000, Jeppesen et al. 2002b). Previous work suggests that submerged plants offer little refuge for zooplankton at both extremes of the nutrient gradient. In low-nutrient lakes, clear water and the scarcity of macrophytes enhance fish predation on zooplankton whereas, under hypertrophic conditions, the refuge effect is weak because of the scarcity or absence of submerged plants and often high densities of planktivorous fish (Jeppesen et al. 1997b). The authors also hypothesise that changes in zooplankton biomass are connected with seasonal changes in the submerged plant density (Jeppesen et al. 1997b).

In the following sections, I will describe three different research lines analysing the relationship between submerged macrophytes and zooplankton under warm(ing) conditions: a) the effects of increased temperatures on submerged plants and zooplankton communities were studied in experimentally warmed ponds during a 1-year-period (paper V and paper in prep. A); b) the consequences of seasonal changes in the refuge capacity of submerged plants for zooplankton were studied in a eutrophic subtropical lake (Lake Blanca, Uruguay, paper VI), and c) the refuge effect of plants along a turbidity gradient was studied during a snap-shot sampling campaign conducted in summer in several Danish and Uruguayan shallow lakes (comparative field study described in papers III and IV). In the last study, we introduced artificial plant beds mimicking different plant architectures (submerged vs. free-floating) in ten shallow lakes along a turbidity and nutrient gradient in Uruguay (30°-35° S) and Denmark (55°-57° N). The diel migration patterns of cladocerans were analysed in half of those lakes in each country, paired in terms of water turbidity (paper III).

a. Response of zooplankton communities to experimental warming

This experiment represented part of the STF-project "Consequences of weather and climate changes for marine and freshwater ecosystems - Conceptual and operational forecasting of the aquatic environment (CONWOY)", conducted by the National Environmental Research Institute and the universities of Aarhus and Copenhagen in co-operation. We monitored the response of freshwater communities to warming using 24 outdoor enclosures (2-m diameter) subjected to three temperature treatments: the IPCC's A2 scenario for the period 2071-2100 downgraded for local Danish conditions, the same A2 +50% increase in temperature; and the control following ambient temperature; in combination with high nutrient (and fish) addition to half of them. The enclosures therefore mimicked north temperate shallow lakes in turbid and clear water states. The set-up and functioning of the experiments are explained in detail in paper V. The results described below correspond to the first year of the experiments (paper in prep. A).

Submerged macrophytes (curly pondweed, *Potamogeton crispus* and elodea, *Elodea canadensis*) established mostly in the low-nutrient treatments, and occurred infrequently in some of the high-nutrients ones. At both nutrient levels the zooplankton communities reflected lakes or ponds with a considerable littoral area. In the low-nutrient enclosures, large-sized cladocerans (mainly *Simocephalus vetulus*) and cyclopoid copepods dominated in terms of biomass. Under high-nutrient conditions, the community consisted of mostly rotifers and small-sized cladocerans (mainly *Chydorus sphaericus*).

We found no significant effects of temperature on the composition of the zooplankton community or the relative biomass distribution of the main groups (cladocerans, copepods and rotifers). We did not find any clear relationship between total and cladoceran biomass with increased temperatures in the high-nutrient treatments (Fig. 4a). Under low-nutrient conditions we found signs of a potential earlier spring peak of cladoceran and copepod biomass with higher temperatures (although not significant in the ANOVA tests, Fig. 4b). In the case of rotifers and copepods we observed indications of a negative response (measured as biomass changes) to higher temperatures in winter. Indirect effects on the zooplankton community, via macrophytes, may occur.

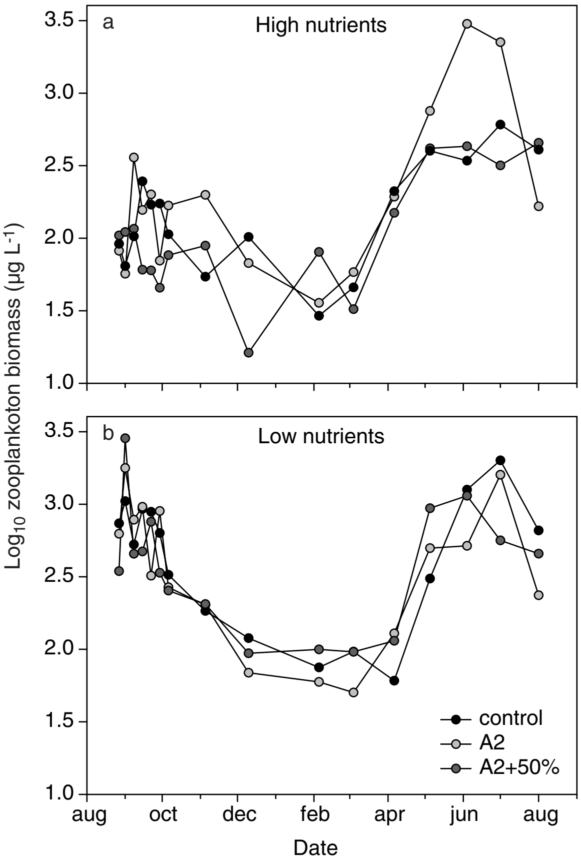

Fig. 4. Total biomass of zooplankton during 1-year experimental warming in ponds, under high (above) and low (below) nutrient conditions. The temperature treatments included a control following ambient temperature, the IPCC's A2 scenario for the period 2070-2100 (Danish conditions), and the previous temperature increased by 50%.

Under low-nutrient conditions cladoceran biomass positively related to plant %PVI (plant volume inhabited, *sensu* Canfield et al. 1984) (R=0.47 p=0.0001). However, we observed signs of an apparent weakening of that relationship with warming.

These results stress the importance of the responses of the submerged plants with higher temperatures for littoral zooplankton. We found that the response of the plants to warming differed between the species. In particular, an earlier and faster growing season of *P. crispus* with higher temperatures took place (Lauridsen et al. in prep.). The results suggest that an earlier growing season and sometimes higher plant biomass might be expected at the community level with warming. Similar results occurred in the only comparable study conducted so far (McKee et al. 2002a, McKee et al. 2002b). These authors found a broad resilience of macrophytes to warming, but also that exotic species could be favoured. In general terms, and in agreement with their findings, the structure of the zooplankton community in our experiments seemed quite resilient to warming, with subtle responses linked to macrophytes and phytoplankton depending on nutrient conditions. However, it must be emphasized that this experimental set up did not fully mimic the response of natural lakes and ponds to warming, because, due to the small size of the enclosures, we fixed fish density (by preventing reproduction), and some trophic dynamics (by not including piscivores).

b. Seasonal changes of refuge effect in a subtropical eutrophic lake

The seasonal variation in the diel horizontal distribution of zooplankton under the scenario of multiple vertebrate and invertebrate predators was analysed in subtropical eutrophic Lake Blanca, Uruguay (paper VI). This lake contains a very well-developed littoral zone hosting submerged (*Egeria densa* and *Ceratophyllum demersum*) and emergent (*Typha latifolia*) plants and by a high abundance of the small omnivorous fish *Jenynsia multidentata* (Río de la Plata one-sided livebearer, local name: overito), the omnivorous shrimp *Palaemonetes argentinus* and the predator invertebrate *Chaoborus* sp (phantom midge) (Mazzeo et al. 2003). The cladoceran community in this lake was very taxon-poor, with *Diaphanosoma birgei* and *Bosmina longirostris* being the most abundant and potentially migrating species. Besides these, also *Moina micrura* and *Chydorus sphaericus* were present.

No significant variation in plant cover was observed during the 1-year study period, with the mean area covered measuring 55% and 46% for emergent and submerged plants, respectively. Fish had the highest densities (CPUE catch per unit effort, using minnow traps) in summer, and shrimps in spring. The seasonal variation in total zooplankton abundance negatively correlated with fish and shrimp CPUE, but not with the densities of *Chaoborus* sp. In particular we observed a strong negative correlation between the density of medium-sized grazers (cladocerans and calanoid copepods) and fish CPUE among seasons (Fig. 5).

The habitat use of *D. birgei* and *B. longirostris* varied among seasons, although the general diel trends of both species were similar most of the year (except summer, Fig. 3 in paper VI). In summer, fish density increased enormously after the main reproductive season. Together with a remarkable decrease in cladoceran abundance (particularly of *B. longirostris*), we found indications of diel vertical migration of *D. birgei* (increased nocturnal densities in open water and among submerged plants). Clearly, no habitat seemed to offer a significant refuge for zooplankters under this enhanced-predation scenario. In contrast, most cladoceran species occurred with higher densities in open water during winter, coinciding with the lowest CPUE of fish and shrimps and the lowest density of *Chaoborus* sp. (Fig. 5).

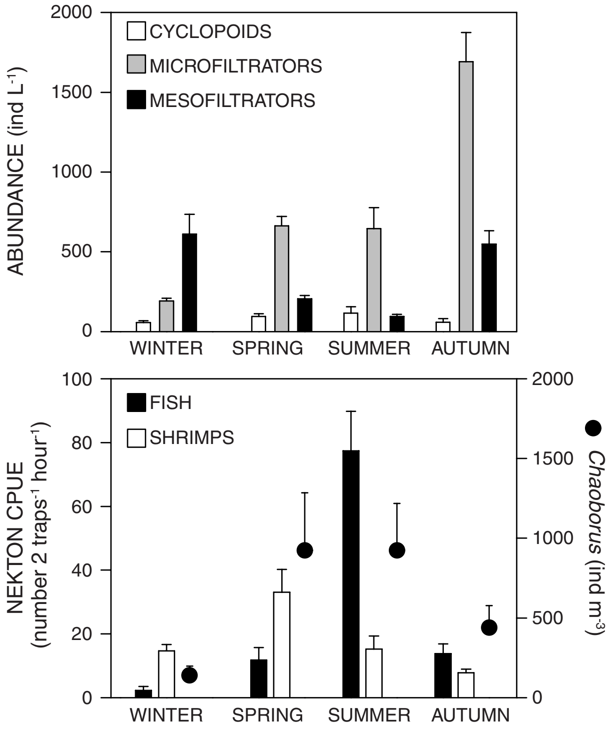

Fig. 5. Seasonal changes in density of zooplankton and potential predators in Lake Blanca (Uruguay). Above: Density of zooplankton: cyclopoid copepods, microfiltrators (rotifers + nauplii), and mesofiltrators (calanoid copepods + cladocerans). Below: Density of nekton (CPUE fish *J. multidentata* + shrimps *P. argentinus*) and the phantom midge *Chaoborus* sp. Data represent whole system averages (error bars= 1 SE). (From paper VI).

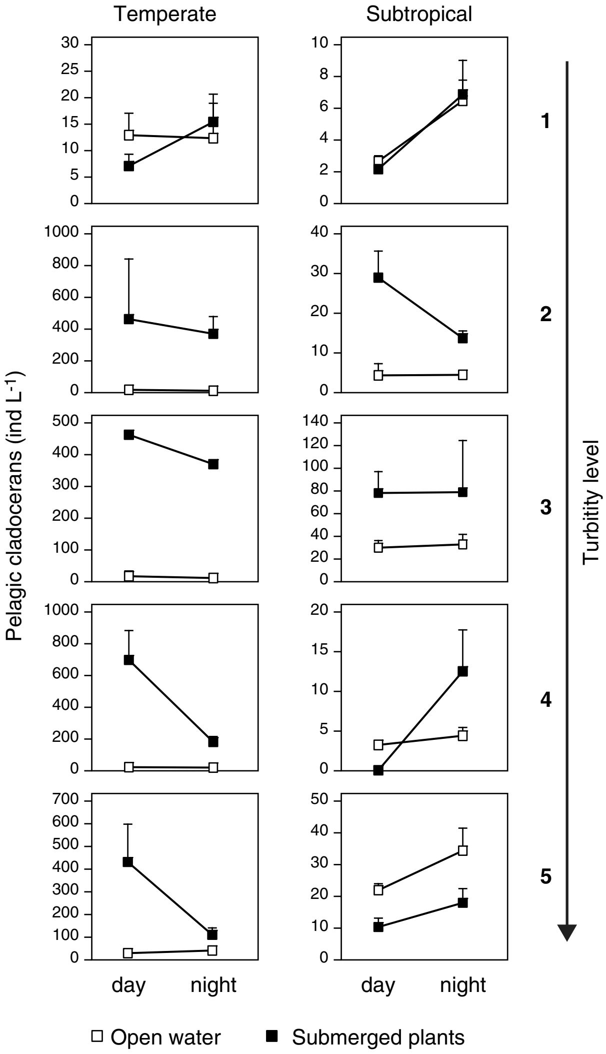

Fig. 6. Diel migration pattern of free-swimming cladocerans in temperate (left) and subtropical (right) lakes along a turbidity gradient (increasing from top to bottom). Lakes in both climate zones were matched in terms of water transparency and shared similar morphometric and physico-chemical characteristics. (Modified from paper III).

We found evidence of significant diel horizontal migration by *Diaphanosoma* and *Bosmina* in autumn and spring, respectively. The direction of this diel horizontal migration (DHM) was reverse as that classically described (Burks et al. 2002), as both cladocerans increased their densities in the submerged plant beds at night-time, while decreasing in open water. The nocturnal occurrence of *Chaoborus* with higher densities in the pelagial or the emergent plants seemed to be the main trigger of this reverse DHM, followed in importance by the small omnivorous fish. The distribution of the third potential predator, shrimps, did not seem to drive the spatial pattern of cladocerans. The highest CPUE of *J. multidentata* occurred in the plants even at night-time. This habitat selection, *J. multidentata* being the top predator in the system, probably depended on the search for food and shelter against piscivorous waterfowl.

According to this field evidence, the seasonal density and the horizontal distribution of grazer zooplankton were apparently conditioned by complex interactions with the potential predators, and not by changes in plant cover as suggested for temperate eutrophic lakes (Jeppesen et al. 1997b). These results indicate that submerged plants in subtropical eutrophic systems might offer some refuge for zooplankton, but only under circumstances of low-intermediate fish and invertebrate predator densities.

c. Refuge effect for cladocerans in subtropical and temperate lakes along a turbidity gradient

In the comparative field study we found that the free-swimming cladocerans displayed, in general, very different diel patterns in both climate zones (paper III). In the temperate lakes, we found higher relative densities of cladocerans during the day (60.7% ±7.4 SE), while densities were higher (61.0% ±9.1 SE) during the night-time in the subtropics. In the temperate zone, the most frequently observed spatial patterns by the population of free-swimming cladocerans fit with the predictions of the classic DHM: the densities in the submerged decreased during the night and increased slightly in the open water (Fig. 6). In the subtropical lakes the spatial distribution of the free-swimming cladocerans differed remarkably. We found clear signs of the classic DHM in only one lake, whereas we found significant evidence of diel vertical migration in three lakes: densities increased in the night-time in all habitats. These DVM patterns occurred at both extremes of the turbidity gradient in the subtropics, while in the temperate zone DHM seemed to occur at all turbidity levels, except in the clearest lake where the total density of free-swimming cladocerans exhibited a reverse horizontal migration pattern (RHM: higher nocturnal densities in the free-floating and submerged plants; lower densities in open water).

The predator *Chaoborus* spp. represented the only taxa that displayed the same behaviour (DVM) under all environmental conditions (climate and turbidity). Otherwise, a broad set of responses was displayed by the same taxa, both across and within each climate zone. In some cases, the behaviour of the individual taxa most susceptible to predation (in terms of size and abundance) differed from the average population pattern (Table 1 in paper III). However, when considering the behaviour of individual taxa, some general patterns emerged. Firstly, the response of the largest cladoceran in each lake was in most cases DVM (in seven out of ten lakes). This evidence suggests that, in most lakes in both climate regions, the largest cladocerans are located on or near the sediments during the day and thereby avoid visual predators (DeStasio 1993). Secondly, DHM represented the commonest migration performed by individual taxa in the temperate lakes (75% of the statistically significant patterns), whereas DVM held this place in the subtropics (71% of the significant responses).

Our results indicate that DVM often occurs simultaneously with DHM, however, DVM seems the commonest response of individual and population cladocerans when the predation risk (or the perception of predation) is very high, while DHM is found under conditions of less predation (or perception) risk. In the temperate lakes, submerged plants acted as refuge for most cladoceran taxa and for the average cladoceran populations under mesotrophic and eutrophic conditions, but not under oligotrophic conditions, which partially agrees with the model proposed by Jeppesen et al. (1997b). Our results also support the hypothesis that DHM is not so prevalent in the subtropics, likely because the high abundances of small littoral fish eliminate the macrophyte refuge (Burks et al. 2002). *Daphnia* cannot survive where abundant fish nullify the refuge provided by the plants (Venugopal and Winfield 1993). Accordingly, almost no large-bodied cladocerans were found in the subtropics (paper IV).

3. Littoral trophic structure and plant architecture in temperate and subtropical shallow lakes

Aquatic plants exert a multiple effect on the structure and functioning of shallow lakes by affecting the communities that live permanently or temporarily in the littoral area. The structure of the fish community in the lake, however, strongly regulates their effects (Jeppesen et al. 1997a). Fish, due to their mobility and flexible feeding behaviour, link the littoral, benthic, and pelagic habitats in a much more significant manner than historically considered (Schindler and Scheuerell 2002, Vander Zanden and Vadeboncoeur 2002), and thus affect the nutrient transport and predator-prey interactions in the littoral and the pelagial.

To test the hypotheses that: 1) fish impact the littoral trophic structure more strongly, 2) trophic cascades are more truncated in the warmer lakes, and 3) plant architecture affects trophic interactions, we introduced artificial plant beds mimicking different plant architectures (submerged vs. free-floating) in ten shallow lakes along a turbidity and nutrient gradient in Uruguay and Denmark. We studied the structure of the key associated communities (fish, zooplankton, macroinvertebrates, and periphyton). The use of artificial substrata may spark debate because it implies the loss of chemical interactions; nevertheless, we wanted to compare the structure and dynamics of associated communities given the environmental factors, and this therefore required identical initial conditions of the substratum quantities and quality. Part of the data was analysed in more detail for a subset of 5 lakes in each country, paired in terms of Secchi disk transparency and with similar size, total phosphorus concentrations, and macrophyte cover within the pairs (paper IV).

a. General trophic characteristics of the littoral area in contrasting climates

Notable differences in littoral trophic structure and dynamics between both climate zones occurred, regardless of the gradient in water transparency and other environmental variables (such as nutrient status and lake area) (Fig. 7).

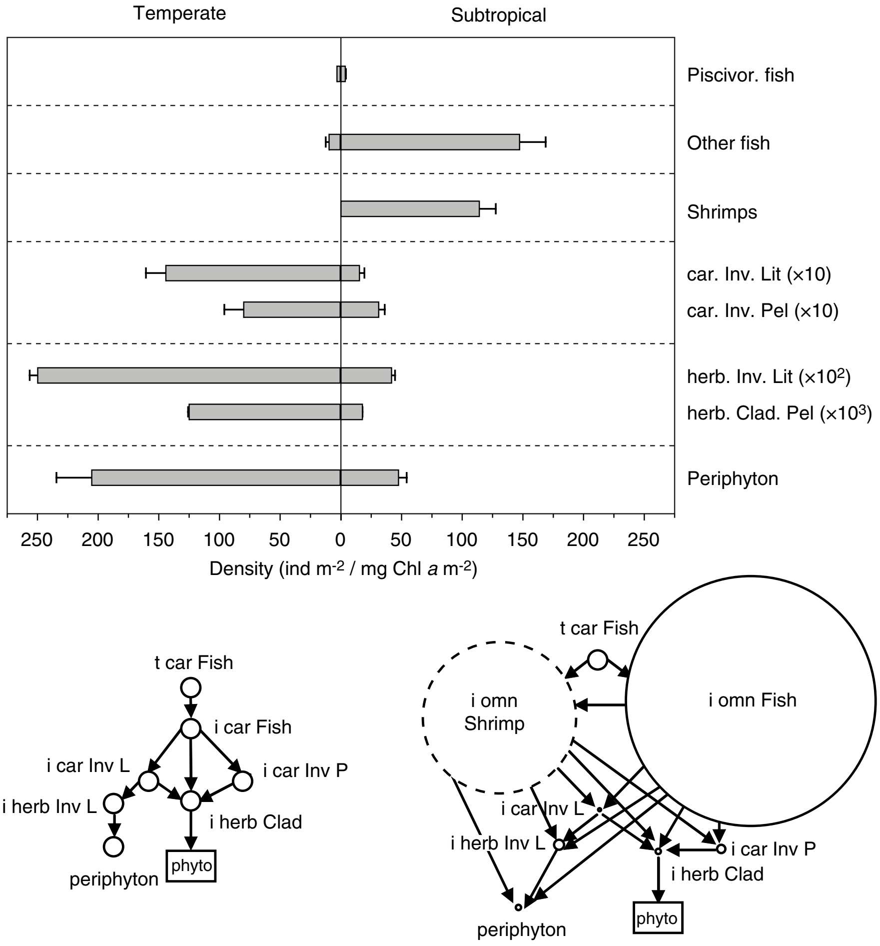

Fig. 7. Main communities' structure in artificial plant beds in temperate (left) and subtropical (right) lakes. Above: Density of potentially piscivorous fish, all other fish, shrimps, littoral predatory invertebrates (car. inv. Lit), pelagic predatory invertebrates (car. inv. Pel), littoral herbivorous macroinvertebrates (herb. inv. Lit), pelagic herbivorous cladocerans (herb. Clad. Pel), and biomass of periphyton. Below: Simplified scheme of trophic interactions among the same trophic groups. The densities in the subtropics are expressed relative to those in the temperate lakes (considered as the unit). Trophic classification (Arim and Marquet 2004): plants (periphyton), intermediate herbivores (i herb), intermediate carnivores (i car), intermediate omnivores (i omn) and top carnivores (t car). Clad: cladocerans, Inv: invertebrates, P: pelagic, L: littoral. Except fish, the same taxa share the same trophic classification in both climate zones. Shrimp relative density is dotted due to shrimp absence in the temperate lakes. Phytoplankton was fixed in the study. Data are sample means of five selected lakes (± 1 SE). (Modified from paper IV).

As expected from other studies (Agostinho et al. 2003), fish species richness in the plant beds was significantly higher in the subtropical lakes. We found a total of 21 fish species among the artificial plant beds in the ten lakes in Uruguay, but only 5 in the Danish lakes. The subtropical fish communities were amply dominated by small cyprinodontids, the most abundant species being the ten-spotted live-bearer (*C. decemmaculatus*) and the one-sided livebearer (*J. multidentata*). The other fish belonged to the perciform (family Cichlidae), siluriform, characiform, and synbranchiform orders. In the temperate lakes, the most abundant species in the plant beds were the cyprinids rudd (*Scardinius erythrophthalmus*) and roach (*Rutilus rutilus*), while perch (*Perca fluviatilis*) occurred most frequently. The piscivore pike (*Esox lucius*) and the mainly benthivore crussian carp (*Carassius carassius*) also appeared, but in very low frequencies (Fig 8). The fish communities in the subtropical lakes were characterised by significantly higher densities, higher biomass, and smaller size. Whereas less than 40% of the temperate fish measured smaller than 3.5 cm standard length, almost 90% did so in the subtropics (Fig. 9). On average, the fish in the plant beds were 11-fold more abundant in the subtropics than in the temperate lakes. Furthermore, extremely high fish densities in the plant beds occurred in the most eutrophic lake in the subtropical set (above 1000 ind m^{-2}). Fish in both countries were classified into trophic groups following the same criteria (using published data) (Table 1). In the subtropical lakes we found five big trophic groups, while in the temperate lakes we found three, including piscivores, which were absent in the former. A much lower relative abundance of potential piscivores (juvenile of adult piscivores) occurred in the plant beds in the subtropics.

By contrast, and against our *a priori* expectations, we found higher richness (families and genera) and significantly higher densities of plant-associated macroinvertebrates in the temperate lakes, both per unit of plant weight and per unit of area of plant cover. We found more than 40 different taxa (mostly identified to genus level) in the temperate systems, and less than 30 in the subtropics (Fig. 10). For example, we found a total of 19 genera of chironomids (4 of those predatory) in the temperate lakes, but only 7 in the subtropical lakes. The size range of the chironomids was large in the temperate systems (from 0.5 to 19 mm), whereas most chironomid larvae in the subtropics had a narrow size range (*ca*. 13-15 mm) (paper in prep. B). We found *ca*. 8-fold higher densities of plant-associated predators, 10-fold of grazers, and 2-fold of collectors in the temperate lakes. However, large-bodied macroinvertebrates, such as shrimps (*P. argentinus*), crabs (*Aegla* sp), and channelled applesnails (*Pomacea canaliculata*),

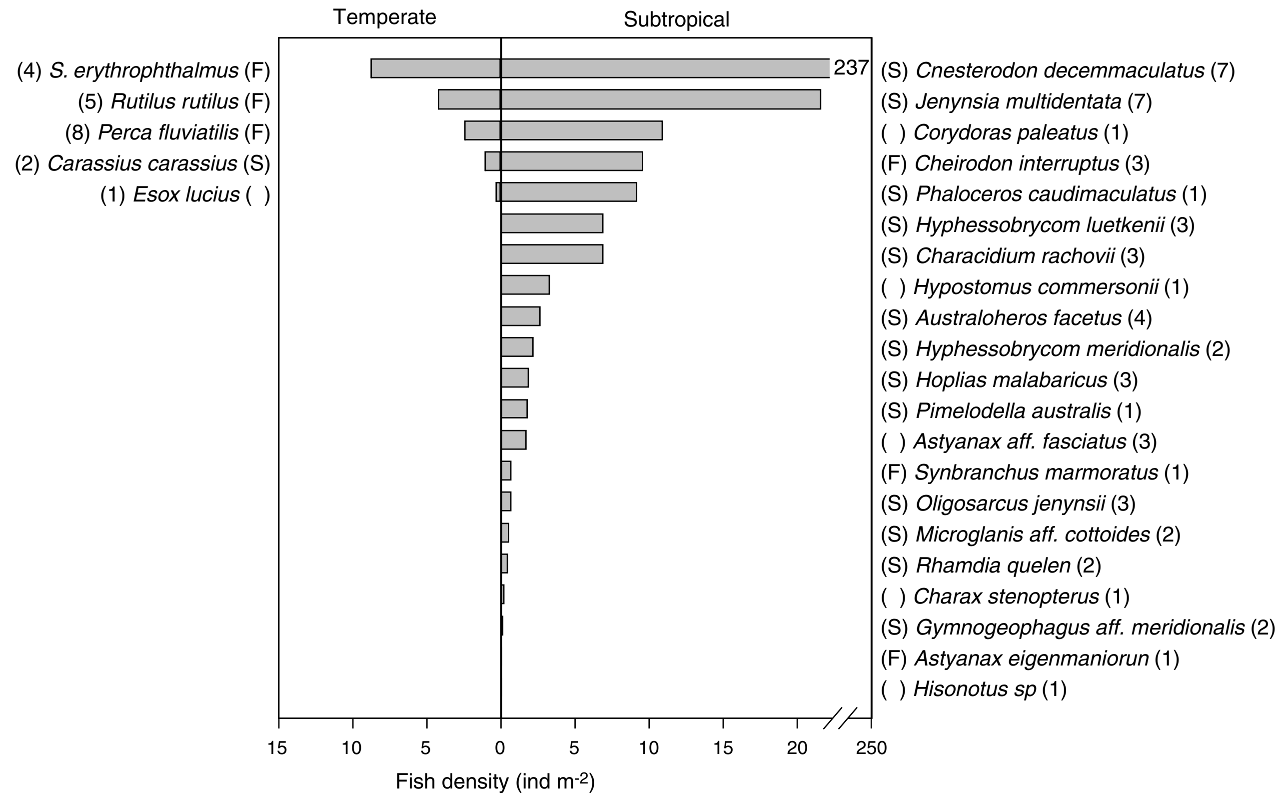

Fig. 8. Mean density of fish species in artificial plant beds in temperate (left) and subtropical (right) lakes, ordered by decreasing density. The letters indicate the habitats with the highest density (F: free-floating, S: submerged, otherwise no preference detected), and the numbers indicate the number of lakes in which each species was present, over a total of 9 and 10, respectively. The means represent the average density considering the number of lakes in which each species was present.

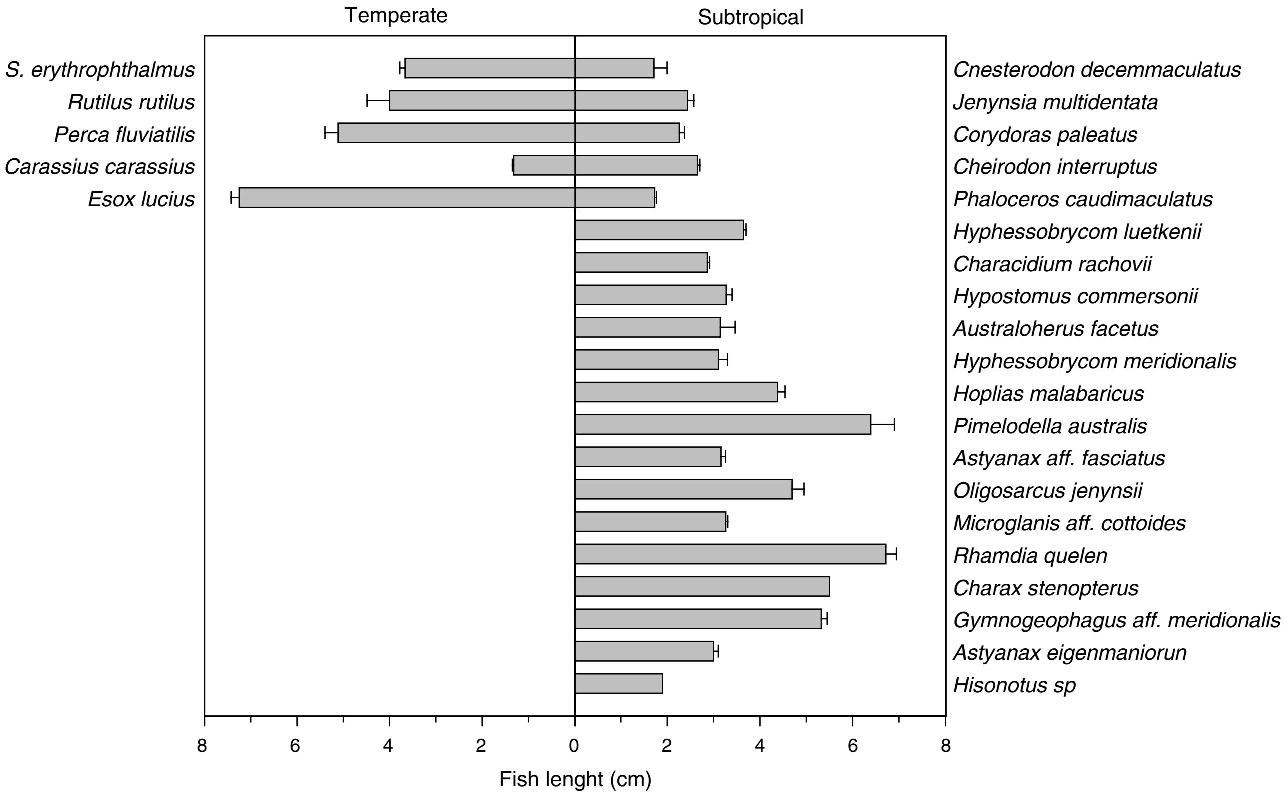

Fig. 9. Size of fish species (mean standard length ± 1 SE) in the plant beds in temperate (left) and subtropical (right) lakes, ordered by decreasing abundance.

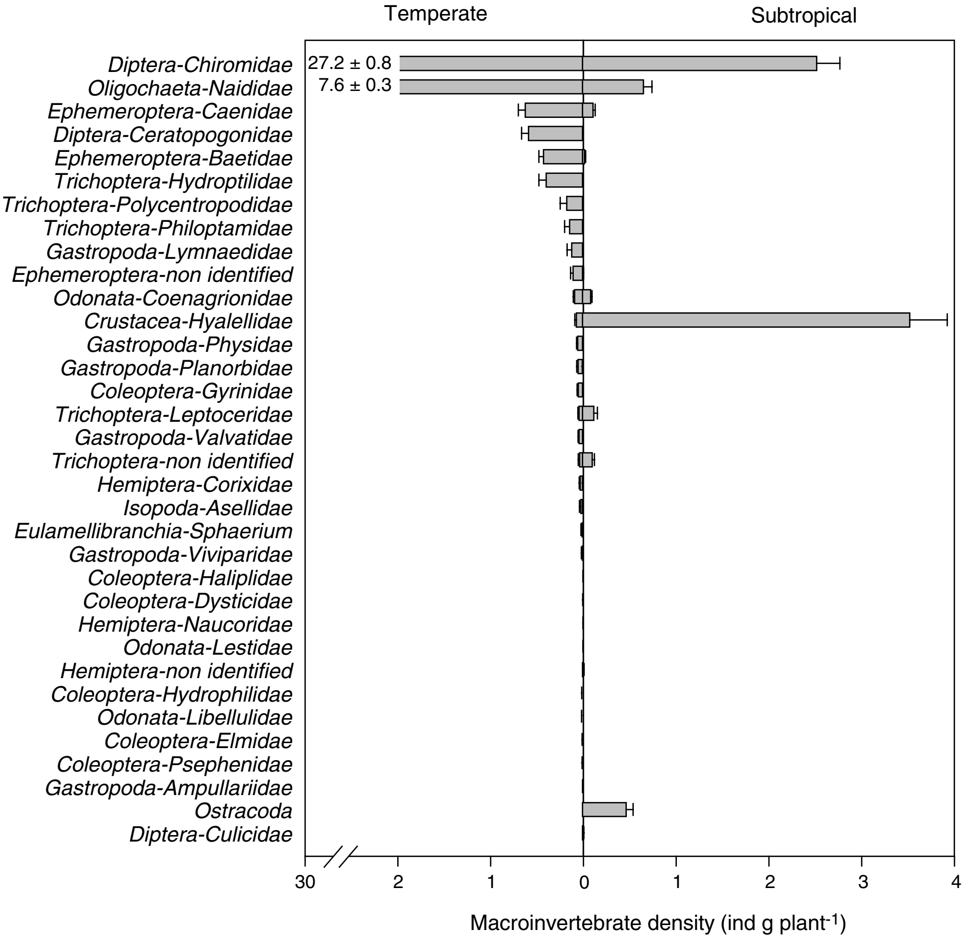

Fig. 10. Mean density of littoral macroinvertebrate families in artificial plant beds in temperate (left) and subtropical (right) lakes. Data represent sample means of 9 and 10 lakes, respectively (± 1 SE).

Trophic group	T	S	Species
Piscivorous	x	-	*Esox lucius*
Omnivore-benthi-piscivorous	-	x	*Australoheros facetus* >3cm
Omnivore-benthi-planktivorous	-	x	*Cnesterodon decemmaculatus* *Jenynsia multidentata* *Cheirodon interruptus* *Phaloceros caudimaculatus* *Hyphessobrycom spp* *A. facetus* <3cm * *Pimelodella australis* *Astyanax spp* *Charax stenopterus*
Omnivore-benthivorous	-	x	*Microglanis aff. cottoides* *Rhamdia quelen* *
Benthi-herbivorous	-	x	*Hypostomus commersonii* *Hisonotus sp*
Benthi-planktivorous	x	x	*Perca fluviatilis* >8cm* (T) *Carassius carassius* (T) *Corydoras paleatus* *Hoplias malabaricus* * *Characidium rachovii* *Symbranchus marmoratus* * *Oligosarcus jenynsii* *
Planktivorous	x	-	*Scardinius erythrophthalmus* *Rutilus rutilus* *Perca fluviatilis* <8cm *
Potentially piscivorous	x	x	*

Table 1. Trophic classification of the fish associated to the artificial plant beds in temperate (T) and subtropical (S) lakes (9 and 10 respectively). We used published data and followed the same criteria: piscivorous= feeding on fish, omnivorous= feeding on primary producers and animals, benthivorous= sediment dwellers and/or feeding on invertebrates, herbivorous= feeding on plants, periphyton or algae, planktivorous= feeding on zooplankton, potentially piscivorous= adults feed on fish. The final classification involves the main items reported in the diet of each species according to the size of the fish found. The species inside each group are ordered by decreasing density (in the respective climate zone).

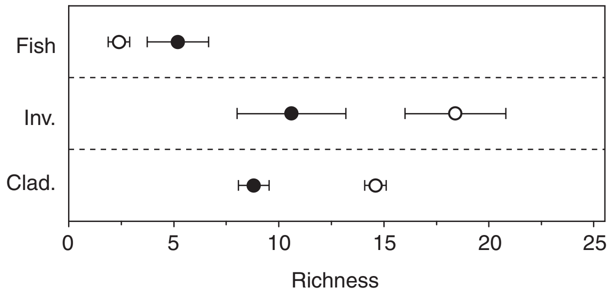

Fig. 11. Diversity cascade in artificial plant beds in temperate and subtropical lakes. Data represent sample means of fish species, littoral macroinvertebrate genera, and cladoceran genera in a subset of five lakes (± 1 SE). (From paper IV).

only occurred in the subtropics. Except for *Pomacea*, no other snails were found in the subtropical lakes, while several small/medium-bodied genera occurred in the temperate systems.

Cladoceran richness and density (total and free-swimming genera) were also higher in the temperate systems. The taxa not found in the subtropics included large-bodied cladocerans, such as the predators *Leptodora kindtii* and *Polyphemus pediculus*, and the plant-attached *Sida crystallina* and *Eurycercus lamellatus*. The typical pelagic cladoceran community in the subtropical lakes was composed of the small-bodied *Bosmina*, *Moina*, *Diaphanosoma* and *Ceriodaphnia* spp, the only large-bodied taxa being *Simocephalus* spp (Fig. 1 in paper III). We found much higher densities of cyclopoid than calanoid copepods in the temperate lakes, while in the subtropics both groups have similar average densities. In most lakes we found some invertebrate predators on zooplankton in the pelagial, thus adding to the predation pressure exerted by fish. The predatory invertebrate *Chaoborus* spp appeared more frequently and with slightly higher densities in the subtropical lakes. The densities of the cladoceran pelagic predators in the temperate lakes followed a pattern quite opposite to that of *Chaoborus* sp (Fig. 2 in paper III).

Interestingly, periphyton biomass was also significantly higher in the temperate lakes, despite the lower solar irradiance and the higher densities of macroinvertebrates (Fig. 7). We found no significant relationship between periphyton biomass (Chl *a*) and nutrient state or water turbidity in the lakes, as has also been reported in other studies (Jones et al. 2002, Vadeboncoeur et al. 2003).

We found signals of a diversity cascade in the littoral zone of the lakes, with different signs across climate zones. More fish species co-occurred with less cladoceran and less macroinvertebrate genera in the subtropical lakes, while the opposite happened in the temperate lakes (Fig. 11). Freshwater lakes show a weaker latitudinal diversity gradient than other ecosystems (Hillebrand 2004). However, several studies show an increase in species richness in many taxon groups (e.g. fish, dragonflies, and beetles) with decreasing latitude, suggesting temperature as the main explanatory factor (Heino 2002). Other environmental factors, such as lake area, are very important for fish richness. However, fish richness increases faster with lake area in the tropics and subtropics than in temperate areas (Amarasinghe and Welcomme 2002). In the case of cladoceran richness, there is still some controversy about the diversity patterns in (sub)tropical versus temperate areas (Dumont 1994, Fernando and Paggy 1997), but the view that cladoceran diversity is low in the tropics is currently being challenged (Sarma et al. 2002). However, regardless of the total number of taxa, large-bodied pelagic cladocerans and particularly predatory genera are usually absent in tropical aquatic systems (Fernando 2002), which agrees with the cladoceran community structure we found in the littoral area of the subtropical lakes. A lower abundance of *Daphnia* spp seems common in the (sub)tropics (Mazumder and Havens 1998, Korínek 2002, Pinto-Coelho et al. 2005), although remarkably rich *Daphnia* communities have been found in the sediment records of particular lakes (Mergeay et al. 2004). The finding of ephippia or remains of *Daphnia* and other large cladocerans in the sediments of (sub)tropical lakes (Karina Jensen unpub. data) indicates that these lakes are able to sustain, but seemingly not to maintain, large cladocerans. Therefore, although very important for cladoceran physiology and competition (Moore et al. 1996), higher temperature itself does not seem to directly explain the low abundance and size of cladocerans in the (sub)tropics. Other environmental factors affected by the high temperatures, such as fluctuations in oxygen concentrations or even anoxia under the plant beds, could affect diversity patterns in warm lakes. However, and in agreement with previous suggestions (Dumont 1994, Fernando 2002), our results strongly support the idea that temperature-related selective forces, namely higher predation, are the most important factors for the observed patterns in the structure of these littoral communities.

b. Effects of plant architecture on fish

More recent studies identify the importance of plant architecture for the spatial use of fish (Lewin et al. 2004, Okun and Mehner 2005).

We found that submerged and free-floating plants exerted different effects on the spatial distribution of fish, with important differences between the climate zones (paper IV & paper in prep. C). Temperate fish seem to display a more pelagic behaviour, as indicated by their preferential use of free-floating plants (with less physical structure in the water column). In contrast, subtropical fish displayed a more littoral behaviour, with a significant preference for the submerged plants. This pattern appeared independent of water turbidity. The more littoral behaviour in the subtropical lakes was displayed by the vast majority of the species found, and *vice versa* in the temperate lakes (Fig. 8). Subtropical shrimps (included here for having a similar ecological role to fish), also exhibited higher densities in the submerged than in the free-floating plants, as had been found in a previous single lake study (Meerhoff et al. 2003).

The number of fish species or the length of the fish did not differ significantly between plant types. The different trophic groups in each climate region did not show significant differences in the use of both plant bed types: all groups had higher densities in the submerged or in the free-floating plants, in the subtropical and temperate lakes, respectively. However, in the subtropical lakes there was a tendency of smaller fish to associate preferably with the submerged plants (Fig. 12), regardless of water transparency or TP gradients. In the temperate set, smaller-sized fish occurred also in the submerged plants in some of the lakes with higher transparency. The occurrence of juvenile or smaller fish in the littoral area can be seen as a trade-off between feeding opportunities and safety. More vulnerable size classes avoid exposed habitats in the presence of predators and use dangerous habitats less than larger fish, at the expense of feeding quality (Werner et al. 1983). Our results therefore suggest that submerged plants represent a better refuge for small fish than free-floating plants in both climate regions. The structural complexity of the habitats can explain more of the spatial distribution of juvenile temperate fish (roach and perch) in the littoral area than several other factors, including the biomass of potential food organisms (Lewin et al. 2004). Habitats with more complex structure reduce the feeding efficiency of some of the most common temperate piscivorous fish (adult perch), thus minimizing the pre-

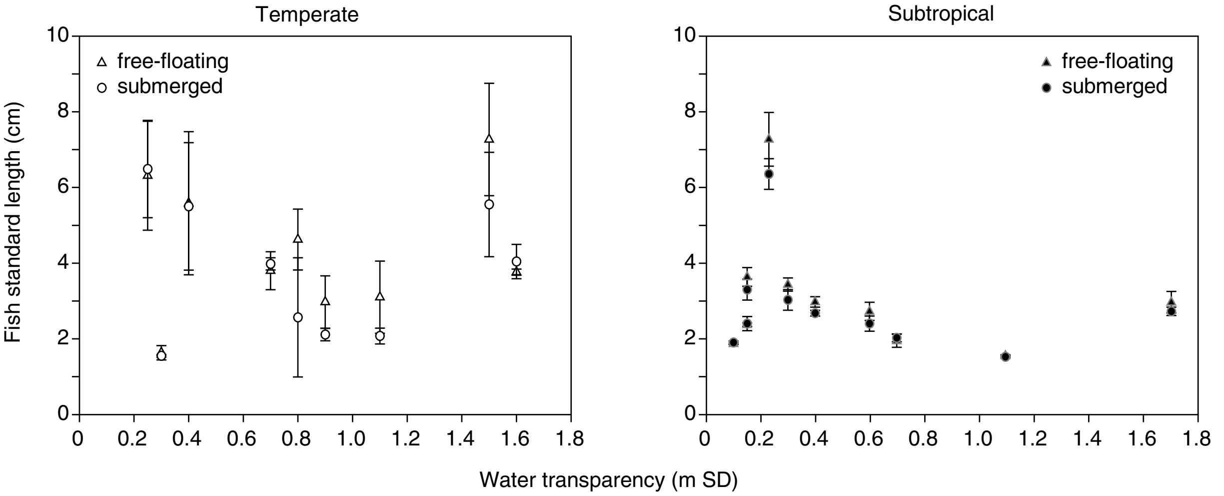

Fig. 12. Mean size of fish associated to free-floating and submerged artificial plants, in temperate (left) and subtropical (right) lakes along a turbidity gradient (Secchi disk depth). Data represent sample means in 9 and 10 lakes respectively (± 1 SE).

dation risk for juvenile or small fish (Persson and Eklöv 1995). In the subtropics, the refuge for the smaller fish seems needed under all environmental conditions, likely due to the very high numbers of fish in the systems. However, we observed that high water turbidity may nullify the refuge advantage of the submerged plants in the temperate lakes. Our field results agree with behaviour experiments where the use by juvenile perch of plants decreased with increasing water turbidity (Snickars et al. 2004), likely because high water turbidity can impair fish predation and therefore protect juveniles (Pekcan-Hekim and Lappalainen 2006).

The spatial distribution of fish can have important consequences for the spatial distribution of their preys, which can modify the expected outcome of direct interactions (Jacobsen et al. 1997, Romare and Hansson 2003). The phenomenon is described as "behavioural cascade" in opposition to the classic "trophic cascade" concept that implies direct effects of fish on abundance of lower trophic levels due to predation on the intermediate levels (e.g. zooplankton and macroinvertebrates). The following sections describe the ways in which plant architecture affected these phenomena in our study.

c. Effects of plant architecture on fish-zooplankton interactions

We found higher densities of cladocerans among the submerged than in the free-floating plants, in both climate zones. The magnitude of the difference in density between plant architectures varied inside each climate region and with turbidity. In the temperate lakes, the use of free-floating plants by cladocerans followed diel trends and densities intermediate between submerged plants and open water. In contrast, in the subtropical lakes the free-floating plant beds had a constantly lower relative density than did the submerged plants or open water, with no significant diel changes. These patterns occurred for both the total free-swimming cladoceran densities and also for individual cladoceran taxa (paper III).

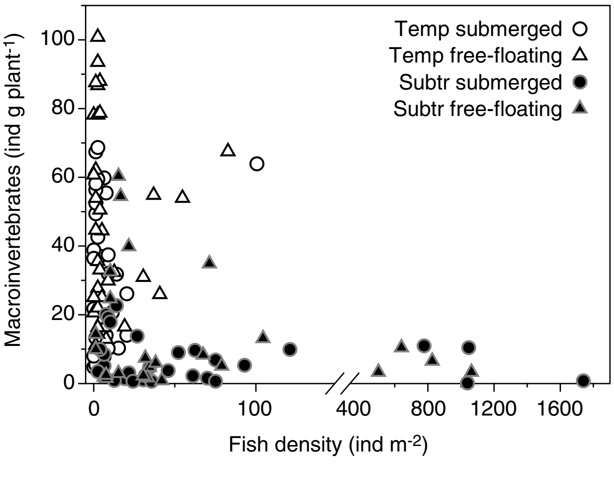

Fig. 13. Relationship between plant-associated macroinvertebrate (i.e. shrimps are excluded) and fish densities in artificial free-floating and submerged plants in temperate (9) and subtropical (10) lakes.

Despite hosting relatively lower densities of fish than the submerged plants, cladocerans in the subtropics particularly avoided the free-floating plants, thus confirming previous single-lake studies with real plants (water hyacinth), where crustaceans occurred in lower densities under free-floating plants than among submerged plants (Meerhoff et al. 2003) or in open water (Brendonck et al. 2003). Our results also give strong field support to the laboratory behavioural experiments (paper I). The daphniid rejection to the free-floating plants seemed to be mediated both visually and chemically (root exudates) in the laboratory experiments (paper I). Chemically-mediated avoidance of plants had also been found in other studies (Burks et al. 2001b). However, the rejection could be only visually mediated in the field experiments, as we used plastic plants. Although these last results give more support to the visually-mediated rejection, they are still inconclusive to clarify the ultimate causes behind this strong avoidance behaviour (such as a relatively high predation risk plus low oxygen concentrations, as occurs under dense real floating plant beds).

In the temperate lakes submerged plants offered better diel refuges for cladocerans than free-floating plants. In the subtropical lakes, a refuge effect of free-floating plants seems completely absent. The field studies described above (papers III, IV & VI) suggest that, in subtropical systems, submerged plants offer only a weak refuge for zooplankton and only under particular circumstances exhibiting low-intermediate fish and invertebrate predator densities. Both field studies indicate the particular lack of refuge effect of submerged plants in summer, when it is most needed due to the increased densities of fish. The predator-avoidance behaviour often described for shallow temperate lakes, i.e. diel horizontal migration (Timms and Moss 1984, Burks et al. 2002), seems thus clearly insufficient in the subtropics. Although there was evidence of diel vertical migration in several subtropical lakes, the density and composition of the cladoceran community indicates that no predator-avoidance behaviour suffices to counteract the effects of the high predation pressure. Refuge-mediated positive effects of aquatic plants on water transparency might therefore be weak or rare in warm lakes.

d. Effects on fish-macroinvertebrates-periphyton interactions

Direct or indirect positive effects of fish on periphyton biomass have been registered under different environmental conditions (Liboriussen et al. 2005). Fish are considered the main determinants in community structure in shallow lakes, through, among other mechanisms, their positive cascading effects on periphyton via invertebrates (Brönmark and Weisner 1992, Jones and Sayer 2003), and the consequent deterioration of the light climate for the submerged plants (Phillips et al. 1978). The strength of trophic cascades in the littoral zone may therefore determine the fate (maintenance or loss) of the plants (Jones and Sayer 2003, Burks et al. 2006).

As expected, the densities of plant-associated macroinvertebrates negatively related to the densities of fish in both countries in our study, but even more so in the subtropical lakes (Fig. 13). The taxonomic diversity and density of the epiphytic macroinvertebrates are related to the growth form of the host plants, but generally their density is positively related with the abundance of submerged plants (Diehl and Kornijów 1998). Our study concurred, as the submerged plants hosted more macroinvertebrates per unit of area of plant cover than the free-floating plants. Interestingly, however, in both countries the free-floating plants had higher densities of plant-associated macroinvertebrates per unit of plant weight. The observed pattern proved consistent along the turbidity gradient, and was particularly pronounced from the intermediate turbidity levels and upwards. The macroinvertebrate community associated to the roots of water hyacinth has been described as abundant and taxon-rich (Takeda et al. 2003). By contrast, in floodplain lakes of the Paraná River system (Argentina), the lowest densities of macrobenthos were found in lakes with a full cover of free-floating plants (Bechara 1996), probably due to low oxygen concentrations. We found no significant difference regarding the total number of macroinvertebrate taxa between plant types inside each climate region. However, we found a different use of the plants by functional groups of macroinvertebrates: whereas collectors and predators had higher densities per unit of plant weight in the free-floating plants in both climate regions, a much higher density of grazers (mainly snails) occurred in the submerged plants in the temperate lakes (Fig. 14). These patterns are likely related to food availability for grazers and collectors (more periphyton biomass in the submerged plants in the temperate lakes; probably better settling conditions for particulate matter under the floating plants), and predation avoidance for predatory invertebrates (more shading under the floating structures).

In the subtropical lakes, the free-floating plants supported a higher biomass of periphyton per unit of weight than did the submerged plants, while the opposite was observed in the temperate lakes. This pattern remained consistent along the turbidity gradient. The plants could affect periphyton development by physical processes. Under dense mats of floating plants, water turbidity usually decreases (Rodríguez-Gallego et al. 2004), both due to decreased light and nutrient availability for phytoplankton, and to the mechanical retention of algae and suspended solids in the dense root network of *E. crassipes* (Poi de Neiff et al. 1994).

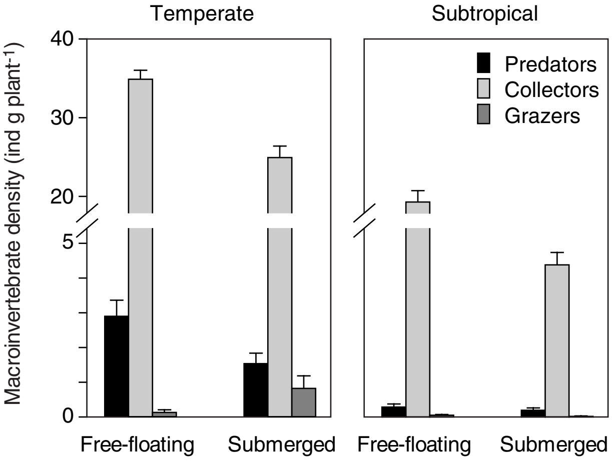

Fig. 14. Density of littoral macroinvertebrate trophic groups in artificial free-floating and submerged plants in temperate (left) and subtropical (right) lakes. Data are sample means of a subset of five lakes (± 1 SE).

Contrary to the expectations based on various studies (Jones and Sayer 2003, Burks et al. 2006), we did not find a negative relationship between the density of grazers and the biomass of periphyton (Fig. 15). Rather, we found signs of a positive relationship between the density of macroinvertebrates (both total and grazers) and periphyton biomass, more in agreement with other findings (Liboriussen and Jeppesen 2006). Moreover, we found much lower periphyton biomass in the subtropical lakes than expected by the release of grazing due to high predation on invertebrates, and by the more positive environmental conditions (more light and higher temperature) for periphyton growth. The net effects of omnivorous consumers on periphyton can vary greatly as several mechanisms act simultaneously, such as consumption, nutrient regeneration, physical stimulation, and trophic cascades (Geddes and Trexler 2003). The observed 4-fold reduction in periphyton biomass in the subtropical lakes seems likely the result of periphyton feeding by the fish and shrimps (maybe enhanced by the applesnails in some of the lakes). Fish could also negatively affect periphyton biomass by producing physical disturbance, as suggested by the opposite patterns of spatial distribution by fish and periphyton between the two types of plants in both climate zones (Fig. 16). This apparently overruled the positive effect of fish on periphyton through the control of the plant-attached macroinvertebrate grazers and potential nutrient regeneration. However, the lower periphyton biomass in the subtropics represents much reduced shading and nutrient and carbon competition with the host plants, compared to the temperate lakes. These results suggest that aquatic plants can potentially develop in the (sub)tropics under more turbid conditions than submerged plants in comparative temperate lakes. We need further experimental studies designed to test this idea.

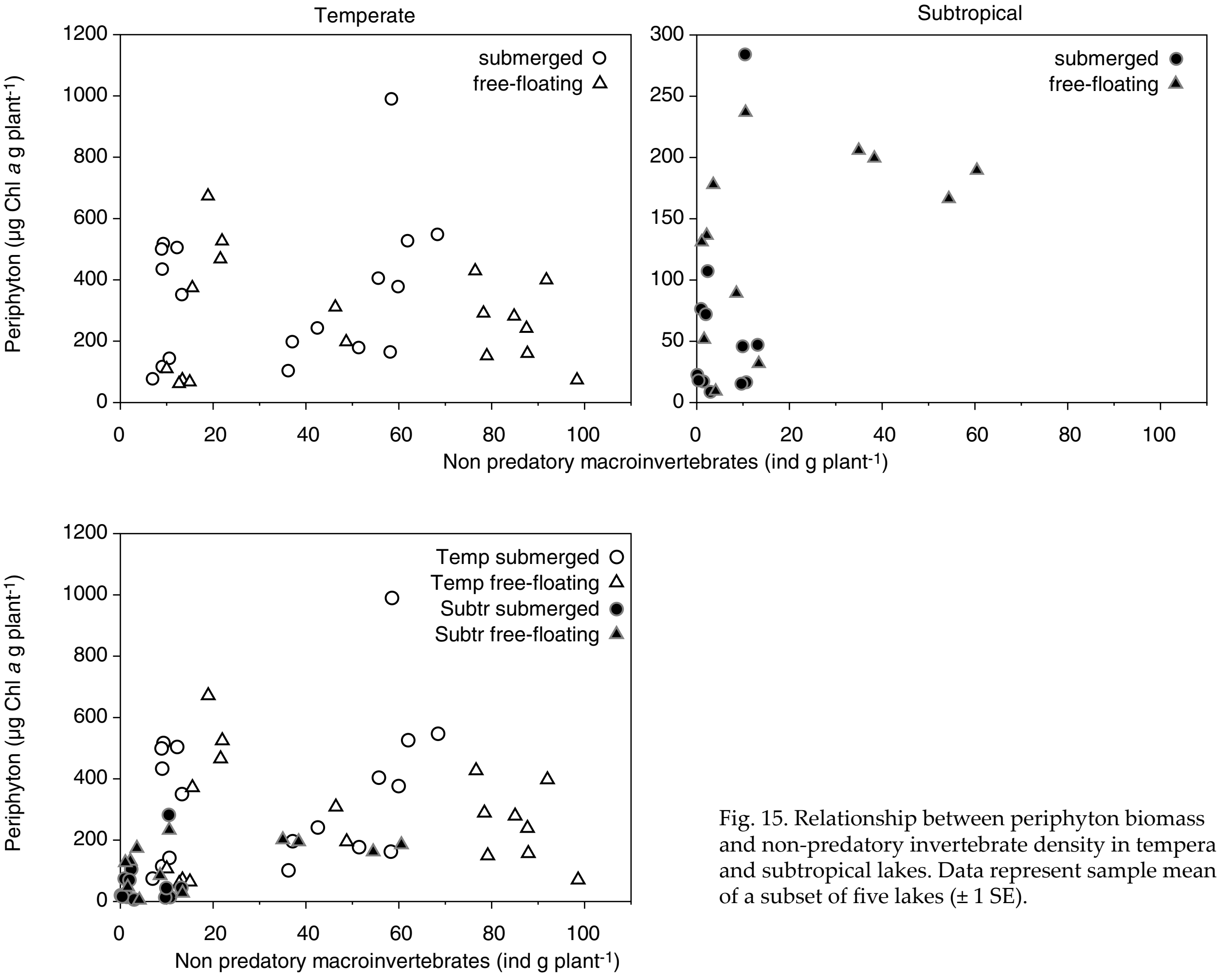

Fig. 15. Relationship between periphyton biomass and non-predatory invertebrate density in temperate and subtropical lakes. Data represent sample means of a subset of five lakes (± 1 SE).

e. The role of plant architecture in trophic and behavioural cascades

Trophic cascades (indirect effects of carnivores on plants mediated by herbivores) may have profound impacts on the structure and functioning of ecosystems. Although described in a variety of environments (Pace et al. 1999), several studies suggest they are stronger in aquatic systems (Strong 1992, Shurin et al. 2002). The occurrence of cascading effects is generally accepted in lakes in the temperate region, although their strength may vary along a nutrient gradient (Jeppesen et al. 2003a).

Several indicators of fish predation pressure (Jeppesen et al. 2002a), involving different trophic groups, indicated a much higher predation pressure in the plant beds in the subtropical lakes (Fig. 17). Support to this assertion included the higher relative density of planktivorous fish, larger snails (and therefore less sensitive to predation by small fish), oligochaetes (relative to chironomids), and *Bosmina* spp (relative to *Daphnia* spp). The lower abundance of zooplankton and macroinvertebrates, the dominance of zooplankton by small-bodied taxa, the more frequent occurrence of large-bodied taxa within the macroinvertebrates, and the contrasting behaviour of cladocerans, indicate that predation pressure is clearly stronger among the plants in the subtropical than in the temperate lakes, as an obvious consequence of the high fish (and likely also shrimps) densities. As we found in this study, large invertebrate omnivores, such as shrimps, can be abundant in the subtropics (Collins 1999). This is probably the result of the small average size of the fish communities. The same phenomenon occurs in brackish lakes in the temperate zone, where *Neomysis integer* coexists with the dominant stickleback *Gasterosteus aculeatus* but is largely suppressed if bigger fish are present. This trophic structure leads to strong cascading effects promoting increased water turbidity despite high abundance of submerged plants (Jeppesen et al. 1994, Jeppesen et al. 1997b).

According to our results, the potential for trophic cascades in the littoral zone seems more truncated in subtropical lakes, as the effects of the high densities of fish in the plants could be traced at all the studied trophic levels, from cladocerans and macroinvertebrates to periphyton.

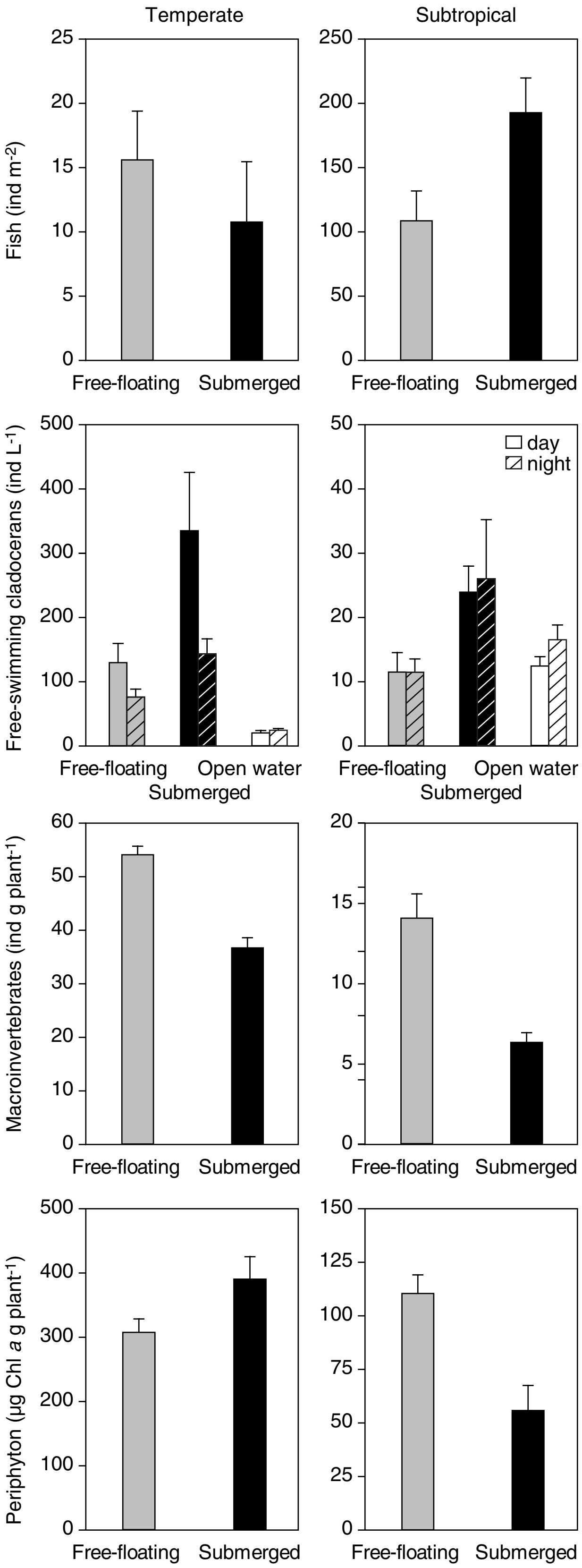

Fig. 16. Spatial distribution of main communities in the submerged and free-floating plant beds in temperate and subtropical lakes. For cladocerans, also the densities in open water and diel variations are shown. The data represent the sample means of a subset of five lakes (within each turbidity level and climate zone) ± 1 SE. (Modified from paper IV).

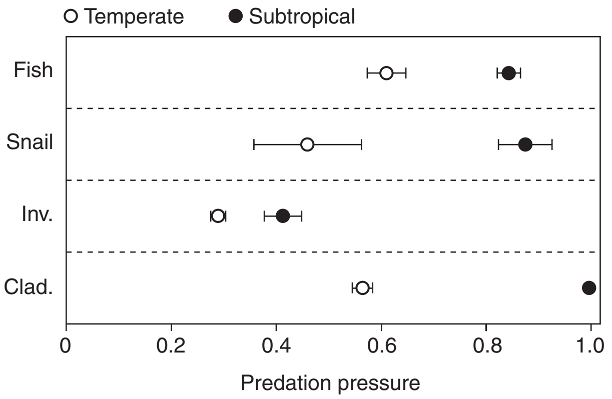

Fig. 17. Predation pressure in the plant beds in temperate and subtropical lakes, considering the density of fish (ratio of planktivores to total fish density), snails (ratio of large snails to total snail density), invertebrates (ratio of oligochaetes to sum of oligochaetes plus chironomids), cladocerans (ratio of *Bosmina* to sum of *Bosmina* plus *Daphnia* density). In all cases, the closer to 1 is the ratio, the higher the predation pressure. The data represent the sample means of a subset of five lakes ± 1 SE, except in the case of the last index, as not even *Bosmina* was found in 2 out of the 5 lakes in Uruguay. (From paper IV).

Our experimental design did not allow a test for "behavioural cascades" separately from "trophic cascades". However, some results indicate the occurrence of behavioural cascades at some trophic levels. In temperate systems, the more abundant fish in the free-floating plants co-occurred with higher densities of macroinvertebrates (per weight of plant), lower densities of pelagic zooplankton and lower biomass of periphyton (opposite pattern in the submerged plants), suggesting a spatial separation between fish and zooplankton (likely behaviourally-mediated), and between invertebrates and periphyton (grazing-mediated). In the subtropical systems, the more abundant fish in the submerged plants co-occurred with lower densities of macroinvertebrates, higher densities of zooplankton and lower biomass of periphyton, suggesting a cascade (predation-mediated) between fish and invertebrates, and fish and periphyton (Fig. 16). We have thus confirmed that the architecture of macrophytes is very relevant to their impacts on trophic dynamics, and that those impacts differ under different climates.

4. Restoration strategies of shallow warm(ing) lakes

On the basis of the results obtained from my PhD, I expect to contribute to the development of more appropriate strategies for the restoration of nutrient-enriched shallow lakes, particularly of those located in tropical and subtropical zones. Below I will present an extended summary of the ideas discussed in a book chapter (paper VII) and in two papers currently in press (papers VIII & IX) that review the current knowledge of lake restoration with focus on the role of nitrogen (N), lake size, and climate.

Much research effort has been directed at the development of restoration techniques to recuperate the clear water plant-dominated state in eutrophic lakes through nutrient control and manipulation of fish communities (Moss et al. 1996). From the 1960s to the 1990s, the strategies to improve the ecological quality of shallow lakes in Europe and North America gradually evolved from simply controlling the input of total phosphorus (TP) towards a more comprehensive and ecosystem-based approach. This includes the control of non-point nutrient sources (mostly from agriculture) and the application of in-lake measures such as dredging, flushing and biomanipulation (Hosper 1997). However, most practical measures still focus on the reduction of the input of TP, particularly from sewage and industrial waste.

After an external loading reduction, the internal TP load often delays recovery due to various mechanisms. Despite this delay, many north temperate lakes have often reached a new equilibrium with respect to TP after <10-15 years, whereas a new equilibrium regarding TN is typically reached after <5-10 years (Jeppesen et al. 2005). The decrease in lake concentrations of TP is most marked in winter, thereafter followed by spring and autumn. Apart from the consequent nutrient control of phytoplankton biomass, increased top-down control due to fast response of the fish community seems often to accompany the external loading reduction. A decline in fish biomass and an increase in the percentage of piscivores and the zooplankton: phytoplankton biomass ratio have been observed in many lakes.

However, despite the historical focus on reducing the input of P, the reduction of N loading seems more important than previously considered. N concentrations higher than 1-2 mg L^{-1}, under moderate to high TP concentrations, seem to trigger the loss of submerged plants (González-Sagrario et al. 2005). The mechanism behind the loss of the plants with high N load appears to be lack of the otherwise usual N-limitation of periphyton growth, which particularly occurs during summer. This high N-stimulated growth of periphyton and phytoplankton can out shade the submerged plants (Phillips et al. 1978). High N load is also associated with lower plant species richness (James et al. 2005). These findings impose a reconsideration of the TP thresholds to be achieved using nutrient control methods. The reduction of N input by the (re-) establishment of wetlands in the catchments might be a very useful (while truly restoring) measure when comprehensive reductions of TP seem unfeasible (paper IX).

All lakes will not necessarily show the same response to the application of these techniques, and understanding the structure and functioning of the particular lake under restoration is therefore needed. Lake size and climate seem key features affecting the outcome of the restoration measures (papers VII & VIII). Cascading effects leading to increased transparency following a reduction in external nutrient load may occur also in relatively large shallow lakes, although far less examples exist allowing a proper comparison. Some lakes have shown resistance to change after loading reductions, either because these reductions have not been sufficient enough, or due to the occurrence of internal resistance mechanisms (chemical, related to internal P load, and biological, related to homeostasis among fish). To speed up the recovery process several physico-chemical and biological restoration methods have been developed for north temperate lakes and these have been used with a varying degree of success. Complementary measures to reduce the internal TP loading, i.e. release of stored P from the sediments (Søndergaard et al. 2002), may include sediment removal. However, this is an expensive measure, especially in large lakes (due to transport and disposal costs). Moreover, sediment removal is likely not a very efficient measure in large lakes because the TP-pool is relatively small due to resuspension-induced washout of particulate P.

Biological measures include selective removal of planktivorous fish (Perrow et al. 1997, Scasso et al. 2001), stocking of young piscivorous fish (Skov and Berg 1999), and implantation or protection of submerged plants (Moss et al. 1996). These measures are often cheap compared to the traditional physico-chemical methods and therefore easier to apply. Transplantation or protection of existing macrophytes to enhance several clear-water stabilising mechanisms seems most useful in small lakes or littoral areas, as the wind-induced disturbance in large lakes likely prevents plant colonization or expansion. The introduction of seeds or plants, with simultaneous protection against waterfowl grazing, may be an interesting supplementary measure in large lakes, as long as it is conducted in sheltered areas. In large lakes, however, fish manipulation might be inefficient due to the enormous fishing (or young piscivores- stocking) effort needed to produce a significant effect. Many of these additional measures to reduce internal nutrient loading (such as chemical treatments, sediment removal or biomanipulation) may be not only costly but also too difficult to apply in large lakes, however, due to the described TP dynamics they may be less needed here.

Besides, the long-term stability of the lakes after biomanipulation is uncertain and highly dependent on the TP and TN loadings in the system. Biomanipulation can be more successful in temperate lakes if TP levels are kept below 0.05-0.1 mg TP L^{-1}. Several northern medium-large lakes have shown a fast recovery response after a reduction in nutrient loading combined with fish biomanipulation. However, recent findings suggest that the stability of the achieved state is weaker than anticipated, and that the lakes are likely to return to the pre-biomanipulation state if no further measures are applied (Jeppesen et al. unpub. data). To maintain the positive results of the fish biomanipulation in the long run, probably more frequent, although not necessarily as intensive, interventions are required. At the same time, an analysis of a set of lakes subjected to different restoration methods (paper IX) suggests that lakes are less resistant to changes with decreasing TP than predicted by the alternative state hypothesis. Thus, the stability of both alternative states (clear and turbid) seems weaker than expected, while the need (and strength) for pushing mechanisms is probably lake-specific.

It is also unclear how appropriate these techniques are outside the climate region (temperate and cold temperate) where they have been developed. Far less data exist on restoration experiences in tropical and subtropical lakes. Some lakes seem to respond positively to external loading reductions, an example being the large Lake Paranoá, in Brazil (discussed in paper VIII), but others have not shown significant responses to a combination of intensive measures, such as Lake Rodó in Uruguay (Scasso et al. 2001). At present, many lakes in warm regions of the globe are suffering severely from eutrophication. Warm lakes often have prolonged growing seasons with a higher risk of long-lasting, and potentially toxic, algal blooms. Eutrophication effects can be expected to worsen even more in the near future, due to the economical development and increasing population in many countries, and likely, also due to climate change. There is therefore an extensive need for gaining new insight into their trophic interactions and potential lake restoration methods.

We still do not fully know how the increases in nutrient loading affect the trophic structure nor the aquatic plant development and the competition among plant types in warm lakes. Inorganic N is usually considered limiting in these lakes, likely due to a higher-temperature enhanced denitrification (Weyhenmeyer et al. submitted). The lack of enough data about the relative importance of P and N in (sub)tropical lake functioning imposes limitations to the strategies for nutrient control.

Moreover, the significant differences between the biological interactions in cold temperate and warm lakes jeopardize the application of the existing biological restoration methods in warm regions. The effects of plants on water transparency are much weaker in many warm temperate and subtropical lakes than in temperate systems (Bachmann et al. 2002). These authors found no difference in the chlorophyll:TP or Secchi depth:TP relationships in shallow lakes in Florida (USA) with low, medium or high submerged plant coverage. Even with abundant macrophytes, there is a higher phytoplankton biomass (measured as chlorophyll) in Florida lakes than in Danish lakes at a given nutrient concentration (paper IX). Previous studies had demonstrated the importance of the structure of the grazer communities (large versus small herbivorous zooplankton) in shaping the different TP-Chlorophyll relationships in temperate versus subtropical lakes (Mazumder and Havens 1998). One of the reasons explaining these patterns, as suggested by the studies conducted in this thesis, may be that macrophytes are not acting as proper refuges for grazer zooplankton in warm lakes, and therefore not leading to a higher share of large cladocerans. Other comparative studies also support this hypothesis (Jeppesen et al. unpub. data).

The characteristics of the main communities in warm lakes (summarised in paper VII), together with the stronger impacts of fish (and other omnivorous consumers) on other trophic levels, as found in this thesis, make it very difficult to predict the effects of fish manipulations. Fish removal (or natural fish kills) might induce positive cascading effects on water transparency. However, these are likely to be only of short-term duration due to fast recovery of the populations (Nagdali and Gupta 2002). Besides, a strong decrease in fish biomass may release invertebrates from predation, and thus enhance invertebrate predation on herbivorous cladocerans (Mumm 1997), affecting the outcome of biomanipulation in warm lakes (Blumenshine and Hambright 2003). The fish biomass or density thresholds allowing the development of large-bodied grazers must therefore be substantially lower than in similar temperate lakes. Furthermore, the lack of massive production systems of piscivorous fish currently prevents the application of biomanipulation in many places in the developing world. Other methods, such as the combined use of adequate harvests of aquatic plants (mostly free-floating if present) and hydraulic management can be useful, though likely insufficient, tools for restoring shallow lakes of small size in tropical and subtropical regions by decreasing the internal nutrient load, as suggested for Lake Rodó, Uruguay (Rodríguez-Gallego et al. 2004).

Warm lakes differ in trophic structure depending on their location in wet or dry regions, and on temperature and precipitation changes. Both (sub)tropical and Mediterranean lakes are very sensitive to changes in water level (Coops et al. 2003, Mazzeo et al. 2003). Hydrological changes can deeply affect water transparency, the development of submerged plant, the structure of the fish communities, and salinity. These characteristics have to be seriously considered when designing restoration strategies for specific lakes. A higher importance of nutrient loading for the functioning of warm lakes than in comparative temperate lakes has been found in field studies in Mediterranean lakes (Romo et al. 2004) and in an experimental study along a latitudinal gradient in Europe (Moss et al. 2004). Regardless of lake size and climate region a drastic reduction of the external nutrient loading seems to be the best way forward for restoring eutrophic lakes. However, the scientific basis on which to decide either restoration (such as the targeted nutrient and fish threshold levels), or management (targeted water level due to water extraction or irrigation) strategies, is still very limited for warm(ing) lakes.

Conclusions

Human activities have reduced the capacity of several ecosystems to cope with external changes and have therefore increased the likelihood of undesired regime shifts (Folke et al. 2004). The effects of aquatic plants on lake functioning are crucial for the resilience of shallow lakes to external changes, such as the human-induced climate warming.

Tropical and subtropical free-floating plants will probably expand their biogeographic distribution as a consequence of increasing winter minimum air temperatures in freshwater systems. In accordance with *Hypothesis 1*, our results indicate that, in the subtropics, large free-floating plants do not promote cascading effects (via fish-zooplankton) leading to increased water transparency, even though they host less fish than the submerged plants (papers I, II & III). In the temperate lakes, free-floating plants hosted more fish than did the submerged plants, and they were clearly not used as refuge by cladocerans (paper III). An expansion of the free-floating plants, especially considering their capacity to constitute an alternative stable state to submerged plants (Meerhoff 2002, Scheffer et al. 2003), would imply negative impacts on trophic dynamics, besides the already known negative effects on general diversity and water qualtiy.

We found no evidence in the experimentally warmed ponds (paper V) directly supporting *Hypothesis 2*, i.e. that warming can lead to a weakening of the macrophyte-dominated clear water state. Although in these experiments we observed some potential effects of increased temperatures on the macrophyte and zooplankton communities, these communities proved quite resilient to warming in general, as has been reported in similar studies (McKee et al. 2003). However, the limitations of the experimental set-up regarding the fish effects prevent the direct extrapolation of these results to natural lakes. The results from the 1-year field study in a subtropical lake (paper VI) and the comparative studies conducted in subtropical and temperate lakes (papers III & IV) contrast the observations in temperate systems that show strong refuge-mediated cascading effects of submerged plants leading to increased water clarity (Jeppesen et al. 2002b). Submerged plants could offer only a very weak refuge for cladocerans, and it seemed clear that no anti-predator behaviour of zooplankton sufficed to counteract the high predation pressure exerted by the fish communities.

Supporting *Hypothesis 3*, our study suggests that, more than other environmental factors, the structure of the typical predator assembly, namely fish, embodies the main reason for the observed different structure of littoral communities in temperate and subtropical shallow lakes (paper IV). As mentioned before, some characteristics in the structure of fish communities in warm shallow lakes from several regions around the world have been recently described (Lazzaro 1997, Beklioglu et al. in press) and reviewed in paper VII. In this thesis we have experimentally confirmed and broadened many of the key differences between warm and temperate lakes regarding littoral trophic structure and dynamics. The differences between climatic regions found in this study seem therefore clearly not the result of "matrix effects" (i.e. comparisons of lakes from different continents), but rather the outcome of climate-related differences in trophic structure. Supporting this statement, *Daphnia* spp appeared scarcely, while fish biomass and the fish:zooplankton biomass ratio were higher in the southern warm lakes in a European study along a climate gradient (Gyllström et al. 2005).

The fish structure in the littoral zone (high diversity, density, and biomass, less piscivory, smaller size, and strong association with the submerged plants), and the very weak capacity of aquatic plants to stabilise these trophic interactions in warm lakes, can have profound effects at community and ecosystem levels. This agrees with empirical data from numerous Florida lakes showing a minor effect of high submerged plant densities on water clarity (Bachmann et al. 2002). However, subtropical lakes may still exhibit high water transparency than could be expected from the described trophic structure and the weakened stabilising role of macrophytes, as was the case of some of the lakes included in this study. Empirical data from shallow lakes along a climate gradient in South America have shown no latitudinal gradient in water transparency (Kosten et al. in prep.). These findings indicate that other processes must operate to maintain clear water in warm lakes. A possible explanation is that higher temperatures may lead to enhanced denitrification (Weyhenmeyer et al. submitted), which potentially may benefit the submerged macrophytes while limiting phytoplankton. These topics deserve further specific research as they are crucial aspects to understanding the potential mechanisms preserving water transparency under warm conditions.

Climate warming may not directly affect submerged plants in a significant manner, but rather other components of the trophic structure of shallow lakes, such as the fish community structure or activity level, as suggested by some experimental studies (Mehner 2000). A warming-related higher impact of fish may strongly affect the resilience of shallow lakes, pushing the lakes in the direction of a higher predation on large-bodied zooplankton and lower grazer control of phytoplankton, and of a decreased capacity of the submerged plants to create and maintain clear-water conditions. The consequent lower resilience of the lakes under higher temperatures may increase their sensitivity to external changes, such as an increase in nutrient loading or changes in water level. The current process of warming, particularly in temperate lakes, may thus impose an enhanced sensitivity to eutrophication, and a threat to the high diversity, clear water state with consequent impoverished ecosystemic and social value.

Furthermore, the cumulative effects of several stress factors on lake ecosystems will be far more serious than those imposed by climate warming and other aspects of global change alone (Schindler and Smol 2006). Therefore the conflicts between conservation and other land uses will likely escalate with climate change. Conservation and restoration strategies should incorporate the substantial differences in lake functioning under different climate regimes, and the likely increased sensitivity of shallow lakes to several external stressors in the future, into today's aims.

Perspectives

Several questions have been addressed in this thesis. We have been able to provide further insight into some of them, but as often happens, we have generated more questions.

In recent years research focusing on the ecology of free-floating plants has substantially increased (e.g. Brendonck et al. 2003, Meerhoff et al. 2003, Scheffer et al. 2003, Van der Heide et al. 2006). However, many aspects requiring further investigations remain, applying small-scale laboratory experiments to geographic gradient studies. Some free-floating plants, such as *E. crassipes* and *Stratiotes aloides* (water soldier), can affect negatively some algae species (Sharma et al. 1996, Mulderij et al. 2005), but further studies are needed to elucidate if free-floating plants can also negatively affect cladocerans. Preliminary experiments took place to analyse the effects of floating plants on life history traits of subtropical *Daphnia* (Fosalba, Noordoven and Meerhoff, unpub. data); however, the results obtained are unclear. The main goal of these investigations is to identify the potential ultimate causes behind the strong aversion found to these plants in the field and in laboratory experiments. Other aspects related to climate change should be addressed experimentally, such as the effects of the increase in CO_2, and its interaction with the increase in temperature, on the performance of large free-floating plants. On a geographical scale, it is important to measure the changes in the distribution and performance of large free-floating plants in the boundaries of their current biogeographic distribution. The factors affecting the competition between the submerged and free-floating plants, and phytoplankton, also deserve much research. Particularly, it seems relevant to analyse how increases in temperature and phosphorus and nitrogen loading can affect the outcome of these competitions, and the consequent cascading impact on trophic dynamics and water quality.

Extensive field surveys, including the study of animal remains from the lake sediments, are crucial to understand the mechanisms behind the low macroinvertebrate and cladoceran diversity we found in the littoral area of subtropical lakes. We hypothesize that also this is the result of the tremendous predation pressure suffered by these communities in the (sub)tropics, although other environmental factors, such as large fluctuations in the oxygen concentrations among the plant beds, may also be important.

The processes, related or not to macrophytes, that help stabilize the clear water state in many warm lakes require intensive research. Our results suggest that the lower biomass of periphyton on the plants may play a significant role in the development or maintenance of macrophyte communities in the (sub)tropics. The factors affecting periphyton growth in warm lakes could be analysed in a series of factorial experiments, under different nutrient loadings (particularly of nitrogen) and fish densities. The role of the large macroinvertebrate grazers, i.e. applesnails and shrimps, in (sub)tropical ecosystems, as well as the factors affecting their feeding behaviour and predation sensitivity, deserve further field and controlled studies. In particular, we need more studies to understand the benthic-littoral-pelagic coupling and the flow of carbon and nutrients inside the system in warm lakes, considering these processes may not be fully accomplished by such depressed macroinvertebrate communities but instead strongly affected by fish. The effects of nitrogen loading and biological availability in warm lakes represent other key research aspect with important applied consequences.

How to address the challenges imposed by climate change on restoration targets and strategies, and the applicability of current restoration techniques to different climate regions in the world, also represent a research and management chapter of increasing importance.

However, despite all the potential for more research into these topics, the most relevant question remain whether the scientific community can cross the bridge and increase the awareness of politicians, managers, and the general public of the present and future effects of global changes. The answers so far are at sight, but we cannot afford to be less than very ambitious in this matter, for there is too much at stake.

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Paper I	**Meerhoff M.**, Fosalba C., Bruzzone C., Mazzeo N., Noordoven W. & E. Jeppesen. 2006. "An experimental study of habitat choice by *Daphnia*: plants signal danger more than refuge in subtropical lakes". *Freshwater Biology* 51: 1320-1330
Paper II	**Meerhoff M**. & N. Mazzeo. 2004. "Importancia de las plantas flotantes libres de gran porte en la conservación y rehabilitación de lagos someros de Sudamérica". Ecosistemas. http://www.aeet.org/ecosistemas/042/revision1.htm
Paper III	**Meerhoff M.**, Teixeira de Mello F., Clemente J. M., Iglesias C., Jensen E., Lauridsen, T.L. & E. Jeppesen. "Effects of climate and habitat complexity on community structure and predator avoidance behaviour of zooplankton in the shallow lake littoral". Submitted to *Freshwater Biology*
Paper IV	**Meerhoff M.**, Clemente J.M., Teixeira de Mello F., Iglesias C., Pedersen A.R. & E. Jeppesen. "Can warm climate-related structure of littoral predator assemblies weaken clear water state in shallow lakes?" Submitted to *Global Change Biology*
Paper V	Liboriussen L., Landkildehus F., **Meerhoff M.**, Bramm M.E., Søndergaard Mo., Christoffersen K., Richardson K., Søndergaard Ma., Lauridsen T.L. & E. Jeppesen. 2005. "Global warming: Design of a flow-through shallow lake mesocosm climate experiment". *Limnology and Oceanography: Methods* 3: 1-9
Paper VI	Iglesias C., Goyenola G., Mazzeo N., **Meerhoff M.**, Rodó E. & E. Jeppesen. "Horizontal dynamics of zooplankton in subtropical Lake Blanca (Uruguay) hosting multiple zooplankton predators and aquatic plant refuges". In press *Hydrobiologia, Special Volume Shallow Lakes*
Paper VII	Jeppesen E., Søndergaard Ma., Mazzeo N., **Meerhoff M.**, Branco C., Huszar V. & F. Scasso. 2005. "Lake restoration and biomanipulation in temperate lakes: relevance for subtropical and tropical lakes". In: Reddy M.V. (ed.) *Restoration and Management of Tropical Eutrophic Lakes*. Science Publishers, Inc., Enfield, USA. pp: 341-359
Paper VIII	Jeppesen E., **Meerhoff M.**, Jacobsen B.A., Hansen R.S, Søndergaard Ma., Jensen J.P., Lauridsen T.L., Mazzeo N. & C. Branco. "Restoration of shallow lakes by nutrient control and biomanipulation- the successful strategy depends on lake size and climate". In press *Hydrobiologia Special Volume*
Paper IX	Jeppesen E., Søndergaard Ma., **Meerhoff M.**, Lauridsen T.L. & J.P. Jensen. "Shallow lake restoration by nutrient loading reduction- some recent findings and challenges ahead". In press *Hydrobiologia, Special Volume Shallow Lakes*

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An experimental study of habitat choice by *Daphnia*: plants signal danger more than refuge in subtropical lakes

MARIANA MEERHOFF,*,†,‡ CLAUDIA FOSALBA,* CARLA BRUZZONE,* NÉSTOR MAZZEO,* WILLEMIJN NOORDOVEN§ AND ERIK JEPPESEN†,‡

**Sección Limnología, Facultad de Ciencias, Universidad de la República. Iguá, Montevideo, Uruguay*

†*National Environmental Research Institute. Vejlsøvej, Silkeborg, Denmark*

‡*Department of Plant Biology, Institute of Biological Sciences, Aarhus University, Ole Worms Allé, Aarhus C, Denmark*

§*Department of Environmental Sciences, Aquatic Ecology and Water Quality Management Group, Wageningen University, Wageningen, The Netherlands*

SUMMARY

1. In shallow temperate lakes, submerged plants often provide refuge for pelagic zooplankton against fish predation, a mechanism with potential strong cascading effects on water transparency and on the entire ecosystem. In (sub)tropical lakes, however, the interaction between aquatic plants and predation may be more complex, particularly because fish density is high within the plant beds in such systems.

2. Using laboratory ‘habitat choice’ experiments, we determined the effects of three (sub)tropical free-floating plants, *Eichhornia crassipes*, *Pistia stratiotes* and *Salvinia auriculata* and the cosmopolitan submerged *Ceratophyllum demersum*, on horizontal movement by the water flea *Daphnia obtusa*. We tested for avoidance of plants in the absence and presence of alarm signals from crushed conspecifics and chemical cues from the fish *Cnesterodon decemmaculatus*, the fish have been subjected to different feeding regimes.

3. In the absence of other stimuli, *D. obtusa* strongly avoided the plants and the crushed conspecifics, as expected. However, the response to fish was insignificant regardless of their previous feeding regime. The avoidance of free-floating plants was more pronounced than that of the submerged plant. Contrary to predictions based on research in temperate lakes, *Daphnia* did not take refuge among the plants but rather swam away from them when exposed simultaneously to plants and alarm signals.

4. We hypothesise that the avoidance of plants by *D. obtusa* may ultimately be attributable to an expectedly higher predation risk within the plants than in the pelagic, because of a high density of associated zooplanktivorous fish in the former. In the (sub)tropics, therefore, aquatic plants and particularly the free-floating ones, may not promote cascading effects via *Daphnia* grazing on phytoplankton as seen in temperate eutrophic lakes.

Keywords: alarm signal, chemical cue, kairomone, predator-avoidance, refuge effect

Introduction

Predation by fish on pelagic zooplankton produces significant impact in lakes because of strong, cascading trophic effects (Brooks & Dodson, 1965; Jeppesen *et al.*, 1997a). In temperate shallow lakes lacking a

Correspondence: Mariana Meerhoff, Sección Limnología, Facultad de Ciencias, Universidad de la República. Iguá 4225, CP 11400, Montevideo, Uruguay.
E-mail: merluz@fcien.edu.uy

hypolimnetic refuge (which favours vertical migration), chemical cues from potential predators lead the large-bodied zooplankton to perform diel horizontal migration (DHM; Burks *et al.*, 2002). The maintenance of a herbivorous zooplankton population that can graze algae has individual, population and even ecosystem consequences (Brönmark & Hansson, 2000; Jeppesen *et al.*, 2002).

In shallow temperate lakes, submerged plant beds provide a daytime refuge for the pelagic zooplankton, which often move to feed in open water at night, when the predation risk from fish is lower (Timms & Moss, 1984; Burks *et al.*, 2002). Both field (Pennak, 1966) and laboratory studies (Pennak, 1973) have shown that plants are avoided by zooplankton, but that the presence of predators induces zooplankters to overcome their repellence and to seek refuge in the vegetation (Lauridsen & Buenk, 1996; Lauridsen & Lodge, 1996). This effect of macrophytes on the interactions between zooplankton and planktivorous fish constitutes an important mechanism by which submerged plants maintain clear water in shallow temperate lakes (Scheffer *et al.*, 1993; Lauridsen *et al.*, 1996). The refuge effect for *Daphnia*, however, varies with the trophic state of the lake (Lauridsen *et al.*, 1999), the structure of the fish community (Jeppesen *et al.*, 1997a) and with several plant characteristics, such as species, architecture, bed size and per cent of the lake volume inhabited (Schriver *et al.*, 1995; Lauridsen *et al.*, 1996). Previous work suggests that at both extremes of the nutrient gradient, submerged plants offer little refuge for zooplankton (Jeppesen *et al.*, 1997b). In low-nutrient lakes, clear water and the scarcity of macrophytes enhance fish predation on zooplankton whereas, under hypertrophic conditions, the refuge effect is weak because of the scarcity or absence of submerged plants and often high densities of planktivorous fish (Jeppesen *et al.*, 1997b). Besides the submerged and floating-leaved plants, other growth forms, such as emergent plants, may be important for the structure and migration patterns of zooplankton (Nurminen & Horppila, 2002; R.L. Burks, H. Michels, M.A. González-Sagrario & E. Jeppesen, unpublished data).

Studies on DHM have so far mainly focused on northern, temperate lakes (Burks *et al.*, 2002), while macrophyte-fish-zooplankton interactions in tropical and subtropical systems remain to be elucidated in more detail (but see Jeppesen *et al.*, 2005; Iglesias *et al.*, in press; Jeppesen *et al.*, in press, a). Such interactions may be more complex in tropical and subtropical lakes (Lazzaro, 1997). Fish zooplanktivory is expected to be stronger because of (i) multiple or continuous reproductive events by fish (Paugy & Lévêque, 1999), (ii) low densities of large, specialist piscivores frequently exhibiting sit-and-wait hunting behaviour (Quirós, 1998), (iii) widespread omnivory (Branco *et al.*, 1997; Yafe *et al.*, 2002) and (iv) high population densities of small and juvenile fish (Mazzeo *et al.*, 2003). Although the necessity of a daytime refuge for zooplankton appears strong in subtropical and tropical lakes, DHM is not so obviously advantageous, because small and juvenile fish are often numerous within macrophyte beds in these lakes (Conrow, Zale & Gregory, 1990; Agostinho, Gomes & Ferreira, 2003; Meerhoff *et al.*, 2003). However, all macrophyte growth forms (emergent, submerged, floating-leaved and large free-floating) can be extremely dense in subtropical and tropical lakes (Talling & Lemoalle, 1998) and thus might potentially provide refuge for the zooplankton against predation despite high fish density. Contrary to temperate lakes, (sub)tropical lakes under eutrophic and hypertrophic conditions may contain a high density of large free-floating plants, which may offer a potential refuge for zooplankton when submerged plants are scarce.

Using laboratory 'habitat choice' experiments, we determined the effects of three free-floating plants, *Eichhornia crassipes* Mart. (Solms) (water hyacinth), *Pistia stratiotes* L. (water lettuce) and *Salvinia auriculata* Aubl (water fern) and the submerged plant *Ceratophyllum demersum* L. (coontail or common horntwort), on horizontal movement by the water flea *Daphnia obtusa* Kurz. We performed these experiments in the absence and presence of alarm signals from crushed conspecifics and chemical stimuli from the planktivorous fish *Cnesterodon decemmaculatus* Jenyns. The free-floating plant species are native to (sub)tropical South America and so is *C. decemmaculatus*, present in Argentina, Uruguay and the south of Brazil (Rosa & Costa, 1993). *Ceratophyllum demersum* is cosmopolitan and widely distributed in subtropical lakes. Based on data from temperate lakes, we would expect daphniids to avoid (i.e. swim away from) the plants in the absence of predation risk or alarm signals, but to seek refuge in the plants when facing risk of predation. However, if predation risk is actually higher in plant beds in (sub)tropical systems, an alternative outcome might be that *Daphnia* would persistently avoid plants.

Methods

Experimental design

We collected *D. obtusa* from Lake Rivera (34°55′S, 56°10′W Montevideo, Uruguay), from patches of open water near the shore. Lake Rivera is a hypertrophic lake with large fluctuations in the density of the omnivorous-planktivorous fish *C. decemmaculatus* (N. Mazzeo, F. Scasso & J. Garcia, unpublished data) and is often covered by free-floating plants (mainly *E. crassipes*) although no submerged plants have been recorded. To be sure of obtaining predator-inducible clones, we cultured the individuals collected in both free-floating and submerged vegetation and allowed *C. decemmaculatus* to feed on the daphniids for about 2 months before the experiments. We used 1.5–2.0-mm egg-carrying daphniids, pre-incubated in the laboratory in 16 h:8 h L : D light chambers, at a constant temperature of 20 °C and fed in excess on dried yeast (daphniids were whitish as a result). We collected the plants from different lakes and thoroughly rinsed them several times in the laboratory to remove any attached organism. We maintained the plants under the natural photoperiod, adding a nutrient-rich solution until the start of the experiments. The fish (adult *C. decemmaculatus*, about 2.5–3.0 cm length) came from a *Daphnia*-free hypertrophic lake. We kept them in aerated aquaria and fed them according to two feeding regimes: half received *Daphnia* and the other half dried *Tubifex* sp., the latter avoiding chemical cues from crushed *Daphnia.*

Each experimental unit consisted of two transparent cylindrical tanks (8-L volume each) connected at the bottom by a 1-m long glass tube with a diameter of 5 cm. The water flow within the set-up permitted the generation of a gradient in the chemical cues (which we confirmed by a dye-study following the same experimental protocol). We used two units at a time, filled with 10-L of spring water, thus avoiding any potential cues prior to the experiments. One unit received the treatment and the other the spring water as a control. The respective treatment was applied in one side of the unit, chosen at random, 30 min before the addition of daphniids, to build up a chemical cue gradient. We then gently added ten large daphniids through a vertical tube (2.0-cm diameter) exactly at the centre of the connecting tube. The treatments (13 in total, Table 1) included: the four plant species covering the whole area of one tank; fish on the two feeding regimes (in a mesh cage in the water but without direct contact with the daphniids); 40 crushed adult *Daphnia* ('alarm signals' *sensu* Pijanowska, 1997); and double treatments: each plant species again in one side and crushed *Daphnia* in the opposite side of the experimental unit. Moreover, to determine whether the behaviour of the daphniids towards the free-floating plants was either chemically or mechanically induced, we used, respectively, suspended filter-paper bags with roots of *E. crassipes* (thus removing the shade effect of an intact plant) and plastic structures mimicking the architecture of the root system and the shade produced by the leaves of

Table 1 Summary of the experiments performed in the study, indicating each treatment and the respective hypothesis to be tested and the methodology employed

No.	Short name	Hypothesis	Method
1	*E. crassipes*	Daphniids avoid plants	Water hyacinth covering one tank
2	*P. stratiotes*	Daphniids avoid plants	Water lettuce covering one tank
3	*S. auriculata*	Daphniids avoid plants	Water fern covering one tank
4	*C. demersum*	Daphniids avoid plants	Coontail covering one tank
5	Roots	Plant avoidance is chemically induced	Suspended roots of *E. crassipes*
6	Plastic	Plant avoidance is mechanically induced	Plastic mimic of *E. crassipes*
7	Fish fed *Tubifex*	Daphniids avoid potential predators	Three caged fish in one tank
8	Fish fed *Daphnia*	Daphniids avoid 'latent' alarm signals	Three caged fish in one tank
9	Alarm signals	Daphniids avoid alarm signals	40 crushed daphniids in one tank
10	Refuge *E. crassipes*	Daphniids seek refuge in the plants	Combination exp 1 + 9
11	Refuge *P. stratiotes*	Daphniids seek refuge in the plants	Combination exp 2 + 9
12	Refuge *S. auriculata*	Daphniids seek refuge in the plants	Combination exp 3 + 9
13	Refuge *C. demersum*	Daphniids seek refuge in the plants	Combination exp 4 + 9

E. crassipes. We did not include leaves in the 'chemical effect' experiment as only a minor part is normally in contact with the water and therefore the chemical cues coming from the root system are more likely to promote the response of *Daphnia*.

After allowing for 15-min acclimation, we recorded the position of individual water fleas at 15, 30, 60 and 90 min after their introduction as being: (i) in the tank without the treatment (interpreted as repellence), (ii) in the tank with the treatment (attraction) or, if in the connecting tube, (iii) repellence if the daphniids were in the half of the tube furthest from the treatment, or attraction if they were in the half closest to the treatment. The same procedure was applied in the control unit (spring water in both tanks) located besides the treatment unit. The experiments took place in a windowless room with a uniform light regime over the units. We replicated each experiment five times over a short period, always using different animals and plants and rinsing the units thoroughly with distilled water before the next experiment. All other conditions, ambient light, and air and water temperatures remained constant.

Statistical analyses

The proportion of daphniids (p) showing repellence (i.e. proportion in the tank opposite to the treatment + corresponding half side of the tube) required transformation [arccosine SQ Root (p)] prior to the statistical analyses (Sokal & Rohlf, 1997). To compare the short and long-term responses to each treatment, we analysed each experiment with paired *t*-tests (treatment *versus* control, considering the proportions of *Daphnia* in the same side of the units) at 15 and 90 min. We acknowledge that these measurements do not represent independent data; however, we wanted to compare the speed of the response beside the long-term one. The consequent relatively large number of tests increases the overall probability of making type I errors. Rather than applying a conservative correction (e.g. Bonferroni), we chose to present the true *P*-values of the tests. Although some of the low *P*-values found could be due to type I errors, the pattern of significances in the tests was consistent and pointed in the same direction within the experiments (whereas type I errors should occur randomly and not in patterns). Moreover, in most cases we found *P*-values even lower than the alpha we should apply based on Bonferroni corrections (e.g. in the first set of experiments the Bonferroni-corrected *P*-value would be $P = 0.0063$, as we performed eight paired *t*-tests).

We tested the differences between particular sets of experiments with one- or two-way repeated measures ANOVA (followed by Tukey HSD if significant differences appeared), after subtracting the transformed proportion of daphniids in each control from the respective treatment. As the RMANOVA requires no temporal trend in the data we excluded the 15 min measurements (see *E. crassipes* in Fig. 1 as example of the temporal pattern in some of the experiments).

Results

Under control conditions, the distribution of daphniids in the experimental unit changed slowly and showed a relatively homogeneous distribution after 30–60 min of the experiments.

Daphnia response to free-floating and submerged plants

In the absence of other stimuli, *D. obtusa* significantly avoided both free-floating and the submerged plants. We found significant differences among the four plant species (one-way RMANOVA $F_{3,16} = 4.84$, $P = 0.0139$), with the repellence by *E. crassipes* being the strongest and that to the submerged plant being the weakest (Fig. 1).

The repellence by *E. crassipes* occurred quickly in our experiment. After 15 min, 90% ± 10% (SD) had swum towards the opposite side ($t_{0.05(4)} = 4.94$, $P = 0.007$). After 30 min and until the end of the experiment, all daphniids had moved away from the plants (90 min: $t_{0.05(4)} = 20.05$, $P < 0.0001$).

A rapid strong repellence also occurred with *P. stratiotes*. After 15 min, 68% ± 26.7% (SD) of the daphniids were located opposite to the plants, while 22% ± 17.9% were in the plant side of the connecting tube ($t_{0.05(4)} = 4.07$, $P = 0.0151$). At the end of the experiment, 96% ± 5.5% were inside the opposite tank ($t_{0.05(4)} = 8.81$, $P = 0.0009$).

The response to the presence of *S. auriculata* occurred quickly and was stronger than that of *P. stratiotes* (Fig. 1). After 15 min, on average 96% ± 8.9% of the daphniids displayed repellence by the plants ($t_{0.05(4)} = 6.6$, $P = 0.0028$), while after 90 min, all had swum away from the plants ($t_{0.05(4)} = 15$, $P = 0.0001$).

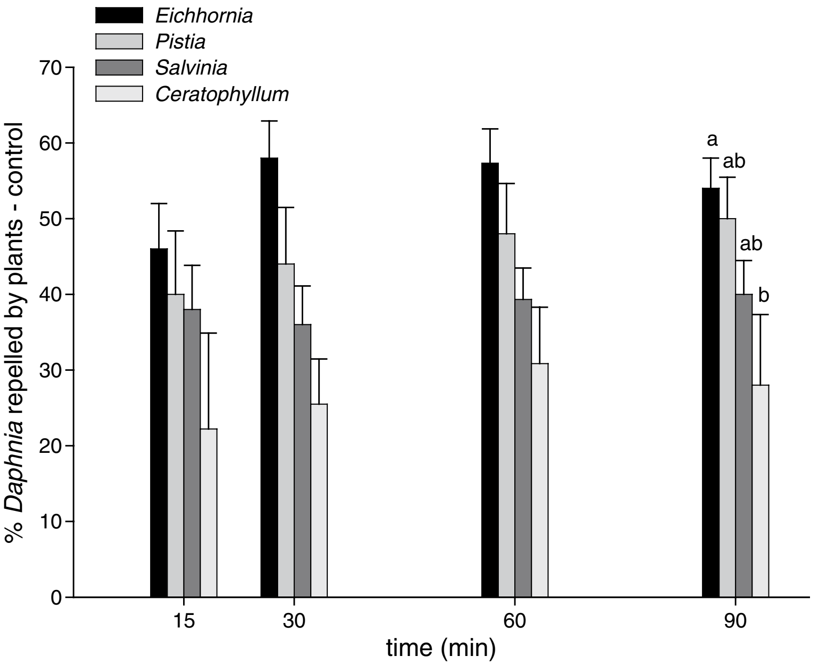

Fig. 1 Net effect of the plants *E. crassipes*, *P. stratiotes*, *S. auriculata* and *C. demersum* on the horizontal movement of *D. obtusa*. Data represent the difference between the proportion of *Daphnia* repelled by the plants and the proportion in the respective side of the control unit (mean ± 1 SE). The weaker the repellent effect of the plant, the closer to 0% is the difference. Small letters over 90-min bars indicate significant differences among treatments for the transformed data, according to RMANOVA and Tukey HSD tests.

In the case of submerged *C. demersum*, the repellence occurred more slowly and was slightly weaker than with the previous plant species. After 15 min, 87% ± 19% (SD) had swum towards the opposite side ($t_{0.05(4)} = 2.14$, $P = 0.0986$). In the long run, most daphniids moved away from the plant (90 min: $t_{0.05(4)} = 3.47$, $P = 0.0254$).

We performed further experiments to elucidate whether the repellence by the free-floating plants was either chemically or mechanically induced, using *E. crassipes* as prototype. We found that both the chemical cues from the roots (90 min: $t_{0.05(4)} = 6.8$, $P = 0.0024$) and the mechanical structure (90 min: $t_{0.05(4)} = 4.97$, $P = 0.0076$) promoted *Daphnia* avoidance. Although the degree of the response to both treatments was similar, daphniids responded more quickly to real roots than to plastic plants (Fig. 2). We found a significant avoidance of roots after 15 min,

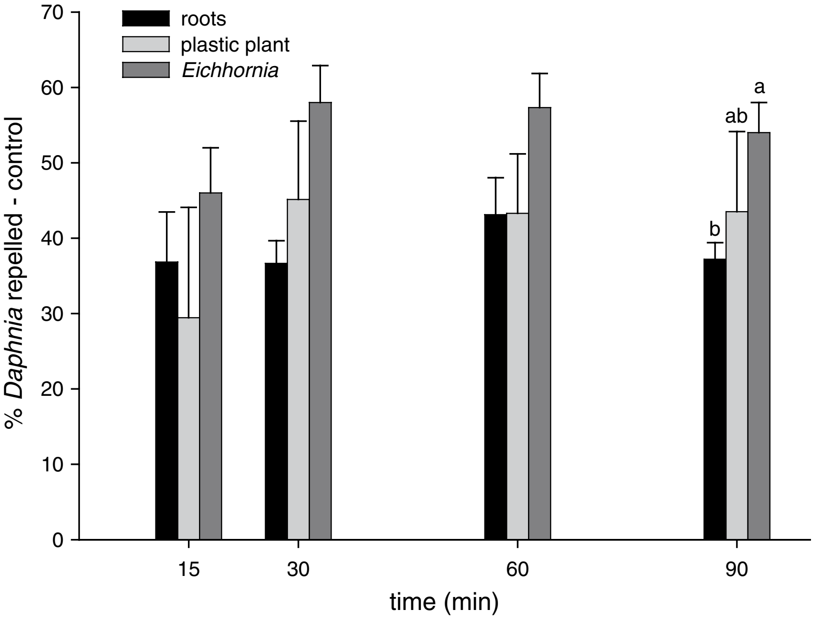

Fig. 2 Horizontal movement of *D. obtusa* in the presence of water hyacinth (*E. crassipes*), water hyacinth roots, and plastic structures mimicking the root system and the shade by the leaves. Data represent the difference between the percentage of *Daphnia* repelled by each treatment and the percentage in the respective side of the control unit (mean ± 1 SE). Small letters over 90-min bars indicate significant differences among treatments for the transformed data, according to RMANOVA and Tukey HSD tests.

but of plastic plants. According to the RMANOVA, complete plants repelled daphniids significantly more than the roots alone ($F_{2,12} = 4.65$, $P = 0.0321$), whereas the effects of the plastic plants did not differ significantly from the other two treatments (Tukey *post hoc* test, Fig. 2).

Daphnia response to fish

Although most daphniids moved to the side with the lowest concentration of fish cues (i.e. the tank opposite to the one with the fish) in several of the replicated experiments, the presence of *C. decemmaculatus* did not significantly affect the behaviour of *D. obtusa*. Most daphniids displayed an almost homogeneous distribution along the unit. The same behaviour occurred for fish fed with *Tubifex* (15 min: $t_{0.05(4)} = 1.08$, $P = 0.3405$; 90 min: $t_{0.05(4)} = 0.81$, $P = 0.4626$) as for those fed with *D. obtusa* ($t_{0.05(4)} = -0.62$, $P = 0.2499$; $t_{0.05(4)} = -0.67$, $P = 0.2991$, after 15 and 90 min, respectively). This means that neither *C. decemmaculatus* itself (fish fed on *Tubifex*) nor fish fed on *Daphnia* (which could be associated with some *Daphnia* alarm substance) repelled *D. obtusa*.

Daphnia response to 'alarm signals'

Although daphniids did not swim away from fish, the chemical cues coming from crushed conspecifics repelled them, although not immediately (Fig. 3). Initially, the distribution was relatively homogeneous (15 min: $t_{0.05(4)} = 1.27$, $P = 0.2722$), but after 30 min more than 40% of the daphniids had moved to the opposite side, while 13% remained inside the tank with the cues. After 90 min, 70.4% ± 10.2% showed repellence while only 25.3% ± 11.2% had moved to the side with the cues ($t_{0.05(4)} = 3.76$, $P = 0.0197$). On the basis of these results, we decided to use crushed daphniids rather than *C. decemmaculatus* cues to trigger refuge search.

Plants as a refuge for Daphnia

To test the hypothesis that *D. obtusa* would overcome their original avoidance of plants when faced with the risk of predation or alarm signals, we placed each of our four experimental plant species and 40 crushed daphniids at opposite sides of the experimental unit. Contrary to our primary expectations, daphniids did not move to the plants in the presence of crushed conspecifics, regardless of the plant species and growth form (Fig. 4).

However, the degree and speed of the response differed with plant species. In the case of *E. crassipes*, 15 min after introduction most daphniids moved towards the side with the crushed conspecifics ($t_{0.05(4)} = -6.36$, $P = 0.0031$) and after 30 min all had swum away from the plants (90 min, $t_{0.05(4)} = -11.2$, $P = 0.0036$). In contrast, in the experiment with *P. stratiotes*, no significant differences occurred after

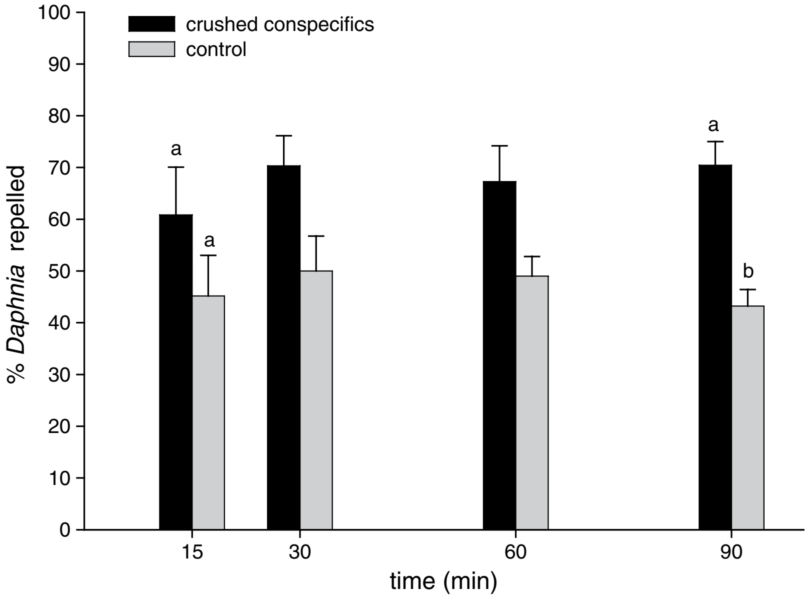

Fig. 3 Response of *D. obtusa* exposed to alarm signals (crushed conspecifics). Data represent the percentage of *Daphnia* repelled by the crushed daphniids (black) compared with the respective side of the control unit (grey) (mean ± 1 SE). Small letters over bars indicate results from paired *t*-tests after transforming the data.

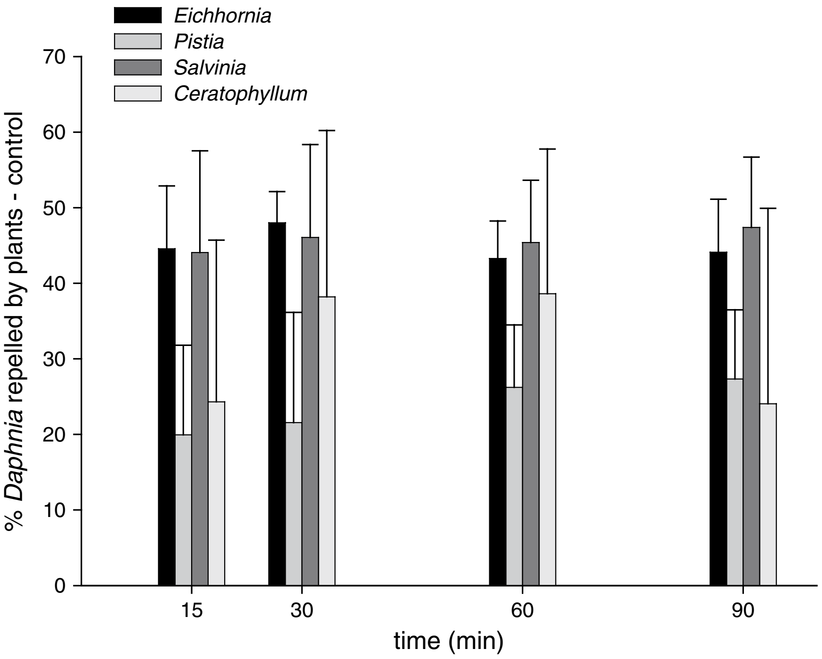

Fig. 4 Choice of *D. obtusa* when exposed simultaneously and on opposite sides of the unit, to crushed conspecifics and *E. crassipes*, *P. stratiotes*, *S. auriculata*, and *C. demersum*. Data show the net repellent effect of the plants, i.e. the difference between the proportions of *Daphnia* repelled by the plants and the proportion in the respective side of the control unit (mean ± 1 SE). If plants were attracting the daphniids ('refuge effect'), the percentage differences would be negative. Treatments did not differ significantly.

15 min compared with the control ($t_{0.05(4)} = -1.95$, $P = 0.122$). With time, instead of searching for the plants, most daphniids (88% ± 4.2%) moved towards the side with crushed *Daphnia* cues (90 min, $t_{0.05(4)} = -4.13$, $P = 0.014$). Also, as with *S. auriculata*, more daphniids moved towards the crushed conspecifics rather than to the plants ($t_{0.05(4)} = -4.8$, $P = 0.009$ and $t_{0.05(4)} = -7.8$, $P = 0.001$; for 15 and 90 min, respectively).

In contrast, in the case of the submerged species, we detected no daphnid preferences (i.e. equal repellence response to both stimuli), as there were no significant differences between the treatment and the control unit, neither at the beginning (15 min, $t_{0.05(4)} = 0.86$, $P = 0.437$) nor at the end of the experiment (90 min, $t_{0.05(4)} = 0.11$, $P = 0.3283$). The variation among the replicates in the presence of crushed conspecifics was much larger than in the presence of the plant *C. demersum* alone.

In most cases, daphnid repellence by the plants was weaker when daphniids were also exposed to crushed conspecifics than when exposed to plants alone (compare Fig. 4 versus Fig. 1). This weaker repellence was more noticeable in the case of *P. stratiotes*. We again compared the repellence by the plants under the scenario of the two simultaneous stimuli and found that the degree of repellence did not then differ significantly between the four plant species (one-way RMANOVA $F_{3,16} = 1.55$, $P = 0.2412$; Fig. 4). Such differences to the response to the plants (Fig. 4 versus Fig. 1), indicate interaction between the factors 'plants species' and 'alarm signals'. We tested for this but found no significant interaction between these factors (two-way RMANOVA $F_{3,32} = 1.17$, $P = 0.3349$).

Discussion

Daphnia obtusa clearly avoided the selected set of plants that are common in (sub)tropical lakes, despite cues to the risk of predation from a very common planktivorous fish and from crushed conspecifics. This is in contrast to the findings for submerged plants in choice experiments (Lauridsen & Lodge, 1996) and field studies (Lauridsen & Buenk, 1996) conducted in north temperate systems. Although the repellence by plants weakened in the presence of alarm signals, our results suggest that the costs of exposure to the plants and particularly to the free-floating species, do not outweigh the benefits of swimming away from predators or alarm signals. These results are more consistent with our alternative hypothesis, that *Daphnia* would avoid the plants, perhaps because of a permanently higher predation risk here.

The behaviour of *Daphnia* in these 'habitat choice' experiments followed our expectations when faced with the plants alone and with crushed conspecifics, but not in the case of the omnivore-planktivore *C. decemmaculatus*. We had expected the fish would repel *Daphnia*, as has been found in studies with several temperate fish species subjected to different food regimes (Stirling, 1995; Von Elert & Pohnert, 2000). *Daphnia* even avoided non-native predators, as long as the predators had previously fed on *Daphnia* (Stabell, Ogbebo & Primicerio, 2003). Moreover, it has been demonstrated that even some *Daphnia* clones coming from fishless habitats can respond to fish kairomones (Boersma, Spaak & De Meester, 1998; Michels & De Meester, 2004).

The fish community of Lake Rivera, where the daphniids were collected, has changed extensively and fish density has ranged from very high to very low (N. Mazzeo & M. Meerhoff, unpublished data). We therefore expected our experimental *Daphnia* population to respond to fish cues. Despite the 2-month exposure of a daphniid population to several cues prior to the experiments, the individuals used in our experiments could potentially have been clones not responsive to fish with behavioural traits. Such interclonal differences have been described for *D. magna* (De Meester, 1993). Alternatively, the short period of our experiments (45 min before recording first daphniid position, 120 min in total) may have been insufficient to generate a high enough concentration of fish cues. However, the response of *D. obtusa* to alarm signals indicates the recognition of a non-specific predation risk (Pijanowska, 1997) and the non-use of the plants as a refuge was evident. This response persisted even when the number of crushed conspecifics was raised from 40 to 100 (M. Meerhoff, C. Fosalba & C. Bruzzone, unpublished data).

Gliwicz & Rykowska (1992) suggested that high predation risk in the littoral zone could be the reason that zooplankton in temperate lakes sometimes avoid the shoreline. Several studies in the subtropics have shown high densities of fish within plant mats, both submerged and free-floating. Stands of submerged and free-floating plants act as important nursery areas for juvenile fish of many species in several subtropical lakes (in Florida, U.S.A., Conrow *et a*l., 1990; Uruguay, Meerhoff *et al*., 2003). The aversion to plants shown in our experiments may not be elicited by the plants themselves, but rather by the characteristically high densities of zooplanktivorous fish seeking refuge or feeding within the vegetation in subtropical lakes. *Daphnia* may use compounds released by macrophytes as a chemical signal (*sensu* Larsson & Dodson, 1993) of a continuous and higher predation risk among plant beds than in the pelagic where the fish density is lower. Also, higher invertebrate predation (Burks, Jeppesen & Lodge, 2001a,b; Van de Meutter, Stoks & De Meester, 2004, 2005) may potentially enhance the effects of such high densities of fish, although evidence is scarce about invertebrate predator abundance and their interaction with fish and zooplankton in the various types of plant communities in lakes in the (sub)tropics (but see González-Sagrario & Balseiro, 2003).

Our results, although obtained from small-scale simple ‘habitat choice’ (one or two chemical cues at a time) experiments, suggest that plants of both life-forms may signal danger rather than potential refuge for daphniids in the subtropics, but that free-floating plants did so to a larger extent than submerged plants. These results concur with field studies where lower densities of crustacean zooplankton occurred under free-floating plants than within submerged plants (Meerhoff *et al.*, 2003 and M. Meerhoff, C. Iglesias & J. Clemente, unpublished data from several lakes). Brendonck *et al.* (2003) also found a lower abundance of zooplankton under mats of *E. crassipes* than in open water in a subtropical lake in Zimbabwe. A differential response of *Daphnia* to the free-floating (strong avoidance) than to the submerged plants (weak avoidance) could be due to several factors. The presence of either free-floating or submerged plants might have different optical consequences, which may be used by daphniids as a proximate cause of migration (as suggested by Siebeck, 1980 and discussed by Ringelberg & Van Gool, 2003 for DVM). Rejection by daphniids of the free-floating plants in our experiments seemed to be mediated both visually and chemically. The relative importance of both mechanisms in a natural environment probably varies with the species involved. The dense structure of *E. crassipes* and *P. stratiotes* roots may hamper movement by daphniids (as suggested for submerged plants by Pennak, 1973). Chemical aversion was more conspicuous in the case of *S. auriculata*, probably because its roots are very small and not easily visible in our experimental set-up. A direct negative chemical effect may occur if some of the chemical compounds released by these plants affected the reproduction or development of *Daphnia*, as Burks, Jeppesen & Lodge (2000) found for the submerged plant *Elodea canadensis* Michx. *Eichhornia crassipes* and the also floating *Stratiotes aloides* L., affect negatively the alga *Scenedesmus*

(Sharma, Gupta & Singhal, 1996; Mulderij, Mooij & Van Donk, 2005), but further studies are needed to elucidate if free-floating plants can also affect *Daphnia* negatively. Lower food quality (because of the shading of phytoplankton) and particularly, detrimental physico-chemical conditions under dense floating mats (such as persistently low oxygen concentration compared to that within beds of submerged plants: Rodríguez-Gallego *et al.*, 2004), may be the complementary causes of the aversion towards this type of plants.

Our results have important implications for understanding the habitat choice and migration patterns of *Daphnia* and probably other cladocerans in shallow (sub)tropical lakes and are supported by field evidence. In subtropical shallow Lake Blanca (Uruguay), the refuge capacity of the submerged plants for zooplankton was weak and changed strongly with seasons and other predator avoidance strategies besides DHM (e.g. vertical migration) seemed to occur (Iglesias *et al.*, in press).

Experimental and field observations in temperate systems have shown strong refuge-mediated cascading effects of submerged plants on the entire ecosystem and on water clarity (Søndergaard & Moss, 1997; Jeppesen *et al.*, 2002). Free-floating and probably also submerged macrophytes (as they seem not to provide a refuge) may therefore have no such positive effect on water transparency in subtropical and tropical lakes. This agrees well with empirical data from numerous Florida lakes showing minor effect of high submerged plant densities on water clarity (Bachmann *et al.*, 2002; Jeppesen *et al.*, in press, b) compared with results from lakes in the north temperate zone (Moss, 1990; Scheffer *et al.*, 1993). However, more experimental and field studies are needed, involving more plant species, other key structuring cladocerans and multiple cues, to elucidate fully the role of macrophytes in warm lakes and their potential cascading effects on water clarity.

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Importancia de las plantas flotantes libres de gran porte en la conservación y rehabilitación de lagos someros de Sudamérica.

Mariana Meerhoff[1,2,3] **y Néstor Mazzeo**[1]

[1] **Sección Limnología, Facultad de Ciencias, Universidad de la República. Iguá 4225, CP 11300, Montevideo, Uruguay.**
[2] **Dep. Plant Ecology, Aarhus University. Nordlandsvej 68, Dk-8240 Risskov, Denmark.**
[3] **National Environmental Research Institute. Vejlsøvej 25, Silkeborg, Denmark**

Las plantas acuáticas presentan un papel muy importante en la estructura y funcionamiento de los lagos someros, y constituyen, por tanto, un elemento clave en el diseño de estrategias de conservación y rehabilitación de estos ambientes. Para algunas formas de vida, como las plantas sumergidas, se conoce ampliamente su influencia en las propiedades fisico-químicas del agua o en la estructura de otras comunidades bióticas, particularmente en regiones templadas. En cambio, aún se desconocen aspectos importantes del papel en los ecosistemas acuáticos de las plantas flotantes libres de gran porte, características de las zonas tropicales y subtropicales. Esta revisión presenta aquellos aspectos conocidos y desconocidos en Sudamérica, de donde son nativas las especies más representativas, y cuáles son las perspectivas futuras en el área básica y aplicada del conocimiento..

Introducción

Los lagos someros presentan diferentes estados alternativos de acuerdo a los grupos de productores primarios que dominan el sistema (Scheffer *et al.*, 1993; Scheffer *et al.*, 2003). Estos ambientes pueden estar dominados por las microalgas o fitoplancton, por plantas sumergidas, o por plantas flotantes libres (**Figura 1**). Las plantas acuáticas condicionan las propiedades fisico-químicas del agua y la estructura de otras comunidades bióticas (por ej. zooplancton y peces) (Jeppesen *et al.*, 1998), mediante la regulación de los intercambios entre los ecosistemas terrestres y acuáticos (Wetzel, 1990; Mitsch y Gosselink, 1993), así como por otros mecanismos descritos en esta revisión.

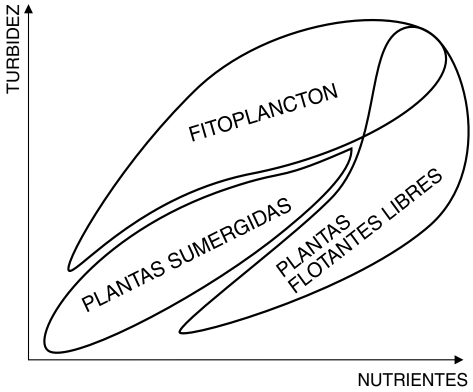

Figura 1. Gradientes de turbidez y nutrientes donde pueden ocurrir los diferentes grupos de productores primarios de un lago somero. Es importante destacar que cada tipo de productor primario puede modificar estas variables, el aumento del fitoplancton incrementa la turbidez y lo opuesto ocurre con las plantas acuáticas.

Existen más de 400 géneros de plantas vasculares reconocidas como acuáticas (Cook, 1990), las cuales se agrupan de acuerdo a su forma de vida en plantas enraizadas y plantas no enraizadas o flotantes libres. El primer grupo incluye plantas emergentes, de

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hojas flotantes, y sumergidas. Las flotantes libres incluyen desde especies pequeñas de los géneros *Azolla* o *Lemna*, hasta especies de mayor porte como *Eichhornia crassipes* (camalote) o *Pistia stratiotes* (repollito de agua) (**Figura 2**), estas últimas nativas de Sudamérica (Cook, 1990). En general todas las formas de vida se encuentran en un amplio gradiente latitudinal en el planeta, excepto las flotantes libres de gran porte, que son características de ambientes tropicales y subtropicales por su gran sensibilidad a las bajas temperaturas del aire y eventos de heladas (Sculthorpe, 1967). A diferencia de otras formas de vida, como la vegetación sumergida (Jeppesen *et al.*, 1998), el papel estructurador y funcional de las plantas flotantes libres de gran porte es escasamente conocido, en particular su rol en las interacciones tróficas directas e indirectas de las zonas litoral y de aguas abiertas (**Figura 3**). El objetivo de la presente revisión es presentar los aspectos conocidos y desconocidos de estos procesos, analizando la conexión entre el conocimiento básico y las estrategias de conservación y rehabilitación de lagos someros en la región.

Efectos de las plantas flotantes libres en sistemas someros de Sudamérica

A nivel mundial, la mayoría de los estudios sobre plantas flotantes libres, especialmente *E. crassipes* y *P. stratiotes*, se relaciona con su crecimiento y capacidad de asimilación de nutrientes (DeBusk y Reddy, 1987), así como con su dispersión y formas de control (Mitchell, 1973). Estas plantas son consideradas las principales malezas acuáticas en sistemas tropicales y subtropicales, tanto en Africa (Cilliers *et al.*, 1996), Asia (Mansor, 1996), Norteamérica (Gutiérrez *et al.*, 1996) y en zonas templado-cálidas de Europa (Moreira *et al.*, 1999) donde son exóticas, como también en partes de Sudamérica (Bini *et al.*, 1999). Además de impactar negativamente múltiples usos de los sistemas (navegación, pesquerías, irrigación, recreación, producción de energía hidroeléctrica y agua potable), esta vegetación podría promover el desarrollo de mosquitos (e.g. Savage *et al.*, 1990) y de otros hospedadores intermediarios de enfermedades como esquistosomiasis (Rumi *et al.*, 2002).

Por otra parte, debido a su "consumo lujurioso" de nutrientes, el camalote y el repollito de agua son empleados en tratamientos de aguas residuales o efluentes industriales a nivel mundial (Vymazal *et al.*, 1998), y en algunos casos

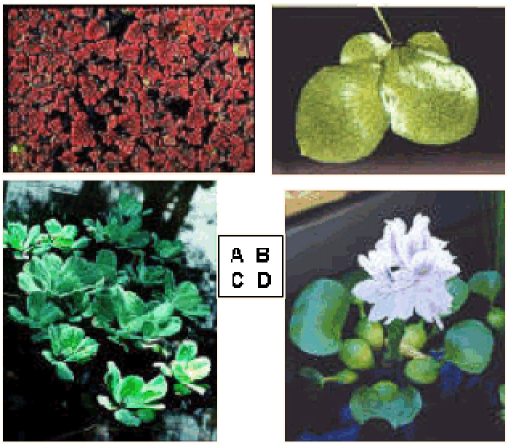
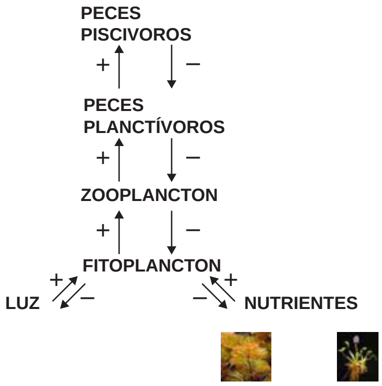


Figura 2. Especies características de plantas flotantes libres pequeñas (A y B) y de gran porte (C y D). A) *Azolla filiculoides*, B) *Lemna gibba*, C) *Pistia stratiotes* y D) *Eichhornia crassipes*.



Efectos	Sumergidas	Flotantes libres
– Disminución de la disponibilidad de luz y nutrientes para el fitoplancton	✓	✓
– Aumento de las tasas de sedimentación de las microalgas	✓	✓
– Reducción de la biomasa fitoplanctónica por retención mecánica del sistema radicular		✓
– Reducción de la resuspensión del sedimento	✓	✓
– Excreción de compuestos alelopáticos	✓	?
– Refugio al zooplancton contra la depredación por peces	✓	?
– Refugio a los peces juveniles contra la depredación (por ej. reducción del canibalismo en poblaciones de peces piscívoros)	✓	?

Figura 3. En la parte superior se indican los factores que condicionan el crecimiento de las microalgas, por ejemplo: disponibilidad de nutrientes (control ascendente) y presión de herbivoría del zooplancton (control descendente). La presión de herbivoría depende a su vez de la relación entre los peces piscívoros y zooplanctívoros. Símbolos: + = efecto positivo, - = efecto negativo. En la parte inferior se comparan los efectos directos e indirectos de las plantas sumergidas y flotantes libres sobre los controles ascendentes y descendentes del fitoplancton. Símbolos: √ = efecto comprobado, ? = escasa evidencia.

en Sudamérica (Dellarrosa *et al.*, 2001). Asimismo, constituyen buenos bioindicadores para el monitoreo de la contaminación con metales (Klumpp *et al.*, 2002) y son ampliamente utilizados como forraje para animales de granja y ración en acuicultura.

En Sudamérica, la mayor parte de las investigaciones sobre las plantas flotantes libres se ha centrado en su relación con la carga de nutrientes. Considerando ambas especies, los estudios sobre *P. stratiotes* son sustancialmente más escasos. Es relativamente abundante la literatura sobre el contenido de nutrientes en los tejidos vegetales (Thomaz y Esteves, 1986), los efectos de estas plantas sobre las características físicas y químicas del agua (Sommaruga *et al.*, 1993; Mazzeo *et al.*, 1995), y sobre las variables ambientales que determinan su distribución, particularmente la concentración de nutrientes y cambios en el nivel del agua (Da Silva y Pinto-Silva, 1989; Walker *et al.*, 1999).

Las interacciones ecológicas de las plantas flotantes libres han sido escasamente estudiadas, patrón aplicable también a las demás formas de vida de las plantas acuáticas en la región (Thomaz y Bini, 2003). Sin embargo, la presencia de plantas flotantes puede afectar fuertemente la trama trófica a través de efectos directos e indirectos sobre distintas comunidades (invertebrados, plancton, peces), tanto litorales como pelágicas.

La interacción plantas flotantes-fitoplancton se ha descrito colateralmente a través de estudios realizados con otros objetivos (Velasco *et al.*, 1999), mientras que muy pocos trabajos han estudiado la comunidad perifítica asociada (Tesolín y Tell, 1996). En sistemas con una cobertura densa, es usual que este tipo de vegetación provoque una disminución de la turbidez del agua, tanto la determinada por una alta biomasa de fitoplancton como por sólidos en suspensión. Además de reducir la penetración de luz y la concentración de nutrientes, y por tanto la producción fitoplanctónica, el sistema radicular de *E. crassipes* puede retener grandes cantidades de biomasa fitoplanctónica y sólidos en suspensión (Poi de Neiff *et al.*, 1994).

La comunidad de macroinvertebrados asociados a las raíces de las plantas flotantes es generalmente muy abundante y presenta una gran riqueza específica (Takeda *et al.*, 2003), incluyendo diversos grupos con predominio de detritívoros, y en particular de oligoquetos (De Marco *et al.*, 2001). La composición y estructura de esta comunidad varía temporal y espacialmente en forma muy significativa, incluso dentro de un mismo sistema (Paparello de Amsler, 1987 a, b) y según el área cubierta por la vegetación. En lagos asociados a la planicie de inundación del río Paraná (Argentina), las biomasas más bajas de macrobentos se registraron en lagos con cobertura total de plantas flotantes (Bechara, 1996). Por otra parte, en el Lago Victoria (Uganda), las mayores densidades y riqueza específica de macroinvertebrados ocurren en las zonas de transición entre las matas de vegetación flotante y las aguas abiertas, disminuyendo en las partes más compactas de las matas (Masifwa *et al.*, 2001), patrón esperable también en Sudamérica. Los macroinvertebrados juegan un papel muy importante en el consumo y descomposición de la vegetación acuática, y constituyen una fuente fundamental de alimento para otras comunidades, particularmente aves y peces.

Precisamente, la enorme importancia de la comunidad de peces en el funcionamiento y estructura de los lagos someros se ha reconocido en años recientes (Jeppesen, 1998). Las distintas formas de vida de las plantas acuáticas afectan diferencialmente la eficiencia de los peces en la captura de las presas y su forma de alimentación (Dionne y Folt, 1991), así como la capacidad de refugio contra depredadores (Persson y Eklov, 1995). Estos efectos sobre la alimentación y uso del espacio de los peces puede determinar cambios de hábitat en el zooplancton (principales herbívoros del fitoplancton) (Romare y Hansson, 2003), generando a su vez efectos indirectos sobre el desarrollo de las microalgas y la transparencia del agua.

En algunos sistemas tropicales y subtropicales con abundante vegetación acuática la distribución espacial de los peces está influida por el pH, la concentración de oxígeno disuelto y la velocidad del agua (Delariva *et al*., 1994), así como la conductividad, la turbidez, y la presencia de otros peces (Fernández *et al*., 1998). En un lago somero e hipereutrófico de Montevideo (Uruguay), la distribución espacial de los peces está condicionada por la forma de vida de las plantas acuáticas presentes (Meerhoff *et al*., 2003). Si bien se observó una alta densidad de peces juveniles y especies de pequeño tamaño tanto en la vegetación flotante libre (*E. crassipes*) como en la sumergida (*Potamogeton pectinatus*), hubo una clara diferencia en el uso de esos hábitats de acuerdo al hábito alimenticio de los peces. Los peces piscívoros (adultos de la castañeta *Cichlasoma facetum* y cabeza amarga *Crenicichla lacustris*) se asociaron fundamentalmente a los camalotes, determinando una mayor presión de depredación sobre los peces más pequeños en ese hábitat (Meerhoff *et al*., 2003) (**Figura 4**). Los individuos juveniles de otras especies piscívoras, como la piraña *Serrasalmus spilopleura*, también se han encontrado en altas densidades entre las raíces de camalotes en Brasil (Sazima y Zamprogno, 1985). Para entender el uso de la vegetación por parte de diferentes tamaños y especies de peces neotropicales, es fundamental cuantificar la complejidad estructural de los distintos hábitats formados por las plantas acuáticas (Agostinho *et al*., 2003).

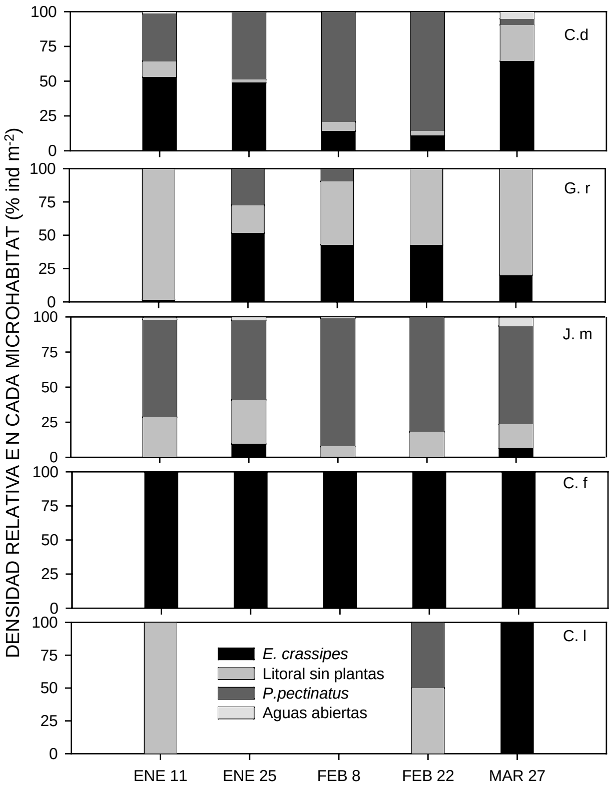


Figura 4. Distribución espacial de peces en un sistema subtropical somero de Uruguay durante la época estival. Los compartimientos analizados fueron litorales sin y con vegetación (*E. crassipes* o *P. pectinatus*) y aguas abiertas (este último no se incluyó debido a la ausencia casi completa de peces). Cd= *Cnesterodon decemmaculatus* (omnívoro-planctívoro), Gr= *Gymnogeophagus rhabdotus* (omnívoro-planctívoro), Jm= *Jenynsia multidentata* (omnívoro-planctívoro), Cf=*Cichlasoma facetum* (omnívoro-piscívoro), y Cl= *Crenicichla lacustris* (omnívoro-piscívoro).

En sistemas templados se ha observado que la vegetación sumergida favorece a las especies de peces piscívoros más comunes, ya que son más eficientes en la captura de sus presas en ambientes estructurados que las especies zooplanctívoras (Persson y Eklov, 1995). En forma similar, el uso de *E. crassipes* por peces piscívoros podría tener distintos efectos en cascada sobre las comunidades de zooplancton y fitoplancton, promoviendo el aumento indirecto del zooplancton de gran tamaño al afectar negativamente la densidad o el comportamiento de los peces zooplanctívoros.

La interacción plantas flotantes-zooplancton prácticamente no se ha estudiado en la región (Meerhoff *et al*., 2003), siendo más abundantes los trabajos que relacionan la estructura de la comunidad zooplanctónica con la presencia de comunidades de plantas acuáticas diversas (Lansac-Thôa *et al*., 2003). En el mismo lago urbano de Uruguay mencionado anteriormente, el zooplancton también presentó una distribución espacial diferente de acuerdo a las formas de vida de las plantas presentes. En comparación con los otros hábitats, se registró una menor abundancia de los organismos de mayor tamaño en las matas de *E. crassipes* (Meerhoff *et al*., 2003) (**Figura 5**). Algunos de los efectos de la vegetación sumergida que contribuyen a una mayor transparencia del agua en sistemas templados, como el refugio para el zooplancton de gran tamaño (Timms y Moss, 1984), parecen no ocurrir en la vegetación flotante de gran porte de acuerdo a dichos resultados de campo (Meerhoff *et al*., 2003) y otros estudios experimentales. Experimentalmente se encontró que *E. crassipes* provoca repelencia sobre *Daphnia*, y aunque los mecanismos no son aún del todo claros, este comportamiento parecería ser mediado químicamente (Meerhoff, datos no publicados).

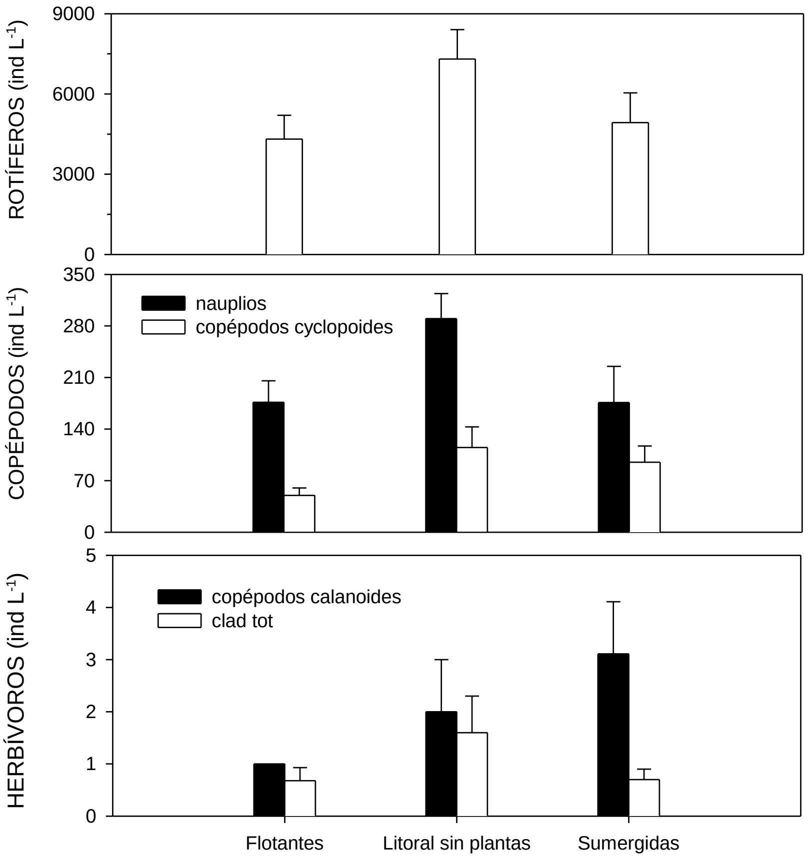


Figura 5. Distribución espacial del zooplancton en el mismo lago y hábitats de la Figura 4, zonas litorales sin y con vegetación (E. *crassipes* o P. *pectinatus*). Los datos representan el promedio y el error típico de la colectas realizadas durante el verano 2000.

Líneas de investigación a profundizar o desarrollar

Pocos trabajos han aplicado un enfoque comunitario o ecosistémico acerca del papel de esta vegetación en el funcionamiento de los lagos, tanto a nivel mundial como en Sudamérica. Los mecanismos que se encuentran detrás de la aparente repelencia provocada por *E. crassipes* sobre el zooplancton, y notoriamente sobre *Daphnia*, no se han elucidado. Se desconoce por tanto si el mismo patrón sería esperable en otras plantas flotantes de gran tamaño, como *P. stratiotes*, y en otras regiones del mundo donde esta vegetación es abundante. Las áreas de investigación relacionadas, ecología del comportamiento y ecología química, están adquiriendo una creciente importancia en los últimos años. Es fundamental realizar estudios experimentales acerca de la influencia de esta vegetación en varios

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aspectos del zooplancton, como el comportamiento, la historia de vida, crecimiento y reproducción. También es importante conocer los posibles efectos aleloquímicos de estas plantas en condiciones naturales, tanto sobre el fitoplancton como otras comunidades.

Los efectos descritos sobre los peces piscívoros (Sazima y Zamprogno, 1985; Meerhoff *et al.*, 2003), requieren para su generalización de más estudios de campo en sistemas con comunidades de peces complejas, así como en escalas temporales variadas para determinar si esos efectos promueven cambios diferenciales en la supervivencia y reproducción de piscívoros y planctívoros. Asimismo, aún se desconoce la influencia de las plantas flotantes libres de gran porte sobre otras comunidades, como las aves. En lugares donde esta vegetación es exótica, se han descrito posibles efectos positivos (Bartodziej y Weymouth, 1995), así como negativos (Rodgers *et al.*, 2001), sobre la alimentación, sustrato y anidación de aves acuáticas.

Implicaciones del manejo de la vegetación flotante libre en la conservación y rehabilitación de lagos someros

El establecimiento de plantas acuáticas sumergidas es tanto una herramienta como un objetivo fundamental en los programas de conservación o rehabilitación, especialmente de los lagos someros templados del Hemisferio Norte (Moss *et al.*, 1996). Esto se debe a que las plantas sumergidas presentan una relación inversa con la turbidez del agua, patrón encontrado en gran cantidad de trabajos durante las décadas de los ´80s y ´90s realizados en Europa, Estados Unidos y Canadá, fundamentalmente. La rehabilitación de lagos someros eutróficos implica en la actualidad no sólo la reducción de la carga externa e interna de nutrientes (procedimiento tradicional), sino también el manejo de las comunidades del propio lago, especialmente de los peces (Shapiro *et al.*, 1975).

Las zonas tropicales y subtropicales se enfrentan a grandes desafíos en el futuro inmediato para revertir las consecuencias de los procesos de eutrofización, los cuales han sido señalados recientemente por Jeppesen y colaboradores (en prensa). En este contexto, es imprescindible entender el rol de la vegetación flotante libre de gran porte en los aspectos relacionados con el consumo de las microalgas, especialmente su papel en la composición y distribución espacial del zooplancton y peces. Esta información nos permitirá establecer si es viable usar esta vegetación no sólo para reducir la disponibilidad de nutrientes en el agua (alcanzable con altas coberturas de la vegetación), sino también para aumentar indirectamente el consumo del fitoplancton (cuyo desarrollo excesivo es la principal consecuencia de la eutrofización). Asimismo, es fundamental entender los cambios que la presencia simultánea de vegetación sumergida y flotante libre origina en la estructura y funcionamiento de los lagos someros, en comparación con la presencia de una sola de las formas de vida.

Además de los aspectos básicos señalados, el notable crecimiento vegetativo de *Eichhornia crassipes* y *Pistia stratiotes*, con los efectos adversos descritos anteriormente, requiere del desarrollo de métodos de confinamiento o de cosecha mecánica continua. En los sistemas subtropicales de la zona límite de su distribución geográfica, el crecimiento de estas plantas está limitado naturalmente en invierno por las bajas temperaturas, por lo que su desarrollo como maleza es menos usual que en sistemas tropicales. Para mantener esta vegetación en los niveles deseados, es imprescindible generar nuevas tecnologías que aumenten la capacidad de control y reduzcan los costos de los dispositivos y maquinaria existentes en la actualidad.

Por último, el aumento en la temperatura provocado por el cambio climático puede promover la expansión geográfica de esta vegetación a zonas donde actualmente está limitada. Este escenario requiere de más investigaciones de campo y estudios experimentales para diseñar medidas de manejo o prevención adecuadas.

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Effects of contrasting climates and habitat complexity on community structure and predator avoidance behaviour of zooplankton in the shallow lake littoral

Mariana Meerhoff [1,2,3]*, Carlos Iglesias[4], Franco Teixeira de Mello[4,5], Juan M. Clemente[3],
Elisabeth Jensen[1], Torben L. Lauridsen[1] & Erik Jeppesen[1,2]

[1] National Environmental Research Institute. Vejlsøvej 25, 8600 Silkeborg, Denmark
[2] Department of Plant Biology, Institute of Biological Sciences, University of Aarhus, Ole Worms Allé, Building 135, 8000 Aarhus C, Denmark
[3] Sección Limnología, Facultad de Ciencias, Universidad de la República. Iguá 4225, CP 11400, Montevideo, Uruguay
[4] Grupo de Investigación en Ecología Básica y Aplicada, Asociación I+D, Montevideo, Uruguay
[5] Grupo de Química Ambiental y Ecotoxicología, Facultad de Ciencias, Universidad de la República.
Iguá 4225, CP 11400, Montevideo, Uruguay

*** Corresponding author**: Email: mm@dmu.dk merluz@fcien.edu.uy

Running title: structure and predator avoidance of zooplankton in warm lakes

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Summary

1. Structural complexity may stabilize predator-prey interactions and affect the outcome of trophic cascades by providing prey refuges. In contrast to deep lakes, where vulnerable zooplankton move vertically to avoid fish predation, in shallow temperate lakes submerged plants often provide a diel refuge for pelagic zooplankton against fish predation, with consequences for the whole ecosystem. Several questions remain about the role of plants in regulating these trophic interactions, such as the effects of plant architecture and the predictability of macrophyte function under different climates.

2. We introduced artificial plant beds in the littoral area of five shallow lakes in Uruguay (30°-35° S) and Denmark (55°-57° N), and analysed the hypothesis that plants act as refuges for zooplankton considering two main factors: climate (temperate *versus* subtropical) and plant architecture (submerged *versus* free-floating) along a water turbidity gradient over which the lakes were paired.

3. We found remarkable differences in the structure (taxon-richness, composition and density) of the littoral zooplankton communities between climate zones. Cladoceran communities were richer and frequently included *Daphnia* spp. and other large-bodied taxa in the temperate lakes, whereas small-bodied taxa typically occurred in the subtropical lakes. The benthic/plant-associated cladocerans were also more diverse in the temperate lakes. The densities of all crustaceans, except calanoid copepods, were significantly higher in the temperate lakes (*ca.* 7-fold higher).

4. Fish and shrimps exerted a stronger predation pressure in the plant beds in the subtropical lakes, with the pelagic invertebrate *Chaoborus* sp. being more abundant than in the temperate lakes. In contrast, predatory cladocerans were frequent, and plant-associated predatory macroinvertebrates were 8-fold more abundant in the temperate lakes. No direct effects of turbidity were observed on any of these communities nor on cladoceran density.

5. Plant architecture affected cladoceran dynamics. The artificial submerged plants hosted significantly more cladocerans than the free-floating plants, which were particularly avoided in the subtropical lakes. Diel horizontal (DHM) and diel vertical (DVM) migration of free-swimming cladocerans occurred simultaneously in both climate zones. DHM was the most frequent pattern observed for both average populations and individual taxa in the temperate lakes. In contrast, in three subtropical lakes we found significant evidence of DVM irrespective of water turbidity, DVM being also the commonest response of individual taxa.

6. Temperature-related selective forces, namely higher fish predation, seem to shape the general structure and dynamics of cladoceran communities in the subtropical lakes. Our results support the hypothesis that DHM is not as prevalent in the subtropics as in temperate lakes. Despite the occurrence of DVM, the density and structure of the cladoceran community indicate that no predator-avoidance behaviour suffices to counteract the predation pressure in the subtropics. Our results indicate that positive effects of aquatic plants on water transparency, via their acting as refuge for zooplankton, may be generally weak or rare in warm lakes.

Introduction

Trophic cascades (indirect effects of carnivores on plants mediated by herbivores) may have profound impacts on the structure and functioning of several ecosystems, particularly so in aquatic environments (Shurin *et al.*, 2002). There is still considerable debate about the ultimate mechanisms causing the variation in the trophic cascades; whether predators indirectly affect the plants by reducing the numbers of herbivores or by inducing anti-predator behaviour in the prey (Lima & Dill, 1990). The last mechanism seems to be more important than the classical numerical effects in several types of ecosystems (Schmitz *et al.*, 2004) In lakes, the spatial distribution of fish can have important consequences for the spatial distribution of their prey and may modify the expected outcome of direct interactions (Romare & Hansson, 2003). However, structural complexity may strongly mediate both competitive and predatory interactions by providing prey refuges, thus stabilizing predator-prey interactions and, potentially, sustaining more diverse communities.

"The plants, though not essential for cladoceran growth, may be necessary to tilt the balance between fish predators and their most vulnerable prey, in favour of the latter" (Timms & Moss, 1984). After this first suggestion, increasing research has focused on testing the hypothesis that aquatic plants provide refuge for large-bodied grazers against fish predation in shallow lakes (Burks *et al.*, 2002). In these systems pelagic zooplankton often migrate horizontally into the littoral area, seeking daytime refuge from predators, primarily fish (Lauridsen & Buenk, 1996; Burks *et al.*, 2002). This refuge effect of submerged macrophytes, enhancing a higher survival of pelagic cladocerans with consequent stronger grazing pressure on phytoplankton, helps maintain water transparency (Timms & Moss, 1984; Scheffer *et al.*, 1993), and indirectly diversity (Declerck *et al.*, 2005), in temperate lakes. Furthermore, the mobility of pelagic zooplankton may enhance the coupling between the pelagic and the littoral habitats (Van de Meutter *et al.*, 2004), as has been indicated for fish (Schindler & Scheuerell, 2002).

By contrast, in deep lakes vulnerable zooplankton typically move to the hypolimnion during the day, where the colder, darker and less oxygenated waters offer refuge from fish visual predation, and move upwards at night (Lampert, 1993; Ringelberg & Van Gool, 2003). The patterns of diel horizontal migration (DHM) seem, however, far less predictable than those of diel vertical migration (DVM) (Burks *et al.*, 2002). The refuge effect of plants for cladocerans, particularly for the important grazer *Daphnia*, varies with the composition of the potential predators (Jeppesen *et al.*, 1997a), as the plants can also provide a refuge for juvenile fish against piscivorous fish predation (Persson & Eklöv, 1995) and host multiple predatory invertebrates (Burks, Jeppesen & Lodge, 2001a). The refuge effect also seems to depend on the architecture (Nurminen & Horppila, 2002), bed size (Lauridsen *et al.*, 1996), density (Burks, Jeppesen & Lodge, 2001b) and volume (Schriver *et al.*, 1995) of the aquatic plants, and the trophic state of the lake (Lauridsen *et al.*, 1999). Under low-nutrient conditions, high transparency and low density of plants enhance the fish predation pressure, whereas, under hypertrophic conditions, the refuge effect is again weak due to the often high densities of planktivorous fish and scarcity of submerged plants (Jeppesen *et al.*, 1997b).

Several key questions remain about the role of macrophytes in regulating these trophic interactions (Burks *et al.*, 2006), the predictability of macrophyte function under different climates being an issue of increasing importance, particularly seen in the light of the current global warming scenario. Most studies on DHM have focused on Northern temperate lakes (Burks *et al.*, 2002). Freshwater lakes located in warmer climate areas differ from temperate lakes in key characteristics regarding the fish community, which likely exerts a stronger predation pressure on the zooplankton compared to similar temperate lakes (Jeppesen *et al.*, 2005; Meerhoff *et al.*, submitted). Concomitantly, large-bodied pelagic cladocerans are rare (Fernando, 2002) while *Daphnia* spp. commonly occur in low abundance in (sub)tropical systems (Mazumder & Havens, 1998; Pinto-Coelho *et al.*, 2005). Some studies suggest a less positive effect of submerged plants as a zooplankton refuge in the subtropics. However, so far, only few studies of eutrophic lakes (Meerhoff *et al.*, 2003; Iglesias *et al.*, in press) and laboratory experiments (Meerhoff *et al.*, 2006) have been conducted. Although far less studied than the submerged plants, other plant architectures (e.g. large free-floating plants) are particularly important in warm climates and are likely to become more important in the future due to the increasing winter minimum air temperatures. In laboratory behaviour experiments, *Daphnia* avoided free-floating plants more than submerged plants, although all plant types were avoided even in the presence of crushed conspecifics (Meerhoff *et al.*, 2006). Yet it is still unclear whether these results can be generalized, i.e., whether *Daphnia* and other cladocerans would respond similarly, especially under other environmental conditions and realistic, multiple cues scenarios.

We analysed the refuge hypothesis of plants for zooplankton considering two main factors: climate (temperate *versus* subtropical) and plant architecture (submerged versus free-floating) along a water turbidity gradient (covering five levels). We hypothesised that the refuge effect for zooplankton would be substantially lower in a warmer climate as a result of the higher densities of zooplanktivorous fish and would decline with decreasing plant structural complexity, and with increasing turbidity.

Methods

Design and sampling methodology

We selected a set of five shallow lakes with contrasting water transparency in Uruguay (30°-35° S) and Denmark (55°-57° N). The lakes were paired in both countries in terms of Secchi disk transparency (1.6, 1.0, 0.7, 0.4, 0.20 m SD) and, within the pairs, had similar size, nutrient concentrations, and macrophyte cover (range: 0-70% PVI, percent volume inhabited *sensu* Canfield *et al.*, 1984).

In each lake we introduced artificial plant beds mimicking submerged and large free-floating plants (four replicates of each). The use of artificial substrata implies the loss of chemical interactions; however, we ensured that initial conditions of substratum amount and quality were identical to properly compare the structure and dynamics of the littoral communities. The modules consisted of 1-m diameter PVC plastic rings with an attached net from which the artificial plants were hanging. Both submerged and free-floating plants were made of the same plastic material (originally green Christmas' trees decorations). In each submerged plant module we used 100 0.8-1.0-m long plants, with an architecture resembling *Cabomba* or *Elodea* spp. (3.5-cm long "leaves"). The free-floating plants (40 per module) consisted of a 15-cm diameter plastic disc, to limit the light passage in a patchy manner, with 2-m long plastic pieces (1.5-cm long "root hairs") arranged to mimic the root network of *Eichhornia crassipes* (Mart.) Solms. Both types of habitat modules therefore had a similar structure (between 80-100 m of plastic material in total) and PVI of 49% and 30% for submerged and free-floating plants, respectively. We placed the habitat modules at 1-m depth in sheltered and plant-free areas. The sampling campaigns were conducted in summer (January 2005 in Uruguay, and July 2005 in Denmark, average water temperature 27 °C and 18 °C, respectively), about four weeks after the introduction of the habitat modules in the lakes, thus allowing periphyton and invertebrates to colonize the plastic structures. The lakes were sampled consecutively.

In open water, we took water samples to analyse total phosphorus and total nitrogen (Valderrama, 1981; Søndergaard, Kristensen & Jeppesen, 1992), chlorophyll-a (Jespersen & Christoffersen, 1987), alkalinity, and measured *in situ* parameters (transparency as Secchi disk depth, PAR light attenuation, pH, temperature, conductivity). We took day and night column samples for zooplankton with a 1.0-m long tube (6-cm diameter) from each module and from four open water sites nearby (pooled total of 8 litres each, filtered through a 50-μm mesh size and preserved with lugol 4%). We removed one "plant" per module to collect the potentially predator macroinvertebrates (sieved through 500-μm mesh size, and preserved in 70% alcohol). In the night, we sampled the fish and shrimps strictly associated to each plant module using a cylindrical net (1.20-diameter, mesh size 0.3 cm) which was previously placed over the sediment and below each module and subsequently lifted quickly from the boat with a 1.5-m long hook. The same modules were used in both countries. Before shipping them, they were washed using high water pressure, disinfected with concentrated chlorine solution, rinsed thoroughly and sun-dried.

We identified all cladoceran zooplankton to genus or species level and counted at least 50-100 individuals of the most abundant taxa. Copepods were counted as cyclopoids or calanoids (including adults and copepodites). We classified the cladoceran genera *Bosmina, Ceriodaphnia, Daphnia, Diaphanosoma, Leptodora, Moina, Scapholeberis, Simocephalus*, and *Polyphemus* as free-swimming.

Statistical analyses

We analysed the effects of climate and plant architecture on each target variable, after matching the lakes by their turbidity level, by applying 3 and 4-way factorial ANOVA (factors: climate, 2 levels; turbidity, 5 levels; plant architecture, 3 levels in the case of zooplankton (plants plus open water); time, 2 levels). To analyse diel spatial changes in the density of individual taxa at each turbidity level, we used 2-way ANOVA (factors: habitats and time). In the case of significant interactions between climate and other factors, we estimated the overall levels of each target variable within climate zones by LSMEANS (PROC MIXED, SAS Institute Inc., 2004), averaging over significant main and interactive effects of turbidity, habitat and time. We classified observed patterns as "classic diel horizontal migration" (DHM) when the densities in the (submerged) plants decreased in the night with a concomitant, even slight, increase in the open water (significant interaction "plant architecture" x "time" in the ANOVA tests). By contrast, we classified patterns as "classic diel vertical migration"(DVM) when we found a nocturnal density increase in all habitats (significant effect of "time"). Diel patterns contrasting those described above were classified as "reverse" (RHM, RVM). However, a nocturnal decrease in all habitats can be interpreted also as extreme DHM, i.e. if the animals dispersed beyond the sampled littoral area, such as into the true pelagic zone. Data were prior $\log_{10}(x+1)$-transformed to fulfil the requirements of homoscedasticity (Cochran's test) and normal distribution of residuals. *Post hoc* analyses were made using Tukey HSD tests.

Results

Community structure and abundance

We found remarkable differences in the structure of the zooplankton communities between climate zones, both as to taxon-richness, composition, and density (Fig. 1). Cladoceran richness was significantly higher in temperate lakes (LSMEANS, $p<0.0001$). *Daphnia* spp., while frequent and abundant in the temperate lakes, appeared scarcely in only one lake in the subtropical set. The free-swimming cladoceran community in the subtropical lakes was typically composed of the small-bodied *Diaphanosoma, Bosmina, Moina,* and *Ceriodaphnia* spp. (ordered by their frequency of occurrence), and in lower densities the large-bodied *Simocephalus* spp. Also the benthic/plant-associated cladocerans included a more diverse community in the temperate lakes, including the large-bodied, plant-attached *Sida crystallina* Müller and *Eurycercus lamellatus* Müller. Also the chydorids *Chydorus*, *Pleuroxus*, *Acroperus*, *Alona* and *Alonella* spp. frequently occurred (Fig. 1). By contrast, in the subtropics, we found less taxa, *Chydorus, Alona,* and *Pleuroxus* spp. being the most representative benthic/plant-associated genera. The density of cladocerans was also significantly higher in the temperate lakes, when considering both total and the free-swimming genera (LSMEANS, $p<0.0001$, on average 7-fold higher) (Fig. 1). The density of free-swimming genera varied from 4.4 (±0.6 SE) to 46.5 (±9.1 SE) ind L^{-1} in the subtropical lakes, and from 24.4 (±3.9 SE) to 284.1 (±65.1 SE) ind L^{-1} in the temperate lakes (whole lake sample means). Also the density of the benthic/plant-associated genera was higher (on average 7-fold) in the temperate lakes, the magnitude of the differences being affected by turbidity and habitats (significant interaction terms in ANOVA test). We found more cyclopoid than calanoid copepods in the temperate lakes (23.1 (±1.9 SE) and 3.2 (±1.0 SE) ind L^{-1}, respectively, whole lake sample means across turbidity levels). However, similar average densities of cyclopoid and calanoid copepods occurred in the subtropics (10.1 (±1.1 SE) and 9.6 (±1.2 SE) ind L^{-1}, respectively, whole lake sample means across turbidity levels). These patterns were maintained for copepodites as well. The number of genera and the density of free-swimming and benthic/plant-associated cladocerans varied along the turbidity gradient (Fig. 2). However, due to the significant interaction between "turbidity" and "climate" or "habitats" in the ANOVA tests, no direct effects of turbidity could be identified.

The structure and density of zooplankton predators also differed substantially between climate zones (Fig. 2). The subtropical fish communities associated with the plant beds were dominated by small-bodied individuals and had significantly much higher densities than in the respective temperate lakes (Meerhoff *et al.*, submitted). The shrimp *Palaemonetes argentinus* Nobilli, which has a broadly omnivorous diet (Collins, 1999) was frequently present in high densities in the subtropics. In almost all lakes in both climate zones we found some invertebrate predator in the pelagial, adding to the predation pressure exerted by fish. The predatory invertebrate *Chaoborus* spp. (phantom midge) appeared more frequently and in slightly higher densities in the subtropical lakes (0.31 ±0.05 SE *versus* 0.19 ±0.04 SE ind L^{-1},

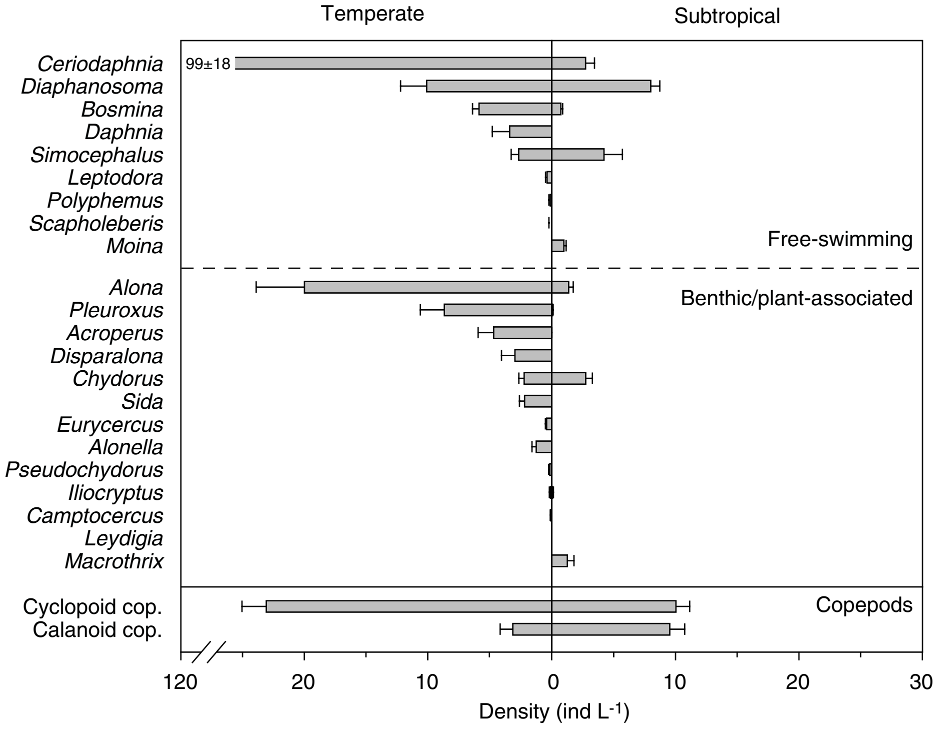

Fig. 1. Mean density of free-swimming and benthic/plant-associated cladoceran genera and adult copepods (calanoid and cyclopoid) in temperate (left) and subtropical (right) lakes. The means represent averages of whole lake and day-night lake average densities (± 1 SE).

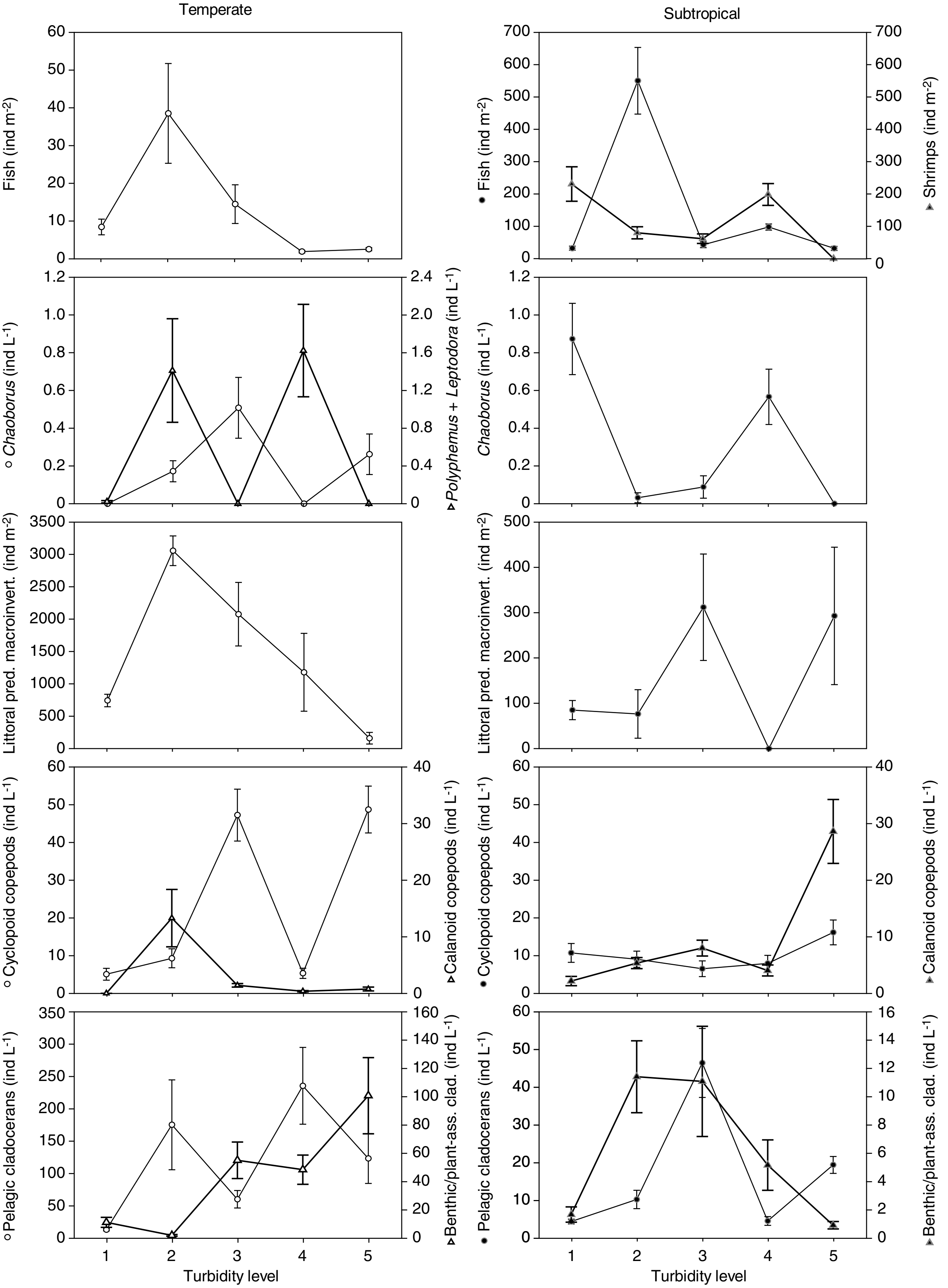

Fig. 2. Changes in mean density (± 1 SE) along a turbidity gradient in artificial plant beds (in all habitats for zooplankton) in temperate (left) and subtropical (right) lakes of: fish (ind m^{-2}), pelagic invertebrate predators (*Chaoborus, Leptodora* + *Polyphemus*, ind L^{-1}), littoral predatory macroinvertebrates (ind m^{-2}), adult calanoid and cyclopoid copepods (ind L^{-1}), and free-swimming and benthic/plant-associated cladocerans (ind L^{-1}). Notice the different scales for both climate zones.

marginally significant effect of climate in ANOVA test). However, the pelagic cladoceran predators *Leptodora kindtii* Focke (giant water flea) and *Polyphemus pediculus* L. occurred only in the temperate lakes and showed a pattern quite opposite to that of *Chaoborus* sp. (Fig. 2). The plant-associated predatory macroinvertebrates were more diverse and had much higher densities (*ca.* 8-fold higher) in the temperate lakes than in the comparative subtropical lakes. For none of the potential predators could abundance be directly related with water turbidity, and neither could cladoceran and copepod densities (Fig. 2).

The role of plant architecture

We found higher densities of cladocerans (total and free-swimming) among the submerged plants, in both climate zones (55.0% ±10.5 SE, and 46.8% ±7.7 SE, in temperate and subtropical lakes, respectively). However, the second highest densities were found among the free-floating plants in the temperate region and in open water in the subtropics. In the temperate lakes, the density of cladocerans among the free-floating plants was intermediate between submerged plants and open water, whereas in the subtropical lakes the free-floating plants were the habitat with a constantly lower relative density (Fig. 3). The benthic/plant-associated cladocerans also exhibited higher densities among the submerged plants in both climate zones (3-way ANOVA, habitat effect, $F_{(2,225)}=82.4$, $p<0.001$). In general, the cyclopoid copepods were more abundant with increasing structure, i.e., highest densities occurred among the submerged plants followed by free-floating plants and, lastly, open water. By contrast, the calanoid copepods presented the opposite pattern in both climate zones.

Most of the potential predators also displayed a differential use of both plant types. Despite frequently showing higher densities in open water, the densities of *Chaoborus* spp. did not differ significantly among habitats in any of the climate zones. *Leptodora kindtii* had higher densities in open water, while *P. pediculus* appeared only in the plant beds and during the day. In the temperate zone fish were more pelagic, as indicated by their stronger association with the free-floating plants, whereas fish significantly associated with the submerged plant beds in the subtropics (3-way ANOVA, interaction plants x climates, $F_{(1,63)}=5.85$, $p=0.018$). The plant-associated predatory macroinvertebrates had higher densities in the free-floating plants (per unit of plant weight), in both climate zones.

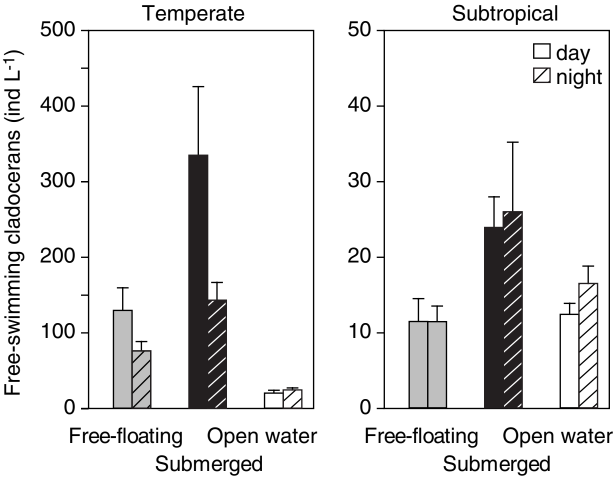

Fig. 3. Diel spatial distribution of free-swimming cladocerans in temperate and subtropical lakes. The data represent the sample means (± 1 SE) of five lakes (average of lake averages).

Diel migration patterns

The diel changes in total densities of the free-swimming cladocerans differed remarkably between climate zones, although the magnitude was affected by the turbidity level (4-way ANOVA, significant 3rd order interaction, $F_{(8,184)}=2.19$, $p<0.0293$). We found higher

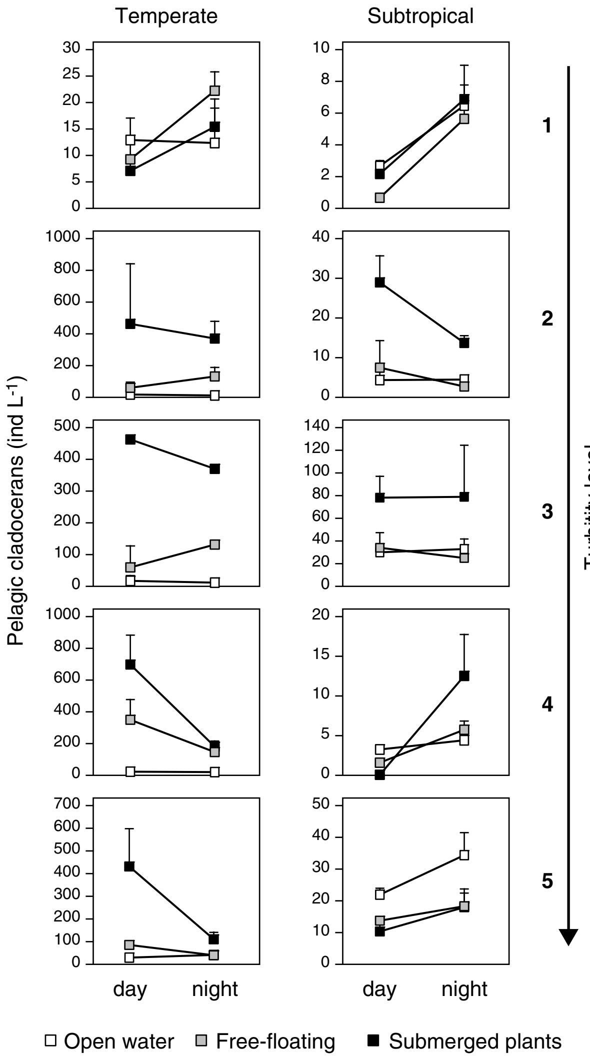

Fig. 4. Diel changes in density in the submerged plants, free-floating plants and open water of the population of free-swimming cladocerans, in temperate (left) and subtropical (right) lakes in lakes along a turbidity gradient (increasing from top to bottom). Lakes in both climate zones were matched by their Secchi disk depth. Notice the different scales for both climate zones.

relative densities of cladocerans during the day in the temperate lakes (60.7% ±7.4 SE) but during the night-time in the subtropics (61.0% ±9.1 SE). In the temperate zone, the most frequently observed patterns fitted with the predictions of the classic DHM: the densities of free-swimming cladocerans in the submerged plants decreased during the night and increased slightly in the open water (Fig. 4, Table 1). In the subtropical lakes, however, we found clear signs of the classic DHM in only one lake, whereas in three lakes we found significant evidence of diel vertical migration. These DVM patterns occurred at both extremes of the turbidity gradient in the subtropics, while in the temperate zone DHM seemed to occur at all turbidity levels except in the clearest lake (Table 1). In this lake, the total density of free-swimming cladocerans exhibited a reverse horizontal migration pattern (RHM: higher nocturnal densities in the free-floating and submerged plants; lower densities in open water).

Cyclopoid copepods often increased their nocturnal densities in all habitats in both climate zones, which fits with the DVM pattern. The calanoids displayed DVM in most temperate lakes, whereas patterns were more diverse in the subtropical lakes (Table 1).

We observed a broad set of responses by the same taxa, both across and within each climate zone (Fig. 5). In some cases, the behaviour of the individual taxa most susceptible to predation (in terms of size and abundance) differed from the average population pattern (Table 1). We could not relate particular behaviour patterns with a taxa being the highest abundant in a lake. However, when considering the behaviour of individual herbivorous cladoceran taxa, some general patterns emerged. Firstly, the most frequent response of the largest taxa in each lake was in most cases DVM (in seven out of ten lakes). In the temperate set, DVM was the pattern displayed by the largest taxa in four lakes, covering all the turbidity levels. In the subtropical set, DVM by the largest present taxa occurred in the last three levels of turbidity. In the clearest lake, the most abundant *Bosmina* sp. showed significant DVM, whereas the slightly larger-bodied but less abundant *Ceriodaphnia* sp. displayed DHM. Secondly, DHM represented the commonest migration performed by individual taxa in the temperate lakes (75% of the statistically significant patterns in the ANOVA tests), whereas DVM was most common in the subtropics (71% of the significant responses). Other spatial patterns, such as those fitting

Table 1. Migration patterns of the average population and the most abundant free-swimming zooplankters in each pair of lakes (temperate and subtropical), along the turbidity gradient (increasing from 1 to 5). The taxa are ordered by decreasing body size. Bold letters indicate significant results in the 2-way ANOVA tests. The total number of each significant pattern by individual herbivorous cladoceran taxa is presented below (non significant patterns in parentheses). Symbols: DVM: diel vertical migration, DHM: diel horizontal migration, RHM: diel reverse horizontal migration, RVM: diel reverse vertical migration, No M: no signs of migration, -: genus absent, d: present in just one habitat or too low numbers for statistical tests, ?: interpreted pattern although the change in density differed from expectations in one habitat.

Turbidity	**1**		**2**		**3**		**4**		**5**	
	Temp.	Sub.	Temp.	Sub.	Temp.	Sub.	Temp.	Sub.	Temp.	Sub.
Clad. population	RHM	**DVM**	DHM?	DHM	**DHM**	No M	DHM?	**DVM**	DHM	**DVM**
Sida	d	-	DVM	-	DVM	-	DHM	-	DVM	-
Eurycercus	d	-	d	-	DHM	-	DHM	-	d	-
Daphnia	-	d	**DHM**	-	DVM	-	DHM?	-	-	-
Simocephalus	d	d	No M	RVM	DHM	DVM	-	-	d	-
Diaphanosoma	d	d	**DHM**	**DHM**	**DHM**	DHM	DHM	**DVM**	DHM	**DVM**
Ceriodaphnia	**DVM**	DHM	No M	-	**DHM**	**DHM**	DHM?	-	DHM	-
Moina	-	-	-	-	-	RHM	-	DVM	-	**DVM**
Bosmina	RHM	**DVM**	d	-	**DVM**	d	**DHM**	**DVM**	**DHM**	-
Calanoid cop.	-	**DVM**	**DVM**	**DHM**	**RVM**	DHM	DVM	**RVM**	**DVM**	RVM
Cyclopoid cop.	**DVM**	DVM	DVM	**DHM**	**DHM**	**DVM**	DHM	DHM	**DVM**	RVM
Predators:										
Leptodora	DVM	-	**RHM**	-	-	-	**RHM**	-	-	-
Polyphemus	-	-	RVM	-	-	-	RVM	-	-	-
Chaoborus	-	**DVM**	**DVM**	**DVM**	**DVM**	**DVM**	-	**DVM**	**DVM**	-
Tot DHM	0	(1)	2	1	2 (2)	1 (1)	1 (5)	0	1 (2)	0
Tot DVM	1	1	(1)	0	1 (2)	(1)	0	2 (1)	(1)	2
Tot RHM	(1)	0	0	0	0	(1)	0	0	0	0
Tot RVM	0	0	0	(1)	0	0	0	0	0	0

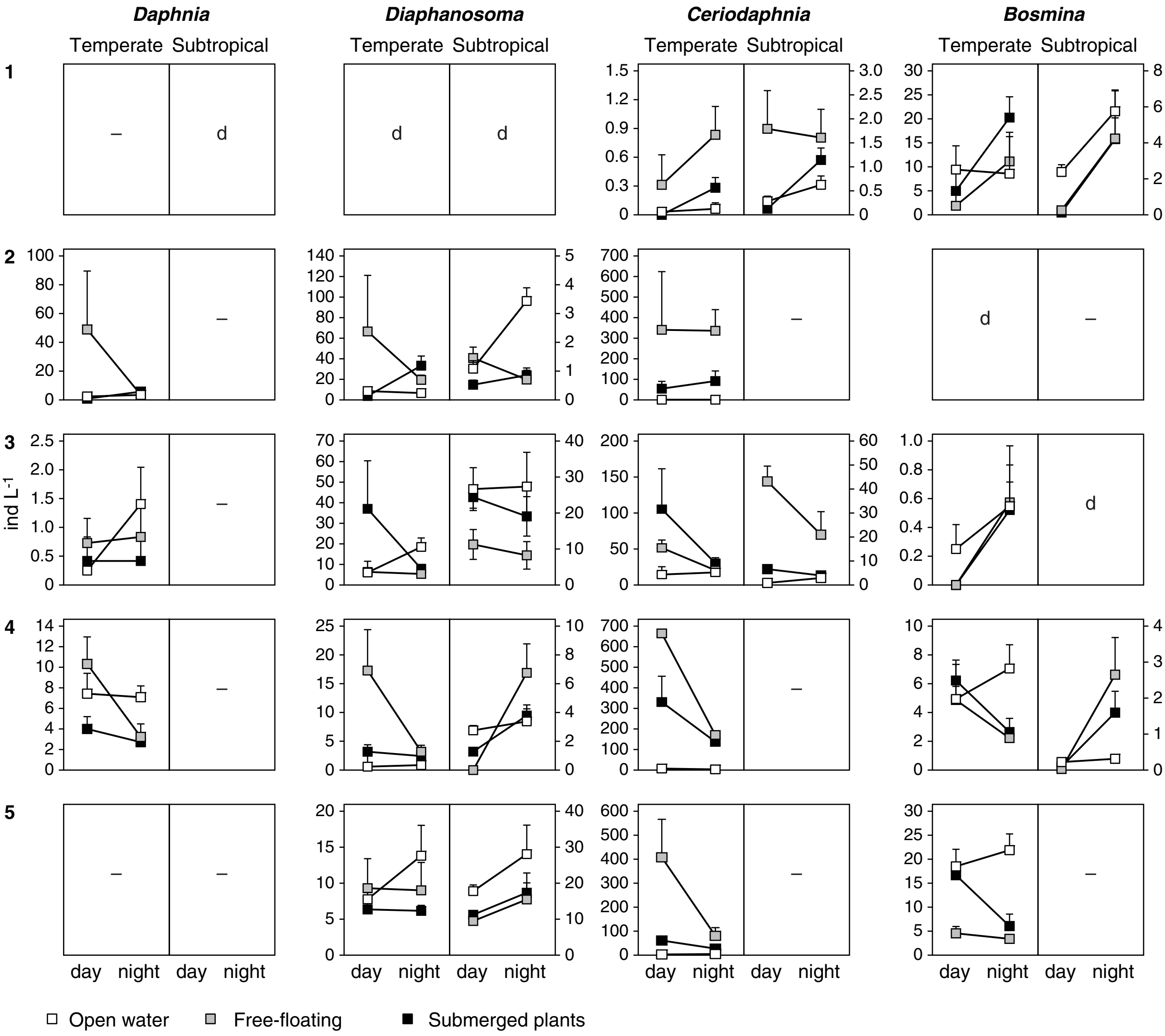

Fig. 5. Diel migration patterns of *Daphnia*, *Diaphanosoma*, *Ceriodaphnia* and *Bosmina* spp. in temperate and subtropical lakes along a turbidity gradient (increasing from top to bottom). The empty boxes indicate the genus was either absent (-) or present in too low numbers to apply statistical tests (d) in that lake.

with reverse horizontal migration and reverse vertical migration, were seldom found (Table 1). In few cases we interpreted the latter pattern as a nocturnal dispersal of the animals beyond the sampled area, i.e. into the true pelagic zone. This was the case for *Ceriodaphnia* sp. in Lake Stigsholm (Denmark, turbidity level 4), where densities in the three sampled habitats decreased by 50% at night-time.

Chaoborus spp. performed DVM under all environmental conditions (climate and turbidity level), with significantly higher densities at night-time. The other pelagic predators, *L. kindtii* and *P. pediculus*, appeared mostly during the day.

Discussion

We found notable differences in zooplankton structure (taxon-richness, density and size) and dynamics between the two climate zones, regardless of the gradient in water transparency and other environmental variables (such as nutrient status and lake area). Subtropical zooplankton communities were characterised by much lower densities and a taxon-poorer composition (at the genus level), leading to a smaller size structure of the cladoceran community than in similar temperate lakes. There is still some controversy about the diversity patterns of cladocerans in (sub)tropical versus temperate areas (Dumont, 1994; Fernando, 2002), but the view that cladoceran diversity is low in the tropics is currently being challenged (Sarma *et al.*, 2002). The cladoceran community structure found in the littoral area of the subtropical lakes concurs with other studies suggesting that large-bodied pelagic cladocerans, and particularly *Daphnia* spp., are infrequent in the (sub)tropics (Fernando, 2002; Mazumder & Havens, 1998; Pinto-Coelho *et al.*, 2005). *Daphnia* cannot survive where abundant fish nullify the refuge provided by the plants (Venugopal & Winfield, 1993). Large-bodied *Daphnia*, even larger than predicted by latitudinal models (Gillooly & Dodson, 2000), can be abundant in subtropical lakes as long as fish are absent (N. Mazzeo & G. Lacerot, unpubl. data). Accordingly, almost no large-bodied cladocerans were found in the subtropical lakes studied. However, remarkably rich *Daphnia* communities have appeared in the sediment records of some lakes (Mergeay *et al.*, 2004), while ephippia and remains of *Daphnia* and other large cladocerans have been found in the sediments of several of the studied shallow lakes in Uruguay (K. Jensen, unpubl. data). This indicates that (sub)tropical lakes are able to sustain, but seemingly not to maintain, large cladocerans (or at least not in high numbers). Although very important for cladoceran physiology and competition (Moore, Folt & Stemberger, 1996), higher temperatures *per se* do not seem to directly explain the low abundance and size of cladocerans in the (sub)tropics. In agreement with previous suggestions (Dumont, 1994; Fernando, 2002), our results support the idea that temperature-related selective forces, namely higher predation, are the most important factors for the observed patterns in the structure of the cladoceran communities.

The same important structural differences between climate regions occurred in the benthic/ plant-associated cladoceran communities, which contrasts the results of a study conducted along a climate gradient in Europe where no climate effect was found (Gyllström *et al.*, 2005). The authors argue, however, that the higher plant %PVI found in warmer lakes could have masked potential climate effects on these particular cladocerans. The lower richness and density of subtropical plant-associated cladocerans in our study may be the result of a higher fish predation pressure inside the plant beds, and also of the lower periphyton biomass (4-fold lower than in temperate lakes, Meerhoff *et al.*, submitted). Due to the sampling device used, we may have underestimated the densities of the strictly plant-associated animals. However, the comparison between both climate regions is valid as we used the same method in all lakes. Interestingly, we found that the relative share of calanoids to total copepod density was higher in the subtropics. A higher relative importance of calanoid copepods in warm lakes often occurs (G. Lacerot, unpubl. data), although this pattern is not universal (Pinto-Coelho *et al.*, 2005).

According to our results, the function of free-floating architecture differs from that of submerged plants, and these impacts may also differ under different climates. We found higher densities of cladocerans among the submerged than among the free-floating plants in both climate zones. In the temperate lakes, the use of free-floating plants by cladocerans followed diel trends and densities intermediate between submerged plants and open water. Despite hosting relatively lower densities of fish than the submerged plants, the subtropical cladocerans particularly avoided the free-floating plants, thus confirming previous single-lake studies using real plants (water hyacinth *E. crassipes*), where crustaceans occurred in lower densities under free-floating plants than among submerged plants (Meerhoff *et al.*, 2003) or in open water (Brendonck *et al.*, 2003). Our results also give field support to laboratory behavioural experiments (Meerhoff *et al.*, 2006), where *Daphnia obtusa* Kurz avoided the free-floating plants to a higher extent than the submerged plants. The daphniid rejection to the free-floating plants seemed to be mediated both visually and chemically (root exudates) in that particular laboratory experiment. Chemically-mediated avoidance of plants has also been found in other studies (Burks *et al.*, 2001b). However, the rejection could only be visually mediated in the field experiments, as we used plastic plants. Therefore, factors other than macrophyte cues promoted the avoidance of cladocerans in our study, likely changes in the light environment, which may be used by cladocerans as a proximate cause of migration as occurs in DVM (Siebeck, 1980; Ringelberg & Van Gool, 2003).

Our results indicate that DHM and DVM may occur simultaneously in shallow lakes, and under different climates. Our study also indicates that different taxa may display different anti-predator behaviours under the same environmental conditions (i.e. same lake), but also that the same taxa may display different anti-predator behaviours under different environmental conditions (i.e. across turbidity and climate). In partial agreement with a proposed model (Jeppesen *et al.*, 1997b), average cladoceran populations and most cladoceran taxa used submerged plants as a diel refuge under mesotrophic and eutrophic conditions in the temperate lakes, but not under oligotrophic conditions where fish predation is expectedly higher (Jeppesen *et al.*, 2003). However, we found evidence of DHM even in very turbid lakes in the temperate region. In the subtropical lakes no relationship between the anti-predator behaviour and water turbidity was observed.

In most documented cases in temperate lakes, diel variation in the risk of predation by planktivorous fish is pinpointed as the main responsible factor (Gliwicz & Rykowska, 1992; Lauridsen & Buenk 1996), although not always required (Wicklum, 1999), for horizontal density gradients or DHM to occur. In our study, we found no thresholds in fish or other potential predator littoral densities leading to particular predator-avoidance behaviours of cladocerans. However, two lines of evidence (i.e. the behaviour of the average population of free-swimming cladocerans under contrasting climates, and the behaviour of the most vulnerable individual taxa in specific lakes), support the idea that DVM represents the commonest response of individual and population cladocerans when the predation risk, or the perception of predation, is very high. In contrast to deep lakes, the absence of stratification in shallow lakes leaves the low light intensity near the bottom as the only refuge for zooplankton, especially in eutrophic lakes. However, our field evidence suggests that the largest cladocerans tended to locate right above the sediment surface during the day, thus avoiding visual predators (De Stasio, 1993), in most lakes in both climate regions. From a broad perspective, our results suggest that DHM occurs under conditions of reduced fish predation (or perception) risk. The importance of fish as the factor promoting DHM in the temperate lakes was evidenced as we found more predacious littoral macroinvertebrates in these systems, which usually decreases the likelihood of DHM (Burks *et al.*, 2001a).

Our results support the hypothesis that DHM is not so prevalent in the subtropics (Burks *et al.*, 2002), but that in contrast DVM may be so. It is striking that subtropical free-swimming cladocerans were more abundant within the submerged plants instead of in open water, considering the high densities of fish among the plants. The plant beds in the subtropical lakes entail a higher fish predation pressure than in the comparative temperate lakes (Meerhoff *et al.*, submitted). We lack data about fish densities in the open water during these field studies; however, fish densities are usually very high in all habitats in the Uruguayan lakes, particularly in summer (Iglesias *et al.*, in press) compared to temperate Danish lakes (Jeppesen *et al.*, 2003). Besides the risk of facing still abundant fish in the pelagic, moving to the open water exposes cladocerans to a high risk of encounter with *Chaoborus* spp. (Iglesias *et al.*, in press), which occurred in high frequency and abundance in the subtropical lakes. In experimental studies with the tropical *Daphnia lumholtzi* Sars, pronounced DVM in the absence of predator cues was observed (Havel & Lampert 2006). The authors suggest that a constitutive response may be more profitable than an inducible response under the continuously strong predation pressure in the tropics. Although DVM represented the commonest behaviour in the subtropical lakes in our study, it was not universal. Our results suggest that this response might be the default under high predation risk conditions, although it may not necessarily be constitutive for all cladocerans. DHM, the predator-avoidance behaviour most frequently described for temperate shallow lakes, seems clearly insufficient in the subtropics due to the high abundances of small littoral fish that eliminate the macrophyte refuge. Despite the occurrence of DVM, the density and structure of the cladoceran community indicate that no predator-avoidance behaviour suffices to counteract the effects of the high predation pressure in subtropical lakes.

Based on laboratory experiments, Meerhoff *et al.* (2006) suggested that plant avoidance may be a constitutive adaptive behaviour of *Daphnia* in subtropical and tropical lakes due to the high predation pressure associated with the plants. Although *Daphnia* was nearly absent in the subtropical lakes, in this study we found higher densities of free-swimming cladocerans in the submerged plants. However, in most lakes we found no evidence of cladocerans moving to the pelagial. Supporting previous single lake investigations (Meerhoff *et al.*, 2003; Iglesias *et al.*, in press), reviews (Jeppesen *et al.*, 2005) and laboratory studies (Meerhoff *et al.*, 2006), our results indicate that refuge-mediated positive effects of aquatic plants on water transparency may be weak or rare in warm lakes. This mechanism would explain the minor effect of high submerged plant densities on water clarity observed in numerous Florida (USA) subtropical lakes (Bachmann *et al.*, 2002) compared to temperate lakes with the same nutrient concentrations (Jeppesen *et al.*, in press).

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Can warm climate-related structure of littoral predator assemblies weaken clear water state in shallow lakes?

Mariana Meerhoff [1,2,3] *, Juan M. Clemente[3], Franco Teixeira de Mello[4,5], Carlos Iglesias[4],
Asger R. Pedersen[1] and Erik Jeppesen[1,2]

[1] National Environmental Research Institute, Vejlsøvej 25, 8600 Silkeborg, Denmark
[2] Department of Plant Biology, Institute of Biological Sciences, University of Aarhus, Ole Worms Allé, Building 135, 8000 Aarhus C, Denmark
[3] Sección Limnología, Facultad de Ciencias, Universidad de la República. Iguá 4225, CP 11400, Montevideo, Uruguay.
[4] Grupo de Investigación en Ecología Básica y Aplicada, Asociación Civil I+D. Iguá 4225, CP 11400, Montevideo, Uruguay.
[5] Grupo de Química Ambiental y Ecotoxicología, Facultad de Ciencias, Universidad de la República. Iguá 4225, CP 11400, Montevideo, Uruguay.

*** Corresponding author**: Email: mm@dmu.dk merluz@fcien.edu.uy

Running title: Shallow lake littoral under warm climates

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Abstract

Shallow lakes, the most abundant lake type in the world, are very sensitive to climatic changes. The structure and functioning of shallow lakes are greatly impacted by submerged plants, and these may be affected by climate warming in various, contrasting, ways.

Following a space-for-time substitution approach, we aimed to analyse the role of aquatic plants in shallow lakes under warm climates. We introduced artificial submerged and free-floating plant beds in five comparable lakes located in the temperate zone (Denmark, 55°-57° N) and the subtropical zone (Uruguay, 30°-35° S), with the aim to study the structure and dynamics of the main associated communities.

Regardless of differences in environmental variables, such as area, water transparency and nutrient status, we found consistent patterns in littoral community dynamics and structure (i.e., densities and composition of fish, zooplankton, macroinvertebrates, and periphyton) within, but substantial differences between, the two climate zones. Subtropical fish communities exhibited higher diversity, higher density, smaller size, lower relative abundance of potentially piscivores within the macrophyte beds, and a more littoral behaviour, compared to otherwise similar temperate lakes. By contrast, macroinvertebrates and cladocerans had higher taxon richness and densities, and periphyton higher biomass, in the temperate lakes. Several indicators suggest that fish predation pressure was much stronger among the plants in the subtropical lakes. The anti-predator behaviour of cladocerans also differed significantly between climate zones.

Submerged and free-floating plants exerted different effects on the spatial distribution of the main communities, the effects differing between the climate zones. In the temperate lakes, submerged plants promoted trophic interactions with potentially positive cascading effects on water transparency, in contrast to the free-floating plants.

The higher impact of fish may result in higher sensitivity of warm lakes to external changes (e.g., increase in nutrient loading or water level changes). The current process of warming may entail an increased sensitivity for temperate lakes to eutrophication and a threat to the high diversity, clear water state.

Introduction

Shallow lakes, the most abundant lake type in the global landscape (Wetzel, 2001), are particularly sensitive to climatic changes (Scheffer, 1998). Climate warming expectantly brings a significant shortening of the duration of ice cover in temperate and cold areas (Smol *et al.*, 2005; Williams *et al.*, 2004), substantial changes in hydrological and thermal regimes in lakes, especially in arid or semiarid areas (Beklioglu *et al.*, in press), as well as local extinction of some species due to physiological stress or via interactions with other species (Schindler, 1997). Shallow lakes possess the ability to support the development of higher aquatic plants over the entire basin, or at least in large sections of their littoral area. Plants affect the biological structure and physico-chemical processes of the littoral zone of lakes, with influences in the entire lake ecosystem (Carpenter & Lodge, 1986; Jeppesen *et al.*, 1997a). In the temperate region, submerged plants stabilize trophic interactions between fish predators and cladoceran and macroinvertebrate preys (Brönmark & Weisner, 1992; Burks *et al.*, 2002; Timms & Moss, 1984) and promote higher water transparency (Canfield et al., 1984) and indirectly biodiversity (Declerck *et al.*, 2005).

However, the role of submerged plants under other climate regimes or under a climate warming scenario is far from clear. Besides being indirectly affected by the effects that climate change will impose on the catchments (e.g. increased run-off of nutrients due to higher precipitation), aquatic plants can be directly affected by the process of warming suffered by the lakes. According to field studies in north temperate lakes, earlier commencement of growing seasons, as predicted by climate change models, would result in greater biomass and distribution of submerged macrophyte communities (Rooney & Kalff, 2000). Some models also describe a warming-related faster development of macrophytes (Asaeda *et al.*, 2001). A model built on the correlation between the winter NAO index and a defined clear water state has predicted a shift in the timing and a higher probability of the occurrence of clear water phases in shallow temperate lakes (Scheffer *et al.*, 2001), linked to an earlier development of submerged plants. In contrast, eutrophication effects may worsen with climate warming as the higher temperatures and lower water level may enhance the sediment release of nutrients, especially of phosphorus (Jeppesen *et al.*, 2003; Mulholland *et al.*, 1997; Søndergaard *et al.*, 2001).

Few studies have tested the effects of increased temperature using an ecosystemic approach in controlled experiments (McKee *et al.*, 2003). Contrary to expectations, they did not find significant interaction effects of nutrients and warming on major freshwater communities, such as invertebrates, zooplankton, and macrophytes (McKee *et al.*, 2002a; McKee *et al.*, 2002b), but instead found increased phosphorus levels and deoxygenation (McKee *et al.*, 2003). Some macrophyte species of north temperate systems seem to be resilient to the predicted increases in temperature, although climate warming might change the proportion of species within macrophyte communities favouring exotic ones (McKee *et al.*, 2002b). However, the activity of small fish increases with warming, which may lead to a stronger predation pressure on zooplankton (Mehner, 2000), and, with it, a weakening of the clear water, macrophyte-dominated state due to an increase in phytoplankton biomass. Furthermore, both fish predation pressure and the importance of nutrient loading increase in warm southern lakes compared to similar temperate lakes, according to an experimental study along a latitudinal gradient in Europe (Moss *et al.*, 2004). Another likely consequence of increasing winter minimum air temperatures in freshwater systems is a broader shift in the geographical range of tropical and subtropical free-floating plants (e.g. water hyacinth *Eichhornia crassipes* (Mart.) Solms), which may constitute an alternative stable state to submerged plant dominance (Scheffer *et al.*, 2003).

There are much fewer studies of the role of aquatic plants in the tropics and subtropics (Thomaz & Bini, 2003). It seems that plants have an extraordinary importance for the fish assemblage in these systems, particularly affecting the number of species (Agostinho *et al.*, 2003) and size distribution in lakes. In particular, the smallest fish species and individuals aggregate in very high numbers in the vegetation (Conrow *et al.*, 1990; Meschiatti *et al.*, 2000), both in submerged and free-floating plants (Meerhoff *et al.*, 2003). Furthermore, fish communities in warmer climates are characterised by a higher share of omnivorous species (Branco *et al.*, 1997; Winemiller, 1990) and multiple or frequent reproduction (Paugy & Lévêque, 1999). Consequently, aquatic plants likely offer a poor refuge for large-bodied zooplankton in warm lakes (Meerhoff *et al.*, 2003), and this may potentially have consequences at the ecosystem level.

By introducing artificial plant beds in similar shallow lakes in the temperate (Denmark, 55°-57° N) and subtropical zone (Uruguay, 30°-35° S) varying along a turbidity gradient, we addressed the hypotheses aquatic plants impact trophic interactions differently according to their architecture (submerged *versus* free-floating) and that the structuring role of submerged plants is affected negatively by warming. Following a space-for-time substitution approach, the knowledge about the role of aquatic plants and trophic dynamics, and consequently on lake functioning, gained from the subtropics may be useful when evaluating the effects of climate warming on temperate lakes.

We hypothesise that a warmer climate can lead to a weakening of the macrophyte-dominated clear water state due to substantial differences in the littoral trophic structure.

Material and Methods

Design and sampling methodology

We selected sets of five shallow lakes with contrasting water transparency in Uruguay (30°-35° S) and Denmark (55°-57° N), with lakes being paired in both countries in terms of Secchi disk transparency (1.6, 1.0, 0.7, 0.4, 0.20 m SD) and with similar size, total nutrient concentrations, and macrophyte cover (range: 0-70% PVI, *sensu* Canfield *et al.*, 1984) within the pairs. The average water temperature during the field campaign was 27°C and 18°C in Uruguay and Denmark, respectively.

In each lake, we introduced eight artificial plant beds mimicking submerged and large free-floating plants (so-called "habitat modules", four replicates of each life-form). The use of artificial substrata may be debatable because it implies the loss of chemical interactions; nevertheless, we ensured that initial conditions of the substratum quantities and quality were identical to properly compare the structure and dynamics of associated communities.

The modules consisted of 1-m diameter PVC plastic rings with the artificial plants hanging from an attached net, and were completely open to water and sediment. We used a total of 3.5 km of originally Christmas' trees decorations to produce the plants. The submerged plants (100 per module, "hairy" 0.8-1.0-m long green plastic pieces) had an architecture resembling *Cabomba* or *Elodea* spp, whereas the free-floating plants mimicked *E. crassipes* (40 per module, 2-m long plastic pieces arranged as the typical root network) and also had a 15-cm diameter plastic disc to limit the light passage in a patchy manner. We placed the habitat modules at 1-m depth in sheltered and plant-free areas. Sampling campaigns were conducted in summer (January 2005 in Uruguay, and July 2005 in Denmark), on average four weeks after the introduction of the habitat modules, allowing periphyton and invertebrates to colonize the plastic structures. Water samples for physico-chemical analyses and *in situ* parameters (transparency as Secchi disk depth, PAR light attenuation, pH, temperature, conductivity) were taken in open water. We sampled zooplankton larger than 50-μm with a 1.0-m long tube (6-cm diameter) from each module and from four open water sites nearby, during the day and again at night-time. We removed one "plant" from inside each module to collect the associated invertebrates larger than 500-μm, and a10-cm long piece of each plant (or "root") from the 10-20 cm depth section to determine the biomass of associated periphyton. The fish (and shrimps) strictly associated to each habitat module were sampled at night-time using a cylindrical net (1.20-diameter, mesh size 0.3 cm) placed over the sediment below each module in the afternoon. We lifted the net from outside the modules using a 1.5-m long stick with a hook, with a very quick manoeuvre. The same modules were used in both countries. Upon removal from the lakes, the modules were washed using high water pressure, disinfected with concentrated chlorine solution for several hours, rinsed thoroughly and sun-dried before shipping in order to remove periphyton and animals attached.

Analysis and statistics

We measured Chlorophyll-*a* (Chl-*a*) (Jespersen & Christoffersen, 1987) and total nutrient concentrations (TN and TP) (Søndergaard *et al.*, 1992; Valderrama, 1981). Fish were identified to species level, counted and measured. We classified as "potentially piscivores" all size classes of the piscivorous species according to bibliographic data. The fish smaller than 3.5 cm SL were classified as "small", this being the adult size of the smallest fish species in the Uruguayan lakes. Invertebrates were counted and identified to genus level, and classified according to their feeding regime (Merrit & Cummings, 1984). We identified all cladoceran zooplankton at least to genus level, and classified genera as littoral or free-swimming. Periphyton biomass was estimated as Chl-*a* concentration (following Jespersen & Christoffersen 1987). The data of macroinvertebrates and periphyton were estimated per unit of plant weight and per unit or plant-covered area. In the case of periphyton, we corrected the estimation by the number of days that the habitat modules remained in the water.

To extract regional effects (Fig. 2), we averaged the data coming from both types of macrophyte beds (weighed average considering the different "biomass" of both plant forms when appropriate), and recalculated all measurements to unify units (expressed in m^2), thus facilitating a comparison across trophic levels. We analysed the effects of climate and habitats (plant architecture) on each target variable turbidity, using turbidity as a matching factor, by applying 3-way factorial ANOVA (factors: climate -2 levels; habitats -2 levels in case of fish, macroinvertebrates and periphyton, and 3 levels in the case of cladocerans; turbidity -5 levels). In the analysis of migration patterns of cladocerans we added time as a fourth factor (2 levels). Data, except number of taxa, were prior $\log_{10}(x+1)$-transformed to fulfil the requirements of homoscedasticity (Cochran's test) and normal distribution of residuals. *Post hoc* analyses were made using Tukey HSD tests. In case of significant interactions between climate and other factors, we estimated the overall levels of each target variable within climate zones by LSMEANS (PROC MIXED, SAS Institute Inc., 2004), averaging over significant main and interactive effects of turbidity, habitat and time.

Results

The role of climate

The taxon-richness of main communities differed remarkably between climate zones. Fish species richness was significantly higher in the subtropical lakes (LSMEANS, $p<0.0001$), with magnitude of the difference affected by turbidity. By contrast, there were sig-

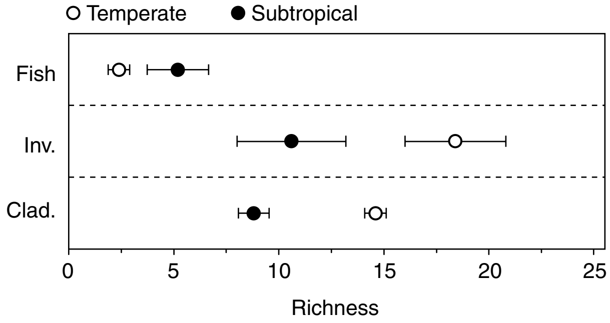

Fig. 1. Taxon-richness of fish (species), macroinvertebrates (Inv., genera) and cladocerans (Clad., genera) in plant beds in temperate and subtropical lakes. The data are the sample means of five lakes (average of lake averages within each turbidity level and climate zone) ± 1 SE.

Fig. 2. Main communities' structure in artificial plant beds in temperate and subtropical lakes.
Left panel: Sample mean density of, from top to bottom, potentially piscivorous fish, all other fish, shrimps, littoral predatory invertebrates (car. Inv. L), pelagic predatory invertebrates (car. Inv. P), littoral herbivorous macroinvertebrates (herb. Inv. L), pelagic herbivorous cladocerans (herb. Clad. P), and biomass of periphyton. All units are ind m^{-2} except for periphyton (mg Chl-*a* m^{-2} plant cover). Notice the different scale for some groups.
Right panel: Simplified scheme of trophic interactions among the same trophic groups. The densities in the subtropics are expressed realtive to those in the temperate lakes (considered as the unit, for being the most known web). The trophic groups were classified as plants (Peri: periphyton), intermediate herbivores (i herb, Clad: cladocerans, Inv: invertebrates), intermediate carnivores (i car, Inv, invertebrates, P: pelagic, L: littoral), intermediate omnivores (i omn), and top carnivores (t car: piscivorous fish) (Arim, Marquet, 2004). Except fish, the same taxa share the same trophic classification in both climate zones. Shrimps relative density is dotted due to shrimp absence in the temperate lakes. Phytoplankton (phyto) box has the same size as it was fixed in the study.

nificantly more genera of littoral macroinvertebrates in the temperate lakes (Fig. 1). However, large taxa of macroinvertebrates, such as shrimps (*Palaemonetes argentinus* Nobilli), channelled applesnails (*Pomacea canaliculata* Lamarck), and crabs (*Aegla* sp), were found only in the subtropics. Cladoceran richness was also higher in the temperate lakes (LSMEANS, p<0.0001, Fig. 1). The "missing" taxa in the subtropics included large-bodied cladocerans, e.g. the predators *Leptodora kindtii* Focke and *Polyphemus pediculus* L., and the plant-attached *Sida crystallina* Müller and *Eurycercus lamellatus* Müller. The typical pelagic cladoceran community in the subtropical lakes was composed of small-bodied taxa.

Also the structure of the main communities differed remarkably between climate zones (Fig. 2). The fish communities in the subtropical lakes were characterised by significantly higher densities, higher biomass, and smaller size. Fish density was significantly higher (range in the paired lakes: 3 to 50 times) in the subtropical lakes (Table 1), with the magnitude of difference depending on turbidity and plant types (significant interaction terms in ANOVA tests). The minimum values in the subtropics were similar to the maximum in the temperate lakes (31.5 and 38.5 ind m^{-2}, respectively). The biomass of fish was also higher (maximum 6 times in the paired lakes), and accordingly, subtropical fish were significantly smaller (Table 1). Whereas only the 38.5% (±13.4 SE) of the temperate fish were smaller than 3.5 cm standard length (SL), 89.8% (±3.4 SE) were so in the subtropics. There were significantly higher densities of plant-associated macroinvertebrates in the temperate lakes (on average 8 times), both per unit of plant weight and per unit of area of plant cover (Table 1, Fig. 2). The density of total and free-swimming cladocerans was also higher in the temperate systems (on average 5.5 times higher), and so was periphyton biomass despite the lower solar irradiance and

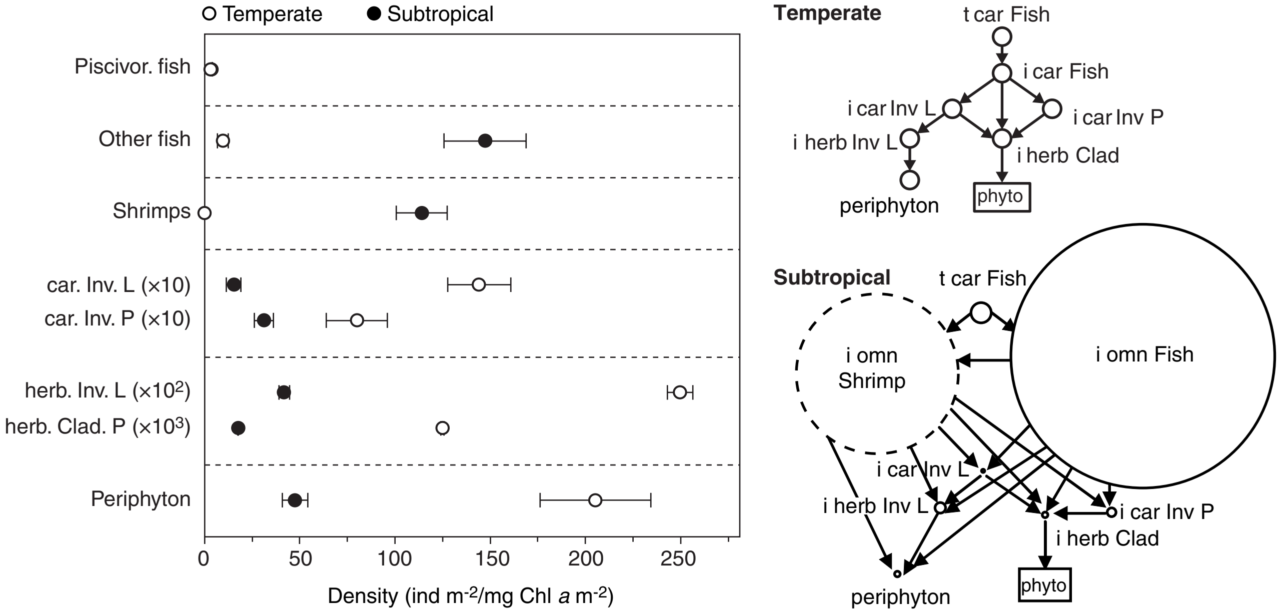

Table 1. Effects of climate on main communities' structure in temperate and subtropical lakes (Climatewise LSMEANS), calculated from statistical models adjusting for the main and interactive effects of turbidity and habitat, and also for time in the case of cladocerans. The data represent estimated population medians and confidence intervals (in italic). Data are presented in m^{-2} of plant cover and in the original units, unless transformation to m^{-2} is straightforward (i.e. zooplankton density per Litre should be multiplied by 1000 to reach densities m^{-3} (m^{-2})).

	Fish			Litt. macroinvertebrates		Cladocerans		Periphyton	
	density ind m^{-2}	biomass g FW m^{-2}	length cm	density ind m^{-2}	ind g plant^{-1}	total ind L^{-1}	pelagic ind L^{-1}	biomass (Chl *a*) mg m^{-2}	µg g plant^{-1}
Temp.	6.0	8.4	4.0	18921	34.5	66.8	44.4	132.2	241.9
	4.7-7.6	*6.1-11.7*	*3.6-4.5*	*16886-21238*	*31.7-37.6*	*59.1-75.5*	*39.0-50.4*	*112.1-155.8*	*207.0-282.6*
Subt.	67.0	25.4	2.2	2298	4.8	12.4	8.25	33.4	32.4
	53.3-81.6	*18.6-34.5*	*2.0-2.5*	*2027-2607*	*4.3-5.3*	*10.8-14.2*	*7-1-9.5*	*26.7-39.3*	*26.7-39.3*
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

the higher densities of macroinvertebrates (on average 4 times higher) (Table 1, Fig. 2).

No direct effects of turbidity or other environmental factors were observed on the target variables, but rather we found an influence of turbidity on the magnitude of the effects of climate (identified as significant interactions between "climate" and "turbidity" in the ANOVA tests). In all cases, the above-described effects of climate overruled the effects of turbidity.

Several indicators of fish predation pressure (modified from Jeppesen *et al.*, 2002) involving different trophic groups, such as fish, snails, macroinvertebrates, and cladocerans, indicated a much higher predation pressure in the plant beds of the subtropical lakes (Fig. 3).

Habitat choice: role of plants

Submerged and free-floating plants exerted different effects on the spatial distribution of the main communities, and most frequently these effects differed between the climate zones (Fig. 4).

Fish showed a strong association with the submerged plants in the subtropical lakes, while in the temperate lakes more fish associated with the free-floating plants (3-way ANOVA, interaction plants x climates, $F_{1,63}$=5.85, P=0.018, for density) (Fig. 4). This pattern was found irrespective of water turbidity. Subtropical shrimps also had higher densities in the submerged plants (2-way ANOVA, plant effects, $F_{1,29}$=11.64, P=0.002).

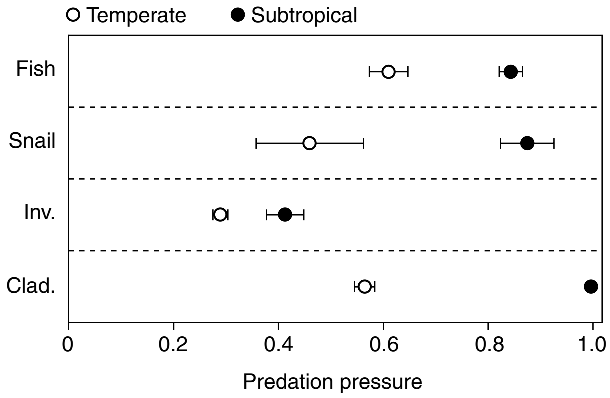

Fig. 3. Predation indexes in the plant beds in temperate and subtropical lakes, considering the density of different taxonomic groups. From top to bottom, Fish: ratio of plankti-benthivores to total fish density, Snail: ratio of large snails to total snail density, Inv.: ratio of oligochaetes to sum of oligochaetes plus chironomids, Clad.: ratio of *Bosmina* to sum of *Bosmina* plus *Daphnia* density. In all cases, the closer to 1 is the ratio, the higher the predation pressure. The data represent the sample means of five lakes (average of lake average within each turbidity level and climate zone) ± 1 SE, except in the case of the last index, as not even *Bosmina* was found in 2 out of the 5 lakes in Uruguay.

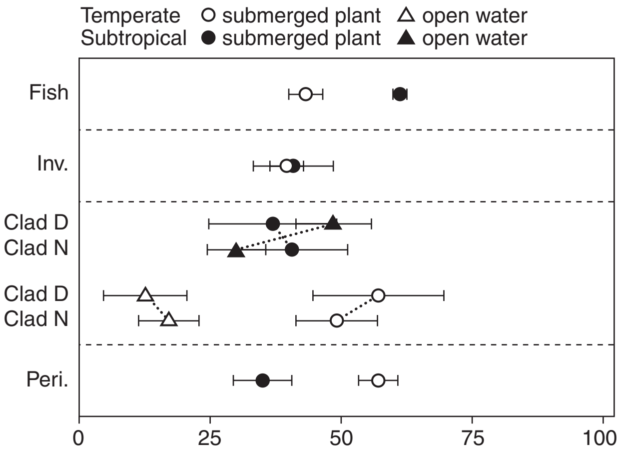

Fig. 4. Spatial use of main communities (free-floating or submerged plants) in the temperate and subtropical lakes, focusing on the relative abundance (%) in the submerged plant beds. From top to bottom, Fish, Inv.: littoral invertebrates, Clad.: free-swimming cladocerans, Peri.: periphyton. For cladocerans, also the relative diurnal (D) and nocturnal (N) densities and the relative densities in open water (triangles) are shown. If there was no preference for plant types, densities should not deviate significantly from 50% (and 33% in the case of cladocerans). The data represent the sample means of five lakes (average of lake averages within each turbidity level and climate zone) ± 1 SE.

We found higher densities of cladocerans among the submerged plants in both climate zones, although the magnitude of the difference varied inside each climate region and with turbidity.

We observed a differential diel use of habitats by cladocerans, although affected slightly by turbidity (4-way ANOVA, $F_{8,184}$=2.19, P<0.0293). In most temperate lakes we observed diel patterns mostly fitting with the predictions of the classic Diel Horizontal Migration (DHM): the densities of free-swimming cladocerans in the submerged plants decreased during the night and increased slightly in the open water (Fig. 4). In contrast, in only one subtropical lake did we find clear signs of the classic DHM. Furthermore, in three lakes we registered significant evidence of Diel Vertical Migration (DVM, increased nocturnal densities in all habitats, fitting the expected pattern: animals move to the bottom during the day and upwards at night, when the predation risk is lower), regardless of water turbidity. In the temperate lakes, the use of free-floating plants by cladocerans was intermediate between open water and submerged plants both in diel trend and densities. In contrast, in the subtropical lakes the free-floating plant beds had constantly lower relative density, with no significant diel changes.

We found higher densities of plant-associated macroinvertebrates per unit of plant weight in the free-floating plants in both countries (Fig. 4), with the magnitude of the difference with the submerged plants influenced by turbidity. In the subtropical lakes, the free-floating plants supported a higher biomass of periphyton per unit of weight than did the submerged plants, while the opposite was observed in the temperate lakes (interaction plants x climates, $F_{1,56}$=16.63, P<0.0001). This pattern was maintained along the turbidity gradient.

Discussion

Regardless of the gradient in water transparency and other environmental variables (such as nutrient status and lake area), we found common patterns in littoral community structure and dynamics within, but large differences between, the climate zones. We found signals of a diversity cascade in the littoral zone of the lakes, with different signs across climate zones: more fish species co-occurred with less cladoceran and less macroinvertebrate genera in the subtropical lakes, while the opposite happened in the temperate lakes. Freshwater lakes show a weaker latitudinal diversity gradient than other ecosystems (Hillebrand, 2004). However, several studies show an increase with decreasing latitude in species richness of many taxon groups (e.g. fish, dragonflies, and beetles), suggesting temperature as the main explanatory factor (Heino, 2002). The expected higher richness with increasing temperature was observed only for the fish community in our study. In agreement with previous suggestions for the zooplankton community (Dumont, 1994; Fernando, 2002), our results support the idea that temperature-related selective forces, namely higher predation, are the most important factors for the observed patterns in the richness structure of the littoral communities.

Some key differences in structure of the fish community between temperate and warm shallow lakes have been summarized recently (Jeppesen *et al.*, 2005; Lazzaro, 1997). High fish diversity among the plants (Agostinho *et al.*, 2003), high abundance (Mazzeo *et al.*, 2003), continuous or frequent reproduction (Paugy & Lévêque, 1999), widespread omnivory (Winemiller, 1990), and low abundances of strictly piscivorous fish (Quirós, 1998), seem common features in warm lakes in different regions of the world. Also, large invertebrate omnivores, such as shrimps, can be abundant in the subtropics (Collins, 1999), probably as a result of the small size of the dominant fish. The same phenomenon occurs in brackish lakes in the temperate zone, where *Neomysis integer* coexist with the dominant stickleback *Gasterosteus aculeatus* but are largely suppressed if bigger fish are present. This trophic structure leads to strong cascading effects promoting increased water turbidity despite high abundance of submerged plants (Jeppesen *et al.*, 1997b; Jeppesen *et al.*, 1994). In our manipulative comparative study we have confirmed for the first time these key differences regarding the littoral fish communities: subtropical lakes have higher diversity, much higher density, smaller size and lower relative abundance of potentially piscivores, and are strongly associated to the submerged plants, compared to similar temperate lakes. The lower abundance of zooplankton and macroinvertebrates, the dominance of zooplankton by small-bodied taxa, the more frequent occurrence of large-bodied taxa within the macroinvertebrates, together with the contrasting behaviour of the free-swimming cladocerans, indicate that predation pressure is stronger among the plants in the subtropical lakes (Fig. 3).

The potential for trophic cascades seems more truncated in subtropical lakes, as the effects of the high densities of fish in the littoral zone could be traced at all the studied trophic levels (Fig. 2). Fish are considered the main determinants in community structure in shallow lakes, through, among other mechanisms, their positive cascading effects over periphyton via invertebrates (Jones & Sayer, 2003), and the consequent deterioration of light climate for the submerged plants (Phillips *et al.*, 1978). To our surprise, we found much lower periphyton biomass in the subtropical lakes than expected by the release of grazing due to high predation on invertebrates, and the more positive environmental conditions (more light and higher temperature) for periphyton growth. The observed 4-fold reduction in periphyton biomass seems likely the result of direct (feeding) or indirect (physical disturbance) activity by the abundant fish and shrimps. This apparently overrules the positive effect of fish through the control of the plant-attached macroinvertebrate grazers. Our results support the hypothesis that widespread omnivory by top or mid-level consumers can limit the potential for cascades to propagate to lower trophic levels (Pace *et al.*, 1999; Polis & Strong, 1996). However, the lower periphyton biomass in the subtropics represents much reduced shading and nutrient and carbon competition with the host plants, which may enhance the chances of the submerged plants to develop under higher turbidity compared to the temperate lakes.

We have confirmed that the architecture of macrophytes is very relevant to their impacts on trophic dynamics, and that those impacts differ under different climates. Despite hosting relatively lower densities of fish than the submerged plants, the free-floating plants were particularly avoided by cladocerans in the subtropics, thus confirming previous single-lake (Meerhoff *et al.*, 2003) and laboratory studies (Meerhoff *et al.*, 2006). In the temperate lakes, free-floating plants hosted more fish than did the submerged plants and were clearly not used as refuge by cladocerans. An expansion of the free-floating plants, especially considering their capacity to constitute an alternative stable state to submerged plants (Scheffer *et al.*, 2003), would imply negative impacts on trophic dynamics, besides the already known negative effects on general diversity and water qualtiy (Talling & Lemoalle, 1998). Submerged plants seemed to offer better refuges for cladocerans, particularly in the temperate lakes. The predator-avoidance behaviour often described for shallow temperate lakes, i.e. diel horizontal migration (Burks *et al.*, 2002; Timms & Moss, 1984), seems clearly insufficient in the subtropics. Although there was evidence of diel vertical migration, the density and structure of the cladoceran community indicates that no predator-avoidance behaviour suffices to counteract the effects of the high fish (and likely shrimp) predation pressure in subtropical lakes. Refuge-mediated positive effects of aquatic plants on water transparency might therefore be weak or rare in warm lakes (Meerhoff *et al.*, submitted).

The differences between climatic regions found in this study seem therefore clearly not the result of "matrix effects" (i.e. comparisons of lakes from different continents), but rather the outcome of climate-related differences in trophic structure. Supporting this statement, *Daphnia* spp appeared scarcely, while fish biomass and the fish:zooplankton biomass ratio were higher in the southern warm lakes in a European study along a climate gradient (Gyllström *et al.*, 2005). Our study suggests that, more than other environmental factors, the structure of the predator assemblies in temperate and subtropical lakes is the main reason for the observed different structure of the other communities, with potential effects at community and ecosystem levels.

Climate warming might not affect submerged plants directly in a significant manner (McKee *et al.*, 2002b) or even promote plant development (Asaeda *et al.*, 2001), but may affect other components of the trophic structure of shallow lakes, such as the fish community structure or activity level, as suggested by some experimental studies (Mehner, 2000). A warming-related higher impact of fish may strongly affect the resilience of shallow lakes (Folke *et al.*, 2004), pushing the lakes in the direction of a higher predation on large-bodied zooplankton and lower grazer control of phytoplankton, and of a decreased capacity of the submerged plants to create and maintain clear-water conditions. This process may explain the empirical data from many Florida (USA) lakes showing minor effect of high submerged plant densities on water clarity (Bachmann *et al.*, 2002) under same nutrient conditions than similar temperate lakes (Jeppesen *et al.*, in press).The consequent lower resilience of the lakes under higher temperatures may increase their sensitivity to external changes, such as an increase in nutrient loading or changes in water level. Supporting this assertion, higher importance of nutrient loading for the functioning of warm lakes than in comparative temperate lakes has been found in field studies in Mediterranean lakes (Romo *et al.*, 2005) and in an experimental study along a latitudinal gradient in Europe (Moss *et al.*, 2004). The current process of warming may thus impose an enhanced sensitivity for temperate lakes to eutrophication, and a threat to the high diversity, clear water state with consequent impoverished ecosystemic and social value.

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Global warming: Design of a flow-through shallow lake mesocosm climate experiment

Lone Liboriussen[1], Frank Landkildehus[1†], Mariana Meerhoff[1,4], Mette E. Bramm[1], Morten Søndergaard[2], Kirsten Christoffersen[2], Katherine Richardson[3], Martin Søndergaard[1], Torben L. Lauridsen[1], and Erik Jeppesen[1,4‡]*
[1]National Environmental Research Institute, Dept. of Freshwater Ecology, Vejlsøvej 25, DK-8600 Silkeborg, Denmark
[2]Freshwater Biological Laboratory, University of Copenhagen, Helsingørsgade 51, DK-3400 Hillerød, Denmark
[3]Dept. of Marine Ecology, University of Aarhus, Finlandsgade 14, DK-8200 Århus N, Denmark
[4]Dept. of Plant Biology, University of Aarhus, Nordlandsvej 68, DK-8240 Risskov, Denmark

Abstract

Shallow lakes are likely to be strongly impacted by climate changes and, in particular, by increased temperatures. To enable realistic experimental studies of the effects of higher temperatures on in-lake processes and dynamics, technologically advanced systems are required. This paper presents design details, operating characteristics, and background information on a currently operating experimental flow-through mesocosm system that allows investigation of the interactions between simulated climate warming and eutrophication and their impacts on biological structure and ecosystem processes in shallow lakes. We use 24 mesocosms to combine three temperature scenarios (one unheated and two heated relative to the Intergovernmental Panel on Climate Change climate scenario A2 and A2 + 50%, respectively) and two nutrient levels (enriched and nonenriched). Planktivorous fish (male sticklebacks, *Gasterosteus aculeatus*) are stocked in accordance with the nutrient level. The water residence time is regulated by the semicontinuous addition of water and is approximately 2.5 mo in each mesocosm. For heating, we use electrically powered heating elements. The heating system has performed well over 16 mo of continuous heating, and seasonal and diurnal temperature variations of the unheated reference mesocosms were paralleled well by the heated mesocosms. The performance of the flow-through system and the heating technique are discussed with special emphasis on strengths, limitations, and potential improvements of the system. To illustrate the performance of the system and its potential, we present data for selected periods on total phosphorus retention in the mesocosms and system primary production and respiration.

During the next century, the global climate is expected to undergo significant changes. The forecasts for the Danish region include a temperature increase of 3°C to 5°C, the increase being most pronounced during autumn and winter, with higher precipitation and more extreme climate events (Christensen and Christensen 2001; Jørgensen et al. 2001). Increased temperatures and higher runoff of nutrients predicted on the basis of the expected increase in precipitation are likely to affect lake ecosystems (Straile et al. 2003; Moss et al. 2003). Moreover, the predicted warmer winters will probably substantially reduce the period with ice cover in the coastal situated lakes. This will, especially in shallow lakes, influence the survival of fish and macrophytes, i.e., the key structuring elements in shallow lakes. Such changes may cascade to other trophic levels and, ultimately, affect lake water quality (Moss 1990; Scheffer et al. 1993; Jeppesen et al. 1997). How shallow north temperate coastal lakes will respond to global warming has been debated in recent years. Some argue for increased possibility for a shift occurring in turbid lakes to a clearwater state due to a stimulated plant growth and reduced predation by fish (Scheffer et al. 2001). Others believe that turbid lakes will remain turbid due to enhanced external and internal nutrient loading and higher predation by fish (Jeppesen et al. 2003; McKee et al. 2003; Moss et al. 2003). Yet, the few field experiments conducted so far show that a temperature increase of a few degrees has a modest effect on the

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Corresponding authors: [*]lol@dmu.dk, [†]fla@dmu.dk, or [‡]ej@dmu.dk

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biological communities, but internal P loading increased (McKee et al. 2003; Moss et al. 2003). Even less is known about the probably different responses of continental situated temperate lakes and subtropical-tropical lakes (Nõges et al. 2002; Jeppesen et al. 2003).

A number of approaches for estimating the effects of climatic changes on lakes exist. These include analyses of interseasonal variations or time series analyses of contemporary data, space-for-time substitution by analyzing data from various climate regions, analyses of paleolimnological records, and controlled experiments in the laboratory and field. All have their potentials and limitations (Schindler 1998; Moss et al. 2003; Battarbee et al. in press).

Several controlled experimental systems have recently been developed to test the effects of global warming on shallow lake ecosystems. Baulch et al. (2003) developed a 700 L lake-based batch enclosure system that is kept open to the sediment. The temperature is controlled by hot water circulated through a network of pipes coiled around the enclosure bottom. The system has run relatively smoothly, although problems in particular in attaining the target temperature difference between the control and the warmed enclosure and controlling the exchange of water between the enclosures and lake have been experienced (Baulch et al. 2003). The latter problem was solved in the land-based batch system described by McKee et al. (2000). That system consists of cylindrical (depth 1 m, diameter 2 m) mesocosms sunk into the ground for insulation, after which sediment was added. Heating was performed by circulating hot water through heating elements placed horizontally on top of the sediment and operating with a sensitivity of ± 0.25°C. Evaporative losses were replaced by deionized water. This system operated smoothly for more than 2 y. However, a drawback of both systems described above is that they are batch systems, whereas natural lakes are typical flow-through systems. Moreover, in both systems, heating elements are fixed in or immediately on top of the sediment to create thermally mixed conditions in the mesocosms without stirring. This construction potentially leads to locally higher temperatures that may influence biogeochemical processes in the sediment (Jensen and Andersen 1992) and, thus, the benthic-pelagic interactions.

Building on the experience of McKee et al. (2000), we have developed a land-based semi-continuous flow-through mesocosm system that takes into account the fact that most shallow lakes are not batch but flow-through systems. Our system allows calculation of mass balances of nutrients and organic matter. Moreover, frequent measurements of oxygen and pH allow estimation of community primary production and respiration, as well as the CO_2 exchange between air and water. Paddles continuously mix the water column in all mesocosms, and the temperature control runs with higher sensitivity than the McKee system. In addition, heating elements are placed above the sediment. The system is equipped with monitoring devices that are supervised by remote control. An alarm system ensures early warning in the case of irregularities in the system functioning. The system allows the simultaneous simulation of warming effects on low nutrient (clear) and enriched (turbid) lakes.

Materials and procedures

General description of the experiment—During spring 2003, we established 24 flow-through outdoor mesocosms in a lowland valley in Central Jutland, Denmark (56°14′N, 9°31′E). The 24 mesocosms allowed a factorial design combining 2 nutrient levels with 3 temperatures in 4 replicates. Aquatic freshwater communities were allowed to establish in the mesocosms before nutrient addition was initiated in early May 2003. By the time heating was initiated (28 August 2003), extensive submerged macrophyte beds had developed in nonenriched mesocosms, while the enriched mesocosms were dominated by phytoplankton or filamentous algae with only sparse macrophyte vegetation. Electrically powered heating is continuously controlled relative to the ambient temperature in the unheated reference mesocosms according to the Intergovernmental Panel on Climate Change (IPCC) climate scenarios A2 (Houghton et al. 2001) and A2 + 50%. Planktivorous fish were stocked in natural densities consistent with the nutrient treatment.

Mesocosm specifications—The mesocosms consist of cylindrical stainless steel tanks 1.9 m in diameter and 1.5 m in total depth (Fig. 1). They are equipped with a flow-through system, where a timer-controlled magnetic valve (Danfoss Group, EV 220B) automatically adds groundwater every sixth hour, while an overflow pipe (diameter = 2.6 cm) drains off excess surface water. A small partly submerged plate (4 × 4 cm) in front of the outflow pipe prevents most snails, floating filamentous algae, and large organic fragments from being washed out of the mesocosm and, also, reduces the risk of clogging the outflow. During winter, the outflow pipe and a small region of water in front of it are kept free of ice by a thermostatically controlled electric warming cable. The flow-through system is thus kept functional throughout the year. Apart from periods of excessive summer evaporation, this flow-system ensures a constant water level of 1.0 m and a total capacity of approximately 2800 L in each mesocosm. The water drained through the overflow pipe is retained in a 500 L covered plastic collection tank placed beside each mesocosm (Fig. 1). Based on the total discharge into these collection tanks, it is possible to calculate the exact water residence time for the individual mesocosms, taking into account water added by the magnetic valve, precipitation, and evaporation. Since the water in the collection tank typically is sampled every 7 to 14 d during the experiment, it can provide integrated information on some of the conservative chemical variables (e.g., iron and total phosphorous) in the water column and permits calculation of highly accurate mass balances.

A 0.1 m layer of washed sand was initially added to each mesocosm and, on top of that, a 0.1 m layer of sediment collected from a nearby nutrient-rich freshwater pond. To remove large fragments of vegetation and avoid uncontrolled

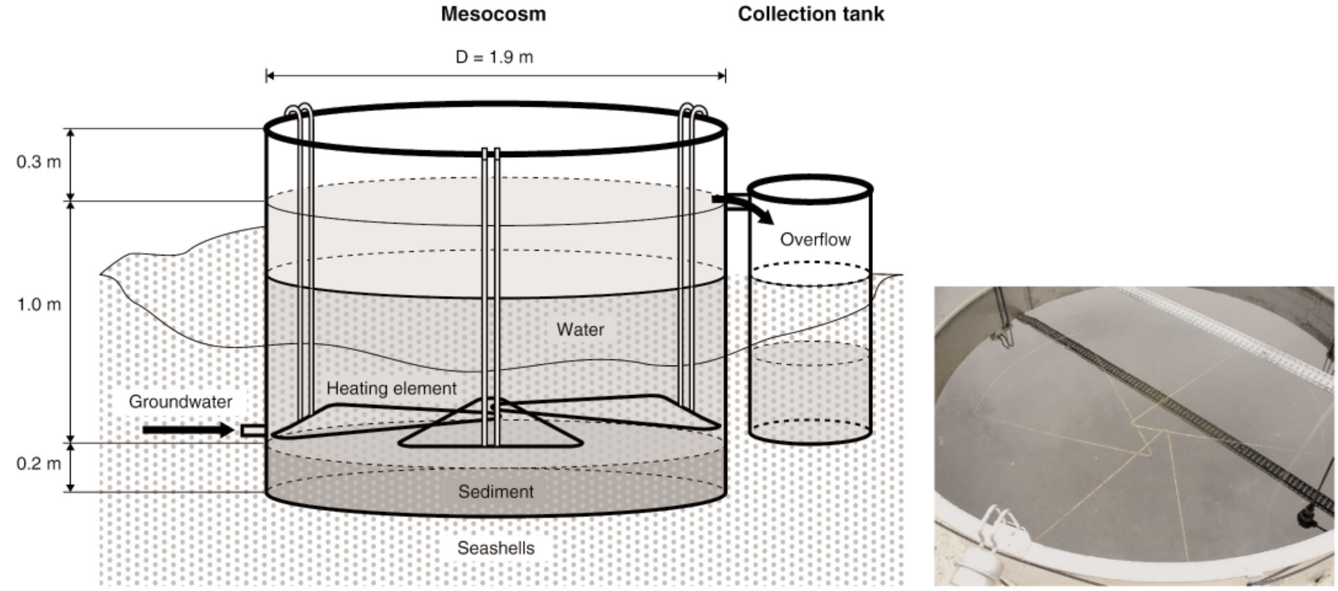

Fig. 1. Illustration and photo of one of the 24 flow-through mesocosms and the collection tank. In the bottom, three heating elements can be seen.

introduction of vertebrates such as fish or amphibians, the sediment was flushed through a net (mesh size: 1 × 2 cm) and drained of excess water before being placed in the mesocosms.

All tanks are located within a 20 × 20 m open area, enclosed at the perimeter by a wire fence and from above by wires strung in rows (distance between wires approximately 0.2 m) over the area (Fig. 2). This prevents the introduction of larger animals and birds to the experimental area. For insulation, the tanks have been embedded into a layer of seashells (*Mytilis edulis*). A major advantage of using seashells as insulation material is that they have a very high drainage capacity. Thus the area between the tanks is dry and accessible even during periods of heavy rain. A fibertex mat covers the shells and on top of that 0.1 m of hard-packed sand provides a stable working area between the tanks and eliminates obnoxious smells from the shells. A metal rim (0.05 m) is fastened around the perimeter of each mesocosm (0.35 m from the ground) to prevent access of smaller animals to the mesocosms. Likewise, a cover on the collection tanks helps prevent them from acting as traps for smaller animals. Inlet and outlet water pipes as well as most electrical wiring have been buried under the shells for insulation.

Heating system—Continuous warming was initiated in late August 2003 and is planned to run until August 2006. Sixteen of the mesocosms are heated under two different warming scenarios: low and high. These are run simultaneously. Three electrically powered (230 V AC) stainless steel heating elements (750 W each) warm the water in each of the heated mesocosms. Unheated mesocosms also contain 'dummy heating elements' that are not connected to the overall heating control system. The heating elements are fixed to the upper rim of the mesocosm and have two bars running parallel to the side before they extend horizontally in an open triangle 0.10 to 0.15 m above the sediment (Fig. 1). This shape was used to distribute the heat evenly near the bottom and to optimize the volume of free water in the middle of the mesocosm and to ensure a minimum of shading on the walls and sediment surface. Placing the heating elements near the bottom of the mesocosms was also predicted to be the optimal setup in terms of its contribution to the vertical mixing of the water within the tank. To supplement this mixing, a paddle-shaped mixer is placed in the upper part of the water column so that there is no visible disturbance of the sediment. The paddles

Fig. 2. Mesocosms to experimentally study how increased temperatures will affect shallow lake systems.

are operated continuously during ice-free periods. However, when the water temperatures approach 0°C, the paddles are stopped so that ice cover and thermal stratification of the water column are allowed to develop.

The mesocosms are grouped in eight blocks of three, including one mesocosm with ambient reference temperature and two mesocosms heated to the low and high warming scenario, respectively. Whether a particular mesocosm is warmed or not was applied in a random design, whereas the blocks were made so that they contain closely sited mesocosms. Within each block, the unheated mesocosm acts as reference for the two heated mesocosms, so that the water temperature in the latter is raised to predescribed target differences over the ambient reference temperature. Temperature control within the distinct blocks, rather than among all mesocosms, is advantageous because a localized failure or breakdown of the temperature recording in one mesocosm can only affect the mesocosms in the same block. It may, however, also potentially induce errors, as a divergence in temperature in only one of the unheated reference mesocosms will, automatically, be transmitted directly to the heated tanks in the block. Natural short-term temperature differences among replicate mesocosms have been observed during the first year of the experiment (August to December: mean 0.0°C to 0.5°C). Nevertheless, as the temperature typically varies with less than 1°C among replicate mesocosms and no systematic differences have been observed to date, it is presumed that these are of minor importance to the overall results.

The heating control system operates through three interlinked components: temperature sensors (PR electronics products: sensor type: Pt 100; maximum error ± 0.15°C at 0°C, temperature transmitter type: TT-5333), a data processor (Siemens S7-ET200 S), and heating elements. Water temperatures are recorded continuously by temperature sensors placed centrally in all mesocosms. Within the data processor, the temperature differences at any given time between warmed and reference mesocosms are compared to the target temperature difference transmitted via a central PC. If the temperature difference is too small, the heating elements in the mesocosm are activated and heating initiated. When the water temperature reaches the target difference to the reference mesocosm, the heating elements are switched off and remain inactive until the temperature difference again drops below the defined target.

In addition to data logging (every 30 min) and temperature control, the computer software has a monitoring function that constantly controls for failures in the information transfer between the computer and the data processor and for temperature deviations from a defined range. Via a local alarm system (FS 944) connected to the computer and the ordinary telephone network, information concerning such errors or notification of power failure is sent to the technician on watch for prompt reaction.

Winter conditions—Controlling the water temperature in the heated mesocosms relative to that of the reference mesocosms presents a problem during winter when the latter become ice-covered. If automatic temperature control continues during periods with severe frost, the heated mesocosms will always remain unfrozen, because the reference water temperature under the ice in the unheated mesocosms will usually remain at 0° to 4°C. Although global warming is anticipated to reduce the frequency and duration of lake ice cover, a scenario where lakes never freeze in Northern Europe is unrealistic according to the predictions of the current climate models (Jørgensen et al. 2001). Therefore, during periods of frost or near-frost, heating is controlled by daily manual adjustments of the target temperature differences according to the prognosis for mean air temperature for the next 24 h. Target temperature differences of 2°C and 3°C at the low and high heating scenario, respectively, are accordingly reduced to only 1°C and 2°C at a mean air temperature of –1°C, while heating is switched off in all mesocosms at mean air temperatures below –3°C. By using the prognosis for mean daily air temperature as reference temperature instead of the actual air temperature, we avoid replication of the considerable temperature fluctuations in air. However, we also eliminate the moderate diurnal variation usually seen for the water temperature. Air temperature is also used as an indicator when stopping the mixing of the water column. At temperatures below zero, the reference mesocosms are no longer mixed and ice cover and thermal stratification are allowed to develop, whereas mixing continues in the heated mesocosms until heating is switched off, according to the criteria described above.

Warming scenarios—IPCC climate scenario A2 (Houghton et al. 2001) and A2 + 50% scaled to local conditions in the region (average over five 25 × 25 km grid cells [pers. comm. O. Bøssing Christensen, Danish Meteorological Institute]) were applied as the low and high warming scenarios, respectively. The climate scenario A2 models actually predict air temperatures. However, because the temperature of surface waters closely follows that of the air in shallow lakes, we chose to use the modeled air temperatures as a surrogate of water temperatures. Warming was calculated as the mean air temperature increases in one particular month with respect to a 30-y reference period (1961 to 1990) and the modeled temperatures in the same month in 2071 to 2100 (Fig. 3). The modeled temperature difference for the A2 scenario is generally higher in August to January (max: 4.4°C in September) than during the rest of the year (min: 2.5°C in June).

Nutrient treatments—Groundwater (total phosphorus: 2 to 20 μg P L^{-1}; total nitrogen: 51 to 70 μg N L^{-1}; total iron: 0.10 to 0.62 mg Fe L^{-1}) collected from a local well is used as inlet water to the flow-through system. To create the two alternative states existing in shallow lake systems, i.e., the plankton dominated (turbid) and the macrophyte dominated (clear) state (Scheffer et al. 1993), half of the mesocosms are enriched with nitrogen and phosphorus while the other half remain unenriched. Throughout the first year, nutrients were added weekly as Na_2HPO_4 and $Ca(NO_3)_2$ solutions with a constant loading of 54 mg P and 538 mg N per mesocosm each week (2.7 mg P m^{-2}

Fig. 3. Modeled monthly temperature increase from the reference period (1961–1990) to 2071–2100 according to the IPCC climate model A2 (gray) and A2 + 50% (dark gray) down-scaled to local conditions around the experimental site. Based on hourly estimates.

d^{-1} and 27.1 mg N m^{-2} d^{-1}). The experiment is still in progress and, depending on the results from the first year, the loading may be changed later in the experiment. The enrichment was initiated in early May 2003 when only sparse submerged vegetation had developed in some of the mesocosms.

Measurements of oxygen and pH—Oxygen and pH probes are rotated among the mesocosms every week and every fourth week, respectively. We use 12 oxygen probes (OxyGuard®, two-wire probe, model 420), and measurements are conducted in all nutrient-enriched mesocosms during 1 week and in all nonenriched mesocosms the following week. At the same time, pH (OxyGuard®, light-duty submersion type connected to a Manta pH measurement system) is measured in six of the mesocosms where oxygen is being measured. A submersible pump (Sicce pumps, model: Micra) connected to the oxygen probe creates continuous horizontal water flow over the membrane and, thus, reduces the risk of instability due to fouling. All probes are calibrated weekly. As for the water temperature, oxygen concentration and pH are measured continuously. However, to reduce the amount of data collected, records are only made every 30 min. The frequent records allow us to describe diurnal variations and to calculate system primary production and respiration using the Odum method (Odum 1956). Re-aeration is a critical factor in these calculations. However, the continuous mixing of the water column allows accurate estimation of the re-aeration coefficient.

Pelagic and benthic communities—Aquatic communities representative of temperate shallow lakes were readily established in the mesocosms before beginning the heating. As inoculum for the benthic populations, sediments from a nutrient-rich plankton-dominated lake (Lake Søbygård) and a less nutrient-rich lake with macrophytes (Lake Stigsholm) were added to the original bulk pool of nutrient-rich pond sediment. In addition to the plankton emerging from resting eggs introduced with the sediment, plankton populations developed from inoculum collected in equal parts from the littoral and pelagic zones of four chemically and biologically different lakes in April 2003. Furthermore, thorough cross-mixing of water among the mesocosms was performed regularly to reduce start community variation among the pelagic communities of the same nutrient treatment. Nevertheless, large snail populations (mainly *Lymnea peregra*) emerged in three mesocosms in the period from May to July, i.e., before initiation of heating. To ensure similar conditions in all mesocosms, we chose to transfer snails to mesocosms with low snail densities.

Macrophytes (mainly *Elodea canadensis* and *Potamogeton crispus*) emerged naturally in most of the nonenriched mesocosms in May to June 2003, whereas sparse shoots of these species appeared in only two of the enriched mesocosms. As the plant populations developed nonuniformly among the nonenriched mesocosms, we manipulated their distribution and coverage before the experiment. In densely vegetated mesocosms, *P. crispus* was thinned out, and intact shoots were transplanted to mesocosms without or with low densities. *E. canadensis* was selectively removed from all mesocosms, but rapid regrowth and colonization prevented complete elimination of this species, and low densities were present in some mesocosms at the start of the experiment. Free-floating and attached filamentous algae developed massively in some of the mesocosms during summer despite our efforts to reduce their abundance during this pre-experimental period. Manipulation of the vegetation was stopped 2 wk before heating was initiated. Yet, 3 to 4 times throughout the experiment sporadically occurring shoots of floating-leaved macrophytes and duckweed were removed from the mesocosms to avoid dominance of floating-leaved plants.

Three-spined sticklebacks (*Gasterosteus aculeatus*) were stocked in the mesocosms in near to natural densities corresponding to the applied nutrient treatment (based on catch per unit effort [CPUE] in gill nets [Jeppesen et al. 2002] in Danish lakes [n = 180]): one individual in nonenriched mesocosms and 12 in enriched mesocosms. The 12 sticklebacks in the enriched mesocosms were added gradually, so that the number increased from mid-May to August (2003) when the heating experiment was initiated. To prevent breeding and, thus, uncontrollable population growth, only sexually mature males with breeding coloration were introduced. This was done because the mesocosms were too small to include piscivorous fish and, in their absence, unnaturally high densities of planktivorous fish were expected to develop if breeding was allowed. Dead or sick fish were replaced when observed. Furthermore, we sought to maintain a constant fish density by additional stocking based on regular visual inspections and the use of minnow traps and catch-recapture estimates. To avoid age-related fish loss, the old fish are replaced by younger males after a year. All sticklebacks came from natural populations and were caught either by traps or drag net.

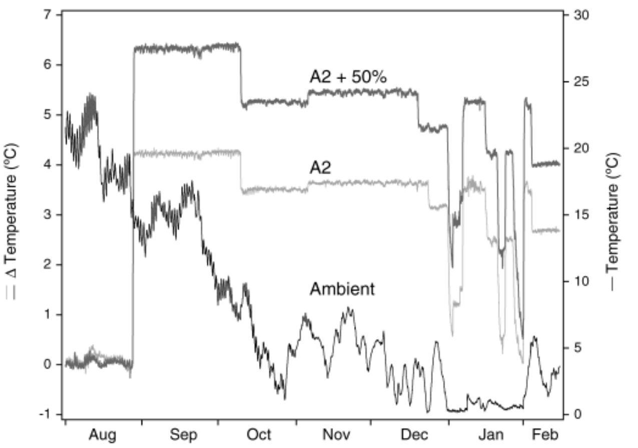

Fig. 4. Temperature difference between the unheated reference mesocosms and those heated according to IPCC climate scenario A2 (gray) and A2 + 50% (dark gray) from August to February. Actual water temperature of the unheated mesocosms is marked in black. Heating was initiated in late August. On frosty winter days (late December to February), the temperature in the heated mesocosms was not raised to a constant difference above the ambient temperature in the reference mesocosm, because the water temperature in the latter would always be ~ 0°C. Alternatively, at mean daily air temperatures, less than 0°C heating was controlled manually by daily adjustments of the temperature difference according to the air temperature. Data logged every 30 min.

Assessment

Both the heating (Fig. 4) and the flow-through system have generally performed well for 16 mo of continuous function. The sensitivity of the heating control system is high, and at the most optimal performance heating cycles run at intervals of 1 to 15 s between on and off, responding to small temperature changes from the target temperature difference. Such frequent cycling allows the heating system to parallel very precisely diurnal and seasonal temperature variation in the control mesocosms. During the first 3 mo with heating, the mean divergence of the measured temperature differences between control and heated mesocosms from the target temperature differences ranged between 0.11°C and 0.26°C (Table 1). In general, the deviation was slightly higher in the high heating scenario. The heating systems previously described by Baulch et al. (2003) and McKee et al. (2000) both controlled temperature by adding hot water through electromagnetic control valves. As pointed out by Baulch et al. (2003), such valves are not suited for continuous cycling between open and closed positions since they will eventually fail due to wear. This limitation, which reduces the sensitivity and accuracy of their systems (deviation from target temperature difference, McKee et al. (2000): margins 0.97°C to –0.63°C, mean = 0.04°C, SD = 0.14; Baulch et al. (2003): mean = –0.5°C), has been overcome in our heating system by using electrical power directly as the heating source.

So far, other freshwater mesocosm experiments on global warming have heated to either a constant temperature (e.g., Beisner et al. 1997), heated to a constant temperature above ambient (McKee et al. 2000; Baulch et al. 2003), or heated only in periods (McKee et al. 2000). By adjusting the temperature difference between the heated and the unheated reference mesocosms every month according to the forecasts of the climate model, seasonal climate warming variation is integrated in our experiment. Heating by on average 1°C to 1.5°C more in August through January than in February through July at the two climate scenarios may be particularly important for the chemical and biological dynamics, because higher temperatures in autumn and winter may extend the growing season of macrophytes, reduce ice covers and the probability of winter anoxia, and increase the survival of various species susceptible to oxygen depletion.

Another strength of our experimental setup compared to most previous mesocosm studies is that the water columns are mixed. Due to weaker surface wave energy mesocosms often tend to have less physical water mixing and, consequently, lower air-water gas exchange than shallow lakes. By constant mixing and semi-continuous inflow of oxygen-rich groundwater to our mesocosms, we attempt to avoid some of the problems frequently seen in unmixed batch system experiments, such as thermal gradients (discussed by Baulch et al. 2003) or total dominance of duckweed (Liboriussen et al. in press)

The flow-through system where each mesocosm has an inlet, an outlet, and a relatively short water residence time was established to mimic the natural passage of water that most lakes

Table 1. Mean temperature difference between unheated reference mesocosms and those warmed to IPCC climate scenario A2 and A2 + 50%, respectively, in August (no heating) to November*

	Scenario A2		Scenario A2 + 50%		
	Target	Measured ± SD	Target	Measured ± SD	*n*
Aug (no heating)	0	0.10 ± 0.10	0	0.04 ± 0.07	1262
Sep	4.39	4.23 ± 0.04	6.59	6.33 ± 0.09	2041
Oct	3.61	3.50 ± 0.03	5.42	5.26 ± 0.04	1253
Nov	3.76	3.64 ± 0.02	5.64	5.45 ± 0.03	2083

*The target temperature difference is calculated as mean temperature increase from 1 month in a 30-y reference period (1961–1990) to the modeled temperatures in the same month in 2071–2100. Measured temperature difference is the actual warming obtained by our heating system. Mean ± SD.

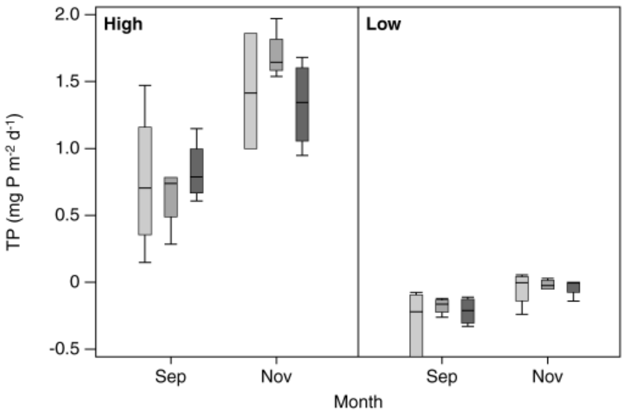

Fig. 5. Box-plot showing examples of mass balances on phosphorus retention in nutrient enriched (high) and nonenriched (low) mesocosms in September and November for unheated reference mesocosms (pale gray) and mesocosms warmed to scenario A2 (gray) and warmed to scenario A2 + 50% (dark gray). Each box shows 10%, 25%, median, 75%, and 90% fractiles.

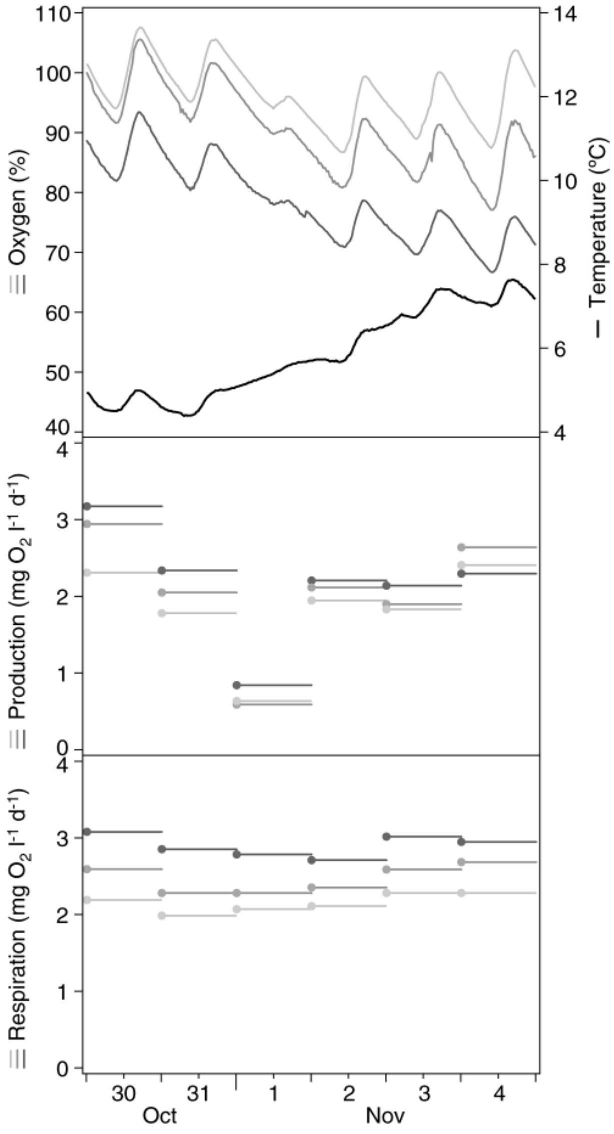

Fig. 6. Example of mean diurnal variation in oxygen saturation and estimated system primary production and respiration in mesocosms being unheated reference (pale gray), warmed to scenario A2 (gray), and warmed to scenario A2 + 50% (dark gray) (*n* = 4). Mean water temperature of the unheated mesocosms is shown in black. All mesocosms are nutrient enriched.

experience. However, constructing a flow-through system that supplies the same volume of water to all 24 mesocosms was not unproblematic and a continuous flow could not be established due to water pressure differences in connected pipes. Supplying water in pulses, as done in this experiment, allows individual adjustments to be made, not only in the duration of the pulses to each mesocosm but also in the frequency and timing of the pulses. Control of the pulse timing assures that water is only supplied to one mesocosm at the time, thus eliminating variation due to reduced water pressure at simultaneous water supply to several mesocosms. Based on measurements of the total discharge to the collection tanks (September 2003 to March 2004), the average water residence time is estimated to be 74 d (ranging from 68 to 85 d among mesocosms). In addition to the impact of small differences in precipitation into and evaporation from the mesocosms, this variation is probably mainly caused by diurnal variations in water pressure and inaccuracy of the adjustments of the water pulses. The accuracy of the water supply could probably be improved by placing a cistern at each mesocosm to be filled with an exact water volume before emptying at defined intervals. However, due to our relatively frequent measurements (7 to 14 d) of the total water output into the collection tank, we may be able to adjust for the differences in our mass balance calculations.

As previously stated, the flow-through system allows us to establish mass balances for some of the conservative chemical variables based on inlet-outlet measurements for the individual mesocosms. An example showing the phosphorus mass balance is given in Fig. 5 for the mesocosms with and without nutrient enrichment, respectively. Nutrient-enriched mesocosms showed positive phosphorus retention, irrespective of temperature, with an increasing trend from September to November. This probably reflects the increased capacity of the sediment to retain phosphorus with decreasing oxygen consumption in the sediment surface due to reduced temperatures. In the mesocosms without nutrient enrichment, phosphorus retention was slightly negative in September at all three temperatures and close to zero in November. This reflects the low inlet concentrations of phosphorus (approximately 12 µg TP L^{-1}) and release from the sediment during summer. Similarly, community primary production and respiration may be calculated from the frequent oxygen monitoring in the mesocosms. As shown in Fig. 6, substantial diurnal variation of the oxygen saturation is evident in all three temperature scenarios. This, along with well-estimated re-aeration caused by constant mixing of the mesocosms, provides us with good opportunities to get year-round information on the metabolic dynamics of the systems and, thus, to describe how heating and nutrients affect systems primary production and respiration. In the period shown, both systems primary production and respiration increased with temperature (Fig. 6).

The use of groundwater as inlet water rather than water from a natural lake may be seen as a weakness of our experiment because groundwater does not carry propagules from upstream waters. However, the main natural water input to many Danish lakes derives either from groundwater or small streams poor in propagules from lentic species. Furthermore, an analysis of metacommunity structure indicates that even in highly interconnected ponds the local communities are often mainly structured by local environmental constraints rather than regional interactions (Cottenie et al. 2003). Lentic propagules in natural lake-inlet waters are thus not only few in numbers, but they can also be expected to have minor effects on the community structure if introduced in natural densities.

The cost of setting up the complete experimental setup including tanks, probes, and the computer-controlled heating and surveillance system has been approximately 140,000 Euro. However, total establishment costs should also include the numerous hours of manpower. Remote access to the computer at the experimental site and, thus, to both past and current data plus several of the vital control functions facilitates easy electronic supervision and allows small adjustments to the software to be made while running the experiment. Despite this advanced technical system, running the experiment is demanding with respect to manpower and requires regular supervision and maintenance of the equipment. Heating expenses are another costly component of the project. On an annual basis, the total electricity consumption of the experiment is approximately 60,000 kWh. This includes electricity supply for heating, mixing, water supply, computer, and control facilities, etc.

Comments and recommendations

We find the system very suitable for describing how changes in temperature and nutrients affect dynamics, storage, and loss of nutrients and carbon in simulated shallow lake system over prolonged time periods and with contrasting trophic structure. Moreover, the design and functional capacity of the flow-through system allow conduction of a range of additional studies not feasible in batch systems, for instance, on how extreme hydrological events or systematic seasonal changes in inflow will affect shallow ecosystems and on the recovery of the ecosystems after such events. The system can also provide both fixed and differential temperature control, just as mixing rate and mixing timing may be minutely controlled. The major drawback of the system is a relatively large variability among mesocosms, particularly at high nutrient loading. Increased replication, albeit costly, would help solve this problem. Another weakness is the relatively small size of the mesocosms. Scale matters (Schindler 1998) and our mesocosms are no exception as we found a tendency for pond forms of, for example, zooplankton to dominate the plankton. Furthermore, relatively high proportions of filamentous algae, more typical for small ponds than for lakes, were observed in the nutrient-enriched mesocosms. In larger systems, such problems could be minimized, and they would also allow stocking of piscivorous fish and, thus, natural development of planktivorous fish. The present system requires restriction of the fish community to single sex small-sized fish to avoid development of unnaturally high fish densities in the absence of piscivores. If the size of the mesocosms were to be increased and the replication strength maintained, alternative heating systems based on wind or sun energy offer an attractive solution. Despite the above-discussed problems and limitations of our technical equipment and of the experiment in general, we believe that the system has a great potential for realistic studies of the effects of global warming and eutrophication on in-lake processes and dynamics in shallow lakes.

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Horizontal dynamics of zooplankton in subtropical Lake Blanca (Uruguay) hosting multiple zooplankton predators and aquatic plant refuges

Carlos Iglesias[1*], Guillermo Goyenola[1], Nestor Mazzeo[1], Mariana Meerhoff[1,2,3], Elena Rodó[1] & Erik Jeppesen[2,3]

[1] Depto. de Ecología. Facultad de Ciencias. Universidad de la República. Iguá 4225 C.P. 11400 Montevideo-Uruguay.
[2] National Environmental Research Institute. Vejlsøvej 25, P.O. Box 314. DK 8600 Silkeborg, Denmark
[3] Department of Plant Biology, University of Aarhus, Ole Worms Alle, DK 8000 Aarhus C, Denmark.

*email: caif@fcien.edu.uy

Key words: spatial use, diel horizontal migration, refuge effect, invertebrate predation, subtropical lakes

Abstract

In the subtropics, the effects of macrophytes on trophic interactions are more complex than in temperate lakes. Fish, particularly the smallest species and individuals, aggregate in high numbers in the vegetation, and a strong predation pressure on zooplankton by shrimps and invertebrates, as *Chaoborus*, can occur in these systems. We studied seasonal and diel changes in zooplankton and their potential predators (both fish and invertebrates) and physical and chemical characteristics among open water and vegetated habitats (emergent and submerged plants), in the subtropical lake Blanca (34°54′S; 54°50′W), a shallow system with an extensive and complex littoral area and high abundance of vertebrate and invertebrate predators on zooplankton. We found differential horizontal distribution of some zooplankton species under the scenario of high abundance of small omnivorous-planktivorous fish and *Chaoborus*, especially in the seasons with intermediate catch per unit effort of fish. We found indications of a diel horizontal migration (DHM) opposite than described for temperate systems, as the two main cladocerans *Bosmina longirostris* and *Diaphanosoma birgei* were found in higher densities in the submerged plant beds during night, in spring and autumn respectively. Although we need experiments to prove DHM, *Chaoborus* seemed to be the main trigger of the apparent DHM, followed by small omnivorous fish. During summer no spatial differences were found likely because of high densities of fish in all habitats. In absence of piscivorous fish, the distribution of fish *Jenynsia multidentata* seemed to be conditioned by food availability and by predation risk of waterfowl. The refuge capacity of aquatic plants for zooplankton in subtropical systems seems weak and with consequent weak or no cascading effects on water transparency, as under very high fish and invertebrate densities (summer) the refuge for zooplankton was lost.

Introduction

The spatial distribution of animals is regarded as a compromise between several costs and benefits, especially the avoidance of predation and the search for optimal feeding and environmental conditions. In freshwater systems, aquatic plants play a relevant role in affecting the spatial distribution of several organisms and shaping predator-prey interactions (Jeppesen et al., 1997a). Submerged macrophytes can often provide daytime refuge for *Daphnia* and other cladocerans from fish predation in shallow temperate lakes (diel horizontal migration (DHM), Timms & Moss, 1984; Burks et al., 2002), although this pattern has not been observed in oligotrophic and mesotrophic lakes (Lauridsen et al., 1999). However, many juvenile fish often use the littoral zone as daytime refuge against predatory fish, which compromise the refuge capacity of macrophytes for zooplankton (Carpenter & Lodge, 1986). Also, invertebrate predators in the pelagic can affect zooplankton populations and DHM, but evidence is scarce yet (Burks et al., 2001; R. Burks et al., unpublished). It has been suggested that the effects of co-existing fish and pelagic invertebrate predators can be additive and increase the likelihood of large zooplankton seeking refuge in the littoral zone (Burks et al., 2002). The net effect of multiple predators, however, seems to depend on the direction of the response of the prey to each predator alone, and their relative risk for that prey. The response of the prey to combined predators would thus be similar to its response to the most risky predator in the pair (Relyea, 2003; Wojtal et al., 2003). Wicklum (1999) suggested that vertebrate and invertebrate predation act alternately because invertebrate predators are themselves subject to fish predation. As a consequence, the impact of invertebrate predation increases as fish predation declines (Mumm, 1997).

In the tropics and subtropics, the effects of macrophytes on trophic interactions are more complex than in temperate lakes, not least because all plant life forms (emergent, submerged, floating-leaved and large free-floating species) can be very abundant (Talling & Lemoalle, 1998), and the predation pressure on zooplankton seems stronger (Jeppesen et al., 2006). Fish are usually present in very high numbers (Scasso et al., 2001), and particularly the smallest species and individuals, aggregate in the vegetation (Meschiatti et al., 2000, Meerhoff et al., 2003). Besides, the zooplanktivorous phantom midge *Chaoborus* seems to be more abundant in tropical lakes (Lewis, 1996). This might reflect that tropical lakes become anoxic in the bottom more quickly than temperate lakes, providing *Chaoborus* with a fish predation refuge for longer periods (Lewis, 1996), which, in turn, permits a stronger predation pressure on zooplankton (Arcifa et al., 1992). Also shrimps can represent important predators on zooplankton in warm regions (Collins, 1999; Collins & Paggi, 1998), probably due to the dominance of small fish species with consequent lower risk of predation on shrimps, as seen for *Neomysis integer* Leach in north temperate brackish lakes with fish communities dominated by small sticklebacks (*Gasterosteus* spp.) (Søndergaard et al., 1997).

Our objectives were to describe the seasonal and diel variation in the horizontal distribution of zooplankton under the scenario of combined vertebrate and invertebrate predators and relate it to variations in predator abundance and the changes promoted by the aquatic plants, in a subtropical shallow lake with a very well-developed littoral zone hosting different plant life-forms and high abundance of small omnivorous fish and invertebrate predators (Mazzeo et al., 2003). Based on knowledge from eutrophic temperate lakes we hypothesised that predation-sensitive zooplankton would seek refuge during the day in those vegetated habitats with less potential predators, and move to open water at night to decrease predation risk. However, an alternative scenario is that the plants are not feasible refuges for zooplankton due to a potential high density of small fish in the plant beds.

Study area

Lake Blanca (34°54’S; 54°50’W) is a shallow (total area=40.5 ha, Z_{max}=3.6m; Mazzeo et al., 2003) lake used for water supply in the main tourist area of Uruguay. It is a warm polymictic (temperature range: 11.3-26.3ºC) and eutrophic lake (in-lake annual mean TP, TN (Valderrama, 1981) and chlorophyll a (Nusch, 1980) concentrations: 93.6, 1010, and 31.9 µg l^{-1}, respectively). A drastic reduction of the lake volume and a consequent loss of piscivorous fish occurred during 1998–1999. Since then, turbid and clear water states have alternated in the system, and the fish community has been dominated by small (13mm<SL<86mm) *Jenynsia multidentata* Jenyns (Anablepidae) (Mazzeo et al., 2003). This is an omnivorous-planktivorous fish (Escalante, 1983; Goyenola et al., unpub. data), with a mean whole lake density=13 ind m^{-2} (density in littoral areas≥100 ind m^{-2}, Mazzeo et al., 2003). The littoral zone of Lake Blanca is rather complex and dynamic, from the shore to the open water area there is an emergent plant zone (*Schoenoplectus californicus* L. and floating islands dominated by *Typha latifolia* L.), followed by submerged plants (*Egeria densa* Planch. and *Ceratophyllum demersum* Planch.), and finally by another zone of emergent plants (only *S. californicus*) (Fig. 1).

Materials and methods

The study was carried out seasonally from southern hemisphere winter 2003 to autumn 2004. The percent volume infested of the submerged plants (PVI, according to Canfield et al., 1984) and the distribution of the emergent plants were estimated from more than 100 sites homogeneously distributed over seven transects that were perpendicular to the maximum length of

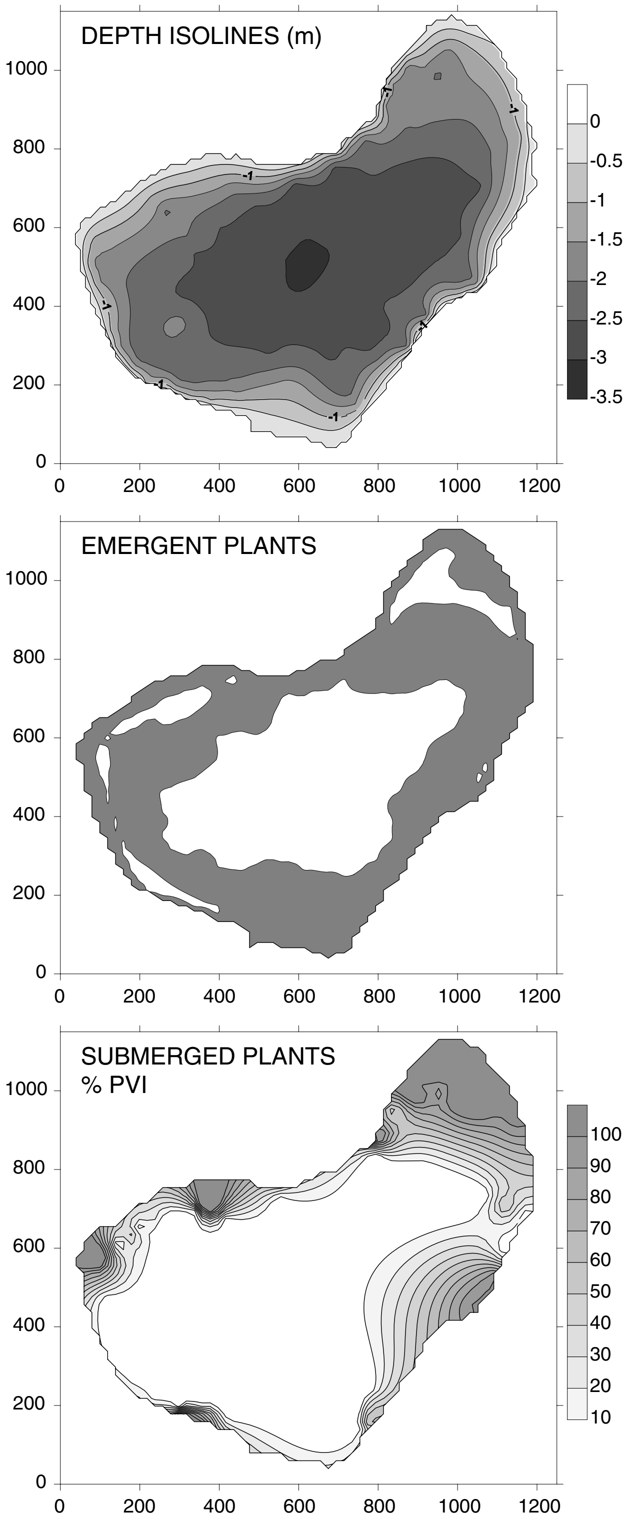

Figure 1. Bathymetric map of Lake Blanca (upper panel), and spatial distribution of aquatic plants: area covered by emergent *Schoenoplectus californicus* (intermediate panel), and % PVI of submerged plants *Egeria densa* and *Ceratophyllum demersum* (lower panel). Distances are marked on the x and y axes (in metres). Data correspond to winter 2003.

the lake and covered all the system. Each season, we mapped the vegetation according to these observations and recorded the positions using a GPS. From the map, we randomly selected five sampling points per strata: emergent plants (EP), submerged plants (SP: PVI ≥25%), and the pelagic or open water area (OW: PVI=0%). Water samples for physical, chemical and plankton analyses were collected with a vertical tube along the whole water column, at midday and midnight. Dissolved oxygen (DO), conductivity, pH, and transparency were registered *in situ*, using Horiba sensors and a Secchi minidisk. The euphotic zone depth (Z_{eu}, 1% of incident light) was determined from the photosynthetic active radiation (Licor Model Li-250). In each habitat we took a 20-litre depth-integrated sample with the tube sampler for zooplankton and *Chaoborus* (filtered through a 50-µm mesh size net). Counts were made according to Paggi & de Paggi (1974). Fish and shrimps were sampled with two minnow traps (40x40x60 cm, 5-cm opening) per station, one close to the surface and one close to the sediment. These catches integrated the periods between sunrise and sunset (day), and sunset and sunrise (night), respectively (capture per unit effort (CPUE) = total number of individuals collected in the two traps per hour).

Data analysis

We used repeated measures 2-way ANOVA (rmANOVA) to detect significant differences within seasons, considering two main factors: a) sampling zones (SP, EP, OW) and b) time as repeated measures (day, night) (Underwood, 1997). In case of relevance, also 1-way ANOVA were performed for specific times (day or night). Non-parametric correlations (Spearman, rs) were performed between relevant variables. When DHM by zooplankton species was detected in the ANOVAs, we tested the overlap between the prey and the potential predators distributions under two scenarios: the “direct” (i.e. day prey-day predator and night prey-night predator distributions) and the “reverse” (i.e. day prey-night predator), using Spearman correlations. In this scenario, the prey is allowed to move while the predator is fixed (in its night location), thus enhancing the detection of migration patterns by the prey (modified from Bezerra-Neto & Pinto-Coelho, 2002).

Results

Effects of the aquatic plants on the physico-chemical variables

The mean area (annual averages) covered by the emergent plants (EP) was 55% and 46% for submerged plants (SP), including some areas where both EP and SP were present (Fig. 1). We did not observe any clear seasonal pattern in coverage of the two plant types (authors, unpub. results). The SP sites had lower DO concentrations than OW and EP in spring (9.35, 10.41 and 10.12 mg l^{-1}, respectively), summer (5.28, 8.12 and

Table 1. Summary of 2-way rmANOVA statistics. Effects of sites, time (day, night) and their interaction, on temperature (T), dissolved oxygen (DO), and pH, along seasons. The differences among sites for water transparency (mini Secchi Disk) correspond to midday (1-way ANOVA). Sites: OW= open water, SP= submerged plants, EP= emergent plants, ordered according to *post hoc* tests. The F values and the respective degrees of freedom are indicated in all cases. Significance level: p>0.1 ns, p<0.05*, p<0.01**, p<0.001***. We indicate the p-value in the marginally significant cases (0.05<p<0.1$^+$).

		SITES		TIME	SITE x TIME
		Day	Night		
T	win	SP=OW=EP 2.34 $_{(2,12)}$ ns	SP=OW=EP 0.35 $_{(2,12)}$ ns	30.2 $_{(1,12)}$ ***	1.85 $_{(2,12)}$ ns
	spr	SP=OW=EP 0.90 $_{(2,12)}$ ns	SP=OW=EP 1.16 $_{(2,12)}$ ns	1.16 $_{(2,12)}$ ns	0.05 $_{(2,12)}$ ns
	sum	SP=EP>OW 9.72 $_{(2,12)}$ ***	SP=OW=EP 2.47 $_{(2,12)}$ ns	2.47 $_{(2,12)}$ ns	1.62 $_{(2,12)}$ ns
	aut	SP=OW>EP 3.08 $_{(2,12)}$ p=0.08$^+$	SP=OW>EP 3.37$_{(2,12)}$ p=0.07$^+$	11.7 $_{(1,12)}$ **	3.46 $_{(2,12)}$ ns
DO	win	OW=SP=EP 0.60 $_{(2,12)}$ ns	OW=EP=SP 1.38 $_{(2,12)}$ ns	34.7 $_{(1,12)}$ ***	0.90 $_{(2,12)}$ ns
	spr	OW=SP=EP 0.15 $_{(2,12)}$ ns	OW=EP>SP 4.95 $_{(1,12)}$ **	66.3 $_{(1,12)}$ ***	1.82 $_{(2,12)}$ ns
	sum	OW=EP>SP 6.87 $_{(2,12)}$ **	OW=EP>SP 45.6 $_{(2,12)}$ ***	41.8 $_{(1,12)}$ ***	9.6 $_{(2,11)}$ **
	aut	OW=EP>SP 25.9 $_{(2,12)}$ ***	OW>EP=SP 5.14 $_{(2,12)}$ **	101.9 $_{(1,12)}$ **	3.9 $_{(2,11)}$ p=0.05$^+$
pH	win	SP=OW=EP 0.2 $_{(2,12)}$ ns	EP=OW=SP 0.9 $_{(2,12)}$ ns	93.6 $_{(1,12)}$ ***	0.8 $_{(2,12)}$ ns
	spr	SP=OW=EP 1.0 $_{(2,12)}$ ns	OW=EP=SP 1.9 $_{(2,12)}$ ns	5.3 $_{(1,11)}$ *	9.2 $_{(2,11)}$ **
	sum	EP=OW=SP 0.5 $_{(2,12)}$ ns	OW=EP>SP 5.3 $_{(2,12)}$ *	0.6 $_{(2,12)}$ ns	2.6 $_{(2,12)}$ ns
	aut	EP=SP=OW 1.0 $_{(2,12)}$ ns	OW=EP>SP 9.8 $_{(2,12)}$ **	33.7 $_{(1,12)}$ ***	7.7 $_{(2,12)}$ **
mSD	win	EP=SP=OW 1.8 $_{(2,12)}$ ns			
	spr	OW=EP=SP 0.3 $_{(2,12)}$ ns			
	sum	SP>EP=OW 10.25 $_{(2,12)}$ **			
	aut	SP>EP=OW 3.1 $_{(2,12)}$ p=0.08$^+$			

8.06 mg l^{-1}) and autumn (8.37, 11.82 and 10.51 mg l^{-1}). DO was always higher during day (Table 1). In SP, the sub-saturation dropped to 44% in summer during night. Intermediate pH values occurred in OW during day, while the same habitat had the highest pH during night (Table 1). The euphotic zone comprised the entire water column. Water transparency was most of the year higher within the submerged plant sites, especially in summer and autumn (Table 1). In summer, the water transparency was 46% higher in SP (35 cm Secchi minidisk) than in OW (21 cm Secchi minidisk), while in autumn the transparency was 34 and 14 cm (Secchi minidisk) in SP and OW respectively.

Structure of main communities

The zooplankton community was numerically dominated by small-sized specimens (mostly nauplii and rotifers) all year round, except in winter when medium-sized filtrators dominated (small cladocerans and calanoid copepods) (Fig. 2). The small *Bosmina longirostris* Müller and *Diaphanosoma birgei* Korinec were the most abundant cladocerans, but the calanoid copepod *Notodiaptomus incompositus* Brian was also common. The maximum total abundance occurred in autumn (2252 ind l^{-1}) and the minimum in spring (856 ind l^{-1}) (whole system averages).

The phantom midge *Chaoborus sp* occurred all year round, but was found only during night. Its abundance varied considerably through seasons, with minimum in winter (100 ind m^{-3}) and maximum during spring and

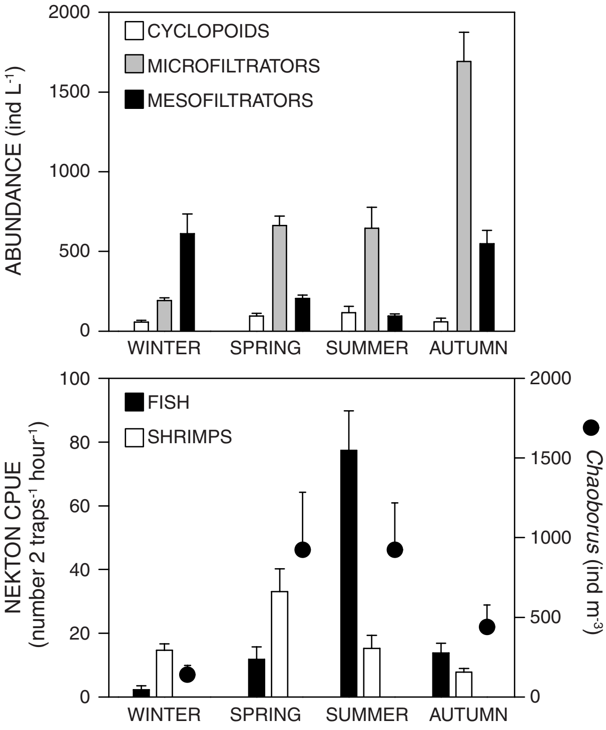

Figure 2. Temporal variation of: Main zooplankton functional groups (according to diet and size): microfiltrators= rotifers + nauplii, mesofiltrators= calanoid copepods + cladocerans (upper panel); Potential vertebrates and invertebrate predators on zooplankton (nekton= fish *J. multidentata* + shrimps *P. argentinus*) (lower panel). Data represent whole system averages (error bars= 1 SE).

summer (*ca.* 1000 ind m^{-3}) (whole system averages, Fig. 2). In terms of numbers, the nekton community was dominated by the fish *J. multidentata* in autumn and summer, when we found the highest CPUE (Fig. 2). The also small omnivorous fish *Cnesterodon decemmaculatus* Jenyns (Poecilidae) occurred in low abundances (<1.5% of total fish density and biomass). The shrimp *Palaemonetes argentinus* Nobili dominated in terms of abundance in winter and spring (Fig. 2).

The interseasonal variation in the total zooplankton abundance was negatively correlated with the CPUE of *P. argentinus* and *J. multidentata* (rs= -0.49, p<0.001; rs= -0.29, p<0.05, respectively), while there was no significant correlation with the abundance of the invertebrate predator *Chaoborus*.

Use of the space of zooplankton and potential predators

The diel use of the different habitats by most zooplankton species varied strongly among seasons. *Bosmina longirostris* appeared mainly in OW in winter (2-way ANOVA $F_{(2,12)}$= 9.9, p<0.05), and in OW and EP in autumn (2-way ANOVA $F_{(2,12)}$= 31.1, p<0.001), during both day and night. We found diel differences only in spring, when *Bosmina*, from an homogeneous distribution during day, became associated with SP and disappeared from OW at night (1-way ANOVA $F_{(2,12)}$= 4.62, p<0.05) (Fig. 3A$_2$). *Diaphanosoma birgei* was collected mostly in OW in winter (2-way ANOVA $F_{(2,12)}$= 136.2, p<0.001), while it was more equally distributed among habitats in spring and summer. In autumn, *Diaphano-*

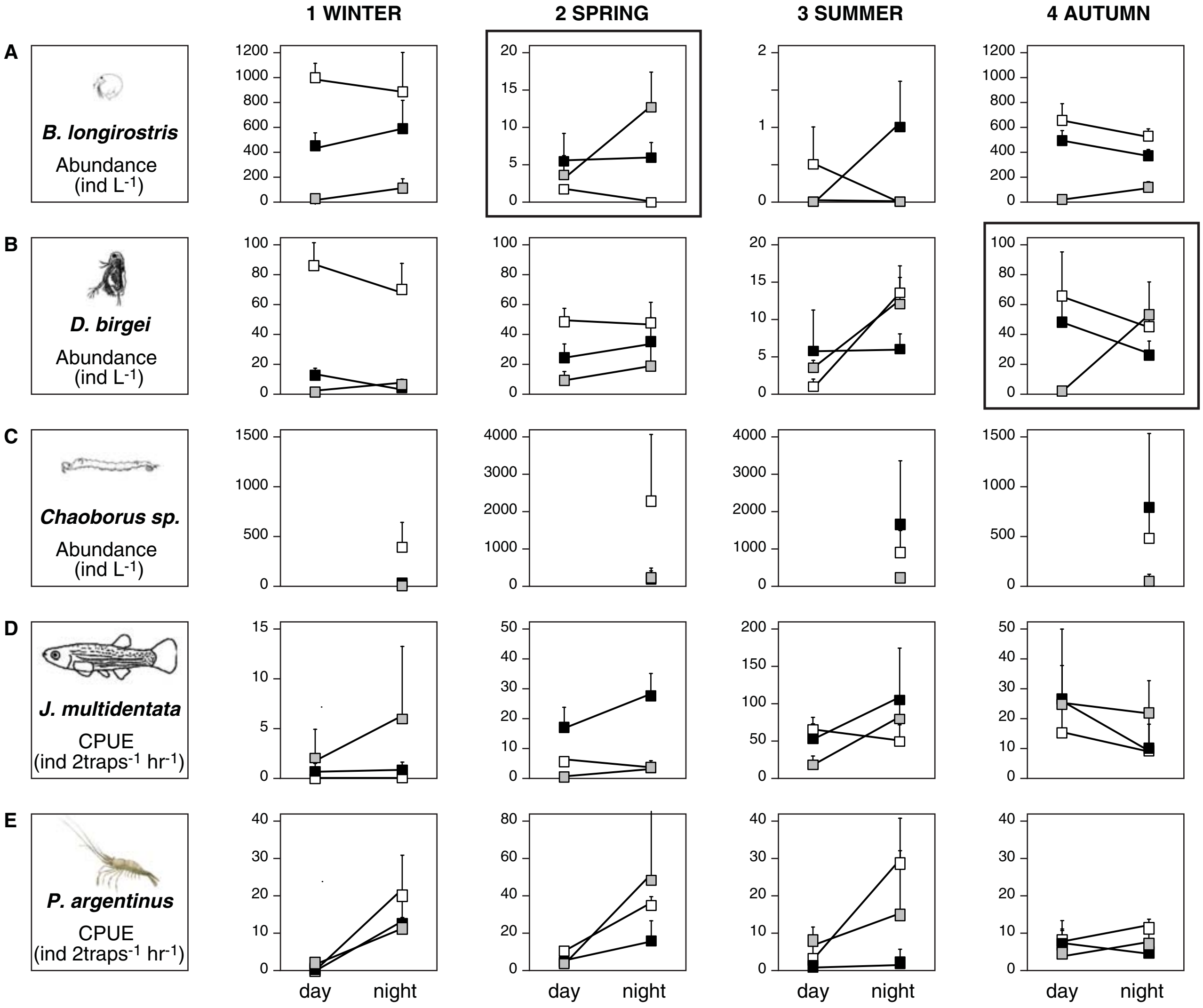

Figure 3. Diel habitat choice in all seasons of *B. longirostris* (A), *D. birgei* (B), and main potential predators: *Chaoborus* (C), *J. multidentata* (D) and *P. argentinus* (E). From left to right, panels correspond to winter (1), spring (2), summer (3), and autumn (4). Symbols: □ = open water, ■ = emergent plants, ▨ = submerged plants. The cases of DHM are bold-framed. Note the different scales in the figures (error bars= 1 SE).

soma had the lowest density within the SP during day (1-way ANOVA $F_{(2,12)}$= 5.21, $p<0.05$), but increased strongly its density in that habitat at night (from 2 to 67 ind l^{-1}, Fig. $3B_4$). *Chydorus sphaericus* Müller was associated with SP in winter (2-way ANOVA $F_{(2,12)}$= 29.4, $p<0.001$) and spring ($F_{(2,12)}$= 37.6, $p<0.001$). We found higher abundances of *Moina micrura* Kurz in OW in winter (2-way ANOVA $F_{(2,12)}$= 4.9 $p<0.05$), and a homogenous spatial distribution during the rest of the year. Cyclopoids had higher abundances within the SP stands during all seasons (2-way ANOVA $F_{(2,117)}$= 12.9, $p<0.001$), while the calanoid *N. incompositus* was homogeneously distributed among the three habitats. Nauplii showed different density patterns with seasons, being higher within vegetation (both plant types) in winter and summer (2-way ANOVA $F_{(2,12)}$= 5.9, $p<0.01$; $F_{(2,12)}$= 6.7, $p<0.01$, respectively).

Chaoborus was found only during night. It was more abundant in OW in winter and in spring (1-way ANOVA $F_{(2,12)}$= 16.4, $p<0.01$; $F_{(2,12)}$= 6.45, $p<0.01$, respectively) (Fig. $3C_{1,2}$). In summer and autumn it was mainly associated with EP and OW (1-way ANOVA $F_{(2,12)}$= 4.3, $p<0.05$; $F_{(2,12)}$=3.5, $p<0.05$, respectively) (Fig. $3C_{3,4}$). *Jenynsia multidentata* tended to occur mainly within the emergent vegetation. In winter, CPUE was higher within SP (though not significantly), while in spring it was collected primarily in EP sites (2-way ANOVA $F_{(2,12)}$= 12.7, $p<0.001$), especially at night (Fig. $3D_2$). In summer, the CPUE was higher at night (2-way ANOVA $F_{(1,12)}$= 9.2, $p<0.05$), and the sequence was OW≥EP>SP during day and EP>SP>OW during night (significant interaction Sites x Time $F_{(2,12)}$= 5.2, $p<0.05$) (Fig. $3D_3$). The spatial distribution in autumn did not show a significant pattern (Fig. $3D_4$).

The third potential predator, the shrimp *P. argentinus*, was collected in winter mostly from OW and during night (Fig. $3E_1$), though not significantly. In spring and summer, the highest CPUE occurred during night (2-way ANOVA $F_{(1,12)}$= 14.93, $p<0.01$ and $F_{(1,12)}$= 9.6, $p<0.01$ respectively). For the summer, the sequence of the densities were SP≥OW≥EP during day and OW>SP>EP at night (significant interaction Sites x Time $F_{(2,12)}$= 4.3, $p<0.05$). In autumn, no statistical differences were found in the habitat preference of shrimps. Only in summer there was an inverse spatial correlation between the CPUE of adult *J. multidentata* and *P. argentinus* (rs= -0.56, $p<0.01$, during night) (Figs. $3D_3$ & $3E_3$).

DHM analysis

We focus here on *B. longirostris* and *D. birgei*, as they were the only species showing significant diel variations between habitats in their densities (in spring and autumn, respectively). In spring the density and distribution of *B. longirostris* during day was positively, though not significantly, correlated with the distribution of *Chaoborus* during night. Contrary, the "direct" (day/day, night/night) correlation of both species during night was negative and significant (rs= -0.59, $p<0.05$) (Figs. $3B_2$ & $3C_2$). In the case of *J. multidentata*, both species were positively but not significantly correlated in the "direct" comparison, while their "reverse" correlation (day prey/night predator) was negative and also not significant (Figs. $3B_2$ & $3D_2$). Besides, the distribution of *B. longirostris* was negatively correlated with DO concentrations in night (rs=-0.72, $p<0.01$). In autumn, the day distribution of *D. birgei* was significant and positively correlated with the distribution of *Chaoborus* during night (rs= 0.81, $p<0.001$), while the "direct" correlation of both species in the night was negative but not significant (rs= -0.33, n.s.). The "direct" correlations between *Diaphanosoma* and *J. multidentata* were positive and significant at night (rs=0.79, $p<0.001$), while the "reverse" correlation was negative but not significant. We did not detect any statistically significant relationship between the distribution of *D. birgei* and the main physico-chemical variables (DO, pH, T). In these intraseasonal analyses, no significant correlation was found between the distribution of these cladocerans and the abundance (CPUE) of shrimps.

Discussion

Predators are thought to be the main factor controlling the spatial distribution, abundance and body size of zooplankton in shallow lakes (e.g. Scheffer, 1998; Burks et al., 2002). In Lake Blanca, the small size of the dominant cladocerans and the dominance by copepods and rotifers likely reflect the extremely high abundance of the potential predators, particularly the omnivorous-planktivorous fish *Jenynsia multidentata*. The most important finding of our study was the lack of fixed patterns in the horizontal distribution of the main zooplankton groups among seasons, particularly of cladocerans. Cyclopoid copepods were associated mainly with submerged plants (day and night) as seen in other studies (Jeppesen et al., 1997b), while calanoids showed a homogeneous distribution.

However, in contrast to most studies from eutrophic shallow lakes in the temperate zone (Burks et al., 2002), the most abundant cladocerans *B. longirostris* and *D. birgei* preferred the vegetated areas (especially the submerged plants) during night and not as expected during day (in seasons when distribution differed significantly). The significantly higher water transparency in sites with plants indicates zooplankton would probably find lower quantity of food resources in these habitats than in open water, which suggests that moving towards the plants was a predator-avoidance behaviour.

The occurrence of DHM (and its reverse direction) in Lake Blanca seemed controlled by two factors related to the potential predators: the temporal variability in fish CPUE, and in the abundance and the habitat use of *Chaoborus*. However, despite the very high fish CPUE, *Chaoborus*, occurring in high densities compared with those of temperate lakes (Liljendahl-Nurminen et al., 2002), seemed to play a more relevant role than fish.

During winter, with the lowest densities of fish and *Chaoborus*, *B. longirostris* and *D. birgei* had the highest abundances and we did not find evidence of DHM as these cladocerans were mostly found in open waters. The reverse DHM occurred during the seasons with intermediate CPUE of fish (spring and autumn). From the "direct" and "reverse" correlation analyses, we can argue that if *Bosmina* did not migrate, from a relatively homogeneous diurnal distribution towards SP at night (in spring), it would encounter with *Chaoborus* in open waters, while with fish in the emergent plants.

In the case of *D. birgei* the DHM pattern seemed more clear than in the case of *B. longirostris*, as day and night densities were balanced in the former. By migrating to SP at night (in autumn), *D. birgei* decreased the encounter rate with *Chaoborus* (which by then mostly appeared among EP), even though the overlapping with *J. multidentata* would increase. Probably, the costs of migrating to the submerged plant beds at night are high, but still lower than of migrating to the pelagic zone where the absence of physical structures would increase the predation efficiency by *Chaoborus* and fish. In summer, *J. multidentata*'s density increased enormously after the main reproductive season. Together with a remarkable decrease in cladoceran abundance, the spatial distribution of *B. longirostris* or *D. birgei* became homogeneous, with no indications of DHM. Clearly, no habitat seemed to offer a significant refuge for zooplankters under this enhanced-predation scenario.

Although we think DHM is the most likely explanation for the observed distributions, we cannot prove it as the sampling method used allows no mass balances to be performed. The pattern, particularly of *B. longirostris*, could also be due to, or enhanced by, differential vertical migration (stronger in the submerged plant sites) or differential predation pressure in the different habitats or their combination. *In situ* and *ex situ* experiments would provide further insights into this subject.

In their review, Burks et al. (2002) did not report examples of diel horizontal migration in the scenario of both pelagic fish and active-at-night invertebrate predators (e.g. *Chaoborus*). Jeppesen et al. (1997a) suggested that DHM induced by invertebrates would probably be more important in lakes with low fish densities, and that the direction of this migration would depend on the habitat choice of the predacious invertebrates (whether pelagic or plant-associated). Likewise Lauridsen et al. (1997) suggested that the reverse DHM by *Daphnia* in the fishless Central Long Lake (USA) could be induced by pelagic nocturnal invertebrates, as *Chaoborus*. R. Burks et al. (unpublished), on the contrary, found direct DHM triggered by *Chaoborus* in a Danish lake with very low abundance of fish, as earlier observed by Kvam & Kleiven (1995) in a Norwegian lake. The advantage of DHM as an anti-predation mechanism depends on both the predators and the refuge capacity of the aquatic plants. Our results can be interpreted as a variation of the additive interaction between vertebrate and invertebrate predators, proposed by Burks et al. (2002). We did not find evidence that the occurrence of invertebrates can lead to higher densities of large-bodied zooplankton, by serving as alternative food for fish, as found by Sagrario & Balseiro (2003) in mesocosm experiments with some of the same predatory species that appeared in Lake Blanca. The physico-chemical differences among sites played a minor role in shaping these spatial patterns. Although we found a negative relationship between the spatial distribution of *B. longirostris* and the oxygen concentration in spring, the differences of DO among sites were low.

Also the predators showed temporal variability in habitat choice, on a diel and seasonal scale. Several authors reported that *Chaoborus* displays vertical migration to avoid fish predation, spending day in the sediments and migrating upwards at night (Davidowicz et al., 1990; Voss & Mumm, 1999). The same behaviour was evidenced in Lake Blanca as *Chaoborus* was only caught during night, a likely less risky time to occupy the water column when visual predatory fish are present.

The habitat selection by *J. multidentata*, as top predator, probably depended on the search for food and shelter from the piscivorous waterfowl. The emergent plant zone is likely a safer place than the submerged plants, as bird attacks can be avoided (e.g. from yellow-billed tern *Sterna superciliaris* Vieillot, olivaceous cormorant *Phalacrocorax olivaceus* Browning present in the lake). The preference of *J. multidentata* for the emergent plants also by night, when predation risk by birds is absent, suggests this fish species also find a comparatively good availability of resources in this habitat. The differences in physical and chemical factors among sites seemed to play a minor role in the horizontal distribution of fish, especially considering that both fish species, *J. multidentata* and *C. decemmaculatus*, have a broad tolerance to different and sometimes extreme environmental conditions (Ortubay et al., 2002).The habitat selection of fish and shrimps seemed to be mutually dependent, as we observed a clear spatial segregation between fish and shrimps. Contrary to earlier findings (Collins 1999), there is no strong evidence that the shrimps were important predators on zooplankton in this lake (G. Goyenola et al. unpublished).

Final remarks

Studies on the behavioural response of preys to combinations of predators are still scarce (Relyea 2003). Based on experimental studies, Van de Meutter *et al.* (2005) proposed that the effects of particular predators on the spatial distribution of a prey (*Daphnia* in that case) can vary strongly in strength and direction, depending on the presence and activity of more than one predator. These authors also suggested that interactions and facilitation among predators can be relevant, and can change with time and environmental characteristics, as

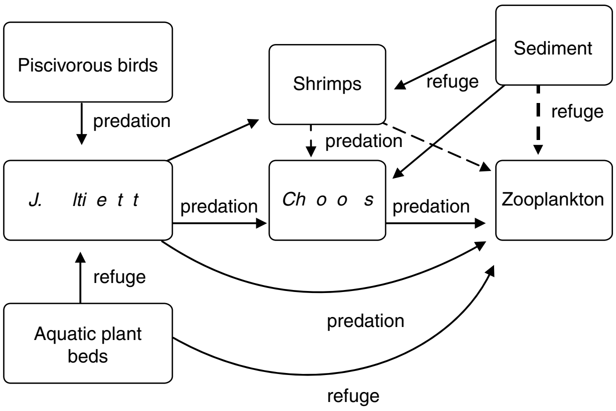

Figure 4. Theoretical model of the main interactions (predation and refuge) among biotic and abiotic components that can influence the spatial distribution of zooplankton and nekton in Lake Blanca. Complete lines correspond to findings of this study while dotted lines to literature data.

we found in Lake Blanca. According to our field evidence, the horizontal distribution of zooplankton was apparently conditioned by complex interactions with the potential predators and the architecture and spatial distribution of the aquatic plants, and indirectly probably also by the activity of piscivorous birds. We propose a conceptual model of the main interactions (predation *versus* refuge) in this lake, to be tested in experiments or under other field conditions (Fig. 4). We suggest that aquatic plants in subtropical shallow lakes offer refuge for zooplankton only at intermediate fish and invertebrate predator densities, and that refuge-mediated positive effects on water transparency therefore might be weak or rare.

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Paper VII

CHAPTER

11

Lake Restoration and Biomanipulation in Temperate Lakes: Relevance for Subtropical and Tropical Lakes

Erik Jeppesen[1,2*], Martin Søndergaard[1], Nestor Mazzeo[3], Mariana Meerhoff[1], Christina C. Branco[4], Vera Huszar[5] and Flavio Scasso[3]

[1]National Environmental Research Institute, Dept. of Freshwater Ecology, Vejlsøvej 25, DK-8600 Silkeborg, Denmark

[2]Dept. of Botanical Ecology, University of Aarhus, Nordlandsvej 68, DK-8230 Risskov, Denmark

[3]Sección Limnología, Departamento de Ecología, Facultad de Ciencias, Iguá 4225, Montevideo, Uruguay

[4]Universidade do Rio de Janeiro, Depto. Ciências Naturais, Av. Pasteur 458, 22290-240 Rio de Janeiro, Brazil

[5]Universidade Federal do Rio de Janeiro, Museu Nacional, Departamento de Botânica, Laboratório de Ficologia. Quinta da Boa Vista, São Cristóvão, Rio de Janeiro, Brazil, 20940-040

ABSTRACT

This chapter gives a broad survey of the responses of temperate lakes to increased nutrient loading (the eutrophication process) and during remediation. Chemically and biologically conditioned resistance may cause a delayed response to the nutrient loading reduction and different methods of overcoming this resistance have been briefly described. Biological restoration methods (termed biomanipulation)

are promising new tools. However, a significant difference in biological interactions in temperate versus subtropical—tropical lakes renders it difficult to directly apply the biological restoration methods. These include often higher dominance and abundance of small fish, higher aggregation of fish in vegetation, higher number of fish cohorts per year, higher degree of omnivorous feeding by fish and less piscivory in subtropical and tropical lakes than in temperate lakes. Unfortunately, very little is known about the trophic dynamics and the role of fishes in warm lakes. Since many subtropical and tropical lakes are heavily eutrophicated, today there is a great need for gaining new knowledge of trophic interactions and possible lake restoration methods for these regions. This is amplified by the fact that the eutrophication problem is expected to increase in the near future due to both the economical development and global warming.

EUTROPHICATION OF LAKES

During the past centuries, increased urbanization and sewage disposal, regulation of wetlands and streams and more intensive farming practices have increased the nutrient loading to many shallow lakes worldwide, not least in the industrialized part of the world. This has resulted in major changes in the biological structure and dynamics of the lakes and often in a noticeable shift from a clear to a turbid state.

A typical succession from temperate lakes in Northern Europe is detailed in Fig. 11.1. At the top of the food web, major changes have occurred in the fish community. At low nutrient concentrations, predatory perch (*Perca fluviatilis*) and pike (*Esox lucius*) dominate the fish community. When the nutrient loading increases, the biomass of fish increases as well. However, a shift occurs to dominance of cyprinids, especially roach (*Rutilus rutilus*) and bream (*Abramis brama*). Roach and bream are partly zooplanktivorous and the predation pressure on zooplankton, therefore, increases, which, in turn, results in a lower grazing pressure on phytoplankton. Changes in the size structure of zooplanktivorous fish towards dominance of small specimens further enhance the predation pressure on zooplankton. In addition, due to competition, perch rarely reach the size of piscivory and, therefore, mainly predate on zooplankton and benthic invertebrates instead. Primarily due to the high fish predation pressure, the biomass ratio of zooplankton to phytoplankton decreases from 0.5-0.8 in mesotrophic lakes to less than 0.2 when phosphorus concentrations are above 0.1-0.15 mg P l^{-1}, the latter figure being so low that zooplankton are incapable of

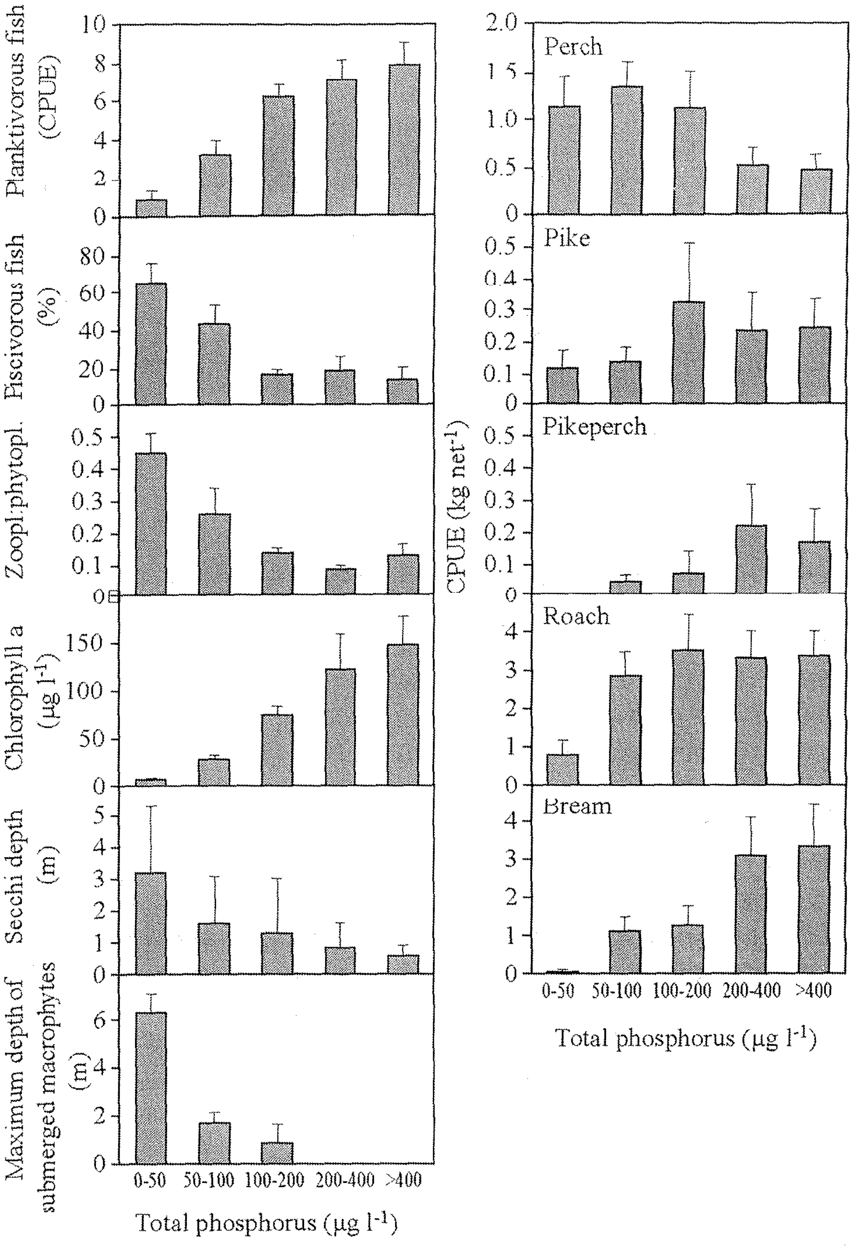

Fig. 11.1 (Left) August biomass of zooplanktivorous fish (measured as CPUE; catch in multiple mesh size gill nets, 14 different mesh sizes 6.25-75 mm) versus summer mean lake water total phosphorus (TP). Also shown are the percentage of piscivorous fish, summer mean (1 May-1 October) of zooplankton : phytoplankton biomass ratio, chlorophyll *a*, Secchi depth and the maximum depth of submerged macrophytes versus the lake water TP-concentration. Mean ± SD of the five TP groups is shown. From Jeppesen et al. (1999). (Right) Biomass (CPUE) of various quantitatively important fish species in Danish lakes versus summer mean TP. The first three species are piscivorous, while the last two are plankti-benthivorous. Modified from Jeppesen et al. (2000)

controlling the phytoplankton, whose turnover time in eutrophic lakes may be 0.5-2 days. With a decreasing grazing pressure by zooplankton and an increased nutrient supply, the biomass of phytoplankton increases, resulting in reduced Secchi depth. In addition, the zooplankton become dominated by small forms, which are less efficient grazers on the large phytoplankton. Large-sized phytoplankton forms, like filamentous cyanobacteria, therefore thrive in many nutrient-enriched lakes. An increase in fish predation may also reduce snail abundance and thus grazing of epiphytes on plants, which also impoverishes the growth conditions for submerged macrophytes. The plants disappear and with it the food source for a large number of herbivorous and macroinvertebrate-eating waterfowl. The result is a lake with a high roach and bream biomass, high abundance of phytoplankton, few or no submerged macrophytes and a greatly reduced density of birds dominated by fish-eating species (Fig. 11.2).

Lessons to be Learnt from Restoration Projects in Temperate Lakes

In recent decades, large efforts have been devoted to combat eutrophication by reducing the external loading of phosphorus in many Western European countries and in North America and, in consequence, loading from sewage and industry sources has declined significantly since the 1970s.

Several lakes respond rapidly and positively to loading reductions; nuisance algal blooming and plankti-benthivorous fish abundance decrease, while the percentage of piscivores increases as do water clarity and submerged macrophyte abundance. A study of 14 recovering lakes in Denmark (Jeppesen et al., 2002) showed that the phytoplankton and fish biomass generally declined, leading to an overall higher zooplankton:phytoplankton ratio, which suggests that enhanced grazing pressure on phytoplankton results in clearer water. Moreover, the biomass of planktivorous fish declined and the share of potential piscivores increased, most likely resulting in a stronger control by piscivores of planktivores. Accordingly, in most lakes, the share of the large-bodied zooplankton *Daphnia* spp., which is particularly sensitive to predation by zooplanktivorous fish, and the body weight of *Daphnia* spp. and other cladocerans generally increased, further enhancing the grazer control of phytoplankton. The improvements in lakewater clarity are, therefore, a result of higher resource control of phytoplankton (lower availability of nutrients) as well as of enhanced predator control of planktivorous fish, cascading to the phytoplankton level via increased grazer control by large-bodied zooplankton. Several other lakes have

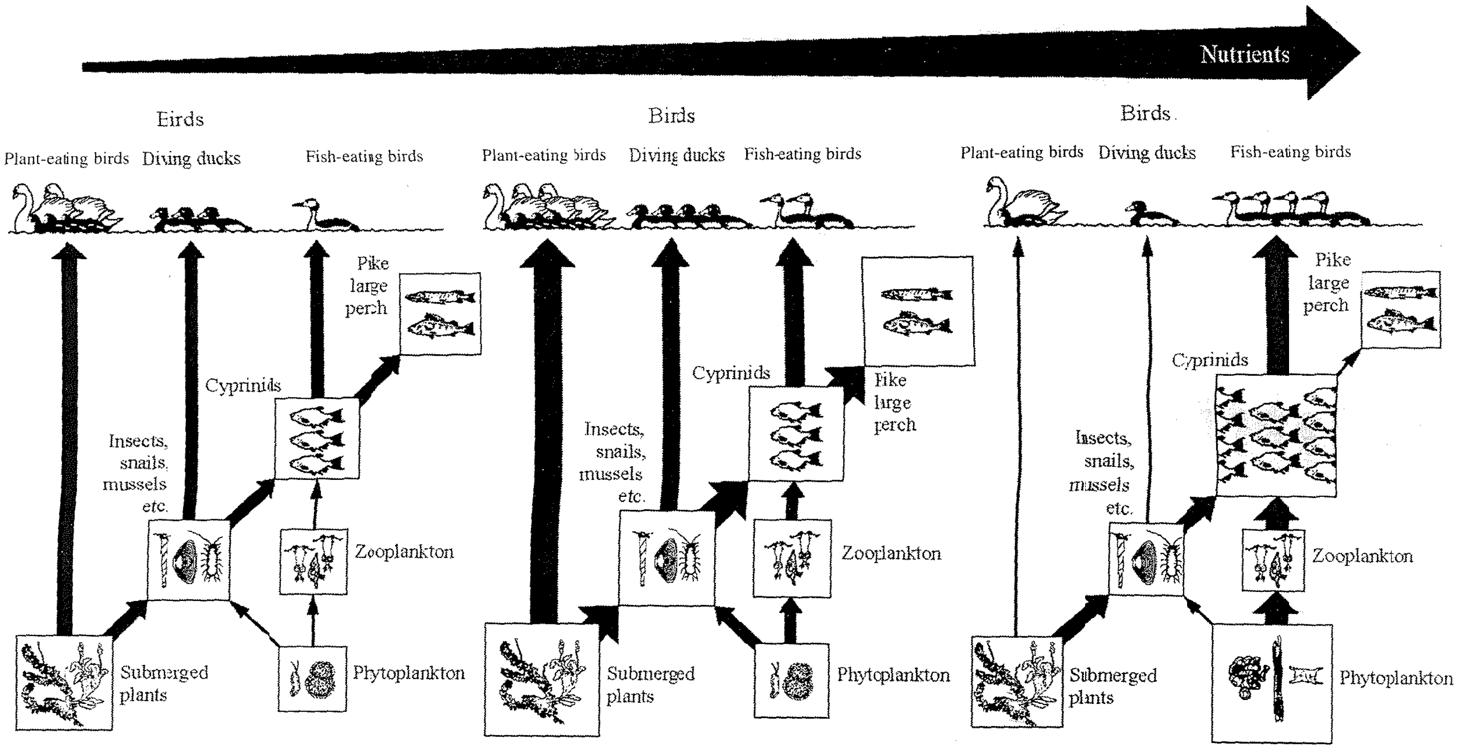

Fig. 11.2 Picture showing how the trophic structure changes along a gradient in nutrient loading in shallow temperate lakes. Low-nutrient lakes are clear with submerged vegetation and a well-balanced pelagic food web. With increasing loading, the lakes first remain clear with higher abundance and production at all trophic levels and a high diversity of waterfowl. At a certain threshold, a shift occurs to a turbid state, with high abundance of zooplanktivorous cyprinids, few piscivores, few zooplankton, high phytoplankton biomass (often with blooms of cyanobacteria in summer), few submerged plants and few waterfowl dominated by piscivores (from Jeppesen et al., 1999, modified from Andersson et al. 1990)

exhibited a swift response to nutrient loading reduction (Edmondson & Lehman, 1981; Bernhardt et al., 1985; Bäuerle & Gaedke, 1998).

However, positive effects cannot always be expected to occur, as many lakes have proven to be highly resistant to loading reductions and have exhibited only little improvement (Sas, 1989, Marsden, 1989). For some lakes, this reflects the insufficient reduction of the nutrient input to trigger a shift to the clear water state. For example, significant and sustaining changes in the biological community and water transparency of shallow temperate freshwater lakes cannot be expected to appear unless the TP concentration has been reduced to a level below 0.05-0.1 mg P l^{-1} (Jeppesen et al., 2000), or for deep lakes below 0.02-0.03 mg P l^{-1} (Sas, 1989). Even when the P loading has been sufficiently reduced, often resistance to improvement is observed. This resistance may be "chemical": P concentrations remain high because of P release from the sediment pool accumulated when loading was high (Søndergaard et al., 2002). Many years may pass before the surplus pool is either released or permanently buried. The duration of this transitional period depends on, for instance, the duration of the period with high TP loading, the residence time and the phosphorus-binding sites (like iron) supplied from the surroundings. In some cases, this transient phase towards a new equilibrium has spanned several decades (Søndergaard et al., 2001; Jeppesen et al., 2003). Various methods have been used to reduce the internal loading of phosphorus (Cooke et al., 1993; Søndergaard et al., 2002), including sediment removal and chemical treatment of the sediment with aluminum or iron salts. In stratified lakes, injections with oxygen or nitrate to the bottom layer or destabilization of the thermocline have also been used.

The resistance may also be "biological". In particular, planktivorous and benthivorous fish contribute to biological resistance in shallow eutrophic lakes (Moss, 1990; Scheffer et al., 1993). A continuously high fish predation pressure prevents both the appearance of large herbivorous zooplankton that would otherwise clear the water as well as diminishes the number of benthic animals stabilizing and oxidizing the sediment. Moreover, excretion of nutrients to overlaying waters from benthic-feeding fish or fish bioturbation of surface sediment may also play a role (Breukelaar et al., 1994; Persson, 1997).

To overcome the biological resistance by fish, various fish manipulation methods have been developed (Benndorf, 1995; Drenner & Hambright, 1999; Søndergaard et al., 2001). One such method is the enhancement of top-down control of phytoplankton by selective removal of planktivorous fish; a method employed worldwide in the temperate zone. Removal of 75-80% of the planktivorous and benthivorous fish stock during a 1-2 year period is recommended to avoid regrowth and to stimulate the growth of potentially piscivorous perch (Perrow et al., 1997; Hansson et al., 1998; Meijer et al., 1999; Mehner et al., 2002). An alternative or supplementary method to fish removal is ample stocking of 0^+ pike (> 1000 $h.^{-1}$) to control newly hatched plankti-benthivorous roach and bream (Prejs et al., 1994; Berg et al., 1997). Others have used stocking of pike perch (*Stizostedion lucioperca*), walleye (*Stizostedion vitreum*) and largemouth bass (*Micropterus salmoides*) (Benndorf, 1995; Lathrop et al., 2002). Opposite to the above-mentioned physico-chemical methods, fish manipulation is often cheap (Jeppesen & Sammalkorpi, 2002) and therefore attractive, though its long-term stability is uncertain. The findings to date indicate that fish manipulation may have a long-term effect in shallow temperate lakes provided that the nutrient loading is reduced to a TP level below 0.05-0.1 mg P l^{-1} in the future state of equilibrium. However, if the nitrogen loading is low, fish manipulation may sometimes have a positive impact at higher TP concentrations (Moss et al., 1994; Jeppesen et al., 1999). The 0.05-0.1 mg P l^{-1} threshold is in accordance with the empirical data appearing in this range (Fig. 11.1). However, temporary effects of fish manipulation can be obtained in lakes with high nutrient concentrations, but it seems unlikely that the effect will prevail in such lakes in the long term unless the abundance of planktivorous fish is repeatedly reduced. The TP threshold for positive effects to occur is most likely lower for deep lakes, but an accurate threshold has to be defined.

CAN THE KNOWLEDGE OF BIOMANIPULATION BE TRANSFERRED TO SUBTROPICAL AND TROPICAL LAKES?

Fish Community

Several factors indicate that fish stock manipulations would not have the same positive effect on the environmental state in tropical lakes as in temperate lakes. First, the richness in fish species is often higher in both tropical and subtropical lakes. South America and Africa have an extraordinarily rich freshwater fish fauna (Sunaga & Verani, 1997; Lévêque & Paugy, 1999). For example, 273 native fish species were found in aquatic environments in 44 × 10^3 km^2 Rio de Janeiro State (Bizerril & Primo, 2001), and in Uruguay (171 × 10^3 km^2), for instance, 51 characid species are found (Reichert, 2002). Many of the fish species show partial niche overlap, which expectedly increases the predator control of prey items (Lazzaro, 1997; Aguiaro & Caramaschi, 1998).

Second, the fish stock in tropical and subtropical lakes is often dominated by omnivorous species that feed on zooplankton but also consume phytoplankton, periphyton, benthic invertebrates, and detritus (species of clupeids, cyprinids, cichlids and poecilids) (Lazzaro, 1997; Branco et al., 1997; Yafe et al., 2002; Aguiaro et al., 2003; Quintans et al., submitted). The subtropical and tropical fish stock that have the potential of feeding on zooplankton may thus attain a higher carrying capacity than obligate zooplanktivores, which augments the potential control of the zooplankton. According to Blanco et al. (2003), the omnivorous structure of fish communities in Mediterranean lakes resembles that described for tropical lakes. Few piscivorous species are present and omnivorous fish generally dominate independently of trophic state. However, the latest research into temperate lakes has shown that many of the important zooplanktivorous fish also consume benthic prey, not least in shallow lakes (Vadeboncour et al., 2002; Jones & Waldron, 2003). Therefore, to a higher degree than hitherto anticipated, also temperate fish may sustain higher biomasses and thus exert a benthic-facilitated top-down control on zooplankton (Vander Zanden & Vadeboncouer, 2002).

Third, compared to North American and European temperate freshwater fish communities, only few large strictly piscivores and small-sized carnivores occur and sit-and-wait predators are often more frequent (Crisman & Beaver, 1990; Quirós, 1998). In addition, some of the omnivorous-planktivorous species can neither be controlled by zooplankton availability (resources) nor by predation, since many are larger than their potential predators (Arcifa et al., 1995; Araújo Lima et al., 1995). Therefore, top-down control by piscivores is most likely weaker in subtropical/tropical lakes than in temperate lakes.

Fourth, recent studies suggest that fish density, but not necessarily biomass, is substantially higher (maybe 1-2 orders of magnitude) in subtropical and tropical lakes in South America than in comparable north temperate lakes (Scasso et al., 2001; Aguiaro et al., 2003; Meerhoff et al., 2003; Mazzeo et al., 2003). Among those are *Cnesterodon decemmaculatus* and *Jenynsia multidentata*, both small omnivorous-planktivorous species that may be very abundant in eutrophic lakes in, for example, Uruguay and North Argentina (Bistoni, 1999). They can account for more than 95% of the fish biomass in a lake, with densities of 97000 and 130000 ind. ha^{-1}, respectively (Scasso et al., 2001; Mazzeo et al., 2003). Moreover, fish reproduction, which in temperate freshwater lakes takes place once a year, occurs throughout the year in many subtropical and tropical lakes (Fernando, 1994; Paugy & Lévêque, 1999) and some species such as *C. decemmaculatus* are viviparous. As small fishes are more zooplanktivorous and have a much higher energy demand per unit of biomass than large fish (Kalff, 2002), the dominance of small fish in such high abundances leads to a higher predation pressure on zooplankton than in temperate lakes, where the effect of juvenile fish is typically strong mainly in mid-late summer (Jeppesen et al., 1997a). Nutrient-rich temperate brackish lakes in Denmark provide an illustrative example of the impact of a prolonged spawning period and dominance of small fish. The fish stock of these lakes is dominated by the small three-spined stickleback (*Gasterosteus aculeatus*) having 2-3 cohorts per year. Accordingly, the predation pressure on zooplankton is substantially higher in brackish lakes than in comparable freshwater lakes dominated by fish with only one annual spawning (Jeppesen et al., 1994, 1997b).

Zooplankton Community Structure

Most likely due to high predation by fish, the zooplankton communities in tropical and subtropical lakes are frequently dominated by small cladocerans (like *Diaphanosoma, Ceriodaphnia* and *Bosmina*) and rotifers, and by juveniles and small copepodites among the copepods (Crisman & Beaver, 1990; Dumont, 1994; Lewis, 1996; Branco et al., 2002, Garcia et al., 2002). When fish are absent large *Daphnia* spp. may sometimes develop (Mazzeo et al., unpublished data). Omnivorous copepods usually dominate in terms of biomass in oligo-mesothrophic systems, whereas microzooplankton prevail in more eutrophic systems. The high temperatures, the daily fluctuations in physical and chemical conditions or sudden environmental changes due to heavy rains may add to the predominance of fast-recovering forms such as protozoans and rotifers in the zooplankton community. The classic control of phytoplankton by large zooplankton in temperate lakes is therefore not usually found in tropical lakes. Besides, the zooplanktivorous phantom midge, *Chaoborus*, seems to be more abundant in tropical lakes, most likely because tropical lakes develop anoxia in the bottom water more quickly than do temperate lakes, which provides *Chaoborus* with a fish predation refuge for prolonged periods (Lewis, 1996). Invertebrate predation by *Chaoborus* and other invertebrates, such as freshwater shrimps (e.g. *Palaemonetes* spp.), could prevent large herbivorous zooplankton from developing and this explains the success of loricated rotifers, or species capable of rapid escape such as *Hexarthra* spp., as well as the high density of copepod nauplii (Branco et al., 2000), even in the absence of zooplanktivorous fishes (Roche et al., 1993). This predation pressure on zooplankton may represent a further limitation of the usefulness of biomanipulation in tropical and subtropical lakes.

Fish–Zooplankton–Macrophyte Interactions

Aquatic plants play a very important structuring role in most freshwater ecosystems (Scheffer et al., 1993, Thomaz & Bini, 2003). In temperate nutrient-rich lakes, not the least submerged plants often act as daytime refuges for zooplankton against fish predators (Timms & Moss, 1984; Lauridsen et al., 1996; Burks et al., 2002). At night, when the risk of predation is lower, zooplankton migrate to the open water for feeding and thereby, contribute to maintaining clearwater conditions in lakes with high macrophyte coverage (Jeppesen et al., 1997a). However, in the tropics and subtropics, the effects of macrophytes on trophic interactions are more complex, as all life forms (emergent, submerged, floating-leaved and large free-floating species) can be extremely prominent. The few studies conducted so far in the subtropics and tropics indicate that fish, particularly the smallest species and individuals, aggregate in high numbers in the vegetation (Conrow et al., 1990; Meerhoff et al., 2003; Branco et al., unpublished data). Studies of the composition of tropical ichthyofauna associated to macrophytes have shown that the life cycle of some species is completely connected to this biotope, examples being small-sized tetragonopterins, characiforms and some cichlids (Sazima & Zamprogno, 1985; Delariva et al., 1994). Many fish species exhibit spatial distribution patterns often connected with zooplankton and predator densities, and also with different macrophyte life forms (Agostinho et al., 1994; Sunaga & Verani, 1997; Meerhoff et al., 2003). Besides, tropical aquatic vegetation can be intensively colonized by periphyton and provide microhabitats for many invertebrates, including chironomids, trichopterans and molluscs (Callisto et al., 1996; Masifwa et al., 2001).

One might, therefore, expect that the vegetation is a poor refuge for large-bodied zooplankton in warm lakes, which seems to be supported by the few field and experimental studies conducted so far (Meerhoff et al., 2003; Meerhoff, unpublished data). Fish aggregation in the vegetation and lack of zooplankton aggregated among plants have also been observed in brackish lakes dominated by small-sized sticklebacks in Denmark (Jeppesen et al., 1997b) and, accordingly, nutrient-rich brackish lakes are turbid, even when macrophyte coverage is high. Likewise, from subtropical freshwater lakes in Florida, Bachmann et al. (2002) showed no positive effect of plants on water clarity and nutrient-rich lakes with high plant biomass were often turbid. These results may well be of general importance for subtropical and subtropical lakes, although more research is needed within this field. Also, contrasting management approaches can alter the role of macrophytes in lake functioning and trophic interactions. While in temperate systems the introduction and development of aquatic plants are considered a key step in a restoration process (Moss et al., 1996), many aquatic plants in the tropics and subtropics are often considered a nuisance and subject to severe eradication measures.

LIMITATIONS OF BIOMANIPULATION IN THE SUBTROPICS AND TROPICS

As can be deduced from above, it seems more difficult to provoke and not least maintain a trophic cascade effect in subtropical and tropical lakes than in temperate lakes, for which the concept of biomanipulation as a restoration tool was developed. Supporting this view, Nagdali & Gupta (2002) found positive, but only short-term cascading effects of a massive (> 80%) kill (due to fungal infection) of the most abundant planktivorous mosquito fish (*Gambusia affinis*) in Lake Naini Tal, India. Zooplankton abundance increased significantly, phytoplankton biomass and productivity declined as did nutrient concentrations, resulting in higher water transparency. However, only four months later, the abundance of mosquito fish, plankton and nutrients had returned to the level recorded in the previous year (Fig. 11.3). In a study performed by Scasso et al. (2001), a slowly increasing abundance of larger species was observed after two years of biomanipulation involving removal of small planktivorous fish, without, however, inducing cascading effects leading to clear-water conditions.

Yet, only few studies have investigated the applicability of the biomanipulation theory to tropical and subtropical freshwater ecosystems, and most of the existing ones have examined food interactions in eutrophic lakes and reservoirs with the aim to control cyanobacterial blooms via enhanced grazing by omnivorous fish such as silver carp (Arcifa et al., 1986; Northcote et al., 1990; Starling, 1993; Jones & Poplawski, 1998; Saha & Jana, 1998). The results obtained indicate that omnivory and strong shifts in fish diet and in the fish and zooplankton composition jeopardize successful biomanipulation (Boon et al., 1994; Arcifa et al., 1995; Starling & Lazarro, 1997; Starling et al., 1998; Boulton & Brock, 2001), but more information on phytoplankton-zooplankton interactions in the tropics is needed before its potential can be fully elucidated. Moreover, the absence of a native piscivorous fish culture in many tropical countries precludes the application of biomanipulation. There is a huge richness of fish species potentially useful for this purpose, but mass production for biomanipulation purposes has not yet been considered. Generally, aquaculture has so far focused on exotic species (i.e. common carp and grass carp) that have negative effects on the water quality and biodiversity.

In many of the experiments undertaken in the temperate zone, an improvement in environmental state has, however, been recorded without the occurrence of a trophic cascade, i.e. without changes in the zooplankton species composition towards higher dominance of large-sized individuals and with it a higher grazing pressure on phytoplankton

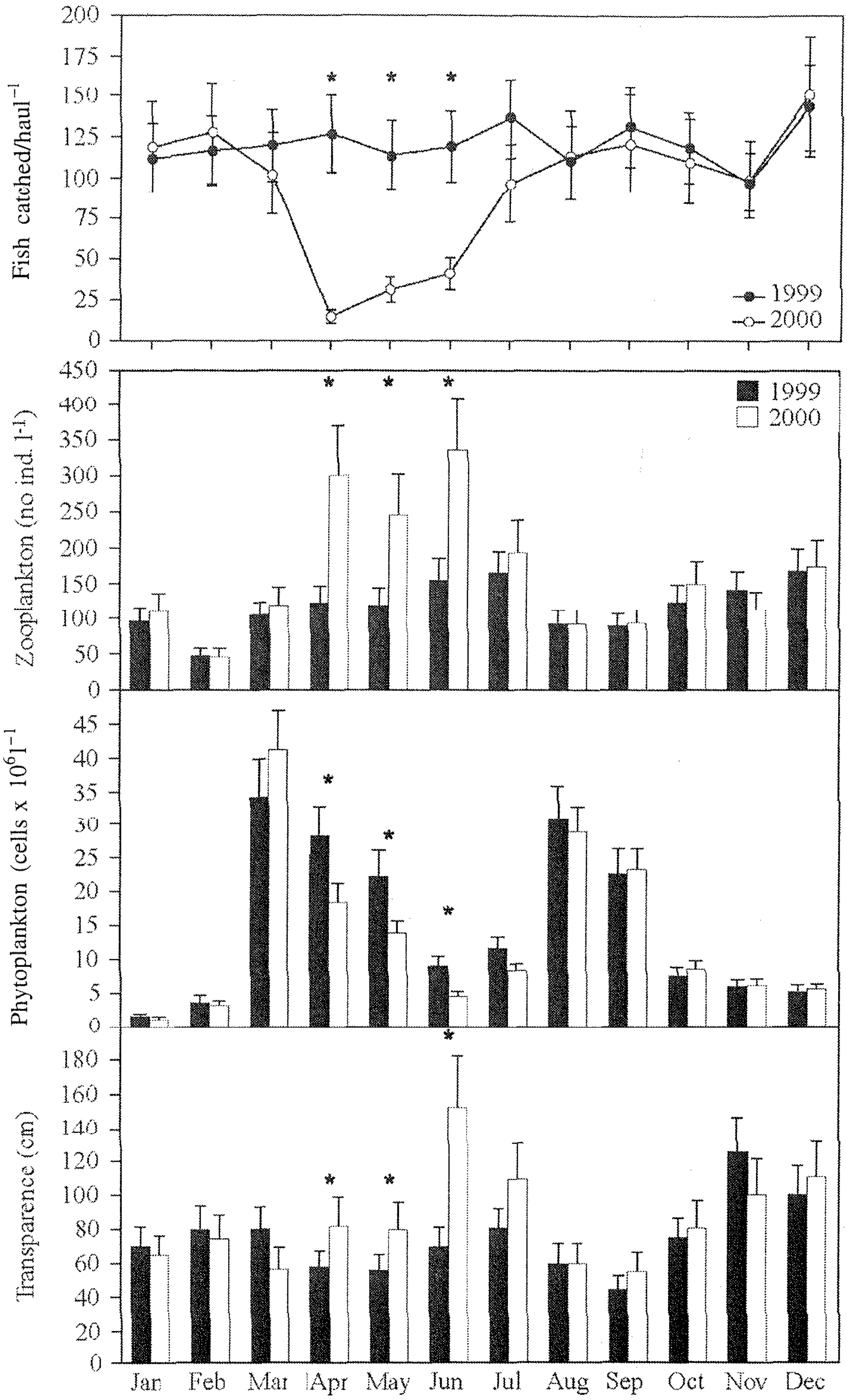

Fig. 11.3 Seasonal variation in fish catch, zooplankton, phytoplankton and Secchi depth in Lake Naini Tal, India, before (1999) and after a major fish kill in April 2000. Note that the fish stock recovered four months after the fish kill and that a short-term cascading effect on zooplankton, phytoplankton and transparency was recorded. Stars indicate significant differences (modified from Nagdali & Gupta, 2002)

(Horppila et al., 1998). Even without such a trophic cascade, a significant reduction has been observed in the occurrence of cyanobacteria, total phosphorus has declined and water clarity increased. These phenomena have been ascribed to reduced release of phosphorus from the sediment, not least due to the lower rate of fish foraging in the sediment following biomanipulation (Horppila et al., 1998). Biomanipulation may, therefore, potentially also reduce the nutrient release from the sediment in tropical lakes, but the dominance of small fish species and the improved growth conditions for cyanobacteria suggest that the effect will not be long lasting. Therefore, a drastic reduction of the external nutrient loading seems to be the best way forward for restoring lakes also in the tropics, but clearly the scientific basis (e.g. nutrient threshold levels) on which to make decisions is yet too limited.

STRONG NEED FOR MORE RESEARCH

As the situation is today, many subtropical and tropical lakes are heavily eutrophied and biodiversity has declined, and in the years to come, it is to be feared that many other lakes in these regions, especially in coastal areas, will follow the same negative developmental pattern both as a consequence of the future economical development and climate change. According to Vitousek et al. (1997), around 60% of the world's population is concentrated in the outer 100 km coastal zone, and disordered urban expansion and agricultural activities have heavily impacted aquatic ecosystems in the developing countries located in tropical and subtropical regions. These already disturbed environments will expectedly face more stressful challenges as a result of increasing human activities and changing meteorological events associated to global warming.

One may hope that the developing countries will learn from the mistakes made in Europe and North America and adopt a farsighted policy and take actions to avoid the eutrophication of present-day high quality lakes and thus the need for initiating expensive restoration projects in the future. However, whether they do so or not, more knowledge is needed to initiate suitable restoration projects of already eutrophied lakes in these regions. This book is an interesting step on the long road to the procurement of such knowledge.

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Restoration of shallow lakes by nutrient control and biomanipulation – the successful strategy varies with lake size and climate

Jeppesen, E.[1,2*], Meerhoff, M. [1,2,3], Jacobsen B. A.[4], Hansen R. S.[4], Søndergaard, M.[1], Jensen, J. P.[1], Lauridsen, T. L.[1], Mazzeo, N.[3] & Branco, C. W. C. [5]

[1] Department of Freshwater Ecology, National Environmental Research Institute, Vejlsøvej 25, DK-8600 Silkeborg, Denmark
[2] Department of Plant Biology, University of Aarhus, Ole Worms Allé 135, DK-8000 Aarhus, Denmark
[3] Sección Limnología, Departamento de Ecología, Facultad de Ciencias, Iguá 4225, Montevideo, Uruguay
[4] Frederiksborg Amt, Technical Department, Amtsgården, Kongens Vænge 2, 3400 Hillerød, Denmark
[5] Departamento de Ciências Naturais, Universidade Federal do Estado do Rio de Janeiro, Av. Pasteur 458, Urca, Rio de Janeiro, Brazil, 22290-040

*Author for correspondence: E-mail: ej@dmu.dk

Key words: lake restoration, large lakes, recovery, nutrient loading reduction, climate

Abstract

Major efforts have been made world-wide to improve the ecological quality of shallow lakes by reducing external nutrient loading. These have often resulted in lower in-lake total phosphorus (TP) and decreased chlorophyll *a* levels in surface water, reduced phytoplankton biomass and higher Secchi depth. Internal loading delays recovery, but in north temperate lakes a new equilibrium with respect to TP often is reached after <10-15 years. In comparison, the response time to reduced nitrogen (N) loading is typically <5 years. Also increased top-down control may be important. Fish biomass often declines, and the percentage of piscivores, the zooplankton:phytoplankton biomass ratio, the contribution of *Daphnia* to zooplankton biomass and the cladoceran size all tend to increase. This holds for both small and relatively large lakes, for example, the largest lake in Denmark (40 km^2), shallow Lake Arresø, has responded relatively rapidly to a *ca*. 76% loading reduction arising from nutrient reduction and top-down control.

Some lakes, however, have proven resistant to loading reductions. To accelerate recovery several physico-chemical and biological restoration methods have been developed for north temperate lakes and used with varying degrees of success. Biological measures, such as selective removal of planktivorous fish, stocking of piscivorous fish and implantation or protection of submerged plants, often are cheap versus traditional physico-chemical methods and are therefore attractive. However, their long-term effectiveness is uncertain. It is argued that additional measures beyond loading reduction are less cost-efficient and often not needed in very large lakes.

Although fewer data are available on tropical lakes these seem to respond to external loading reductions, an example being Lake Paranoá, Brazil (38 km^2). However, differences in biological interactions between cold temperate versus warm temperate-subtropical-tropical lakes make transfer of existing biological restoration methods to warm lakes difficult. Warm lakes often have prolonged growth seasons with a higher risk of long-lasting algal blooms and dense floating plant communities, smaller fish, higher aggregation of fish in vegetation (leading to loss of zooplankton refuge), more annual fish cohorts, more omnivorous feeding by fish and less specialist piscivory. The trophic structures of warm lakes vary markedly, depending on precipitation, continental or coastal regions locations, lake age and temperature. Unfortunately, little is known about trophic dynamics and the role of fish in warm lakes. Since many warm lakes suffer from eutrophication, new insights are needed into trophic interactions and potential lake restoration methods, especially since eutrophication is expected to increase in the future owing to economic development and global warming.

Introduction

Efforts in the last 20–30 years have attempted to combat eutrophication by reducing external phosphorus (P) loading in Western Europe and North America. Sewage and industrial loading has declined, whereas nutrient input from diffuse sources often remains high, particularly in countries with intensive agriculture (Van der Molen & Portielje, 1999; Kronvang et al., 2005).

Many lakes have responded positively to nutrient loading reductions (Sas, 1989; Marsden, 1989; Jeppesen et al., 2005c). An analysis of European and North American lakes based on 35 case studies, including long time-series data, revealed that, although internal P loading does delay recovery, new equilibria with respect to TP were reached in most lakes after <10–15 years, marginally dependent on lake retention time and depth (Jeppesen et al., 2005c). In comparison, response times to reduced N loading are typically <5 years. These findings also apply to shallow Danish lakes after a reduction in mainly external P loading (Søndergaard et al., 2002a, 2005; Jeppesen et al., 2002, 2005a,b). In these lakes, TP has declined during all seasons, particularly in winter, followed by spring and autumn, while weaker effects in late summer can be ascribed to internal P loading. Changes in chlorophyll *a* follow TP, indicating that nutrient constraints are important in determining seasonal behaviour. Diatoms declined precipitously in winter and spring. Non-heterocystous cyanobacteria declined throughout the summer, primarily in early summer, while heterocystous cyanobacteria increased in late summer and autumn. Green algae declined throughout the year but least so in mid-summer. In Barton Broad, a shallow lake in the UK, Phillips et al. (2005) observed a similar pattern with strong effects on diatoms in spring and a decline first in non-heterocystous cyanobacteria and secondly, albeit slower, in heterocystous genera after 15–20 years. Thus, there is clear evidence for enhanced resource control of phytoplankton after an external loading reduction.

However, enhanced top-down control by zooplankton may also play a role. Fish biomass often declined in the Danish lakes and the percentage of piscivores often increased. Similar observations were made in the study of 35 North American and European lakes in recovery (Jeppesen et al., 2005c); in 82% of the lakes for which data were available, fish biomass declined with TP, while the piscivore proportion increased in 80% of the lakes. This is surprising given the high longevity of the dominant species. While there were no changes in zooplankton biomass, the contribution of *Daphnia* to the biomass and mean individual biomass of cladocerans increased most notably during summer. Moreover, the zooplankton:phytoplankton biomass ratio, and probably phytoplankton grazing, rose. This is suggested also by the cladoceran size increase. Higher abundance of large-bodied zooplankton, particularly in summer, may reflect that increased piscivory leads to stronger predation control of young-of-the year (YOY), primarily planktivorous, fish. This reduces predation on zooplankton during summer, when YOY fish are abundant and forage in the pelagic zone. Enhanced abundance of submerged macrophytes or filamentous algae in some lakes may also enhance survival of large-bodied zooplankton as they can serve as a daytime refuge from predation (Burks et al., 2002).

A north temperate example: recovery of Lake Arresø, Denmark

The positive response to external nutrient loading reduction is not restricted to small lakes. Here we elaborate in more detail on the response of Arresø to nutrient loading reduction. Arresø is the largest lake in Denmark (40 km^2). It is shallow (mean depth 3.1 m, max depth 5.9 m) and has a 215 km^2 catchment area. The retention time is long (1.3 to 10.3 y, mean 1989–2003: 3.5 y) compared with most Danish shallow lakes. The lake is near the coast and exposed to prevailing north-westerly winds and, thus, wave-induced resuspension (Kristensen et al., 1992). Based on intensive sampling (every 2–8 hours) during different seasons in 1991, and modelling, resuspension was calculated to occur 50% of the time and to reduce Secchi depth to <0.5 m on average (Kristensen et al., 1992). The lake was loaded heavily with P and N from the catchment, particularly P from the upstream city of Hillerød. TP loading decreased from 0.68 g P m^{-2} y^{-1} in 1989 to 0.16 g P m^{-2} y^{-1} for 1996–2003, and TN decreased from 13 to 11 g N m^{-2} y^{-1} (Jacobsen et al., 2004). The reduction mostly was due to improved sewage treatment, establishment of artificial lakes on the main inlet stream, and possibly to reduced fertilization in the catchment. Nutrient load reduction led to substantial improvements in the lake. TN, TP, chlorophyll *a* and the total biomass (and especially of green algae) of phytoplankton have declined markedly (Fig. 1). Diatom biomass has tended to increase with associated effects on silicate concentration, whereas the trend for cyanobacteria is ambiguous. Algal biomass changes cannot be ascribed entirely to enhanced nutrient control, since there have been marked zooplankton community changes and potential grazing, most likely due to fish community changes. Biomasses of the dominant plankti-benthivorous bream (*Abramis brama*) (Fig. 2) and benthic ruffe (*Gymnocephalus cernua*) are decreasing, whereas zander (*Sander lucioperca*), a potential predator, is increasing, implying enhanced top-down control on prey fish. Accordingly, zooplankton biomass and the share of large-sized cladocerans have increased substantially. This can be explained by reduced predation pressure on zooplankton, since the algal biomass was dominated by edible

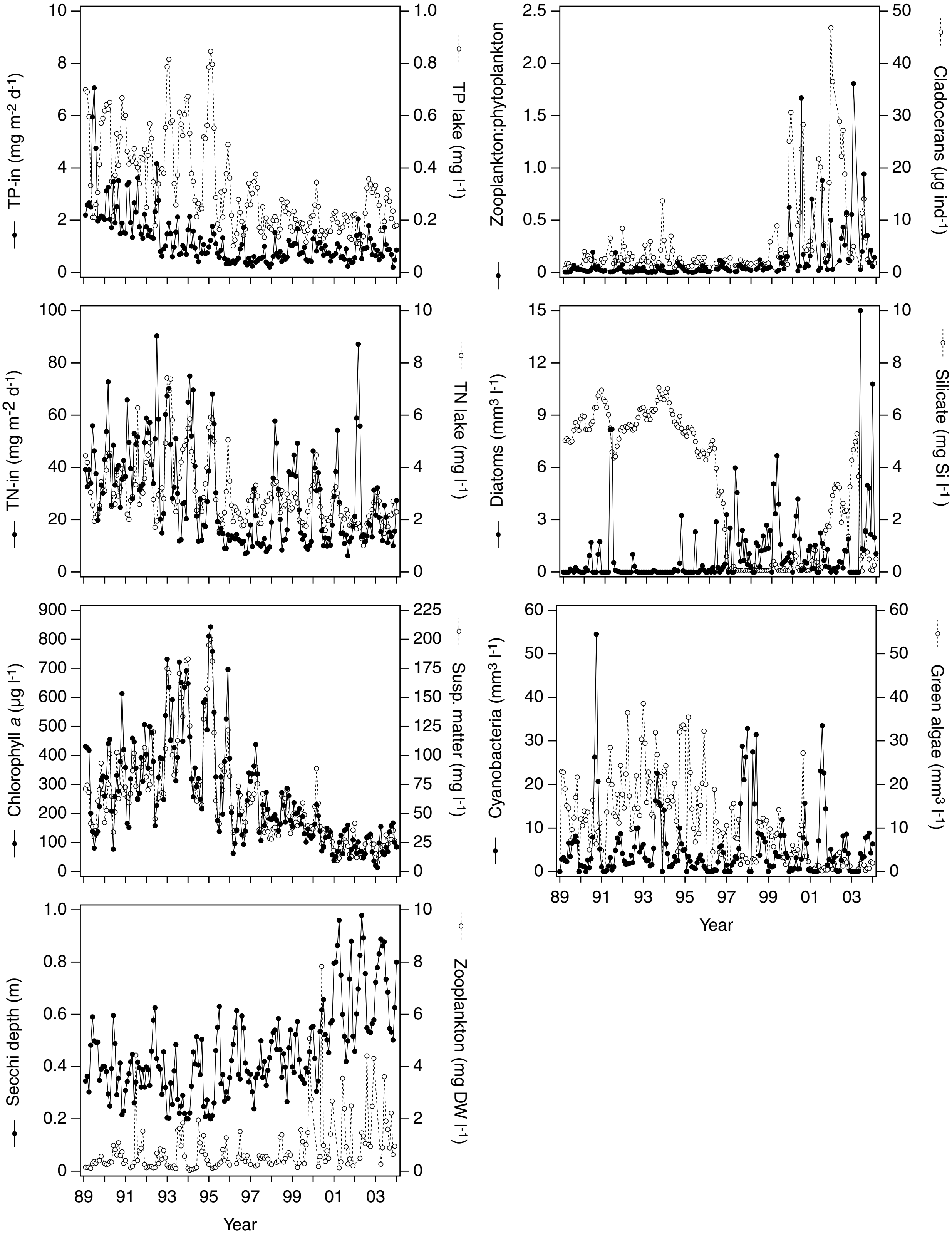

Figure 1: Monthly mean data on various environmental variables in Arresø. TP_{in} and TN_{in} are the external loading of the lake. Zooplankton:phytoplankton is the biomass (mg DW) ratio.

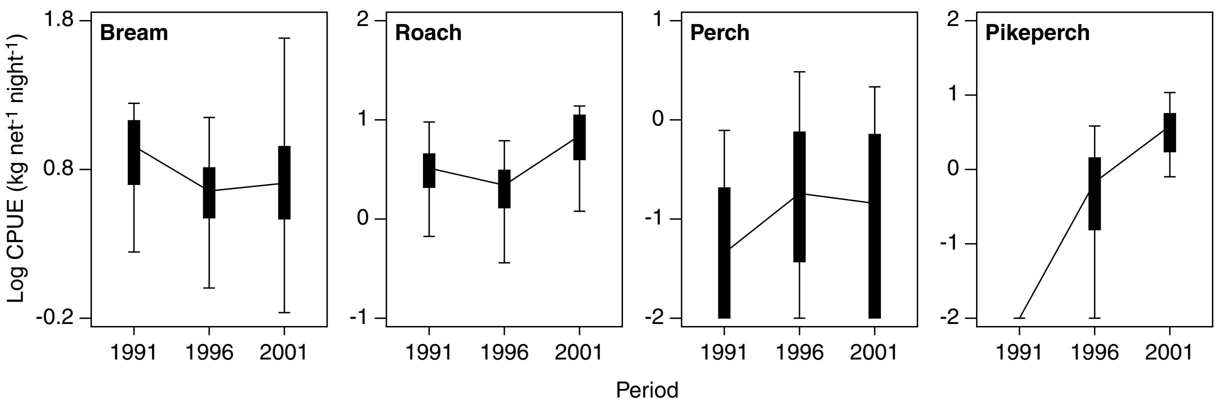

Figure 2: Box plot (10, 25, 75, 90% percentiles) of log (base e) transformed catch per net per night in Arresø using 52 nets distributed in the lake according to Jeppesen et al. (2004).

green algae when biomass was low and the zooplankton were dominated by small forms. With increased zooplankton biomass the grazing pressure on phytoplankton has intensified, and the phytoplankton face simultaneous, enhanced resource control due to lower P and N concentrations. Grazing control is strengthened by the shift to large-sized cladocerans *Daphnia hyalina* and *D. galeata* (Fig. 1), which have broader feeding capabilities than previously dominant *Bosmina* spp and *Chydorus sphaericus*. Following the increase in zooplankton biomass and cladoceran size, and the zooplankton:phytoplankton ratio, water transparency has improved (Fig. 1), emphasizing the role of enhanced grazer control of phytoplankton.

Seasonal phytoplankton biomass reduction and Secchi depth increases are larger in spring, autumn and winter than in summer (Fig. 3). This also is true for the zooplankton:phytoplankton biomass ratio and cladoceran body mass, suggesting that changes in grazing pressure are important. The minor effect in summer coincides with fish larvae exploiting the pelagic in Danish lakes (Jeppesen et al., 2004). Moreover, during this period, nitrate is low (in all years) (Fig. 3), which likely may have dampened P loading reduction effects.

However, loading still remains too high to promote a clearwater state with macrophytes, which prevailed in the 1900's per paleolimnological analyses (Klein, 1989).

Accelerating the recovery of northern temperate lakes

Positive effects may not occur after nutrient input reduction. Several lakes have been resistant to loading reductions and shown slow recovery (Sas, 1989; Marsden, 1989). For some lakes, this reflects insufficient nutrient input reduction to promote a clearwater state. For example, significant and sustained changes in the biological community and water transparency of shallow, temperate, freshwater lakes may not appear unless TP is reduced below 0.05–0.1 mg P l^{-1} (Jeppesen et al., 2000) or, for deep lakes, 0.02–0.03 mg P l^{-1} (Sas, 1989). Even when P loading has been reduced sufficiently, resistance to improvement often is observed. This resistance may be "chemical", P concentrations remain high due to release from the sediment pool accumulated when loading was high (Søndergaard et al., 2002a). Various methods have been used to reduce internal P loading (Cooke et al., 1993; Søndergaard et al., 2002a,b), including sediment removal and chemical treatment with aluminum or iron salts. In stratified lakes, oxygen or nitrate injections to the bottom layer or destabilization of the thermocline have been used (Cooke, 1993). For large, shallow lakes, it is debatable whether sediment removal and chemical treatment are feasible methods. Sediment removal is expensive, particularly for large lakes due to transport and disposal. Moreover, sediment resuspension and redistribution may hamper removal of the upper, nutrient-rich sediment layers. Resuspension also is a problem with chemical methods. P bound to aluminum or iron may return to the water on windy days and loosely bound P made available to phytoplankton. Finally, the sediment P pool often is small in large lakes because resuspension leads to washout of particulate TP (unless hydraulic retention time is long) and low organic net sedimentation, the latter due to high mineralization. Thus, the usefulness of sediment removal or treatment is more limited in large than small lakes. The fast response of Arresø was attained without sediment removal.

The resistance also may be "biological". Planktivorous and benthivorous fish contribute to biological resistance in shallow eutrophic lakes (Moss, 1990; Scheffer et al., 1993). High fish predation prevents the appearance of large herbivorous zooplankton, which would otherwise clear the water, and diminishes benthic invertebrates, which stabilize and oxidize the sediment. Moreover, nutrient excretion to overlaying waters by benthic-feeding fish or fish bioturbation of surface sediment may play a role (Breukelaar et al., 1994; Pers-

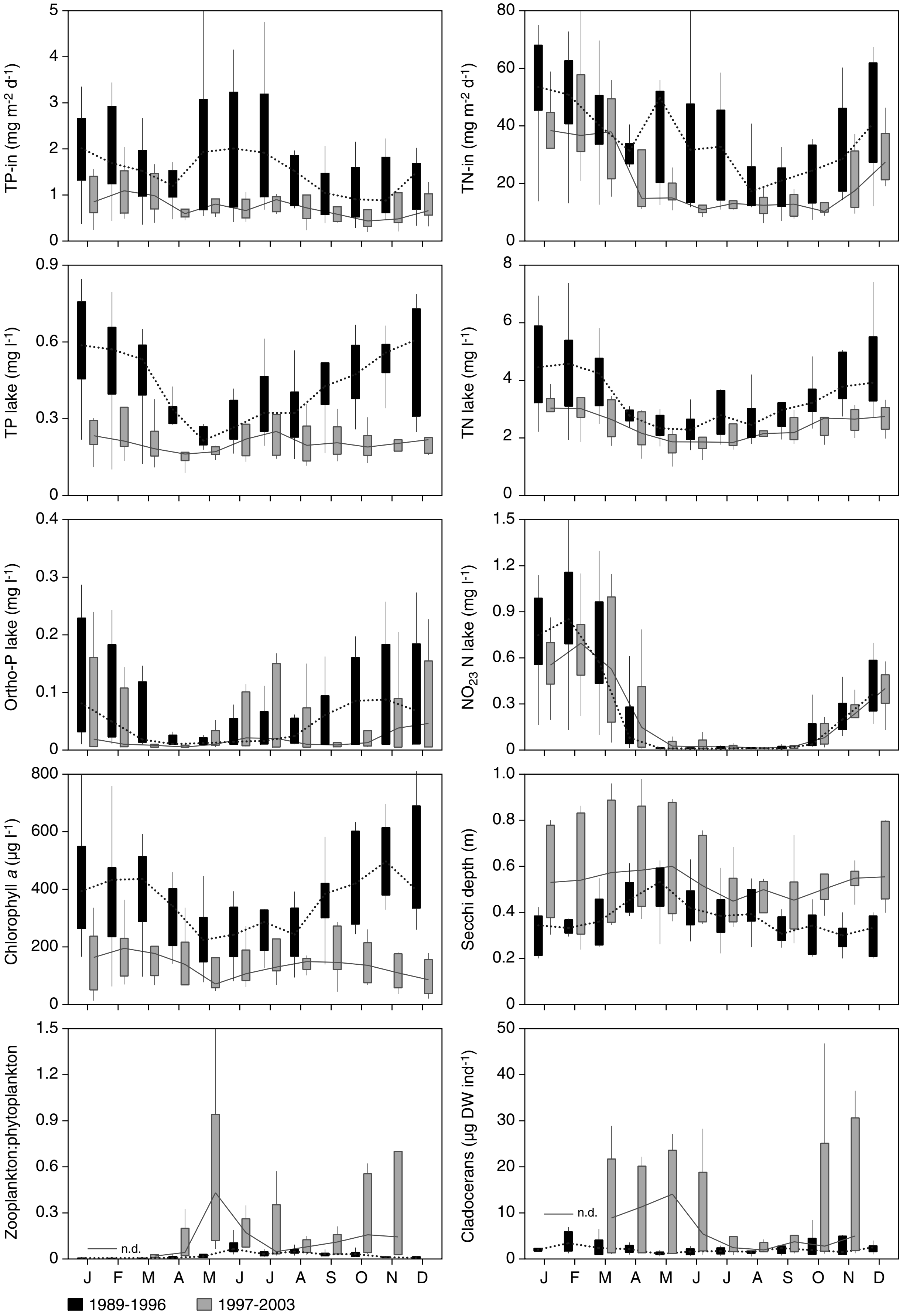

Figure 3: Box plot (10, 25, 75, 90% percentiles) of selected environmental variables in Arresø during the first (1989–1996) and the last part of the study period (1997-2003). n.d. means no data in Jan-Feb for 1997-2003. See also legend of Fig. 1.

son, 1997). To overcome biological resistance, various fish manipulation methods have been developed (Benndorf, 1995; Drenner & Hambright, 1999; Søndergaard et al., 2001). One method is enhancement of top-down control of phytoplankton by selective removal of planktivorous fish; a method employed extensively in the temperate zone, but only just beginning in the subtropics (Scasso et al., 2001) and other warmer climate zones, such as Mediterranean arid lakes (Beklioglu & Tan, personal communication). Removal of 75–80% of the planktivorous and benthivorous fish stock over 1–2 years is recommended to avoid regrowth and stimulate growth of potentially piscivorous perch (Perrow et al., 1997; Hansson et al., 1998; Meijer et al., 1999). An alternative or supplementary method to fish removal is stocking of 0^+ pike to control newly hatched plankti-benthivorous roach and bream (Prejs et al., 1994; Berg et al., 1997, Skov & Berg, 1999) though the results are ambiguous (C. Skov et al., unpublished data). Others have stocked pikeperch, walleye (*Stizostedion vitreum*) and largemouth bass (*Micropterus salmoides*) (Benndorf, 1995; Lathrop et al., 2002). In contrast to chemical engineering methods, fish manipulation often is cheap (Jeppesen & Sammalkorpi, 2002) and therefore attractive, though its long-term stability is uncertain. Findings to date indicate that fish manipulation may have a long-term effect in shallow temperate lake if the TP loading is reduced to a level that leads to a lake concentration below 0.05–0.1 mg P l^{-1} in the future equilibrium state. However, if N loading is low, fish manipulation may have a positive impact at higher TP concentrations (Moss et al., 1994b; Jeppesen et al., 1999). The 0.05–0.1 mg P l^{-1} threshold is in accordance with empirical data (Jeppesen et al., 2000). Temporary fish manipulation effects can be obtained in lakes with high nutrient concentrations, but it seems unlikely that effects will persist in the long term, unless planktivorous fish are reduced repeatedly.

It is unlikely that fish manipulation will be feasible for very large lakes (say > 500 km^2). First, it is difficult to obtain a 'sledgehammer' effect on the target fish population in very large lakes. The fish cannot easily be removed fast enough to compensate for their annual reproductive rate. Changes in the fish stock may simply become difficult to discern unless the fishing effort is substantial. Second, recent studies indicate that the fish population responds quickly to a nutrient loading reduction (Jeppesen et al., 2005a,b,c). This may render fish stock intervention less attractive in very large lakes as the natural changes may occur at higher speed than those invoked by biomanipulation in such lakes. Third, fish stocking is expensive for large lakes. However, biomanipulation combined with nutrient loading reduction has yielded a fast response (higher transparency, fewer cyanobacteria and in some cases extensive growth of submerged macrophytes) in medium large lakes, examples being Lake Vesijärvi, Finland (109 km^2), Lake Wolderwijd, Holland (27 km^2), and Lake Christina, USA (16 km^2) (Hanson & Butler, 1990; Peltonen et al., 1999; Meijer et al., 1999).

Delay in recovery and re-establishment of submerged vegetation after nutrient loading reduction have often been noted (Sas, 1989; Cooke et al., 1993; Jeppesen & Sammalkorpi, 2002; Jeppesen et al., 2005c). The delay in plant appearance may be due to lack of seed banks, waterfowl grazing, limited grazing on periphyton or too high turbidity (Søndergaard et al., 1996; Mitchell & Perrow, 1997; Lauridsen et al., 2003). Construction of exclosures to protect macrophytes against waterfowl grazing to improve plant re-establishment has been used as an alternative or supplementary restoration tool to fish manipulation (Cooke, 1993, Jeppesen & Sammalkorpi, 2002). The exclosures enable macrophytes to grow in a grazer-free environment where they can spread seeds, turions or plant fragments and thereby augment colonization. Moreover, they serve as a daytime refuge for zooplankton. Plant refuges as a restoration tool is probably most useful in small lakes and in the littoral zone of other lakes where colonization is restricted to the near shore areas due to light limitation in the deeper main lake. Waterfowl aggregate in littoral emergent zones, which provide shelter and nest sites, and may impart higher grazing pressure on plants. Plants as a refuge have greater effect in nutrient-rich lakes where plant density often is highest and prey fish are confined to the pelagial (Jeppesen et al., 1997; Blindow et al., 2000, Burks et al., 2002). Transplantation or protection of plants (in sheltered beds) and seeds may be useful in large shallow lakes if pre-studies reveal that (re)-colonization is seed-limited (Weisner & Strand, 2002; Ke & Li, in press). However, it is important to evaluate whether wind (wave) disturbance will prevent colonization. In large lakes effort should be concentrated on sheltered areas where plants can colonize gradually, as seen in large (27 km^2) Lake Veleuwe (Meijer et al., 1999).

Warm lakes

Few long-term lake recovery studies after reduced external loading exist for tropical and subtropical lakes: here we present an example from Brazil. Lake Paranoá (15°48'S, 47°47'W) is a shallow, urban, man-made lake (area 38 km^2, mean depth: 13 m, retention time: 0.9 y). It was created in 1959 for recreational purposes, hydroelectric power production, to increase the humidity of the region, as well as to receive wastewater and run-off from the city of Brasilia. The first plankton study recorded dominance of desmids and large-bodied cladocerans (*Daphnia* and *Ceriodaphnia*) (Oliveira & Krau, 1970). Later, phytoplankton biomass was dominated by *Cylindrospermospsis raciborskii*, which remained dominant in subsequent decades (Cronberg, 1976; Pinto-Coelho & Giani, 1985; Branco & Senna, 1991). *Microcystis aeruginosa* blooms occurred periodically during the end of the dry season (Branco & Senna, 1994). Besides the permanent bloom of filamentous cyanobacteria (*C. raciborskii*), eutrophic conditions were also indicated by small-bodied zooplankton (mainly rotifers and small cladocerans, such as *Diaphanosoma birgei* and *Bosmina* spp.), low Secchi depth (< 50 cm) and high levels of nutrients and chlorophyll *a* (Branco & Senna, 1996; Branco & Cavalcanti, 1999, Fig. 4). Aquatic macrophytes, such as free-floating *Eichhornia crassipes* and *Pistia stratiotes*, also spread in some areas. Eleven fish species colonized the lake from the catchment. In addition, the lake was stocked with exotic fish species, such as bluegill sunfish (*Lepomis macrochirus*), black-bass (*Micropterus salmoides*), carp (*Cyprinus carpio*), Congo (*Tilapia rendalli*) and Nile (*Oreochromis niloticus*) tilapias, and the piscivorous tucunaré (*Cichla ocellaris*) from the Amazon Basin (Ribeiro et al., 2001). In the late 1980s, tilapia dominated and accounted for 50% of total fish biomass. In subsequent years, the lake was stocked with tambaqui (*Colossoma macropomum*) and tamoatá (*Callychthys callychthys*), both from the Amazon Basin and with high commercial value.

In 1993–1994, two tertiary sewage treatment plants capable of processing 2400 l s^{-1} were constructed in addition to a complementary sewage collecting system. Resultant decrease in external point-source of TP loading, from 6.3 g m^{-2} y^{-1} in 1989 to 0.48 g m^{-2} y^{-1} in 1998 (Pereira & Cavalcanti, 1997; Burnett et al., 2001), led to decreased mean TP, and also total Kjeldahl nitrogen (TKN) loading and concentration decreased (Fig. 4). Chlorophyll *a* values decreased (Fig. 4), and Secchi depth attained 2.4 m in 1999. Important planktonic community level changes were observed from 1996 to 1999: (1) decreased cyanobacteria dominance and replacement of *C. raciborskii* by green algae and diatoms by late 1998; (2) short-term dominance of colonial floating green algae (*Botryococcus braunii*), replacing *M. aeruginosa* in some hypertrophic areas in 1996; (3) re-appearance of *Ceriodaphnia cornuta*, *Daphnia gessneri*, other small cladocerans, such as *Moina micrura* and *Bosminopsis deitersi* (Elmoor-Loureiro et al., 2004), and *Notodiaptomus cearensis*, an oligotrophic calanoid copepod species (Padovesi-Fonseca et al., 2001). Other strategies, such as reducing the retention time of the lake and fish population management, were used to generate further improvements. An echo-sounding campaign in 1998 revealed a fish stock of 1,500 tons, with tilapia constituting up to 90% of fish biomass. Laboratory studies on fish feeding rates on lake plankton and P excretion rates, plus an assessment of planktivorous fish impacts in enclosures, indicated that tilapia overpopulation control would prevent accumulated P from being available to primary production, especially due to bottom feeding (Starling, 1993a, 1993b; Starling & Rocha, 1990). Tilapia control was initiated in the most eutrophicated region by legalizing commercial cast-net fisheries. Continuous improvement of lake environmental conditions was confirmed in early 2000 by lower chlorophyll *a* values, enhanced water transparency, and decreased fish biomass to 800 tons. Tilapia dominance declined, allowing recovery of a heterogeneous fish population, including native omnivores and piscivores, and some exotic species, all of which contributed to enhancement of large zooplankton populations.

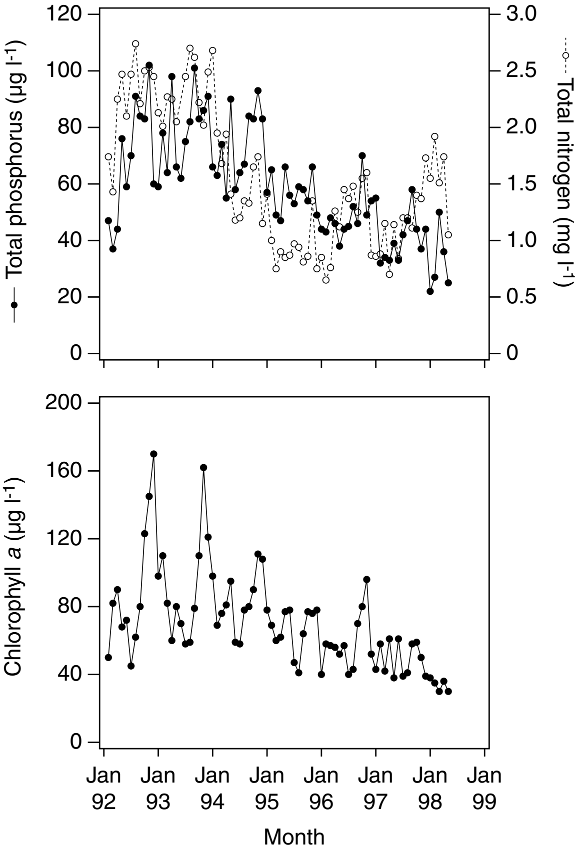

Figure 4: Total phosphorus, total Kjeldahl nitrogen (TKN) and chlorophyll *a* in Lake Paranoá, Brazil, before and after (1993–94) a major reduction of nutrient loading.

Trophic dynamics and biomanipulation in warm lakes

While results from Lake Paranoá illustrate the importance of reducing external loading to restore subtropical and tropical lakes, it is difficult to evaluate effects of fish manipulations in the lake. It is debatable whether fish manipulation methods used in cold temperate lakes can be employed in warm temperate, subtropical and tropical lakes. Several factors indicate that fish stock manipulation may not have the same positive effects in warm lakes (Moss et al., 2004; Jeppesen et al., 2005d): a) fish species richness often is higher and many fish show partial niche overlap, which should increase predator control of prey (Lazzaro, 1997; Aguiaro & Caramaschi, 1998); b) fish stocks often are dominated by omnivores independent of trophic state (Lazzaro, 1997; Branco et al., 1997; Yafe et al., 2002; Blanco et al., 2003; Mazzeo et al., unpublished data) and generally few piscivores are present (Quirós, 1998); c) fish density, but not necessarily biomass, is higher (1–2 orders of magnitude) (Scasso et al., 2001; Aguiaro et al., 2003; Mazzeo et al., 2003; Meerhoff et al., 2003); d) fish reproduction occurs throughout the year (Fernando, 1994; Paugy & Lévêque, 1999) and many species are viviparous and/or show parental care (Lorier & Berois, 1995), assuring higher juvenile survival rates. Therefore, top-down control by piscivores probably is weaker in warm lakes. Since small fish are more zooplanktivorous and have higher energy demand per unit biomass (Kalff, 2002), small fish in high abundance leads to higher predation pressure on zooplankton.

Accordingly, zooplankton communities in tropical and subtropical lakes often are dominated by small cladocerans (i.e. *Diaphanosoma*, *Ceriodaphnia* and *Bosmina*), rotifers, and copepod juveniles and nauplii (Dumont, 1994; Lewis, 1996; Branco et al., 2002, Garcia et al., 2002; Lacerot et al., unpublished data). Large *Daphnia* spp. may appear in early spring and autumn in warm-temperate lakes (Romo et al., 2005) or, if fish are absent, for longer periods in subtropical lakes (Mazzeo et al., unpublished data). Omnivorous copepods usually dominate zooplankton biomass in oligo-mesotrophic systems, whereas microzooplankton prevail in eutrophic systems. Therefore, classic control of phytoplankton by large zooplankton usually is not found in warm lakes. However, the numerous microzooplankton in these systems may control nanophytoplankton, thus favouring larger cyanobacteria. Some works demonstrate that cyclopoid copepods can control diatoms, rotifers can graze small green algae, and particulate matter and associated bacteria is an important food resource for zooplankters in tropical lakes (Infante, 1978; González et al., 2005). The zooplanktivorous phantom midge *Chaoborus* also seems to be more abundant in tropical lakes (e.g. Aguilera & Goitia, 1999), perhaps because bottom water anoxia is more common, which may provide *Chaoborus* with a fish predation refuge (Lewis, 1996) and allow strong predation pressure on zooplankton (Arcifa et al., 1992). Finally, shrimps (e.g. *Palaemonetes argentinus* or *Macrobrachium borelli*) also can be important predators on zooplankton considering their high abundance, food preferences and several reproductive events from spring to summer (Boschi, 1981; Collins, 1999; Collins & Paggi, 1998).

Aquatic plants play an important structural role in most freshwater ecosystems (Moss, 1990; Scheffer et al., 1993), also in the tropics (Thomaz & Bini, 2003). In temperate nutrient-rich lakes, submerged plants act as daytime refuges for zooplankton against fish predators (Timms & Moss, 1984; Lauridsen et al., 1996; Burks et al., 2002). At night, large zooplankton move out to the open water to feed, thereby contributing to clearwater conditions in lakes with high macrophyte coverage (Jeppesen et al., 1997). However, in the tropics and subtropics, effects of macrophytes on trophic interactions are more complex, since all forms (i.e. emergent, submerged, floating-leaved and large free-floating species) can be prominent. Macrophytes are extremely important to fish in the tropics and subtropics. Removal of *Chara* spp beds in an Itaipú reservoir resort (Santa Helena, Brazil) with the purpose to decrease piranha (*Serrassalmus marginatus*) population led to decreased fish abundance and species richness from 25 to 10 species (Agostinho et al., 2003; Fig. 5). Studies comparing different habitats in tropical and subtropical lakes indicate that the smallest fish species and individuals aggregate in all vegetation forms (Conrow et al., 1990; Meschiatti et al., 2000; Meerhoff et al., 2003; Meerhoff et al. unpublished data). Densities above 140 ind m^{-2} of a single, small-sized species were found in submerged plant beds in a subtropical lake (Meerhoff et al., 2003). Therefore, submerged vegetation in warm lakes may be a poor refuge for large-bodied zooplankton, particularly cladocerans (Meerhoff et al., 2003; Meerhoff et al., in press). An inverse diel horizontal migration pattern (aggregating in the pelagial during day and among plants at night) was found for *Bosmina longirostris* and *Diaphanosoma birgei* during spring and autumn in a subtropical lake without piscivores (Lake Blanca, Uruguay; Iglesias et al., accepted). The preference of small omnivorous fish for emergent and submerged plant beds during day and the occurrence of *Chaoborus* in the pelagic at night determined the spatial distribution observed. In accordance with the suggestion of a poor refuge effect for zooplankton in warm lakes, no

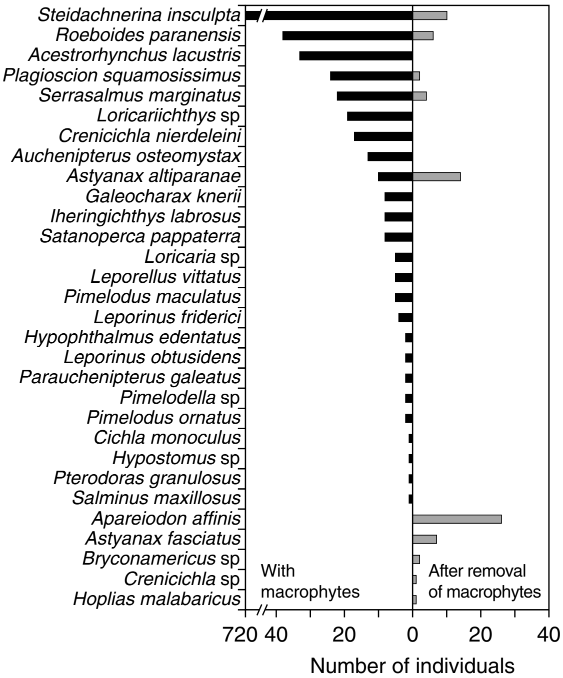

Figure 5: Specific composition of fish assemblages in Santa Helena, Itaipú Reservoir (Paraná, Brazil) before (1987) and after (1988) a substantial removal of submerged macrophytes. Reproduced with kind permission from Agostinho et al. (2003).

positive effect of plants on water clarity was found in nutrient-rich subtropical lakes in Florida, and nutrient rich lakes with high plant biomass often were turbid (Bachmann et al., 2002).

Considering the higher fish density in tropical systems and lack of refuges for zooplankton among macrophytes, fish biomass thresholds allowing major grazing pressure by zooplankton may be lower and fish reductions therefore have to be very large compared to temperate lakes to allow large-bodied grazers, like *Daphnia* spp, to dominate (Scasso et al., 2001; Mazzeo et al., unpublished data). The efficiency of decreasing planktivorous fish biomass by fish removal is further hampered by continuous and/or viviparous reproduction in many (sub)tropical species (Scasso et al., 2001 and Fig. 6). Therefore, it may be more difficult to provoke and maintain a trophic cascade effect in subtropical and tropical lakes than in temperate lakes. Accordingly, positive, but only short-term, cascading effects of a massive (>80%) fish kill (due to fungal infection) of the dominant planktivorous mosquito fish (*Gambusia affinis*) were found in Lake Naini Tal, India (Nagdali & Gupta, 2002). Zooplankton abundance increased, phytoplankton biomass and productivity declined, as did nutrient concentrations, resulting in higher water transparency. However, four months later, mosquito fish, plankton and nutrients had returned to previous levels.

In subtropical and tropical lakes, stocking of herbivorous silver carp (*Hypophthalmus molitrix*) has, with some success, been used to combat potentially toxic cyanobacteria (Starling et al., 1998; Datta & Jana, 1998). However, silver carp also consume zooplankton (Wu et al., 1997, Starling et al., 1998, Xu & Zie, 2004), which reduces grazing on small-sized phytoplankton. Therefore, it is unlikely that silver carp stocking, which is a symptom treatment rather than a restoration, will lead to a shift to a clear, macrophyte dominated stage (Matyas et al., 2003). Introduction of strictly piscivorous fish might be the only efficient biomanipulation strategy in (sub)tropical regions to control planktivorous fish. Ongoing mesocosm experiments using the sit-and-wait piscivore tararira (*Hoplias malabaricus*) support this hypothesis (Mazzeo et al., unpublished), but clearly more experiments are needed in this field before any firm conclusions can be drawn.

However, it is important to notice trophic structure varies among warm lakes, depending on whether they are situated in wet or dry regions and temporal variations in temperature and salinity, and this may have implications on the restoration strategy as well. For example, tropical lakes are particularly sensitive to hydrological changes, which strongly affect their structure and functioning. In Cochabamba, Bolivia (17°S), switches from phytoplankton to macrophyte dominance in Lake Alalay have been associated to an increase in water level (Cadima, 1997). Higher densities and richness of zooplankton and fish were found during the low water season in several shallow lakes in Bolivia (Pouilly et al., 1999; Rejas & Maldonado, 2000). Water level changes also have strong effects on macrophyte abundance and water clarity in Turkish lakes (Beklioglu et al., 2003). These lakes, as well as northern Greek lakes, are, though warm in summer, subjected to cold winters, leading to a mixed fish community, often with low species richness compared to the subtropical and tropical lakes, and dominance of northern species (Beklioglu et al., 2003; Zalidis et al., 2002). These Turkish warm-water lakes form an intermediate state between the north temperate lakes and winter warm temperate lakes and the subtropical/tropical lakes, which have to be taken into account in connection with restoration initiatives (Beklioglu et al., 2003; Beklioglu & Tan, submitted). Likewise, saline lakes are typically species poor (Williams, 1998) and, like in the subtropical lakes, submerged macrophytes appear to have little effect on water clarity in saline lakes (Moss, 1994a; Jeppesen et al., 1994) and form special cases.

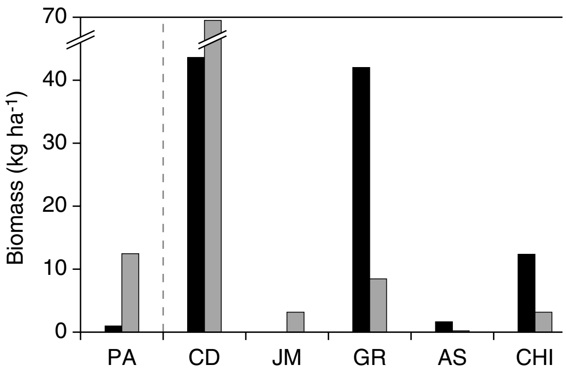

Figure 6: Changes in the biomass of nekton at the beginning (black) and end (white) of summer 2003 in biomanipulated Lake Rodó (Uruguay) after an intensive programme of planktivorous fish removal. The biomanipulation had little effect om shrimps (*Paleomonetes argentinus*, PA) and viviparous fish, such as *Cnesterodon decemmaculatus* (CD) and *Jenynsia multidentata* (JM), while more effective on the short term for oviparous species: *Gymnogeophagus rhabdotus* (GR), *Astyanas* sp (AS) and *Cheirodon interruptus* (CHI). Moreover, no effect was observed on total fish biomass (from F. Quintans, unpublished data).

In many experiments in temperate lakes, improvement in environmental state was observed without a trophic cascade after biomanipulation, i.e. without zooplankton species composition changes towards dominance of large-sized individuals and, consequently, higher phytoplankton grazing (Horppila et al., 1998). However, reductions in cyanobacteria and TP were observed, and water clarity increased. These phenomena may be due to reduced sediment P release and fish foraging following biomanipulation (Horppila et al., 1998). Biomanipulation also may reduce sediment nutrient release in tropical lakes, but the dominance of small fish species and better growth conditions for cyanobacteria suggest that effects may be temporary. Other methods, such as the combined use of aquatic plant harvests (mostly free-floating, if present) and hydraulic management, can be useful, though insufficient, for restoring small, shallow lakes in warm regions by decreasing internal nutrient loads (Rodríguez-Gallego et al., 2004). Sediment removal might be more attractive in such lakes, since P-content per unit volume of sediment exceeds that in the water column and plants (Søndergaard et al., 2003). However, drastic reduction of external nutrient loading seems to be the best method for restoring small or large subtropical and tropical lakes, but the scientific basis (e.g. nutrient threshold levels) to make decisions remains limited. Focus may well have to be on reducing external N loading as nitrogen has often been found to be the limiting nutrient for phytoplankton growth in tropical lakes (Lewis, 1996). Focus on nitrogen, in addition to phosphorus, is also relevant for temperate shallow lakes, as recent studies indicate that submerged macrophyte recovery after loading reduction is more likely to occur at moderately high TP when TN loading is low (González-Sagrario et al., 2005; James et al., 2005; Jeppesen et al., 2005b).

Conclusions

For small and large shallow lakes, the best strategy for improving the environmental state is external nutrient loading reduction. Like small lakes, large shallow lakes also may respond strongly and quickly to loading reduction. For smaller lakes resistant to nutrient loading reduction, physico-chemical and biological methods may enhance recovery. However, these methods are unlikely to be feasible in large shallow lakes. Sediment removal and chemical treatment, in particular, are less attractive because: a) sediment transport and storage on land (if removed) will be expensive; b) there is a large risk of sediment redistribution from resuspension; c) the sediment P pool often is relatively small in large lakes. Fish manipulation also may be impractical because: a) the large size makes it difficult to obtain a strong effect on the target fish population; and b) fish population is among the first to respond to nutrient loading reduction, and natural changes in fish stock become difficult to discern in large lakes. If needed, transplantation/protection of aquatic plants (in sheltered beds) and seeds may be useful in large shallow lakes, the latter if pre-studies reveal that (re)-colonization is seed-limited. However, it is important first to evaluate whether wind (wave) disturbance will prevent colonization where seeds or plants are added.

Differences in biological interactions in cold temperate versus warm temperate-subtropical-tropical lakes make it difficult to apply biological restoration methods to warm lakes. Warm lakes often have prolonged growth seasons with a larger risk of long-lasting algal blooming and dense floating plant communities, higher dominance and abundance of small fish, higher fish aggregation in vegetation (loss of zooplankton refuge), more fish cohorts per year, and more omnivory by fish and less specialist piscivory. However, trophic structure varies among these lakes, depending on whether they are situated in wet or dry regions and temporal variations in temperature and salinity and this have implication for the restoration strategy.

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Shallow lake restoration by nutrient loading reduction – some recent findings and challenges ahead

Erik Jeppesen[1,2], Martin Søndergaard[1], Mariana Meerhoff[1,2,3], Torben L. Lauridsen[1] and Jens Peder Jensen[1]

[1] National Environmental Research Institute, Dept. of Freshwater Ecology, Vejlsøvej 25, DK-8600 Silkeborg, Denmark
[2] Dept. of Plant Biology, University of Aarhus, Ole Worms Allé, Building 135, DK-8000 Aarhus C, Denmark
[3] Depto. de Ecología. Facultad de Ciencias. Universidad de la República. Iguá 4225 C.P. 11400 Montevideo-Uruguay.

Corresponding author: Erik Jeppesen, ej@dmu.dk

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Abstract

Shallow lakes respond to nutrient loading reductions. Major findings in a recent multi-lake comparison of data from lakes with long time series revealed: that a new state of equilibrium was typically reached for phosphorus (P) after 10-15 years and for nitrogen (N) after <5-10 years; that the in-lake Total N: Total P and inorganic N:P ratios increased; that the phytoplankton and fish biomass often decreased; that the percentage of piscivores often increased as did the zooplankton:phytoplankton biomass ratio, the contribution of *Daphnia* to zooplankton biomass, and cladoceran size. This indicates that enhanced resource and predator control often interact during recovery from eutrophication. So far, focus has been directed at reducing external loading of P. However, one experimental study and cross-system analyses of data from many lakes in north temperate lakes indicate that nitrogen may play a more significant role for abundance and species richness of submerged plant than usually anticipated when total phosphorus is moderate high. According to the alternative states hypothesis we should expect ecological resistance to nutrient loading reduction and P hysteresis. We present results suggesting that the two alternative states are less stable than originally anticipated. How global warming affect the water clarity of shallow lakes is debatable. We suggest that water clarity often will decrease due to either enhanced growth of phytoplankton or, if submerged macrophytes are stimulated, by reduced capacity of these plants to maintain clear-water condition. The latter is supported by a cross-system comparison of lakes in Florida and Denmark. The proportion of small fish might increase and we might see higher aggregation of fish within the vegetation (leading to loss of zooplankton refuges), more annual fish cohorts, more omnivorous feeding by fish and less specialist piscivory. Moreover, lakes may have prolonged growth seasons with a higher risk of long-lasting algal blooms and at places dense floating plant communities. The effects of global warming need to be taken into consideration by lake managers when setting future targets for critical loading, as these may well have to be adjusted in the future. Finally, we highlight some of the future challenges we see in lake restoration research.

Introduction

During the past 20-30 years much effort has been directed, not least in Europe and North America, at combating the eutrophication of shallow lakes by reducing external phosphorus (P) loading. Loading with sewage and industrial waste has declined, but the nutrient input from diffuse sources has remained high, particularly in intensively cultivated countries (Van der Molen & Portielje, 1999; Kronvang et al., 2005). Many lakes have responded positively to nutrient loading reductions; however, delayed recovery has often been observed, the causes being internal P loading or biological resistance (Sas, 1989; Marsden, 1989; Jeppesen et al., 2005). In the present paper we first describe the highlights of a recent cross-system analysis of 22 long-term studies of shallow lakes recovering from eutrophication. Then we discuss the role of nitrogen (N) for recovery, resilience and how climate change might influence lake ecosystem state and the expected nutrient targets for shifts to good or high ecological state. Finally, we identify some important research areas for the future within the field of lake recovery and restoration.

A recent analysis of 22 case studies on re-oligotrophication

A recent a cross-system analysis was conducted on long term data series from one North American and 21 shallow European lakes (mostly north temperate lakes) in recovery from eutrophication (Jeppesen et al., 2005). It was found that internal P loading as expected delayed recovery. However, a new equilibrium with respect to total phosphorus (TP) was typically reached after <10-15 years, and the response time was only marginally dependent on hydraulic retention time. The response time to N loading reduction was typically <5-10 years. As loading reductions have mainly focused on P, the TN:TP and inorganic N:ortho-P ratios have increased (Fig. 1), often markedly. Lake TP typically declined during all seasons, but particularly in winter, followed by spring and autumn. Changes in chlorophyll *a* followed those of TP, indicating that nutrient constraint (resource control) has been a key factor for the changes in seasonal dynamics of phytoplankton biomass (Fig. 1). However, enhanced top-down control by zooplankton in the recovery process may also play a role. Thus in most lakes for which fish data were available the catch-per–unit-effort (test-fishing or commercial catches) by weight declined, often substantially, while the proportion of piscivores increased. In accordance with the expected cascading effects of such changes in the fish community, the zooplankton:phytoplankton biomass ratio (and thus probably also phytoplankton grazing) rose in many lakes, while the contribution of *Daphnia* to the total biomass (Fig. 1) and mean individual biomass of cladocerans increased. It must be emphasised, though, that the zooplankton data mainly derived from north temperate lakes. A different scenario is likely for warm temperate and (sub)tropical lakes where fish abundance, and likely then also the predation pressure on large-bodied zooplankton, is higher (Meerhoff et al., 2003; Romo et al., 2005; Jeppesen et al., in press).

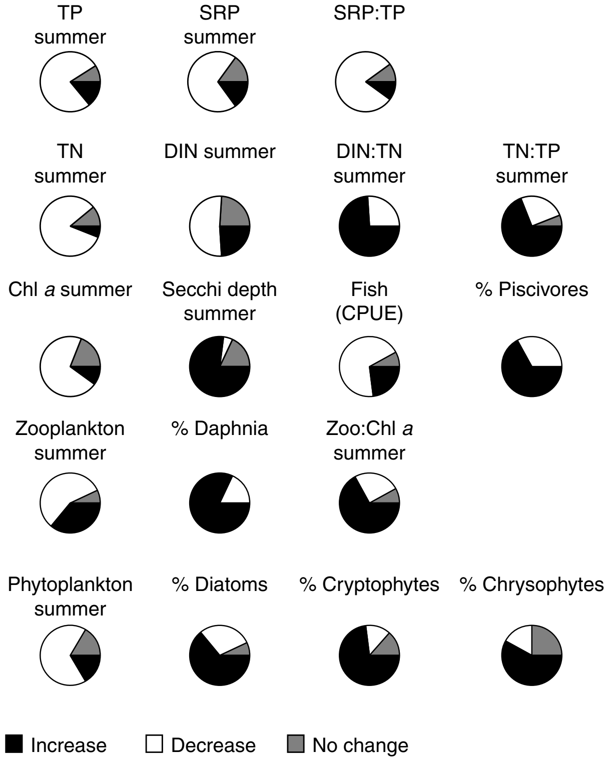

Figure 1. Some major results of a multi-lake (22 lakes) comparison of responses to nutrient loading reduction. The pies show direction of changes (% of lakes) during recovery 5-25 years) of a series of biological and chemical variables, all summer means (May 1 – October 1 in cold temperate lakes and May 1 - Nov 1 in warm temperate and subtropical lakes). DIN is dissolved inorganic N, SRP is soluble reactive phosphorus, CPUE is catch-per-unit-effort based on test-fishing or in a few cases commercial catches. Zooplankton and phytoplankton are total biomass and total biovolume, respectively, % *Daphnia* is contribution of *Daphnia* to the total zooplankton biomass and % of algae taxa their contribution to the total biovolume. Zoo is zooplankton biomass. (Modified from Jeppesen et al., 2005).

Nitrogen reduction – do we have to consider it?

In most studies, recovery follows a reduction in P loading, as P is generally considered the most important limiting nutrient in lakes (Schindler, 1977). However, some recent studies in shallow lakes emphasise also the role of N (Moss, 2001; González-Sagrario et al., 2005; James et al., 2005). Enclosure experiments run at various combinations of P and N in a shallow Danish lake indicated that the risk of loss of submerged macrophytes was high when N was above 1.2-2 mg l^{-1} and P higher than 0.1-0.2 mg l^{-1} (González-Sagrario et al.,

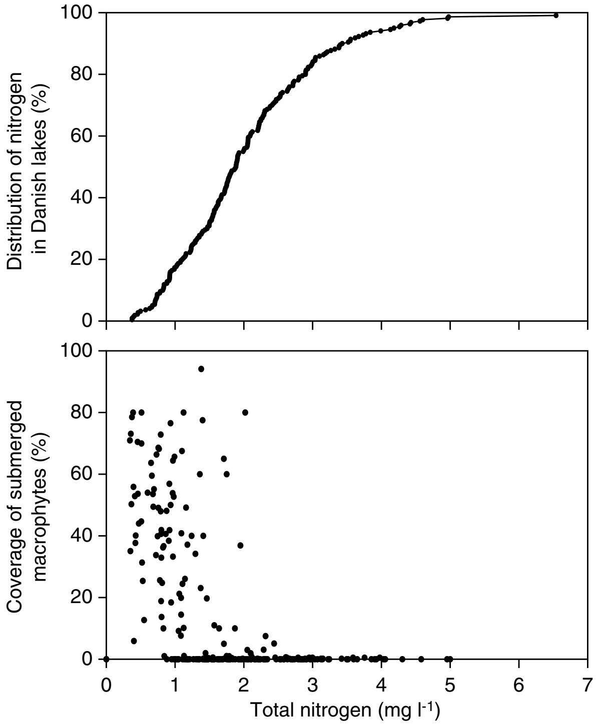

Figure 2. The distribution of summer averages of nitrogen in 204 Danish lakes (left, upper) and the coverage of submerged macrophytes in late summer in 44 Danish lakes (246 lake years) (left, lower) along a nitrogen gradient. All lakes have a mean depth lower < 5 m and a lake area >5 ha (from González-Sagrario et al., 2005).

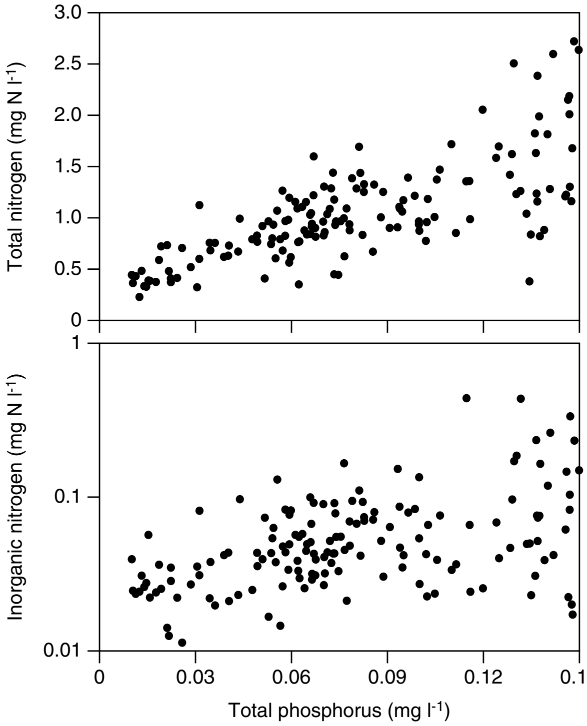

Figure 3. The concentration of nitrogen (total nitrogen upper and inorganic nitrogen lower) along a phosphorus gradient. The analysis comprises only lakes with a summer average inorganic N:P ratio in the lake water <7 (56 lakes in the analysis (152 lake years)). All lakes have an area>5 ha, a mean depth <5 m and a total phosphorus concentration< 0.15 mg l^{-1}.

2005). However, at low P concentrations, variations in N loading had no impact. In accordance with these results, data from 44 Danish lakes (and 246 lake years) suggest that submerged plants typically disappear at concentrations of 1-2 mg N l^{-1} when TP is moderately high (González-Sagrario et al., 2005; Fig. 2). These N concentrations, which apparently trigger the loss of macrophytes are low compared to the present concentrations in Danish lakes (Fig. 2).

But why should these plants be lost at high N loadings? In eutrophic shallow lakes, nitrate is often low during late summer due to high denitrification and low summer input of nitrogen, and TP is high due to internal loading (Søndergaard et al., 2005). Nitrogen limitation of periphyton or filamentous algae on plant surface and phytoplankton is therefore likely to occur during mid-summer. Thus when N loading is high growth of these algae may be stimulated and potentially then outshade the submerged macrophytes. To gain further insight, we depicted, in an N:P diagram, summer data from the 44 lakes with an inorganic N:P ratio below 7, where algae are likely N limited if the inorganic N concentrations are low (Smith, 1983), and focused on the P concentrations at which a shift to a clear-water state may typically occur, i.e. <0.15 mg P l^{-1} (Jeppesen et al., 1990) (Fig. 3). Lakes with inorganic N:P <7 are detected within the 0.5-2 mg TN l^{-1} interval (Fig. 3), i.e. where submerged macrophytes actually disappear (Fig. 2). Moreover, the concentration of inorganic N in these lakes usually remains <0.1 mg l^{-1} when TP is between 0.015-0.12 mg l^{-1}, meaning that N limitation of algae is likely within this TP range. If, instead, we used data from late summer (15 July - 15 August) when submerged macrophyte biomass typically reaches a seasonal maximum and nitrate its seasonal minimum, the evidence of N limitation would be even stronger.

N not only seems to affect the abundance of submerged plants. Studies conducted in Polish and British lakes have further shown that plant species richness declines with increasing winter nitrate concentrations (James et al., 2005). Development of a particular or few species may cause large interannual variations in plant abundance, as indicated by results from Danish lakes dominated by a single species, such as rigid hornwort (*Ceratophyllum demersum*) or Canadian pondweed (*Elodea canadensis*) (Søndergaaard et al., 1997; Lauridsen et al., 2003). In some years the lakes may be nearly filled up with plants, while in other years plants may be completely absent (Søndergaard et al., 1997), with great risk of the lakes reverting to a turbid state.

All these results may indicate N to play a more significant role than usually anticipated. They also indicate that it may be difficult to obtain a clear-water state in shallow lakes at medium-high P concentrations if the

N concentration in summer exceeds 1-2 mg N l^{-1}. If P can be reduced to low concentrations, N is not likely to be of major importance, but in agriculturally impacted landscapes it may be difficult to reach P concentrations sufficiently low to eliminate the impact of N. One may therefore perceive N reduction as an additional measure to obtain good water quality in lakes where sufficiently high P reduction is difficult to achieve. An obvious possibility of reducing the N input is (re-) establishment of wetlands in the lake catchment, as these have a high capacity to remove N. However, we must emphasise that the results presented only allow preliminary conclusions and need further testing. Moreover they only cover north temperate shallow lakes of the size 5-3900 ha and intermediate to high P concentrations. In ponds and small lakes (< 5 ha) submerged macrophytes may well be abundant at higher N concentrations (authors' unpublished data). This may also be true in lakes – not only ponds but also larger lakes - in other geographical areas (see discussion in González-Sagrario et al., 2005).

P hysteresis in the recovery phase – do we need to push the systems?

According to the alternative state hypothesis (Scheffer et al., 1993), we should expect not only chemical resistance to nutrient loading reduction due to internal P loading but also ecological resistance and P hysteresis, i.e. the shift from turbid to clear occurs at a lower P concentration when P is decreasing than from clear to turbid when P is increasing. When approaching the P range of alternative states after nutrient loading reduction, a push (e.g. biomanipulation) may help shifting the lakes to the clear state (Moss, 1990; Jeppesen et al., 1990; Scheffer et al., 1993). An example of P hysteresis comes from Lake Veluwe (Meijer et al., 1999). One of the factors that, according to the theory, is expected to contribute to ecological resistance and P hysteresis is homeostasis among fish due to high longevity and persistence of some of the key species dominating in the turbid state, such as bream (*Abramis brama*) and various carp species. They then continue to control zooplankton and stir up suspended sediment a long time after the external nutrient loading and P concentration has been reduced. However, analyses of data from Danish lakes (Jeppesen et al., 2002) and the 22 lakes (see above) showed relatively fast responses towards reduced abundance of plankti-benthivorous fish (including bream) and a higher proportion of predatory fish.

Besides enhancing internal loading, resuspension of sediment detritus accumulated during the eutrophication period is also considered to contribute to P hysteresis as water clarity is reduced. However, a study of 15 Danish shallow lakes showed major reduction in suspended matter during recovery and an almost proportional reduction in chlorophyll *a*, detritus and inorganic suspended matter. This indicates that resuspension of old detritus, accumulated during the eutrophication period, does not hinder an improvement of water transparency in Danish lakes <3900 ha. (Jeppesen et al., 2003), though it is debatable whether the same holds true for very large lakes (Bachmann et al., 1999; Coveney et al., 2005).

Delayed response of submerged macrophytes due to lack of propagules, waterfowl and fish grazing, poor substrate conditions and fish control of snails (indirectly contributing to periphyton-induced out-shading of the plants) are also considered to create hysteresis (Moss, 2001). The multi-lake comparison of the 22 lakes (Jeppesen et al., 2005) suggested different response patterns. In some lakes no colonisation was observed despite raised water clarity, while in others a sudden increase was seen, supporting the hypothesis. However, in some cases abundance, coverage, plant volume inhabited (PVI) or depth distribution of submerged macrophytes increased gradually concurrently with the reduction in in-lake P.

In view of the above, it can be discussed whether P hysteresis is as important for lake recovery as suggested in the alternative state theory (Scheffer et al., 1993). Some of the arguments for P hysteresis seem less strong than originally thought. To gain further insight into the impact of P hysteresis, we analysed a series of Danish lakes with long-term data during the recovery phase (Fig. 4A). We found major reductions in chlorophyll *a* (Chl *a*), which largely followed TP in lakes subjected only to external nutrient loading control, indicating that P hysteresis is of minor importance. In biomanipulated (Fig. 4B) lakes an abrupt drop in Chl *a* and TP was seen after biomanipulation, followed in some cases by an increase some years later. In these cases there was evidence that the Chl *a*:TP ratio was lower immediately upon biomanipulation when Chl *a* reached low concentrations, which is in accordance with expectations in the P hysteresis hypothesis. However, Lake Arreskov became unstable shifting between the clear and turbid state as seen in several other Danish case studies of biomanipulation (Lauridsen et al., 2003). Large year-to-year variations between low and high Chl *a* and TP are also seen in some of the lakes with no or minor changes in external loading, such as Lake Stigsholm and Lake Hornum (Fig. 4C), indicating that the "stability" of the clear-water and turbid states may not be as strong as predicted for the TP range where alternative states are to be expected. We further plotted Chl *a* against TP for the lakes presented in Fig. 4A,C and seven other Danish non-biomanipulated shallow lakes with long time series (Fig. 5). While one lake showed a tendency to a stepwise decrease of the Chl *a*:TP ratio at a specific P threshold, the overall impression is that Chl *a* follows TP, though the year-to-year variation could be high in the P range of alternative states as seen also in several lakes elsewhere (Mitchell, 1989; Moss et al., 1990; Perrow et al., 1994; Blindow et al., 1993).

Our results therefore indicate that resistance to changes with decreasing TP concentrations are less

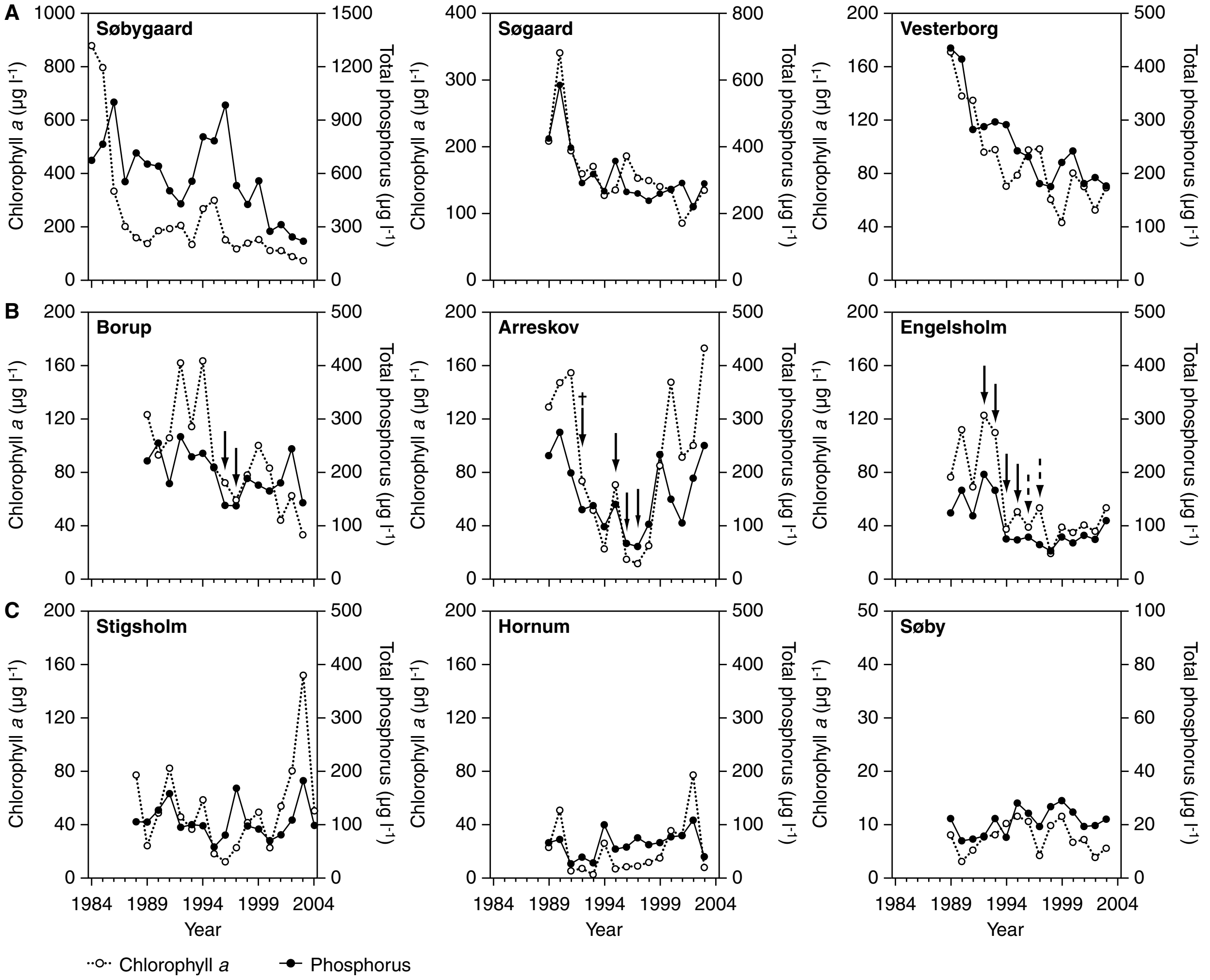

Figure 4. Time series of summer mean (1 May - 1 October fortnightly samplings) concentration of chlorophyll *a* (Chl *a*) and total phosphorus in the lake water of 9 shallow Danish lakes following: A) external nutrient loading reduction only, B) external loading reduction and biomanipulation conducted in the years indicated by arrows, and C) lakes with minor or no reduction in external loading. Note the different scale on axes. The arrows indicate years of fish removal or fish kills (+), if broken then only little effort was used to remove fish that particular year.

strong than predicted by the alternative state hypothesis and the stability of the two states within the window of alternative states weaker. In northern temperate lakes a shift to the clear state may occur gradually, concurrently with the reduction in internal P loading and the in-lake P. Whether additional tools such as biomanipulation are needed to push the system from turbid to clear has therefore to be evaluated carefully in each case. Certainly, many experiments involving a reduction in fish biomass have led to abrupt changes from turbid to clear (Hansson et al., 1998; Meijer et al., 1999; Mehner et al., 2002) (Fig. 4B) and to reduced internal P loading, having thus reinforced the recovery, at least in the short term. So far, the long-term perspectives are less promising when based on a single event to change the system from turbid to clear (Meijer et al, 1999; Olin et al., 2005; authors' unpublished results). More focus should be on how to stabilise the lake ecosystem to get long term success of biomanipulation (Mehner et al., 2002), perhaps by less intensive but more frequent manipulation of the fish stock. Methods to improve establishment and growth of submerged macrophytes may be needed if plants do not emerge after a rise in water clarity, as has been the case for several lakes (Köhler et al., 2005, Jeppesen et al., 2005). However, a reduction of the external nutrient load appears as the most straightforward strategy to improve the ecological status of shallow eutrophic lakes.

Climate change – impact on the future ecological state?

How changes in climate will affect the response to loading reduction and the new ecological state at steady state is debatable. Few studies have tested the effects of increased temperature and nutrient-enrichment using an ecosystem approach in controlled experiments

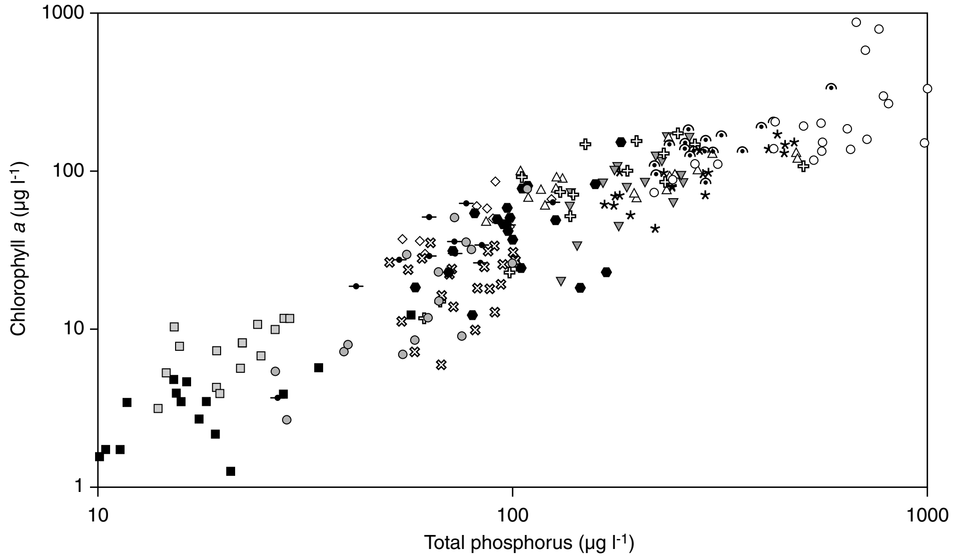

Figure 5. Summer mean (1 May - 1 October fortnightly samplings) chlorophyll *a* versus total phosphorus in non-biomanipulated shallow lakes with long time series. Each symbol represents one lake and each point the summer average for one of the years included.

(McKee et al., 2003, Liboriussen et al., 2005). McKee et al. (2003) showed that warming did not promote phytoplankton blooming but increased P concentrations and de-oxygenation. Long-term field data show that effects of warming on water quality can be contradictory and the net effect therefore difficult to predict (Carvalho & Kirika, 2003). Here, we first discuss how climate change, mostly warming, may affect the shallow lake ecosystem and, secondly, the potential consequences for the future ecological state of recovering lakes and possible adaptation strategies for managers.

A possible scenario is that macrophyte growth is stimulated by climate warming (Scheffer et al., 2001) due to the higher temperature, and in the Mediterranean region due to reduced water table as well (Beklioglu & Tan, submitted). Moreover, unless compensated by higher precipitation induced N loading, nitrate in summer will probably be lower in the future due to enhanced denitrification (Weyhenmeyer et al., submitted), which potentially may be beneficial for the macrophytes as outlined above. Therefore, shallow lakes may potentially shift from the phytoplankton to the macrophyte dominated state. However, the role of submerged macrophytes for water clarity differs along the climate gradient. While high abundance of plants is synonymous with clear water in most north temperate non-humic lakes, the effect is much weaker in warm temperate (Romo et al., 2005) and subtropical lakes (Bachmann et al., 2002). Thus, Bachmann et al. (2002) found no difference in the chlorophyll:TP or Secchi depth:TP relationships in shallow lakes in Florida with low, medium high or high plant coverage (COV) or plant volume infested (PVI).

We conducted a comparative study of relationships between Chl *a*, nutrients and submerged plant abundance in lakes from Florida and Denmark, and the results showed Chl *a* at a given nutrient concentration to be overall higher in Florida lakes than in Danish lakes when macrophytes are abundant (Fig. 6). Accordingly, the slopes for COV and PVI in multiple regressions, including also N and/or P as independent variables and Chl *a* as dependent variable, were much lower for Florida lakes (Table 1). One reason for these lower slopes may be that macrophytes are not acting as proper refuges for zooplankton in warm lakes. Several studies in subtropical and tropical lakes have shown that fish, particularly the smallest species and individuals, aggregate in high numbers in the vegetation in subtropical and tropical lakes (Conrow et al., 1990; Meerhoff et al., 2003) and some are even confined to this biotope (Sazima & Zamprogno, 1985; Delariva et al., 1994). Fish aggregation in the vegetation and lack of zooplankton aggregation among plants have also been observed in brackish lakes dominated by small-sized sticklebacks (*Gasterosteus aculeatus*) in Denmark and eutrophic brackish lakes thus remain turbid even when macrophyte coverage is high (Jeppesen et al., 1997). Besides, in shallow lakes in the tropics and subtropics, fish zooplanktivory is stronger due to multiple or continuous reproductive events (Paugy & Lévêque, 1999), lower densities of large specialist piscivores with sit-and-wait hunting behaviour more frequent among them (Quirós, 1998), widespread omnivory (Branco et al., 1997, Yafe et al., 2002), and high population densities (but not necessarily high biomass) of fish, particularly of small and juvenile individuals (Mazzeo et al., 2003). Accordingly, (sub)tropical lakes are usually dominated by small forms of zooplankton (Crisman & Beaver, 1990; Meerhoff et al, 2003) that exert low grazing pressure on phytoplankton. Although more studies are needed to elucidate the differences in predation on zooplankton, it may be concluded that this community structure and common habitat use of fish may be one of the key factors determining the weaker effect of submerged plants on water clarity in warm lakes. We may therefore expect that north temperate lakes move in the

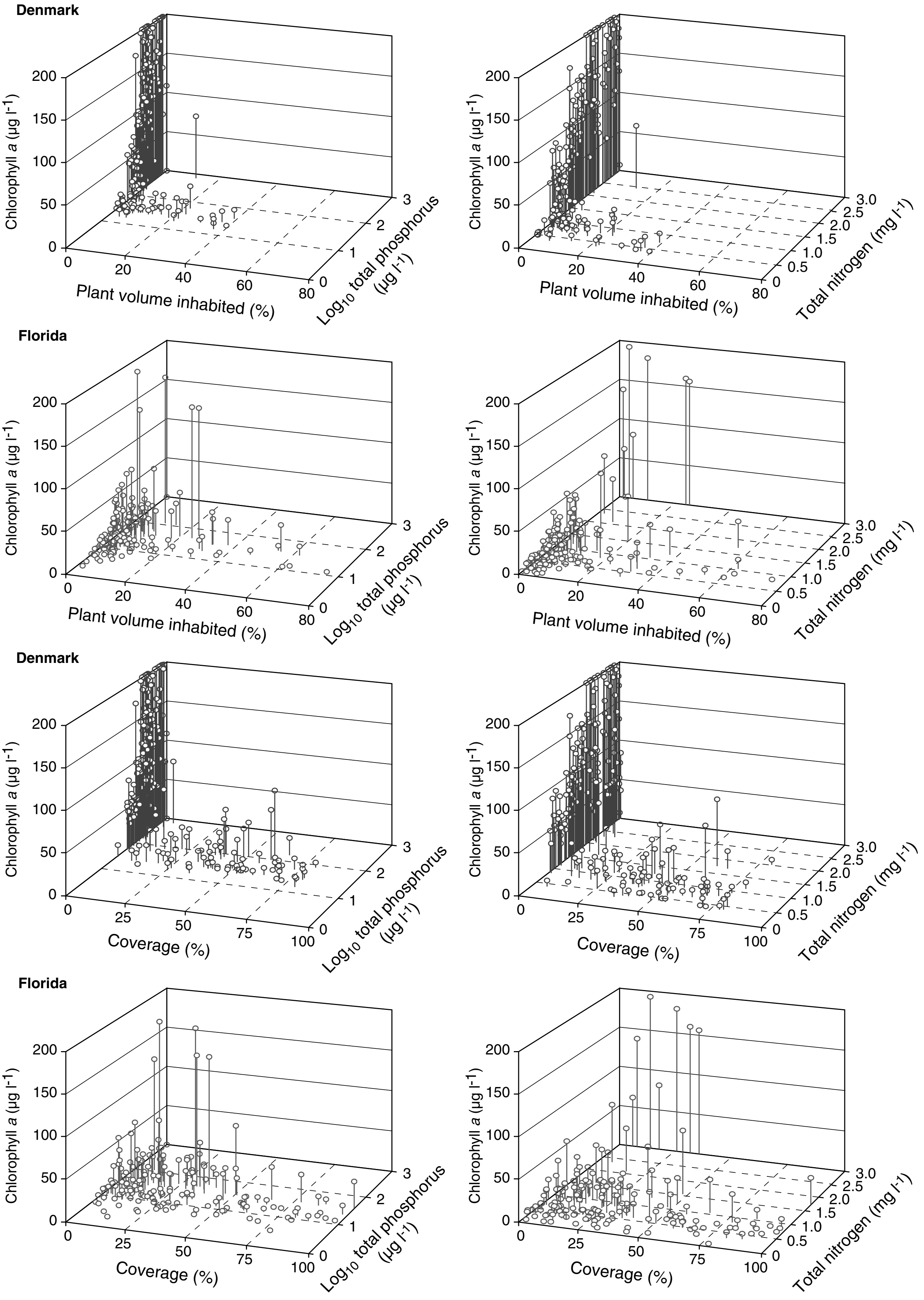

Figure 6. Chlorophyll a in the lake water at contrasting concentrations of total nitrogen and total phosphorus and coverage (A) and percentage of water occupied by plants (PVI, sensu Canfield et al., 1984) of submerged macrophytes in Florida and Danish shallow lakes in summer. Abbreviation at 200 µg l^{-1} chlorophyll a in some of the Danish lakes.

	Intercept	Log$_{10}$ (TP)	Log$_{10}$ (TN)	Log$_{10}$ (Cov)	Log$_{10}$ (PVI)	r^2	p <	n
COV								
DK	1.23±0.33	1.47±0.14		-0.73±0.08^{a}		0.78	0.0001	217
FL	-0.63±0.26	2.54±0.16		-0.25±0.12^{b}		0.61	0.0001	175
DK	4.03±0.10		2.01±0.25	-0.88±0.09^{a}		0.75	0.0001	227
FL	3.20±0.18		2.50±0.18	-0.38±0.13^{b}		0.53	0.0001	175
DK	1.58±0.33	1.17±0.15	1.07±0.25	-0.58±0.09^{a}		0.80	0.0001	217
FL	0.71±0.31	1.71±0.19	1.31±0.20	-0.29±0.11^{b}		0.68	0.0001	175
PVI								
DK	-0.24±0.37$^{n.s.}$	2.07±0.16			-0.69±0.15^{a}	0.78	0.0001	179
FL	-0.86±0.22	2.58±0.16			-0.22±0.12^{b}	0.60	0.0001	175
DK	3.59±0.09		3.42±0.26		-0.77±0.15^{a}	0.77	0.0001	189
FL	2.97±0.13		2.57±0.18		-0.36±0.14^{b}	0.53	0.0001	175
DK	1.06±0.40	1.28±0.20	1.90±0.33		-0.45±0.14^{a}	0.81	0.0001	179
FL	0.53±0.28$^{n.s.}$	1.74±0.19	1.35±0.20		-0.32±0.11^{a}	0.69	0.0001	175

Table 1. Relationship between chlorophyll *a* (μg l^{-1}) and total nitrogen (TN) and phosphorus (TP) and coverage (COV,%+1) and Plant Volume Infested (PVI, % +1) (all log transformed, base e) in a series of lakes from Denmark (DK) and Florida (FL) in summer. All slopes (± Std. error) on COV and PVI were significantly different among the two countries except for PVI together with nitrogen and phosphorus. Tests for differences in slope on COV and PVI between DK and FL are shown (a,b different; a,a not).

direction of a decreased capacity of the submerged plants to create and maintain clear-water conditions and of a higher predation on large-bodied zooplankton and lower grazer control of phytoplankton. Supporting this view, Gyllström et al. (2005) found the fish CPUE (multi-mesh sized gillnets) to zooplankton biomass ratio to increase and the zooplankton:phytoplankton biomass ratio to decrease from cold to warm shallow temperate lakes in Europe.

An alternative scenario to increasing macrophyte growth is stronger dominance of phytoplankton, as phytoplankton growth may be stimulated by higher internal P loading, which is highly sensitive to temperature in shallow lakes (Jensen and Andersen, 1992, Søndergaard et al., 2003; Jensen et al., 2006). Moreover, the growing season is extended and the risk of prolonged periods with blooming of noxious cyanobacteria most likely increases. Phytoplankton dominance may be further stimulated as the above outlined changes in fish community composition, size distributions and habitat choice may enhance predation on zooplankton and thus release phytoplankton from grazing. Moreover, in north temperate lakes higher disturbance of sediment is to be expected if cyprinids as predicted (Lehtonen, 1996) and likely also carp (*Cyprinus carpio*) become more abundant in the future warmer climate. In north-west Europe, higher precipitation combined with reduced winter frost enhance nutrient loss from cultivated fields, not least during autumn and winter (Andersen et al., submitted; JRC, 2005). In Southern Europe the risk of drought or low water concentrations in inland waters will increase and salinisation become a more widespread phenomenon due to increased evaporation and likely also enhanced consumption of water for irrigation purposes (Williams,1998 ; Zalidis et al., 2002). Less precipitation in these areas means lower nutrient loading to lakes, but this is not expected to compensate for the negative consequences of water loss. Nutrients will likely be up-concentrated and eutrophication increase (Zalidis et al., 2002). Moreover, at intermediate nutrient concentrations saline lakes tend to be more turbid than freshwater lakes at similar nutrient concentrations (Jeppesen et al., 1994).

Apart from the effects of warming, both phytoplankton and macrophyte production are expected to increase with increased CO$_2$ concentrations (Schippers et al. 2004a, b), at different rates according to variations in external conditions, such as nutrient status and alkalinity. Besides, local extinctions and the arrival of higher-temperature tolerant species will further complicate the final state and function of shallow lakes. Complications may also occur if changes in seasonality affect key interactions among different trophic levels (e.g. among fish, zooplankton and phytoplankton) leading to mismatch that may cascade through the food-web and ultimately affect the ecological state of the lake (Boersma et al, 1996; Benndorf et al, 2001; Winder & Schindler 2004).

However, we suggest that the most probable outcome of the effects of climate change, at least in Europe, is that the eutrophic lakes, whether phytoplankton or macrophyte dominated, will reach a more turbid state and, in southern Europe, become more saline as well. If true, it may therefore be more difficult to fulfil the present-day objectives set for the ecological state of the lakes without undertaking additional efforts to reduce nutrient loading to levels lower than the present-day expectations. This demands better management practise, including optimised fertiliser use and less intensive farming and improved sewage treatment. Moreover, re-establishment of lost wetlands will enhance N loss (particularly) and the P retention capacity, leading to reduced eutrophication. Re-establishment of buffer zones along streams may also reduce the nutrient input. To reduce the risk of salinisation, species loss, eutrophication and lowering of the water table of shallow lakes in Southern Europe, restrictions on human use of water are needed, which may imply less intensive agriculture in sensitive areas, improved recycling of water within catchments, increased efficiency with which water is allocated among different uses, and drought control.

Some challenges ahead

Although new knowledge of how shallow lakes respond to nutrient loading reduction has been gained recently, a large number of questions are still unresolved and need further consideration. Examples are:

Role of nitrogen. How important is nitrogen loading for the presence and absence of submerged macrophytes? If nitrogen is of importance, as recent studies suggest, then what are the causal mechanisms? How does the threshold concentration for plant disappearance vary with lake size, depth, trophic state, plant composition and climate?

Resistance, P hysteresis, alternative states. When do we expect P hysteresis and when not? Can P hysteresis sometimes be attributed to the fact that P loading reduction is usually not accompanied by a strong (if any) N loading reduction (apparent P hysteresis)? How is hysteresis influenced by climatic changes? Is it less strong in subtropical and tropical lakes with natural fish populations?

What is the role of the benthic-pelagic coupling and benthic facilitation for resistance to changes in loading, P hysteresis and existence of alternative states?

Fish manipulation. When is fish manipulation required to shift the lakes from turbid to clear? What are the long-term perspectives? Do we need repeated manipulations in the early phase to avoid strong perturbations that may eventually shift them back to the turbid state? How important is the risk of accumulating a ticking P bomb after biomanipulation (higher P accumulation in the sediment after manipulation) and how can we prevent its explosion?

Macrophyte growth. Which factors are most important for delaying the colonisation of plants after loading reduction – sediment content, waterfowl and fish grazing, limited amount of propagules and seeds, shading by periphyton and phytoplankton (including lack of grazing due to predation on snails and zooplankton, respectively)? How can we reinforce growth of macrophytes – sediment removal, protection against grazers, addition of seeds, change of water level or biomanipulation of fish? How can macrophytes be maintained, once established, and are some macrophyte communities more stable than others?

Role of climate. For northern shallow lakes more research is needed on the role of climate change on lake ecosystems not least on the effect of changes in seasonality on nutrient dynamics, trophic interactions and potential for shifts to a clear state after nutrient loading reduction. Little is known about how lakes in the subtropics and the tropics respond to nutrient loading reduction and about additional methods to reinforce the improvement of the ecological state. To what extent can we transfer knowledge about recovery from eutrophication from temperate lakes to the (sub)tropics and vice-versa? How realistic are the present-day objectives set for the ecological state of shallow lakes under the global warming scenario?

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National Environmental Research Institute
Frederiksborgvej 399
PO Box 358
DK-4000 Roskilde
Denmark
Tel: +45 4630 1200
Fax: +45 4630 1114

Management
Personnel and Economy Secretariat
Monitoring, Advice and Research Secretariat
Department of Policy Analysis
Department of Atmospheric Environment
Department of Marine Ecology
Department of Environmental Chemistry and Microbiology
Department of Arctic Environment

National Environmental Research Institute
Vejlsøvej 25
PO Box 314
DK-8600 Silkeborg
Denmark
Tel: +45 8920 1400
Fax: +45 8920 1414

Monitoring, Advice and Research Secretariat
Department of Marine Ecology
Department of Terrestrial Ecology
Department of Freshwater Ecology

National Environmental Research Institute
Grenåvej 14, Kalø
DK-8410 Rønde
Denmark
Tel: +45 8920 1700
Fax: +45 8920 1514

Department of Wildlife Ecology and Biodiversity

The structuring role of macrophytes on trophic dynamics
in shallow lakes under a climate-warming scenario

The effects of climate warming on the role of free-floating and submerged plants on trophic dynamics were studied by means of controlled laboratory and outdoor experiments and comparative field studies in a series of subtropical and temperate shallow lakes. In the subtropics, the typical warm-climate large free-floating plants, and submerged plants as well, do not seem to promote cascading effects generating increased water transparency, which contrasts with observations made in temperate shallow lakes. Our results suggest that a warming-related higher impact of fish may strongly affect the resilience of shallow lakes.

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