CASCADING EFFECTS OF PREDATORS IN TEMPERATE AND SUBTROPICAL SHALLOW LAKES

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PhD Thesis 2010

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Abstract: With a climate warming perspective, the present thesis aimed to compare the functioning of shallow lakes in contrasting climate zones by conducting a series of field mesocosm experiments and field surveys of the food web structure and trophic interactions in lakes located in subtropical Uruguay and temperate Denmark. The structuring role of fish was proven experimentally in both climate regions, with strong cascading effects on the pelagic food webs and particularly so in the subtropics. Together with higher fish diversity, trophic webs in warmer lakes were generally one trophic level shorter than their temperate counterparts. We argue that the widespread omnivory of fish across the food web explains the shorter food webs and the weakness of cascading effects, seldom reaching phytoplankton, in real lakes in the subtropics, thus having profound effects on ecosystem functioning. The complexity of trophic interactions in warmer systems may therefore weaken important positive feedback mechanisms, known from temperate lakes, and thereby decrease the stability and resilience of the clear water state. The evidence from subtropical lakes may, with caution, provide indications of the responses to be expected with warming in currently cold ecosystems.

Keywords: Climate warming, shallow lakes, fish structuring role, subtropical lakes, trophic interactions, trophic web structure

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Content

> "My momma always said, life was like a box of chocolates.
>
> You never know what you're gonna get."
>
> Forrest Gump

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List of included papers

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Iglesias, C., Mazzeo, N., Teixeira de Mello, F., Goyenola, G., Fosalba C., García. S. & E. Jeppesen. 2008 Field and experimental evidence of the effect of *Jenynsia multidentata* Jenyns (Cyprinodontiformes, Anablepidae) on the size distribution of zooplankton in subtropical lakes. Freshwater Biology. 53:1797-1807.

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Mazzeo, N., **Iglesias, C.**, Teixeira de Mello, F., Borthagaray, A., Fosalba, C., Ballabio, R., Larrea, D., Vilches, J., García, S., Pacheco J.P, & E. Jeppesen 2010. Trophic cascade effects of *Hoplias malabaricus* (Characiformes, Erythrinidae) in subtropical lakes foodwebs: a mesocosm approach. Hydrobiologia, 644: 225-235.

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Iglesias, C., Meerhoff, M., Johanson, L.S., Vianna, M., Mazzeo, N., Pacheco, J.P., Teixeira de Mello, F., Goyenola, G., González-Bergonzoni, I., Lauridsen, T.L., Søndergaard, M., Davidson, T.A. & E. Jeppesen. Food webs are more truncated in subtropical than temperate shallow lakes: Implications of fish omnivory in warm lakes. Manuscript.

1 Summary

Shallow lakes are important ecosystems that are directly related to and highly influenced by humans. These systems have long been studied by scientists. Eutrophication, the process of nutrient enrichment, and its consequences for whole-lake functioning, increased the scientific curiosity, which after years of field, experimental and modeling research resulted in the hypothesis of alternative states for shallow lakes. In a few words, this hypothesis states that over a wide range of nutrient concentrations, a shallow lake can present clear or turbid water with dominance of either macrophytes or phytoplankton, depending on a series of feedback mechanisms of which some are related to physico-chemical processes, while others reflect the biological interactions. Of key importance is the structure of the fish communities. If piscivores are abundant, they may control the planktivorous fish stocks with cascading effects, via zooplankton, resulting in low phytoplankton biomass, high water transparency and potentially abundant submerged macrophytes. If planktivorous fish dominate, however, grazing by zooplankton is typically low and phytoplankton biomass high. Accordingly, the lake is turbid and often without submerged macrophytes. This knowledge led to the development of a number of biological restoration techniques called 'biomanipulation', often involving a re-structuring of fish communities to achieve the desired clear water state. However, the alternative state hypothesis is based on research from northern temperate lakes and until recently knowledge of the functioning of warmer shallow lakes has remained limited. The studies conducted so far indicate that the functioning of warm lakes does not resemble that of the more well-studied temperate systems. Actually, the differences in fish community structure appear so prominent that the interactions described for temperate lakes might be markedly different in subtropical lakes. However, with the ongoing global warming, temperate lakes are being threatened not only by eutrophication, but also by rising temperatures that might affect the ecological dynamics and functioning in a direction similar to the current functioning of warmer lakes. Thus, seen in this context, understanding of the functioning of warm lakes becomes useful in two ways. On the one hand, the theoretical framework based on north temperate lakes can be tested on (sub)tropical lakes and, on the other hand, the new knowledge on trophic interactions in warm lakes may provide information about how temperate lakes will respond to the rising temperature in a future warmer world.

The present thesis aims to compare the functioning of both temperate and subtropical shallow lakes by conducting a series of field experiments and field surveys of the trophic web structure and trophic interactions in lakes located in contrasting climatic zones (subtropical Uruguay and temperate Denmark). We studied the occurrence of *Daphnia* in some subtropical lakes with contrasting fish densities and in egg banks in the surface sediment of 18 shallow lakes (Paper I). Also, in several lakes in both countries, we performed predator-exclusion mesocosm experiments (Paper III) and field studies of food webs tracked by stable isotopes analyses (Paper VI). Finally, in Uruguay, we conducted an outdoor trophic-cascade mesocosm experiment (Papers II & IV) and an extensive sampling of organisms belonging to various trophic levels during contrasting seasons (Paper V).

The structuring role of fish seemed highly important in both climatic zones. It has been previously shown that fish body size is smaller, abundance is higher and the littoral habitat use stronger in the subtropics. However, our results demonstrated other series of differences in fish community structure

and functioning between climate regions. Together with the widespread prevalence of omnivory in the subtropics, the predation pressure on primary consumers is higher. This leads to dominance of small-bodied zooplankton. However, medium- and large-sized zooplankton can occur in subtropical lakes, when fish predation is removed. When the fish predation pressure was extremely low in fishless Lake Rivera (Uruguay), a large amount of large-bodied *Daphnia* appeared, which is in accordance with the findings of *Daphnia* resting eggs in an important number of lakes and with the occurrence of *Daphnia* in our mesocosm experiments when released from fish predation (Papers I & III). This strongly suggests that predation, more than metabolic constraints, is of key importance for the dominance of small-bodied zooplankton in the (sub)tropics (Paper I). Regardless of climatic zone, our experimental results showed that fish affected the composition, biomass and total abundance of zooplankton (Papers I, II & III). Moreover, in the subtropics, the experimental addition of a top predator fish led to a trophic cascade down to the phytoplankton level, similar to the response under fish free conditions, while mesocosms holding only prey fish had low zooplankton but high phytoplankton biomass (Paper IV). We did not find experimental evidence that potential predator macroinvertebrates should affect zooplankton structure in both climatic zones, as we had hypothesized. We did not find as clear effects of either fish or potentially predatory macroinvertebrates on the littoral food webs as seen for the pelagic webs (Paper III). Fish negatively affected plant-associated macroinvertebrate abundance in both climatic zones, but did not reach the periphyton level. We claim that the poor colonization of the artificial plant beds (enclosure effect) and the significant absence of snails may be responsible for the absence of a clear cascading effect. Potentially predatory macroinvertebrates (*Palaemonetes argentinus* in subtropical and *Gammarus lacustris* in temperate lakes) had no effect on plant associated-macroinvertebrates or periphyton. We present evidence (Paper V) that the cascading effects observed under experimental conditions are weaker in natural subtropical lakes than in the temperate region, which may be attributed to differences in food web architecture between the two climatic zones (Paper VI). Trophic webs in subtropical lakes are generally one trophic level shorter than in their temperate counterparts, despite the higher fish diversity, and fish show a higher degree of omnivory than temperate lakes. Thus, intermediate consumers make a very large contribution to subtropical food webs, acting as integrators of the different carbon sources and enhancing the transfer of the basal carbon to the next trophic positions.

Our findings contribute to the growing knowledge that the structure and functioning of subtropical shallow lakes are more complex than those of similar lakes in temperate regimes and that some of the mechanisms behind the stabilization of the clear water phase appear to be weakened by the complex trophic interactions in sub(tropical) lakes. This again may have strong implications for the management of temperate lakes that are facing a warming process.

Dansk resumé

Lavvandede søer er vigtige økosystemer, der er i høj grad udsættes for menneskelig påvirkning. Denne søtype har længe været genstand for videnskabelig interesse, blandt andet fordi de er talrige og har været genstand for særlig kraftig eutrofiering. Mange års forskning omfattende feltarbejde, forsøg og modellering har ført til hypotesen om alternative tilstande i lavvandede søer. Kort beskrevet siger denne hypotese, at en lavvandet sø – over et bredt næringsstofniveau – kan være i enten en klar eller en uklar tilstand med dominans af enten undervandsplanter eller planteplankton, der begge kan været markant påvirket af en række feedback-mekanismer, hvoraf nogle er relateret til fysisk-kemiske processer og andre styret af de biologiske samfund. Af afgørende betydning er fiskesamfundenes sammensætning. Findes der mange rovfisk i systemet, kan de kontrollere de planteædende fisk, hvilket kan have en kaskadeeffekt – via dyreplanktonet – nedad i systemet til planteplanktonet, og søen kan fastholdes i en klarvandet tilstand med mange undervandsplanter. Er der derimod få rovfisk, og er søerne næringsrige, bliver de domineret af planteplankton, fordi byttefiskene holder dyreplanktonet nede, og dermed bliver græsningen på planteplanktonet lille. Denne erkendelse førte også til udvikling af metoder til sørestaureringer ved 'biomanipulation', og en ofte benyttet metode er, at fiskebestanden justeres med henblik på at opnå kaskadevirkning og dermed en klarvandet tilstand. Hypotesen om alternative tilstande er baseret på resultater fra nordligt beliggende tempererede søer, og indtil for nylig har vores viden om mekanismerne og muligheder for at restaurere søer ved biomanipulation i varmere, lavvandede søer været begrænset. Hidtidige undersøgelser tyder dog på væsentlige forskelle mellem de biologiske samspil i varme søer og de mere velundersøgte tempererede systemer. Specielt er fiskesamfundenes sammensætning og størrelsesfordeling så markant forskellig, at de beskrevne interaktioner og feedback-mekanismer kendt for de tempererede søer sikkert ikke er gældende for de varme søer. Med den globale opvarmning trues de tempererede søer ikke alene af eutrofiering, men også af de direkte effekter af stigende temperaturer. Studier af varme søer kan derfor være nyttig af to grunde. For det første giver det en mulighed for at teste teorien om alternative ligevægtstilstande på de varme søer, og for det andet kan ny viden om trofiske interaktioner i varme søer give informationer om, hvordan tempererede søer vil reagere i fremtiden i takt med den globale opvarmning.

Målet med denne afhandling har været at sammenligne biologiske samfund og samspil i tempererede og subtropiske lavvandede søer baseret på en række felteksperimenter samt undersøgelser i naturlige søer beliggende i to forskellige klimatiske zoner (Uruguay og Danmark). Vi undersøgte forekomst af *Daphnia* i søer i Uruguay, som havde en meget forskellig tæthed af fisk, og i overfladesediment (hvileæg) i 18 lavvandede søer (Artikel I). I flere søer i begge lande udførte vi eksperimenter i indhegninger med og uden fisk og med og uden potentielle makroinvertebrat-prædatorer (Artikel III). Vi gennemførte også undersøgelser af fødekæderne i forskellige søer baseret på analyser af stabile isotoper (Artikel VI & V). Endelig gennemførte vi mere specifikke felteksperimenter i subtropiske søer til vurdering af betydningen af rov- og byttefisk (Artikel II & IV) og en ekstensiv undersøgelser gennem sæsonen af en række organismer repræsenterende forskellige trofiske niveauer (Artikel V).

Fiskesamfundet havde stor indvirkning i begge klimazoner. Det har tidligere været vist, at fiskene gennemgående er mindre, forekommer i højere tæthed og er mere tilknyttet littoralzonen (undervandsplanterne) i de subtropiske søer. Vores resultater viste dog en række andre forskelle i fiskesamfundenes sammensætning og samspil mellem de to klimatiske zoner. Vi fandt, at mange af fiskearterne er "altædende" i de subtropiske søer (Artikel VI), og dette – samt dominansen af små former i høj tæthed – fører til høj prædation på primærkonsumenterne, inklusiv dyreplanktonet. Middelstore og store dyreplanktonarter kan dog forekomme i subtropiske søer, når prædation fra fisk mangler eller er lav. Således forekom *Daphnia* i høj tæthed i den næsten fiskefrie Lake Rivera, hvilket stemmer godt overens med, at vi fandt hvileæg af *Daphnia* i overfladesedimentet i mange af søerne og i eksperimenterne uden fisk (Artikel I & III). Dette giver stærk evidens for, at det er prædation og ikke fysiologiske begrænsninger, der fører til dominans af små dyreplanktonarter i varme søer (Artikel I). I en række eksperimentelle undersøgelser viser vi, at kaskadevirkningen i vandfasen i de subtropiske søer er af samme størrelsesorden som i de tempererede søer (Artikel I, II & III).

Eksperimenter under subtropiske forhold viste endvidere, at en tilførsel af fiskeædende fisk førte til en trofisk kaskade via dyreplankton til planteplanktonet, der var af samme størrelse, som hvis fisk ikke var til stede, mens indhegninger, som kun havde byttefisk, havde lav dyreplanktonbiomasse og høj biomasse af planteplankton (Artikel IV). Vi fandt ikke eksperimentel evidens for, at potentielle makroinvertebrat-prædatorer påvirkede dyreplanktonet, hverken i de subtropiske eller tempererede søer, som vi ellers havde forventet. Vi fandt heller ikke så klare effekter af fisk eller potentielle invertebrat-prædatorer på de littorale fødenet, som vi fandt i pelagiet (Artikel III). Fisk påvirkede de plantetilknyttede makroinvertebrater markant i begge klimazoner, men der var ingen effekt på perifyterne. Det kan skyldes en "indhegningseffekt", da f.eks. snegle helt manglede. De potentielle invertebrat-prædatorer (*Palaemonetes argentinus* i de subtropiske og *Gammarus lacustris* i tempererede søer) havde ikke nogen effekt på de plantetilknyttede makroinvertebrater eller på perifyterne. Vore resultater (Artikel V) tyder endvidere på, at kaskadevirkningen i naturen er langt mindre udtalt end i eksperimenterne og mindre i subtropiske søer end i tempererede søer, hvilket tilskrives forskelle i fødekædernes sammensætning (Artikel VI). Fødekæderne i de subtropiske søer var således generelt et trofisk niveau lavere end i de tempererede søer på trods af en højere fiskediversitet, og fiskene var gennemgående langt mere altædende end i de tempererede søer. Intermediære konsumenter har derfor stor betydning i fødekæderne i subtropiske søer, hvilket sikrer en effektiv udnyttelse af fødekilderne og en effektiv videreførelse af stof op gennem fødekæden.

Vores resultater peger på, at de biologiske samfund og samspil er mere komplekse i subtropiske lavvandede søer end i tilsvarende tempererede søer, og at det tilsyneladende mindsker chancen for at stabilisere den klarvandede tilstand.

2 Introduction

2.1 The role of predation in shallow lake food webs under different climates

Aquatic communities and food webs are structured by the available resources and by predation (Carpenter et al., 1987), classically known as bottom-up and top-down driving forces (McQueen et al., 1986). The importance of fish predation for the structuring of the freshwater zooplankton community is well documented, particularly in temperate shallow lakes in Europe and North America. Fluctuations in planktivorous fish abundance may induce major shifts in the size distribution of zooplankton (Hrbáček et al., 1961; Brooks & Dodson, 1965) or behavioural shifts (Timms & Moss, 1984; Schriver et al., 1995; Lauridsen & Lodge, 1996; Burks et al., 2002; Romare & Hansson, 2003).

Limnological studies have traditionally focused on the communities and the trophic interactions in the pelagic habitat rather than on the benthic and littoral habitats (Carpenter et al., 2001; Vadeboncoeur et al., 2002). However, littoral processes are often highly important for whole-lake functioning (Carpenter & Lodge, 1986; Jeppesen et al., 1997; Jeppesen, 1999), particularly because fish and zooplankton move between the habitats and thus constitute an important linkage between the pelagic, benthic and littoral zones (Vander Zanden & Vadeboncoeur, 2002; Jones & Waldron, 2003). Littoral areas are typically characterized by the presence of aquatic macrophytes, which play an important structuring role in most freshwater ecosystems (Jeppesen et al., 1998) and directly and indirectly affect the water transparency and important trophic interactions (Moss, 1990; Scheffer et al., 1993; Jeppesen et al., 1998). Macrophytes compete with phytoplankton for nutrients and light, but may also 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 clear water conditions in lakes with high macrophyte coverage (Burks et al., 2002).

However, in warm climate lakes, the impact of macrophytes on water clarity is apparently substantially reduced (Jeppesen et al., 2007b; Kosten et al., 2009). One reason may be that macrophytes do not function as proper refuges for zooplankton (Meerhoff et al., 2006; Meerhoff et al., 2007b). The subtropical littoral fish communities are characterized by higher species richness, higher densities, higher biomass, greater trophic diversity (with predominance of omnivores and often lack of true piscivores (Branco et al., 1997; Quirós, 1998), and smaller body size than in similar temperate lakes (Teixeira de Mello et al., 2009). Experiments have described important effects of common planktivorous fish (*Odontesthes bonariensis* Valenciennes; *Jenynsia multidentata* Jenyns) on subtropical zooplankton community structure (Boveri & Quirós, 2007), while other studies deal with zooplankton behavioural shifts related to predation risk (Meerhoff et al., 2007b; Trochine et al., 2006; Iglesias et al., 2007). The effects of fish predation in warmer climate regions are less well understood (Meerhoff et al., 2007a), but various evidence suggests that fish zooplanktivory is stronger here.

As a consequence, the zooplankton communities of tropical and subtropical shallow lakes are continuously exposed to high predation pressure by small omnivorous–planktivorous fish (Fernando, 1994; Lazzaro, 1997; Pinel-Alloul et al., 1998) and often also to large macroinvertebrate predators (e.g. shrimps and the phantom midge *Chaoborus*), which seem to be of great importance (Boschi, 1981; Collins & Paggi, 1998; Collins, 1999; Iglesias et al., 2007; González-Sagrario et al., 2009). Consequently, the zooplankton are often dominated by small cladocerans, copepod nauplii and rotifers (Crisman & Beaver, 1990; Dumont, 1994; Branco et al., 2002; Garcia et al., 2003; Havens et al., 2007; Meerhoff et al., 2007b, Kruk et al., 2009), resulting in an often low grazing pressure on phytoplankton.

Recent works have also shown a substantially lower abundance of plant-associated macroinvertebrates among plant beds in warm lakes than in similar temperate lakes (Meerhoff et al., 2007a). By consuming plant-attached macroinvertebrate grazers, such as snails, fish may indirectly enhance periphyton growth, as suggested by experiments (Brönmark & Weisner, 1992; Jones & Waldron, 2003; Liboriussen et al., 2005a) and field data (Jones & Sayer, 2003); however, there are some studies showing no effects (Bertolo et al., 2000). Meerhoff et al. (2007a) also found higher biomass of periphyton on the plants in the temperate lakes despite higher abundances of grazing macroinvertebrates and potentially poorer growth conditions (i.e. lower temperature and light). The reason may be that the subtropical fish and shrimps feed on the periphyton to a larger extent than the temperate fish. Thus, fish (at least several species) may partly become primary consumers and move down their trophic position, contrary to fish in temperate regions. This fact positively reinforces high fish abundance, but also implies that the food webs are likely more truncated in the subtropics than in temperate shallow lakes.

2.2 Potential implications in a climate warming scenario

The potential effects of climate warming on shallow lakes are being investigated using different approaches, such as long-term historical and paleolimnological data (Battarbee, 2000), model simulations (Scheffer et al., 2001), controlled experiments (McKee et al., 2003; Liboriussen et al., 2005b), and application of the space-for-time substitution approach in field studies (Jeppesen et al., 2003; Moss et al., 2004; Meerhoff et al., 2007a). The last approach implies that the results from experimental manipulations and parallel field studies conducted in similar ecosystems of different climates may provide much needed data to improve our understanding of the potential effects of warming on the functioning of the currently colder ecosystems.

Climate models predict that the mean annual global surface temperature will increase by another 1-3.5 °C by the year 2100, warming being more pronounced at higher latitudes (Rouse et al., 1997), but also of significance in subtropical regions (Mulholland et al., 1997). Predictions are that temperatures will rise 3 to 5 °C in most parts of Europe (IPCC, 2007). Moreover, some works also predict even warmer scenarios promoted by positive feedbacks between warming and atmospheric CO_2 concentrations (Scheffer et al., 2006).

Shallow lakes and ponds appear to be quite sensitive to climate warming because they have a large surface area to volume ratio, and hence closely mirror main climatic temperatures (McKee et al., 2002a). The extent of the changes is likely very variable; some species may be entirely excluded from warmer systems due to physiological stress, or via interactions with other species, while for others species some key life-history traits may be altered (Schindler, 1997). Nevertheless, some major freshwater communities, such as macrophytes, macroinvertebrates and zooplankton, so far seem quite resilient to experimental warming (McKee et al., 2002a; McKee et al., 2002b), but strong physico-chemical changes may occur (McKee et al., 2003). Some evidence suggests that warming will exacerbate the effects of eutrophication (Jeppesen et al., 2007a) and that fish appear to be particularly sensitive to warming (Jeppesen et al., 2010). The narrow temperature tolerance of some fish and the indirect effects of warming causing oxygen depletion near the sediments have been pointed out as the main reasons for the loss of fish populations in mesocosm experiments (*Gasterosteus aculeatus*, Moran et al., 2010). Therefore, the different scenarios potentially resulting from climate warming seem very diverse and perhaps contradictory; on the one hand, fish activity may be enhanced and their winter survival may increase, resulting in higher zooplankton consumption (Balayla et al., 2010) and phytoplankton development (Moss et al., 2004; Jeppesen et al., 2005); but, on the other hand, if fish abundance declines the zooplankton will be released from fish predation and might thus exert a higher control of phytoplankton crops (Scheffer et al., 2001).

3 Research approach and questions

In this thesis, I have aimed at further elucidating the role of fish and potentially predatory macroinvertebrates in the trophic structure of shallow lakes under different climates, with special focus on pelagic (i.e. fish-zooplankton-phytoplankton) and littoral (i.e. fish-macroinvertebrate-periphyton) interactions. For this purpose, and in a complementary way, we have combined controlled experiments at different scales (outdoor and in-lake mesocosm experiments) with comparative field studies of trophic webs in subtropical (Uruguay: 30º-35º S) and temperate (Denmark: 55º-57ºN) shallow lakes. All these studies have aimed at providing complementary evidence to enhance our knowledge in order to answer the following research questions:

Q1) Do typical and abundant small planktivorous fish structure the zooplankton community in subtropical shallow lakes?

I aim at answering this question in Papers I and II.

Q2) Does the response of zooplankton and littoral macroinvertebrates to predation differ between temperate and subtropical lakes? Do these differences cascade down the trophic web resulting in differences in phytoplankton and periphyton biomass?

I aim at answering these questions in Paper III.

Q3) Can piscivorous fish promote trophic cascading effects in warm lakes (i.e. control the planktivorous fish, thereby allowing zooplankton to control phytoplankton crops), as has been observed in temperate lakes?

I aim at answering this question in Papers IV and V.

Q4) Are the food webs more truncated in subtropical lakes? Are subtropical food webs fuelled by periphyton to a larger extent than temperate food webs?

I aim at answering these questions in Paper VI.

4 The structuring role of fish and cascading effects in the littoral and the pelagic food webs

The trophic cascade hypothesis (Carpenter & Kitchell, 1996) has been frequently applied to temperate lakes involving many experimental studies and field research (Carpenter & Kitchell, 1996; Scheffer & Jeppesen, 1998; Jeppesen et al., 1998; Moss et al., 1998). In a few words, this hypothesis states that changes at one trophic level can transfer downwards and thereby affect other levels that are not directly connected. For example, an increase in planktivorous fish biomass would indirectly result in a greater biomass of phytoplankton, whereas an increase in the biomass of piscivorous fish would result in a reduction of phytoplankton biomass (Fig. 1). The trophic cascade effects of native fish in South American tropical and subtropical lakes are, however, less well studied and sometimes their very occurrence has been extensively debated (Lazzaro, 1997; Jeppesen et al., 2005; Jeppesen et al., 2007b).

In temperate climates, in contrast, the impacts of fish on ecosystem functioning are well documented (Moss et al., 1996; Carpenter & Kitchell, 1996, Jeppesen et al., 1998). Depending on the food web structure fish

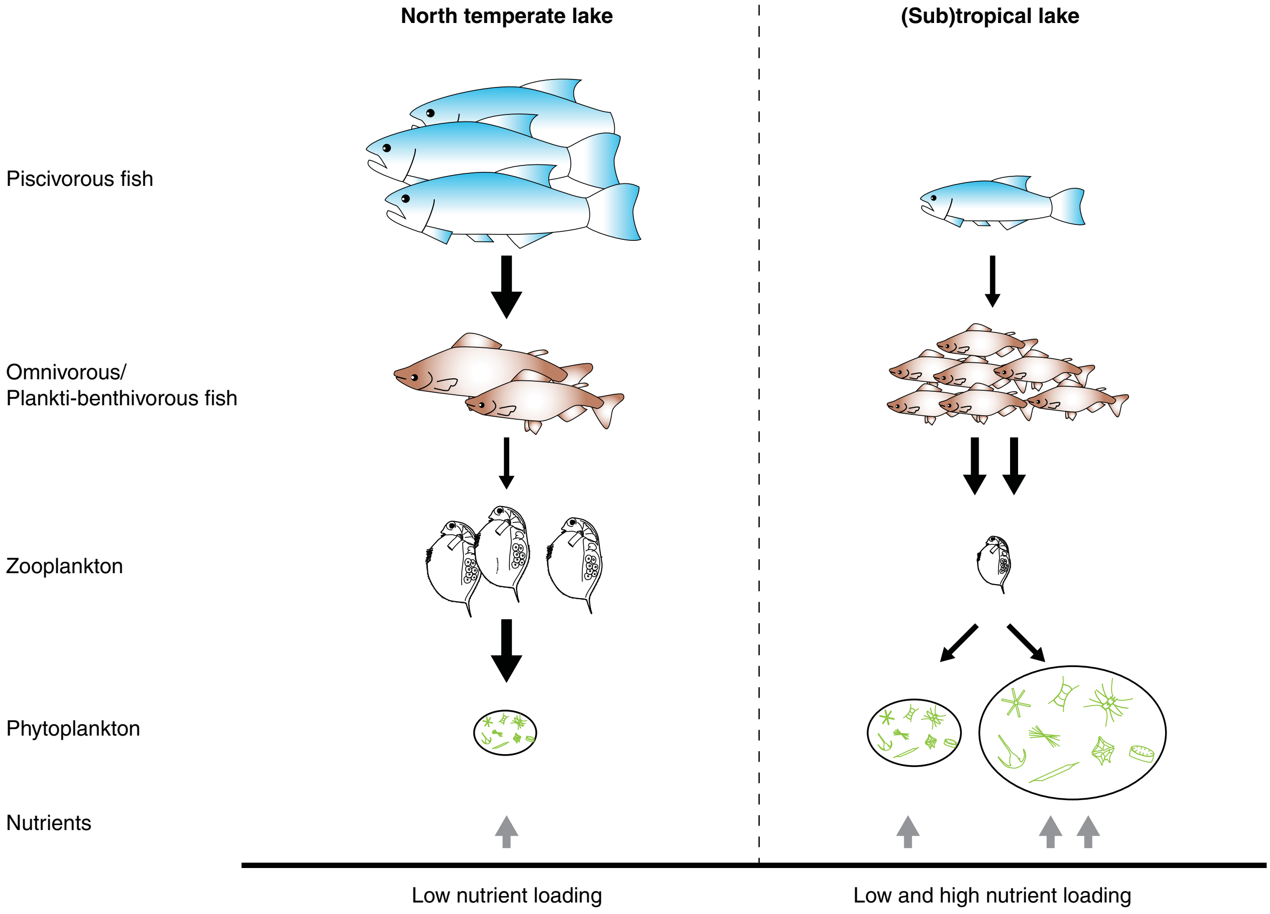


Figure 1. Conceptual model showing trophic structure changes among non-eutrophied temperate and non-eutrophied and eutrophic subtropical lakes. In north temperate non-eutrophied lakes, plankti-benthivorous fish are controlled by piscivorous fish, releasing the predation on large-bodied zooplankton (*Daphnia* spp.). Therefore, grazing on phytoplankton is relatively high and the clear water phase is maintained. In subtropical lakes, however, lakes are dominated by numerous small omnivorous fish and grazing on zooplankton is negligible with little effect on phytoplankton development. (Modified from Jeppesen et al., 2010).

may promote and maintain either the clear or the turbid water state of shallow lakes (Scheffer et al., 1993). In such temperate systems, planktivorous fish may induce major shifts in the size distribution (Hrbácek et al., 1961; Brooks & Dodson, 1965) and sometimes also in the behaviour of zooplankton (Timms & Moss, 1984; Lauridsen & Lodge, 1996; Burks et al., 2002; Romare & Hansson, 2003). Modifications of the fish community have been used as a tool to improve the lake water quality of eutrophic temperate lakes (biomanipulation, *sensu* Shapiro et al., 1975). By direct removal of planktivores or addition of piscivores, positive effects on water transparency have been achieved and re-establishment of aquatic vegetation has occurred in many temperate European shallow lakes, although the long-term success of such measures is debated (Jeppesen et al., 2007a; Søndergaard et al., 2008).

Fish predation seems to be much stronger in warm lakes than in comparable temperate lakes (Meerhoff et al., 2007a), likely due to the characteristics of the subtropical fish assemblages described above (Teixeira de Mello et al., 2009). In this sense, Gillooly & Dodson (2000) found that the mean body length of limnetic cladocerans decreases from cold temperate to tropical regions, in both the northern and the southern hemispheres, though these authors claimed that physiological constraints were of key importance for this latitude gradient in size. Lacerot et al. (submitted) found a similar pattern in cladoceran size in a comprehensive study of shallow lakes covering a latitudinal gradient in South America (5°- 55°S) and attributed it to changes in the size of fish.

Several pieces of evidence strongly support the idea that fish predation is the key structuring factor for the dominance of small zooplankton in warm (or lower latitude) lakes (Papers I, II, III). Based on the combined study of resting eggs of *Daphnia* spp. that appeared in the sediments of 18 Uruguayan lakes and the experimental fish exclusion experiments (Box 1, Paper I), we provide evidence that large-sized cladocerans (e.g. *Daphnia* spp.) may occur even at high temperatures (Fig. 2). Contrarily to Moore et al. (1996), who suggested that the lower abundance and smaller mean sizes of *Daphnia* in warmer regions could be related to a lower upper thermal tolerance of these organisms, we registered the presence of *Daphnia* at water temperatures of 28ºC in two out of three subtropical lakes after two weeks of fish exclusion (Paper I). The average body size of cladocerans seems also to be directly affected by fish (Figs. 2 & 5, Papers I, III), and likely only indirectly by temperature. The mean size of *Daphnia* (*D. obtusa*) in a subtropical fishless lake (Lake Rivera, Montevideo, Uruguay) exceeded the mean body size of cladocerans expected for the latitude 35° (Gillooly & Dodson, 2000). In contrast, in a similar subtropical lake with

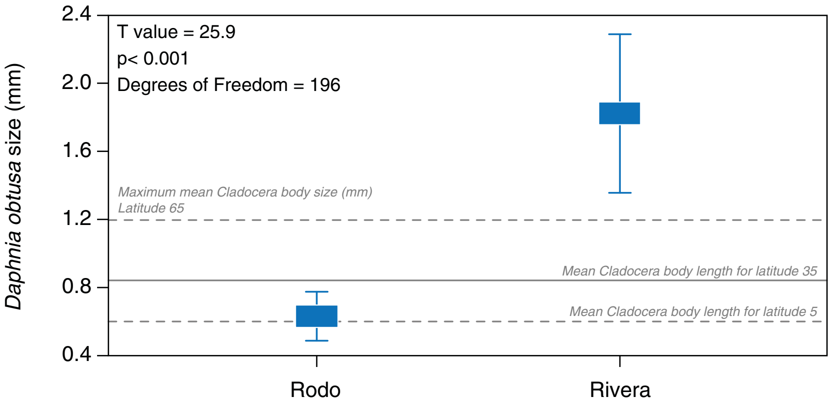


Figure 2. Field evidence from two urban subtropical Uruguayan lakes. Body length of *D. obtusa* in Lake Rivera and Lake Rodó. Above: mean values (±1 SE) of all females analyzed in relation to the body length expected from Gilloly & Dodson (2000); the statistics correspond to unpaired t-test. Below: distribution of frequency of body sizes. Modified from Paper I.

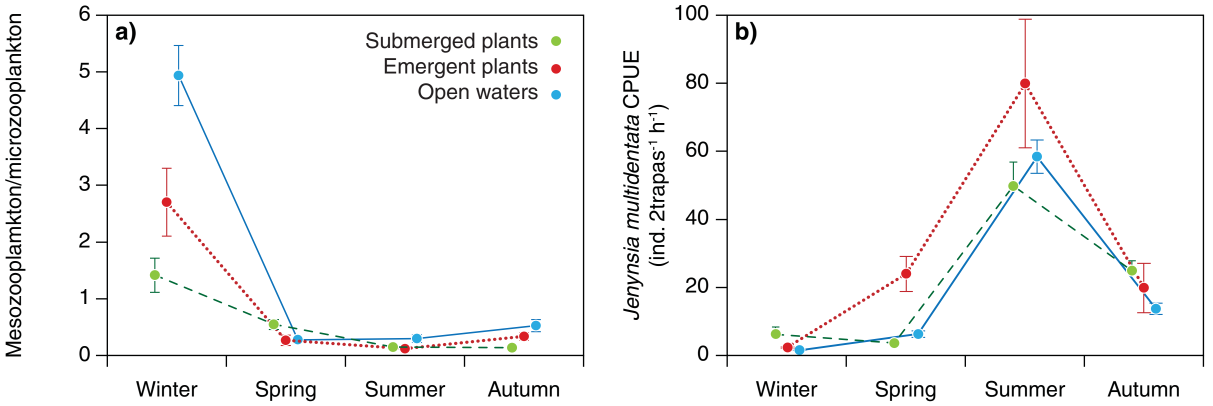


Figure 3. Seasonal variation in: (a) herbivorous mesozooplankton/microzooplankton abundance ratio and (b) the CPUE of the omnivorous planktivorous fish *Jenynsia multidentata*; in submerged plants (green), emergent plants (red) and open waters (blue). Error bars represent ± 1 SE. Mesozooplankton = cladocerans + calanoid copepodites and adults, microzooplankton = rotifers +nauplii. Modified from Paper II.

high fish densities (Lake Rodó, Montevideo, Uruguay) the mean sizes of *Daphnia* were smaller than expected (Gillooly & Dodson, 2000), most likely as a result of the strong predation by fish (Fig. 2). Other cladocerans (e.g. medium-sized *Diaphanosoma*) may also be present during colder periods of the year, coinciding with comparatively low fish abundance or activity (Fig. 3, Paper II). When the typical dominance by small-sized individuals within the fish community (Teixeira de Mello et al., 2009) disappears, either due extensive fish kills (Paper I), experimental manipulation (Papers III and IV) or even natural forces (Lacerot et al., submitted), the structure of zooplankton may shift towards a cladoceran dominated community (in terms of abundance and biomass, Fig. 4).

With the experimental design shown in BOX 1, we aimed at testing the effects of both common fish and of large (and potentially predatory) macroinvertebrate species on the zooplankton. We found that even in a highly structured environment (high artificial %PVI in Paper III, compared to low %PVI in Paper IV) and with high availability of other food items for the fish (e.g. macroinvertebrates and periphyton attached to artificial plants), subtropical zooplankton were very sensitive to fish, with strong implications for community structure and cladoceran mean body sizes. In contrast to the strong effects of fish, both alone and together with macroinvertebrates, potentially predatory macroinvertebrates alone (i.e. shrimps, *Palaemonetes argentinus*) did not induce significant effects on the zooplankton communities in the subtropical lakes (Fig. 4, Paper III). The experimental results in the temperate lakes were similar in nature to those found in the subtropical lakes. Cladoceran abundance was also significantly reduced in the treatments with fish. The (potentially) predatory macroinvertebrates (in this case *Gammarus lacustris*), like shrimps in subtropical lakes, had no negative effects on the zooplankton (Fig. 4, Paper III).

The grazing pressure of zooplankton, estimated as the zooplankton : phytoplankton biomass ratio, decreased significantly in treatments with fish in all lakes in both climatic zones (Fig. 5). This decrease led to enhanced phytoplankton biomass in the treatments with fish in both climatic zones (Fig. 5). Our experiment highlights the role of predators, particularly fish, as an important structuring force in the pelagic food webs of shallow lakes, in both temperate and subtropical climates. We attribute the significant decrease in phytoplankton biomass to the concomitant increase in zooplankton biomass observed only when fish were absent (Fig. 5).

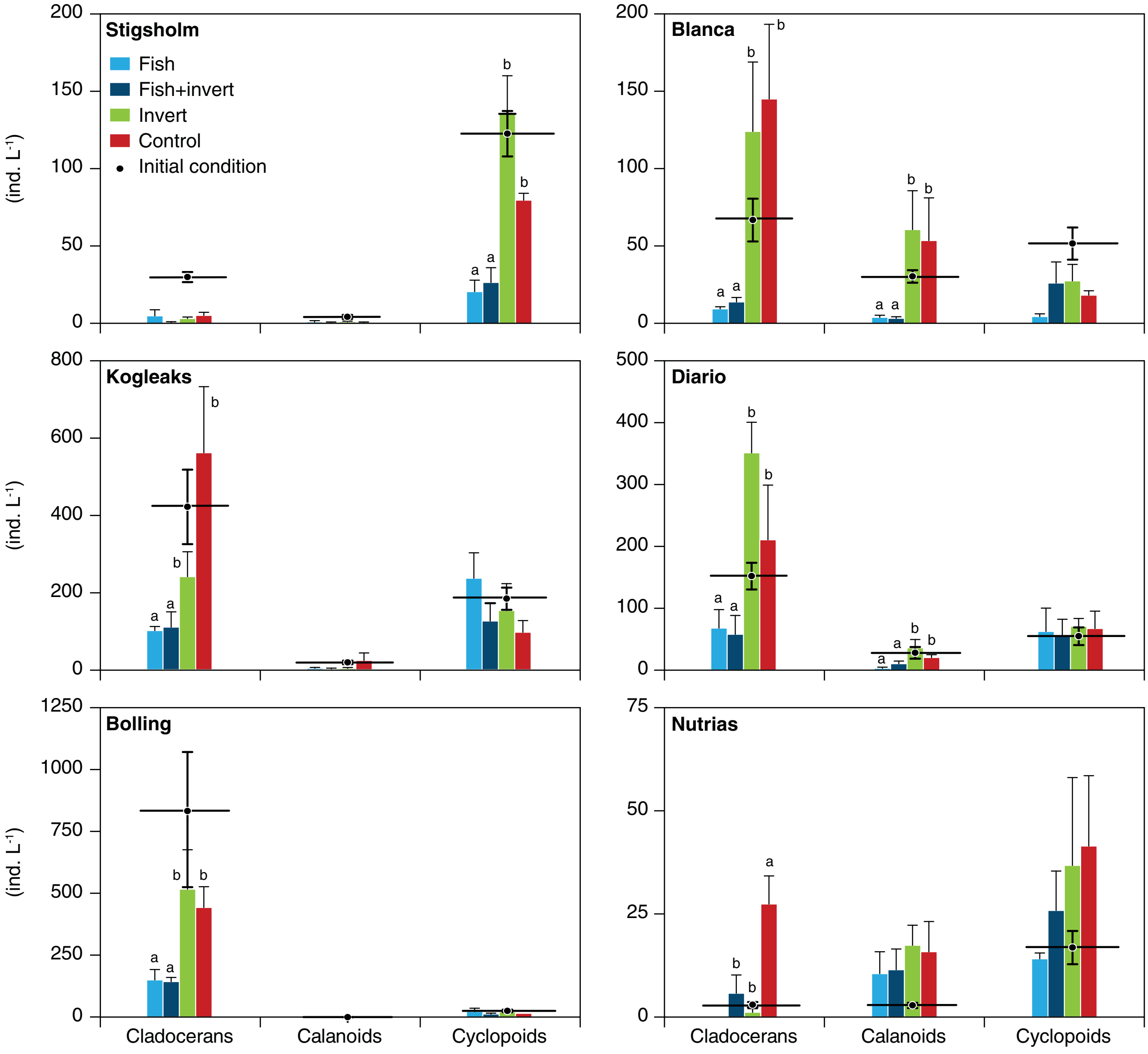


Figure 4. Effects of experimental treatments on the abundance of zooplankton in Uruguayan (right) and Danish (left) lakes. Horizontal lines represent initial conditions (just before treatment assignment and 15 days after fish exclusion), while columns show final conditions (49 days after fish and macroinvertebrate addition). Error bars represent ± 1 standard error. Please note the different scales on the y axes. Letters indicate similar groups as shown by the Tukey post hoc tests when ANOVA tests indicated significant differences. Modified from paper V.

A clear effect of planktivorous fish on 3-level trophic webs appeared from the experimental studies, as suggested earlier from empirical field data (Persson et al., 1991; Persson et al., 2001; Jeppesen et al., 2007b; Iglesias et al., 2007; Meerhoff et al., 2007a) and, potentially, the removal of planktivorous fish could be used to enhance grazing on algae and thereby create a clearwater state (Gulati, 1990; Hansson et al., 1998a; Søndergaard et al., 2007, 2008). However, removal of planktivorous fish will likely only have short-term effects in the field in subtropical lakes due to fast recovery of the fish population (Jeppesen et al., 2007b). We therefore added an extra trophic position to test the potential cascading effects of piscivores (BOX 2). Our experimental results were conclusive in that we did find trophic cascading effects of piscivorous fish. *Daphnia obtusa* abundance decreased in the treatment with omni-planktivorous fish (*Jenynsia multidentata*), while it remained high in the control and the piscivorous fish treatments (Fig. 6, Paper IV). Moreover, a cascading effect of the piscivorous *Hoplias malabaricus* on phytoplankton was detected after the first 11 days of

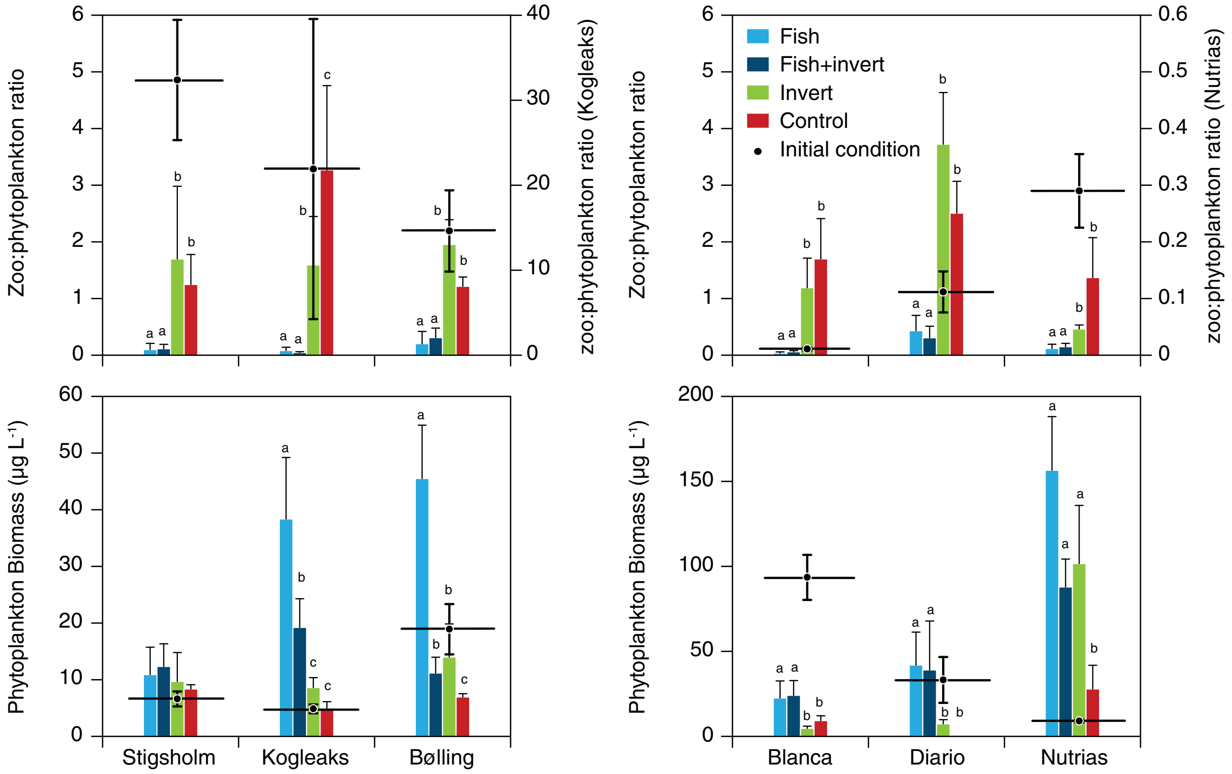


Figure 5. Effects of treatments on phytoplankton biomass and zooplankton grazing pressure (lower panels) biomasses in subtropical (right) and temperate (left) lakes. Horizontal lines represent initial conditions (just before treatment assignment and 15 days after fish exclusion), while columns show final conditions (49 days after fish and macroinvertebrate addition). Error bars represent ± 1 standard error. Please note the different scales on the x axes. Letters indicate similar groups according to Tukey's post hoc tests. Modified from paper III.

the experiment and lasted for a period of one month (Fig. 7, Paper IV). However, the strong cascading effect triggered by piscivorous fish that we observed under experimental conditions (Papers II & IV; Sinistro, 2010; Fontanarrosa et al., 2010) is not typically observed in whole-lake studies from the subtropics (Kruk et al., 2009; Pacheco et al., 2010; Fig. 8, Paper V), as otherwise seen in non-eutrophied temperate lakes (Carpenter & Kitchell, 1996; Jeppesen, 1998; Scheffer & Jeppesen, 1998). In a series of four subtropical shallow lakes, varying in trophic state and water transparency (Paper V), the abundance of planktivorous fish was not related to the seasonal changes in piscivorous fish abundance (Fig. 8), and the seasonal changes in zooplankton abundance were negatively related to variations

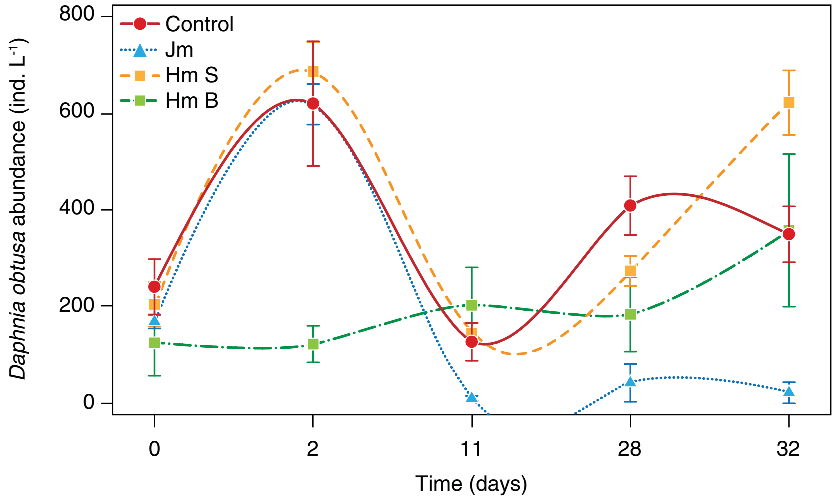


Figure 6. Temporal changes in *Daphnia obtusa* abundance. The graph includes the mean of three replicates and the error bars (±1SE). Control: two trophic levels food web treatment (phytoplankton + zooplankton); JM: three trophic levels food web treatment (phytoplankton + zooplankton + *J. multidentata*); HM s and HM b = four trophic level treatment with small *H. malabaricus* and big *H. malabaricus* specimens, respectively, (phytoplankton + zooplankton + *J. multidentata* + *Hoplias malabaricus*).
Modified from paper IV.

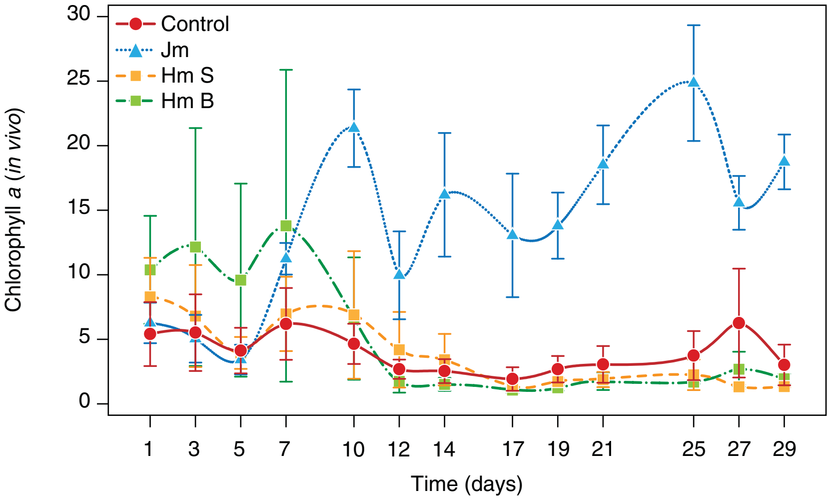


Figure 7. Temporal variation in phytoplankton biomass estimated by fluorescence in vivo. The graphs include the mean of three replicates and the error bars (±1SE). Control: two trophic levels food web treatment (phytoplankton + zooplankton); JM: three trophic levels food web treatment (phytoplankton + zooplankton + *J. multidentata*); HM s and HM b = four trophic level treatment with small *H. malabaricus* and large *H. malabaricus* specimens, respectively, (phytoplankton + zooplankton + *J. multidentata* + *H. malabaricus*).
Modified from Paper IV.

in the abundance of planktivorous fish. In addition, in all cases zooplankton density and phytoplankton biovolume exhibited the same seasonal variations (Fig. 8), and no relationships were found between piscivorous fish and water transparency or between piscivorous fish abundance and phytoplankton (Paper V). These findings all indicate an overall low top-down effect of piscivorous fish in these subtropical lakes.

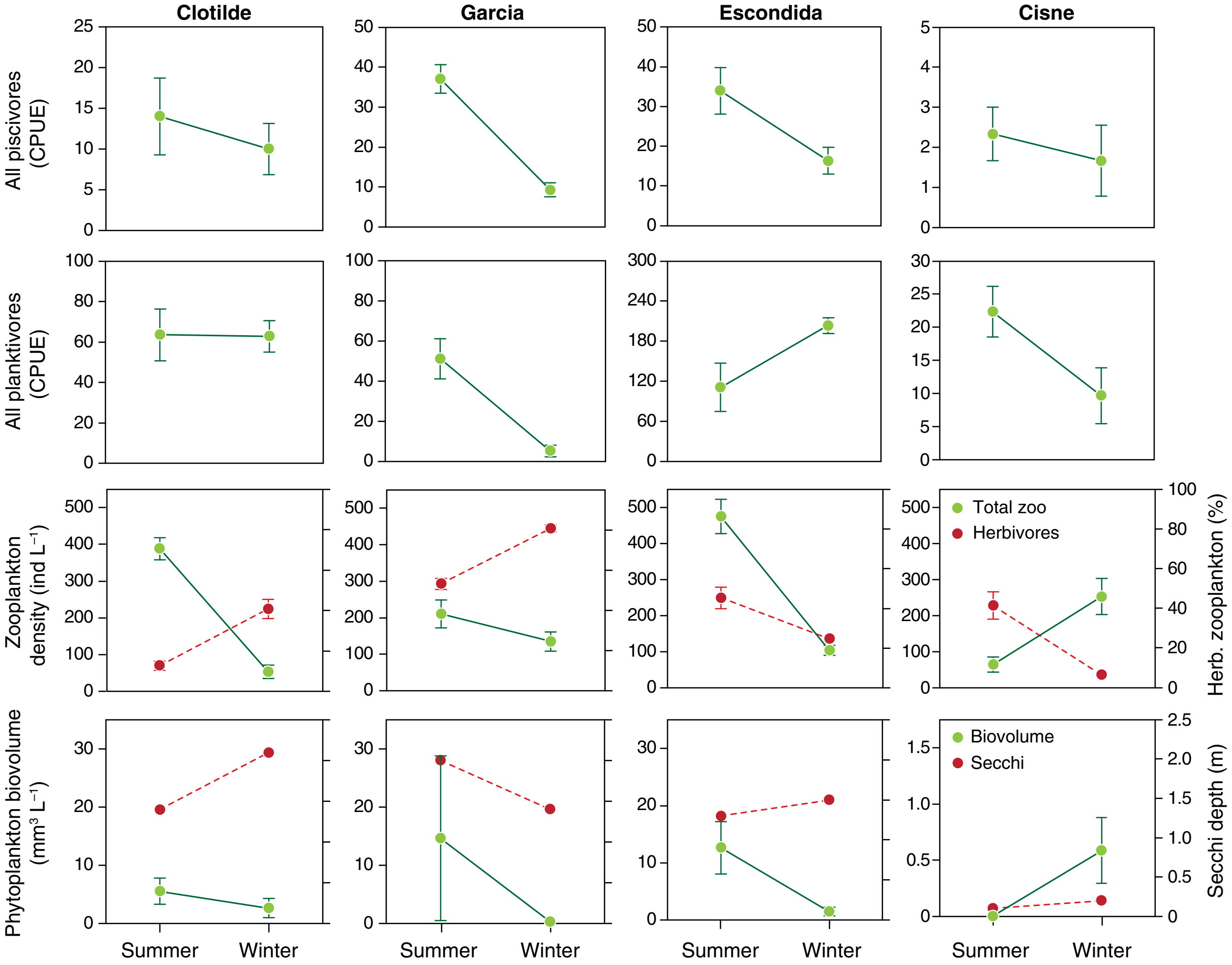


Figure 8. Temporal variation in cascading effects on 4 subtropical lakes. From the top, all 4 trophic levels are represented, piscivores and planktivores CPUE, zooplankton abundance and phytoplankton biovolume and Secchi depth for summer and winter. Cascading effects are evidenced at the 3 superior levels of the trophic webs with a very strong link in the middle of the webs between planktivores and zooplankton but a weak link between zooplankton and phytoplankton. This kind of interactions resulted in absence of a clear positive cascading effect from piscivores to phytoplankton. From Paper V.

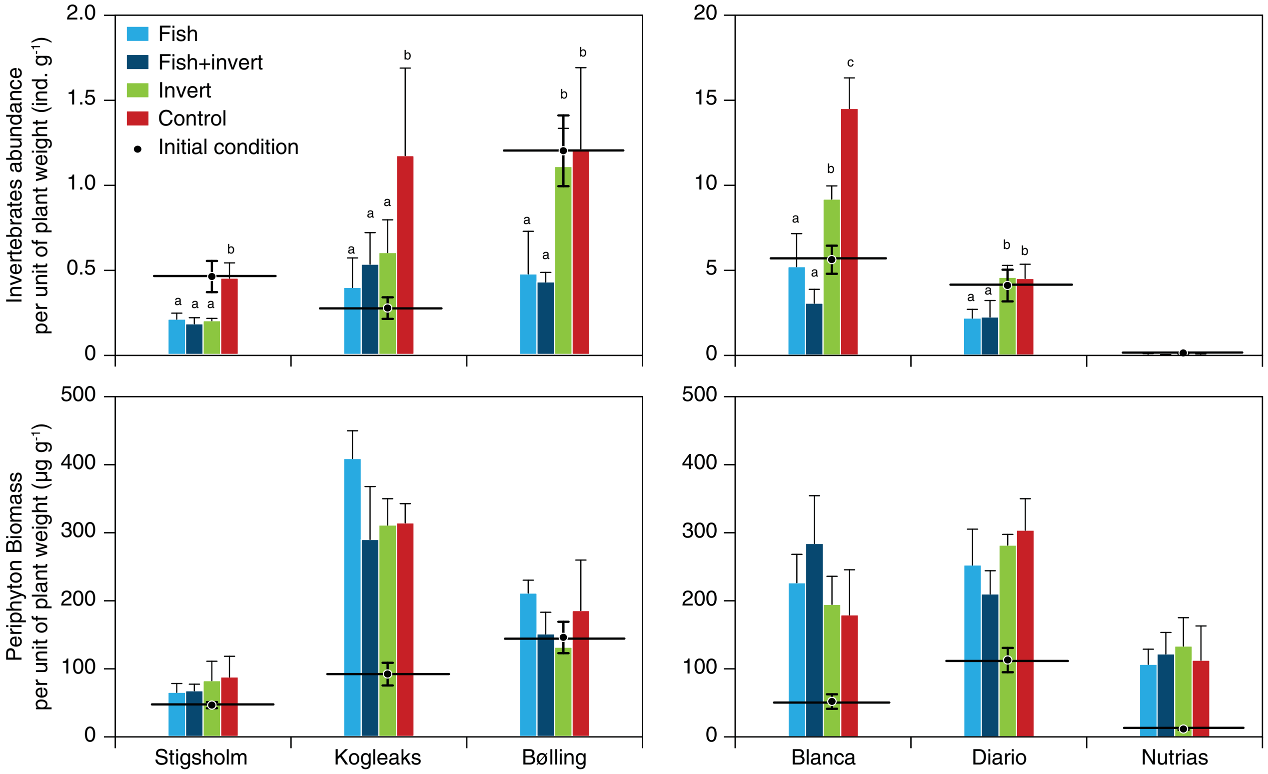


Figure 9. Density of plant-associated macroinvertebrates (upper panels) and periphyton biomass (lower panels) in subtropical (right) and temperate (left) lakes. Horizontal lines represent initial conditions (just before treatment assignment and 15 days after fish exclusion), while columns represent final conditions (49 days after fish and macroinvertebrate addition). Error bars represent ± 1 standard error. Please note the different scales on the x axes. Letters indicate similar groups according to Tukey's post hoc tests. Modified from Paper III.

In contrast to the consistent, clear findings for the pelagic interactions, the so-called littoral pathway (Schindler & Scheuerell, 2002; Vadeboncoeur et al., 2002) was more complex. We found no direct or cascading effects from fish to periphyton in any of the two climate zones (Fig. 9). This apparently contradicts the hypothesis that in subtropical lakes fish and/or shrimps could be the explanation for the differences in periphyton biomass, as previously suggested (Iglesias et al., 2007; Meerhoff et al., 2007a) as a consequence of a high degree of omnivory and the littoral habitat preference of most subtropical fish (Teixeira de Mello et al., 2009). In contrast, in temperate regions the lack of effects of fish on periphyton seems to be relatively common (Hansson, 1992; Bécares et al., 2008) and has also been observed in mesocosm experiments (Brönmark & Vermaat, 1998; Bertolo et al., 2000; Fig. 9, Paper III).

While we used closed mesocosms with fixed fish densities, the previous experiment by Meerhoff et al. (2007a) used open plant beds, allowing greater disturbance and potentially stronger grazing effects of high numbers of fish than our manipulated densities. However, the differences may also reflect an oversimplification of our subtropical predator assemblages, with lower total densities and much lower richness than under natural conditions (2 spp compared to approx. 10 spp per lake, Kruk et al., 2006), likely leading to greater availability of alternative, more nutritious, food sources such as zooplankton and macroinvertebrates. Evidence of the effects of fish on the littoral food webs is thus controversial, and more complex experiments resembling natural taxon diversity and densities are needed before we can draw any firm conclusions on the functioning of littoral food webs.

We conclude that trophic cascades can be observed under experimental conditions in the subtropics – to a similar extent as in temperate lakes. These trophic cascades seem particularly pronounced in the pelagic food webs involving omni-planktivorous fish, zooplankton and phytoplankton. Moreover, in our outdoor experiment including a piscivore we also obtained strong cascading responses. However, these apparently promising results must be interpreted with caution, as several whole-lake studies conducted so far (including the results presented here) indicate that the responses in natural lakes do not fully resemble the experimental results and that the cascading effects in the natural lakes are much less significant.

BOX 1 (Paper III)

Trophic cascades under contrasting climates – a mesocosm experiment

Methods

We selected three shallow lakes in Uruguay (30–35°S) and Denmark (55–57°N). Eight mesocosms were placed along each side of a specially constructed bridge that allowed sampling with minimum disturbance. The water level inside the mesocosms varied between 0.8 m and 1.1 m during the course of the experiment, approximating a total water volume of 1000 litres. Prior to the establishment of the mesocosms, all natural vegetation was carefully removed from the area. Fish were prevented from entering by adding a fine mesh net to the bottom of each enclosure during the construction. In each mesocosm an artificial plant bed mimicking submerged plants was established. The modules were kept floating on the water surface and consisted of 1.2 m diameter PVC plastic rings with the artificial plants attached to a net. The submerged plants (120 per module) were made of "hairy" 1.0 m long plastic pieces (originally green Christmas tree garlands) with an architecture similar to that of macrophytes such as *Myriophyllum* sp.

Design

A 4×4 factorial design with four replicates per treatment was set up adding, respectively, F: omni-planktivorous fish; F+INV: planktivorous fish + omnivorous macroinvertebrate; INV: omnivorous macroinvertebrate, and CON: no fish/no macroinvertebrate added (control). As our aim was to test the importance of the predators' assemblage, in each country we used two common and abundant fish species and a typical omnivorous macroinvertebrate:

		Fish sp. 1	Fish sp. 2	Macroinvertebrates
Subtropical	Name	*Cnesterodon decemmaculatus*	*Jenynsia multidentata*	*Palaemonetes argentinus*
	Density*	50 (42)	40 (33)	120 (100)
Temperate	Name	*Gasterosteus aculeatus*	*Perca fluviatilis*	*Gammarus lacustris*
	Density*	12 (10)	6 (5)	240 (200)

*values correspond to number of individuals added and (density m^{-2})

Zooplankton, plant-associated macroinvertebrates, phytoplankton and periphyton were sampled at initial conditions and 2 months later, together with the complete array of physico-chemical variables under consideration. Abundance and biomass were determined and compared among treatments and between countries.

BOX 2 (Paper IV)

Cascading effect of piscivores in subtropical mesocosm experiments

Methods

A 1-month experiment in six outdoor 3800 L mesocosms (3 × 2 × 0.60 m) was conducted in April to May to test direct and indirect effects of common subtropical fish species, the piscivore *Hoplias malabaricus* and the planktivore *Jenynsia multidentata*, on zooplankton and phytoplankton community structure. The bottoms of the mesocosms were covered with washed river sand and they were filled with groundwater. 30 % of the total surface area was covered with the free-floating plant *Eichhornia crassipes*. The experimental units were allowed to settle for 2 months prior to initiation of the experiment to enable development of phytoplankton and zooplankton. High nitrogen (N) and phosphorous (P) inputs were maintained, to prevent any confounding effects of nutrient limitation, by weekly additions of 5 mg L^{-1} N and 5 mg L^{-1} P to each unit, as commercial fertilizer NPK (15-15-15) ISUSA, total nitrogen (TN), P_2O_5 and K_2O, respectively.

Design

Four different treatments were randomly assigned to the experimental units:

1. Control: no fish, zooplankton + phytoplankton (n=3)
2. JM: *J. multidentata* (9 ind m^{-2}) + zooplankton + phytoplankton (n=3)
3. HMs: *H. malabaricus* with SL≤15cm. (0.5 ind m^{-2}) + *J. multidentata* + zooplankton + phytoplankton (n=3)
4. HMb: *H. malabaricus* with SL>30 cm. (0.5 ind m^{-2}) + *J. multidentata* + zooplankton + phytoplankton (n=3)

5 Food web architecture in subtropical and temperate shallow lakes

Understanding of food web dynamics has become crucial for the elaboration of theoretical frameworks as well as of management strategies, such as manipulation of fish communities (biomanipulation) in temperate shallow lakes. Most frequently, fish represent the central core of aquatic food webs. Their role is often complex as they may occupy different trophic positions over time due to ontogenetic diet shifts or differences in available resources (Persson & Hansson, 1999). Several properties of food webs are directly or indirectly affected by ambient temperature, and differences between the architecture of food webs in subtropical and temperate climates are thus to be expected (Vander Zanden & Fetzer, 2007).

While several fish species in the temperate zone display some degree of omnivory (Vadeboncoeur et al., 2002; Vander Zanden & Vadeboncoeur, 2005), subtropical fish assemblages are generally believed to display widespread omnivory (Lazzaro, 1987; Winemiller, 1990; Jeppesen et al., 2010). Omnivory, defined here as feeding on more than one trophic level (*sensu* Pimm & Lawton, 1978), can theoretically promote both a stabilizing and a destabilizing effect on ecosystems, depending on where it occurs in the food chain (Yodzis, 1984; Pimm, 1982). Theoretical linear models predict that a change in one trophic position should positively affect every second link below in the trophic web, and this assumption is the basis for the trophic cascading effects observed in temperate shallow lakes (Carpenter and Kitchell, 1996) and the biomanipulation techniques involving fish community manipulation (Shapiro et al., 1975; Hansson et al 1998a). Experimental approaches have shown that these kinds of responses can be over simplistic and only partially explain the observed patterns (Persson et al., 2001); among others, omnivory may obscure the predicted linear response depending on the vulnerability of the prey (Hansson et al., 1998b). For example, in the link between planktivorous fish and a vulnerable prey like *Daphnia*, omnivory may result in a strong predation pressure leading to a decrease in the population of *Daphnia* (Stein et al. 1995, Paper I). The strong effect of omnivorous fish on keystone groups such as large Cladocerans (Hansson et al., 2004) may diminish the resilience of the clear water state in shallow lakes (Scheffer et al., 1993).

The trophic web length is another food web emergent property that potentially may affect ecosystem stability (Pimm, 1982; Post & Takimoto, 2007). Recent works (Post & Takimoto, 2007; Vander Zanden & Fetzer, 2007) suggest that resource availability plays only a minor role in determining the trophic web length in natural systems, particularly in temperate lakes, except under extremely low resource conditions (Post, 2002a). Trophic web length may also be affected by temperature, as energetic limitations may be more important in warmer climates (McNab, 2002). In such situations, the potential predators often have to feed on a wide range of prey items (potentially from different trophic levels) to satisfy their energy demand.

Apart from determining food web interactions in the lakes in different climate zones, we aimed at testing whether subtropical trophic webs are shorter or more truncated than temperate webs (Paper VI), as a consequence of different fish community structure and trophic interactions (Fig. 10, Meerhoff et al., 2007a). We used carbon and nitrogen stable isotope analyses to compare the food web structures in a total of nine shallow

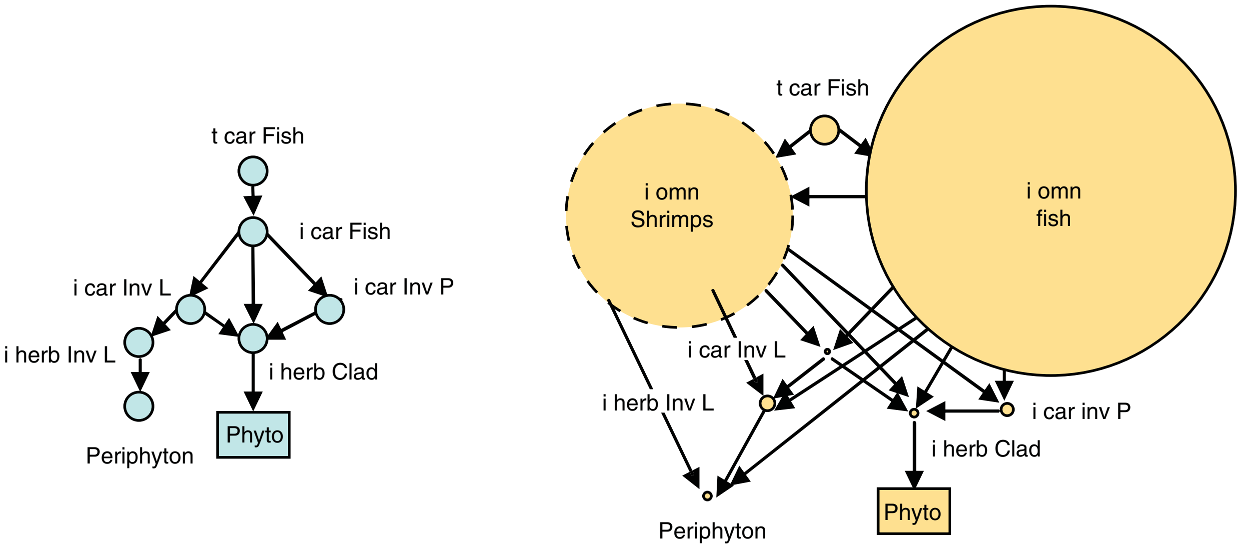


Figure 10. Simplified scheme of trophic interactions in temperate and subtropical lakes based on the measured densities of individuals. The densities in the subtropics are expressed relative to those in the temperate lakes (considered as the unit, for being the most known web). The shapes proposed there are quite similar to the ones we describe here using stable isotopes techniques to construct the food webs. Used Abbreviations, phytoplankton (Phyto), 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). Modified from Meerhoff et al., (2007a).

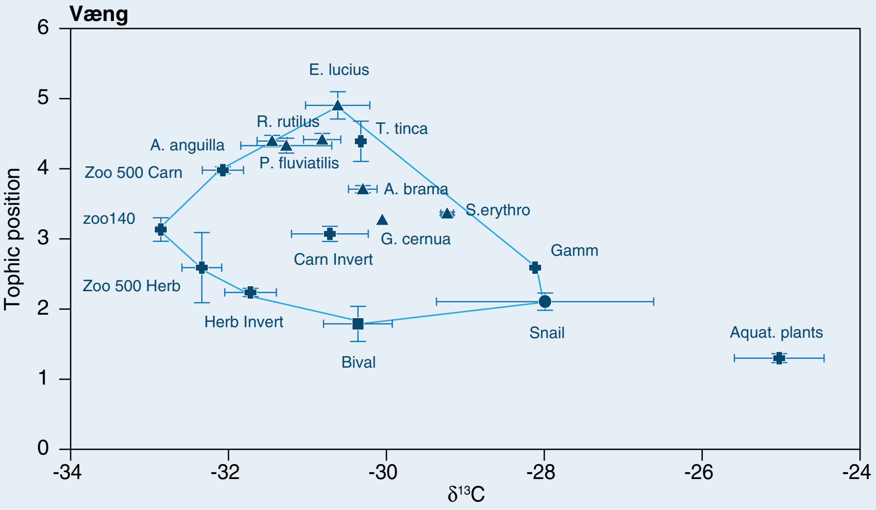
lakes, 5 in subtropical (Uruguay, 30–35 °S) and 4 in temperate (Denmark, 55–57 °N) climate zones (described in Table 1 of Paper VI). This approach complements well the experiments already described and contributes to further elucidate the differences in the functioning of the trophic webs (Cabana & Rasmussen, 1996; Vander Zanden & Rasmussen, 2001; Jones & Waldron, 2003) of shallow lakes in the two climate zones.

Nitrogen and carbon isotopes are often used to study trophic web structure and sources and fluxes of energy and carbon in aquatic systems. Both have two stable (i.e. not radioactively decaying) isotopes, $^{12}C/^{13}C$ and $^{14}N/^{15}N$ (the lighter being the more abundant in both cases), and are useful because biological processes often 'prefer' one isotope over the other. This 'preference' may cause changes in the stable isotope ratio (so-called fractionation) between adjacent trophic levels in a typical trophic web (e.g. a consumer compared to its prey). An increase of about 2-3 in the $\delta^{15}N$ values among each trophic position usually occurs, whereas hardly any fractionation for carbon is found. Plants, on the contrary, show little fractionation for N (ranges from –5 to +5) but high fractionation for C (ranges from –10 to –25; Fry, 2006). Thus, N isotopic fractionation is used to determine the position in the trophic web (e.g. primary, secondary, tertiary consumer), while carbon isotopic signals are used to determine main carbon sources. It may thus be determined whether and to what extent a certain trophic web is mostly based on phytoplankton or periphyton (Fry, 2006). Trophic web structures or architectures can be compared after estimating the trophic position of each individual (following Post, 2002b), the Trophic Web Length (TWL: maximum trophic position in the system) and several other community-wide metrics (Box 3; Layman et al., 2007).

Supporting our hypothesis, the trophic webs in the set of subtropical shallow lakes were shorter than those in the temperate lakes. Temperate fish species showed higher trophic position (apical positions usually occupied by, for instance, pike, *Esox lucius*), resulting in longer TWL in the temperate lakes. The subtropical fish at the highest trophic level in our study

BOX 3 (Paper VI)

Community-wide metrics of trophic webs based on SIA



Nitrogen and carbon isotopes are the isotopes most frequently used in aquatic ecology to study trophic web structure and energy and carbon sources and fluxes. They are useful because biological processes often 'prefer' one isotope over another. This 'preference' causes changes in the stable isotope ratio (fractionation) between one trophic level and the next. An increase of about 3 in the $\delta^{15}N$ values from one trophic level to the next has been seen, whereas hardly any fractionation for carbon occurs. Results are reported in δ notation as parts per thousand (‰) differences from the recognized standard (Pee Dee Belemnite for ^{13}C and atmospheric N_2 for ^{15}N):

$$\delta X=[(R_{sample}/R_{standard})-1\ ] \times 1000$$

where X is the isotope of interest (^{13}C or ^{15}N) and R is the ratio of this isotope to the lighter one ($^{13}C/^{12}C$ or $^{15}N/^{14}N$).

Wide-metrics were calculated as:

%Cont-Lit $= [\delta^{13}C_{consumer} - \delta^{13}C_{pelagic})/(\delta^{13}C_{littoral} - \delta^{13}C_{pelagic})] \times 100$

measures relative contribution of different carbon sources, where $\delta^{13}C_{consumer,}$ $\delta^{13}C_{pelagic}$ and $\delta^{13}C_{littoral}$ are the mean $\delta^{13}C$ of the consumer, pelagic and littoral sources.

Trophic Position (Tr-Po) $=2+[(\delta^{15}N_{consumer} - \delta^{15}N_{base})/2.98]$ measures the Tr-Po of each species.

$\delta^{15}N_{consumer}$ and $\delta^{15}N_{base}$ are the isotopic signatures of consumer and averaged baseline organisms, 2.98 is the expected ^{15}N fractionation per trophic level and 2 is the trophic level of the baseline organism.

Trophic web length (TWL) = Maximum Tr-Po for each lake.

Total and per Trophic Level $\delta^{13}C$ range (CR, CR2, CR3) = difference between the most $\delta^{13}C$-enriched and the most $\delta^{13}C$-depleted values in the entire trophic web (CR) and for each TL (e.g. CR2 was calculated by the extreme $\delta^{13}C$ values registered for individuals belonging to the second trophic level).

Total area (TA) = The convex hull area given by all species in the $\delta^{13}C$-$\delta^{15}N$ biplot. TA represents a measure of the total amount of niche space occupied by the trophic web.

Mean Nearest neighbour distance (NND) = mean of the Euclidean distances to the nearest neighbour (NND) of each species in the biplot. NND is a measure of the overall food web density of species packing.

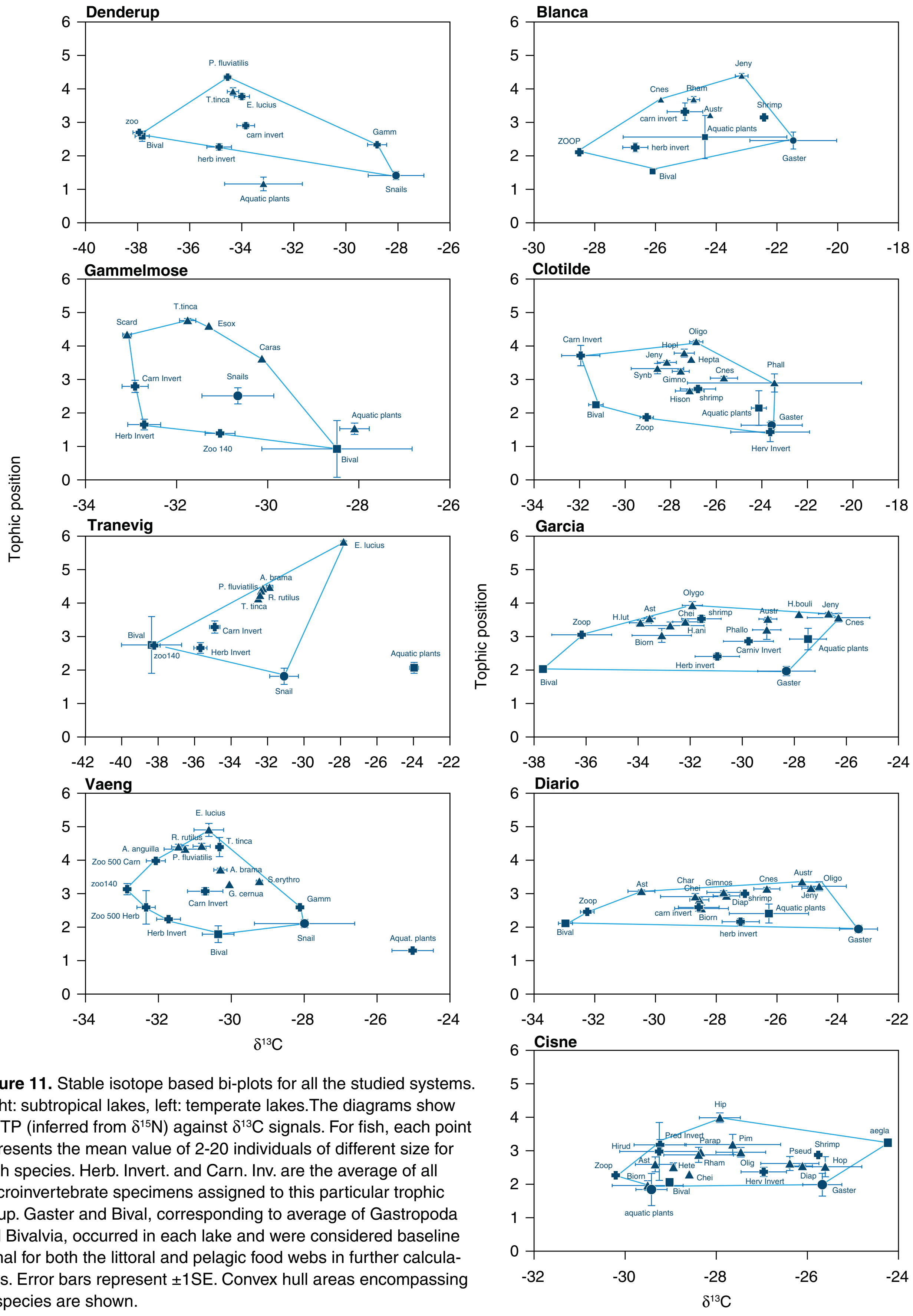


Figure 11. Stable isotope based bi-plots for all the studied systems. Right: subtropical lakes, left: temperate lakes.The diagrams show the TP (inferred from $\delta^{15}N$) against $\delta^{13}C$ signals. For fish, each point represents the mean value of 2-20 individuals of different size for each species. Herb. Invert. and Carn. Inv. are the average of all macroinvertebrate specimens assigned to this particular trophic group. Gaster and Bival, corresponding to average of Gastropoda and Bivalvia, occurred in each lake and were considered baseline signal for both the littoral and pelagic food webs in further calculations. Error bars represent ±1SE. Convex hull areas encompassing all species are shown.

were typically one trophic position lower than in the temperate lakes. This implies that one step of energy transfer through the food web is missing (Fig. 2 in Paper VI). Besides, most subtropical fish species were at the same trophic position in most lakes (Paper VI). In addition, some of the large-

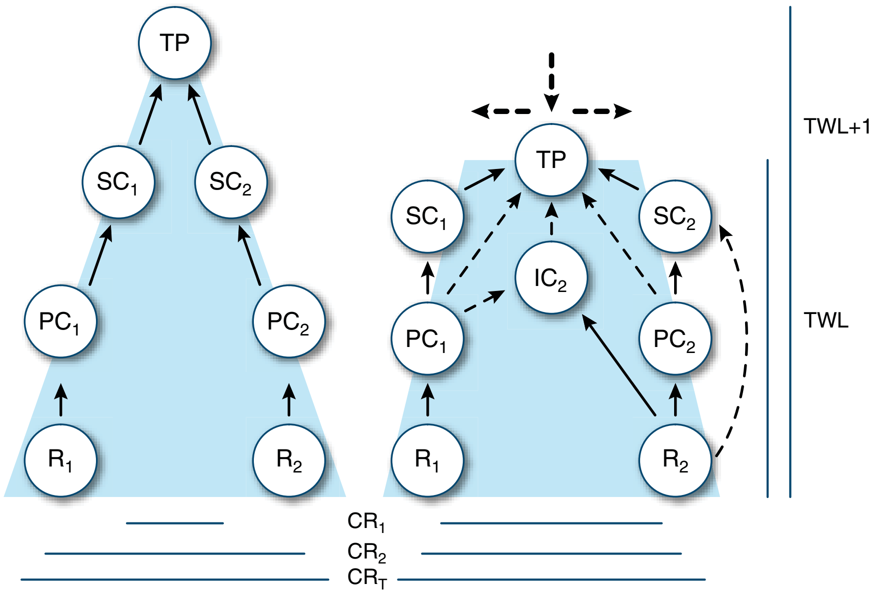


Figure 12. Conceptual models of trophic web functioning in temperate (left) and subtropical (right) lakes inferred from $\delta^{13}C$-$\delta^{15}N$ bi-plots and community-wide metrics. According to our results, the temperate systems can be depicted by a multi-chain omnivory model, with a top predator integrating different carbon sources that fuel the web (in the scheme, R_1 and R_2 represent phytoplankton and periphyton, respectively). In subtropical lakes, multi chain omnivory alone cannot depict the observed trophic web shapes (convex hull shapes) and a combination of simple chain (dotted) with multi-chain (full) models can explain the metrics and the shapes observed. Subtropical trophic webs are shorter than temperate ones, because of the lack of linearity due to the omnivory of fish in the top of the chain. The arrows above the model indicate the lowering of one Tr-Po occurring concomitantly with a widening of the carbon range reaching the 3rd level of the chain. As a result, almost all fish species end up by occupying the same trophic positions. Abbreviations: PC, Primary consumers. IC, Intermediate consumers. SC, Secondary consumers. TP, top predators. CR T, total carbon range, CR_1 and CR_2 carbon range that reaches the respective trophic positions. TWL, trophic web length. From Paper VI.

sized fish species, usually classified as piscivores or omni-benthi-piscivores (Teixeira de Mello et al., 2009; Paper V), had trophic positions similar to those of much smaller-bodied fish species, and in some cases even to those of macroinvertebrate predators. These results strongly suggest that most subtropical fish species are omnivores, potentially predating on fish, but also often or regularly feeding at one or more trophic levels down the trophic web (Fig. 11). Similar patterns have been identified in previous works based on stomach content data (Lazzaro, 1997; Teixeira de Mello et al., 2009; Jeppesen et al., 2010) and are also evident from a recent stable isotope study of a set South American lakes (Lacerot et al., submitted). In contrast to the trophic position achieved by the predator fish species in the temperate lakes (such as pike and perch, *Perca fluviatilis*), it seems unlikely that any of the subtropical predatory fish could be considered strictly piscivorous. Shrimps, often described as zooplankton predators (Collins & Paggi, 1998; González-Sagrario et al., 2009; González-Sagrario & Balseiro, 2010) and used in our mesocosm experiment (Paper III), in most cases appeared as second level consumers in the periphyton carbon source pathway.

Our results also suggest that the food webs of both climate zones are fuelled by a wide range of carbon sources following a similar pattern (i.e. similar the mean $\delta^{13}C$ range (CR) and the relative contribution of periphyton to each trophic level (% Cont-Lit; Table 1, Paper VI). In both climate regions the food webs seemed fuelled mostly by periphyton (% Cont-Lit > 50 %).

Moreover, we observed an important difference in the shapes of the food webs (Fig. 12), also suggesting that the energy pathways differ slightly between climate zones. Adjusted convex hull areas in the temperate lakes resembled the typical chain-like trophic architecture (Vadeboncoeur et al., 2005) often characterizing pelagic food chains (Sprules & Bowerman, 1988). Multi-chain omnivory (Vadeboncoeur et al., 2005, Fig. 12) may still be underlying in our set of temperate lakes, as reflected by the important littoral contribution and CR values registered. However, our estimated metrics suggest that the spread of carbon range that reaches the third trophic positions in temperate lakes is diminished in comparison with the range at the base of the trophic web and especially when compared with the observed pattern in subtropical systems. The subtropical fish species, in contrast, exhibit a strong niche or feeding overlap (indicated by a smaller nearer neighbour distance (NND) than in temperate food webs, Paper VI, Table 1). Our findings indicate that differential food web functioning under contrasting climatic zones reflects changes in the degree of omnivory by the top predator (“omnivory mechanism”, Post & Takimoto, 2007) and also the presence of redundant fish species (“addition model”, Post & Takimoto, 2007) in warm food web lakes. Strong evidence of how these differences affect ecosystem functioning is emerging and suggests that the theoretical framework and experience from temperate lakes cannot fully explain the functioning of subtropical lakes, and therefore cannot be directly applied to subtropical shallow lakes.

6 Conclusions and Perspectives

From the combination of experimental and empirical evidence, we have verified the strong role of fish predation in shaping the structure of several key communities and the food webs in shallow lakes, in both temperate and subtropical climate scenarios, but particularly in the subtropics. We present evidence that medium- and large-sized zooplankton can occur in subtropical lakes provided that fish predation is removed. Although high-temperature induced physiological constraints may still play a role, our diverse field and experimental evidence collectively supports the hypothesis that fish are the key factor determining the dominance of small-sized zooplankton in warm shallow lakes (Papers I & II).

We found clear experimental effects of a – similar to natural, but simplified – fish assemblage on zooplankton community structure (i.e. composition, biomass and total abundance) in both climates (Paper III). Fish effects were strong enough to cascade down to the phytoplankton level, thus leading to greater phytoplankton biomass in the presence of fish in both climate zones.

Contrarily to our *a priori* expectations, but at least in part due to aspects of our experimental design, the abundance of plant-associated macroinvertebrates was indeed affected by fish in both climate zones; however, periphyton biomass did not respond to predator presence and tended to increase in all the lakes (Paper III).

Finally, we proved that under experimental conditions the addition of a top predator fish can promote clear cascading effects reaching the phytoplankton level, and that even-levelled trophic webs (2 and 4 levels in our case) showed similar responses (Paper IV). However, whole-lake field data from several subtropical lakes indicate that most often there is no connection between the biomass or activity of the potential piscivorous fish and phytoplankton biomass (and consequently water transparency) (Paper V).

The analysis of the architecture of the trophic webs in temperate and subtropical lakes confirmed some patterns previously hypothesized (Meerhoff et al., 2007a; Jeppesen et al., 2010). According to our results, temperate systems can be depicted by a multi-chain omnivory model (Vadeboncoeur et al., 2005), with a top predator integrating different carbon sources that fuel the web (mainly represented by phytoplankton and periphyton). In subtropical lakes, a combination of simple-chain omnivory and multi-chain omnivory models may better explain the community metrics and the shapes observed there (Paper VI). Supporting previous suggestions (Meerhoff et al., 2007a), subtropical trophic webs were indeed shorter than the temperate ones due to the omnivory of fish across the food web, also at the top position. Intermediate consumers occurred massively in the subtropical food webs, acting as intermediate integrators of the different carbon sources (Post & Takimoto, 2007) and enhancing the transfer of the basal carbon to the next trophic positions.

Both SIA and field evidence suggest that the widespread omnivory of subtropical fish species causes strong overlap of trophic niches, high redundancy and absence of a so-called keystone species. In poorer fish assemblages, such as those typically occurring in temperate lakes, changes in the density of just one species may result in a whole-system response (keystone species concept, Mills et al., 1993). Clearly, this is not expected in subtropical lakes, where the space that a species may release will be rapidly occupied by another species, thus weakening any possibility of cascading effects to propagate all along the food web.

This main difference in trophic web architecture may lead to profound effects on ecosystem functioning. For instance, widespread omnivory resulting in a constant predation pressure on zooplankton weakens the link between zooplankton and phytoplankton in warmer lakes.

Our results thus support previous suggestions that top-down positive effects might be weak or absent in warmer systems (Meerhoff et al., 2007a; Jeppesen et al., 2007b; Lacerot et al., submitted) due to the structure of fish assemblages and particularly to the omnivory. The positive experimental effects found in relatively simple experiments may be a consequence of an over simplification of the more complex subtropical food webs. Such complexity is also a consequence of the higher taxon diversity of South American fish assemblages.

These patterns have deep theoretical and practical implications. The alternative states hypothesis (Scheffer et al., 1993) and fish biomanipulation techniques (Shapiro et al., 1975; Hansson et al., 1998a) are based on a series of positive feedbacks and the occurrence of a more or less linear trophic web. The above-mentioned previous findings in warm lakes, together with the results of this thesis (i.e. greater degree of niche overlap, shortening of the TWL due to the lack of a true piscivore at the top of the chain, and a close coupling between littoral and pelagic habitats), strongly support the idea that the complexity of trophic interactions in warmer systems may inhibit the occurrence of some positive feedbacks while decreasing the stability and resilience of the clear water state (Kosten et al., 2009). Future research, in particular experimental approaches, should aim to reflect the complexity of natural subtropical food webs. However, more research conducted in a larger number of lakes and covering a wider temperature range is also needed.

In a climate warming perspective, this evidence suggests that stronger fish effects are likely to occur with an increasing mean ambient temperature, since recent findings suggest that fish omnivory (as consumption of plant material) increases with increasing temperature (Jeppesen et al., 2010), and particularly with the occurrence of milder winters. Such a permanent control on zooplankton will reduce the window of opportunity for large-sized herbivorous zooplankton to reach relevant densities to control phytoplankton biomass. However, transfer of evidence from subtropical to present-day cold but in future warmer lakes demands caution, although such data may serve as a good indication of a potential response to be expected in ecosystems with warming.

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Manuscript 1

HIGH FISH PREDATION IS THE KEY FACTOR FOR DOMINANCE OF SMALL-BODIED ZOOPLANKTON IN WARM LAKES – EVIDENCE FROM LAKES, FISH EXCLOSURES AND SURFACE SEDIMENT

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Submitted

High fish predation is the key factor for dominance of small-bodied zooplankton in warm lakes – evidence from lakes, fish exclosures and surface sediment

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Abstract

Body length of limnetic cladocerans decreases from cold temperate to tropical regions, in both the northern and the southern hemispheres. The reasons for a declining size with increasing temperature have been debated extensively and are still not conclusive, as it could reflect both direct (e.g. physiological) or indirect (e.g. increased predation) effects. To provide further information on the role of fish, we compiled results obtained by three different approaches: (i) field observations from two subtropical lakes with contrasting fish abundance, (ii) fish exclusion experiments conducted in-lake mesocosms in three subtropical lakes, and (iii) analyses of the egg bank in the surface sediment of a number of subtropical shallow lakes. When fish predation pressure disappeared in Lake Rivera, large-bodied *Daphnia* appeared and the zooplankton:phytoplankton biomass ratio was high, indicating a high grazing pressure on phytoplankton, while small-sized cladocerans were abundant in the "reference" lake, Lake Rodó, hosting a normal high abundance of fish. Likewise, relatively large cladocerans (e.g. *Daphnia* and *Simocephalus*) appeared in fishless mesocosms after only two weeks, most likely hatching from resting egg banks in the surface sediment. Accordingly, 9 out of 18 Uruguayan shallow lakes had resting eggs of *Daphnia* in their surface sediment, despite that this genus was only recorded in a snap-shot summer sample in three of the lakes. The results showed that medium and large-sized zooplankton can occur in subtropical lakes when fish predation is removed. Our results collectively confirm the hypothesis that predation, rather than high-temperature induced physiological constraints, is the key factor determining dominance of small-sized zooplankton in warm lakes.

Introduction

Cladocerans are typically the most important planktonic herbivores in freshwater lakes (Brooks & Dodson, 1965) and are capable of consuming as much as 80-100% of the phytoplankton biomass (Lampert, 1988; Jeppesen et al., 1997; Shurin et al., 2006). Thus, cladocerans occasionally exert strong control of algal standing crops, even at high nutrient concentrations (Jeppesen et al., 1998). However, in nutrient-enriched north temperate lakes, zooplankton dominance often switch from big-sized individuals (like *Daphnia* spp) to smaller-bodied zooplankters (Jeppesen et al., 2000), most likely due to high fish predation (Brooks & Dodson, 1965; Gliwicz, 2003), triggering a shift in dominance from *Daphnia* to small-sized cladocerans like *Diaphanosoma* and *Bosmina*.

In subtropical and tropical lakes, small zooplankters are particularly dominant even at low nutrient concentrations (Fernando et al., 1987; Dumont, 1994; Meerhoff et al., 2007a; Havens et al., 2009). Accordingly, the mean body length of limnetic cladocerans decreases from cold temperate to tropical regions, in both the northern and the southern hemispheres (Gillooly & Dodson, 2000). In their study, no large-bodied species were observed in the subtropical and tropical waters, a latitudinal pattern that could not be attributed to spatial variation in primary production, nutrient regeneration or oxygen availability (Gillooly & Dodson, 2000). Lacerot et al. (submitted) found a similar latitudinal pattern in cladoceran body size in a study of shallow lakes in South America, covering a latitudinal gradient from Natal in Brazil to Tierra de Fuego in Argentina (5°- 55°S).

The reasons for a declining size with increasing temperature have been debated extensively and no conclusive answers have been found. Gillooly & Dodson (2000) noted that the strong correlation of body size with lake temperature might reflect both direct (e.g. physiological) or indirect (e.g. increased predation) temperature effects. They concluded, however, that differences in water temperature were the major driver behind this pattern and argued that the upper thermal tolerance of large cladocerans (ca. 30° C) is often exceeded in subtropical and tropical lakes. Growth, reproduction and respiration rates of zooplankton, as well as size at maturity, are also influenced by temperature (Moore et al., 1996; Weetman & Atkinson, 2004), whereas food requirements of cladocerans increase more steeply with body size at elevated temperatures (Hardy & Duncan, 1994). However, increased temperature might also have several indirect effects, such as changes in food quality and increasing size-selective predation, by allowing an earlier onset of fish reproduction (Moore et al., 1996). Recently, several authors have argued that higher fish predation, more than temperature, is responsible for the reduced size structure of the zooplankton community towards the tropics (Jeppesen et al., 2007; Meerhoff et al., 2007b; Havens et al., 2009; Havens & Beaver, in press; Lacerot et al., submitted). Tropical and subtropical fish assemblages are usually dominated by small omnivorous fish and often show a replacement of large predatory fish to smaller forms that are less efficient at controlling large coarse fish (Lazzaro, 1997; Kruk et al., 2009; Jeppesen et al., 2010). *Daphnia* and other large-bodied cladocerans may, therefore, be controlled all year-round or at least in the warmer (i.e. reproductive) seasons by fish (Lazzaro, 1997; Pinel-Alloul et al., 1998; Jeppesen et al., 2010). Moreover, in contrast to north temperate lakes (Burks et al., 2002), submerged macrophytes do not seem to be an efficient daytime refuge for zooplankton (Meerhoff et al., 2006), as fish abundance, and thus predation, among the plants is high (Meerhoff et al., 2007a), which further enhances the predation risk for large-bodied zooplankton in such lakes. Plant-associated macroinvertebrates can also predate and control zooplankton in subtropical shallow lakes, contributing further to a weakening of the refuge effect promoted by vegetation in the subtropics (González Sagrario et al., 2009; González Sagrario & Balseiro, 2010).

Experimental studies conducted in summer in subtropical Uruguay have shown that, despite high temperatures, stocked *Daphnia* may become the dominant zooplankton as long as small omnivorous-planktivorous fish are absent or controlled by piscivores, causing strong cascading effects on phytoplankton and water clarity (Iglesias et al., 2008; Mazzeo et al., 2010). These results further advocate for the predation hypothesis for dominance of small-bodied zooplankton in the (sub)tropics.

Here, we aim at providing further evidence supporting the hypothesis that fish, rather than temperature, is the key controlling factor for the typically small range size of cladocerans in the subtropics. We combine results obtained by three different approaches: (i) field observations from two subtropical lakes with contrasting fish abundance, (ii) fish exclusion experiments conducted in mesocosms in three subtropical lakes in summer, and (iii) analyses of the egg bank stored in the surface sediment of 18 subtropical shallow lakes.

Materials and Methods

Field studies – Comparison of two urban hypertrophic, subtropical lakes

Lake Rivera and Lake Rodó are small (5 and 1.5 ha, respectively), shallow systems (mean depths 0.8 and 1.7 m, respectively) located within two city parks of Montevideo in Uruguay (34°55´S, 56°10´W). Both lakes are mainly used for recreational and cultural activities, but are eutrophic as a result of a high nutrient input resulting from human activities (mean annual TP: 2.4 and 0.2 mg L^{-1} and TN: 11.5 and 2.8 mg L^{-1}, in Rivera and Rodó respectively). Lake Rivera has received a substantial amount of sewage and shows great interannual variation in the occurrence of free-floating plants (mainly *Eichhornia crassipes* and *Spirodela intermedia*). Lake Rivera usually has high fish densities (mainly of the dominant species *Cnesterodon decemmaculatus*). However, in the year 1999 an extensive fish kill (likely due to elevated ammonia concentrations) occurred and a severe decrease of fish density was registered from summer to autumn, resulting in almost fishless conditions (Mazzeo et al., 2000). Lake Rodó, in contrast, typically has a high density of fish all year-round (Scasso et al., 2001). Here, we compare the fish and zooplankton structure in the two lakes during 1999 when Lake Rivera was almost fishless, while Lake Rodó had a diverse fish community dominated by omnivores-planktivores and high fish densities (Table 1).

We applied the same sampling strategy in both systems. We assessed fish communities by point sampling electro-fishing (Perrow et al., 1996) in spring and summer 1999, at around 100 points along transects covering the whole lake (one electric burst per point).

Table 1. Body size (mean standard length (SL) and range) and feeding preferences (as described in published literature) of fish and shrimps in Lake Rodó, indicating the publications used. Macroinvertebrates include all groups except *M. borelli* and *P. argentinus*, which were included as a separate category (shrimps)/which are shown separately.

	Cnesterodon decemmaculatus 1.4 cm SL 0.8-3.5 cm	*Jenynsia multidentata* 2.4 cm SL 1.0-6.1 cm	*Australoheros facetus* 3.1 cm SL 1.0-13.0 cm	*Gymnogeophagus rhabdotus* 2.7 cm SL 0.9-7.0 cm	*Chrenicichla scotii* 7.9 cm SL 2.4-14.5 cm	*Synbranchus marmoratus*	*Macrobrachium borellii*	*Palaemonetes argentinus*
Detritus	✓		✓	✓			✓	✓
Phytoplankton	✓							
Periphyton		✓	✓	✓				
Zooplankton	✓	✓	✓	✓	✓		✓	✓
Macroinvertebrates	✓	✓	✓	✓	✓	✓	✓	✓
Shrimps		✓	✓		✓	✓	✓	✓
Fish		✓	✓		✓	✓	✓	
References	1, 2, 3, 4	1, 2, 3, 4	1, 5, 6, 7	5	8	9-10	11	12

1. (Ringuelet, 1975), 2. (Escalante, 1983), 3. (Hartz et al., 1996), 4. (Quintans et al., 2009), 5. (Yafe et al., 2002), 6. (Escalante, 1984), 7. (Ruiz et al., 1992), 8. (Lobón-Cerviá et al., 1993), 9. (Meschiatti & Arcifa, 2002), 10. (Mérigoux & Ponton, 1998) ??Ponton, 1998), 11. (Collins & Paggi, 1998), 12. (Collins, 1999b).

Phytoplankton and zooplankton were seasonally collected with a 5-l Patalas sampler at three random points (triplicate) in the pelagic area, covering the whole water column. We estimated phytoplankton biovolume according to Hillebrand et al. (1999). We calculated mean body size of zooplankton after measuring at least 20 individuals of each species in each lake, and particularly for cladocerans we used the total body length of adult females. For the calculation of biomass, we followed length-weight regressions according to Bottrell et al. (1976) and Ruttner-Kolisko (1977). We applied analysis of variance (ANOVA) to test statistical differences, either considering one factor (lakes) or two factors (lakes and seasons). To meet assumptions, we prior (ln x+1 or square root) transformed data and checked the homogeneity of variances (Cochran´s test) and the normal distribution of the residuals (by visual inspection). We compared body size of cladocerans (defined as the body length of *Daphnia obtusa*) between lakes with Student t-test. The relationship between variables was explored with linear and non-linear regressions, considering all the replicates in each lake.

Fish exclosure-mesocosm experiments

The experiments were carried out in summer 2008 in three subtropical shallow lakes in Uruguay, Lakes Diario (34°54´S, 55°00´W), Blanca (34°53´S, 54°50´W) and Nutrias (34°40´S, 54°17´W), covering a wide range of turbidity and submerged plant coverage (Table 2).

Previous works on these lakes have shown that the zooplankton community of all three lakes consisted mainly of rotifers and nauplii, whereas the abundance of large-sized cladocerans (like *Daphnia* or *Simocephalus*) was low or missing and particularly low during summer (Iglesias et al., 2007., 2008; Kruk et al., 2009). In each lake, we used eight 1000-L 1-m high plastic bags that were open to the atmosphere and fixed into the bottom to ensure isolation from outside water and allow full contact between water and sediment. Fish were prevented from entering the mesocosms by adding a fine mesh (size: 1 mm) to the bottom during the set-up.

Table 2. Main limnological characteristics of the 18 Uruguayan lakes (in alphabetical ordered) sampled for *Daphnia ephippia*. The stars (*) show the three lakes where the fish exclusion experiment took place. Abbreviations indicate: values for *Daphnia* abundance in water, Dap W (ind. L^{-1}), *Daphnia ephippia* in sediment sample, DapEphi (μg DW^{-1}); Maximum depth, Z_{max} (m); Secchi depht, SD (m) Total Phosphorus, TP (μg L^{-1}), Total Nitrogen, TN (μg L^{-1}) and Conductivity, K (μS cm^{-1}). Plant Volume Infested, PVI (%). Fish species, Ast: *Astyanax* sp.; Bry: *Bryconamericus iheringi*, Cal: *Callichthys callichthys*, Char: *Charax stenopterus*, Rach: *Characidium rachovii*, Chei: *Cheirodon* sp., Cren: *Chrenicichla scotii*, Aust: *Australoheros facetus*, Cnes: *Cnesterodon decemmaculatus*, Cory: *Corydoras paleatus*, Cyph: *Cyphocharax voga*, Diap: *Diapoma terofalli*, Gymno: *Gymnogeophagus* sp., Hepta: *Heptapterus* sp., Hyph: *Hyphessobrycon* sp., Hypo: *Hypostomus commersoni*, Jen: *Jenynsia multidentata*, Mima: *Mimagoniates inequalis*, Odo: *Odontesthes bonariensis*, Oligo: *Oligosarcus jenynsii*, Para: *Parapimelodus valenciennis*, Phallo: *Phalloceros caudimaculatus*, Pime: *Pimelodella australis*, Plata: *Platanichtys platana*, Pseudo: *Pseudocorynopoma doriaei*, Rham: *Rhamdia quelen*, Syn: *Synbranchus marmoratus*. Environmental data were published in Kruk et al. (2006) and Kruk et al. (2009).

Lake	DapW	DapEphi	Area*	Zmax*	SD**	TP**	TN	K*	PVI**	Fish species*
Aguada	2	0	0.5	3.95	0.4	43.0	975	63	100	Chei, Aust, Cnes, Hepta, Hyph, Phal, Rham
Barro	0	10.1	22	4.90	0.3	32.8	884	187	0	Gymno, Jen, Pime
Blanca*	0	10.6	60	3.43	0.7	51.9	1017	223	13	Cnes, Jen, Austra, Rham, Pime
Chaparral	0	0	2.5	8.73	0.7	47.2	598	40	74.5	Chei, Jen
Chica	0	4.8	3	46.48	0.1	90.5	1164	68	0	Cnes, Cory, Jen, Phal, Symb
Cisne	0.11	0	127	4.17	0.1	413.0	1048	327	0	Ast, Char, Chei, Aust, Cory, Cypho, Diap, Gymno, Hop, Hypo, Hyso, Oligfo, Parap, Rham
Clotilde	0.001	7.4	29	4.11	1.8	27.7	451	70	28.5	Ast, Aust, Char, Rach, Cnes, Cory, Hop, Hyph, Hypo, Jen, Mima, Oligo, Phal, Pime, Rham, Symb
Diario*	0	0	101	1.32	0.6	75.8	825	272	34	Bryco, Rach, Cren, Cichla, Cnes, Cory, Diap, Gymno, Hyph, Jen, Odon, Oligo, Plata, Rham
Escondida	0.14	1.5	14	1.12	1.1	24.2	489	215	16	Aust, Cnes, Hop, Jen, Symb
Garcia	3.89	0	13.5	0.00	1.6	29.8	332	129	5	Aust, Ast, Cnes, Cypho, Hepta, Hyph, Jen, Oligo, Phal, Rham, Symb
Mansa	0	2.8	5	4.51	1.0	184.2	1534	135	68.5	Rach, Cnes, Cory, Cypho, Hop, Jen, Oligo, Phal
Moros	0	0	2.5	13.98	1.0	28.7	437	180	14	Cichla, Cnes, Cypho, Hepta, Hyph, Symb
Nueva	0	0	0.5	6.96	0.6	60.9	1160	67	90	no fish was found
Nutrias*	0.15	6.3	24	3.34	0.5	99.8	1136	30	0	Ast, Chei, Cnes, Cory, Jen, Oligo, Rham
Ponderosa	0	0	0.5	9.37	0.9	86.5	888	60	65	Chei, Jen, Rham, Symb
Redonda	0	56.6	6	1.55	2.0	23.9	514	73	58	Cal, Rach, Cnes, Jen, Phal, Symb
Techera	0	8.2	3	13.67	0.7	37.9	1681	134	96	Cypho, Jen, Phal
Pajarera	0	0	0.5	13.81	0.3	179.8	2691	150	0	Ast, Rach, Chei, Cnes, Hop, Hyph, Phal, Rham, Symb

In each mesocosm we introduced an artificial plant bed (120 1-m long plants) mimicking submerged *Myriophyllum* or *Cabomba* spp (as in Meerhoff et al., 2007a), and fish were stocked in half of these (4 randomly assigned replicates). We added a combination of the two commonest omnivorous-planktivorous fish species in the region, *C. decemmaculatus* (40 individuals) and *Jenynsia multidentata* (50 individuals), according to reported average natural densities of these species in vegetated habitats (Teixeira de Mello et al., 2009). The mesocosms were left undisturbed for two weeks prior to adding the fish and to conducting the first sampling

(T_0), allowing the zooplankton community to develop from the contemporary pool and from resting stages in the sediment. Two months later, we carried out a second sampling (T_F) to determine the fish effects on zooplankton assemblages.

We collected zooplankton for quantitative analysis with a pump, integrating different depths and zones inside each mesocosm (without prior mixing of water). Besides, we took one sample from outside the mesocosms ("reference lake samples"). In all cases, we filtered a subsample of eight litres of water through a 50-mm mesh size net and fixed the samples with acid Lugol. We counted the samples following Paggi & José de Paggi (1974). Copepods were identified and grouped as calanoids and cyclopoids, and cladocerans as free-swimming and plant-associated following Meerhoff et al. (2007a). We estimated mean body size of crustacean zooplankton after measuring 20 individuals of each species present in each sample. We analysed the data in each lake using Student t-test, with fish presence or absence as factor.

Surface sediment samples of subtropical lakes

In 18 shallow lakes located along the Uruguayan coast, we took surface sediment samples (3 replicates per lake) from the deepest point of each lake by free diving (top 5 cm, likely representing the last 2-10 years; García-Rodríguez et al., 2004; Vandekerkhove et al., 2005). The lakes varied in trophic state, fish densities, and other limnological characteristics (Table 2, for methodological details and further data, see Kruk et al., 2009).

We obtained the resting eggs after heating approximately 5 g (wet weight) of homogenised surface sediments in 50 ml of 10 % KOH for 20 minutes and kept them cold (4 °C) for a maximum of two weeks, until we performed the counting. We quantified the resting eggs of *Daphnia* that were retained on a 140-µm mesh sieve using a binocular microscope (100x, Leica MZ12) and an inverted light microscope (320x, Leitz Labovert FS).

Results

Field studies - Comparison of two urban hypertrophic, subtropical lakes

The structure of the zooplankton community differed strongly between the two lakes, the almost fishless Lake Rivera showing a lower taxonomic richness but larger-bodied cladocerans than Lake Rodó. All the species of Lake Rivera, except for a couple of rotifer species (*Brachionus urceolaris* and *B. rubens*), were found also in Lake Rodó. The cladocerans *Moina micrura* and *D. obtusa* were registered in both lakes, while *Diaphanosoma birgei* and *Alona* sp. were registered only in Lake Rodó. Zooplankton biomass differed significantly between the two lakes for rotifers, total cladocerans and *D. obtusa* (one-way ANOVA $F_{1,16}$= 31.4, p<0.001; $F_{1,16}$=31.9, p<0.001, $F_{1,16}$=80.4 p<0.001, respectively). In Lake Rivera, cladocerans dominated the zooplankton community in terms of biomass, except in summer when the copepod biomass was higher (Fig. 1). *Daphnia obtusa* was the species with the highest contribution to total biomass. The higher abundance of *D. obtusa* occurred from autumn to spring, and males were observed in winter and spring. In contrast, in Lake Rodó small and medium-sized cladocerans had the highest contribution to total biomass in winter (due

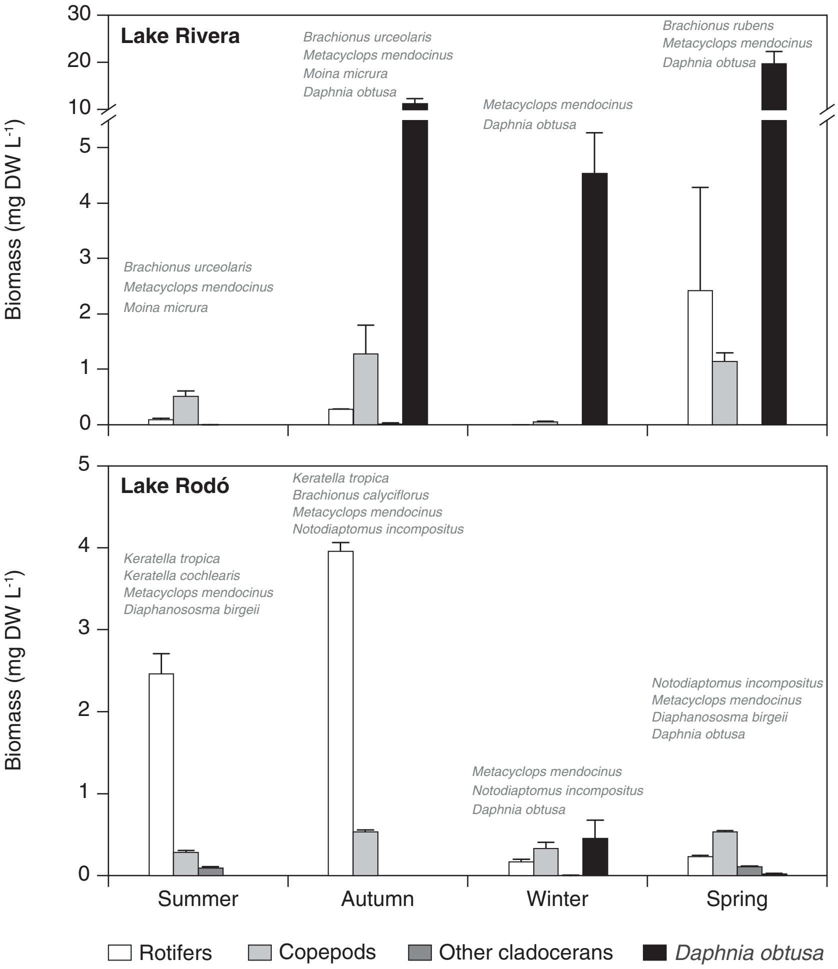

Figure 1. Field evidence from two urban subtropical Uruguayan lakes. Biomass of main zooplankton groups in Lake Rivera (fishless) and Lake Rodó, indicating the species with the highest contribution to the biomass of each group (mean ±1SE). Note the different scales in each lake.

to *D. obtusa*), while copepods dominated in spring; nevertheless total biomass was ca. 10 times lower than in Lake Rivera (Fig. 1). The mean, minimum and maximum body size of the cladocerans in the lakes Rivera and Rodó were 1.85, 0.23, 3.74 mm and 0.52, 0.29, 0.81 mm, respectively. Moreover, *D. obtusa* was significantly larger in Lake Rivera than in Lake Rodó (T-test t_{196}=25.9, $p<0.01$; Fig. 2). The mean size of *D. obtusa* in Lake Rivera exceeded the mean size of cladocerans expected for the latitude 35° (Gillooly & Dodson 2000); in contrast, in Lake Rodó mean sizes were smaller than expected (Gillooly & Dodson 2000, Fig. 2).

We found only three fish species in Lake Rivera: *C. decemmaculatus*, *Gymnogeophagus rhabdotus* and *Synbranchus marmoratus*, and the latter two species with only one individual each. Total biomass was extremely low (<1.0 kg ha^{-1}). *C. decemmaculatus* reached a density of 1,170 ind ha^{-1} in January (summer) and only 78 ind ha^{-1} in September (spring). Lake Rodó, in contrast, exhibited higher species richness (8 species), a total biomass of 20.0 kg ha^{-1} and dominance by small-bodied fish. The total estimated density of *ca.* 100,200 ind ha^{-1} was 100 times higher than in Lake Rivera. The most important species were *C. decemmaculatus* (96 % of total abundance, 32 % total biomass) and *Australoheros facetus* (37% of total biomass). The piscivorous *C. lacustris* accounted for 11 % of the total biomass. Besides, shrimps (*Palaemonetes argentinus* and *Macrobrachium borelli*) occurred in high densities only in Lake Rodó, maximum total biomass and density being 8 kg ha^{-1} and 145,0 ind ha^{-1}, respectively. All the fish species found in Lake Rodó, except for adult *C. lacustris*, were omnivore-zooplanktivores (Table 1), and so were the shrimps (Collins, 1999a; Collins & Paggi, 1998).

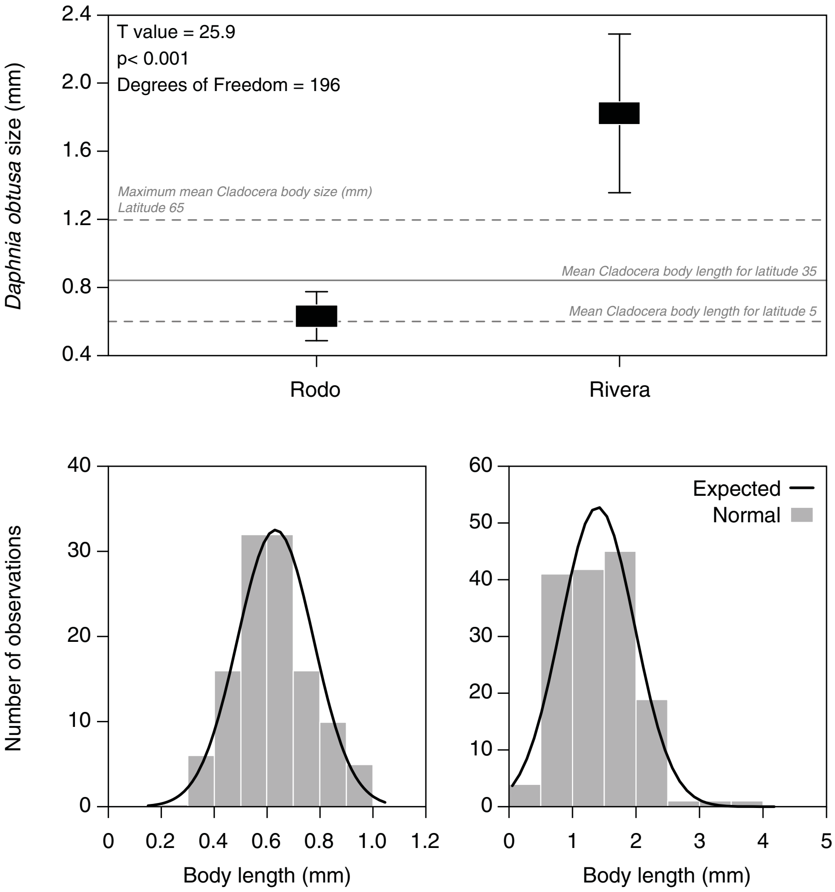

Figure 2. Field evidence from two urban subtropical Uruguayan lakes. Comparison of mean body length of *D. obtusa* in Lake Rivera and Lake Rodó, indicating the body length as expected from the Gillooly & Dodson (2000) latitudinal gradient study. Above: mean values (±1 SE) of females. Below: distribution of frequency of *Daphnia* body sizes.

Phytoplankton biomass (Chl-a) was usually higher in Lake Rivera, except during winter (ANOVA, interaction between lakes and seasons, $F_{3,16} = 188.2$, $p<0.001$). The 10-fold decrease in algal biomass during winter in Lake Rivera coincided with the highest percentage of cladocerans in terms of abundance and biomass. We found a negative exponential relationship between cladoceran abundance and Chl-a ($r^2=0.79$, $p<0.001$). In Lake Rodó, large-sized cyanobacteria dominated most of the year (e.g. *Raphidiopsis mediterranea* in summer, *Sphaerocavum brasiliensis* in autumn and *Planktothrix agardhii* during winter and spring).

Fish exclosure-mesocosm experiments

Cladocerans were in all cases more diverse and abundant inside the exclosures than in the reference lake samples, both considering plant-associated and free-swimming species (Fig. 3). Remarkably, large-sized cladocerans (*Daphnia)* suddenly became part of the zooplankton community in Lake Nutrias and even occurred in high densities in clear Lake Diario. In Lake Blanca large-sized *Simocephalus* sp. appeared, being recorded for the first time since 2000 despite quite extensive sampling campaigns during the previous years (Fig. 3). Two months after fish introduction, the zooplankton composition had reverted to the earlier dominance of small-sized individuals inside the mesocoms with fish. Cladocerans became rare again, while in the fishless mesocosms cladocerans (together with calanoid copepods) were still common. The effect of fish on these two zooplankton groups was significant in the three lakes. The abundances of cladocerans plus calanoids were 70 and 367 ind L^{-1} (Lake Diario, T-test $t_6= 5.0$, $p<0.01$), 16 and 306 ind L^{-1} (Lake Blanca, $t_6= 2.5$, $p<0.05$) and 12 and 44 ind L^{-1} (Lake Nutrias, $t_6= 5.8$, $p<0.01$) in the mesocoms with and without fish, respectively. The size

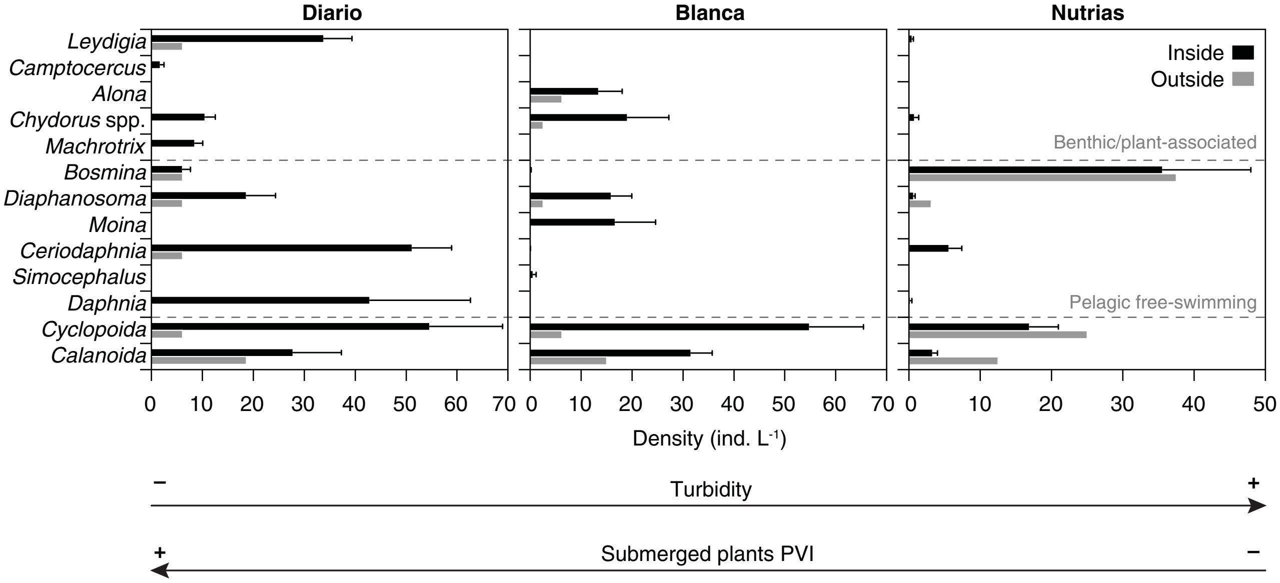

Figure 3. Experimental evidence from mesocosms in three subtropical Uruguayan lakes. Mean density (± 1 SE) of free-swimming and plant-associated cladocerans and copepods (calanoid and cyclopoid) inside fish exclosures (black) and from lake (outside) samples (grey) at initial conditions.

structure of the zooplankton assemblages also differed remarkably with the presence of fish; the mesozooplankton (i.e. cladocerans+calanoid copepods) to microzooplankton (i.e. rotifers+ nauplii) density ratios were much higher in the fishless mesocoms in all three lakes. The ratios varied between mesocosms with and without fish: 0.25 and 0.70 (Lake Diario, T-test $t_6 = 3.2$, $p<0.01$); 0.30 and 1.12 (Lake Blanca, $t_6 = 3.7$, $p<0.01$), and 0.43 and 2.58 (Lake Nutrias, $t_6 = 2.4$, $p<0.05$), respectively.

Pelagic cladocerans were particularly sensitive to fish, in some cases totally disappearing or, alternatively, becoming significantly smaller in the fish treatments (Fig. 4). Also, large plant-associated species (e.g. individuals/species of the genus *Simocephalus*) were affected, either in occurrence or in mean body size. Contrarily, small plant-associated cladocerans (e.g. *Chydorus* spp.) and copepods (both calanoids and cyclopoids) appeared to be less affected by fish. We only found differences in body size for copepods in the clearest lake, Lake Diario (T-test $t_6 = 2.9$, $p<0.01$; $t_6 = 2.6$, $p<0.05$, for cyclopoids and calanoids, respectively).

Surface sediment samples

Many limnological features and fish community structure varied among the 18 studied systems (Table 2). *Daphnia* spp. ephippia were found in the surface sediment of nine of the lakes, the average density being 6.0 individuals g DW^{-1} (Table 2). Remarkably, only in four of those nine lakes did *Daphnia* occur in both the sediment and the water, appearing in very low numbers in the summer water samples (<5 ind L^{-1}, Table 3; Kruk et al., 2009). In two of the lakes, *Daphnia* spp. was registered in water samples, but ephippia were not found in the sediments. Moreover, *Daphnia* also occurred in our fish exclosure experiment in Lake Diario, even though it was not found in sediments or water samples during our snap-shot approach. In fact, in contemporary samples *Daphnia* was found only in 33% of the lakes, but this value rose to 72 % when including evidence of their presence from ephippia data or the fish exclosure experiments.

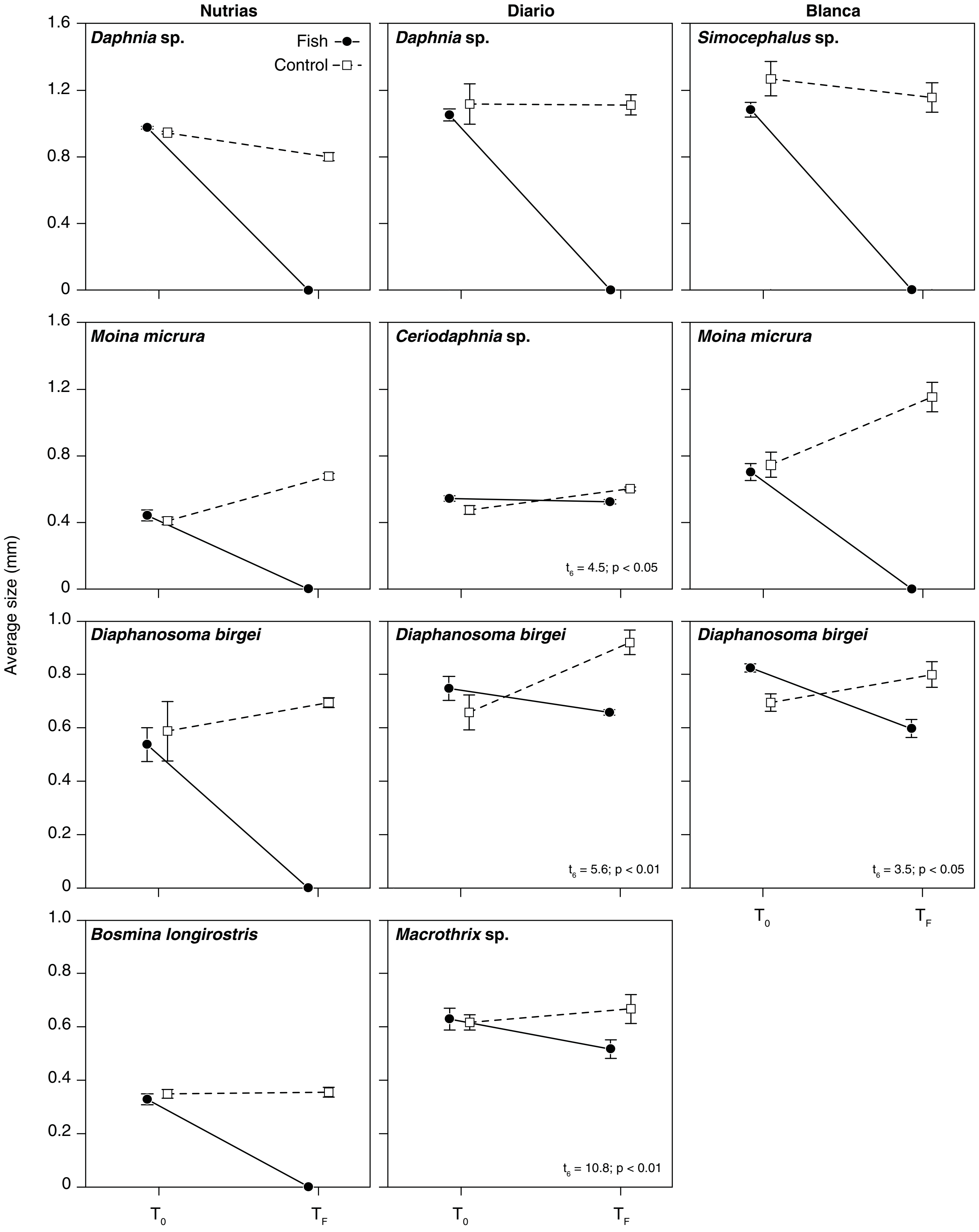

Figure 4. Experimental evidence from mesocosms in three subtropical Uruguayan lakes. Initial (T_0) and final (T_2) average body size (mm) of the main cladoceran genera found in each lake in mesocosms with and without fish. Statistics correspond to Student T-test analyses. Note the different scales and that some species disappeared in some mesocosms; their mean size was set to 0 in the figure.

Discussion

Our results, obtained from different approaches, support the hypothesis that fish predation is the key structuring factor for the dominance of small zooplankton in subtropical lakes. When the predation pressure was extremely low in fishless Lake Rivera, large numbers of large-bodied *Daphnia* appeared and phytoplankton biomass was markedly reduced. In contrast, small-sized cladocerans were constantly abundant in the "reference" lake, Lake Rodó, hosting high abundances of small fish. Likewise, relatively large cladocerans (e.g. *Daphnia* and *Simocephalus*) appeared in mesocosms after only two weeks in a fish-free environment, most likely hatched from resting eggs in the surface sediments. Accordingly, nine of 18 surveyed subtropical shallow lakes had resting eggs of *Daphnia* in the surface sediments, although *Daphnia* had been found in water snap-shot summer samples only in four of them (Table 2).

The seasonal dynamics of cladocerans in Lake Rodó followed a pattern often seen in lakes and reservoirs in Uruguay. Medium (and seldom large-sized) cladocerans are only found in moderately high densities by the end of winter and spring, and sometimes in autumn, coinciding with periods of low abundance or activity of fish (Fabian, 1993; Scasso et al., 2001; Mazzeo et al., 2003; Iglesias et al., 2007). In contrast, we found a 2-fold higher annual average biomass in the almost fishless Lake Rivera. A similar seasonal pattern as that typical of lakes in Uruguay (except Lake Rivera) has also been observed in warm lakes in the Mediterranean region (Romo et al., 2004) and in subtropical US, Florida (Havens & Beaver, in press). While the shift to small-sized zooplankton in spring and summer could be a physiological consequence of increased temperatures, it also coincides with the time when small omnivorous-planktivorous fish reproduce and shrimps start hatching and reach the highest densities (Iglesias et al., 2007). Both shrimps and the dominant fish species have multiple reproductive events starting in spring, and recruit high numbers of potentially zooplanktivorous individuals in spring to early summer (Boschi, 1981; Lorier & Berois, 1995; Garcia et al., 2004; Ituarte et al., 2007).

A predation effect was not only evidenced by the lower biomass of large-bodied zooplankton. *Daphnia* mean size in fishless Lake Rivera was significantly larger and exceeded the mean size expected for the latitude 35° and average water temperature (18.7 °C) (according to Gillooly & Dodson, 2000), but also exceeded the mean size expected at the highest latitude (65°). It also exceeded the largest size recorded by Lacerot et al. (submitted) in the latitude gradient study of 83 shallow lakes in South America (82 lakes had fish, and only one cold lake was fishless).

Fish effects were also evident in our experimental approach, particularly in the case of cladocerans and specifically on the pelagic or free-swimming species (i.e. *Daphnia*, *Simocephalus*, *Ceriodaphnia, Diaphanosoma*, *Bosmina* and *Moina*, Fig. 4). Copepods and small plant-associated cladocerans (e.g. *Chydorus* spp.) appeared to be less sensitive to fish predation, with fish effects being notable only in the lake with clearest water conditions (Lake Diario). However, we also found effects on medium to large-sized plant-associated taxa, such as *Macrothrix* sp., even in the more turbid lakes, Lake Blanca and Nutrias. These findings agree with the direct fish effects (and only indirect temperature effect) suggested by Lacerot et al. (submitted) in their South American latitudinal analysis. The average size of cladocerans in the mesocosms with fish fell in the range previously reported in that work (Lacerot et al., submitted) for South American shallow lakes with fish, while the average cladoceran size registered in the mesocosms without fish exceeded it.

The lower zooplankton richness of Lake Rivera compared to the otherwise similar Lake Rodó agrees with the patterns found in fish-free habitats reviewed by Gliwicz (2003). The absence of fish predation can enhance the monopolization of resources by single herbivorous species after excluding all inferior competitors, thus leading to a lower overall richness. This response has been observed in many systems of different trophic state (from ultra-oligotrophic to hypertrophic conditions) and also in hypersaline habitats (Gliwicz, 2003). However, Kruk et al. (2009) did not find any relationship between fish abundance (or richness) and the richness of zooplankton or cladocerans in the 18 shallow lakes studied in Uruguay, presumably reflecting a narrow fish abundance gradient. All these results, from zooplankton richness to biomass and body size, support the idea of fish predation being of key importance for the dominance of small forms in warm lakes. Studies in tropical Lake Naini Tal (India) by Nagdali & Gupta (2002) recorded a shift to large-bodied zooplankton and a higher zooplankton:phytoplankton biomass ratio following a massive (>80%) kill, due to fungal infection, of the most abundant planktivorous mosquito fish (*Gambusia affinis*). However, four months later the abundance of mosquito fish and also plankton and nutrient levels had returned to the levels recorded in the previous year, which indicates that fish play a key role for the dominance of small-bodied zooplankton and a low grazing pressure on phytoplankton in this tropical lake.

Further evidence for the predation hypothesis comes from reservoirs, lakes and ponds in tropical African lakes. Mergeay et al. (2004) found a clear relationship between changes in *Daphnia* species composition (identified from ephippia in the sediment) and historical information on fish abundance and stockings in shallow Lake Naivasha (Kenya). Moreover, contemporary and surface sediment analyses of 40 standing water bodies in the highlands of Kenya, covering small ephemeral pools to large permanent lakes, showed presence of *Daphnia* in 70% of the lakes (Mergeay et al., 2006). In the Uruguayan lakes, *Daphnia* appeared in the surface sediment in half of the lakes, but only in 1/3 of the water samples. Similar findings were obtained in a set of 32 Ethiopian highland reservoirs (Mergeay et al., 2006), with *Daphnia* appearing in the sediments of 62% of the waterbodies, but only in 40% of the water samples. A higher occurrence of *Daphnia* in the sediments is to be expected as sediments integrate several years, while the snap-shot samples are representative of summer when *Daphnia* densities are typically low. The relatively high percentage of *Daphnia* occurrence in the combined sediment-contemporary samples suggests that large-bodied zooplankton are indeed present in many warm lakes (if not all) despite the high mean water temperatures. The typically high densities of fish seem, however, to be able to substantially reduce the densities of large-bodied zooplankton.

For water bodies with similar high predation risks and nutrient states, it is likely that zooplankton may still be somewhat smaller in warm lakes than in their colder counterpart, due to the direct effect of temperature on metabolism and physiological constraints (particularly on *Daphnia*). However, evidence is so far lacking. Our results add further evidence of the hypothesis (Jeppesen et al., 2005) that higher predation, mostly from fish (Iglesias et al., 2008; Meerhoff et al., 2007b; Mazzeo et al., 2010) but potentially also from some macroinvertebrates (Iglesias et al., 2007; Trochine et al., 2006; González Sagrario et al., 2009), rather than physiological constraints, is the key factor for the dominance of particularly small-sized zooplankton in warm lakes.

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Manuscript 2

FIELD AND EXPERIMENTAL EVIDENCE OF THE EFFECT OF *JENYNSIA MULTIDENTATA*, A SMALL OMNIVOROUS–PLANKTIVOROUS FISH, ON THE SIZE DISTRIBUTION OF ZOOPLANKTON IN SUBTROPICAL LAKES

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Field and experimental evidence of the effect of *Jenynsia multidentata*, a small omnivorous–planktivorous fish, on the size distribution of zooplankton in subtropical lakes

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SUMMARY

1. Small cladocerans, copepod nauplii and rotifers often dominate the zooplankton community in tropical and subtropical lakes. This is probably because of high predation pressure by small omnivorous–planktivorous fish, but experimental evidence is scarce.
2. This study used two approaches to test the effect of the small omnivorous–planktivorous fish species *Jenynsia multidentata*, which is frequently abundant in (sub)tropical eutrophic lakes in South America, on the size distribution of zooplankton. In Lake Blanca (Uruguay), which lacks any piscivores, we sampled seasonally for both fish and zooplankton. We also conducted an outdoor mesocosm experiment with treatments containing or lacking *J. multidentata*.
3. Together, the empirical and experimental data suggest that *J. multidentata* predation plays an important role in modulating the size structure of the zooplankton community in subtropical lakes. In the absence of *J. multidentata*, stocked large-sized zooplankters like *Daphnia obtusa* were abundant in the experiments, while small-sized zooplankton dominated in the presence of fish, as they did in the lake itself from spring to the end of the season.

Keywords: *Jenynsia multidentata*, predatory, subtropical shallow lake, zooplankton structure

Introduction

The importance of fish predation for structuring the freshwater zooplankton community is well documented, particularly in temperate lakes in Europe and North America, and the presence or absence of planktivorous fish is known to induce major shifts in the size distribution of zooplankton (Hrbacek *et al.*, 1961; Brooks & Dodson, 1965) or behavioural shifts (Timms & Moss, 1984; Schriver *et al.*, 1995; Kairesalo, Tátrai & Luokkanen, 1998; Burks *et al.*, 2002; Romare & Hansson, 2003). Far less is known about lakes in warmer climatic regions. A recent study has shown the impact of *Odontesthes bonariensis* (Valenciennes, 1835) predation on subtropical zooplankton community structure (Boveri & Quirós, 2007), and other studies have focused on zooplankton behavioural shifts related to predation risk (Meerhoff *et al.*, 2006, 2007a,b; Trochine, Modenutti & Balseiro, 2006; Iglesias

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et al., 2007). The zooplankton communities of tropical and subtropical shallow lakes are most often dominated by small cladocerans, copepod nauplii and rotifers (Crisman & Beaver, 1990; Dumont, 1994; Branco *et al.*, 2002; Garcia *et al.*, 2002; Havens, East & Beaver, 2007), probably due to high predation pressure by small omnivorous–planktivorous fish (Fernando, 1994; Lazzaro, 1997; Pinel-Alloul *et al.*, 1998) and by large invertebrate predators (Boschi, 1981; Collins & Paggi, 1998; Collins, 1999; Iglesias *et al.*, 2007). However, experimental evidence is scarce.

Variations in abundance or foraging activity of planktivorous fish also influence seasonal patterns in the size distribution of herbivorous zooplankton in (sub)tropical lakes. Mazzeo *et al.* (2003) found that a shift to dominance of small sized specimens (rotifers) in the zooplankton in summer in Lake Blanca coincided with an increase in abundance of *Jenynsia multidentata* (Jenyns, 1842). Likewise, in Lake Rodó (Montevideo, Uruguay), a hypereutrophic urban shallow lake, Scasso *et al.* (2001) found the summer decline in mesozooplankton abundance to be related to fish activity, in this case principally represented by *Cnesterodon decemmaculatus* (Jenyns, 1842) and *J. multidentata*. Also in a Spanish warm temperate lake (Lake Albufera), Romo *et al.* (2005) attributed the lower abundance or absence of larger-bodied zooplankton from late spring to autumn to high fish predation. Others have argued that low abundance or absence of *Daphnia* in warmer regions may relate to a lower upper thermal tolerance of these organisms (Moore, Folt & Stemberger, 1996). Daily physical and chemical fluctuations and sudden environmental shifts (e.g. due to heavy precipitation leading to large variations in water level) may also favour dominance of small forms (for a review see Jeppesen *et al.*, 2005a).

Among the many small fish species in subtropical lakes in the south eastern region of South America, the 'one-sided livebearer' *J. multidentata*, is one of the most common omnivorous–planktivorous fish (Ringuelet, 1975), often occurring in high densities in (hyper)eutrophic shallow lakes (Scasso *et al.*, 2001; Mazzeo *et al.*, 2003; Garcia *et al.*, 2006). *Jenynsia multidentata* can feed on zooplankton, phytoplankton, periphyton, invertebrates and also on detritus (Escalante, 1987; Koblitz & Andreata, 1996; Marti *et al.*, 2006). Its distribution ranges from Rio de Janeiro (Brazil: 43°15′W, 22°15′S) to Uruguay and Argentina (67°20′W, 39°47′S) (Ghedotti, 1998). It exhibits a wide tolerance of several environmental variables (being euryhaline, eurythermic, euryoic) (Thormahlen de Gil, 1940; Gómez, 1993; Menni, Gómez & López Armengol, 1996; Betito, 2006), allowing the species to inhabit many aquatic systems (Bistoni *et al.*, 1999; Kruk *et al.*, 2006). In shallow lakes that lack piscivores *J. multidentata* may reach extremely high abundances (Mazzeo *et al.*, 2003), often associated with aquatic plants (Mazzeo *et al.*, 2003; Iglesias *et al.*, 2007; Meerhoff *et al.*, 2007a,b). It is viviparous (Siccardi, 1940), reproducing throughout the year in the tropics (Novaes & Andreata, 1996) and in spring and summer in subtropical and temperate regions (Turner, 1957; Garcia *et al.*, 2004).

The aim of the present study was to assess the impact of an omnivorous–planktivorous fish on zooplankton size distribution in a subtropical shallow eutrophic lake, using both an empirical and an experimental approach. We hypothesized that the zooplankton community would be dominated by medium to large sized herbivorous individuals (cladocerans and calanoid copepods) only in the absence or at low abundance or activity of predatory fish.

Methods

Field survey

The field data were collected four times from winter 2003 to autumn 2004. Both fish and zooplankton communities were sampled in a shallow lake that lacks piscivorous fish. Lake Blanca (Maldonado, Uruguay, 34°54′S; 54°50′W) is a shallow (total area = 69.1 ha, Z_{max} = 3.6 m), warm polymictic (temperature range: 11.3–26.3 °C) and eutrophic lake (in-lake annual mean total phosphorus (TP), total nitrogen (TN) and chlorophyll *a* concentrations: 94, 1010 and 31.9 μg L^{-1}, respectively). Since a dramatic reduction in lake volume during 1997–98 (Mazzeo *et al.*, 2003), eliminating most of the native species, the fish community has been dominated by small (13 mm < SL < 86 mm) *J. multidentata*.

For each sampling season, the plant volume infested (PVI, *sensu* Canfield *et al.*, 1984) of submerged and emergent vegetation coverage was mapped. Five sampling points within each of three microhabitats were randomly selected: emergent plants (EP), submerged plants (SP = PVI ≥25%), and open water without plants (OW). Water samples for zooplankton quantification were collected with a vertical tube enclosing the whole water column at midday and midnight and averaged before analyses. In each habitat 20 L of water were filtered through a 50 μm mesh size net and subsequently preserved in 4% formaldehyde.

Zooplankton were classified to species level and counts were made according to Paggi & de Paggi (1974). For each habitat the abundances of microzooplankton (nauplii + rotifers) and mesozooplankton (small cladocerans + calanoid copepodites + adults) were determined and the mesozooplankton/microzooplankton (meso/microzooplankton) ratio calculated.

Quantitative sampling of the fish community was performed using two unbaited minnow traps (double cone, 60 × 80 cm, 5 cm opening, mesh 0.5 mm, volume = 40 L) per station, one placed close to the surface and one close to the sediment. The catches integrated the periods between sunrise and sunset (day) and sunset and sunrise (night), respectively. Fish were classified to species level and capture per unit effort was calculated (CPUE = total number of individuals collected over the period in two traps, per hour).

Use of trap samples for dietary analyses is not advisable due to abnormal feeding conditions inside the traps and post-catch digestion (Windell & Bowen, 1978). For diet analyses, point-sample electric fishing (Perrow, Jowitt & González, 1996) was undertaken using Sachs Elektrofischfanggerate GmbH D-88299 Leutkirch equipment. In each considered habitat (submerged plants, emergent plants and open waters), five samples were taken at noon and midnight. Each sample integrated catches of nine pulses of 5 s taken in random transects (mesh seine 0.5 mm). Fish were fixed in 10% formaldehyde. This technique prevents regurgitation; digestion stops quickly and the stomach content is well preserved (Bowen, 1983; APHA, 1998).

Length–age relationship was established for each sex of *J. multidentata* from the apparent shift of the modes in a time series of length–frequency samples (modal progression analysis) using the FiSAT II software based on the results of Bhattacharya's method (n = 8574 fishes, s.i. > 2; Gayanilo *et al.*, 2005). It was assumed that the length of the fish of a certain age is normally distributed (Sparre & Venema, 1997).

Gut content analysis of 151 randomly chosen *J. multidentata* belonging to all sampled seasons was performed under dissecting microscope. To test differences in the use of trophic resources, three age (by length) groups were considered: group 0 (0–9 months), group 1 (12–21 months) and group 2 (≥24 months). Presence/absence data were registered and frequency of occurrence of each item was calculated (Hyslop, 1980). Trophic level, defined as position in the food chain assessed by the number of energy-transfer steps to reach that level (Begon, Townsend & Harper, 2006), was estimated from the percent contribution of each item (SIMPER; Clarke, 1993; Clarke & Warwick, 1994) using the approach of TrophLab (Pauly *et al.*, 2000). The same methodology was followed for *C. decemmaculatus*.

Mesocosm experiment

An experiment in six outdoor 3800 L mesocosms (3 × 2 × 0.60 m) was conducted from 9 April to 11 May 2005 to test the effect of *J. multidentata* on the zooplankton community. The bottoms of the mesocosms were covered with washed river sand and they were filled with groundwater. Thirty per cent of the total surface area was covered with *Eichhornia crassipes* (Mart) Solms. The experimental units were allowed to settle for 2 months prior to initiation of the experiment to allow phytoplankton and zooplankton to develop. High nitrogen and phosphorous inputs were maintained, to prevent any confounding effects of nutrient limitation, by weekly additions of 5 mg N and 5 mg P to each unit, as commercial fertilizer NPK (15-15-15) ISUSA, TN, P_2O_5 and K_2O, respectively.

To evaluate the predation effect on medium and large-sized cladocerans, *Daphnia obtusa* Kurz specimens were cultured and added 2 months before the initiation of the experiment. To ensure uniform initial conditions in the experimental units, up to 2000 L from each mesocosm were distributed among the other five mesocosms (400 L to each). This homogenization procedure was done using a special pump allowing interchange of water, phytoplankton and zooplankton.

Initial samples were taken at T_0 after homogenization and just before randomly assigning two different treatments to the experimental units: no-fish: zooplankton + phytoplankton (n = 3) and fish: *Jenynsia* *multidentata* (9 ind m^{-2} collected from Lake Blanca) + zooplankton + phytoplankton ($n = 3$). To prevent predation of fish by birds, the mesocosms were covered by a plastic net.

Water samples for analysis of zooplankton and nutrients (TN and TP; Valderrama, 1981) were collected throughout the water column with a tube at midday, integrating eight sites per mesocosm without previous mixing of water. For zooplankton quantitative analysis, 15 L of water were filtered through a 50 μm net and the specimens fixed with acid Lugol. Counts were made according to Paggi & de Paggi (1974) and abundances were expressed as ind L^{-1}. Animals were grouped as microzooplankton (nauplii + rotifers) and mesozooplankton (cladocerans + calanoid copepodites and adults) and the mesozooplankton/microzooplankton ratio was calculated. Dissolved oxygen, conductivity and pH were recorded *in situ* in the central part of the mesocosm using Horiba sensors.

Statistical analyses

Field data were analysed using ANOVA with season as factor (four levels: winter, spring, summer and autumn), and considering each habitat (submerged plants, emergent plants and open waters) separately, Tukey *post hoc* tests were used. We thus assumed the four seasons to be independent datasets, which is likely for organisms with fast turnover, such as zooplankton. To test for ontogenetic difference in use of food resources, analysis of similarity (ANOSIM, Clarke, 1993; Clarke & Warwick, 1994) test (999 permutations; $P_{\mathrm{global}} < 0.05$) over Raup-Crick similarity matrix for presence/absence data (Raup & Crick, 1979) was performed considering the age groups defined *a priori*. Student *t*-test for each sampling date was performed for physical, chemical and biological mesocosm data to investigate statistical differences between fish and no-fish treatments. Assumptions of the statistical tests were verified, Cochran *C*-tests were used to check variance homocedasticity, while normality was tested using Kolmogorov–Smirnov test and by visual inspection of residuals. When violations were detected, square root transformations (x or $x + 1$) were performed. The correlation between the seasonal variation of mesozooplankton abundance and fish CPUE was analysed using non-parametric Spearman (rs).

Results

Field survey

The zooplankton community was dominated by microzooplankton, except in winter when mesozooplankton was important as well. Microzooplankton abundance peaked in autumn (1691 ind L^{-1}), while minimum numbers occurred in winter (191 ind L^{-1}). Nauplii dominated the microzooplankton in winter, while rotifers were most abundant during the other seasons. *Keratella cochlearis* Gosse was the most abundant rotifer in the system year round, but *Fillinia longiseta* Ehrenberg and *Polyarthra* sp. Ehrenberg were also common, principally in summer and autumn. Among the mesozooplankton, the most abundant cladoceran species were *Bosmina longirostris* Müller and *Diaphanosoma birgei* Korinec. Cladocerans were homogeneously distributed in spring (n.s., OW = PE = PS) while they in winter and summer occurred in higher abundances in the open waters followed by the emergent plants ($F_{2,12} = 19.1$, $P < 0.001$ and $F_{2,12} = 15.3$, $P < 0.001$, respectively, OW > PE > PS, Fig. 1a). In autumn they were more abundant in the submerged plants ($F_{2,12} = 6.1$, $P < 0.05$. PS > PE = OW, Fig. 1a). The calanoid copepod *Notodiaptomus incompositus* Brian was also common and was homogeneously distributed all year round among the three habitats (Fig. 1b).

The meso/microzooplankton ratio varied strongly among seasons in all three habitats. Statistical differences among habitats were found in winter and autumn when the highest values occurred in open water ($F_{2,12} = 13.0$, $P < 0.001$ and $F_{2,12} = 7.0$, $P < 0.01$, respectively. OW > PS = PE, Fig. 1c), while no statistical differences were detected in spring and summer. Considering the seasonal variation of the ratio, winter values were an order of magnitude higher than the other seasons ($F_{3,36} = 86.8$, $P < 0.0001$. Winter > spring = summer = autumn, Fig. 1c).

The fish community was dominated by *J. multidentata*. The highest and lowest catches occurred in summer and winter, while intermediate catches occurred in spring and autumn (2-way ANOVA $F_{3,36} = 40.3$, $P < 0.001$; Fig. 1d). In winter, CPUE was higher in the submerged plants ($F_{(2,12)} = 17.5$, $P < 0.001$; SP > EP > OW), while in spring fish were collected primarily in the emergent plant sites ($F_{(2,12)} = 18.1$, $P < 0.001$; EP > SP = OW for spring

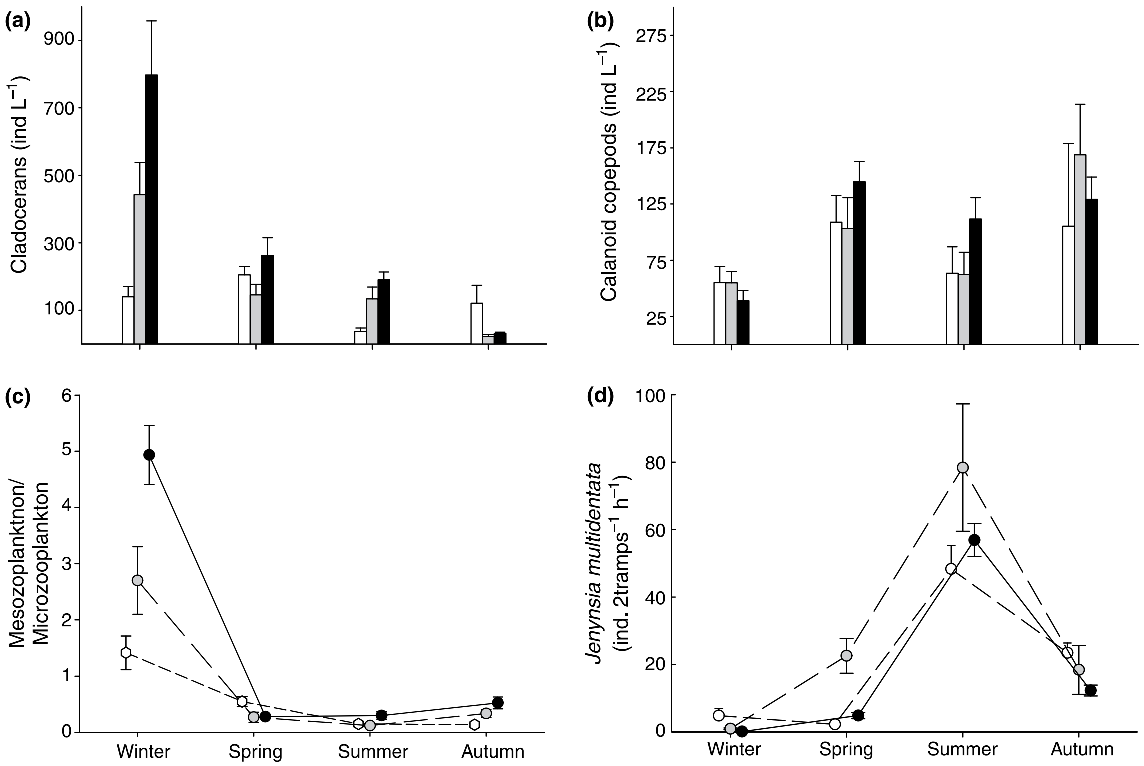

Fig. 1 Seasonal variation in: (a) cladoceran abundance; (b) calanoid copepod abundance (adults + copepodites), (c) herbivorous mesozooplankton/microzooplankton abundance ratio and (d) the CPUE of the omnivorous planktivorous fish *Jenynsia multidentata*; in submerged plants (open), emergent plants (grey) and open waters (black). Error bars represent ±1SE. Mesozooplankton = cladocerans + calanoid copepodites and adults, microzooplankton = rotifers + nauplii.

and summer respectively). In summer and autumn no differences among habitats were detected.

Young of the year (YOY) individuals of *J. multidentata* were absent in winter samples, recruitment started in spring and continued until autumn. The other fish species captured, the small omnivorous *Cnesterodon decemmaculatus* (Jenyns, 1842; Poecilidae), occurred in low abundance (<1.5% of total fish density and biomass).

Mesozooplankton abundances were correlated negatively with the CPUE of *J. multidentata* in all the considered habitats (rs = −0.69, $P < 0.0001$; rs = −0.58, $P < 0.0001$; rs = −0.63, $P < 0.0001$ for PS, PE and OW, respectively).

Gut content analysis confirmed the previously reported omnivorous–planktivorous diet habits of *J. multidentata*. Mesozooplankton constituted the most frequent item ($F_o = 0.81$). The diet also included insects (mainly Diptera, *Chaoborus* sp. and chironomids) and basal items of trophic level 1 (e.g. periphyton, phytoplankton, plant remains, sediments and/or detritus) (Table 1). Shrimps and fish were infrequent items. The trophic level was 3.0 ± 0.4 (mean ± SE).

All comparisons of food choices between age groups showed significant differences (Table 2). *J. multidentata* exhibit a clear ontogenetic diet shift, mesozooplankton contributed *c.* 90% of gut content of age group 0, while it represented *c.* 70% for group 1

Table 1 Items found in the stomach contents of *Jenynsia multidentata* from Lake Blanca. Frequency of occurrence (F_0 *sensu* Hyslop, 1980) and percent contribution of each item by age group (SIMPER *sensu* Clarke, 1993)

		% contribution		
Item	F_0	Group 0	Group 1	Group 2
Mesozooplancton	0.811	87.0	69.2	40.3
Diptera	0.336	9.0	22.6	36.4
Periphyton	0.315	3.9	7.9	18.2
Shrimp	0.098	0.1	0.3	5.1
Fish	0.014	0.0	0.0	0.0*

*Very close to 0.

Table 2 ANOSIM comparison of food choices among three different age groups of *Jenynsia multidentata* from Lake Blanca

Comparison	*R*	*P*
Global analysis	0.139	0.01
0 versus 1	0.088	0.02
1 versus 2	0.268	0.02
0 versus 2	0.388	0.02

Group 0: 0–9 month, group 1: 12–21 month, and group 2: >24 month old fish, respectively.
The *R* statistic generated by ANOSIM is 0 when there is no difference between groups.
Increasing positive values mean greater differences between groups.

declining to less than 40% in group 2 (Table 1). Almost the entire *C. decemmaculatus* catches were obtained in SP (79%), and its gut contents consisted only of basal items (i.e. trophic level = 2; $n = 15$).

Mesocosm experiment

No fish kill occurred during the experiment as all fish stocked were recovered from the mesocosms at the end of the experiment. A major decline in *D. obtusa* abundance occurred shortly after the mesocosms were stocked with *J. multidentata* (Fig. 2a), significant differences being observed from day 11, when *Daphnia* disappeared from one of the fish tanks and abundances of 3.3 and 20.0 ind L^{-1} were registered in the other two. On day 32 *Daphnia* was absent in two of the three tanks, while it reached 196 ind L^{-1} in the last replicate. In the no-fish treatment we found abundances of 198 ± 108 and 275 ± 119 ind L^{-1} (mean ± SE) on days 11 and 32, respectively (n.s., $t_4 = 4.6$, $P < 0.05$, $t_4 = 4.2$, $P < 0.05$, on day 0, 11 and 32, respectively (Fig. 2a,b). Concomitantly, in the fish treatment, microzooplankton (particularly rotifers) rose in abundance from 10 ± 2 to 299 ± 96 ind L^{-1} (mean ± SE), being higher than in the no-fish treatment from day 11 until the end of the experiment ($t_4 = -5.5$, $P < 0.01$, $t_4 = -2.8$, $P < 0.05$, at time 11 and 32, respectively). From initially similar meso/microzooplankton ratios, the ratio dropped in the fish treatments, but increased in the no-fish treatments, resulting in significant differences among treatments from day 11 until the end of the experiment (n.s.,

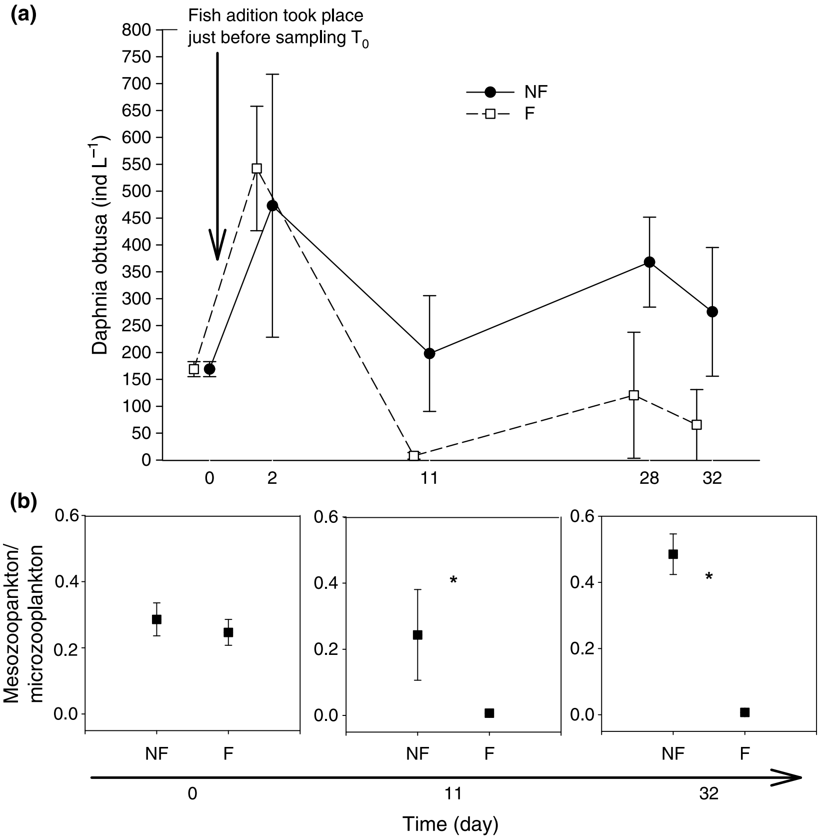

Fig. 2 (a) Changes in *Daphnia obtusa* abundance (top graph) during the experiment. NF = no-fish treatment, $F = 9$ ind m^{-2} fish treatment, error bars represent 1 SE. Sampling was conducted on the same dates in the two treatments, but NF has been shifted in the figure to clarify patterns. (b) Experimental variation in the herbivorous mesozooplankton/microzooplankton ratio is shown at start-up (day 0) and day 11 and 32. *significant differences between treatments. Mesozooplankton = cladocerans + calanoid copepodites and adults, microzooplankton = rotifers + nauplii. Mean (three replicates average), ±1 SE.

$t_4 = 3.2$, $P < 0.05$, $t_4 = 6.4$, $P < 0.01$, at time 0, 11 and 32, respectively, Fig. 2b).

No significant differences were detected for physical and chemical variables among treatments during the experiment. The temperature varied from 13.1 to 19.7 °C, dissolved oxygen from 8.2 to 12.9, pH values were always around 9, while conductivity was near 500 μS cm^{-1}. The supply of TP and TN ensured no bottom-up limitation during the experiment and no statistical differences were detected among treatments: TP rose from 2.6 ± 0.1 to 7.2 ± 0.5 mg L^{-1}, while TN varied from 2.1 ± 0.3 to 22.6 ± 0.9 mg L^{-1} on day 0 and 32, respectively.

Discussion

Both field and experimental data suggest a strong effect of *J. multidentata* on the size distribution of zooplankton. The zooplankton community in Lake Blanca was dominated by microzooplankters, particularly rotifers, and this is consistent with previously reported results for eutrophic-hypereutrophic (sub)tropical lakes (Crisman & Beaver, 1990; Dumont, 1994; Branco *et al*., 2002; Garcia *et al*., 2002; Jeppesen *et al*., 2005a; Havens *et al*., 2007). The small size of the cladocerans (mainly *B. longirostris* and *D. birgei*), the absence of large bodied specimens (e.g. *Daphnia* spp.) and the dominance of rotifers are consistent with what would be expected due to size selective predation on larger zooplankton by abundant zooplanktivorous fish.

A clear seasonal variation in meso/microzooplankton ratios was found in the lake in all three habitats considered. In winter, when fish catches were low (2.3 fish in 2 traps h^{-1}) higher ratios were found, reflecting the highest mesozooplankton and the lowest microzooplankton abundances observed. The ratio was an order of magnitude lower during spring when catches of *J. multidentata* started to increase and YOY fish appeared in the lake, and during the rest of the seasons when catches were high (up to 40 times winter catches). Zooplankton constitutes *c*. 90% of the gut content of YOY fish, which was more than twice as high frequency as in 2-year-old specimens (Table 1), High abundances of YOY fish in spring (G. Goyenola, unpubl. data) and likely also a higher fish metabolism may therefore explain the strong effect on zooplankton in spring despite relatively minor changes in fish CPUE from winter to spring. The seasonal variation in herbivorous zooplankton size fractions accords with previous findings from subtropical and warm temperate lakes, such as those for Lake Blanca (Mazzeo *et al*., 2003) and Lake Rodó in Uruguay (Scasso *et al*., 2001), and in Lake Albufera in Spain (Romo *et al*., 2005). The omnivorous habit of *J. multidentata*, shown by gut content analyses, may be responsible for a high carrying capacity of this species. Therefore, it is to be expected that top-down control of *J. multidentata* on mesozooplankton will be high. Furthermore, *J. multidentata* was abundant not only in the open water, but also among plants, leaving little opportunity for cladocerans to seek refuge from predators among vegetation as otherwise reported from temperate shallow lakes (Burks *et al*., 2002; Meerhoff *et al*., 2007b).

The role of fish for structuring the zooplankton community and size structure is confirmed by the experimental results. *Jenynsia multidentata* exerted a strong predation pressure on cladocerans, particularly *D. obtusa*. Following a decrease in *Daphnia* abundance, rotifers became significantly more abundant in the fish treatments and accordingly the meso/microzooplankton ratio decreased substantially, while the ratio remained high in the no-fish treatments (Fig. 2). The ratios estimated were higher in the experiment (fish treatments) than in the lake, and also that *D. obtusa* occurred in one of the fish mesocosm but not in the lake. These differences probably reflect a higher abundance of fish in the lake. We used 9 fish m^{-2}, the density previously reported in Laguna Blanca (Mazzeo *et al*., 2003), but recent studies have reported higher densities, around 25–42 fish m^{-2} (Meerhoff *et al*., 2007a,b).

Our field and experimental results show that at reduced fish (in our study *J. multidentata*) abundance or activity, large-sized zooplankton, including *D. obtusa*, are able to reach high abundances. In accordance with these results, Mazzeo *et al*. (2000) observed an extremely high abundance of *D. obtusa* in Lake Rivera (hypertrophic system located in Montevideo, Uruguay) and short-term clear-water conditions after an extensive fish kill caused by high ammonia levels.

Considering the habitat preferences of *J. multidentata*, its often high abundance and its ability to persist at low oxygen levels and in turbid water conditions, it is likely that this species (together with other small fish species) reinforces the eutrophication process in nutrient enriched subtropical lakes in South America.

Control of the abundance of small omnivorous–planktivorous fish is therefore a key issue in the rehabilitation of such lakes. Experience from temperate lakes suggests that reductions in nutrient loading may lead to lower abundances of planktivorous–benthivorous fish and a higher proportion of piscivorous fish (Jeppesen *et al.*, 2005b). To reinforce recovery, fish manipulations have been used in temperate lakes (Meijer *et al.*, 1994; Benndorf, 1995; Hansson *et al.*, 1998; Mehner *et al.*, 2002). Previous studies have shown, however, that in contrast to temperate lakes active removal may have only a short term effect in warmer climates, mainly because a viviparous fish population (e.g. *Jenynsia multidentata* or *Cnesterodon decemmaculatus*) recovers fast even after extensive fish removal (Jeppesen *et al.*, 2005a, 2007). In (sub)tropical lakes, fish reproduce several times a year (Fernando, 1994; Lazzaro, 1997; Pinel-Alloul *et al.*, 1998), whereas temperate fishes typically reproduce only once a year. Piscivorous fish stocking may be an alternative biomanipulation strategy in subtropical systems, not least when predatory fish are missing or their abundance is low. However, this remains to be tested.

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CASCADING EFFECTS PROMOTED BY FISH AND MACROINVERTEBRATES ON FOOD WEBS OF SHALLOW LAKES IN DIFFERENT CLIMATE ZONES – A MESOCOSM EXPERIMENT

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Cascading effects promoted by fish and macroinvertebrates on food webs of shallow lakes in different climate zones – a mesocosm experiment

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Summary

1) Shallow lakes are often characterized by the presence of aquatic plants, which may impact lake functioning and water transparency, particularly via affecting trophic cascades promoted by fish. Recent studies have indicated major differences in the structuring role of plants in temperate and subtropical and tropical lakes, though firm experimental evidence is scarce.

2) To test for differences in the cascading effects promoted by fish and macroinvertebrate predators in temperate and subtropical shallow lakes, we conducted an in-lake 49-day long mesocosm experiment in three pairs of lakes in Uruguay (30º–35 ºS). and Denmark (55º–57 ºN). We used two mostly planktivorous fish species and one omnivorous macroinvertebrate species, alone and combined and in numbers resembling their natural densities, and analysed changes in zooplankton, phytoplankton, littoral macroinvertebrates and periphyton.

3) We found clear effects of fish on the pelagic food webs in both climate zones. Zooplankton structure was affected by fish, and particularly cladocerans and calanoid copepods were drastically diminished. These effects cascaded down to the phytoplankton and resulted in higher phytoplankton biomass in the fish treatments in both locations. Macroinvertebrate predators had no distinctive effect on zooplankton and phytoplankton irrespective of climate zones.

4) The effects of predators on the littoral food web were partly unexpected. As expected, the abundance of plant-associated macroinvertebrates was significantly affected by fish in both climate areas. However, contrary to our expectations, periphyton biomass did not vary among treatments and in general tended to increase in all the lakes.

5) The experiments demonstrated that fish, more than macroinvertebrate predators, determine the structure of the pelagic and littoral herbivorous communities in both subtropical and temperate shallow lakes, with visible cascading effects on phytoplankton, but not so on periphyton. Thus, littoral pathways seem more complex than anticipated, particularly for the subtropical lakes.

Introduction

Aquatic communities and food webs are typically structured by available resources and predation (Carpenter, Kitchell, Hodgson et al., 1987), classically known as bottom-up and top-down driving forces, respectively (McQueen, Post and Mills, 1986). Different food webs may occur within aquatic systems (e.g. pelagic, littoral), which may be strongly linked by the movement of fish, some macroinvertebrates and zooplankton (Vander Zanden and Vadeboncoeur, 2002), particularly in shallow lakes. The impact of fish predation on freshwater zooplankton is well documented, and the occurrence of planktivorous fish is known to induce major shifts in the size distribution (Hrbácek, Dvorakova, Korinek et al., 1961; Brooks and Dodson, 1965). and sometimes the behaviour of zooplankton, especially in temperate lakes (Timms and Moss, 1984; Lauridsen and Lodge, 1996; Burks, Lodge, Jeppesen et al., 2002; Romare and Hansson, 2003). By also consuming plant-attached macroinvertebrate grazers, such as snails, fish may indirectly enhance periphyton growth, as suggested by experimental results (Brönmark and Weisner, 1992; Jones and Waldron, 2003: Liboriussen, Jeppesen, Bramm et al., 2005). and field data (Jones and Sayer, 2003); although others have found no fish effects on periphyton (Bertolo, Lacroix, Lescher-Moutoué et al., 2000).

However, fish predation seems to be even stronger in warm lakes than in comparable temperate lakes (Meerhoff, Clemente, Teixeira de Mello et al., 2007a), likely due to the typically higher densities, greater trophic diversity (with predominance of omnivores). and smaller body size (Lazzaro, 1997, Teixeira de Mello, Meerhoff, Pekcan-Hekim et al., 2009). Recent experimental studies have shown important effects of planktivorous fish predation on subtropical zooplankton (Boveri and Quirós, 2007; Iglesias, Mazzeo, Goyenola et al., 2008), while other studies have focused on the zooplankton structure in field surveys (Meerhoff, Iglesias, Teixeira de Mello et al., 2007b; Castro, Marques and Goncalves, 2007). and behavioural shifts related to the risk of predation from fish (Meerhoff, Fosalba, Bruzzone et al., 2006; Meerhoff et al., 2007b, Iglesias, Goyenola, Mazzeo et al., 2007). or by predacious littoral macroinvertebrates (González-Sagrario, Balseiro, Ituarte et al., 2009). A common feature in subtropical lakes is that mesozooplankton often is dominated by small cladocerans, nauplii and rotifers (Crisman and Beaver, 1990; Dumont, 1994; Branco, Rocha, Pinto et al., 2002; Meerhoff et al., 2007b; Kruk, Rodríguez-Gallego, Meerhoff et al., 2009), resulting in a overall low grazing pressure on phytoplankton. Moreover, Meerhoff et al. (2007a). found a substantially lower abundance of plant-attached macroinvertebrates and lower biomass of periphyton on artificial plants in subtropical than in similar temperate shallow lakes, arguably because fish feed on the periphyton to a larger extent than in temperate lakes. Hence, fish would partly become primary consumers, implying that food webs are more truncated in the subtropics (Meerhoff et al., 2007a).

By affecting these prey-predator interactions, macrophytes may influence importantly the whole-lake functioning (Carpenter and Lodge, 1986; Jeppesen, Jensen, Søndergaard et al., 1997). As a result, submerged plants often promote positive effects on water clarity (Scheffer et al., 1993), particularly in temperate shallow lakes. At warm climate conditions, such effects seem substantially reduced (Jeppesen, Meerhoff, Jacobsen et al., 2007), not least because macrophytes do not act as proper refuges for large zooplankton (Meerhoff et al., 2006; Meerhoff et al., 2007b). but host small fish instead (Conrow, Zale and Gregory, 1990; Meerhoff, Mazzeo, Moss et al., 2003; Teixeira de Mello et al., 2009).

In the above mentioned study, Meerhoff et al. (2007a). used open plant beds that allowed disturbance by waves and potentially attracting high numbers of fish from a large lake area. However, such an experimental design does not permit disentanglement of effects between different predators, such as fish and macroinvertebrates (typically shrimps), and between predation and mechanical disturbance. In the present study we focus on the role of fish and macroinvertebrate predators on the trophic structure jn closed experimental mesocosms under different climates. We emphasise on pelagic (i.e. fish-zooplankton-phytoplankton). and littoral (i.e. fish-plant-associated macroinvertebrates-periphyton). trophic interactions. Based on the earlier findings, we hypothesized that a fish and macroinvertebrate effect can directly affect primary consumers and cascade down the trophic web, resulting in facilitation for phytoplankton, irrespective of climatic zone, but most notably in the subtropics. We further hypothesized that in subtropical lakes, fish, and probably macroinvertebrates (shrimps). as well, negatively affect the development of periphytic algae by direct consumption, being higher here than in temperate lakes.

Methods

We selected three shallow lakes in Uruguay (30-35°S, lakes Blanca, Diario and Nutrias). and Denmark (55-57°N, lakes Kogleaks, Stigsholm and Bølling), with approximately the same gradients in environmental variables including submerged macrophyte cover (range: 0-70 % plant volume inhabited, PVI, sensu Canfield, Shireman, Colle et al. (1984)). (Canfield, Shireman, Colle et al., 1984), salinity, pH, turbidity, total nutrients and phytoplankton chlorophyll-a (Chl-a). range (Table 1). We carried out the mesocosm experiments during summer 2008, in both the southern (January-April). and northern hemispheres (August-October).

We manipulated the abundances of two mostly planktivorous fish species and one omnivorous macroinvertebrate species in 16 transparent cylindrical PVC enclosures (diameter 1.2 m). in each of the six lakes (Table 2). The mesocosms were kept open to the atmosphere and fixed by a metallic ring penetrating ca. 0.30 m into the sediment, thus ensuring isolation from the outside water. A hard plastic ring and several rubber bands mounted to poles secured the top ca. 0.40 m above the water surface. We placed the mesocosms along both sides of a specially constructed bridge enabling us to take the samples with minimum disturbance (Fig. 1). The water level within the enclosures varied between 0.8 and 1.1 m during the course of the experiment, approximating a total water volume of 1000 litres. Prior to the establishment of the mesocosms, we carefully removed all natural vegetation from the area. Fish were prevented from entering the bags by a small-sized mesh net placed on the bottom of each mesocosm during the set-up.

Table 1. Main limnological parameters of the lakes where the experiment was conducted. Shaded subtropical Uruguayan lakes (30°-35°S) and non-shaded temperate Danish lakes (55°-57°N), showing mean dissolved oxygen (DO$_2$), pH, water turbidity (NTU), percentage of volume inhabited by submerged plants (% PVI), concentration of phytoplankton chlorophyll-a (Chl-a) at the beginning and end of the experiments, and distinctive traits of the lakes, including submerged plant % PVI and total nitrogen (TN) and total phosphorus (TP) concentrations.

	Diario		Blanca		Nutrias		Kogleaks		Stigsholm		Bølling	
	start	**end**	**start**	**end**	**start**	**end**	**start**	**end**	**start**	**end**	**start**	**end**
Temperature (°C)	28.0	20.5	22.5	21.4	27.0	20.2	16.6	8.3	15.1	10.4	14.7	13.9
DO$_2$ (mg L^{-1})	9.7	7.0	6.7	10.4	7.4	8.4	5.7	9.1	10.5	9.3	8.7	7.6
pH	8.6	7.5	8.4	7.61	6.14	5.8	6.9	7.5	8.4	7.5	7.1	7.2
Conductivity (mS cm^{-2})	566	617	318	316	75	82	595	542	210	185	116	116
Turbidity (NTU)	6.3	20	14.2	18.2	26.3	39.7	12.4	2.1	3.6	3.9	14.7	17.3
Phyto. Chl-a (µg L^{-1})	15.7	9.6	46.5	56.1	15.7	4.7	8.9	4.7	11.4	8.2	8.4	10.7
Distinctive traits	Partly brackish		Cyanobacteria blooms		Humic		Partly brackish		Cyanobacteria blooms		Humic	
PVI (%)	>75%		<25%		0%		ca. 50%		ca. 50%		0%	
TN (µg L^{-1})	970		1391.5		670		2330		2275		1600	
TP (µg L^{-1})	89.2		65.9		122.5		214.2		55		182.8	

Table 2. Fish and macroinvertebrate predator species and numbers added to the mesocosms in the treatments F: Planktivorous fish; F+INV: Planktivorous fish + macroinvertebrates, and INV: Omnivorous macroinvertebrates. In both the F and F+INV treatments two fish species were added (final densities, as ind. m^{-2}, are given in parenthesis). Densities of predators were taken from literature: *Teixeira de Mello et al. 2009; +Liboriussen et al. 2005; ¤ Wilhelm and Schindler 1999.

		Fish sp. 1	**Fish sp. 2**	**Macroinvertebrates**
Subtropical	Name	*Cnesterodon decemmaculatus*	*Jenynsia multidentata*	*Palaemonetes argentinus*
	Density	50 (42)*	40 (33)*	120 (100)*
Temperate	Name	*Gasterosteus aculeatus*	*Perca fluviatilis*	*Gammarus lacustris*
	Density	12 (10)+	6 (5)*	240 (200)¤

To each mesocosm we introduced an artificial plant bed mimicking submerged plants (120 per module, PVI% =75%). The plants were made of "hairy" 1.0-long plastic pieces (originally green Christmas tree garlands). with an architecture similar to that of macrophytes such as Myriophyllum or Cabomba spp. (as in Meerhoff et al., 2007b). The plants were introduced to the mesocosms two weeks before the first sampling to allow periphyton and invertebrates to colonize the plastic structures.

We ran a 4 × 4 factorial design (with four replicates per treatment). adding, respectively, F: only planktivorous fish; F+INV: planktivorous fish + omnivorous macroinvertebrates; INV: only omnivorous macroinvertebrates and CON: no predators added (control). (Table 2). The treatments were

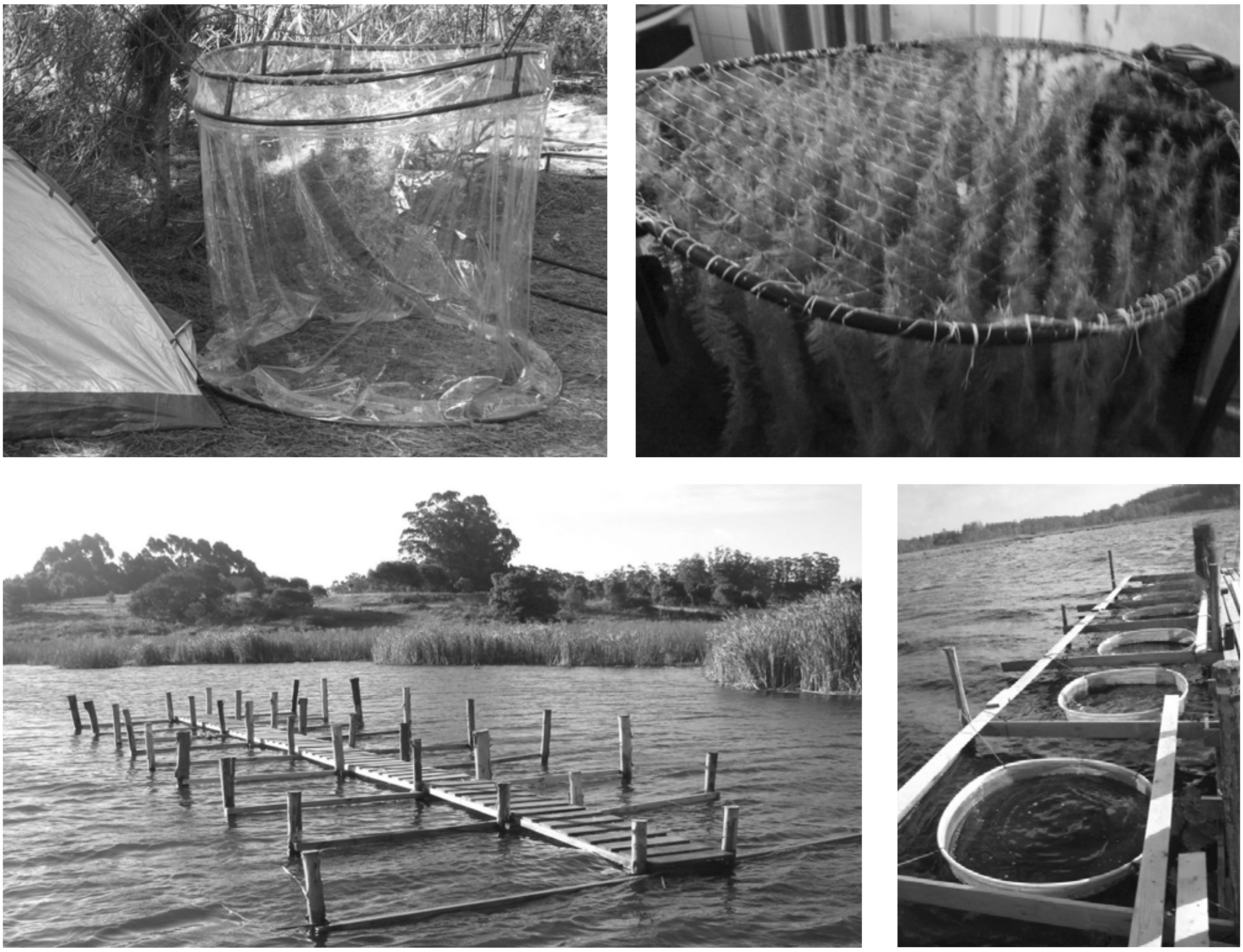

Figure 1. Eight plastic enclosures were fixed on each side of a bridge and an artificial plant bed was introduced to all enclosure two weeks before sampling to allow periphyton and macroinvertebrates to colonize the plastic structures.

randomly assigned among the 16 mesocosms. In each country we used two typically abundant fish and one macroinvertebrate species, since we focused on the potential cascading effects of an assemblage of predators. We selected both the species and the abundances to be added as to resemble their natural conditions according to available literature (Teixeira de Mello et al., 2009; Liboriussen et al., 2005; Wilhelm and Schindler, 1999; Iglesias et al., 2007). (Table 2). In all cases, we included individuals from both sexes and smaller than 7.0 cm (standard length), to assure predominance of zooplankti-benthivory feeding habits.

We took water samples for physico-chemical analyses and measured in situ parameters immediately prior to (T0). and seven weeks (49 days). later (TF). in all lakes, apart from one where some mesocosms were broken by the wind during a storm (Lake Bølling, Denmark). Since we had conducted an extra sampling campaign four weeks after T0, we considered the results from this campaign to represent the final conditions for Lake Bølling. We collected depth-integrated water samples for the analysis of phytoplankton, zooplankton and nutrients (total and dissolved phosphorus and nitrogen), using a pump that integrated different depths and zones inside each mesocosm. For the quantitative analysis of zooplankton, we filtered 6 - 8 L of water through a 50-µm mesh net and fixed the filtrate with acid Lugol. We performed counts (according to Paggi and José de Paggi, 1974), and classified the identified cladocerans as free-swimming (pelagic). and plant-associated (benthic). (following Meerhoff et al., 2007b). and other zooplankters just as rotifers, calanoid and cyclopoid copepods (both juvenile and adults). and nauplii.

We measured 20 individuals of all the crustaceans occurring in each sample from the initial and final sampling campaigns and determined the biomass of total zooplankton (dry weight, DW). using the length/weight relationships of (Bottrell, Duncan, Gliwicz et al., 1976). Dumont, Van de Velde and Dumont (1975). As an indirect measure of zooplankton grazing impact on phytoplankton, we estimated the zooplankton to phytoplankton biomass ratio (hereafter zoo:phyt). Phytoplankton Chl-a was measured spectrophotometrically following cold ethanol extraction (Jespersen & Christoffersen, 1987). For the determination of the zoo:phyt ratio, we converted the Chl-a concentration to phytoplankton DW using a Chl-a:C ratio of 30 and a DW:C ratio of 2.2 (Jeppesen, Søndergaard, Kanstrup et al., 1994).

We removed one "plant" from each mesocosm (avoiding the outer part for potential wall effects). and shaked it inside a 250-µm mesh-sized net to collect the plant-associated macroinvertebrates. The animals were preserved with alcohol (70%). for identification and later classified according to their feeding habits following literature. Another plant was carefully removed, rinsed with purified tap water and shaken inside a plastic bottle to determine the biomass of associated periphyton in the laboratory (as Chl-a, Jespersen and Christoffersen, 1987). We recaptured the added fish and macroinvertebrate predators after the final sampling (TF). by electrofishing inside the mesocosms.

Statistical analyses

We performed one-way ANOVA analyses to test for initial and final differences among treatments (i.e. F, F+INV, INV, and CON), in the biomass and abundance of zooplankton, phytoplankton, plant-associated macroinvertebrates, periphyton and the calculated ratios. We used Tukey's HSD test for the post hoc comparisons. Prior to the analyses, we tested for homoscedasticity and normal distribution of residuals using the Cochran's C test and visual inspection of fitted values, respectively. When needed, we transformed the data by square-root or 4th root to homogenize variances. We tested for differences in the initial conditions between countries using Student T-test.

Results

Initial conditions

Water temperature was the physico-chemical variable varying most distinctively between the climate zones. Total nitrogen was higher in the temperate lakes (T-test t$_{89}$= 3.61, $p<0.001$), while we found no major differences in TP (Table 1). After the two colonization weeks, initial conditions were similar in the two countries regarding taxon richness and composition of plant-associated macroinvertebrates; however, mean total abundance was seven times higher in the subtropical lakes (t$_{89}$= 3.64, $p<0.001$). Also, mean total zooplankton abundance and taxon richness were similar in both countries before the introduction of predators (non significant T-test), whereas composition differed greatly. Total cladocerans were four times more abundant (t$_{89}$= 10.5, $p<0.01$), and particularly the abundance of free-swimming cladocerans was on average six times greater (t$_{89}$= 12.5, $p<0.01$). in the mesocosms from the temperate lakes (Table 3). *Daphnia* spp. occurred in the three temperate lakes (with densities of 135, 8.1 and 3.6 ind L^{-1} in lakes Kogleaks, Stigsholm and Bølling, respectively), but also in two subtropical lakes (43.0 and 5.20 ind L^{-1}, in lakes Diario and Nutrias, respectively). We found, on average, a five times greater phytoplankton biomass (t$_{89}$= 4.3, $p<0.001$). in the subtropical mesocosms, whereas periphyton biomass was two times greater in the temperate mesocosms (t$_{83}$= 2.8, $p<0.001$; Table 3).

Predators-zooplankton-phytoplankton interactions

After the 49-day long experimental period, we observed strong fish effects on zooplankton abundance (Fig. 2). and phytoplankton biomass (Fig. 3).

Table 3. Animal and plant variables in temperate and subtropical lakes at initial conditions (i.e. after 15 days of fish exclusion). Values represent averages of 16 enclosures in three lakes per climate zone. Standard errors are given in parentheses. T.R. Taxon richness. P.I. predation impact index, for invertebrates calculated as the ratio of oligochaetes relative to the sum of oligochaetes and chironomids (in densities), and for zooplankton as the ratio of microzooplankton (nauplii plus rotifers) relative to total zooplankton densities. In both cases, the closer to 1 the ratio, the higher the predation pressure.

	Littoral Macroinvertebrates			Zooplankton							Phytoplankton	Periphyton
					Total		Total clad.		Pelagic clad.		Biomass	Biomass
	T.R.	P.I.	ind g plant^{-1}	T.R.	P.I.	ind L^{-1}	T.R.	ind L^{-1}	T.R.	ind L^{-1}	µg L^{-1}	µg g plant^{-1}
Subtrop.	4.2 (0.4)	0.8 (.04)	5.1 (.85)	7.5 (0.4)	0.8 (.02)	1079.7 (94.8)	3.9 (0.3)	83.2 (11.2)	2.4 (0.2)	33.4 (5.4)	49.2 (8.7)	55.0 (8.8)
Temp.	4.2 (0.2)	0.3 (.05)	0.6 (.09)	8.7 (0.2)	0.6 (.04)	1390.8 (176.3)	4.5 (0.2)	314.9 (58.7)	2.4 (0.1)	172.5 (47.0)	12.7 (3.5)	94.9 (11.1)

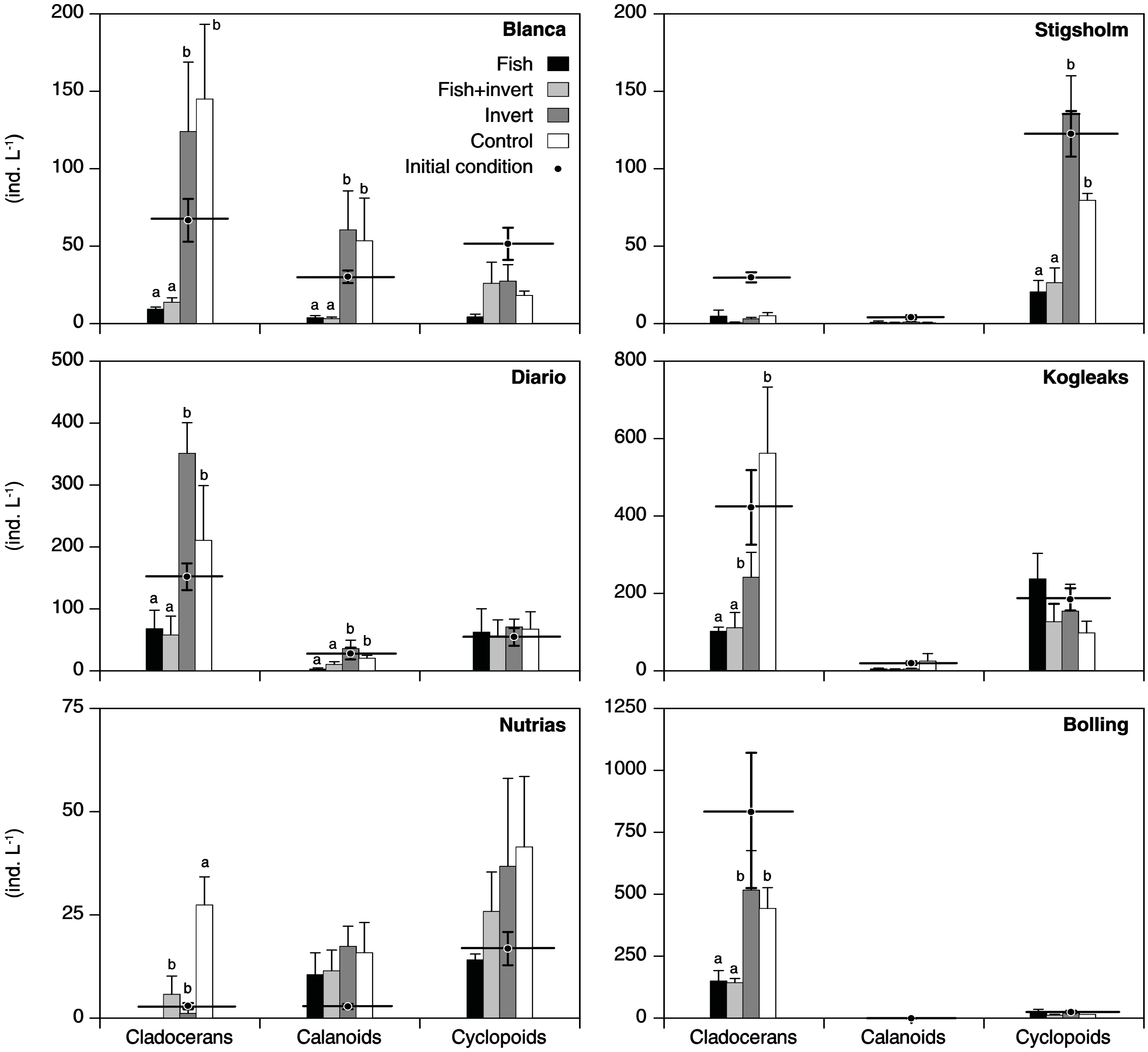

Figure 2. Effects of experimental treatments on the abundance of zooplankton in Uruguayan (left) and Danish (right) lakes. Horizontal lines represent the initial conditions (just before treatment assignment and 15 days after fish exclusion), while columns show the final conditions (49 days after fish and macroinvertebrate addition). Error bars represent ± 1 standard error. Please note the different scales on the y-axes. Letters indicate groupings based on Tukey's post hoc tests when ANOVA tests were significant.

in both climatic areas. Fish, both alone and together with macroinvertebrate predators, promoted strong effects on zooplankton, whereas macroinvertebrates alone generally did not trigger significant effects (Fig. 2).

In all the Uruguayan lakes we found statistically significant lower cladoceran abundances in the presence of fish predators (ANOVA $F_{3,12}$=4.7, p<0.05; $F_{3,12}$=4.4, p<0.05 and $F_{3,12}$=9.8, p<0.01, for lakes Blanca, Diario and Nutrias, respectively, Fig. 2). Daphnia spp. were completely excluded from the fish mesocosms (F and F+Inv), but still occurred in the fishless treatments; however, their abundance fell from 43±20 to 5.1±1.1 and from 5.2±1.3 to 2.14±0.2 ind L^{-1}, in lakes Diario and Nutrias respectively. The abundances of calanoid copepods were also significantly lower in the F and F+INV treatments in Lake Blanca ($F_{3,12}$=8.4, p<0.01). and Lake Diario ($F_{3,12}$=11.9, p<0.01). Copepod nauplii were negatively affected by fish only in Lake Blanca ($F_{3,12}$=5.5, p<0.05), whereas cyclopoid copepods never seemed to be affected by fish or macroinvertebrate predators in the subtropical lakes.

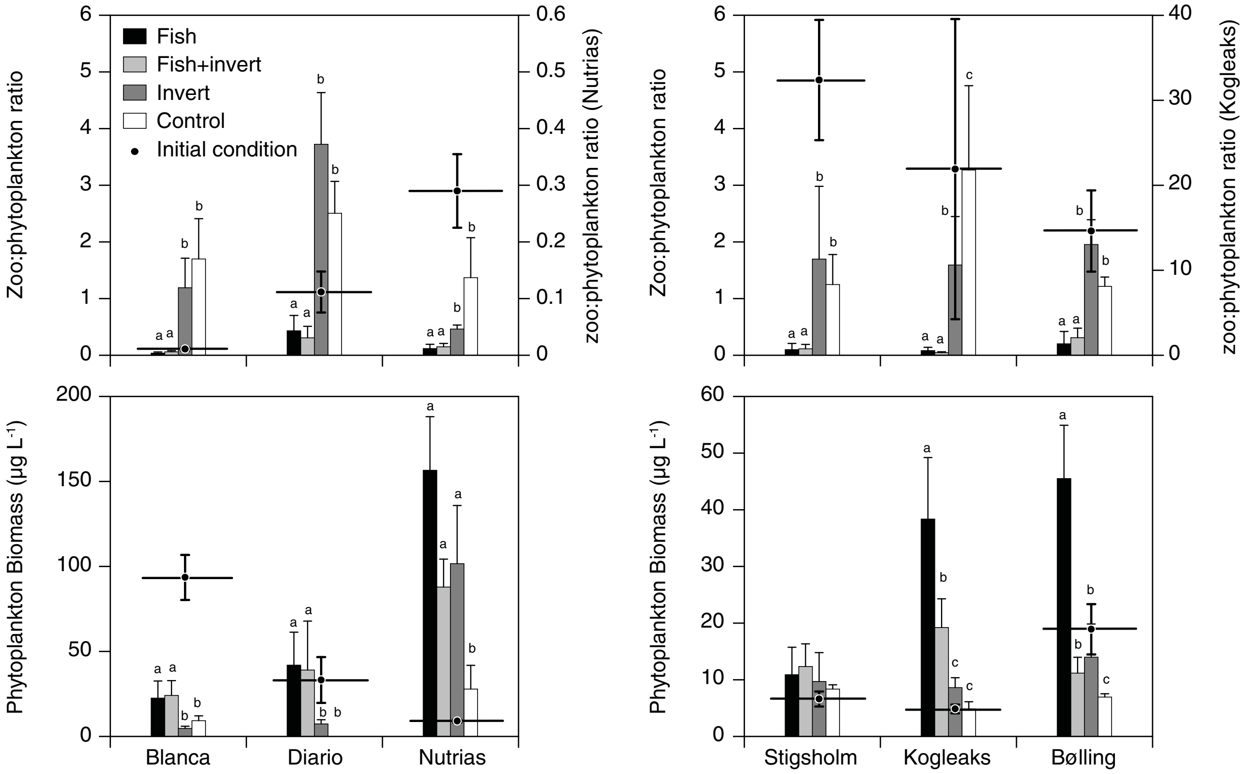

Figure 3. Effects of treatments on phytoplankton biomass and zooplankton grazing pressure (lower panels) and biomasses in subtropical (left) and temperate (right) lakes. Horizontal lines represent initial values (just before treatment assignment and 15 days after fish exclusion), while columns show final values (49 days after fish and invertebrate addition). Error bars represent ± 1 standard error. Please note the different scales on the y axes. Letters indicate groupings according to Tukey's post hoc tests when ANOVA tests were significant.

In contrast, phytoplankton biomass (measured as Chlorophyll-a). was higher in the treatments with fish ($F_{3,12}=3.3$, $p<0.05$; $F_{3,12}=10.34$, $p<0.01$ and $F_{3,12}=4.3$, $p<0.01$, in lakes Blanca, Diario and Nutrias, respectively, Fig. 3). Only in Lake Nutrias, did macroinvertebrates alone (Inv). affect the phytoplankton biomass, the effect being similar as to those in the treatments with fish (Fig. 3).

Similarly, in two Danish lakes we found statistically significant effects of fish (both alone, F, and together with Gammarus lacustris, F+INV). on cladoceran abundances ($F_{3,12}=6.6$, $p<0.01$ and $F_{3,8}=8.2$, $p<0.01$, in Lake Kogleaks and Lake Bølling, respectively). In Lake Stigsholm, in contrast, cladoceran abundances decreased in all treatments. Contrarily to the observed pattern in subtropical lakes, here Daphnia followed the same pattern as total cladocerans in all the lakes. In the three Danish lakes, calanoid copepods occurred in abundances so low that no statistical analysis could be performed. Cyclopoid copepods and nauplii decreased significantly only in the treatments including fish and only in Lake Stigsholm (F and F+INV, $F_{3,12}=15.7$, $p<0.001$ and $F_{3,12}=4.3$, $p<0.05$). (Fig. 2), and the macroinvertebrate predator alone (Inv). did not affect zooplankton.

Phytoplankton biomass was also greater here in the treatments including fish, and particularly in those with fish only, in the same lakes where zooplankton was affected (i.e. in lakes Kogleaks and Lake Bølling, $F_{3,12}=6.1$, $p<0.001$; $F_{3,10}=9.3$, $p<0.01$, respectively). In Lake Stigsholm, however, phytoplankton biomass did not differ among treatments, despite presumed differences in grazing pressure (Fig. 3).

In all the lakes in both climatic areas, the grazing pressure of zooplankton, estimated as the zoo:phyt biomass ratio, decreased significantly in both treatments with fish, but not in the treatment with only macroinvertebrate predators (Fig. 3). Despite similar trends among treatments, the estimated grazing pressure varied greatly among lakes, being extremely low in all the treatments in Lake Nutrias (Uruguay). and quite high in all the treatments in Lake Kogleaks (Denmark, Fig. 3)

Predators-plant associated macroinvertebrates-periphyton interactions

The patterns of littoral interactions were more complex than the pelagic ones, with predator effects reaching the plant-associated macroinvertebrates, but not the periphyton level. Fish (F and F+INV), but sometimes also the predatory macroinvertebrates alone (INV), decreased the densities of plant-associated macroinvertebrates.

Lake Nutrias had an extremely low abundance of macroinvertebrates throughout the entire study period, preventing statistical analyses. The density of macroinvertebrates was thus significantly greater in the absence of predators in the other two subtropical lakes ($F_{3,12}=14.0$, $p<0.001$ and $F_{3,12}=6.4$, $p<0.01$, in lakes Blanca and Diario, respectively, Fig. 4). Fish effects were stronger than shrimp effects (as evidenced in the post hoc tests), which were only noticeable in Lake Blanca (Fig. 4). The most frequent taxa in the subtropical lakes were Oligochaeta, Chironomidae and Ostracoda, but Hirudinea and Gasteropoda also appeared.

Similarly, in the three temperate lakes the effects of predators were significant ($F_{3,12}=4.3$, $p<0.05$; $F_{3,8}=4.3$, $p<0.05$; and $F_{3,12}=5.6$, $p<0.05$ in Lakes Kogleaks, Bølling and Stigsholm, respectively, Fig. 4). Here, in contrast, the effects of the macroinvertebrate predator were similar to the effects of fish in two of the lakes (as evidenced in the post hoc tests, Fig. 4). The macroinvertebrate communities of the temperate lakes were also dominated by Oligochaeta and Chironomidae, whereas Ostracoda were also important in Lake Kogleaks and Lake Bølling, and Trichoptera and Isopoda (e.g. Asellus sp.). also occurred in Lake Stigsholm.

In the six lakes (both climate zones), periphyton biomass increased at the end of the experiment, with no significant effects of treatments (Fig. 4). Particularly, a lack of relationship between the densities of plant-associated macroinvertebrates and periphyton biomass was evidenced in Lake Nutrias, where periphyton density was lowest despite the almost complete absence of macroinvertebrates (Fig. 4).

Discussion

Our experiment highlights the role of predators, and particularly of fish, as a strong structuring force, particularly in pelagic food webs in both temperate and subtropical shallow lakes. We detected a significant decrease in phytoplankton biomass together with an increase in zooplankton biomass only when fish were absent. In the fish treatments, a cascading effect was evidenced by a reduction of the potential grazing (the zoop:phyt ratio decreased and significantly differed from fishless treatments). and a significant increase in phytoplankton biomass in both climatic zones.

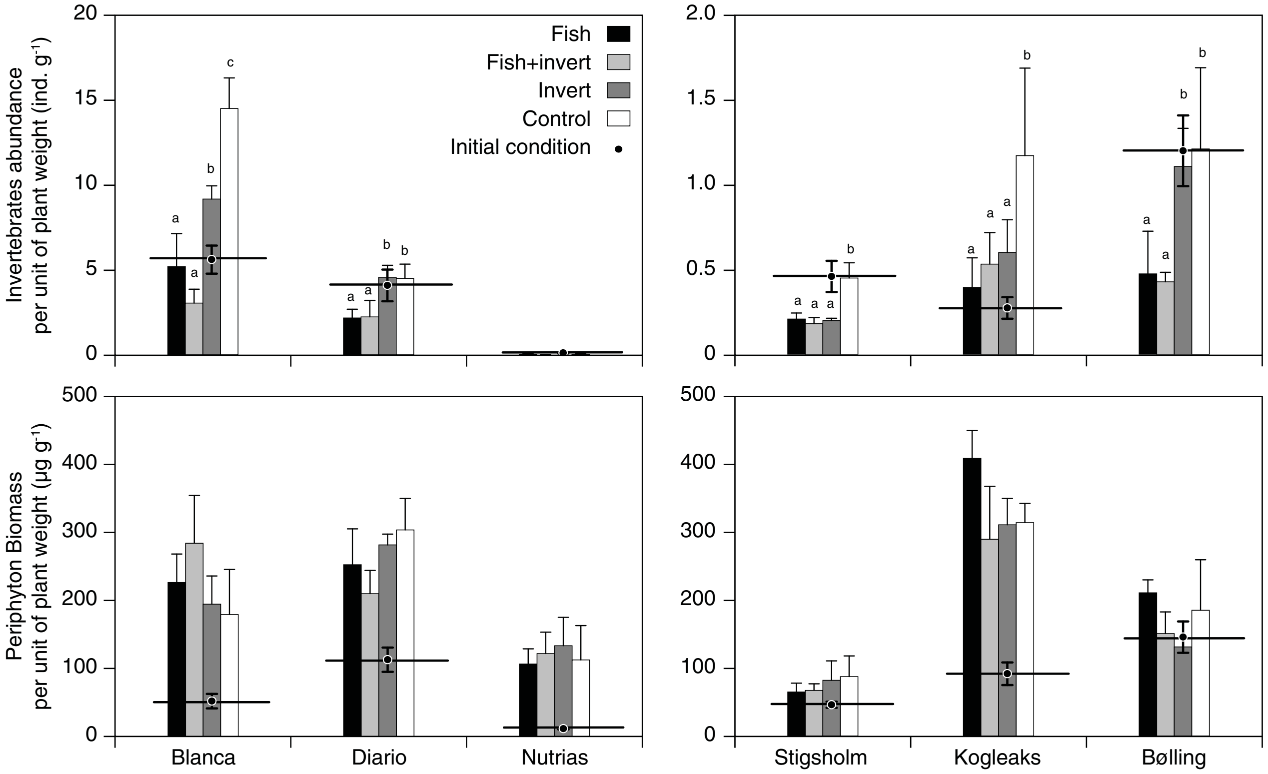

Figure 4. Density of plant-associated macroinvertebrates (upper panels) and periphyton biomass (lower panels) in subtropical (left) and temperate (right) lakes. Horizontal lines represent initial values (just before treatment assignment and 15 days after fish exclusion), while columns represent final conditions (49 days after fish and invertebrate addition). Error bars represent ± 1 standard error. Please note the different scales on the y axes. Letters indicate groupings according to Tukey's post hoc tests when ANOVA tests were significant.

It has long been debated why (sub)tropical zooplankton communities are commonly dominated by small-sized individuals and large-bodied species are often absent from the water (e.g. Fernando, 1994; Gillooly & Dodson, 2000). Besides the above-mentioned effects on zooplankton biomass, large cladocerans occurred in the mesocosms (particularly of *Daphnia* spp.). at water temperatures of 28ºC, as a response to the exclusion of fish. Our findings suggest a top-down driven structure of zooplankton, fish predation being the responsible factor for the observed patterns, as suggested also by other works in warm areas (Nagdali and Gupta, 2002; Meerhoff et al., 2007a; Iglesias et al., 2008; Sinistro, 2010; Fontanarrosa, Chaparro, de Tezanos Pinto et al., 2010; Iglesias et al, submitted).

Our results also suggest that a highly structured environment (high artificial PVI of ca. 75%). and the availability of other food items (such as invertebrates and periphyton attached to the artificial plants). do not suffice to counteract the effects of fish predation on zooplankton in warm lakes. This contrasts with previous findings in temperate lakes where a threshold of >20% PVI has been identified experimentally to offer a significant refuge effect for zooplankton against fish predation (Schriver, Bøgestrand, Jeppesen et al., 1995). Similarly, and despite noticeable effects of fish, large-bodied zooplankton reached comparatively higher densities in the temperate lakes than in the same treatments in the subtropical lakes, supporting our hypothesis that fish effects are even stronger in warm lakes.

The clear cascading effects promoted by fish under experimental conditions in the subtropics (Iglesias et al., 2008; Mazzeo et al., 2010; Sinistro, 2010). are, however, not seen in whole-lake studies from this climate zone (Kruk et al., 2009; Pacheco, Iglesias, Meerhoff et al., 2010; Gelós, Teixeira-de Mello, Goyenola et al., 2010). to the same extent as under temperate conditions (Carpenter and Kitchell, 1996; Jeppesen et al., 1997). The typically high fish densities, widespread omnivory (Winemiller, 1990; Teixeira de Mello et al., 2009; Iglesias et al., in prep.). and preferential use of macrophytes as habitats (Meerhoff et al., 2007a), together with possible physiological constraints to cladocerans (Weetman and Atkinson, 2004), have been pointed out as the main reasons for a continuously strong effect of fish, moderating strong relationships between zooplankton and phytoplankton in whole lakes under warm conditions. Contrary to expectations based on previous field studies, neither the shrimp *P. argentinus* in the subtropics (Collins and Paggi, 1998). nor the amphipod *G. lacustris* in the temperate lakes (Wilhelm and Schindler, 1999). triggered a change in zooplankton abundance and phytoplankton biomass (unless together with fish). In contrast, González-Sagrario et al. (2009). have experimentally identified *P. argentinus* as a strong predator on zooplankton. The different results can perhaps be attributed to a higher plant PVI (ca. 75 % in our investigation and only 10 % in their work), which may decrease the feeding efficiency of the shrimps (though clearly not of the subtropical fish).

Our second hypothesis focused on the so-called littoral pathway (Schindler and Scheuerell, 2002; Vadeboncoeur, Vander Zanden and Lodge, 2002). We did find a clear effect of predators on plant-associated macroinvertebrates, as previously suggested for temperate (Diehl and Kornijów, 1997; Jones and Sayer, 2003). and subtropical shallow lakes (Meerhoff et al., 2007a). As in the case of zooplankton, a fish effect was evidenced in both countries and in all lakes; however, only in temperate lakes did the macroinvertebrate predator *G. lacustris* seem to affect macroinvertebrate abundances as well. In the subtropics, a similar predator effect of shrimps was not clearly evidenced.

In contrast to our expectation, however, we found no cascading effects of any of the predators on periphyton biomass in any of the lakes. This suggests the absence of indirect cascading effects and also the absence of direct (i.e. feeding). effects of fish in our experiments. In temperate lakes the lack of effects of fish on periphyton seems to be common in lakes (Hansson, 1992; Bécares, Gomá, Fernández-Aláez et al., 2008). and in mesocosms experiments (Brönmark & Vermaat, 1998; Bertolo et al., 2000). In contrast, in the subtropics we expected a direct negative effect of fish on periphyton, as has previously been suggested (Iglesias et al., 2007; Meerhoff et al., 2007a; Jeppesen et al., 2010), due to the omnivory and habitat preference of most subtropical fish (Teixeira de Mello et al., 2009). The different response in the study of Meerhoff et al. (2007a). and ours, although the experiments were conducted in the same type of lakes, may reflect the different experimental set-up applied. While our experiment used closed mesocosms with fixed fish densities, Meerhoff et al. (2007a). used open plant beds, allowing greater disturbance and stronger grazing effects of potentially higher numbers of fish than our manipulated densities. The lower mean densities of plant-associated macroinvertebrates in our study (particularly in the temperate lakes). may, in contrast, indicate poorer colonization conditions inside our mesocosms than in their open beds. This poor colonization, particularly by snails, might have been an important factor preventing the occurrence of indirect positive effects from fish on periphyton, as previously reported for temperate systems (Brönmark and Vermaat, 1998; Jones and Sayer, 2003). In the subtropics, in contrast, the lack of (negative). effects may reflect an oversimplification of our predator assemblages, with lower total densities and much lower richness than under natural conditions, leading to a greater availability of alternative, more nutritious, food sources such as zooplankton and macroinvertebrates. The evidence is thus controversial, and more complex experiments resembling natural taxon diversity and densities are needed before we can draw any firm conclusions on the functioning of littoral food webs.

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Manuscript 4

TROPHIC CASCADE EFFECTS OF *HOPLIAS MALABARICUS* (CHARACIFORMES, ERYTHRINIDAE) IN SUBTROPICAL LAKES FOOD WEBS: A MESOCOSM APPROACH

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PRIMARY RESEARCH PAPER

Trophic cascade effects of *Hoplias malabaricus* (Characiformes, Erythrinidae) in subtropical lakes food webs: a mesocosm approach

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Abstract While the cascading effect of piscivorous fish on the pelagic food-web has been well studied in north temperate lakes, little is known about the role of native piscivores in warm lakes. Here, the fish communities are typically characterized by high abundances of small, omnivorous fish exerting a high predation pressure on the zooplankton. We conducted a 1-month replicated mesocosm experiment at subtropical conditions to test the effects of piscivorous (*Hoplias malabaricus*) fish on phytoplankton biomass and water transparency. Our experimental design comprised two (phytoplankton + zooplankton), three (phytoplankton + zooplankton + planktivores) and four (phytoplankton + zooplankton + planktivores + piscivores) trophic levels. We designed two different four trophic level treatments, one with juveniles of *H. malabaricus* (<15 cm) and the other with adults (>30 cm), to evaluate the strength of the effects of juveniles and adults. A major trophic cascade response was observed. In the planktivores treatment, chlorophyll *a* (Chl *a*) and turbidity significantly increased, while total zooplankton abundance (especially *Daphnia obtusa*) and water transparency decreased. In both *H. malabaricus* treatments and in the two trophic levels control, the opposite pattern was observed; thus, Chl *a* and turbidity decreased, while zooplankton abundance and water transparency increased. The differences observed reflected the strong control on the planktivore *Jenynsia multidentata* by both sizes of *H. malabaricus*, propagating down through the trophic web. *Hoplias malabaricus* is widely distributed in South America and may, therefore, be a good candidate for restoration by biomanipulation in eutrophic lakes of subtropical and tropical regions. However, detailed investigations at whole-lake scale are needed to determine its potential.

Keywords *Hoplias malabaricus* · *Jenynsia multidentata* · Trophic cascade effects · Eutrophication · Fish · Biomanipulation · Restoration

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Introduction

Piscivorous fish can reduce the predation pressure on zooplankton by preying upon planktivorous fish with cascading effects on the phytoplankton (de Bernardi, 1981; Carpenter et al., 1987). This hypothesis, known as the trophic cascade effect (Carpenter & Kitchell, 1996), was developed for temperate lakes and many experimental studies and field research have provided supporting evidence of the phenomenon (Carpenter & Kitchell, 1996; Jeppesen, 1998; Moss et al., 1998; Scheffer & Jeppesen, 1998). The trophic cascade effects of native piscivores in South American tropical and subtropical lakes are, however, less well studied (Lazzaro, 1997; Jeppesen et al., 2005; Jeppesen et al., 2007).

The smallest size of limnetic cladocerans of South America occurred in the subtropical and tropical zones, increasing to the maximum size in cold temperate regions (50–60°C) in both the northern and southern hemispheres. This spatial pattern could result from direct (e.g. physiological) or indirect (e.g. increased predation) effects (Gillooly & Dodson, 2000). Recent experimental evidence supported the role of the fish predation pressure (Iglesias et al., 2008, Okun et al., 2008) that also influences the spatial distribution and the horizontal migration pattern of the zooplankton (Iglesias et al., 2007; Meerhoff et al., 2007). The high abundance, small size and multiple reproductive events of several native omnivorous–planktivorous fish in South America result in a high predation pressure on zooplankton throughout the year (Fernando, 1994; Lazzaro, 1997; Pinel-Alloul et al., 1998; van Leeuwen et al., 2007; Iglesias et al., 2008; Attayde & Menezes, 2008; Jeppesen et al., in press). The omnivorous habit of these small fish implies that their population can be maintained by alternative food sources, especially when the zooplankton population is drastically diminished, thereby uncoupling the predator–prey dynamics (Branco et al., 1997; Lazzaro, 1997; Yafe et al., 2002).

In warmer regions of South America, use of removal of planktivorous fish as a biomanipulation tool to restore eutrophic lakes has proven successful only with some oviparous native fishes, *Cheirodon interruptus* and *Astyanax* spp. (Characidae) or *Gymnogeophagus rhabdotus* (Cichlidae), but ineffective with viviparous small fishes like *Jenynsia multidentata* (Anablepidae) (Jeppesen et al., 2007). Contrarily with biomanipulation predictions, *J. multidentata* populations rose at the end of extensive fish removal programme (Jeppesen et al., 2007), due to its remarkable reproductive capacity (Goyenola, 2008) and the elimination of the main competitors. Introduction of native piscivorous fish might be an alternative. The piscivores may control the abundance of small viviparous–planktivorous fish, which would allow the development of zooplankton and indirectly would increase the consumption of the phytoplankton biomass. The experimental evidence to confirm this hypothesis in tropical and subtropical regions is lacking.

We conducted a mesocosm experiment to evaluate how the piscivory of the widely distributed South American *Hoplias malabaricus* (Erythrinidae) affects plankton structure and water quality. We addressed three questions: (1) Does *H. malabaricus* effectively control *J. multidentata* (omnivorous–planktivorous), allowing development of large-bodied zooplankton, like *Daphnia*? (2) Does the *H. malabaricus* (piscivore) effect cascade through the trophic web, leading to lower phytoplankton biomass and higher water transparency? (3) Are there differences in the trophic cascade effects between juveniles (standard length (SL) <15 cm) and adults (SL >30 cm) of *H. malabaricus*?

Materials and methods

Selected species

Hoplias aff. *malabaricus* is a widely distributed (Central and South America) ambush predator (Saint-Paul et al., 2000; Ferreira, 2007). According to many authors (Dergam et al., 1998; Bertollo et al., 2000), *H. malabaricus* represents a set of species in several neotropical watersheds. Therefore, it is common to refer it as *Hoplias* aff. *malabaricus*.

The taxa complex is active mainly by night and inhabits principally vegetated areas of both lentic and lotic systems. *Hoplias* aff. *malabaricus* reaches a maximum size of about 62 cm (Ringuelet et al., 1967), starts the reproductive period with a SL between 16 and 20 cm (Azevedo & Gomes, 1942). Several separated spawning events take place during the reproductive season laying between 700 and

3000 eggs on every event (Azevedo & Gomes, 1942). It exhibits diet shifts during its ontogeny, individuals below SL = 20 mm being planktivorous, but becoming insectivorous at around 100–120 mm, and strictly piscivorous at SL >160 mm (Azevedo & Gomes, 1942; Oliveros & Rossi, 1991; Galvis et al., 1997; Meschiatti & Arcifa, 2002). In this sense, not only fish was the predominant food items of *Hoplias* aff. *malabaricus* adults in the Paraná river floodplain, but also shrimps were ingested, along with Coleoptera, Ephemeroptera, Hemiptera, Odonata and other insects, as well as, occasionally, organic detritus and plant (Peretti & Andrian, 2004). *Hoplias* aff. *malabaricus* exhibits the smallest overlap with other native piscivores from South America (Luz-Agostinho et al., 2008), due to its restricted habitat use (structured area associated with riparian forest or aquatic plants) and its ambush strategy.

Jenynsia multidentata is a small omnivorous–planktivorous fish (86 mm maximum SL registered by Goyenola, 2008). Its distribution ranges between the tropics of Capricorn to the latitude 40°S (Ferriz & López, 1987; Ghedotti & Weitzman 1996; Novaes & Andreata, 1996). It is one of the most common Cyprinodontiforms of several aquatic ecosystems in the southeast of South America (Ringuelet, 1975; Hued & Bistoni, 2001; Kruk et al., 2006; Marti et al., 2006). In accordance with the tolerance of *J. multidentata* towards several environmental factors, it is considered as a eurithermic, eurihaline and eurioic species (Thormahlen de Gil, 1949; García Romeu et al., 1964; Gómez, 1993; Menni et al., 1996; Betito, 2006). *Jenynsia multidentata* inhabits the pelagic and the littoral areas, but it is mainly found among the vegetation (Iglesias et al., 2007). Experimental and field evidence has earlier shown that *J. multidentata* has a strong impact on the distribution of cladocerans (Iglesias et al., 2007) and on the composition of the zooplankton community (Iglesias et al., 2008).

The cladoceran *Daphnia obtusa* has a worldwide distribution and is typically found in temporary pools and small lakes (Peters & de Bernardi, 1987; Benzie & Hodges, 1996). *Daphnia obtusa* rapidly colonizes new habitats and dominates the community during the first stages of the succession process (Louette & De Meester, 2007). This cladoceran species is one of the largest herbivore of our study region, with up to 3.7 mm as maximum recorded length (Mazzeo et al., 2000).

Experimental design

Hoplias malabaricus and *J. multidentata* specimens were collected from natural lakes (Río Santa Lucía basin and Laguna Blanca–Maldonado, respectively) and cultured in mesocosms (area = 6 m^2, volume = 3800 l) with sediment and free-floating plants (*Eichhornia crassipes*) for 3 months before initiating the experiment (Fig. 1). Also *Daphnia obtusa* was cultured. The food items supplied to *H. malabaricus* were: Characidae (*Astyanax* spp., *Pseudocorynopoma doriae, Oligosarcus jenynsii*, *Cheirodon interruptus*, *Charax stenopterus*), Curimatidae (*Steindachnerina biornata*, *Cyphocharax voga*), *Cnesterodon decemmaculatus* (Poeciliidae) and *J. multidentata*. *Jenynsia multidentata* was fed with zooplankton and macroinvertebrates (mainly dipterans larvae), and *D. obtusa* with yeast and phytoplankton.

The mesocosm experiment was carried out in the Uruguayan countryside (34°41′12″ S, 56°35′07″ W) with a mean annual air temperature of 17.5°C (range −11 to 44°C) and an annual mean precipitation of 1100–1200 mm (Koppen classification: humid subtropical region with warm summer) from 9 April to 11 May 2005. Twelve 3800 l plastic experimental units (3 × 2 × 0.60 m), whose bottoms were covered with river sand, were filled with water from the Raigón aquifer and kept undisturbed for 2 months to

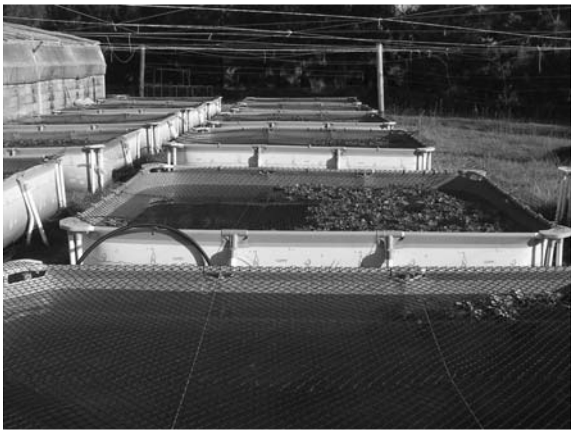

Fig. 1 Experimental mesocosms, twelve 3800 l plastic experimental units (3 × 2 × 0.60 m). Each mesocosm was filled with groundwater from the Raigón aquifer, including sediment and free-floating plants. The treatments were randomly assigned

allow stabilization and phytoplankton development, 20% of total surface being covered by *Eichhornia crassipes* (Fig. 1).

Two months before initiating the treatment assignment (end of January), *Daphnia obtusa* were added to each of the 12 mesocosms. Immediately prior to introducing the fishes, water was mixed by interchanging water between the mesocosms (2000 l from each, using a special water pump that did not disturb the plankton organisms) to obtain homogeneous start-up conditions. The four different treatments were randomly assigned (triplicate) to the experimental units:

1. Control: no fish, zooplankton + phytoplankton ($n = 3$)
2. JM: *Jenynsia multidentata* (9 ind m^{-2}) + zooplankton + phytoplankton ($n = 3$)
3. HMs: Small specimens (juveniles) of *Hoplias malabaricus* (SL $\leq$15 cm, 0.5 ind m^{-2}) + *J. multidentata* (9 ind m^{-2}) + zooplankton + phytoplankton ($n = 3$)
4. HMb: Big specimens (adults) of *Hoplias malabaricus* (SL >30 cm, 0.5 ind m^{-2}) + *J. multidentata* (9 ind m^{-2}) + zooplankton + phytoplankton ($n = 3$)

The selection of fish densities was based on an average of several shallow lakes examined in Uruguay (Mazzeo et al., 2003; Kruk et al., 2006) and the Pampa region (SALGA Project, unpublished data). In order to prevent fish predation by birds the mesocosms were covered with a plastic net. Nitrogen (N) and phosphorous (P) were added (N:P = 1) to each experimental unit. Measured values at initial and final sampling dates were 2.6 (±0.05) and 7.2 (±0.28) mg l^{-1}, and 3.2 (±0.40) and 23.3 (±0.24) mg l^{-1}, for total phosphorus (TP) and total nitrogen (TN), respectively. The artificial nutrient scenario selected avoided the limitation of phytoplankton growth by bottom-up mechanisms and the potential nutrient competition with the free-floating plants. Water temperature range during the experiment was 15–20°C.

Homogeneous initial conditions for temperature (18.3 ± 0.1°C), water transparency (mini Secchi disk, 22.7 ± 0.9 cm), pH (9.1 ± 0.1), conductivity (582 ± 2 $\mu S\ cm^{-1}$), dissolved oxygen (11.1 ± 0.3 $\mu g\ l^{-1}$), TN and TP, and biological variables (algal biomass: 49 ± 11 $\mu g\ l^{-1}$ and *D. obtusa* abundance: 241 ± 55 ind l^{-1}) were verified by one-way ANOVA (main effect 'treatment', four levels: Control, JM, HMs and HMb), and no statistical differences appeared ($P > 0.05$). All the values indicated above are mean and standard errors for all treatments and replicates.

Sampling and analysis

All water samples for chemical and plankton analyses were collected (two times per week) throughout the water column with a tube at noon, integrating eight sites per mesocosm. The mesocosms were divided into eight fixed quadrants, including all the micro-habitats (open water and free-floating beds). Dissolved oxygen (DO), conductivity (K), pH, water transparency, turbidity and chlorophyll *a* in vivo (Chl-a) were recorded in situ using Horiba sensors, mini Secchi disk and fluorometer (Aquafluor, Turner Designs), respectively. In this case, the values were recorded every 2 days. Chlorophyll *a* was determined using the Nusch (1980) method (ethanol hot). TN and TP were determined according to (Valderrama, 1981).

For quantification of zooplankton, 15 l were filtered through a 50-μm net and fixed with acid Lugol. Counts were made according to Paggi & de Paggi (1974). Twenty individuals of *D. obtusa* were measured from each treatment at the initial and the final sampling. *Daphnia obtusa* biomass was determined using the length/weight relationships according to Bottrell et al. (1976). The impact of grazers on phytoplankton was estimated by calculating a potential grazing pressure index (PGP), assuming that the daily consumption of cladocerans is equivalent to their biomass (Schriver et al., 1995) and they only feed on phytoplankton. Thus, PGP is the ratio between *D. obtusa* biomass and phytoplankton biomass.

The phytoplankton composition was analysed both at the beginning and at the end of the experiment, on acid Lugol (1%) fixed samples. Counts were made with sedimentation chambers (10 and 20 ml) in an inverted microscope based on the criteria of Ütermöhl (1958). Biovolume was calculated according to Hillebrand et al. (1999).

The habitats of the mesocosm (open water, free-floating beds and bottom) selected by *J. multidentata* and *H. malabaricus* were visually checked at midday and midnight. At the end of the experiment, all the mesocosms were emptied to quantify the final density of *J. multidentata* and to verify whether all the piscivores survived.

Statistical analysis

Analysis of variance for repeated measures (ANOVA*mr*) was performed to test differences among treatments (control, JM, HMs and HMb) (Underwood, 1997). If relevant, one-way ANOVA analyses were also performed for specific sampling times. When necessary, data were log $(x + 1)$, square root or 4th root transformed to homogenize variances. Homoscedasticity, sphericity assumption and the normal distribution of residuals were tested by Cochran's C test, Mauchley Sphericity test and the visual inspection of fitted values, respectively. Post hoc comparisons were performed with Tukey's test. The relationships among several responses were analysed by non-linear regressions ($y = a + be^x$ and $y = a + b\ln x$), multiple regression was performed between water transparency versus phytoplankton biomass and turbidity. The significance levels considered in all the statistical analyses carried out were <0.05, <0.01 and <0.001.

Results

Hoplias malabaricus effectively controlled the population of *J. multidentata*, the maximum percentage of the 54 introduced specimens re-captured after finishing the experiment was 33% for all HM replicates. Moreover, in several replicates of HM treatments (independently of the size) *J. multidentata* was completely eliminated. During the experiment, the small omnivorous–planktivorous fish use the open water and *E. crassipes* stands in the mesocosm without *H. malabaricus*. On the contrary, in the HM treatments, the daytime activity of *J. multidentata* was substantially lower,and they remained within the *E. crassipes* stands. The bigger specimens of *H. malabaricus* remained together during the day, generally inactive at the bottom just under the free-floating plants. At night, each individual selected one corner of the mesocosm and adopted a typically sit-and-wait position, tail close to the bottom and the head in direction of the water surface. The juveniles (HMs treatment) not only showed a less-structured behaviour, but also exhibited more activity during the night.

We found trophic cascade effects of both piscivorous fish sizes which were tested in this experiment. *Daphnia obtusa* abundance decreased in the JM treatments, while it remained high in the control and the *Hoplias* treatments (Fig. 2A). Significant differences among treatments occurred on day 11 and remained so until the end of the experiment

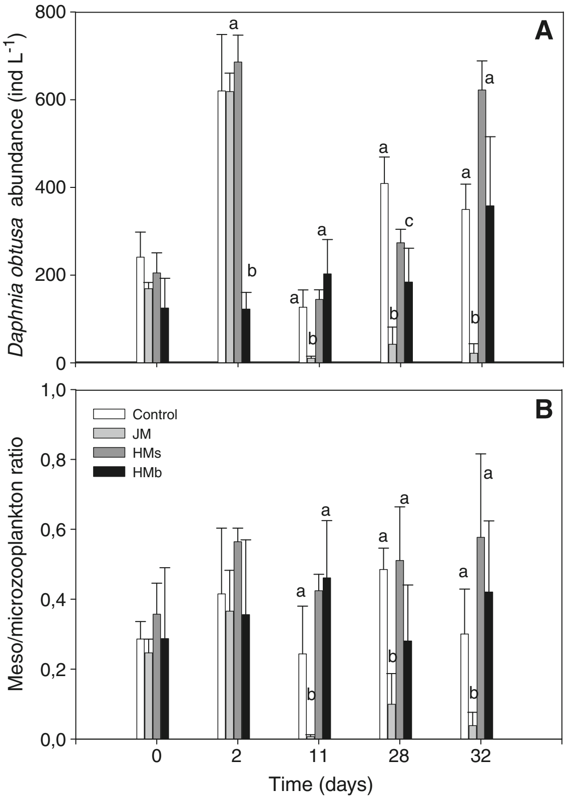

Fig. 2 **A** Temporal changes in *Daphnia obtusa* abundance and **B** the herbivorous mesozooplankton/microzooplankton ratio. Mesozooplankton = cladocerans + calanoid (copepodites + adults), microzooplankton = rotifers + nauplii. The graphs include the mean of three replicates and the standard error bars. Control: two trophic levels food web treatment (phytoplankton + zooplankton); JM: three trophic levels food web treatment (phytoplankton + zooplankton + *J. multidentata*); HMs and HMb = four trophic levels treatment with small *H. malabaricus* and big *H. malabaricus* specimens, respectively, (phytoplankton + zooplankton + *J. multidentata* + *Hoplias malabaricus*). The *rm*ANOVA results for *D. obtusa* and meso/microzooplankton ratio (interactions treatments × time) were $F_{(12,\ 32)} = 10.5$, $P < 0.001$ and $F_{(12,32)} = 2.7$, $P < 0.01$, respectively. a, b and c indicate the results of the Tukey post hoc tests

(Fig. 2A). Both *Hoplias* treatments (HMs and HMb) differed significantly from the JM treatment, but not from the control or relative to HM size (Fig. 2A). The indirect positive effect of piscivores on *D. obtusa* was followed by a decrease in the abundance of other cladocerans species (i.e. *Simocephalus* spp and *Alona* spp). An inverse nonlinear relationship between *D. obtusa* and the other cladocerans species (in terms of abundance) was observed ($r^2 = 0.22$, $F_{(1,59)} = 16.3$, $P < 0.001$). The abundance of rotifers and cyclopoids increased in the JM treatments compared to the control, HMs and HMb, especially after day 11. Accordingly, a clear effect of piscivores was observed in the meso/microzooplankton ratio (in terms of abundance) when we finished the experiment, being significantly smaller in the JM treatment ($F_{(3,8)} = 4.1$, $P < 0.05$) than in the HMs, HMb and control treatments (Fig 2B), while no statistically significant differences were found among the latter three treatments.

The indirect effects of *Hoplias* on algal biomass were notable, both algal biomass estimators (Chl *a* in vivo and Chl *a* determined in the lab) showed the same treatment responses. Mean Chl *a* in JM ranged from 50 $\mu g\ l^{-1}$ (as the initial condition) to 350 $\mu g\ l^{-1}$ at the end of the experiment, from 67.1 to 29.1 $\mu g\ l^{-1}$ in the control, and from 39.5 to 4.4 $\mu g\ l^{-1}$ and from 61.2 to 11.8 $\mu g\ l^{-1}$ in HMs and HMb, respectively. Statistical differences among treatments for Chl *a* in vivo ($F_{(3,8)} = 5.6$, $P < 0.05$) were detected on day 11 and onwards, while no differences were observed between HMs and HMb (Fig. 3).

In all treatments during the whole period, the phytoplankton community was dominated by the green algae *Scenedesmus quadricauda*, both in terms of abundance (85.9 ± 7.5 and 95.5 ± 3.2% (SD), initial and final times, respectively) and biovolume (77.4 ± 13.4 and 93.3 ± 3.6%, initial and final times, respectively). The piscivores treatments and control showed at the end significantly lower levels for both abundance attributes ($F_{(3,8)} = 18.1$, $P < 0.001$; $F_{(3,8)} = 17.3$, $P < 0.001$, abundance and biovolume, respectively), without composition modifications. Besides, no significant differences in abundance and/or biovolume were registered in the post hoc analysis among the control, HMs and HMb. From initially similar values of the grazing pressure index (PGP), the ratio dropped in the JM treatment, resulting in significant differences among JM and the other

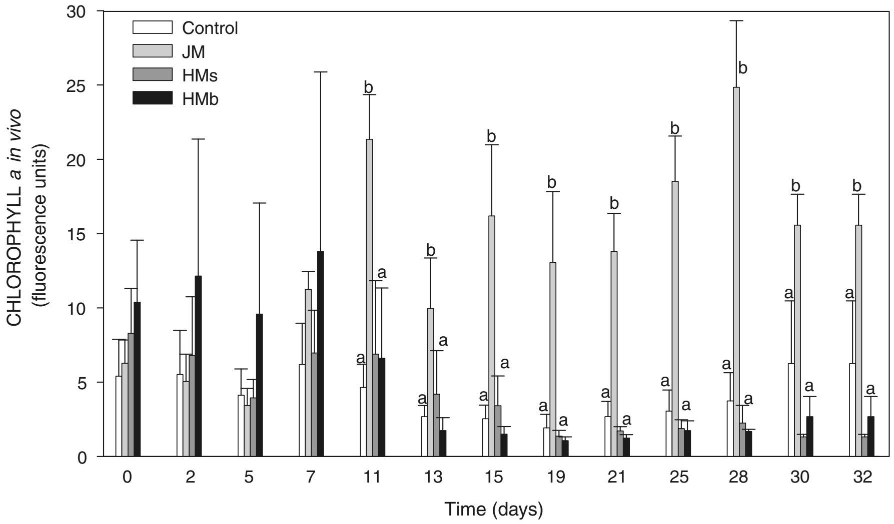

Fig. 3 Temporal variation in phytoplankton biomass estimated by fluorescence in vivo. The graphs include the mean of three replicates and the standard error bars. Control: two trophic levels food web treatment (phytoplankton + zooplankton); JM: three trophic levels food web treatment (phytoplankton + zooplankton + *J. multidentata*); HMs and HMb = four trophic levels treatment with small *H. malabaricus* and big *H. malabaricus* specimens, respectively, (phytoplankton + zooplankton + *J. multidentata* + *H. malabaricus*). The *rm*ANOVA result (interactions treatments × time) was $F_{(36,\ 96)} = 2.3$, $P < 0.001$. a and b indicate the results of the Tukey post hoc tests

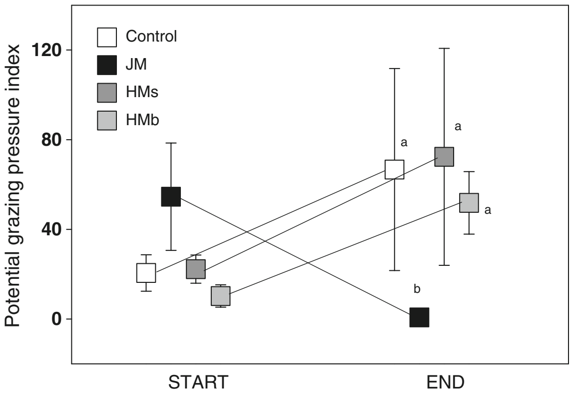

Fig. 4 Initial and final values of the potential grazing pressure index during the experiments. The graphs include the mean of three replicates and the standard error bars. Control: two trophic levels food web treatment (phytoplankton + zooplankton); JM: three trophic levels food web treatment (phytoplankton + zooplankton + *J. multidentata*); HMs and HMb = four trophic levels treatment with small *H. malabaricus* and big *H. malabaricus* specimens, respectively, (phytoplankton + zooplankton + *J. multidentata* + *H. malabaricus*). a and b indicate the results of the Tukey post hoc tests

treatments at the end of the experiment ($F_{(3,8)} = 5.9$; $P < 0.05$, Fig. 4).

The *Hoplias* effect also resulted in differences in various physical and chemical water characteristics. A decrease in water transparency was found in JM (mini Secchi disk depth decreased from 25 to 18.5 cm), while it increased from 23 to 36.5 in the control, from 21.5 to 52.5 and 21.5 to 50 cm in HMs and HMb, respectively. Significant differences among treatments were observed after day 15 until the end of the experiment (Fig. 5).

A multiple regression between water transparency versus microalgae biomass and turbidity was found ($r^2 = 0.62$, $F_{(2,\ 153)} = 124.5$, $P < 0.001$), both beta were significant (−0.23 and −0.63, respectively). Similarly, DO and pH showed nonlinear relationships to the phytoplankton biomass ($r^2 = 0.36$, $F_{(1,\ 154)} = 83.2$, $P < 0.001$; $r^2 = 0.55$, $F_{(1,\ 154)} = 177.5$, $P < 0.001$). No significant differences between treatments were found for nutrients (TP and TN).

Discussion

Our experimental results showed a trophic cascade response promoted by both the tested size classes of *H. malabaricus* as well as adverse effects of *J. multidentata* on the water transparency. When the omnivorous–planktivorous fish *Jenynsia multidentata* was the top predator, as in the three trophic levels treatment, Chl *a* increased due to a reduction in medium and large sized herbivores (in our experiment *Daphnia obtusa*). The opposite pattern was observed in the piscivores treatments. The zooplankton and phytoplankton structures of our experiment, dominated by medium-sized herbivores and palatable algal species, conditioned the fast cascade responses that remained stables until the end. The experimental evidence of our control treatment agrees with field observations in some shallow, hypertrophic and subtropical lake without fish, that showed a high abundance of *D. obtusa* and low phytoplankton biomass (Mazzeo et al., 2000) during spring and autumn.

In subtropical regions like Uruguay, removal of planktivores appears to be an ineffective restoration strategy under eutrophic conditions, as they are dominated by fast reproducing small omnivorous, sometimes viviparous fish species (e.g. *J. multidentata*) that recover rapidly after extensive removal (Jeppesen et al., 2007). Omnivorous–planktivorous fishes reproduce several times a year (Fernando, 1994; Lazzaro, 1997; Pinel-Alloul et al., 1998) in the tropic and subtropics whereas temperate fishes typically reproduce once a year (van Leeuwen et al., 2007). Some authors have suggested that piscivorous fish stocking could be a potential alternative biomanipulation strategy in subtropical systems (Jeppesen et al., 2007; Iglesias et al., 2008), while others suggest that a fish community structure prevailing in warm lakes may prevent trophic cascading effects (Lazzaro, 1997; Meerhoff et al., 2007).

Our results showed that *H. malabaricus* appears to be a good candidate for biomanipulation. The fact that both the tested sizes classes of *H. malabaricus* effectively promoted clear water conditions suggests that biomanipulation using small *H. malabaricus* specimens is feasible, and that this control (on small planktivorous fishes) will continue although individuals may reach bigger sizes. We had expected stronger effects of the small-sized *H. malabaricus*, because the larger-sized specimens probably predate on fishes larger than *J. multidentata*. However, field evidence suggests that the experimental results also were detected under natural conditions with a diverse

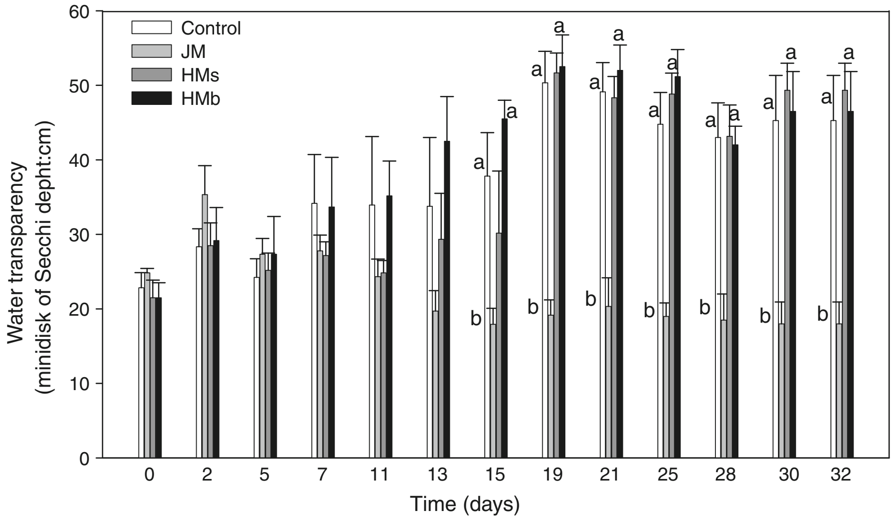

Fig. 5 Temporal changes in water transparency estimated by Secchi minidisk depth) during the experiment. The graphs include the mean of three replicates and the standard error bars. Control: two trophic levels food web treatment (phytoplankton + zooplankton); JM: three trophic levels food web treatment (phytoplankton + zooplankton + *J. multidentata*); HMs and HMb = four trophic level treatment with small *H. malabaricus* and big *H. malabaricus* specimens, respectively, (phytoplankton + zooplankton + *J. multidentata* + *H. malabaricus*). The *rm*ANOVA result (interactions treatments × time) was $F_{(36,\ 96)} = 3.1$, $P < 0.001$. a and b indicate the results of the Tukey post hoc tests

community of fish preys. The feeding patterns of *H.* aff. *malabaricus* from the high Paraná river floodplain demonstrated that the small prey fish (average SL = 5.0 cm) were intensely preyed upon by specimens up to 45 cm (Almeida et al., 1997). In the south east of Brazil, Mazzoni & Soares da Costa (2007) found no ontogenetic differences for the intestinal coefficient and food categories consumed by *H. malabaricus*. Whereas significant differences occurred as regards prey sizes, positive correlation between predator and prey size was observed. In accordance with this field evidence, the smallest specimens of *H. malabaricus* feeding on small fishes had an SL = 11 cm in the south of Uruguay (Teixeira-de Mello et al., 2006), but under cultured conditions, even *H.* aff. *malabaricus* juveniles (SL = 5 cm) were able to predate on fish (*Cnesterodon decemmaculatus*) (Teixeira-de Mello et al., unpublished data). This capacity may substantially reduce the costs of the piscivores introduction as biomanipulation can be implemented with small individuals.

Hoplias aff. *malabaricus* is a voracious piscivore feeding on numerous fish species and even birds (Ringuelet et al., 1967) and small mammals (Teixeira-de Mello unpublished data), which may enhance its carrying capacity. It exhibits parental care of hatchings (Ringuelet et al., 1967) and tolerates very turbid and hypoxic conditions (Rantin & Johansen, 1984; Polez et al., 2003), which are common features of eutrophic systems. These characteristics, considered as the key factors for its successful colonization of almost all water ecosystems in the region, may also be useful in the biomanipulation of eutrophic ecosystems.

Bistoni et al. (1995) observed that food consumption and the digestion rate of *H. malabaricus* are the highest in summer and much lower during the cold seasons. A recent study by Petry et al. (2007) experimentally tested the effect of temperature on the prey consumption rate of *H. malabaricus* specimens with sizes of 17.5–24.7 cm SL. They found that prey consumption ceases completely between 14 and 18°C and demonstrated that maximum prey consumption occurs at 30°C, when four times more prey is consumed than at 18 °C, the consumption being twice as high at 22, 26 and 34°C. When taking maximum summer water temperature into account, almost all the subtropical and tropical regions of South America belong to this temperature range (20–35°C). Thus, maximum prey consumption takes place during the reproductive season of small native omnivorous–planktivorous fish species like *J. multidentata* (Turner, 1957; García et al., 2004; Goyenola, 2008), when predation on zooplankton is usually high.

Our experimental results cannot be directly transferred to whole-lake systems without further tests due to the simplified conditions and small scale. Another scenario with an increased complexity of the food webs and spatial heterogeneity in subtropical and tropical regions may add other constraints whose effects need further examination. *Hoplias malabaricus* and *J. multidentata* inhabit the same spatial niche in natural systems; but how this may affect the predator–prey interaction cannot be elucidated from our study. *Hoplias malabaricus* prefers structured environments (macrophytes stands) where the number of potential prey is high and effective ambush predators reach favorable conditions (Almeida et al., 1997; Luz-Agostinho et al., 2008). *Jenynsia multidentata* live and feed among submerged and emergent plants (Goyenola, 2008). A possible scenario is that *H. malabaricus* by preying upon *J. multidentata* in the littoral areas will make this zone a safer place for the zooplankton, with cascading negative effects on the phytoplankton. However, large-scale or whole-lake experiments must be designed and undertaken to fully elucidate the potential of using *H. malabaricus* stocking for restoration of subtropical lakes in South America.

Finally, the ongoing focus on sustainable use of aquatic resources offers the chance to combine the nutrient loading reduction and the traditional inland fisheries management techniques for the rehabilitation/restoration of eutrophic lakes (Mehner et al., 2004). *Hoplias malabaricus* is one of the target species of most commercial and recreational fishing, stocking and controlled harvesting could be a successful strategy, combining ecosystem and fisheries management. The spatial preferences of *H. malabaricus* for littoral zones must be considered, or else, the probability of success of massive introduction in ecosystems without littoral regions would be very low.

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Manuscript

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SEASONAL AND DIEL CHANGES IN FISH ACTIVITY AND POTENTIAL CASCADING EFFECTS IN SUBTROPICAL SHALLOW LAKES WITH DIFFERENT WATER TRANSPARENCY

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SHALLOW LAKES

Seasonal and diel changes in fish activity and potential cascading effects in subtropical shallow lakes with different water transparency

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Abstract Fish play a key role in the functioning of shallow lakes. Simultaneously, fish are affected by physical in-lake factors, such as temperature and water transparency, with potential changes in their cascading effects on other communities. Here, we analysed the fish community structure and fish activity in four subtropical shallow lakes, varying in trophic state and water transparency, to assess changes promoted by temperature (i.e. summer and winter) and the light regime (i.e. day and night). We used a passive method (gillnets) during the day- and at night-time to detect changes in fish activity, but also sampled the littoral zone (during night) by point sample electrofishing to obtain a better description of the fish assemblage and habitat use. We observed different fish assemblages in the two seasons in all lakes. We captured more fish species and also obtained higher numbers (CPUE with nets) in summer. Contrary to our expectations, the visually oriented Characiformes were the most captured fish regardless of water transparency, at both day-time and night-time. We also found higher fish CPUE at night-time in all lakes. However, the differences between night and day decreased with decreasing transparency, being lower in the least clear lake, Lake Cisne. The nocturnal increase in fish CPUE (including visually oriented species) suggests that darkness serves as a good refuge for fish in shallow subtropical lakes, even at the likely cost of a lower feeding efficiency during the night. The importance of darkness seems to decrease with decreasing water transparency. We also argue that cascading effects of changes in the activity of piscivorous fish (seasonal changes in piscivores CPUE), when omni-planktivorous fish are indeed affected, may eventually reach the zooplankton, but

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may not be strong enough to reach the phytoplankton, regardless of water transparency.

Keywords Omnivorous fish · Water transparency · Night · Winter · Temperature

Introduction

Fish play a fundamental role in the functioning of shallow lakes through a series of physico-chemical (e.g. nutrient release, sediment turbation) and biological mechanisms (e.g. competition, predation). Fish may affect diversity and water transparency by indirect trophic cascading effects occurring in both pelagic and littoral zones (Jeppesen et al., 1997). By consuming zooplankton and plant-attached macroinvertebrate grazers, fish may indirectly enhance phytoplankton and periphyton biomass (Carpenter & Kitchell, 1993; Jones & Sayer, 2003; Liboriussen et al., 2005), thus increasing water turbidity. Besides, fish may often promote similar effects by affecting the spatial distribution of their prey through processes known as behavioural cascades (Romare & Hansson, 2003; Schmitz et al., 2004). In contrast, fish may be influenced by physical in-lake factors, such as temperature, turbulence and water transparency (Stoner, 2004), which may moderate their habitat use and activity level.

Water temperature strongly affects fish metabolism, nutritional needs and movement (Linlokken & Haugen, 2006). Metabolic processes rates (e.g. nutrient excretion rates) generally increase between 1.5 and 2.5 times with every 10°C increase in water temperature (reviewed by Vanni, 2002). The subtropical *Hoplias malabaricus* (local name “tararira”), one of the largest piscivores of South America, consumes more food in summer than in winter (Petry et al., 2007). The nutritional needs of fish are also affected by temperature. With increasing water temperatures, temperate roach (*Rutilus rutilus*) and rudd (*Scardinius erythrophthalmus*) increase their consumption of plant material (Prejs, 1984).

Temperature also affects fish activity; hence, a temperature rise leads to a higher attack coefficient and reduced prey handling times together with an increase in swimming speed (Persson, 1986). The greater fish activity may lead to a higher probability of encounter with the nets (Hamley, 1975) and thus an increased catchability in passive gears (such as gill nets). All these changes are species-dependent, with each species presenting an optimum temperature (Persson, 1986).

Underwater light, on the other hand, has diel and seasonal fluctuations and depends on both water colour and transparency (Johnsen, 2003). Low water transparency may negatively impact visually hunting fish, but may, contrarily, favour those fish that use other sensorial organs to find their prey (Aksnes & Giske, 1993; Pekcan-Hekim, 2007). Those basically visually oriented fish species may thus be favoured in clear water systems, being more active during the day. In contrast, the non-visual fish species may be more abundant in more turbid systems (Rodríguez & Lewis, 1997; Tejerina-Garro et al., 1998) and/or display higher nocturnal activity. Planktivorous fish can also be favoured in turbid waters, since they become less vulnerable to predation by piscivores, while their feeding efficiency on zooplankton is not significantly affected (De Robertis et al., 2003). In temperate shallow lakes, fish often change their spatial distribution within the ecosystem after changes in trophic state and water transparency and consequent changes in predation risk (Jacobsen et al., 2004; Pekcan-Hekim & Lappalainen, 2006).

The fish predation pressure on prey communities, such as zooplankton, macroinvertebrates or even small fish, can thus differ under different temporal scales (i.e. diel and seasonal), and environmental characteristics (such as water transparency). This may result in changes in fish cascading effects on primary producers and, ultimately, on ecosystem state and function.

In shallow subtropical lakes, however, there is still scarce evidence regarding the potential cascading effects of changes in fish assemblages. A few experimental studies have analysed changes on a simplified trophic chain using, however, only a single fish species (e.g. Boveri & Quirós, 2007; Iglesias et al., 2008; Okun et al., 2008). Also, a few studies conducted in single lakes have focused on the effects of changes in fish assemblages on other communities after the occurrence of a strong perturbation (e.g. biomanipulation, Scasso et al., 2001; fish kills, Nagdali & Gupta, 2002). Comparative field experiments in a series of lakes, but conducted only during summer, have suggested that fish predation pressure is stronger and wider and the potential for cascading effects, therefore, weaker in subtropical than in similar temperate lakes (Meerhoff et al., 2007a, b). Besides being relevant from a theoretical viewpoint, these differences in trophic cascading effects under different climates can have profound implications for the management strategies of lakes used as water sources.

Here, we analysed the changes in fish community structure and activity in four subtropical shallow lakes, varying in trophic state and water transparency, to assess changes promoted by temperature (i.e. summer and winter) and light regime (i.e. day and night) in the fish community and the consequent potential effects on lower trophic levels of the pelagic food web. We expected that fish activity (expressed as abundance captured with gill nets) would be reduced in winter regardless of water transparency and the dominant fish species. Second, we hypothesised that visually oriented fish species would be caught in higher numbers during the day, mostly in the clear lakes. On the other hand, we expected that non-visual fish species would be more frequent at night-time in all lakes and even at daytime in the least clear lakes. Third, we expected that the decrease in water transparency would lead to smaller differences between day and night captures. Finally, we expected that the seasonal or diel changes in piscivorous fish activity would have a negligible effect on the lower trophic levels.

Materials and methods

Study area

The four studied shallow lakes (Cisne, Escondida, Clotilde and García) are located along the coast of Uruguay (from 34.75°S–55.83°W to 34.29°S–53.80°W). All lakes, except for Cisne, originated during the Holocene after marine transgression (García-Rodríguez et al., 2004). Three lakes are used, and one is being considered to be used, as drinking water sources for regional towns. The lakes encompass a gradient in water transparency (from 0.20 to 1.80 m Secchi disc), area (from 17 to 200 ha.) and nutrient concentrations (Table 1).

In all lakes, sections of the littoral are covered by emergent macrophytes, whereas submerged plants occur only in Escondida and Clotilde (Table 1); *Ceratophyllum demersum*, *Myriophyllum aquaticum* and *Potamogeton* sp. in Escondida and *Potamogeton illinoensis* in Clotilde.

Sampling and analysis

The sampling campaigns were conducted in February–March (summer) and August (winter) 2006, one campaign per season (Table 1). We took samples for water chemistry (e.g. alkalinity (APHA, 1985), chlorophyll-a (Nusch, 1980), total and dissolved nutrients (Valderrama, 1981) and phyto and zooplankton from

Table 1 Main limnological parameters of the lakes, showing surface area, maximum depth (*z* max, summer and winter average) and mean summer and winter water temperature, dissolved oxygen, pH, water transparency (Secchi depth), percentage of volume inhabited by submerged plants (%PVI), concentration of chlorophyll *a* (Chl *a*), total nitrogen (TN) and total phosphorus (TP)

	Clotilde		García		Escondida		Cisne	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Area (ha)	17.7		5.2		20.8		257.2	
z max (m)	3.0		2.0		3.7		2.7	
Temperature (°C)	25.7	11.1	24.5	10.7	25.8	11.9	21.9	10.9
DO_2 (mg l^{-1})	7.1	8.5	8.5	8.3	7.5	9.3	10.8	10.1
pH	7.1	6.6	7.7	6.5	7.5	6.6	6.9	6.8
Secchi (m)	1.4	2.1	2.0	1.4	1.3	1.5	0.1	0.2
PVI (%)	5.2	<5	0	0	23	<20	0	0
Chl *a* (μg l^{-1})	1.6	2.8	7.2	2.3	8.9	5.5	5.0	4.4
TN (μg l^{-1})	624.0	588.3	540.7	608.3	447.3	631.7	1068.7	1055.0
TP (μg l^{-1})	19.0	42.4	29.0	62.4	47.7	66.4	610.3	611.1

three randomly selected shore-to-shore transects with a PVC tube (10-cm diameter, length adjusted to lake depth). We mixed the water from five equidistant points per transect into a bulk sample; therefore, each transect represented a sampling replicate. We measured in-lake variables in situ at the deepest point in each lake (e.g. dissolved oxygen, temperature, conductivity, etc., Table 1). The percentage of the lake volume inhabited by submerged plants (%PVI, Canfield et al., 1984) was estimated as the %plant cover multiplied per plant height and divided by water depth in a series of points over transects that covered the whole lake area.

We sampled the fish applying two techniques. First, we used a passive method to detect changes in fish activity (Hamley, 1975; Portt et al., 2006). For this purpose, we placed three multi-mesh gill nets (30 × 1.5 m; mesh sizes: 15, 20, 30, 35, 40 and 50 mm knot to knot) perpendicular to the shore, covering both pelagic and littoral zones. The nets were left for 2 h after sunset (night-time) and for 2 h after sunrise (day) (capture per unit of effort, CPUE: ind. net^{-1} 2 h^{-1}). We chose these periods to maximise capture, since the early hours of both day and night frequently exhibit the highest fish activity in many Uruguayan lakes (F. Teixeira-de Mello, unpublished data). The relatively short length of exposure (2 h) was chosen to avoid saturation of the nets (Olin et al., 2004). The captures with this method represent the combined changes in fish activity and abundance (Olin et al., 2004). Second, to obtain a better description of the fish assemblages, we sampled the littoral zone after sunset via point sample electrofishing (active method) (Sacks Elektricfischfanggerate GmbH type FEG 1000), applying 10 short bursts of electricity (by triplicate) per lake. All fish were euthanised with an overdose of anaesthetic (2-Phenoxy-ethanol, 1 ml l^{-1}) and preserved with formaldehyde. In the laboratory, we weighed (g), measured (standard length, mm) and identified to the highest possible taxonomic resolution each fish individual. Fish were also classified trophically using an extensive review of published literature, and following the classification of Teixeira-de Mello et al. (2009) as omnivore-benthivorous (thereafter OB), benthi-planktivorous (BP), omnivore-benthi-herbivorous (OBH), omnivore-benthi-planktivorous (OBP), omnivore-benthi-piscivorous (OBPis), piscivorous (Pis) and potentially piscivorous (PP), incorporating into that classification the groups omnivore-planktivorous (OP, e.g. *Parapimelodus valenciennis*, a pelagic filter-feeding) and detritivorous (D, e.g. *Cyphocharax voga,* sediment consumer). Besides, fish species were classified as visual or non-visual according to literature (e.g. Rodríguez & Lewis, 1997; Tejerina-Garro et al., 1998).

Zooplankton and phytoplankton were counted under stereomicroscope. Zooplankton was identified to species and classified according to main groups (cladocerans, copepods, rotifers and nauplii). Phytoplankton biovolume was calculated for each phytoplankton taxon by multiplying the population density by the mean volume (according to Hillebrand et al., 2004) (Pacheco et al., 2010).

Data analysis

In most analyses, we used the gill net data, that provide insight into both fish activity and population abundance (Portt et al., 2006) (hereafter we use the term ‘abundance’ for CPUE). We analysed the fish assemblage structure (i.e. richness, abundance, biomass, mean body size) in each lake by 2-way ANOVA tests, with season (winter, summer) and time (day, night) as main factors. We checked the assumptions using the Kolmogorov–Smirnov (normality) and the Levène test (homoscedasticity). In case of significant differences in the ANOVA tests, we performed multiple comparisons applying Tukey post hoc tests. We also performed linear regressions (Pearson) between relevant variables. We conducted statistical tests with the Statistica 6.0 package.

Results

Fish community composition: fishing methods and transparency gradient

In total, we caught 30 fish species, 13 families and 5 orders (Table 2). The two different sampling techniques yielded different fish assemblages within some of the lakes. Besides, the efficiency of electrofishing and gill nets used at different hours varied according to trophic groups (Fig. 1). Moreover, we caught more fish with the electrofishing method (littoral zone) than with the gill nets (that mostly covered the pelagic zone). Thus, in total we found 355, 93, 972 and 25

Table 2 Fish species captured in all systems (Clotilde, García, Escondida, Cisne), indicating trophic classification (combination of main diet items and feeding habitats, as *O* omnivory, *B* benthivory, *P* planktivory, *Pis* piscivory, *H* herbivory, *D* detritivory, see text and Teixeira-de Mello et al., 2009 for the classification system) and feeding mode (visual: v and, non-visual: nv)

Order & family	Species	Feeding habit	Mode	Clo	Gar	Esc	Cis
Characiformes							
Characidae	*Astyanax eigenmanniorum*	OBP	v	ne			ne
Characidae	*Astyanax cf. fasciatus*	OBP	v	n			n
Characidae	*Astyanax* sp.	OBP	v	e			e
Characidae	*Charax stenopterus*	OBP	v	ne			ne
Characidae	*Cheirodon interruptus*	OBP	v	n		e	e
Characidae	*Diapoma terofali*	OBP	v	n			e
Characidae	*Hyphessobrycon anysitsi*	OBP	v	e			
Characidae	*Hyphessobrycon luetkenii*	OBP	v	ne	ne		e
Characidae	*Hyphessobrycon meridionalis*	OBP	v	n			
Characidae	*Hyphessobrycon* sp.	OBP	v				e
Characidae	*Oligosarcus jenynsii*	BP <11 cm OBPis >11 cm	v	ne	ne	n	ne
Crenuchidae	*Characidium rachovii*	BP	v	ne			ne
Curimatidae	*Cyphocharax voga*	D	v	e	e		ne
Erithrynidae	*Hoplias malabaricus*	OBPis >9 cm Pis >16 cm	v	e		e	n
Cyprinodontiformes							
Anablepidae	*Jenynsia multidentata*	OBP	v		e	e	
Poeciliidae	*Cnesterodon decemmaculatus*	OBP	v	e	e	e	
Poeciliidae	*Phalloceros caudimaculatus*	OBP	v	e	e		
Perciformes							
Cichlidae	*Australoheros facetus*	OBP <3 cm OBPis >3 cm	v	e	e	e	
Cichlidae	*Gymnogeophagus meridionalis*	OBP	v	e			
Siluriformes							
Callichthyidae	*Corydoras paleatus*	BP	nv			e	
Heptapteridae	*Heptapterus mustelinus*	OBP	nv		e		
Heptapteridae	*Heptapterus sinterigium*	OBP	nv		e		
Heptapteridae	*Pimelodella australis*	OBP	nv			e	e
Heptapteridae	*Rhamdia quelen*	OBPis	nv	n	n		n
Loricariidae	*Hisonotus* sp.	OBH	nv	e	e		
Loricariidae	*Hypostomus commersoni*	OBH	nv				e
Pimelodidae	*Parapimelodus valenciennis*	OP	nv				n
Synbranchiformes							
Synbranchidae	*Synbranchus marmoratus*	BP	nv		e	e	e

Also, it is indicated whether species were caught with nets (n), electrofishing (e), or both methods (ne)

individuals with electrofishing versus 101, 197, 23 and 84 individuals with gill nets in Clotilde, García, Escondida and Cisne, respectively. With electrofishing, we captured small-bodied fish species that often take refuge within macrophytes (e.g. *Phalloceros caudimaculatus*, *Cnesterodon decemmaculatus*, *Hyphessobrycon luetkenii*) or lay on the sediment (e.g. *Synbranchus marmoratus*).

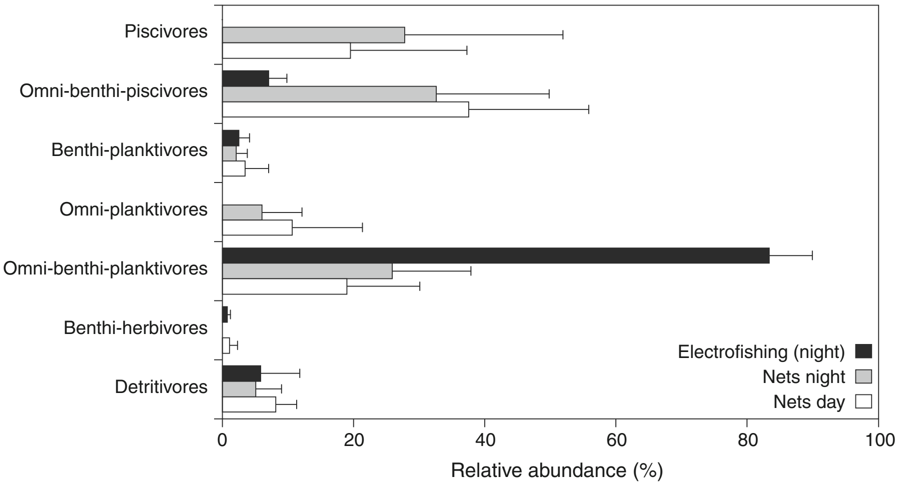

Fig. 1 Comparison of fish trophic groups captured with different gear: gill nets (day, night sampling) and electrofishing. Data represent the mean percentage of each trophic group over the total captures per fishing gear in each lake (summing up winter and summer captures), averaging across the lakes (±SE)

With the nets, we caught larger and potentially more active fish (e.g. *Oligosarcus jenynsii* and *Astyanax eigenmanniorum*). For instance, the visually oriented OBP is *O. jenynsii* (pike-characin, local common name 'dientudo') was the most captured species with the nets (13.85 ± 2.03 cm SE, average standard length), whereas the small OBP *C. decemmaculatus* (10-spotted live-bearer, local common name 'madrecita') was the most frequently captured species with electrofishing (1.95 ± 0.71 cm SE, average standard length) (Fig. 1). This last species is usually too small (typically <3.0 cm S.L.) to be caught with the nets.

In both clear lakes, Clotilde and García, Characiformes (visually oriented) was the most abundant fish group, both in nets and electrofishing. Siluriformes (non-visual) appeared in low abundances (1 and 3%, respectively, of the catches with nets). In the clear Lake Clotilde, we captured more tetra fish (*H. luetkenii*) in the littoral zone (electrofishing) than in the pelagic zone (nets), even individuals that were large enough to be caught in the nets (i.e. >2.8 cm S.L.).

In Lake Escondida, the nets caught only few individuals, the piscivorous *H. malabaricus* (i.e. >16 cm S.L., Table 2) being the most abundant (23 individuals in total). In contrast, in the littoral zone of this lake (electrofishing), *C. decemmaculatus* was the most abundant species (500 individuals in total). In the least clear system, Lake Cisne, also Characiformes dominated in terms of abundance (CPUE with nets) followed by Siluriformes (which accounted for 39% of total abundance, with nets).

Effect of season and time on fish community (capture with gill nets)

Except for Lake Escondida, species richness was significantly greater in summer than in winter (Table 3). We also found different assemblages in each season in some lakes (Fig. 2). For instance, *Parapimelodus valenciennis* predominated in winter (in Lake Cisne), while other species, such as *Charax stenopterus* and *A. eigenmanniorum*, predominated in summer (Lakes Cisne and Clotilde). However, only in Lake García we detected a significant seasonal difference in fish abundance and biomass (greater in summer, Table 3). In both extremes of the transparency gradient, Lake Clotilde and Lake Cisne, we registered a trend towards increased biomass and abundance in summer (Fig. 3). We found no significant differences in the average size of fish between seasons in any of the lakes (Table 3; Fig. 3).

In the clearest lakes (i.e. Clotilde and García), we found a significant difference between time of the day on species richness (greater at night-time, Table 3; Fig. 3). In Lake Clotilde, the piscivorous *O. jenynsii* appeared in lower numbers during the night in summer, while the abundance of smaller-bodied tetras (local name 'mojarras': *A. eigenmanniorum*, *A. cf. fasciatus*, *H. luetkenii*, *Charax stenopterus*, and *Cheirodon interruptus*) increased (Fig. 2). This pattern led to a (marginally) significant difference in the average size of fish between day and night (2-way ANOVA, Table 3, with smaller fish at night-time) and between summer and winter (n.s. ANOVA, with smaller fish in summer) in this lake.

Table 3 Effects on season (summer: S, winter: W), time (day: D, night: N) and interaction, on fish richness, abundance, biomass and length in the four lakes as captured with nets, ordered by decreasing water transparency

Variables	Factor	Season (winter, summer)				Time (night, day)				Season × time		
	Lake	*F*	d.f.	*P*	Post hoc	*F*	d.f.	*P*	Post hoc	*F*	d.f.	*P*
Richness	Clotilde	4.57	1, 8	0.06	S > W	10.28	1, 8	*	N > D	1.14	1, 8	ns
	García	8.10	1, 8	*	S > W	8.10	1, 8	*	N > D	0.90	1, 8	ns
	Escondida	0.13	1, 8	ns	S = W	1.13	1, 8	ns	N = D	0.13	1, 8	ns
	Cisne	8.04	1, 8	*	S > W	0.04	1, 8	ns	N = D	0.43	1, 8	ns
Abundance	Clotilde	2.73	1, 8	ns	S = W	3.29	1, 8	ns	N = D	0.08	1, 8	ns
	García	27.68	1, 8	***	S > W	31.11	1, 8	***	N > D	14.73	1, 8	**
	Escondida	1.71	1, 8	ns	S = W	0.01	1, 8	ns	N = D	0.09	1, 8	ns
	Cisne	1.18	1, 8	ns	S = W	<0.01	1, 8	ns	N = D	0.27	1, 8	ns
Biomass	Clotilde	0.36	1, 8	ns	S = W	0.89	1, 8	ns	N = D	1.59	1, 8	ns
	García	13.4	1, 8	**	S > W	25.01	1, 8	**	N > D	10.08	1, 8	*
	Escondida	1.48	1, 8	ns	S = W	0.11	1, 8	ns	N = D	0.10	1, 8	ns
	Cisne	0.65	1, 8	ns	S = W	0.96	1, 8	ns	N = D	<0.01	1, 8	ns
Standard length	Clotilde	0.63	1, 7	ns	S = W	4.93	1, 7	0.06	N = D	0.72	1, 7	ns
	García	1.41	1, 8	ns	S = W	1.03	1, 8	ns	N = D	0.26	1, 8	ns
	Escondida	0.05	1, 3	ns	S = W	0.71	1, 3	ns	N = D	0.11	1, 3	ns
	Cisne	1.09	1, 6	ns	S = W	<0.01	1, 6	ns	N = D	0.51	1, 6	ns

Summary of 2-way ANOVA tests, showing *F*-values, degrees of freedom (d.f.), *P*-value and a summary of the post hoc Tukey tests when relevant

Significance levels: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, $0.05 < P < 0.1$ shown, *ns* $P > 0.1$

Again, only in García we observed significant differences between day and night in fish abundance and biomass; as well as a significant interaction between time and season (Table 3).

In Lake Escondida, we found a greater (not significant) biomass during the night (Fig. 3), mainly due to variations in *H. malabaricus* numbers. In contrast, in the least clear system (Cisne), we found very small (albeit insignificant) differences in abundance or biomass (as CPUE) between day and night during both seasons (Table 3; Fig. 4). Here, the visually oriented fish occurred in higher numbers at all times, although some non-visual species, such as the OBH *Hypostomus commersoni* and the pelagic filter-feeding *P. valenciennis*, occurred during both day and night.

Potential cascading effects of changes in fish structure and activity

The phytoplankton structure varied among the lakes, Lake Clotilde being the richest in terms of species and functional groups, and Lake Cisne the poorest and with lowest biomass (Pacheco et al., 2010). The phytoplankton biovolume was greater in summer in all lakes, except for Cisne, where it decreased (Fig. 5).

The zooplankton assemblages were, in all lakes, characterised by small-sized organisms, with dominance of rotifers and nauplii in terms of abundance and extremely low numbers of *Daphnia* spp. As with phytoplankton, in all lakes except for Cisne, total zooplankton numbers increased in summer (though not significantly in all cases). In the clearest lakes, Clotilde and García, the relative density of herbivorous zooplankton (i.e. calanoid copepods and cladocerans) decreased in summer (from 41 to 13% and from 81 to 53%, respectively), while the opposite occurred in both Lakes Escondida and Cisne (from 25 to 45% and 8 to 41%, respectively) (Fig. 5). In three out of four lakes, we found no significant relationship between zooplankton attributes (total densities and proportion of herbivores) and active fish attributes (total abundance, biomass and mean length of fish captured by nets). Only in Lake García did we find significant (positive) relationships between total

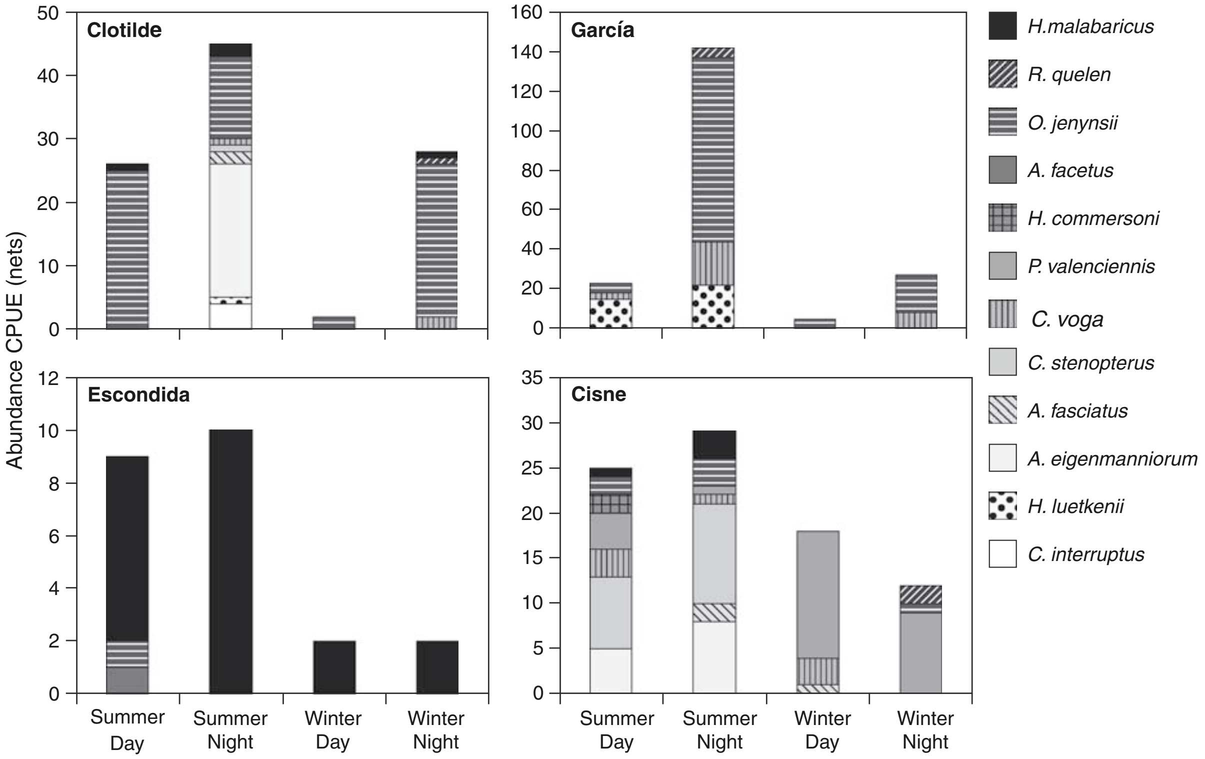

Fig. 2 Diel and seasonal changes in fish species composition and abundance (as CPUE with nets) in the four lakes (ordered by decreasing transparency from left to right and top to bottom). Please note the different scales

zooplankton densities and fish abundance and biomass (adjusted Pearson $r^2 = 0.77$, $P < 0.013$, and $r^2 = 0.68$, $P < 0.02$, respectively).

To evaluate the potential impact of changes in activity and abundance of all potential piscivores on planktivorous fish, we summed up the fish captured with both fishing gears and for both day and night (integrated CPUE of ind. net^{-1} 4 h^{-1} + ind. $(10 \text{ electric bursts})^{-1}$). In none of the lakes were planktivorous fish significantly related to the seasonal changes in piscivorous fish (no significant regressions), although in Escondida and Clotilde both groups varied in opposite directions (Fig. 5). The seasonal changes in planktivores were, in contrast, sometimes significantly and negatively related to the zooplankton level (total density, in Lakes Escondida and Cisne: $r^2 = 0.60$, $P < 0.043$ and $r^2 = 0.62$, $P < 0.040$, respectively; and relative density of herbivores, in Lake García: $r^2 = 0.51$, $P = 0.066$). However, the seasonal changes in zooplankton density were very seldom positively related to changes in water transparency (Secchi depth) (Lake Clotilde, $r^2 = 0.85$, $P < 0.005$, and Lake Cisne $r^2 = 0.68$, $P < 0.026$, for relative density of grazers and total zooplankton, respectively) and negatively related to changes in phytoplankton biovolume (Lake García, not significantly) (Fig. 5). In all cases, zooplankton total density and phytoplankton biovolume followed the same seasonal variations (Fig. 5). Except for the positive relationships between piscivores and Secchi depth but also with phytoplankton biovolume in Lakes García and Escondida, respectively, no significant relationships were found between piscivorous fish and water transparency or phytoplankton.

Discussion

We observed a different structure (i.e. richness, composition, biomass, abundance) of the fish assemblages between seasons in the four lakes. In agreement with our first expectations, we collected more fish (as CPUE with nets) in summer in all the lakes. Despite this, the 'season effect' was not statistically

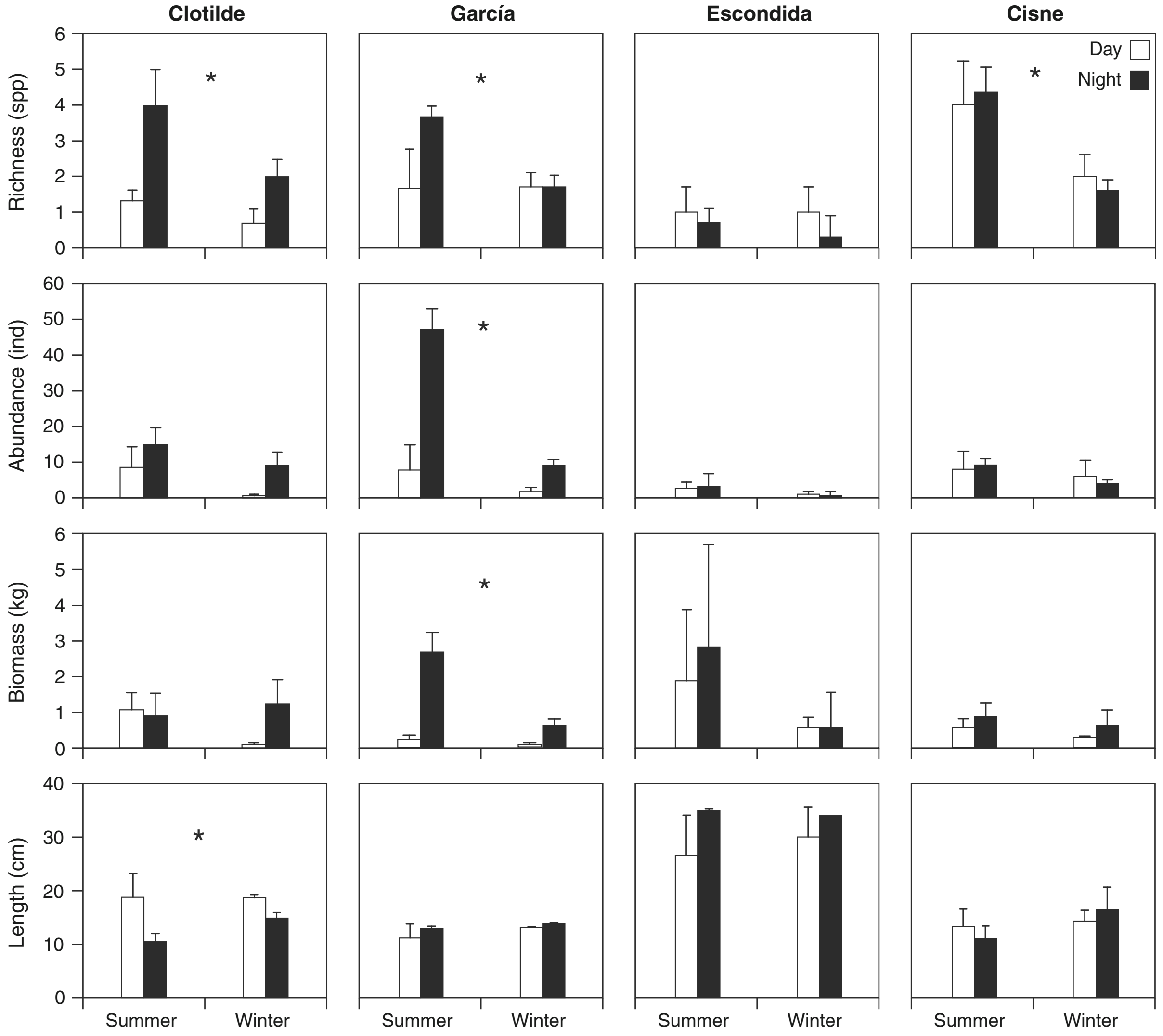

Fig. 3 Main characteristics of the fish assemblages in the four lakes, showing seasonal (summer–winter) and diel (day–night) mean values (±SE) of richness (species captured net^{-1} 2 h^{-1}), abundance (ind. net^{-1} 2 h^{-1}), biomass (kg net^{-1} 2 h^{-1}), and standard length (cm). *Stars* (*) indicate significant results in 2-way ANOVA tests (see Table 3)

significant in all cases; however, the observed trends were clear, suggesting greater fish activity in summer. Since fish are thermo-dependant animals (Linlokken & Haugen, 2006), the higher the water temperature, the greater their metabolic rate and activity level. The large temperature difference between seasons (>10°C) likely had differential effects not only on the different fish species within the assemblages, according to the optimal temperature of each species (Persson, 1986; Hölker, 2003), but also on biological interactions such as competition and predation. Interestingly though, in most lakes we found more species in summer than in winter. The appearance of more species with a smaller mean body size in Lakes Clotilde (most transparent) and Cisne (least transparent) led to a smaller (though not significant) mean body size in summer than in winter in these lakes.

Surprisingly, despite the differences in water transparency, we observed only small differences in fish composition considering their feeding mode. In partial agreement with our second hypothesis, the relative abundance (CPUE with nets) of the non-visual Siluriformes was higher in the least clear Lake Cisne than in the other lakes. This finding is in

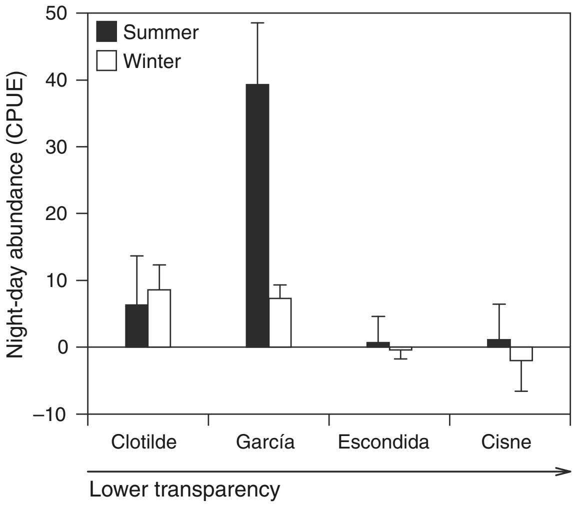

Fig. 4 Difference between mean night and day CPUE (ind. net^{-1} 2 h^{-1}, ±SE) in summer and winter along a decreasing transparency gradient, from left to right

accordance with previous studies (e.g. Rodríguez & Lewis, 1997; Tejerina-Garro et al., 1998). Opposite to our expectations, the visually oriented Characiformes predominated during both day and night in the clear water lakes. However, the few Siluriformes individuals captured (such as the catfish *Rhamdia quelen*) occurred in the night-time (and only in winter in Lakes Clotilde and Cisne), implying a rather nocturnal behaviour. We, therefore, found no evidence to support our hypothesis that visual feeders increase their activity in clear lakes during the day. In fact, all fish species appeared in higher numbers in the night; thus, the species appearing during the day increased their activity at night and new additional species occurred. We also observed a trend of higher fish biomass at night-time. This result could be the consequence of two different processes: either more individuals are more active (with a concomitant increase in numbers and no changes in mean body size) or larger-bodied individuals become more active (with a concomitant increase in size and not necessarily in numbers) at night-time. The first scenario occurred in the clearest Lake Clotilde where we captured more (small-bodied) tetra fishes in the night. This might suggest a nocturnal decrease in pressure by the visually oriented predators, promoting a behavioural response by small fish that become more active during the night. In Lake García, both the abundance (as CPUE) and mean body size increased in the night (particularly in summer) due to the increase in numbers of the OBP is *O. jenynsii*.

In agreement with our third hypothesis, we observed a reduction in the differences between night and day for fish CPUE with a decrease in water clarity, particularly in winter. In summer, the pattern was similar in all lakes, except for Lake García, because of the above-mentioned occurrence of high abundance of *O. jenynsii*. This pattern could potentially combine changes related to feeding strategy, as visually oriented species are less efficient in turbid waters (e.g. Pekcan-Hekim & Lappalainen, 2006), and/or to a reduced anti-predator behaviour in turbid waters (e.g. Snickars et al., 2004). However, since all fish assemblages were dominated by visual feeders, we would expect a nocturnal reduction in fish activity (i.e. a negative difference between night and day). The occurrence of higher numbers of all fish species at night-time, even the visually oriented species, may suggest that a strong refuge is needed in these systems, even at the cost of a likely decrease in feeding efficiency in the darkness. The need for refuge seemed particularly important in the clear water lakes, where the night–day differences were larger. A strong need for refuge by subtropical fish has been suggested by previous studies carried out in similar lakes (Meerhoff et al., 2007a; Teixeira-de Mello et al., 2009), where extremely high densities of (small-bodied) fish were found within artificial submerged plants compared to other, less structured, habitats. Similarly, higher densities of small fish aggregated among the submerged and emergent vegetation than in open water, even at night-time, in eutrophic Lake Blanca (Maldonado, Uruguay) (Iglesias et al., 2007). In contrast, in shallow clear water temperate lakes, juvenile planktivorous fish (e.g. roach) often aggregate in highly structured habitats during the day, but partly move into open waters at night (Lewin et al., 2004). In line with this suggestion, we captured high numbers of small-sized individuals with the electrofishing method in the littoral zone of the clear water lakes. This might reflect a greater food offer, but most likely better refuge possibilities for the small-bodied fish in the littoral area. Behavioural models predict that, given different foraging returns and predation risks in different habitats, the vulnerable individuals will choose the habitats with the lowest mortality risk (Kramer et al., 1997). The nocturnal increase in fish

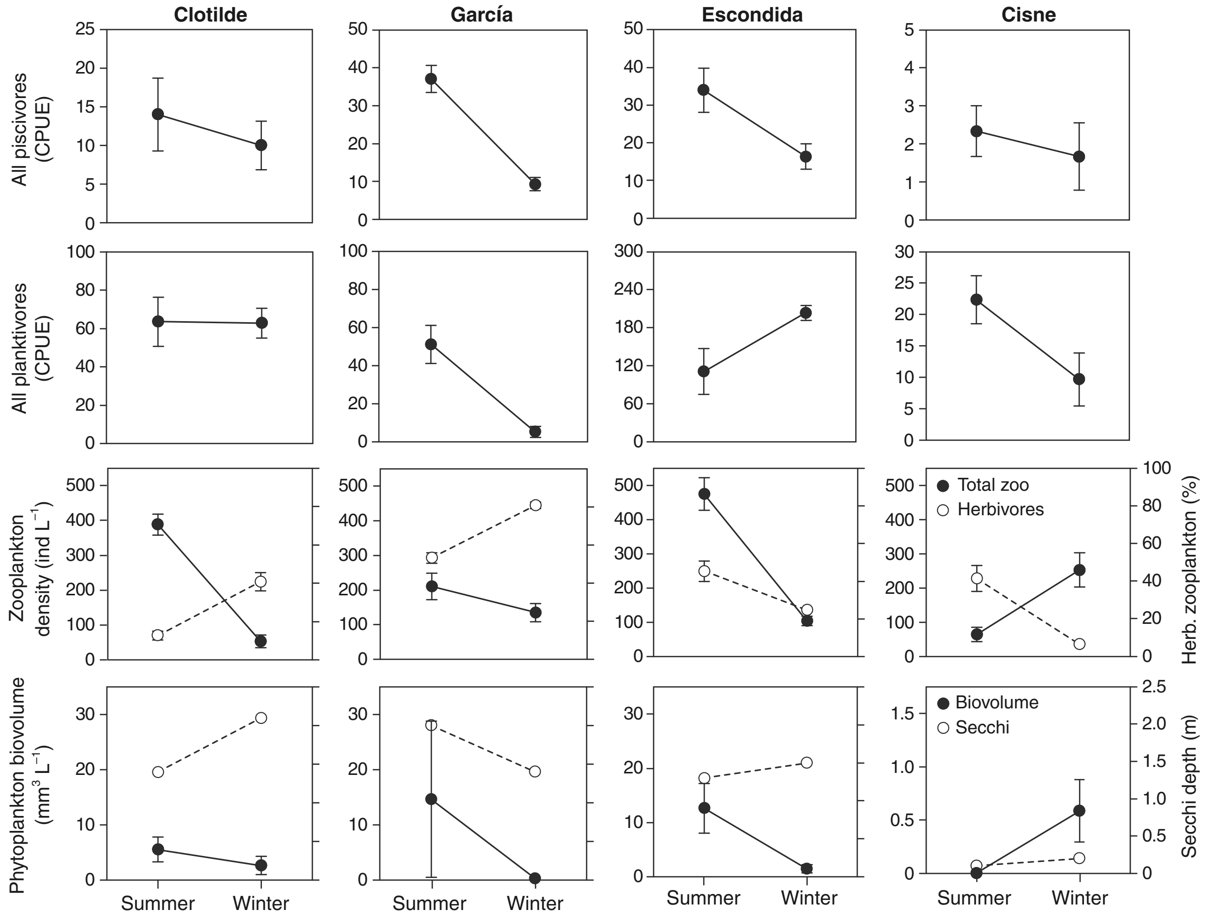

Fig. 5 Cascading effects of seasonal changes in mean (±SE) piscivorous fish (OBPis + Pis) abundance and activity, as integrated CPUE: nets + electrofishing, on planktivorous fish (OBP + OP + BP), zooplankton density (total and % of herbivores: cladocerans + calanoid copepods), and phytoplankton biovolume and water transparency (as Secchi depth). Please note the different scales

activity, as found in our study, suggests that the combination of highly structured habitats (as in Meerhoff et al., 2007a; Teixeira-de Mello et al., 2009) and also darkness represent the best refuge for fish in shallow subtropical lakes, the latter decreasing in importance with decreasing water transparency.

Also other factors may affect our findings. Some authors argue that fish might be able to see and thus avoid the nets during the day under high water transparency conditions (Hansson & Rudstam, 1995; Olin et al., 2004). However, we caught more fish in the night also in the most turbid lake (in summer), which suggests that our findings reflect greater nocturnal activity rather than visual avoidance of the nets during the day.

Our study analysed both fish abundance and activity as a response to changes in temperature, and the consequent potential cascading effects on other communities. Supporting other studies (e.g. Lazzaro, 1997; Meerhoff et al., 2007a; but see Iglesias et al., 2008 for opposite experimental results), our field results also suggest that the potential for trophic cascading effects in the subtropics is weak. In only one lake (García), the changes in planktivorous fish activity seemed to weakly reach the phytoplankton level via effects on herbivorous zooplankton; with cascading effects thus involving three trophic levels. In another lake (Escondida), piscivores and planktivores varied in opposite direction (although not significantly) with effects reaching the zooplankton but not the phytoplankton level (again, only three trophic levels were involved). Therefore, downwards propagation of effects from top predators, in those cases where omni-planktivorous fish seemed affected, could eventually reach zooplankton (either densities and/or the proportion of grazers), but did not seem strong enough to affect the phytoplankton biomass, regardless of water transparency or trophic state. The widespread omnivory (Polis & Strong, 1996), a likely strong benthic subsidy, and the high specific and functional richness of subtropical fish likely explain the truncated cascading effects in these lakes (Meerhoff et al., 2007a).

These findings are important to consider when estimating the potential success of biomanipulation or other management measures in lakes that are used as drinking water sources. As also suggested in other studies, reduced nutrient loading rather than fish manipulation is likely to be a more successful strategy to maintain high water quality in warm lakes (e.g. Jeppesen et al., 2007, 2009; Meerhoff et al., 2007a). The long-term success of combining both management measures in warm lakes still remains to be elucidated.

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6

FOOD WEBS ARE MORE TRUNCATED IN SUBTROPICAL THAN TEMPERATE SHALLOW LAKES: IMPLICATIONS OF FISH OMNIVORY IN SUBTROPICAL SHALLOW LAKES

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Food webs are more truncated in subtropical than temperate shallow lakes: Implications of fish omnivory in subtropical shallow lakes

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Running title: Food webs in shallow lakes of contrasting climates

Keywords: benthic-pelagic coupling, trophic position, stable isotopes

Summary

The length of trophic webs may depend, among other factors, on temperature, as energetic limitations are potentially stronger in warm climates. A richer diet (i.e. more diverse food items), required to cover the higher energetic demands of the potential predators there, may result in lower trophic positions of the top consumers. On the other hand, many characteristics of the subtropical fish assemblages are somehow related to the higher ambient temperature, such as multiple or long reproduction seasons, small mean body sizes and high densities. With the predicted changes in temperature created by climate warming the food web structure and dynamics of ecosystems, as well as ecosystem functioning, may be affected in various ways.

To analyse the potential effects of warming on trophic webs in shallow lakes we compared the food web structure of main communities in 5 subtropical (Uruguay, 30–35°S) and 4 temperate (Denmark, 55–57°N) shallow lakes using stable isotopic analyses.

Supporting our hypothesis, we found that the trophic webs in subtropical shallow lakes were, on average, one trophic position shorter than their temperate counterparts. Our results strongly suggest that subtropical fish species, even at the top of the food webs, are actually omnivores (i.e. predating on fish, but also feeding at one or more trophic levels down the trophic web) and exhibit a strong degree of niche or feeding overlap. Besides, intermediate consumers make a very large contribution to subtropical food webs.

The ranges of carbon sources fuelling the food webs of both climate regions were similar, and the periphytic pathway seemed equally important (similar littoral contribution). However, food webs had different and distinct shapes in each climatic zone. Temperate food webs may be represented by a triangular shape, depicted by a multi-chain omnivory model, with a top predator integrating different carbon sources that fuel the web (phytoplankton and periphyton, respectively). In subtropical lakes, multi-chain omnivory alone cannot explain the trapezoid-shaped trophic webs, and a combination of simple chain with multi-chain omnivory models is proposed.

These differences in food web architecture may have profound effects on ecosystem functioning, with warming affecting the resilience and stability of the clear water phase of shallow lakes typically occurring with longer, chain-like trophic webs.

Introduction

The relationship between trophic web length (TWL) and ecosystem stability has long been debated. Some believe that shorter trophic webs are more stable (Pimm, 1982), while others hold the opposite view (e.g. Sterner et al., 1997). The length of trophic webs may depend on temperature, as energetic limitations potentially play a stronger role in warm climates (McNab, 2002). A richer diet (i.e. more diverse food items), needed to cover the energy demands of the potential predators there, may result in lower trophic positions of top consumers. Such a pattern has been found in riverine tropical ecosystems (Jepsen & Winemiller, 2002) and is considered a plausible explanation for potentially predatory fish occupying lower trophic positions in South American shallow lakes at lower latitudes (Lacerot et al., submitted). Some works (Post, 2002b; Vander Zanden & Fetzer, 2007) have suggested that resource availability has little impact on TWL in natural systems, being important only under extremely low resource conditions, particularly in temperate lakes (Post, 2002). Further evidence is provided by Vander Zanden and Fetzer (2007), who found that on the global scale lake and stream TWL showed only weak or no relationships with ecosystem size, mean annual air temperature or latitude, although lake and stream food chains tended to be longer at high latitudes compared to the tropics.

Many characteristics of the subtropical and tropical fish assemblages are directly or indirectly related to the higher ambient temperature (Lazzaro, 1997), such as multiple or long reproduction seasons, small body sizes and high densities (Meerhoff et al., 2007a; Teixeira de Mello et al., 2009). Furthermore, omnivory (i.e. feeding at more than one trophic level) seems to be a predominant characteristic of subtropical fish assemblages (Lazzaro et al., 2003), which may also affect ecosystem functioning depending on its occurrence within the food web (Yodzis, 1981; Pimm, 1982).

Climate models predict that mean annual global surface temperature will have increased by 1.8–4.0ºC by the year 2100 (IPCC, 2007), most pronouncedly at higher latitudes (Rouse et al., 1997), but noticeably also in subtropical regions (Mulholland et al., 1997). Such predicted changes in temperature may influence the food web structure of ecosystems in various ways. Fish assemblages are particularly sensitive to temperature changes, with cascading effects on trophic structure and interactions. In northern cold shallow lakes, warming has allowed greater survival of fish during winter (Balayla et al., 2010) and increased the activity of small individuals (Hill & Magnuson, 1990; Jackson et al., 2007), which may, in turn, lead to a stronger predation pressure on zooplankton (Mehner, 2000) and affect the consumption of the primary producers. Using a space for time substitution approach (Jeppesen et al., 2003; Moss et al., 2004; Meerhoff et al., 2007a, b), we aim to increase our understanding of the consequences of warming on ecosystem functioning.

To analyse the potential effects of warming on trophic webs in shallow lakes we compared stable isotopic values of main communities in 5 subtropical (Uruguay, 30–35°S) and 4 temperate (Denmark, 55–57°N) shallow lakes. Nitrogen and carbon isotopes are most frequently used in aquatic ecology to study trophic web structure and energy, carbon sources and fluxes (Cabana & Rasmussen, 1996; Vander Zanden & Rasmussen., 2001; Jones & Waldron, 2003). The primary aim was to test whether trophic webs are shorter in subtropical than in temperate shallow lakes due to the pronounced omnivory at the top of the subtropical food webs. Secondly, we aimed to detect the strength of the relative contribution of periphyton (littoral) and phytoplankton (pelagic) to sustain higher trophic levels under contrasting climate scenarios. We hypothesized that the benthic or littoral pathway (i.e. periphyton-fuelled) would be stronger in warm lakes.

Methods

Study sites

We selected 5 shallow lakes located along the east coast of Uruguay covering a wide range in trophic state and water transparency (Table 1). Based on previous data they were considered representative of the typical variability of Uruguayan shallow lakes (Kruk et al., 2009). For the climate comparison we used existing data at the National Environmental Research Institute from a subset of shallow lakes in Denmark, comparable as to trophic state and main limnological characteristic (Table 1).

Field sampling

All samples represent the end of the growing season (summer) in both countries. In Uruguay, sampling was performed in March 2009. All lakes were shallow and at both locations covered a wide range in area, nutrient level and plant coverage (Table 1). We conducted an intensive sampling campaign in different habitats (i.e. pelagial and littoral) of taxa representing all trophic levels and potential carbon sources (i.e. phytoplankton, periphyton and macrophytes). We collected zoobenthos and plant-attached macroinvertebrates with a 200 µm mesh sweep net.

Each macrophyte species was carefully collected and a piece of each species was then vigorously shaken in tap water to remove attached algae, obtain periphyton suspension and a clean piece of macrophyte for stable isotopes analysis (SIA). We collected zooplankton and seston (mainly phytoplankton) samples by filtering water through a 50 and a 12 µm mesh, respectively, integrating water from different depths and from different open water parts in each lake. Afterwards, we filtered the periphyton and phytoplankton suspensions through a Whatman GF/C filter (1.2 µm). All samples were rapidly frozen (-18ºC) and transported to the laboratory.

Table 1. Main limnological features of the subtropical (shaded) and temperate (clear) study lakes. Area (Ha); maximum depth, Z_{max} (m); Secchi depth, SD (m), and summer values of temperature (°C), total phosphorus, TP (µg L^{-1}), total nitrogen, TN (µg L^{-1}) and conductivity, K (µS cm^{-1}) are shown together with Plant Volume Infested, PVI (%) and phytoplankton biomass, Chl-a (µg L^{-1}).

Area	Cisne	Blanca	Diario	Garcia	Clotilde	Vaeng	Gammelmose	Denderup	Tranevig
Area	127	60	101	13.5	29	15	1.3	4.6	2.7
Z_{max}	3.2	2.5	1.7	2	3.1	1.7	1.6	1.9	0.9
SD	0.4	0.64	1.05	1.7	1.8	0.9	1.3	1.8	0.75
Temp	13.1	19.6	19.2	16.3	17.6	17	16.4	16.3	16.6
pH	7.1	7.4	7.3	6.32	7	7.9	8.1	8.1	7.6
Cond	210	320	348	142	360	268	595	171	71
Chl a	6	38.6	10	2	2.3	65.8	78.5	7.2	37.6
PVI	0	13	40	5	28	0	3	27	44
TP	413	51.9	75.8	29.8	27.7	113	157	54	60.5
TN	1048	1017	825	332	451	1018	2212	664	1040

We sampled fish using both multi-mesh-size gillnets and electrofishing. Minimum three 30 m long and 1.5 m deep nets (including 2.5 m sections of 12 different mesh sizes from 5 to 55 mm, knot to knot distance) were randomly distributed at sunset and left until sunrise. At sunset, we also conducted extensive electrofishing along littoral areas to capture fish species and size classes not available from the gillnet catches. We classified the fish to species level after which they were measured (standard length SL, 0.1 cm) and weighed (fresh weight, 0.01 g). We removed a piece of flank muscle for isotope analysis from ten individuals, if available, of each fish species, covering their size distribution. On a depth integrated water sample we measured total phosphorus and total nitrogen according to Valderrama (1981) in each of the nine lakes during the sampling period. The same protocol was followed in both countries.

Sample processing for isotopic analysis and data analysis

In the laboratory, we freeze-dried and fine powdered the fish flank muscle, snails and bivalves tissue and macroinvertebrates for SIA. We kept the samples separated in new clean Eppendorf tubes, until ca. 1 mg (weighed to 0.01 mg precision) was loaded into tin capsules and sent to the UC Davis Stable Isotope Facility (University of California, USA) for determination of stable isotopes. Determination of the food web structure of each lake was conducted by plotting $\delta^{15}N$ against $\delta^{13}C$ values for all available organisms (Fry, 1991).

We estimated the relative contribution of the two alternative carbon sources or pathways (littoral and pelagial, based on periphyton and phytoplankton, respectively) according to Vander Zanden and Vadeboncouer (2002):

$$\%\ \text{Cont-Lit} = 100 \times [(\delta^{13}C_{\text{consumer}} - \delta^{13}C_{\text{pelagic}}) / (\delta^{13}C_{\text{littoral}} - \delta^{13}C_{\text{pelagic})}]$$

The relative contribution of periphyton (% Cont-Lit) was then calculated: $\delta^{13}C_{\text{consumer}}$ is the mean $\delta^{13}C$ of the consumers in the pelagic (i.e. bivalves) and littoral zones (i.e. gastropods). We used primary consumers also as isotopic endpoints, as the model assumes no fractionation for $\delta^{13}C$, and also used them as baseline (following Post, 2003) (i.e. trophic level=2). Consequently, we used snails and bivalves as baseline, as representative of the littoral (periphytic) and pelagic (seston) based food web and estimated the trophic position of each individual following Post (2002):

$$\text{Trophic position} = [(\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{base}}) / 2.98] + 2$$

where $\delta^{15}N_{\text{consumer}}$ and $\delta^{15}N_{\text{base}}$ are the isotopic signatures of each individual and of the averaged (bivalves and snails) baseline organisms; 2.98 is the expected $\delta^{15}N$ fractionation per trophic level (Vanderklift & Ponsard, 2003) and 2 is the theoretical trophic level of baseline organisms (Post, 2002). We estimated the TWL as the maximum trophic position for each lake. We also studied community-wide metrics (sensu Layman et al., 2007) to look for climatic distinctive characteristics of food webs. Together with TWL and % Cont-Lit, we analyzed: i) carbon range (CR), both total and per trophic level (CR, CR2, CR3), as the difference between the most $\delta^{13}C$-enriched and the most $\delta^{13}C$-depleted values in the trophic web and for each distinctive trophic position; ii) total area (TA), measured as the convex hull area given by all species in the δ^{13}C-δ^{15}N bi-plot, and iii) the mean nearest neighbour distance (NND), as the mean of the Euclidean distances to the nearest neighbour of each species in the bi-plot. CR gives an indication of the amplitude of the carbon resources being used, TA represents a measure of the total amount of niche space occupied by the trophic web and NND indicates a redundancy of species with similar trophic ecology (for further description of these metrics, see Layman et al., 2007).

We calculated the last two parameters using PAST software. We tested for differences between climate zones on the measured trophic web attributes (i.e. TWL, %Cont-Lit, CR, TA and NND) using Mann-Whitney non-parametric test.

Results

The trophic webs in subtropical shallow lakes were indeed shorter than the temperate ones (Table 2). In the temperate lakes, typically one or two fish species obtained a higher trophic position (usually pike, *Esox lucius*) than the highest position typically reached by subtropical fish (Fig. 1), resulting in a higher TWL (Table 2). The subtropical lakes in our study had on average one less trophic position (Fig. 1).

Mean fish richness was higher in the subtropical than in the temperate lakes (9.5±1.5 SE and 5.3±1.1 SE, respectively). Within the subtropical fish assemblages, several potentially piscivorous species occurred (*Australoheros facetus* (Jenyns), *Hoplias malabaricus* (Bloch), *Oligosarcus Jenynsii* (Gunter), Rhamdia quelen (Quaoy & Gaymarel) and *Synbranchus marmoratus* (Bloch) (Table 3). In every lake at least two potential piscivorous species appeared; however, not always reaching a piscivorous size (e.g. A. facetus

Table 2. Fish species richness (FR) and community-wide metrics from subtropical (above) and temperate (below) lakes were calculated based on the distribution of species in the δ^{13}C-δ^{15}N bi-plots (Fig. 1). Trophic web length (TLW), maximum trophic position for each lake, carbon range (CR), percentage of littoral primary production fuelling food webs (% Cont-Lit), total area (TA), convex hull area encompassed by all species and mean nearest neighbour distance (MNN) values are shown. Median and range for each climate area are provided together with results of statistical analysis testing for differences between countries (Mann-Whitney non parametric tests). Lakes are ordered by decreasing FR.

Lake	FR*	TLW*	CR	CR 2	CR 3*	%CONT LITT	TA	NND*
Cisne	13	4.0	7.7	4.5	3.5	53.5	10.1	0.4
Diario	11	3.4	9.7	9.7	5.9	54.7	9.2	0.4
Garcia	11	3.9	9.9	9.4	7.6	64.5	15.5	0.6
Clotilde	9	4.1	8.5	7.7	5.1	50.7	16.0	0.6
Blanca	4	4.4	7	7	3.4	46.1	10.7	0.7
Uy median	11	4.0	8.5	7.7	5.1	53.5	10.7	0.6
Range	4-13	3.4-4.4	7.0-9.9	4.5-9.7	3.4-7.6	46.1-64.5	9.2-16.0	0.4-0.7
Vaeng	8	5.1	4.9	4.3	2.6	52.7	8.6	0.6
Tranevig	6	5.8	10.5	7.3	0.6	58.3	16.1	0.9
Gammelmose	4	4.6	4.6	4.4	3.0	98.2	10.2	1.2
Denderup	3	4.4	9.9	9.9	4.1	31.6	15.4	0.6
Dk median	5	4.8	7.4	5.9	2.8	55.5	12.8	0.8
Range	3-8	4.4-5.8	4.6-10.5	4.3-9.9	0.6-4.1	31.6-98.2	8.6-16.1	0.6-1.2
Z_{value}	13	4.0	7.7	4.5	3.5	53.5	10.1	0.4
P	11	3.4	9.7	9.7	5.9	54.7	9.2	0.4

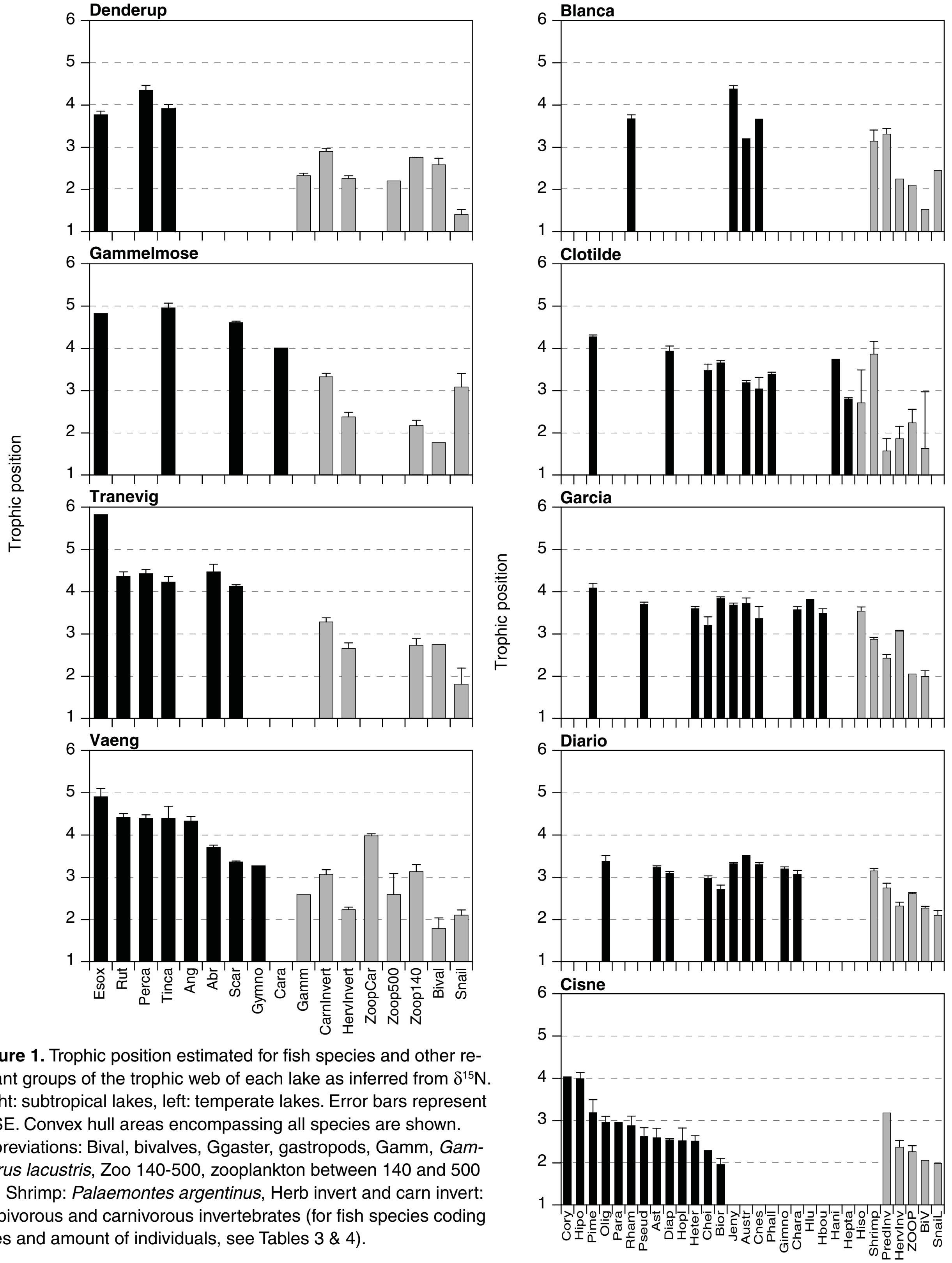

Figure 1. Trophic position estimated for fish species and other relevant groups of the trophic web of each lake as inferred from δ^{15}N. Right: subtropical lakes, left: temperate lakes. Error bars represent ±1SE. Convex hull areas encompassing all species are shown. Abbreviations: Bival, bivalves, Ggaster, gastropods, Gamm, *Gammarus lacustris*, Zoo 140-500, zooplankton between 140 and 500 µm, Shrimp: *Palaemontes argentinus*, Herb invert and carn invert: herbivorous and carnivorous invertebrates (for fish species coding sizes and amount of individuals, see Tables 3 & 4).

and *R. quelen* in Lake Blanca). Several relatively small-sized omnivorous species occurred, *Jenynsia multidentata* (Jenyns) and *Cnesterodon decemmaculatus* (Jenyns) being the most abundant (Table 3).

To our surprise, *H. malabaricus* (Bloch) never occupied the top of the subtropical food webs, but instead held the same trophic position as small-sized omnivores (Fig. 2). In contrast, *J. multidentata*, usually considered an omni-planktivore, exhibited high mean δ^{15}N values in all the systems

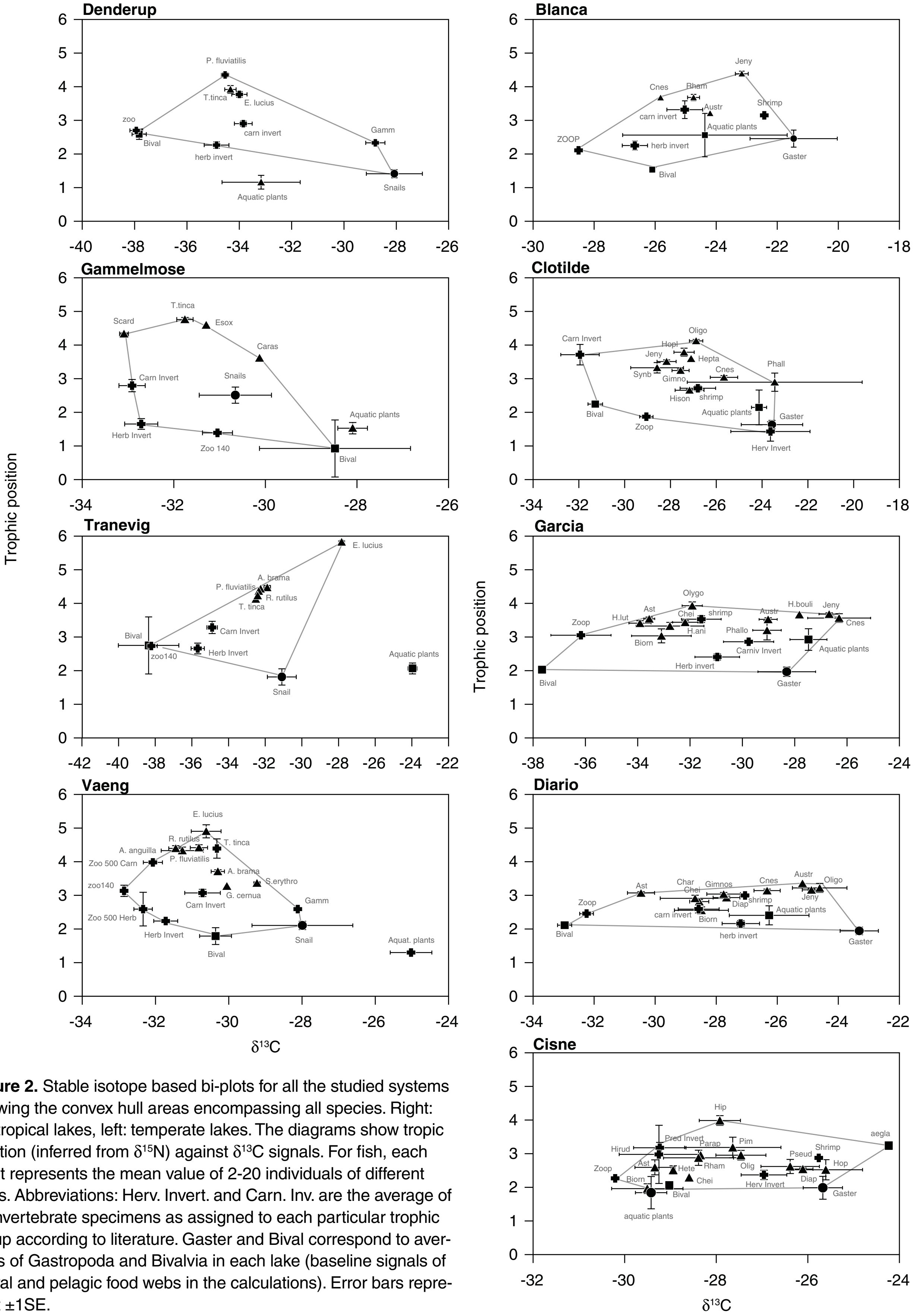

Figure 2. Stable isotope based bi-plots for all the studied systems showing the convex hull areas encompassing all species. Right: subtropical lakes, left: temperate lakes. The diagrams show tropic position (inferred from $\delta^{15}N$) against $\delta^{13}C$ signals. For fish, each point represents the mean value of 2-20 individuals of different sizes. Abbreviations: Herv. Invert. and Carn. Inv. are the average of all invertebrate specimens as assigned to each particular trophic group according to literature. Gaster and Bival correspond to averages of Gastropoda and Bivalvia in each lake (baseline signals of littoral and pelagic food webs in the calculations). Error bars represent ±1SE.

(Table 1). Shrimps appeared in all the lakes, and particularly *Palaemonetes argentinus* was present in four of them where it occupied the 3rd trophic position along with predatory macroinvertebrates and several omnivorous fish species. Remarkably, their $\delta^{13}C$ values indicated a %Cont-lit that

Table 3. Fish species found in each Uruguayan lake, indicating the amount (n), the minimum (min), maximum (max) and mean body lengths used for the stable isotopes analyses, and estimated trophic position (T_R-P_o).

	Cisne					Blanca					Clotilde					Diario					Garcia				
Species	**n**	**min**	**max**	**mean**	**T_R-P_O**	**n**	**min**	**max**	**mean**	**T_R-P_O**	**n**	**min**	**max**	**mean**	**T_R-P_O**	**n**	**min**	**max**	**mean**	**T_R-P_O**	**n**	**min**	**max**	**mean**	**T_R-P_O**
*Australoheros facetus**						1			2.6	3.34						1			3.1	3.69	10	2	11	5.8	3.62
*Hoplias malabaricus**	3	5	5.4	5.2	3.23						5	24	34	30.2	4.31										
*Oligosarcus jenynsii**	2	9.7	14.2	12	3.66						9	14.5	19	16.9	4.65	10	7	20.5	12.7	3.55	10	3.6	15	8.6	4.02
*Rhamdia quelen**	4	4.6	5.2	4	3.59	6	3	5	3.9	3.69															
*Synbranchus marmoratus**											6	7.5	34	24.3	3.85										
Characidium rachovii																6	2.2	3.5	2.7	3.24					
Corydoras paleatus	1			3.5	4.03																				
Astyanax sp.	8	3.5	8.6	5.8	3.30											3	1.9	2.2	2	3.4	10	1.6	7	3.5	3.63
Cheirodon interruptus	1	4			2.76											10	3.2	4.7	4	3.16	6	4	5	4.7	3.53
Cnesterodon decemmaculatus						1			1.8	3.79	10	1.5	3	2.4	3.57	10	1.9	3.5	2.6	3..47	3	1.7	3	2.5	3.66
Diapoma terofali	10	3	5.4	4.6	3.25											10	4.5	7.1	5.6	3.26					
Gymnogeophagus cf. meridionalis											15	1.8	6	3.7	3.78	8	1.5	6.5	3.4	3.37					
Hyphessobrycon anisitsi																					2	3.6	3.7	3.7	3.53
Hyphessobrycon boulengeri																					1			3.7	3.76
Heptapterus mustelinus											1			2.6	3.9										
Heterocheirodon yatai	2	3.8	4.3	4.1	3.22																				
Hyphessobrycon luetkeni																					8	2.6	7.3	5	3.51
Jenynsia multidentata						11	1.7	5.5	3.6	4.38	9	1.8	3.9	3.6	4.06	10	1.8	3.7	2.8	3.49	10	2	6.9	4.3	3.78
Phalloceros caudimaculatus											2	1.8	2.5	2.2	3.42						3	1.5	2.7	2.2	3.21
Pimelodella australis	2	3.5	4.5	4	3.89																				
Pseudocorynopoma oriae	6	3.2	5.8	4.5	3.29																				
Steindachnerina biornata	10	4.2	17	9.9	2.67											9	10.5	14	11.7	2.55	6	2.5	16.5	10.1	3.13
Hipostomus commersonii	2	4	4.2	4.1	4.69																				
Hisonotus sp.											5	4	6.5	5.2	3.2										
Parapimelodus valenciennis	1			15.1	2.95																				

exceeding 60% in all the lakes, even reaching 80% in Lake Blanca, indicating a strong important reliance on periphytic primary production.

Temperate fish assemblages were more species poor than those in the subtropical lakes (Table 4), but the same trophic groups were found. Potential piscivores were abundant and included pike *(E. lucius L.)*, perch (*Perca*

Table 4. Fish species found in each Danish lake, indicating amount (n), minimum (min), maximum (max) and mean body lengths used for the stable isotope analyses, and estimated trophic position (T_R-P_o).

Species	**Denderup**					**Vaeng**					**Gammelmose**					**Tranevig**				
	n	**min**	**max**	**mean**	**T_R-P_O**	**n**	**min**	**max**	**mean**	**T_R-P_O**	**n**	**min**	**max**	**mean**	**T_R-P_O**	**n**	**min**	**max**	**mean**	**T_R-P_O**
Abramis brama						28	4.1	35.1	9.2	3.68						11	8.5	44	24.2	4.47
Esox lucius	4	8.5	17	14.4	3.37	7	17	89.9	33.8	4.87	1			38	4.59	1			50.8	5.82
Perca fluviatilis	32	6.5	31	16.2	3.95	43	5.9	24.3	12.7	4.36						17	5.2	21.1	13.5	4.35
Rutilus rutilus						60	2.4	25.6	15	4.38						12	6.9	28.5	14.7	4.34
Scardinius erythropthalmus						31	3.9	17.9	9	3.33	6	8.5	27	12.9	4.33	21	2	23.5	13.5	4.13
Carassius carassius											1			15	3.61					
Gymnocephalus cernua						1			7	3.26						4	21.8	41.8	34.6	4.23
Tinca tinca	7	35	53.5	45.4	3.52	2	37	45.1	41	4.36	8	39	54	45.4	4.76					
Anguilla anguilla						9	29	57.3	44.3	4.3										

fluviatilis L.) and eel (*Anguilla anguilla L.*) (Table 4). Pike was the apical species in two out of the four lakes with values close to the 6th trophic position, and in none of the lakes it was lower than the 4th. In the other two lakes, either only one individual (38 cm of total length) was caught (Lake Gammelmose) or pike mainly consisted of juveniles (TL ranged from 8.5 to 16.5 cm of total length). Remarkably, both perch and tench (*Tinca tinca*) exceeded pike in trophic position in Lake Denderup, likely reflecting the overall small size of pike in this lake (Table 4). Several fish species showed high trophic positions (around 4) which were only occasionally observed in the subtropical lakes.

The overall mean $\delta^{13}C$ carbon range of the systems (CR) was slightly (though not significantly) greater in the warmer lakes, being 8.7 in subtropical and 7.5 in the temperate lakes (Table 2). The CR range per distinct trophic level did not differ significantly between countries at trophic position=2, but at 3 the CR was twice as wide in the subtropical lakes (U-test p= 0.05; Table 2).

Temperate trophic webs typically had a triangular shape as indicated by the convex hull area (Fig. 2). Contrarily, the subtropical webs were trapezoid-shaped (Fig. 2), being shorter and wider at the 3rd trophic position (Fig. 3). However, TA did not differ between climate zones and the NND values measured were smaller in the subtropical species (see also Fig. 2).

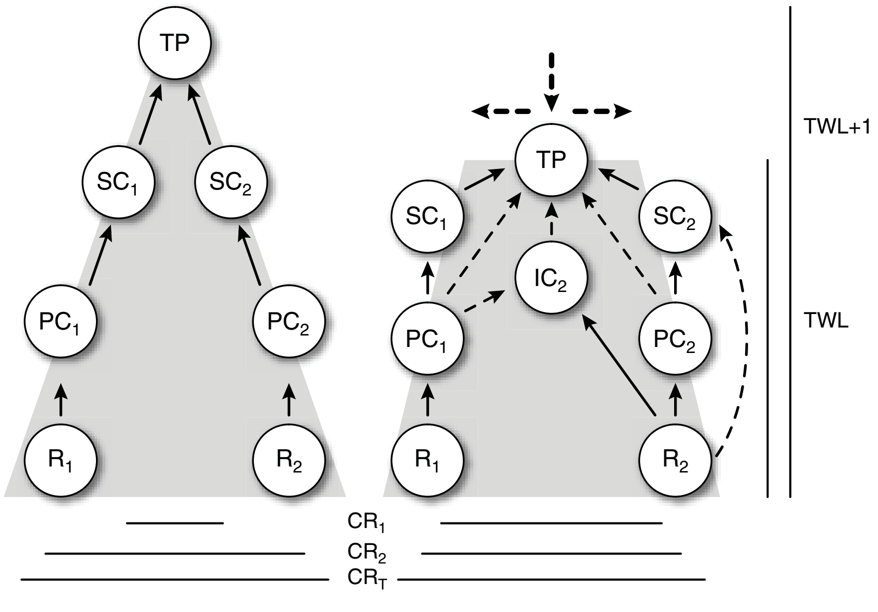

Figure 3. Conceptual models of trophic web functioning in subtropical and temperate lakes inferred from $\delta^{13}C$-$\delta^{15}N$ bi-plot and community-wide metrics. Abbreviations: R_1 and R_2 phytoplankton and periphyton, respectively. The arrows above the model indicate the lowering of one trophic position occurring concomitantly with a widening of the carbon range that reaches the 3rd level of the chain. Used Abbreviations: PC, Primary consumers. IC, Intermediate consumers. SC, Secondary consumers. TP, top predators. CR_T, total carbon range, CR_1 and CR_2 carbon range that reaches the respective trophic positions. TWL, trophic web length.

Discussion

Supporting our hypothesis, we found that the trophic webs in subtropical shallow lakes were shorter than their temperate counterparts. Notwithstanding that large-sized fish species, usually classified as piscivores or omni-benthi-piscivores (Teixeira de Mello et al., 2009; Gelós et al., 2010), were present, their isotopic values indicated that their position in the trophic web is similar to that of much smaller fish species, and in some cases even to predatory macroinvertebrates. Our results strongly suggest that these subtropical fish species are actually omnivores, predating on fish, but also feed at one or more trophic levels down the trophic web (Fig. 3). A similar pattern has been observed in previous works relying, however, mainly on stomach content data (Lazzaro, 1997; Teixeira de Mello et al., 2009; Jeppesen et al., 2010). Our data concur with recent evidence also using stable isotopes analyses (Lacerot et al., submitted). In contrast to the predatory fish species in the temperate lakes that achieved high trophic positions (principally pike and perch), it seems unlikely that any of these subtropical predatory fish could be considered strictly piscivorous. Remarkably, the highest trophic position in the subtropical lakes was occupied by a variety of species that, based on stomach content data, are often classified as mostly non-predatory (see Teixeira de Mello et al., 2009, for such classifications). In particular, the small-sized J. multidentata, an omnivore-planktivorous species that seldom feeds on fish (Iglesias et al., 2008), exhibited one of the highest trophic position in our set of subtropical lakes. Shrimps, often described in the literature as zooplankton predators (Collins & Paggi, 1998; González-Sagrario et al., 2009), in most cases appeared as second level consumers and strongly relied on periphyton carbon sources, as could be expected from their usual association to vegetated littoral areas (Iglesias et al., 2007; Meerhoff et al., 2007b; Teixeira de Mello et al., 2009).

Different carbon sources (phytoplankton and periphyton) were seemingly exploited to a similar extent (i.e. similar CR) in both climate zones. Together with the absence of differences in the littoral contribution (% Cont-Lit), this finding suggests that at both locations food webs are fuelled by a wide range of carbon sources. Reliance on periphyton as a carbon source has been previously observed in temperate lakes (Vander Zanden & Vadeboncoeur, 2002; Jones & Waldron, 2003), stressing the importance of fish mobility for integrating the habitats (pelagic and littoral) (Schindler & Scheuerell, 2002). This observation does not support our a priori expectation of subtropical food webs being more fuelled by periphyton than temperate food webs, a hypothesis based on the very strong association of subtropical fish to vegetated areas and the comparatively lower biomass of periphyton in such habitats, as compared with similar temperate shallow lakes (Meerhoff et al., 2007a). However, even if the contribution of periphyton to the food web is similar in the two climates, the connection between fish and periphyton was indeed closer in the subtropics. The estimated metrics (CR2 & 3) suggest that the width of carbon range that reaches the third trophic positions in temperate lakes is shorter compared to the range at the base of the trophic web, and especially when compared with the observed patterns in subtropical systems. We speculate that this is a reflection of widespread omnivory and higher fish diversity in the latter, leading to a lower degree of mixing of carbon sources (i.e. maintenance of more pathways), contrasting with the greater mixing of carbon sources observed in the temperate lakes at the second and third trophic positions. We observed an important difference in the shape of the food webs (shown by the convex hull shapes and community-wide metrics), also suggesting that the energy pathways may be different between climate zones. Adjusted convex hull areas were typically triangular in the temperate lakes, with a single predator species (apical species) at the top (most often pike), higher maximum trophic position and several steps (one extra than in the subtropical lakes) of energy transfer. These shapes resembled the typical chain-like trophic architecture that often characterizes pelagic food chains (Sprules & Bowerman, 1988).

These differences in trophic web architecture may have profound effects on ecosystem functioning (Post & Takimoto, 2007). Our analysis of the architecture of the trophic webs in temperate and subtropical lakes has confirmed some patterns previously hypothesized based on composition and abundances of different trophic groups (Meerhoff et al., 2007a; Jeppesen et al., 2010). According to our results, temperate systems can be depicted by a multi-chain omnivory model (Vadeboncoeur et al., 2005), with a top predator integrating different carbon sources that fuel the web (mainly represented by phytoplankton and periphyton and showing a mixed $\delta^{13}C$ value). In subtropical lakes, a combination of simple-chain omnivory and multi-chain omnivory models may better explain the community metrics and the shapes observed there (particularly shortening the TWL and exhibiting a wider CR at the third trophic positions). Our findings support previous suggestions (Meerhoff et al., 2007a) that subtropical trophic webs were indeed shorter than the temperate ones due to the omnivory of fish across the food web, including the top position. Intermediate consumers make a very large contribution to subtropical food webs, acting as intermediate integrators of the different carbon sources (Post & Takimoto, 2007) and enhancing the transfer of the basal carbon to the next trophic positions.

Apart from typically higher densities and species richness compared to similar temperate lakes (Teixeira de Mello et al., 2009), subtropical fish assemblages thus exhibit a strong degree of niche or feeding overlap (indicated by the smaller NND metrics than in temperate food webs, Table 2). Subtropical food webs therefore reflect a higher degree of omnivory by the top predator (“omnivory mechanism”, Post & Takimoto, 2007) and also the presence of redundant fish species (“addition model”, Post & Takimoto, 2007). This evidence accords with previous suggestions based on stomach content analyses that omnivory is a general characteristic of subtropical fish assemblages (Teixeira de Mello et al., 2009; Jeppesen et al., 2010; González-Bergonzoni et al., in prep.), determining a high redundancy and the absence of a so-called keystone species. Richer and redundant fish assemblages may result in a constant predation pressure over zooplankton, explaining the weak link between zooplankton and phytoplankton in warmer lakes observed in field studies (Kruk et al., 2009; Gelós et al., 2010), but contrasting findings in experimental, simple web-based studies (Iglesias et al., 2008; Mazzeo et al., 2010; Iglesias et al., submitted).

Strong evidence of how these differences affect ecosystem functioning is emerging and suggests that the theoretical framework and experience from temperate lakes cannot fully explain the functioning of subtropical lakes and therefore cannot be directly applied to subtropical shallow lakes. Previous findings (Meerhoff et al., 2007a; Jeppesen et al., 2007), together with our results (i.e. greater degree of niche overlap, shortening of the TWL due to lack of a true piscivore at the top of the chain, and a close coupling between littoral and pelagic habitats), strongly support the hypothesis that the complexity of trophic interactions in warmer systems may, in part, counteract some of the positive feedbacks between trophic length and ecosystem functioning, typically observed in temperate lakes (Scheffer et al., 1993). At the same time, the stronger complexity may decrease the stability and resilience of the clear water state in shallow subtropical lakes, as well as in lakes experiencing a warming process, as suggested from comparative analyses of lakes data from different climate regions (Moss et al., 2004; Meerhoff et al., 2007a; Kosten et al., 2009).

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CASCADING EFFECTS OF PREDATORS IN TEMPERATE AND SUBTROPICAL SHALLOW LAKES

With a climate warming perspective, the present thesis aimed to compare the functioning of shallow lakes in contrasting climate zones by conducting a series of field mesocosm experiments and field surveys of the food web structure and trophic interactions in lakes located in subtropical Uruguay and temperate Denmark. The structuring role of fish was proven experimentally in both climate regions, with strong cascading effects on the pelagic food webs and particularly so in the subtropics. Together with higher fish diversity, trophic webs in warmer lakes were generally one trophic level shorter than their temperate counterparts. We argue that the widespread omnivory of fish across the food web explains the shorter food webs and the weakness of cascading effects, seldom reaching phytoplankton, in real lakes in the subtropics, thus having profound effects on ecosystem functioning. The complexity of trophic interactions in warmer systems may therefore weaken important positive feedback mechanisms, known from temperate lakes, and thereby decrease the stability and resilience of the clear water state. The evidence from subtropical lakes may, with caution, provide indications of the responses to be expected with warming in currently cold ecosystems.

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