Effects of climate on size structure and functioning of aquatic food webs

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Thesis

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Gissell
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Table of contents

Cover image: The Golden Fish by Paul Klee (1925) Kunsthalle, Hamburg

Summary

In aquatic food webs, the role of body size is notoriously strong. It is also well known that temperature has an effect on body size. For instance, Bergmann's rule (1847) states that body size increases from warm to cold climates. This thesis addresses the question how climate shapes the size structure of fish and zooplankton communities, and how this affects the strength of the trophic cascade from fish to plankton. I combine three different approaches: a space-for-time substitution study of data from the 83 shallow lakes distributed along a latitudinal gradient in South America, simple mathematical models to explore climate effects on the dynamics of trophic interactions, and an experimental analysis of trophic interactions using outdoor mesocosms.

First, in chapter 2, I analyze how the size structure of fish communities changes with latitude and show that lakes along this climatic gradient tend to be dominated by either small or large fish. Moreover, I show that this pattern suggests a catastrophe fold in the body size structure of fish communities, with higher probabilities of a small fish-dominated state at warmer climates. This suggests that factors that tend to reduce fish body size such as fishing for the largest individuals could change a large-fish dominated state into a small-fish dominated state more easily in warmer climates. In chapter 3 I present an analysis of a smaller set of lakes, in which we estimated the trophic position of fish using stable isotopes (^{15}N). This revealed that while in cold lakes large fish forage higher in the food web than small fish, in tropical lakes large fish forage at equal or lower trophic levels than small fish. This pattern suggests that top-down control of small fish by large fish may be weaker towards warmer climates.

Chapter 4 shows that, just as in fish, the body size of zooplankton decreases from higher to lower latitudes. This pattern is stronger for Cladocera and Calanoid copepods than for smaller organisms such as rotifers. Also, the size gradient is strong for pelagic species but not for macrophyte-associated species. These patterns suggest that fish predation (to which larger pelagic species are particularly sensitive) may be an important driver of the patterns. Indeed, differences in pelagic cladoceran body length were correlated to the proportion of small fish in the lakes. In chapter 5 we used a model to analyze the potential implications for zooplankton of two characteristics of fish in warm regions: continuous reproduction and omnivory. Continuous reproduction leads to permanent presence of small fish, while omnivory boost their overall abundance. Our results indicate that while both aspects lead to reduced chances of top-down control of phytoplankton, continuous fish reproduction by itself may in principle lead to the reduction of large-sized *Daphnia* populations and reduce chances of top-down control of phytoplankton. This suggests that in temperate shallow lakes, seasonality in fish reproduction might be essential to leave a window of opportunity for the zooplankton in early spring resulting in a clear-water phase in which zooplankton drives

phytoplankton to very low densities. In chapter 6, we experimentally analyze the ability of the zooplankton typically found in subtropical lakes to control phytoplankton, under different levels of fish predation. Experimentally altered zooplankton densities did not affect phytoplankton densities, indicating that the present zooplankton community had little top-down effect at all on the phytoplankton communities we tested. The experiments also revealed that very low fish densities were enough to eliminate large zooplankton from the communities.

In Chapter 7 I link the results presented in the different chapters. I argue that top-down control of phytoplankton tends to be weaker in warmer climates due mostly to a cascading effect of the temperature-driven increase in small fish densities leading to a lack of larger zooplankton in warm lakes. However, I also point out a range of questions that would need to be addressed if we wish to understand the causality behind the patterns better.

Samenvatting

In aquatische voedselwebben speelt de lichaamsgrootte van de organismen een belangrijke rol in de stroom van energie en materiaal. Vele factoren zijn van invloed op de lichaamsgrootte, waarvan vooral temperatuur een oude bekende is. Volgens de stelling van Bergmann (1847) neemt de lichaamsgrootte af naarmate het klimaat warmer wordt. In deze dissertatie wordt onderzocht hoe het klimaat de grootte verdeling van vis- en zoöplanktongemeenschappen vormt en wat de impact is op de trofische cascade van vis naar plankton. Hiervoor zijn drie verschillende benaderingen gekozen:

1) Een 'ruimte-voor-tijd'-substitutiestudie van ondiepe meren verdeeld over een latitudinaal transect in Zuid-Amerika.
2) Eenvoudige mathematische modellen om klimaatseffecten op trofische interacties te exploreren.
3) Een mesocosm experiment waarin trofische interacties onderzocht zijn.

In Hoofdstuk 2 wordt de grootteverdeling van visgemeenschappen langs het latitudinaal transect in Zuid-Amerika geanalyseerd. Uit de analyse volgt dat de ondiepe meren over deze klimaatgradiënt of gedomineerd worden door kleine vissen, of door grote vissen. Het patroon levert een sterke aanwijzing voor alternatieve evenwichten in de lichaamsgrootteopbouw van visgemeenschappen: een situatie gedomineerd door kleine vissen en een situatie gedomineerd door grote vissen. De kans dat kleine vissen domineren neemt toe met de temperatuur. Een consequentie hiervan is factoren die de visgrootte reduceren, zoals het vissen op de grootste exemplaren, een systeem makkelijker doen omslaan naar een door kleine vissen gedomineerd systeem in een warm klimaat dan een kouder klimaat.

In Hoofdstuk 3 worden de resultaten gepresenteerd van een deel van de ondiepe meren uit de latitudinale transect, waarin de trofische positie van de aanwezige vissen is bepaald met behulp van stabiele isotopen (^{15}N). Hieruit kwam naar voren dat grotere vissen in koudere meren hoger in het voedselweb forageren dan kleinere vissen, terwijl in warmere meren de grotere vissen of op het gelijke niveau, of zelfs lager in het voedselweb forageerden dan de kleine vissen. Dit betekent dat de top-down controle van kleine vis door grote vis waarschijnlijk zwakker is in een warmer klimaat dan in een kouder klimaat.

Hoofdstuk 4 laat zien dat, net als bij vissen, de lichaamsgrootte van zoöplankton ook afneemt van een hogere naar een lagere breedtegraad. Het patroon is sterker voor Cladocera en Calanoïde roeipootkreeftjes dan voor kleiner zooplankton, zoals raderdiertjes. Verder is de groottegradiënt duidelijk voor pelagische soorten, maar niet voor waterplant geassocieerde watervlooien. Deze observaties doen sterk vermoeden dat vispredatie, waarvoor met name de grotere pelagische soorten kwetsbaar zijn, een belangrijke sturende factor is. Deze

veronderstelling wordt ondersteund door het feit de verschillen in lichaamsgrootte van pelagische Cladocera correleren met het aandeel kleine vissen in de meren.

In Hoofdstuk 5 wordt verder ingegaan op de mogelijk gevolgen voor zoöplankton van twee karakteristieke eigenschappen van vissen in een warmer klimaat: continue reproductie en omnivorie. Daar waar een continue reproductie resulteert in een permanente presentie van kleine vissen, leidt omnivorie tot een hogere abundantie. Onze model-resultaten tonen aan dat weliswaar beide eigenschappen in een lagere top-down controle van fytoplankton resulteren, maar dat de continue reproductie alleen al voldoende is om een reductie van populaties van grote *Daphnia* (een belangrijke grazer op fytoplankton) te bewerkstelligen en daarmee de kans op top-down controle van fytoplankton drastisch te reduceren. Dit betekent dat in ondiepe meren in gematigde streken de seizoensdynamiek in visreproductie een kans biedt aan zoöplankton om in het voorjaar het fytoplankton tot zeer lage hoeveelheden te reduceren; een fenomeen dat bekent staat als de "clear-water-phase".

In Hoofdstuk 6 wordt experimenteel onderzocht of het zoöplankton wat karakteristiek is voor subtropische meren in staat is om het aanwezige fytoplankton effectief te begrazen. Hiertoe zijn zowel de vispredatie als de zoöplanktonconcentraties experimenteel gemanipuleerd. Echter veranderde zoöplanktonconcentraties hadden geen effect op verschillende fytoplanktongemeenschappen wat de geringe impact van subtropisch zoöplankton op het fytoplankton onderstreept. De experimenten lieten zien dat een lage visdichtheid al voldoende is om het grotere zoöplankton te elimineren.

De resultaten uit de verschillende hoofdstukken worden in Hoofdstuk 7 bijeen genomen en gekoppeld. Een belangrijke conclusie is dat de top-down controle van fytoplankton in een warmer klimaat zwakker is dan het goed beschreven effect in een kouder klimaat in gematigde streken, doordat in warmere meren groot zoöplankton ontbreekt als gevolg van een temperatuur gedreven dominantie van kleine vissen. Tot slot worden in dit hoofdstuk een aantal vragen geformuleerd voor verder onderzoek naar de causaliteit achter de in deze dissertatie gevonden patronen.

1. Introduction

> *"A journey is a person in itself, no two are alike, and all plans, safeguards, policies and coercion are*
> *fruitless. We find after years of struggles that we do not take a trip, a trip takes us"*
> *John Steinbeck*

Body size determines the scale at which organisms interact with their physical world (Hilldrew *et al.*, 2007). It is therefore important across all levels of organisation, from the individual to the ecosystem (Peters, 1983; Hilldrew *et al.*, 2007; Woodward *et al.*, 2005; Gaston & Blackburn, 2000). At the organism level body size influences metabolic requirements (Elton, 1927; Brown *et al.*, 2004), food choices and rates of consumption (Brose *et al.*, 2006; Brooks & Dodson, 1965; Mittelbach, 1981), nutrient recycling (Hall *et al.*, 2007) and susceptibility to predation (Brooks & Dodson, 1965). At the level of communities and food webs, body size distributions affect the strength of trophic interactions (Emmerson & Raffaelli, 2004) and are correlated with patterns of diversity and abundance (Fenchel & Finlay, 2004; Cohen *et al.*, 2003; Hillebrand & Azovsky, 2001).

In aquatic food webs, the role of body size is notoriously strong. Classical studies like the work of Brooks & Dodson (1965) show the importance of body size and size-selective predation in structuring plankton communities. Moreover, trophic cascades, which are well documented in aquatic ecosystems, are largely based on hierarchical body size differences (but see Jones & Jeppessen, 2007 for an analysis of this topic).

It is well known that temperature has an effect on body size. Already in Darwin's days Bergmann formulated his famous rule (Bergmann, 1847) stating that small-sized species dominate in warm climates while large species are more common at colder regions. Indeed this has been well documented in later studies (Blackburn *et al.*, 1999). The explanation of this pattern is not completely resolved, although various mechanisms have been suggested to play a role. Importantly, in the case of ectotherms, higher maturation rates with increasing temperature produces smaller adult body sizes (Atkinson, 1994; Atkinson & Sibly, 1997). In the face of climate change, there is renewed interest in the question how food web structure and function might be impacted by an increase in temperature.

This thesis addresses the question how climate affects food webs and trophic interactions in shallow lakes. While this is a broad and complex issue, I will focus on the question how climate shapes the size structure of fish and zooplankton communities, and how this affects the strength of the trophic cascade from fish to plankton.

I combine three different approaches: a space-for-time substitution study of data from the 83 shallow lakes distributed along a latitudinal gradient in South America, simple mathematical models to explore climate effects on the dynamics of trophic interactions, and an experimental analysis of trophic interactions using outdoor mesocosms.

Central to this thesis are the latitudinal data obtained in the framework of the SALGA project (South America Lake Gradient Analysis). This project is a joint effort of scientists from several South American and European countries (Argentina, Belgium, Brazil, Denmark, Netherlands and Uruguay) with the aim of understanding what is the effect of climate on the structure and functioning of shallow lakes. We studied 83 lakes along a latitudinal gradient from 5°S to 55°S along the east coast of South America (Figure 1.1). All lakes were located at less than 500 m of altitude and had a similar size and depth. We chose lakes within each climatic region that varied in nutrient level. Average air temperatures for the lakes ranged from 27.6°C in Rio Grande do Norte (Brazil) to 4°C for tundra lakes in Tierra del Fuego (Argentina) (New *et al.*, 2002). All lakes were sampled once during summer or during the dry season (in the tropics), between November 2004 and March 2006. The same team, using the same set of equipment, and following one protocol, sampled all lakes. In each lake we measured the main physical and chemical variables both in the water column and in the sediment, and sampled the communities of bacteria, protozoa, phytoplankton, zooplankton, fish and macrophytes. While in this thesis I report on findings with respect to the structure of zooplankton and fish communities, additional results from the SALGA project are reported in two other PhD theses: one focusing on the question how climate might affect the probability of lakes to be dominated by algae or submerged macrophytes and another thesis analyzing the relationship between climate and the phytoplankton community.
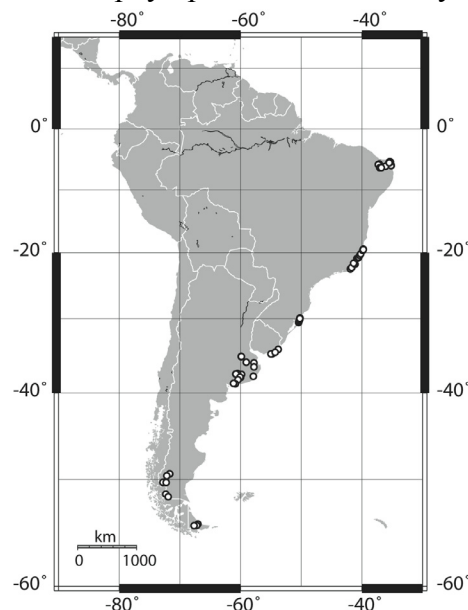

Figure 1.1 Distribution of the 83 lakes sampled for the SALGA project across South America.

1.1 Outline of the thesis

The first two chapters after the introduction (Chapters 2 and 3) deal with size patterns in the fish community revealed by analysis of our field data along the climatic gradient. First (chapter 2), I analyze how the size structure of fish communities changes with latitude. A minimal model is used to illustrate how underlying feedback mechanism might cause the particular pattern we find.

Chapter 3 contains an analysis of field data of a smaller set of lakes distributed along the climatic gradient, in which we estimated the trophic position of differently sized fish using stable isotopes (^{15}N). Results indicate that the classical hierarchical size distribution of fish communities disappears in warmer climates.

Chapters 4, 5 and 6 focus on the zooplankton community size structure, and on the role of zooplankton as both predator and prey in food webs. Chapter 4 shows that the average body size of zooplankton decreases from cold to warm regions, and shows how this pattern is related to fish size structure and other variables. We also highlight how the latitudinal size pattern differs between zooplankton groups. In chapter 5 we use a model to analyze the potential implications for zooplankton of two often-cited characteristics of fish in warm regions: continuous reproduction and omnivory, assuming that multiple reproductive events in a year would lead to permanent presence of small fish, while omnivory would boost their abundance.

Chapter 6 contains the results of experiments designed to analyze the ability of the zooplankton typically found in subtropical lakes to control phytoplankton, under different levels of fish predation. Finally, in chapter 7 I summarize the main conclusions and offer some perspectives for future work.

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2. Dominance by the small as an alternative stable state in fish communities

Gissell Lacerot, Egbert H. van Nes, Sarian Kosten, Erik Jeppesen, Néstor Mazzeo, Cajo J.F. ter Braak, Franco Teixeira de Mello, Christina C. Branco & Marten Scheffer

2.1 Summary

The collapse of Newfoundland Atlantic cod (Myers *et al.*, 1997) and the shift from Cichlids to Nile Perch in Lake Victoria (Goudswaard *et al.*, 2008; Kolding *et al.*, 2008) illustrate that fish communities may occasionally go through a radical transition between a persistent state dominated by large predatory fish, and one dominated by small fish. Models suggest that these states may represent alternative attractors (Claesen & De Roos, 2003; De Roos & Persson, 2002; De Roos *et al.*, 2003) but empirical evidence for this view is scarce. We analyzed the size structure of fish communities in 82 lakes along a climatic gradient in South America, and show statistically that lakes tend to be dominated by either small or large fish, with the probability of the latter decreasing in warmer climates. Our analysis suggests that alternative body size attractors in fish communities may be widespread. An implication is that pressures such as fishing or eutrophication may induce critical transitions between big fish and small fish dominated states. In addition our results show that the chances of switching to a small fish dominated state will be higher in a warmer climate.

2.2 Introduction

The lack of recovery of Newfoundland cod decades after the closure of fisheries has raised the question whether fishing may push communities to an alternative stable state that represents an irreversible trap. Indeed, models suggest plausible mechanisms that might keep collapsed stocks of large predatory fish from recovering. The idea is that such large predators promote conditions under which their populations thrive but loose that capacity if their stocks fall below certain critical levels. There are a few different mechanisms proposed for this positive feedback. One specific mechanism is that size selective predation by large fish indirectly promotes the production of suitably small sized prey (De Roos & Persson, 2002; De Roos *et al.*, 2003). Another possibility is that a sufficient stock of large adults of such piscivores is needed to control smaller species that prey on their eggs, larvae of juveniles or compete with their offspring for food (Walters & Kitchell, 2001; Carpenter *et al.*, 2007). Either way, loss of the large predators is hypothesized to lead to an excessive abundance of smaller species that block predator recovery.

If true, a reduction of the dominant small fish populations should be a way to invoke a reverse shift back to a large predator dominated system. Indeed, in a Swedish lake experimental reduction of a prey fish population has been shown to allow recovery of stable predator dominance (Persson *et al.*, 2007). On a larger scale, the abrupt shift to Nile perch dominance in Lake Victoria has been attributed to a similar mechanism (Kolding *et al.*, 2008). Nile perch remained sparse upon its introduction in the 1960's but sharply expanded to dominate the lake more about three decades later (Kolding *et al.*, 2008) (Figure 2.1). It is thought that cichlids controlled Nile perch for decades, until fisheries and eutrophication suppressed cichlids enough to allow Nile perch to escape from control by predators (Kolding *et al.*, 2008).
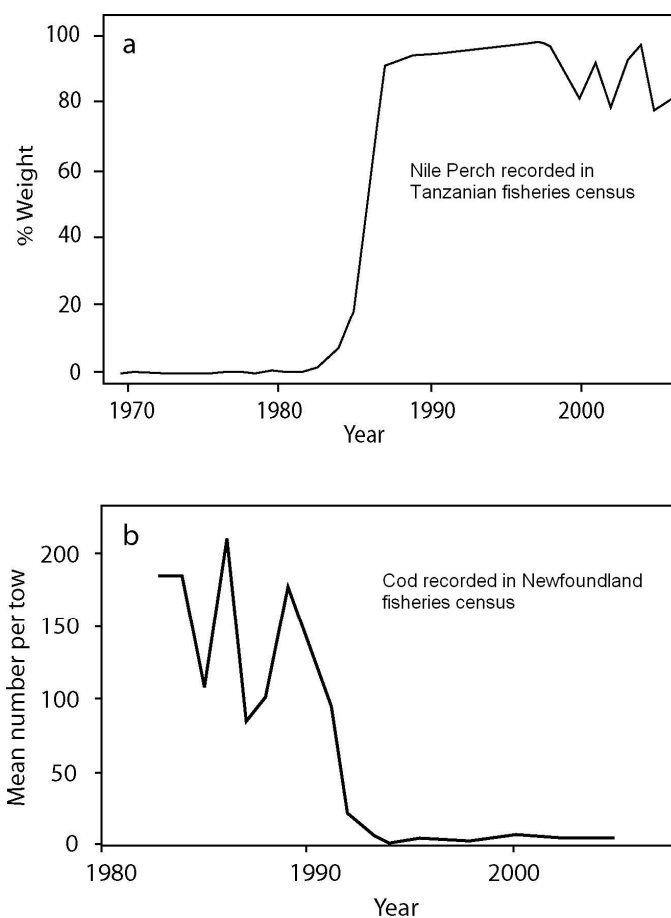

Figure 2.1 Two examples of abrupt transitions in fish communities. Panel a, the shift to dominance by Nile Perch in Lake Victoria reflected in Tanzanian survey data (Redrawn from Kolding *et al.*, 2008). Panel b, the collapse of northern Newfoundland cod (NAFO divisions 2J3KL) recorded in Canadian research vessel surveys (Redrawn from Stares *et al.*, 2007).

Although no formal proofs, these examples of shifts between large predator dominance and small fish dominance fit with the hypothesis that those states can represent alternative

attractors. However, there is no evidence for the idea that this might indeed be a widespread property of fish communities so far. Two key predictions from the theory can be tested provided that we have consistent data from a large set of distinct communities (Scheffer & Carpenter, 2003). Firstly, the size structure of the community plotted against a conditioning factor known to affect the system should reflect a catastrophe fold. This implies in practise that the effect of the conditioning factor is better described by two separate relationships than by one. Secondly, the probability of being in a small fish dominated state should decrease towards a critical threshold point in the catastrophe fold where the small fish dominated state ceases to exist. This pattern is an implication of the inevitable shrinking of the basin of attraction around the small fish dominated state towards the critical threshold.

Here we report on results of a massive effort to characterize size structure of fish communities in a series of lakes to test this prediction. As a conditioning factor we chose latitude, as it is known that warm conditions tend to favour small fish over large fish in lakes (Jeppesen *et al.*, 2007; Texeira de Mello *et al.*, 2009). We systematically sampled 82 lakes that were similar in size and depth (Kosten *et al.*, 2009) (see Table S2.1 in supplementary section 2.6), across a latitudinal gradient from the tropics to the tundra along the east coast of South America (see methods section 2.4). Since fishing effort cannot be reliably kept constant across lakes we analyzed the proportional composition, reflected by the fraction of small fish (body length < 10 cm).

2.3 Results and Discussion

To explore evidence for an underlying catastrophe fold, we tested whether the relationship between the percentage of small fish and latitude is better described by one or by two regression lines, using latent class regression, a finite mixture model approach (see methods section 2.6). This method is designed to find homogeneous classes in observations with a mixture of different classes without prior knowledge or assumptions about the class membership of each observation. It appears that the likelihood corrected for the number of parameters (BIC, Bayesian Information Criterion) was better for a mixture of two regression lines than for a single line (or a mixture or 3-5 lines) (see Table S2.2 in supplementary section 2.6). We also tested whether the latent-class membership of the observations is related to a "concomitant variable" (Dayton & Macready, 1988), in this case latitude. Indeed if we simultaneously fit a concomitant variable model, the fit improves further (Figure 2.2a). Moreover this analysis shows that the probability of being small fish dominated increases towards the tropics (Figure 2.2b). We obtained similar results when we excluded lakes with exotics species from the analysis (see 2.6.2 in supplementary section 2.6).

To check how the fitted lines relate to what might theoretically be expected we constructed a minimal model capturing the essence of the mechanism of the interaction between a population of large fish (*L*) and a logistically growing population of small fish (*S*).

$$\frac{dS}{dt} = r_S S\left(1 - \frac{S}{K}\right) - gS\frac{L}{L+h} - f_S S$$

$$\frac{dL}{dt} = egS\frac{L}{L+h}e^{-\alpha S} - (f_L + m_L)L \tag{1}$$

This model is based on the classical model for a predator foraging on a logistically growing prey (Rosenweig & MacArthur, 1963). We assumed that the large fish are predators with a sigmoidal functional response for their consumption of small fish. We further assume the negative effect of small fish on the growths of large fish to be an exponential term (HilleRisLambers, *in press*) (exp(-α *L*)) in which α is a parameter indicating the strength of the competition between young fish. Both small fish and big fish have a mortality term representing the effect of fisheries (resp, f_L and f_S). The model was rescaled to reduce the number of parameters (see method section 2.4). Depending on the parameter settings the model does have alternative attractors. As temperature has a negative effect on the average body size of ectotherms related most likely to a relatively steep increase of energy demands with temperature (Atkinson, 1994), we explored the potential effects of rising temperature on the interaction by plotting the equilibria against the rescaled overall loss rate $(f_L+m_L)/r_S$ of large fish (Figure 2.3 and Table S2.3 in supplementary section 2.6). For comparability we depict the result in terms of the percentage of small fish.

It can be seen that the lines fitted to the data are well in line with the prediction by the minimal model, suggesting that the character of the underlying catastrophe fold is consistent with what might be expected from the hypothesized mechanisms. Obviously, the minimal model we used is a rather strong oversimplification and clearly the prediction that there are no large fish at all in the small fish dominated state is not realistic. More detailed models, describing the various specific mechanisms suggested in different studies to cause the negative effect of small fish on large fish might well give somewhat different predictions.
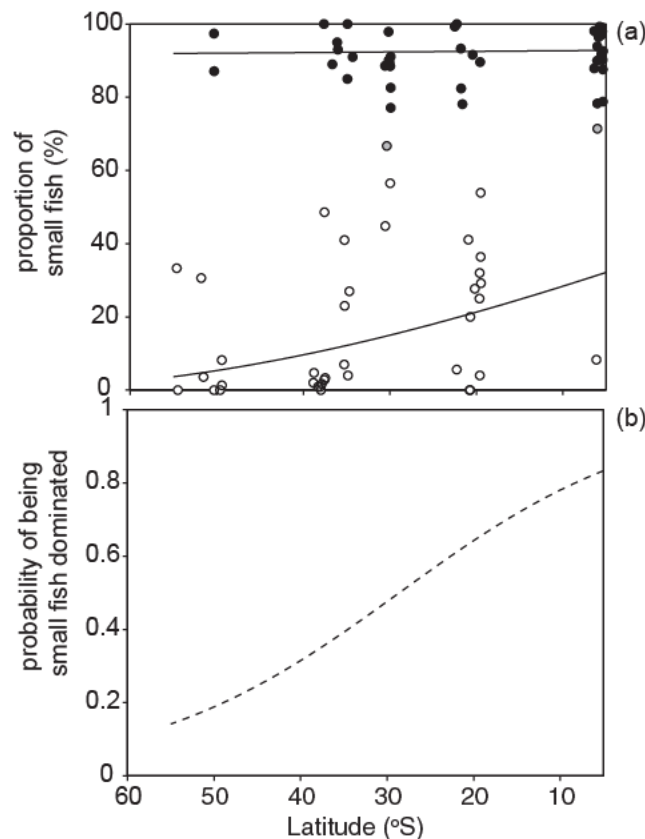
Figure 2.2 The best model (based on BIC) describing the relation between latitude and the proportion of small fish with posterior class membership of the observations (black circles: small fish dominated, white circles: large fish dominated, gray circles: unsure (posterior probability<0.9 for class membership). (a) all lakes (b) the probability of being in a small fish dominated state determined by adding a concomitant variable model. Latent class regression on arcsine-sqrt transformed data, both data and model were transformed back for this figure.

Most likely, the data will not allow us to distinguish between such refined differences in underlying mechanisms. However, the results of our latent class analysis of the data do imply strong evidence for the hypothesis that fish communities for this wide range of lakes have two alternative attractors, and that the probability of dominance by small fish increases markedly with temperature. This complements incidental observations of shifts as in Lake Victoria and the Newfoundland Cod, and the experimental evidence from a Swedish lake showing that fishing may indeed induce such a shift to an apparently stable alternative state.

Our results based on lakes ranging from the tropics to the tundra imply that alternative stable size structures are probably not a peculiarity of particular fish communities, but may be the rule rather than an exception. A major implication is that drivers such as fishing, eutrophication, loss of macrophyte coverage or climatic change may appear to have relatively

moderate effects until a critical point where they can induce sharp shifts to an alternative stable state that can be difficult to reverse.
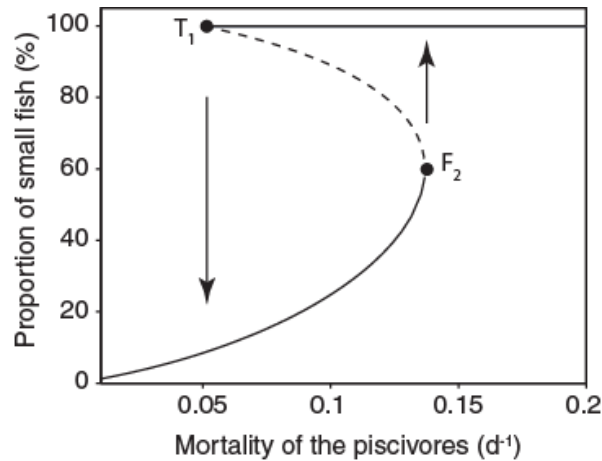

Figure 2.3 Equilibria of the rescaled model (Eq. 2) as a function of the parameter *fL* representing losses of the population of large fish, assumed to increase with temperature. Dotted lines represent unstable equilibria. The points T1 and F2 represent transcritical and fold bifurcations respectively. (The figure is produced for $\alpha = 1$; $K = 5$; $fS = 0.1$; $fL = 0.1$; $g = 1$)

2.4 Methods

Sampling. We sampled the fish community of each lake according to a stratified random sampling with multi-mesh-size gillnets (Appelberg, 2000). Each net was 30 m long and 1.5 m deep and consisted of 12 different mesh sizes ranging from 5 mm to 55 mm (knot to knot), randomly distributed in 2.5 m sections. The number of nets placed in each case varied between 2 and 5, depending on lake area. Nets were distributed between pelagic and littoral areas. We counted; measured and weighed all fish caught and calculated the proportion of fish smaller than 10 cm. All lakes were sampled once by the same team between November 2004 and March 2006.

Line fitting. We used the R package FlexMix (version 2.2-3) (Leisch, 2004; Grün & Leisch, 2008) for the latent class analysis (R version 2.8.1). This package uses the expectation-maximization (EM) procedure to find the best fit for a fixed number of latent classes. We compared different models with 1-5 classes, with the Bayesian Information Criterion (BIC), to find a parsimonious model with the best fit. Before analysis, the fractions of small fish were arcsine-square root transformed to approach a Gaussian distribution. We used latitude as dependent variable. Subsequently, we added latitude as a concomitant

variable and fitted a logistic model for the membership of the classes to the concomitant variable. The model with the lowest BIC and the posterior class membership is plotted in Figure 2.2. In the online supplementary section 2.6 we present the same analysis for the dataset excluding the lakes with exotics species.

The model. We rescaled the model (equation 1) to reduce the number of parameters. We substituted $\tau = \dfrac{t}{r_s}$; $L^* = h\,L$; $S^* = \dfrac{h}{e}S$ and defined the following compound parameters

$$K^* = \frac{eK}{h}; \; g^* = \frac{g}{r_s}; \; f_S^* = \frac{f_S}{r_S}\frac{h}{e}; \; f_L^* = \frac{\left(f_L + m_L\right)}{r_s}; \alpha^* = \alpha\frac{h}{e}.$$

The rescaled model then reads (dropping asterisks for convenience):

$$\frac{dS}{dt} = S\left(1 - \frac{S}{K}\right) - g\,S\frac{L}{L+1} - f_S S$$

$$\frac{dL}{dt} = g\,S\frac{L}{L+1}e^{-\alpha S} - f_L L \tag{2}$$

This rescaled model is functionally equivalent to the model of HilleRisLambers (*in press*). The model was analysed using GRIND for MATLAB (http://www.aew.wur.nl/UK/GRIND/), which uses a Runga Kutta solver. The stable equibria in Figure 2.3 were determined by simulation. The unstable branches were determined by following the unstable equilibrium numerically in GRIND.

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2.6 Supplementary section Chapter 2

2.6.1 Main characteristics of studied lakes and results of latent-class regression analysis and minimal model for all lakes.

Table S2.1 Main characteristics of the lakes studied (modified from Kosten *et al.*, 2009)

	Range	Mean	Medium
Area (km^2)	0.09 – 2.53	0.62	0.46
Mean depth (m)	0.5 – 4.5	1.8	1.6
Average air temperature during warmest month (^{o}C)[a]	8.2 – 28.7	23.2	23.5
Conductivity (μScm^{-1})	37.8 - 4930	527	167
Total nitrogen (mg N l^{-1})	<0.1 – 25.8	1.8	0.4
Total phosphorus (mg P l^{-1})	0.02 – 9.14	0.27	0.10
Chlorophyll-a (μg l^{-1})	0.6 - 2889	79.6	4.7
PVI (%)	0 – 81	10.5	0.5

[a.] Monthly means over the period 1961-1990 New *et al.*, 2002

Table S2.2 Latent-class regression analysis with correction by number of parameters (BIC) and addition of latitude as a concomitant variable.

	1 class	2 classes	3 classes	4 classes	5 classes
Regression without concomitant variable	114.5	95.2	105.7	107.2	118.9
Regression with latitude as concomitant variable		87.4	96.2	101.8	120.8
Classes only	130.3	95.3	90.8	101.3	101.3
Classes with concomitant variable		82.9	79.6	89.9	95.6

Table S2.3 Parameters of the best model.

Without concomitant	Intercept	P	Slope	p
Class 1	0.99	<0.001	-0.017	<0.001
Class 2	1.33	<0.001	-0.001	0.56 ns
With concomitant variable	**Intercept**	**P**	**Slope**	**p**
Class 1	1.30	<0.001	-0.00031	0.85 ns
Class 2	0.64	<0.001	-0.0082	0.04
Concomitant Class 2	-1.95	0.002	0.068	<0.001

2.6.2 On the presence of non-native species in our dataset.

Even though the presence of exotic species in our lakes was not restricted to a specific climatic region it was more common at high latitudes, where many naturally fishless lakes have been introduced with salmonid species (Reissig *et al*., 2006; Pascual *et al*., 2002). Thus, we also analyzed whether these large-sized salmonids could potentially influence the observed pattern. After eliminating all lakes with presence of exotic species we repeated the analysis and saw that the proportion of small fish in a mixture model with two classes still explains the data better than one class (see Figure S2.1). However, more than two lines could also explain data, but this could be expected as we shortened our latitudinal gradient (see Figure 2.1)

Table S2.4 Latent-class regression analysis with correction by number of parameters (BIC) and addition of latitude as a concomitant variable.

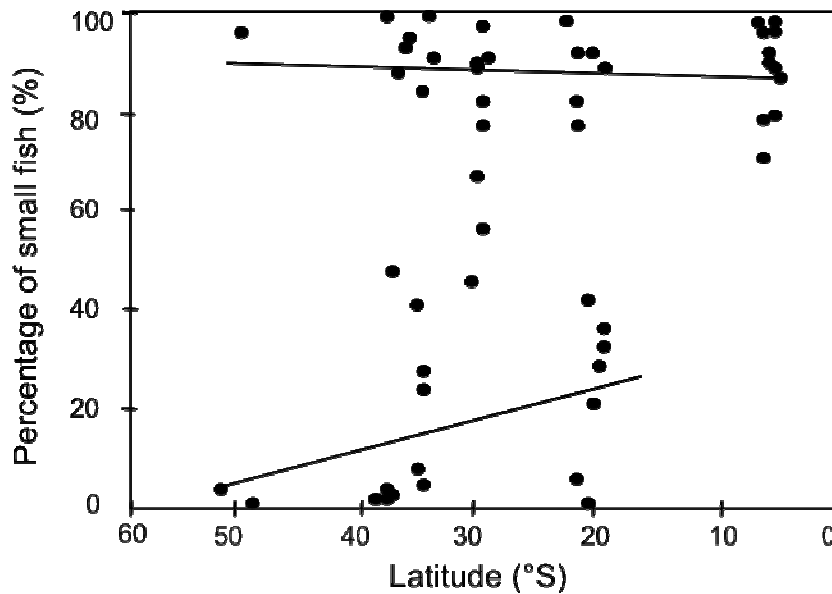|	1 class	2 classes	3 classes	4 classes	5 classes
Regression without concomitant variable	91.9	74.9	69.9	77.9	77.9
Regression with latitude as concomitant variable		75.9	80.0	73.6	87.5
Classes only	95.7	83.8	75.0	73.1	83.8
Classes with concomitant variable		72.1	69.3	78.2	89.4

Figure S2.1 The best model (based on BIC) describing the relation between latitude and the proportion of small fish with posterior class membership of the observations (black circles: small fish dominated, white circles: large fish dominated, gray circles: unsure (posterior probability<0.9 for class membership). Latent class regression on arcsine-sqrt transformed data, both data and model were transformed back for this figure. Lakes with exotic fish were removed from the analysis

2.6.3 References

Kosten, S., Lacerot, G., Jeppesen, E., da Motta Marques, D., van Nes, E. H., Mazzeo, N. & Scheffer, M. (2009). Effects of Submerged Vegetation on Water Clarity Across Climates. *Ecosystems,* **12**, 1117–1129.

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3. Larger fish forage lower in the food web in warmer lakes

Gissell Lacerot, Sarian Kosten, Raquel Mendonça, Erik Jeppesen, Jose Luiz de Attayde, Néstor Mazzeo, Franco Teixeira-de Mello, Gilbert Cabana, Matías Arim, David da Motta Marques, José H. C. Gomes & Marten Scheffer
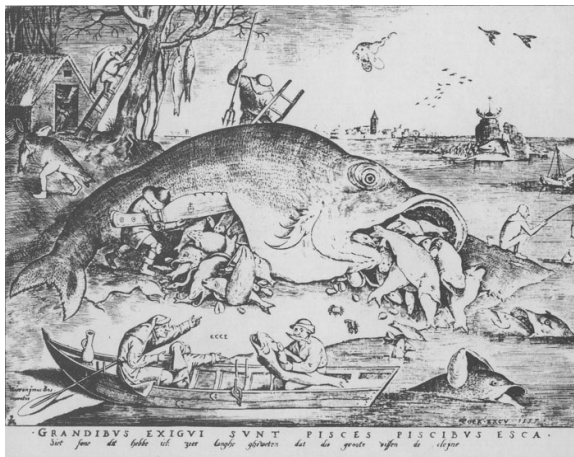
Pieter van der Heyden after Pieter Bruegel the Elder. Big Fish Eat Little Fish (1557). Harris Brisbane Dick Fund (1917). MET Museum

"Look son, I have long known that the big fish eat the small"

3.1 Summary

Body size is usually considered a good indicator of trophic position in fish communities (Jennings *et al*., 2001; Cohen *et al*., 1993; Akin & Winemiller, 2008). Indeed, the proverbial wisdom that *Big Fish Eat Little Fish* is well in line with data from marine systems (Jennings *et al*., 2001) where systematic removal of large individuals can be interpreted as fishing down the foodweb (Pauly *et al*., 1998), with consequent cascading effects on lower trophic levels (Worm & Myers, 2003). Such trophic cascades are also well documented in temperate lakes (Carpenter & Kitchell, 1996; Brett & Goldman, 1996); however, the food web in (sub)tropical lakes may work differently (Jeppesen *et al*., 2005; Jeppesen *et al*., 2007). Here, we analyze fish communities along a climatic gradient and show that the classical correlation between body size and trophic position disappears in warmer climates where large fish appear to be feeding systematically on lower trophic levels. This fits well with the recent experimental finding (Behrens & Lafferty, 2007) that the benefits for omnivorous fish of including more animal matter in their diet may disappear at higher temperatures. Our results imply that top-down control of small fish by large fish may decrease systematically towards warmer climates.

3.2 Introduction

In aquatic foodwebs most predators are not markedly selective, but gape-size puts a limit to the size of the prey (Cohen *et al.*, 1993). Indeed, the proverb *"Big Fish Eat Little Fish"* famously depicted by the Flemish painter Pieter Bruegel the Elder (ca. 1525/30–1569) reflects well how food web relations are often arranged in fish communities. An implication is that selective fishing on the largest individuals causes a systematic alteration of trophic structure leading to suppression of top predators with often-profound effects on the lower trophic levels (Worm & Myers, 2003). The biomass of small fish may increase, resulting in a decrease of large herbivorous zooplankton and an increase in microalgae biomass (Worm & Myers, 2003; Scheffer *et al.*, 2005; Frank *et al.*, 2005). While for marine systems debate remains over the causal relationships behind trophic cascades, the phenomenon is well elucidated for lakes (Carpenter & Kitchell, 1996). In fact, manipulation of fish communities is now a commonly used tool in temperate regions to reduce phytoplankton biomass through a cascade of effects from fish over zooplankton to algae (Gulati *et al.*, 1990; Søndergaard *et al.*, 2008). However, less is known about the functioning of foodwebs in subtropical and tropical lakes. Results so far suggest that reduction of top predator fishes does not have the same strong effects in warmer lakes as in temperate lakes (Jeppesen *et al.*, 2005), probably due to the different structure of the foodwebs (Meerhoff *et al.*, 2007; Teixeira de Mello *et al.*, 2009; Havens *et al.*, 2009; Layman *et al.*, 2005)¸ and because herbivory and omnivory appear to be more important (Wooton & Oemke, 1992; Lazzaro, 1997; Attayde & Menezes, 2008).

To check whether the trophic structure of fish communities does indeed change systematically with climate, we analyzed fish communities of 17 similar shallow lakes located along a latitude gradient from 5°S to 54°S in South America (supplementary information is given in section 3.6 Table S3.1). All lakes were sampled using multi-mesh-size gillnets, and the nitrogen stable isotope ratio (δ^{15}N) was used to characterize the relative trophic position of individual fish (see methods). δ^{15}N can be used as an indicator of trophic position, because it changes continuously with each level in the foodweb (Cabana & Rasmussen, 1994). To analyse how trophic position escalated with body size, we focused on the slope of the relationship between δ^{15}N and the log body length for each individual lake (as from now the δ^{15}N-size slope).

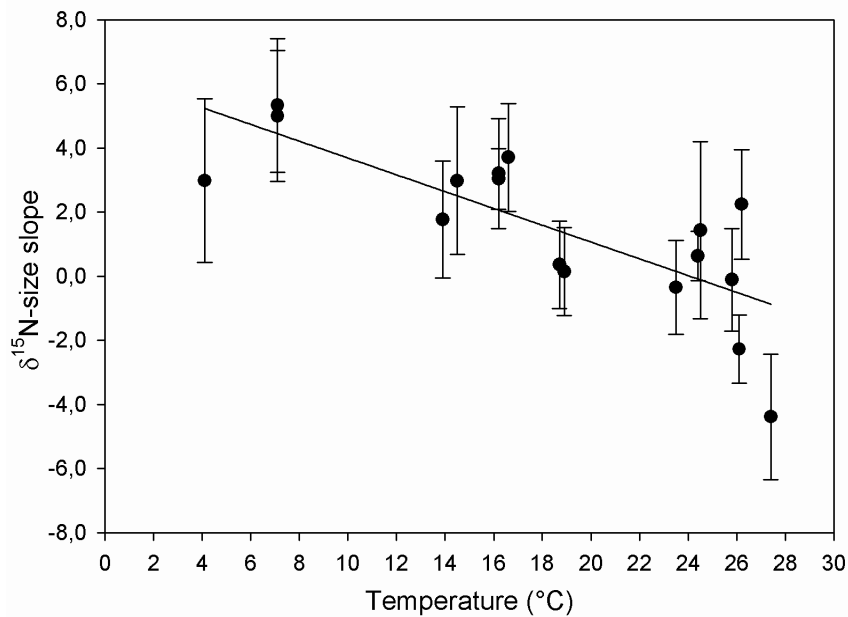**Figure 3.1** δ^{15}N-size slope relation for each lake against the average regional temperature (°C) at the lake location (R^2= 0.588, p=0.0003, n=17). Bars indicate the standard error of the slope.

3.3 Results and Discussion

If we plot the δ^{15}N-size slopes of the regressions obtained from the individual lakes against their latitude and average regional temperature (Figure 3.1, see also supplementary section 3.6 Figure S3.1), a striking pattern emerges. In colder lakes trophic position increases with body size, but in tropical lakes this relationship disappears entirely. This is reflected in a systematic decrease in the δ^{15}N-size relationships found in individual lakes, but also Spearman correlation coefficients decrease and even become negative at higher temperatures (see Figure S3.2 in the supplementary section 3.6). Stepwise regression analysis of the δ^{15}N-size slope against average regional temperature and a range of other factors including lake area, total phosphorus (TP), percentage of lake volume occupied by macrophytes (PVI), the average and maximum fish length and fish species richness (S) yielded a model with only temperature accounting for a remarkable 60% of the variation (δ^{15}N-size slope= -0.205 T + 5.785; R^2=0.627, p=0.009).

The isotopic signature provides an objective indication of the average trophic level at which an individual has foraged. As an additional check on the emerging patterns we also examined trends in the diet of the species based on available literature. This analysis reveals that species with documented omnivorous diets become relatively more abundant in warmer climates, while the relative importance of piscivores decreases with temperature (Figure 3.2)

31

($r^2_{Spearman}$= 0.499, p=0.04 for CPUE omnivorous fish and temperature). Another clear trend is found in the overall average and maximum body size of individual fish (Figure 3.3). Warmer lakes tend to have smaller fish ($r^2_{Spearman}$= -0.782 for average length and $r^2_{Spearman}$= -0.802 for maximum length, p=0.0001). These results are well in line with the idea that body size of ectotherms is smaller in warmer environments (Atkinson, 1994) and fits with earlier suggestions that small fish tend to be dominant in warm lakes (Teixeira de Mello *et al.*, 2009; Wootom & Oemke, 1992). It could be argued that the change in slope along the latitude gradient might be caused by a systematic change in the size range of the fish population analysed for isotopes, due to dominance of smaller fish in the warm lakes geographic region. However we found no relation between the fish size range in each lake and latitude ($r^2_{Spearman}$= 0.431 p=0.084) or temperature ($r^2_{Spearman}$= -0.438 p=0.079) (see also Figure S3.3a-b in supplementary section 3.6).

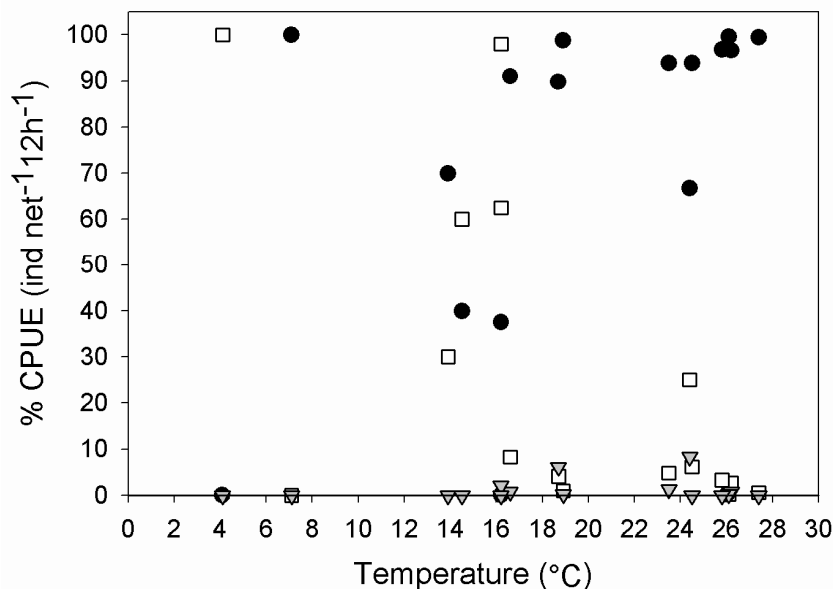**Figure 3.2** Food habits composition in % CPUE: individuals. net^{-1}. $12h^{-1}$ against the average regional temperature at the lake location (°C). Black circles: omnivores, White squares: piscivores, Grey triangles: others (iliophagus and herbivores)

To check if the presence of exotic species affects this pattern, we repeated our analysis excluding all non-native species. Introduction of exotic fish species is common in many aquatic systems and our lakes are no exception. We found non-native species in 7 lakes (supplementary section 3.6 Table S3.2). The presence of exotic species was not restricted to a specific climatic region or functional group, and usually represented a small fraction of the total catch per unit of effort (CPUE) (see supplementary section 3.6 Table S3.2). Analysis of the exotic-free data set showed slightly weaker relations but maintained the same trend (see

supplementary section 3.6 Figures S3.4a-b and Figure S3.5). This suggests that the systematic relationship between trophic size structure and climate is a robust phenomenon, which does not depend upon differences in the role of introduced species.

The key question remains which factors might explain the systematic change in size and trophic structure of freshwater fish communities with climate. One hypothesis is that energetic limitations play an increasingly important role at higher temperatures. Larger animals have higher energetic demands (McNab, 2002). Food availability decreases as we go up the food web due to the limited efficiency with which energy is transferred from one trophic level to the next, implying that the energetic demands of large animals are more easily met for herbivores than for carnivores (Elton, 1927; Arim *et al.*, 2007). While gape-limitation tends to result in carnivores being larger, energy limitation is thus expected to promote the opposite pattern (Arim *et al.*, 2007). In ectothermic animals such as fish, energy demands go up steeply with temperature (Atkinson, 1994). Therefore, energy limitation could potentially explain why in warmer lakes average and maximum body sizes are smaller and why potential predators do not forage as high in the food web as they do in cold lakes.
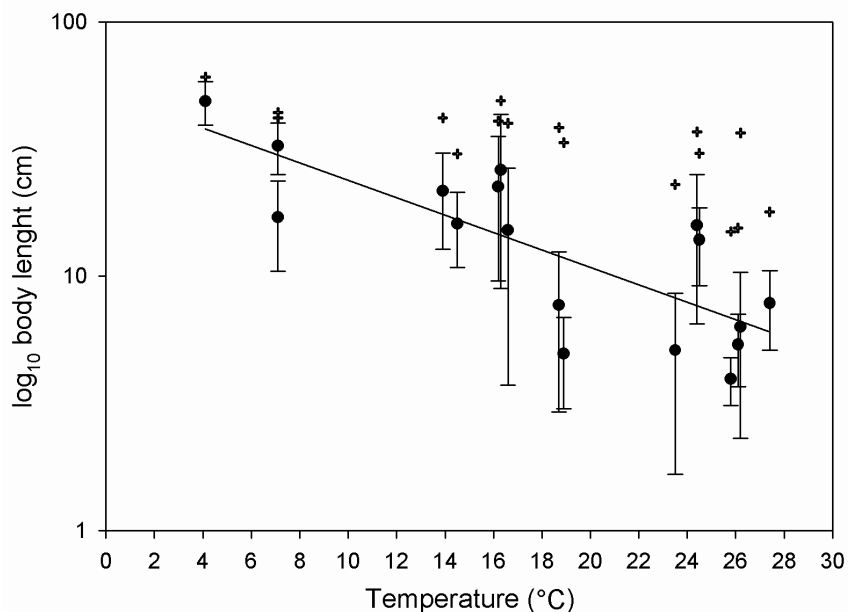

Figure 3.3 Average body length (log scale) of individual fish for each lake against their average regional temperature (R^2=0.608, p=0.0001, n=17) + shows maximum fish length (cm) for each lake. Bars indicate standard deviations.

Another potential explanation is that plant material may be more easily assimilated by fish at warmer temperatures, as suggested by an experimental study where adding animal matter to the diet of an omnivorous fish promoted performance at low but not at high temperatures (Behrens & Lafferty, 2007).

Whatever the mechanistic explanation may be, our results indicate that the classical image that the big fish eat the small does not hold for lakes in warm climates. This implies that top-down control of small fish is likely to be substantially weaker than under temperate conditions. As a consequence small fish may well exert a higher predation pressure on zooplankton, resulting in reduced top-down control on phytoplankton in warm climates. This shift in trophic structure is of more than academic interest, as managing the trophic cascade in lakes is an important part of the toolbox for combating excessive phytoplankton growth.

3.4 Methods

Sampling We sampled the fish community of each lake according to a stratified random sampling with multi-mesh-size gillnets (Appelberg, 2000). Each net was 30 m long and 1.5 m deep and consisted of 12 different mesh sizes ranging from 5 mm to 55 mm (knot to knot), randomly distributed in 2.5 m sections. We took representatives of different sizes within all species found and recorded their length and weight and took a white muscle sample for stable isotope analysis. If the fish were too small to obtain enough material (<6 cm), we used whole body minus head, tail, skin and guts. All samples were stored on ice immediately and frozen within a couple of hours. All remaining fish caught were counted, measured and weighed. Fish abundance was estimated as the average catch among nets and expressed as catch per unit of effort (CPUE; individuals net^{-1} 12 hour^{1}).

Total phosphorus concentration was determined using a continuous flow analyzer (Skalar Analytical BV) following NNI protocols (1986) with the exception of the UV/Persulfate destruction which was not executed beforehand but integrated in the system.

Submerged macrophyte coverage was estimated based on observations of macrophyte presence/absence at 20 random points in the lake combined with coverage estimations of macrophytes at 13-47 points (average 22) equally distributed on 3 to 8 parallel transects. The number of transects varied with the shape and size of the lake. Observations were made from a boat using a grapnel when water transparency was insufficient to get a clear view of the bottom. The percentage of the lake's volume filled with submerged vegetation (PVI) was determined analogously to classical studies (Canfield *et al.*, 1984). First, we calculated the PVI of the individual sampling locations by multiplying the coverage percentage by the

average length of the macrophytes divided by the depth. The PVI of the entire lake (PVI) was calculated by multiplying the area of the lake covered by macrophytes (m^2) with the average height of vegetation in the vegetated locations that were sampled (m) divided by the total volume of the lake (m^3).

All lakes were sampled by the same team between November 2004 and March 2006.

Stable isotope analysis For the nitrogen stable isotope analysis, fish muscle was freeze-dried and powdered, and 0.5-mg-aliquots were placed in tin capsules. The capsules were loaded into an elemental analyzer for combustion (Carlo Erba EA). The nitrogen gas (N_2) generated from the combustion was purified in a gas chromatographic column and passed directly to the inlet of a gas isotope ratio mass spectrometer (Delta Plus, Finnigan Mat). The nitrogen isotopic ratio was expressed as $\delta^{15}N$ in relation to the atmospheric N_2 standard.

Data analysis. We estimated the slope ($\delta^{15}N$-size slope) from the regression between fish $\delta^{15}N$ and log body length for each lake using least squares method. Fish abundance (CPUE) was divided in 4 functional groups according to their diet (omnivores, iliophagus, herbivores and piscivores) based on literature (Mazzeo *et al.*, unpublished). Non-parametric Spearman correlations were applied to check for relations between latitude, regional temperature, fish body length (both average and maximum length), fish length range and fish CPUE according to diet groups. Multiple stepwise regression analysis was used to evaluate the influence of latitude, temperature, lake characteristics and fish community characteristics on the $\delta^{15}N$-size slope. Data were transformed with log x or log(x +1) to approach normality. Statistical results were considered significant at *p*<0.05. All statistical analyses were performed on SPPS for Windows v. 15.0 (SPSS Inc., Chicago, IL, U.S.A.).

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3.6 Supplementary section for Chapter 3

Table S3.1 Latitude, longitude and limnological characteristics of the 17 lakes studied. Average regional temperature (T), average depth (Z), area, total phosphorus (TP), percentage of lake volume occupied by submerged vegetation (PVI) and number of fish species (S)

Latitude (°S)	Longitude (°W)	T (°C)	Z (m)	Area (Ha)	TP (mg. l^{-1})	PVI (%)	S
5.377	35.381	26.2	2.36	61.49	0.03	0	9
5.581	35.491	26.1	1.96	24.07	0.13	0	5
6.047	35.188	25.8	3.55	91.21	0.07	0	6
6.403	37.067	27.4	2.73	87.47	0.07	0	4
19.511	39.764	24.4	2.21	49.92	0.02	2.88	8
19.617	39.847	24.5	2.31	67.34	0.03	9.55	5
21.809	41.131	23.5	3.07	75.76	0.09	17.47	10
29.910	50.220	18.7	1.34	50.63	0.07	2.64	14
30.147	50,260	18.9	1.71	116.32	0.14	0	14
34.295	53.805	16.6	2.45	16.72	0.14	16.44	7
35.229	59.935	16.2	0.88	32.83	0.24	1.13	6
35.245	59.856	16.2	1.14	31.33	0.18	74.04	5
37.866	57.895	13.9	1.20	114.32	0.28	0	5
38.837	60.862	14.5	1.35	78.20	0.52	0	6
49.409	71.703	7.1	0.99	8.90	0.07	73.37	2
49.417	71.682	7.1	2	39.38	0.26	0.30	2
54.295	67.014	4.1	0.93	55.23	0.06	63.3	1

Table S3.2 Exotic species found in our study, their location and relative presence in the fish community. * lake removed from analysis in the non-native species dataset. Percentage in total catch per unit of effort (% CPUE)

| Scientific name (author)	Common name	Number of lakes	Latitude (°S)	Functional group	% in total CPUE
Cichla ocellaris (Bloch & Schneider, 1801)	Tucunaré	2	6.047	Benthic-pelagic	1.3
			6.403	Piscivorous	0.6
Cyprinus carpio (Linnaeus, 1758)	Common carp	1	38.837	Benthic-pelagic Omnivorous	6.9
Oreochromis niloticus (Linnaeus, 1758)	Tilapia	1	6.403	Benthic-pelagic Omnivorous	1.2
Salmo trutta (Linnaeus, 1758)	Brown trout	1	54.295	Pelagic Piscivorous	100*
Percichtys (Perca) trucha (Valenciennes, 1833)	Perch	2	49.417	Benthic-pelagic Omnivorous	0.8
			49.409		82.6
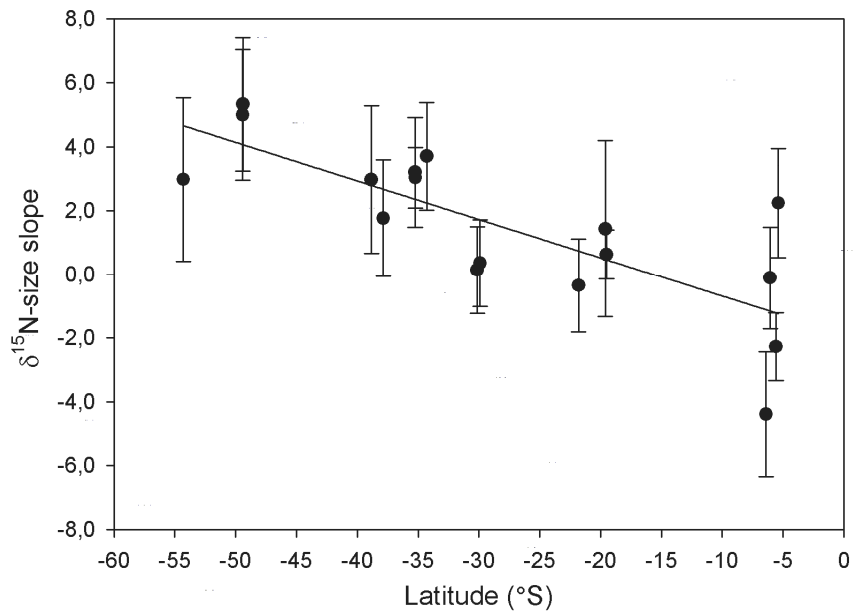
Figure S3.1 δ^{15}N- size slope relation for each lake against their latitude (R^2=0.593, p=0.0003 n=17). Bars indicate the standard error of the slope.

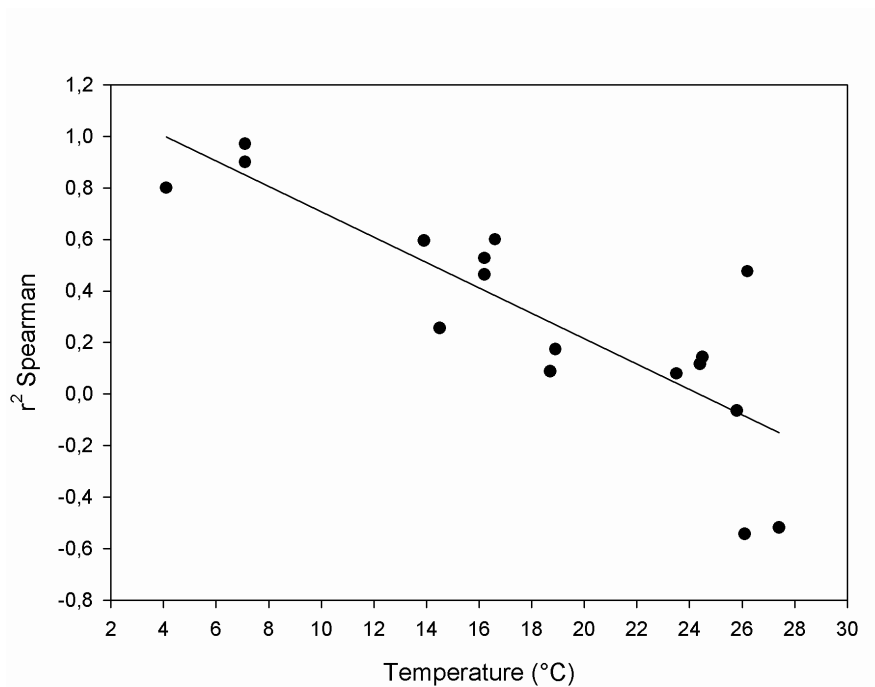**Figure S3.2** Spearman correlation coefficients between log body length and δ^{15}N for each lake against their average regional temperature (R^2=0.677, p=0.0001, n=17)
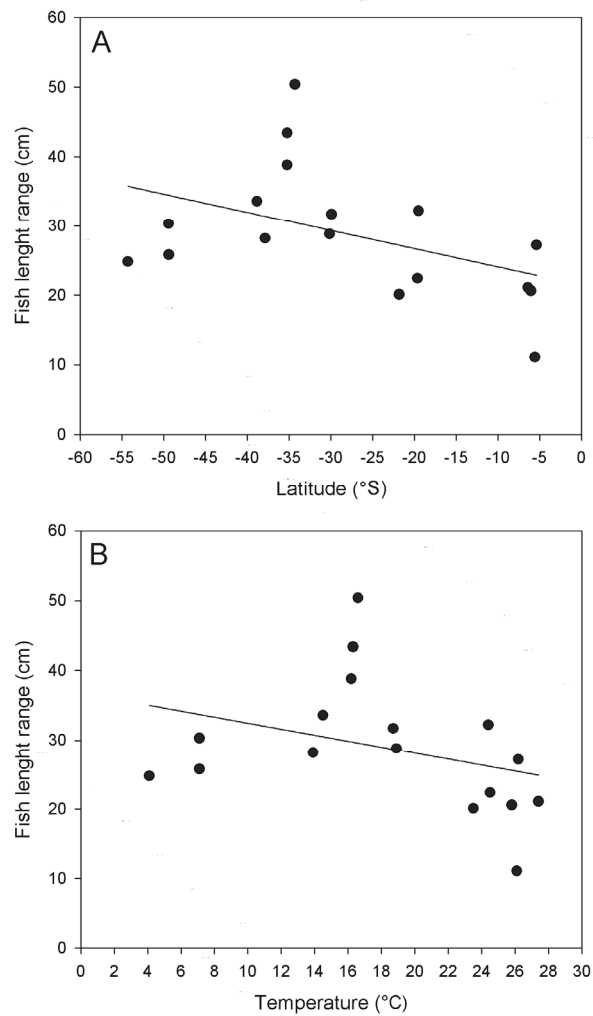
Figure S3.3 Fish length range (cm) for all lakes studied against **A.** their latitudinal position (R^2= 0.203, p=0.069, n=17), **B.** the average regional temperature from lake location (R^2= 0.116, p=0.181, n=17)
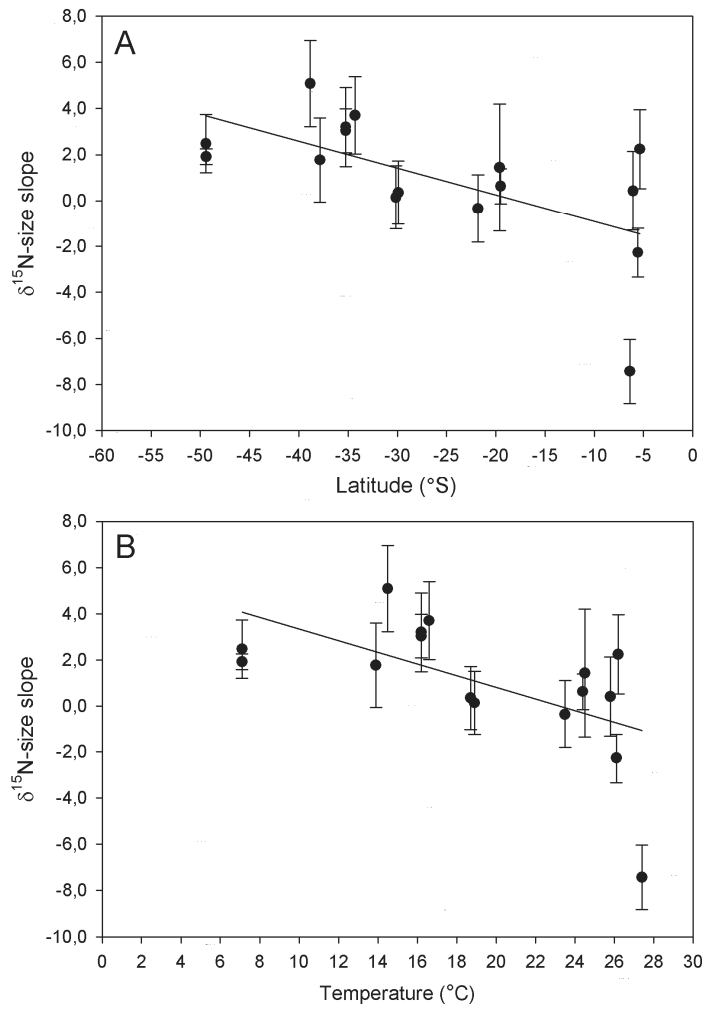
Figure S 3.4 δ^{15}N-size slope relation for the studied lakes against **A**. their latitudinal position (R^2= 0.374, p=0.012, n=16), **B**. the average regional temperature from lake location (R^2= 0.334, p=0.019, n=16). Bars indicate the standard error of the slope. Non-native species excluded from the analysis.
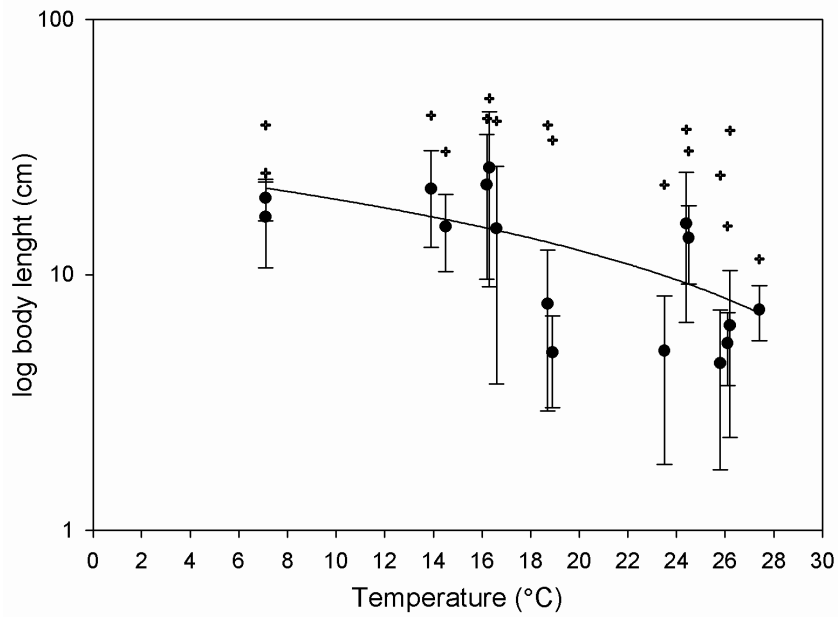
Figure S3.5 Average body length (log scale) of individual fish for each lake against their average regional temperature. Non-native species excluded from the analysis (R^2=0.440, p=0.0053). + shows maximum fish length (cm) for each lake. Bars indicate standard deviations.

4. Fish size explains zooplankton size along a latitudinal gradient in South America

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4.1 Summary

It has been reported that zooplankton species in lakes and ponds from warm climates are generally much smaller than in temperate regions. Several potential explanations for this pattern have been suggested including a temperature-mediated increase of physiological rates, lower food quality and stronger predation pressure towards the tropics. However, few studies have tested these alternative explanations. We analyzed zooplankton body size distributions in detail for 81 shallow lakes along a latitudinal gradient in South America (5-55°S) and explored how these distributions were related to fish communities, vegetation abundance and a range of other limnological variables. In line with earlier results, we found that the average body size of the zooplankton community decreased from higher to lower latitudes. However, body size patterns differed among the taxonomic groups with little size variation for calanoid and cyclopoid copepods and none for rotifers. Also, cladocerans associated to benthic habitats and vegetation showed no latitudinal size gradient, whereas pelagic cladocerans showed a pronounced latitudinal gradient in body size. Not surprisingly, average regional temperature correlated strongly to the latitudinal body-size patterns. However, the proportion of small fish in the lake (<10 cm) explained a remarkably high proportion of the variance too. Small fish are notorious predators on zooplankton, and were relatively more abundant in warmer lakes. The correlation of body size to the fish, together with the fact that the zooplankton size gradient is limited to groups that are notoriously susceptible to fish predation, suggests that size-dependent predation rather than a physiological temperature effect is the dominant force in causing the observed latitudinal gradient in body size of cladocerans.

4.2 Introduction

Zooplankton shows broad latitudinal patterns in body size. In warm climates zooplankton species are generally much smaller than in colder regions, and particularly large herbivorous species such as *Daphnia* seem less diverse or even absent at lower latitudes (Dumont & Segers, 1994; Fernando *et al*., 1987; Gillooly & Dodson, 2000; Tims & Morton, 1998).

This pattern is of more than academic interest as body size affects the functional position of zooplankton in aquatic ecosystems. It determines their vulnerability to predation by fish (Brooks & Dodson, 1965) as well as their food preference and grazing rates (Cyr & Curtis, 1999; Gliwicz & Lampert, 1990; Tessier *et al*., 2001). Therefore, body size is closely related not only to their susceptibility to top-down control, but also to competitive abilities of zooplankton (Gilbert, 1988; Pace & Vaque, 1994; Vanni, 1986) and their effectiveness as predators of the phytoplankton community (Dawidowicz, 1990; Jeppesen *et al*., 1999).

So far, the cause of the latitudinal pattern in zooplankton body size remains unclear, although several possible explanations have been proposed. Temperature is the most obvious factor differing between climatic regions, and it is associated with reduced body size in various ectotherms (Atkinson, 1994; Atkinson & Sibly, 1997). Several zooplankton life-history parameters such as growth, reproduction and respiration rates as well as size at maturity are influenced by temperature (Moore *et al*., 1996; Weetman & Atkinson, 2004). Increased metabolic rates with temperature might favor smaller zooplankton body sizes over larger forms. For example, it has been shown that tropical Cladocera have an order of magnitude higher food requirements than temperate cladocerans (Duncan, 1989) and that at high temperatures, their food requirements increase steeply with body size (Hardy & Duncan, 1994). Also, food limitation in warmer conditions may be promoted by restrictions in food quality if those are associated to climate. For instance, fast-growing zooplankton species such as *Daphnia* have high phosphorus requirements and are more susceptible to P-deficient algae than slower-growing species (i.e. copepods) (Elser *et al*., 2000; Urabe *et al*., 1993; Urabe *et al*., 1997).

Currently, the most cited hypothesis for the reduction in zooplankton size with latitude is related to the predation pressure exerted by fish communities (Lazzaro, 1997; Jeppesen *et al*., 2005; Jeppesen *et al*., 2007; Meerhoff *et al*., 2007a; Havens *et al*., 2009). Even though there has been an increase in comparative studies in lakes among different climatic regions, few studies have systematically analyzed variables related to both bottom-up and top-down control over large latitudinal gradients (i.e. Gyllström *et al*., 2005).

Here we aim to contribute to bridging this knowledge gap by exploring the latitudinal pattern of the body size in different groups of zooplankton in relation to a data on the size structure of the fish communities and other characteristics of lakes we sampled along a climate gradient.

4.3 Methods

We sampled 81 shallow lakes between latitudes 5°S and 55°S in South America. All lakes were shallow (maximum mean depth= 4.5 m) and small to medium-sized (9-253 Ha). Average air temperatures for the lakes ranged from 27.6°C in Rio Grande do Norte (Brazil) to 4°C for tundra lakes in Tierra del Fuego (Argentina) (New *et al.*, 2002). All lakes were chosen to differ as much as possible in their trophic state in each climatic region. Lakes were sampled once during summer or dry season (tropical lakes), between November 2004 and March 2006. In all cases we followed one sampling protocol, executed by the same team.

Each lake was surveyed for physical, chemical and biological characteristics in the water column as well as the sediment. Water samples for nutrient and plankton communities analysis were taken with a tube, at 20 random points in each lake, and integrating the whole water column. The 20 points included both pelagic and macrophyte areas.

Nutrient samples. At each pelagic point, two liters of the depth-integrated sample were pooled in a bulk sample. Water from this bulk sample was used for various nutrient analysis including total phosphorus (TP). At the laboratory, TP concentrations were analyzed with a continuous flow analyzer (Skalar Analytical BV) following NNI protocols (1986; 1990), with exception of the UV/Persulfate destruction, which was not executed beforehand but integrated in the system.

Zooplankton, fish and macrophytes samples. At all points (both pelagic and macrophyte areas), two liters of the depth-integrated sample were pooled into a bulk sample for quantitative zooplankton analysis (final volume=40 liters). This sample was filtered through a 50μm sieve and preserved in a 4% formaldehyde solution. Additionally, we took an extra qualitative sample from several net tows at different points in the lake and preserved it in 4% formaldehyde solution. Zooplankton body length (mm) was estimated by measuring a minimum of 20 specimens of each rotifer species and at least 50 specimens for each copepod and cladocera species found.

The fish community was sampled according to a stratified random method with multimesh-gillnets (Appelberg, 2000). Each net was 30 m long and 1.5 m deep and consisted of 12 different mesh sizes ranging from 5 mm to 55 mm, randomly distributed in 2.5 m sections. Fishes caught were counted, measured and weighted in the field, and specimens taken for taxonomy analysis. Otherwise, the whole catch was preserved in 10% formaldehyde solution for later analysis.

Submerged macrophyte coverage was estimated based on observations of macrophyte presence/absence at 20 random points in the lake combined with coverage estimations of macrophytes at 13-47 points (average 22) equally distributed on 3 to 8 parallel transects. The number of transects varied with the shape and size of the lake. Observations were made from a boat using a grapnel when water transparency was insufficient to get a clear view of the bottom. The percentage of the lake volume filled with submerged vegetation (PVI) was determined analogously to Canfield *et al.* (1984). First we calculated the percentage at each sampling location by multiplying the coverage percentage with the average length of the macrophytes and divided it by the depth. Then the PVI was calculated by multiplying the area of the lake covered by macrophytes (m^2) with the average height of vegetation in the vegetated locations that were sampled (m), divided by the total volume of the lake (m^3).

Data analysis. Zooplankton species were classified in 4 taxonomic groups (rotifers, cladocerans, calanoid copepods and cyclopoid copepods). For each taxonomic group, species were also assigned to two different habitats based on literature (pelagic or benthic/macrophyte-associated). For each group we calculated the average geometric mean body size (μm). For the analysis of body size variations with latitude, lakes were grouped in 10 intervals of 2°S each (Table 4.1).

For each lake, we calculated the proportion of fish smaller than 10 cm in the fish community, from now on the percentage of small fish (PSF). This percentage was calculated to assess the effect of fish on zooplankton irrespective of their feeding habits, as for many of the fish species found diet data is scarce.

Table 4.1 Latitudinal intervals considered in the study and number of lakes in each one.

Latitude (°S)	N lakes
5-6	19
19-20	13
21-22	6
29-30	12
34-35	8
36-37	9
38-39	4
49-50	5
51-52	2
54-55	3

To check which dependent variables in our dataset contributed to explain zooplankton body size differences with latitude, we performed stepwise multiple regression analysis. To approach normality, data was transformed with log x, log(x+1) or arcsine[square root (%+1/100)] in the case of percentage variables (PVI and PSF). Results were considered

significant at *p<0.05*. All statistical analyses were performed on SPPS v.15.0 (SPSS Inc., Chicago, IL, U.S.A.).

4.4 Results

The average body size of the zooplankton community in our lakes varied between 126 and 342 μm and decreased from higher to lower latitudes (Table 4.3). Body size patterns differed among the taxonomic groups. Calanoid and Cyclopoid copepods showed little size variation among the latitudinal intervals (Table 4.2). The presence of large-sized species from the Genus *Boeckella* increased average body size at the highest latitudes (Table 4.2). The body size of rotifers did not differ significantly among the latitudinal intervals considered (Table 4.3).

Table 4.2 Average body size and range (μm) for copepods and cladocera genera found in the study. Only genera found in two or more lakes are shown. BM=benthic/macrophte- associated species. P=pelagic

	Average body size	Range
Cyclopoid copepods		
Acanthocyclops (P)	459.8	802-300
Eucyclops (BM)	334.9	368-300
Mesocyclops (P)	423.3	767-275
Metacyclops (P)	520.6	812-355
Microcyclops (P)	411.8	604-330
Thermocyclops (P)	404.6	594-235
Tropocyclops (P)	374.0	554-275
Calanoid copepods		
Boeckella (P)	899.7	1893-235
Notodiaptomus (P)	706.9	1263-385
Cladocera		
Alona (BM)	236.6	445-200
Alonella (BM)	229.6	274-200
Bosmina (P)	304.5	435-212
Bosminopsis (P)	229.3	300-185
Ceriodaphnia (P)	438.0	639-249
Chydorus (BM)	265.2	461-200
Daphnia (P)	1174.9	1815-654
Diaphanosoma (P)	609.6	974-431
Ephemeroporus (BM)	283.7	300-207
Latonopsis (BM)	1678,3	1800-1548
Macrothrix (BM)	358.8	481-236
Moina (P)	497.7	705-356
Pleuroxus (BM)	555.1	600-410
Sarsilatona (BM)	670.0	700-640
Simocephalus (BM)	1092.3	1277-1000

On the other hand, Cladocera showed pronounced differences in body size, increasing from lower to higher latitudes with some evidence for a minimum at latitudinal intervals between 20°S and 30°S (Figure 4.1a, Table 4.3). For this group we also found differences depending on the habitat. Species associated with benthic/macrophyte habitats did not show significant variations in body size along the latitudinal gradient (Table 4.3). For this group larger body sizes were related to the presence of *Latonopsis* or *Simocephalus* species, which were found in lakes throughout the latitudinal range (Table 4.2). In contrast, the strongest differences in body size were found for the pelagic cladocerans, which were substantially larger towards the colder end of our gradient than in warmer lakes (Figure 4.1b, Table 4.3).
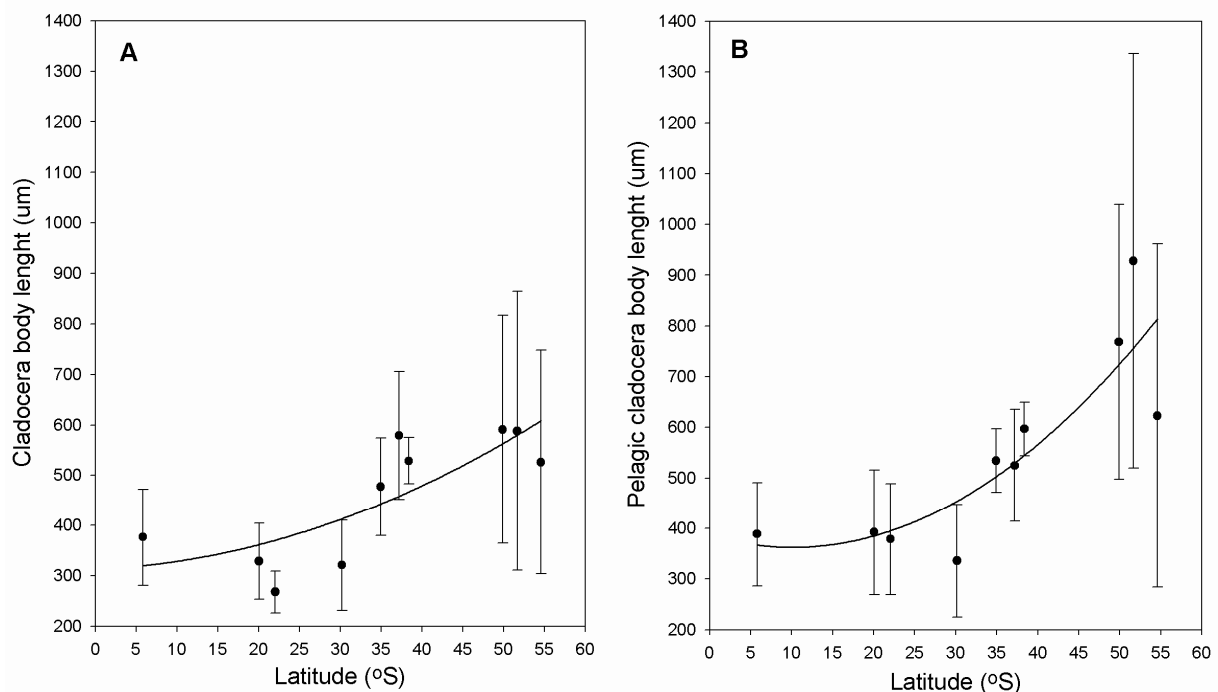

Figure 4.1 Variation of average body lenght (μm) with latitude for **A**. Cladocera and **B**. Pelagic Cladocera. Lakes are grouped in intervals of 2° of latitude. Bars indicate standard deviations.

To explore potential causality behind the latitudinal patterns in body size we did stepwise multiple regression analyses offering log total phosphorus, log lake area, the arcsine-transformed percentage of volume occupied by plants (PVI), the arcsine transformed percentage of fish smaller than 10 cm (PSF) and average regional temperature as independent variables. Average regional temperature correlated most strongly to overall mean zooplankton body size (Table 4.4). The inclusion of a square term for the regional temperature further indicates a minimum body size at intermediate latitudes. Again, results differed widely between taxonomic groups. The variation in body size of Cyclopoid and Calanoid copepods was poorly explained by the variables considered (Table 4.4), although lake PVI was

negatively related to body size of cyclopoids, and the size of calanoid copepods decreased with the percentage of small individuals in the fish community (PSF) (Table 4.4).

Table 4.3 Second order polynomial model fits to the average body size for all zooplankton together and for each taxonomic group against latitude. In the case of Cladocera, groups associated to pelagic versus benthic/macrophyte habitat are also shown.

|	R^2	F	P	Model
All zooplankton	0.564	4.534	0.055	$-2.914lat+0.069lat^2+235.860$
Rotifers	0.565	4.548	0.064	$-1.152lat+0.025lat^2+139.235$
Cyclopoid copepods	0.090	0.352	0.715	$-3.041lat+0.053lat^2+381.661$
Calanoid copepods	0.605	5.359	0.039	$-15.006lat+0.313lat^2+787.660$
Cladocera	0.633	6.036	0.030	$-0.699lat+0.086lat^2+312.864$
Pelagic Cladocera	0.734	9.678	0.010	$-4.631lat+1.484lat^2+385.980$
Benthic/macrophyte associated Cladocera	0.027	0.095	0.910	$-1.307lat+0.005lat^2+321.965$
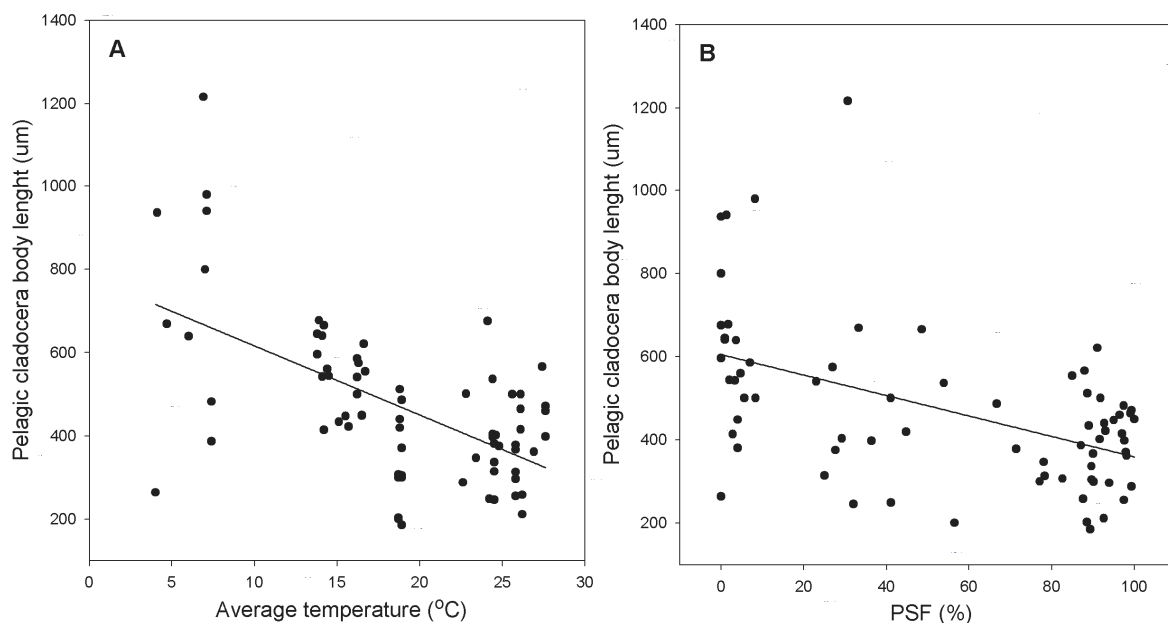
Figure 4.2 Relation between body size of pelagic cladocera and **a**. average regional temperature (R^2=0.341, F=36.21, p<0.0001) and **b**. the proportion of small fish (PSF) (R^2=0.253, F=23.69, p<0.0001).

As in the case of the regressions against latitude, explained variance of body size was highest for cladocera, temperature being the most important explanatory variable (Table 4.4). Again, patterns differed between plant-associated and pelagic species. The proportion of submerged macrophytes in the lake was the only variable associated with the body size of benthic/macrophyte- associated cladocera (Table 4.4). For pelagic species the PSF in the lake was included together with temperature as the dominant explanatory variables (Figure 4.2a-b,

Table 4.4). Average regional temperature and PSF were positively correlated ($r_{Spearman}$=0.437 $p<0.001$). In summary, while temperature was correlated to cladoceran body size along the latitudinal gradient, the fraction of small fish could explain a significant portion of the variation too, in particular for pelagic cladocera (Figure 4.2).

4.5 Discussion

The body size pattern we found for cladocera along our extensive latitudinal gradient is in line with what has been shown by Gillooly & Dodson (2000), from an analysis of data taken from different studies. However, the systematic data we collected on other groups of plankton, and on fish communities, vegetation abundance and several other limnological variables now puts us in the position to explore more of the potential causality behind this intriguing pattern.

Table 4.4 Stepwise multiple regression analysis for the log average body size (μm) for different groups of zooplankton against log-transformed phosphorus (TP), log-transformed lake area (Area), arcsine-transformed percentage of plant coverage (PVI), arcsine-transformed percentage of small fish (PSF), and average regional temperature (T) and its square (T2). Variables were included at a significance level of $p<0.05$. An equation for rotifers is not given, as there was no significant relationship to any of the variables considered.

Variable	Intercept	TP	Area	PVI	PSF	T	T2	F	p	R^2 adj
Zooplankton	2.671 (<0.001)	0.28 (0.039)				-2.57 (<0.001)	2.41 (<0.001)	7.18	<0.001	0.20
Cyclopoid copepods	2.620 (<0.001)			-0.27 (0.023)				5.43	0.023	0.06
Calanoid copepods	2.870 (<0.001)				-0.27 (0.030)			4.90	0.030	0.06
Cladocera	3.151 (<0.001)	0.41 (0.002)				-1.77 (0.003)	1.44 (0.021)	11.51	<0.001	0.30
Pelagic Cladocera	2.899 (<0.001)				-0.30 (0.010)	-0.37 (0.002)		17.31	<0.001	0.32
Benthic/ macrophyte associated Cladocera	2.444 (<0.001)			0.36 (0.008)				7.73	0.008	0.12

The first indication for possibly important role of fish communities in explaining the zooplankton body size variation along the climate gradient is simply the correlation we found between the fraction of small fish in the community and cladocera body size. In fact, it is remarkable that we could explain such a significant proportion of the body size variation with latitude using only a rough proxy for fish predation pressure such as the estimated proportion of small individuals in the fish community. A negative impact of fish predation on

zooplankton body size has been well documented (Brooks & Dodson, 1965; Hrbáček *et al*., 1961; Jeppesen *et al*., 2004; Jeppesen *et al*., 1997), and various studies have provided evidence for the idea that predation on lake zooplankton by fish might be particularly strong in warmer climates (Gyllström *et al*., 2005; Jeppesen *et al*., 2007; Meerhoff *et al*., 2007a). This is most likely related to the fact that in warm regions fish communities tend to be dominated by small-sized individuals (Teixeira De Mello *et al*., 2009; Chapter 2 this thesis), characterized by high growth and reproductive rates (Blanck & Lamoroux, 2007; Van Leeuwen *et al*., 2007). As many of the small fish species in warmer regions will not only consume zooplankton but also algae and detritus (Nilssen, 1984; Winemiller, 1990), they do not only compete with zooplankton for resources, but can also reach extremely high abundances (Meerhoff *et al*., 2007a; Teixeira De Mello *et al*., 2009). Another aspect that may promote the densities of such fish is the fact that communities from warm lakes have few real piscivores (Quirós 1998). Compared to predators in temperate regions, the few generalist predators in warm lakes occupy lower trophic positions in the food web, therefore exerting a less effective top-down control on smaller fish (Layman *et al*., 2005; Chapter 2 this thesis). In conclusion, the idea that fish predation may play a key role in causing the observed decrease in zooplankton body size towards the tropics is consistent with what we know about latitudinal gradients in fish community structure and functioning.

Obviously we cannot exclude that other mechanisms play a role too in causing the body size gradient. In fact, in 83% of 109 studies on ectotherm animals of various kinds, larger sizes are found at cooler temperatures (Atkinson, 1994). This suggests that there may be more general mechanisms at play. One explanation could be that, as temperature increases, organisms with larger body sizes run into problems to meet their energetic demands, which could make smaller forms competitively superior (Moore *et al*., 1996; Weetman & Atkinson, 2004). Indeed, it has been suggested that tropical cladocera might need much higher food concentrations than temperate species to compensate for their higher metabolic rates (Duncan, 1989). However, performing experiments to test temperature effects on differently sized *Daphnia* and *Ceriodaphnia,* Achenbach & Lampert (1997) found no influence of temperature on the outcome of competition other than the usual effects of body size.

Another possibility might be that there is a latitudinal gradient in food quality for zooplankton. It has been suggested that low phosphorus contents of phytoplankton (Elser *et al*., 2000; Ferrão-Filho *et al*., 2003) as well as a large percentage of filamentous cyanobacteria (De Mott *et al*., 2001; Gliwicz, 1990) may pose problems to large cladocerans. Our lakes show no systematic gradient in nutrient limitation (Kosten *et al*., 2009). The percentage of cyanobacteria in the phytoplankton communities tends to be somewhat higher in warmer lakes though (Huszar *et al*., unpublished), and could potentially play a role.

While, physiological effects of temperature and of food quality cannot be excluded, there are other arguments that indicate a dominant role of fish predation as a driver of the body size pattern in zooplankton. Importantly, the decrement with latitude was strong for species typical of pelagic environments (e.g. *Daphnia*, *Moina* and *Ceriodaphnia*), but absent for species of comparable size usually associated with benthic/macrophyte habitats (i.e. *Latonopsis* and *Simocephalus*). Also the calanoid copepods in which we found a slight latitudinal fish-correlated size gradient are all typical of pelagic environments. It is generally acknowledged that vulnerability to fish predation is less in vegetation beds than in the pelagic environment (Burks *et al*., 2002), although in warmer lakes this refuge effect may be counterbalanced by high densities of fish encountered between the macrophytes (Meerhoff *et al*., 2007b). In any case, while food quality might in principle differ between habitats, it is hard to imagine how physiological temperature would affect only some of the taxonomic groups (or habitat groups) of zooplankton and not others, even if size ranges of these groups overlap.

A final important argument against the idea that temperature or food poses a limitation to zooplankton body size is the observation that zooplankton size structure typically shifts markedly towards larger sizes if fish density is sufficiently reduced in subtropical (Iglesias *et al*., 2007; Mazzeo, unpublished data) or tropical lakes (Okun *et al*., 2007; Pinto-Coelho, 1998).
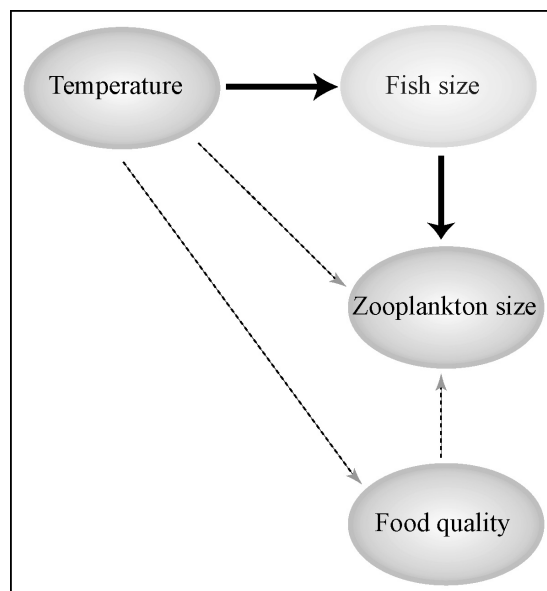

Figure 4.3 Hypothesized causality behind the latitudinal gradient in cladoceran body size. Heavy arrows represent strong effects, dashed arrows correspond to weak or unknown effects. We propose that a temperature related dominance by small individuals of fish communities in warmer lakes leads to a strong predation pressure on zooplankton, which selectively eliminates larger individuals.

In conclusion, our results suggest that size-dependent predation is a dominant force causing the observed latitudinal gradient in body size of lake cladocerans. This does not imply that other factors cannot play a role too. Experimental work would be needed to unravel potential confounding effects of other factors such as temperature and food quality. It seems quite likely that temperature is ultimately the driver of the gradient in zooplankton body size. However, causality may be indirect. In fact, physiological effects of temperature may well shape the latitudinal gradient in fish size (Chapters 2 and 3 of this thesis). We propose that the gradient in zooplankton body size may thus result from a cascading effect of the temperature driven increase in the density of small fish towards warmer climates (Figure 4.3).

4.6 References

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5. Reduced top-down control of phytoplankton in warmer climates can be explained by continuous fish reproduction

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5.1 Summary

Large herbivorous zooplankton and spring clear-water phases occur rarely in tropical lakes. It has been hypothesized that this can be explained by latitudinal differences in the structure of the trophic cascade. Many tropical fish species reproduce throughout the year and are omnivorous. Both properties could reduce the chances for large zooplankton to cause a clear-water phase. Continuous reproduction leads to permanent presence of planktivorous young individuals, and thus a continuously high predation pressure on large zooplankton. On the other hand, omnivory could allow fish to be more abundant, leading to a higher predation pressure on zooplankton. Data to distinguish between effects of continuous reproduction and omnivory boosted abundance are scarce. Here, we use a simple model to indicate that continuous fish reproduction in the tropics by itself can reduce the chances for top-down control of phytoplankton by *Daphnia* even if the carrying capacity for small fish would not be boosted by omnivory. The model predicts that only at very low fish densities, clear-water phases are possible, but these tend to occur at irregular times of the year. We also analyzed the effect of change in the mean annual temperature. This model analysis suggests that in temperate regions where fish reproduction is pulsed, climatic warming would tend to promote the chances for top-down induced clear-water phases.

5.2 Introduction

Top-down control of phytoplankton by large herbivorous zooplankton has been a major focus of research over the past decades. In many temperate lakes such top-down control causes the well-known spring clear-water phase (CWP) (Lampert *et al.*, 1986; Luecke *et al.*, 1990; Rudstam *et al.*, 1993; Sarnelle, 1993; Hanson & Butler, 1994; Townsend *et al.*, 1994; Jurgens & Stolpe, 1995). Remarkably, in tropical lakes chances for an effective top-down control over phytoplankton seem to be lower than in temperate lakes (Fernando, 1994; Lazzaro, 1997). Indeed, zooplankton species in tropical lakes and ponds are generally much smaller than in temperate zones and large herbivorous zooplankton of the genus *Daphnia* seems to be rare or absent at lower latitudes (Green, 1972; Burgis, 1973; Green, 1976; Hebert,

1978; Deevey *et al.*, 1980; Dumont, 1980; Fernando, 1980a; Fernando, 1980b; Fernando, 1980c; Foran, 1986; Fernando *et al.*, 1987; Dumont, 1994; Gillooly & Dodson, 2000). A better understanding of the mechanisms behind this apparent decrease in the chances for top-down control of phytoplankton, and thus the occurrence of CWPs, towards the tropics is important as it may enhance our ability to predict the implications of climate change for lake ecosystems. Several potential explanations for the pattern have been suggested.

One line of thought has been that high temperatures alone would prevent *Daphnia* from controlling phytoplankton at lower latitudes (Moore *et al.*, 1996). Cladoceran mean body size across latitudes is related to temperature, the mean body size being largest at mean annual water-body temperatures of 6-8 °C (corresponding to temperate regions, 50-60 °N) and smaller in colder and warmer temperatures (Gillooly & Dodson, 2000). This could be related to direct effects of temperature on these organisms, reducing growth rates and size at maturity (Moore *et al.*, 1996). However, this explanation is contradicted by laboratory experiments showing that large *Daphnia* are rather tolerant to high temperatures (Mitchell & Lampert, 2000; Giebelhausen & Lampert, 2001). Another factor that could be involved is the quality of food for *Daphnia*, which may decrease towards lower latitudes as the relative share of cyanobacteria may increase at higher temperatures (e.g., Weyhenmeyer, 2001). However this seems unlikely to be the main explanation of the latitudinal gradient in top-down control as some low-latitude lakes are not dominated by cyanobacteria, but still lack large *Daphnia*.

Probably the most important line of reasoning now is that stronger top-down control of large zooplankton by fish at lower latitudes is the main underlying mechanism (Jeppesen *et al.*, 2005). Good quantitative data on fish communities along a latitudinal gradient are scarce. Hence the top-down argument is inferred indirectly from two observations: 1) many fish species at low latitudes are omnivorous, and therefore not limited by zooplankton seasonal dynamics, as they can also feed on phytoplankton and detritus, thus reaching higher carrying capacities than strict zooplanktivores (Stein *et al.*, 1995; Lazzaro, 1997; Pinel-Alloul *et al.*, 1998) and 2) many fish species at low latitudes reproduce in several reproductive events during the year (Fernando, 1994; Lazzaro, 1997; Pinel-Alloul *et al.*, 1998) resulting in continuously high numbers of young individuals (Dumont, 1994; Jeppesen *et al.*, 2005) that maintain a high predation pressure on zooplankton during the whole year.

Although it seems plausible that omnivory may boost fish biomass resulting in stronger predation pressure on *Daphnia*, it is not *a priori* clear that this 'apparent competition'-like mechanism (Holt, 1977) should be stronger in the tropics. Omnivory is also common among temperate fish. Indeed, the dense fish community in temperate eutrophic shallow lakes is typically largely sustained by benthic food (Lindegaard, 1994) and many of these fish are really omnivorous causing a high predation pressure on zooplankton (Jeppesen

et al., 1996; Scheffer, 1998). While omnivory may help sustaining high biomasses of potentially planktivorous fish in the tropics, there has been no systematic study of the relative importance of this mechanism in temperate and tropical habitats so far. However, the difference between continuous reproduction in the tropics and annual pulsed reproduction of most temperate fish is well documented (Fernando, 1994; Lazzaro, 1997; Pinel-Alloul *et al*., 1998). It is not *a priori* obvious under which conditions this could really prevent *Daphnia* induced CWPs in the tropics. Generally, it is not enough to demonstrate that certain processes become stronger at higher temperatures, as temperature affects virtually all rates of biological and chemical processes, which makes the net effect on ecosystem dynamics quite unpredictable (Norberg & DeAngelis, 1997).

Here we explore a model of a seasonally forced simple food chain in an attempt to tease apart how the chances for clear-water phases would theoretically depend upon seasonality, the average fish predation pressure and temperature. Our results indicate which of the mechanisms is important and suggest questions and directions for future research.

5.3 Model description

Our model is based on a simple periodically forced algae (*A*) - zooplankton (*Z*) model, which has been shown to produce the occurrence of CWPs and other common patterns in shallow lakes with remarkable realism (Scheffer *et al*., 1997). The rationale behind the basic formulation has been described in various earlier contributions (Gragnani *et al*., 1999; Scheffer & Rinaldi, 2000; Scheffer *et al*., 2000; Malchow *et al.,* 2002). In absence of zooplankton the algae population grows logistically. The functional response of the zooplankton grazing the algae is Holling type II (Monod function). We accounted for spatial heterogeneity of zooplankton to obtain more realistic dynamics (Scheffer & De Boer, 1995). In this approach the algae-zooplankton cycles are stabilized by an import of algae from areas of the lake that are not affected by zooplankton grazing. Algae in these ungrazed parts are assumed to be at their carrying capacity (*K*), which is related to the productivity of the lake. The grazed algae are converted into zooplankton with efficiency *e*. In absence of predation by fish, the natural background mortality of the zooplankton is exponential with probability *m* per day. Predation of zooplankton by fish is modelled with a sigmoidal functional response (Holling type III). The basic formulation of the model is given in system (1) (symbols, see Table 5.1):

$$\frac{dA}{dt} = \mu_T \varphi_T(t) r A \left(1 - \frac{A}{\varphi_L(t)K} \right) - \mu_T \varphi_T(t) g Z \frac{A}{A + h_A} + i(\varphi_L(t)K - A) \left.\begin{matrix}\\\\\\\\\end{matrix}\right\}$$

$$\frac{dZ}{dt} = e\mu_T \varphi_T(t) g Z \frac{A}{A + h_A} - \mu_T \varphi_T(t) m Z - \mu_T \varphi_T(t) \varphi_F(t) F \frac{Z^2}{Z^2 + h_z{}^2}$$

(1)

We assume that the carrying capacity of algae (K) varies only with seasonally fluctuating light levels, defined by the forcing function $\varphi_L(t)$. The following parameters are assumed to be directly influenced by seasonal fluctuating temperature: the growth rate of algae (r), consumption rate of algae by zooplankton (g) and death rate of zooplankton (m). These are multiplied by the function $\varphi_T(t)$ and the mean temperature μ_T. The predation pressure on zooplankton by fish (F) is affected by both temperature and seasonality of reproduction and is thus multiplied by the functions $\varphi_F(t)\varphi_T(t)$ and the mean temperature μ_T. Although our model is functionally identical to the parent version (Scheffer *et al.*, 1997), we introduced the mean temperature μ_T and modified the formulation of the seasonal forcing function to:

$$\varphi_p(t) = 1 - a_p cos\left(\frac{2\pi t}{365} \right)$$

(2)

This function controls three different aspects of seasonality: light ($p=L$), temperature ($p=T$) and fish reproduction ($p=F$).

We will use the same relative amplitude for light and temperature and set $a_T = a_L$ in all our simulations, because data from Lewis (1987) suggests that these are very similar. The default parameter values are the same as the defaults in the parent model (Scheffer *et al.*, 1997). The new model formulation however, requires that some parameter values are converted (Table 5.1) as they are now defined as the yearly average value, instead of the yearly maximum value, thus we divided by the mid-summer value of the seasonal forcing in temperate regions (i.e. 1.7).

Table 5.1 Parameter dimensions and default values for system (1) (taken or adapted from Scheffer *et. al*, 1997).

Parameter	Value	Dimension	Description
R	0.3	d^{-1}	maximum algal growth rate
K	6	mg DW l^{-1}	algal carrying capacity
G	0.25	d^{-1}	maximum grazing rate of algae by zooplankton
h_A	0.6	mg DW l^{-1}	half-saturation algal concentration for zooplankton grazing
I	0.01	d^{-1}	inflow rate of algae from the ungrazed parts
E	0.6	-	efficiency of food conversion to growth
M	0.09	d^{-1}	mortality rate of zooplankton
h_Z	0.5	mg DW l^{-1}	half-saturation zooplankton concentration for
F	-	mg DW l^{-1}d^{-1}	maximum predation rate of zooplankton by fish
T	-	d	day of the year
a_T	0.2[1] or 0.7[2]	-	amplitude of temperature effects
a_L	0.2[1] or 0.7[2]	-	amplitude of light effects
a_F	0 or 0.7[3]	-	amplitude of fish reproduction pulse
μ_T	1	-	mean temperature effect

[1]: the value for the situation in tropical lakes

[2]: the value for temperate lakes

[3]: 0/ 0.7 for no/ yes reproduction pulse

5.4 Results

Under the assumption that the rates of biological processes double at every increase of 10°C (e.g., Scheffer *et al.*, 2001), and if the mean temperature is about 10-15 °C higher in the tropics (latitude 15° versus 55°) (Lewis, 1987), μ_T (which scales the effect of mean temperature) is approximately 2-4 times higher in the tropics than in temperate zones. The mean light intensity is not much higher in the tropics (Lewis, 1987), so this value is not varied in our analysis. We define a CWP in the model as a period when the algae concentration is ten times lower than the mean algae concentration during the summer ($t = 91–274$) (*cf.* Scheffer, 1998). The seasonal amplitudes of the effects of temperature (a_T) and light intensity (a_L) are assumed to be similar. Both will be much smaller in the tropics than in temperate zones. For the tropics both amplitudes are set to 0.2 based on mean amplitudes of water temperature and irradiance at latitude 15° (Lewis, 1987). For the temperate regions we used a value of 0.7 based on Scheffer *et al.* (1997) and Lewis (1987). Though this implementation of seasonality is very simple, it produced realistic seasonal patterns for temperate lakes (Scheffer *et al.*, 1997). Figure 5.1 shows the seasonal dynamics of system when continuous fish reproduction is assumed and all parameters are set at their default values. The parameter that causes the different dynamics is the predation by fish (F). In both the temperate and the tropical situation, the dynamics can vary from only a winter dip in the population density of algae to a winter dip plus one or more CWPs during summer. In the tropics, the difference between the summer and winter biomass is smaller and at low fish densities the number of cycles per year

much larger. Moreover, these cycles are less predictable in the tropics, because there is usually no phase locking, which means that the timing of the cycles differs each year.
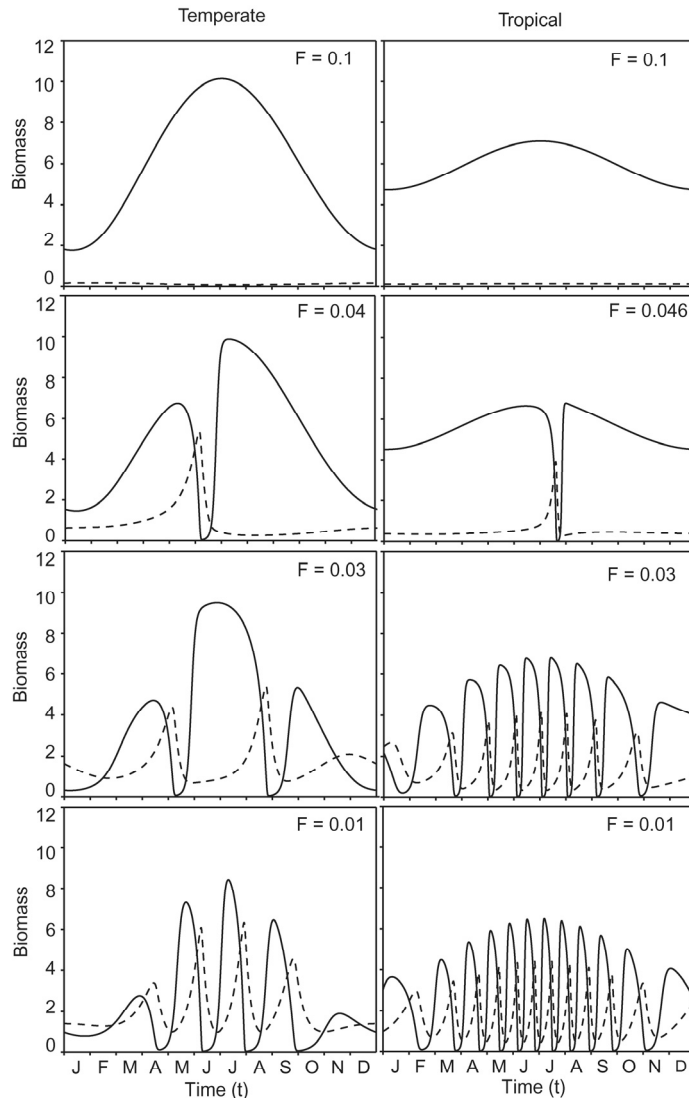

Figure 5.1 The yearly dynamics of the algae and zooplankton populations in temperate (left) and tropical lakes (right) under continuous fish reproduction. All parameters are at the default values as given in Table 5.1 except the value for predation by fish (*F*) (similar as in: Scheffer *et al.*, 1997). Algae and zooplankton are represented by drawn and dashed lines respectively.

To show how seasonality of fish reproduction ('pulsed reproduction') affects the reaction of the system to latitudinal gradients in temperature and light regimes, two bifurcation diagrams were computed (Figure 5.2). The surfaces in this figure connect the critical values of fish predation pressure *F* (i.e. maximum tolerated predation pressure of fish) for different values of a_T and a_L (scaling of the seasonal amplitude) and μ_T (scaling the effect of mean temperature). Above the surfaces, CWPs are absent, and below the surfaces, one or

more CWPs occur, which can be phase-locked (repeating the same patterns each year) or not. The latter occurs when seasonal forcing is weak (low a_L and a_T) (Scheffer *et al.*, 1997). A more precise description of the effects of seasonal forcing on phase locking can be seen in figure 5.3. This figure shows the effect of carrying capacity (*K*) and fish predation pressure (*F*) in different climates on the number of CWPs and whether or not the model is phase locked. Spring CWPs typically occur in temperate lakes with a high carrying capacity (interpretable as eutrophic conditions). With lower carrying capacities, the model predicts that there are no clear-water phases. With full seasonal forcing (temperature, light and fish reproduction), the same pattern repeats each year. With weaker seasonal forcing (no seasons and no seasonal reproduction peak), an area exists without phase locking. Temperature alone seems to have little effect: only the number of CWPs increases clearly with higher temperatures (Figure 5.3 left and right panels). Remarkably, phase locking also determines the way in which the cycles are lost at high fish densities. If the same pattern repeats each year, with increasing fish densities the number of cycles typically reduces, until there is only one clear-water phase left (Figure 5.4 a-c). Without phase locking, the patterns are very different. With increasing fish densities, the amplitude of the cycles reduces until it seems barely a wrinkling on the seasonal sine pattern (Figure 5.4 d-f). Note also that without phase locking the cycles are lost in a very small parameter range.
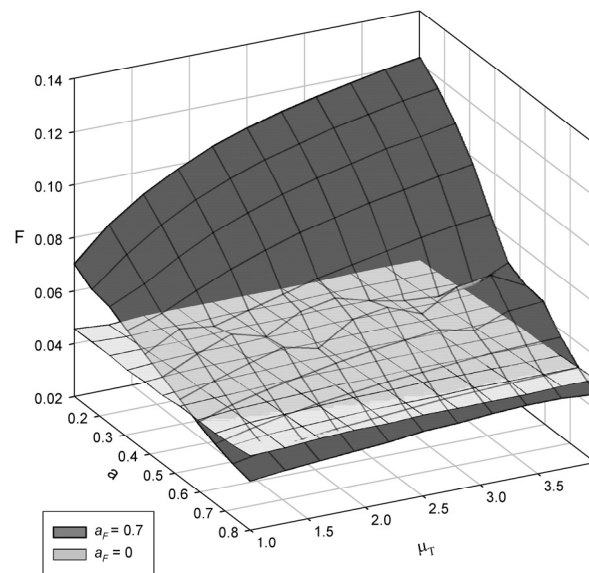

Figure 5.2 Predicted critical mean fish predation pressure (*F*) for allowing a CWP as a function of seasonal amplitudes ($a = a_T = a_L$) of light and temperature (varied simultaneously) and mean temperature (scaled by μ_T). Seasonal reproduction of the fish leads to the dark shaded separating surface and continuous reproduction to the light-separating surface. CWPs occur in the simulations below the separating surfaces.

If pulsed reproduction of fish is included in the model ($a_F = 0.7$), the model predicts that CWPs occur at higher fish densities in the tropics (high μ_T and low a), compared with temperate zones (low μ_T and high a). However, if fish is assumed to reproduce continuously, CWPs are restricted to situations with low fish densities, rather independently of mean temperature or seasonality in temperature and light. This shows that the fish reproduction pulse is an important factor in the model.
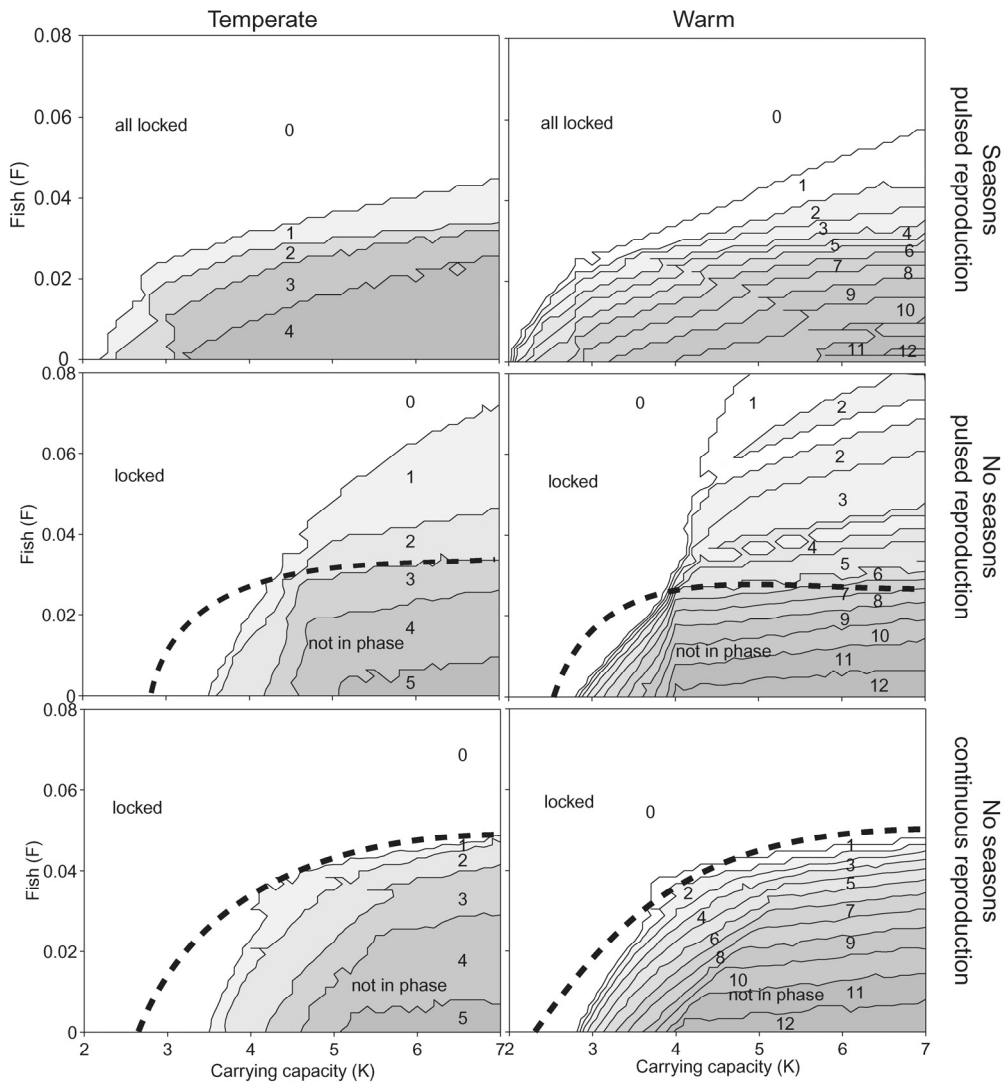

Figure 5.3 The mean number of CWPs per year. The values indicate the number of CWPs of an area. CWPs were calculated by counting periods when the algae concentration is ten times lower than the mean algae concentration during the summer ($t = 91–274$) and dividing these by the number of simulated years (50). The thick dashed line separates areas with and without phase locking, which was determined by visual inspection of runs. Parameters: Temperate: $\mu_T = 1$; Warm: $\mu_T = 3$; seasons and pulsed reproduction: $a_T = 0.7$, $a_L = 0.7$ and $a_F = 0.7$; no seasons and pulsed reproduction $a_T = 0.2$, $a_L = 0.2$ and $a_F = 0.7$; no seasons and continuous reproduction $a_T = 0.2$, $a_L = 0.2$ and $a_F = 0$
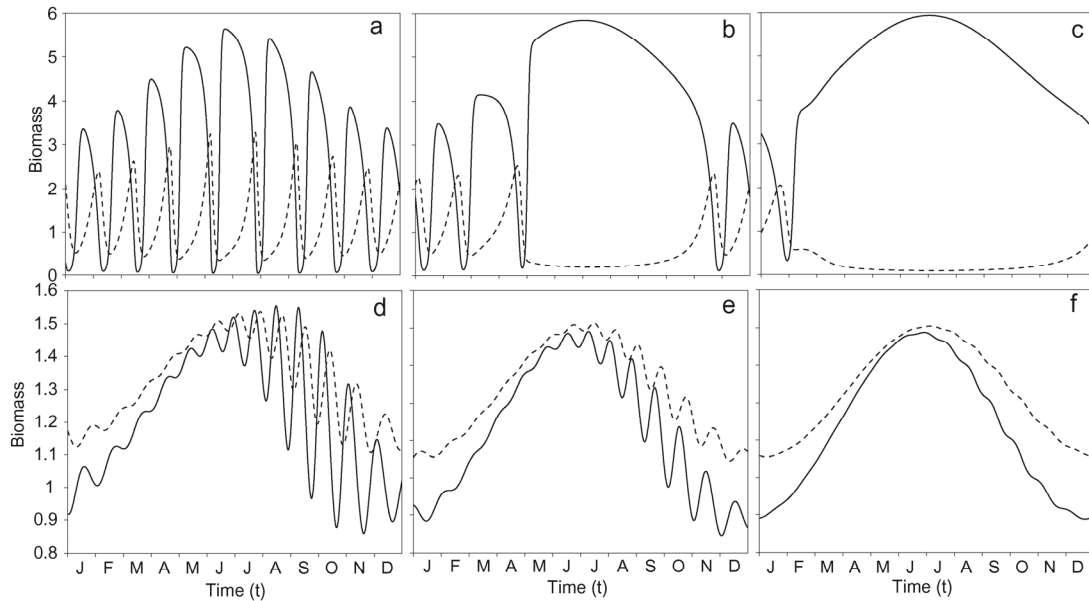
Figure 5.4 Two ways of losing cycles with increasing fish biomass in the model. The examples are from the warm situation with reproduction pulse of fish ($\mu_T = 3$, $a_F = 0.7$, $a_L = 0.2$ and $a_T = 0.2$). Upper panels: in a high productive ($K=5$) situation the number of cycles reduces gradually a. $F = 0.02$, b. $F = 0.04$, c. $F = 0.08$. Lower panels: in a low productive ($K=3$) system the amplitude of the predator-prey cycles reduces to zero. In this situation there is no phase locking. d. $F= 0.0160$, e. $F = 0.0162$, g. $F=0.0165$.

5.5 Discussion

In this paper, we introduced a simple model with seasonal forcing to gain more insight into the apparent lack of clear-water phases in tropical lakes. We use this model as a first step towards understanding of this phenomenon. The model results suggest that continuous fish reproduction in the tropics is an important factor. In contrast, higher temperatures and lower seasonal variation in temperature and irradiance seem to be unlikely explanations as these factors can promote CWPs in the model. The model results also indicate that the shift in reproductive regime is unlikely to be the sole explanation, as the predicted critical mean fish predation level for CWPs does not differ much between the tropics and the temperate parameter settings.

Our conclusions are in line with observations in a Indian lake (Nagdali & Gupta, 2002), which illustrates the importance of fish in tropical lakes. In this lake, 80% of the fish community was killed by a fungal infection. Immediately, this event was followed by an increase in water clarity and zooplankton density. However, the continuous fish reproduction makes this system resilient, and the fish community restored very rapidly within 3-4 months.

Our model analysis suggests also that there still is a possibility for periodicity in (sub)tropical systems if fish densities are low. While this disagrees with most observations that phytoplankton is almost constant in tropical lakes (e.g., Schelske *et al.*, 2003; Gonzalez *et al.*, 2004), we found time series of two subtropical South-African reservoirs that both show seasonal patterns in zooplankton (Hart, 2004). These lakes were monitored in bi-weekly intervals for a period of 10 years. Though large *Daphnia* species were present in relatively low numbers during the full year, some other species (e.g. *Diaphanosoma excisum*) were clearly restricted to a part of the year (in this case to the summer).

We tried to minimise the complexity of our model. This way, a thorough analysis of this model is possible. However, this approach has also disadvantages, as many mechanisms may be oversimplified or discarded (Van Nes & Scheffer, 2005). One has to bear in mind that in reality there are more mechanisms important than described in our simple model. The model focuses on top-down effects of large zooplankton species, but there may be other reasons for a collapse of phytoplankton in spring. Suggested other mechanisms include nutrient depletion (Huppert *et al.*, 2002; Van Nes & Scheffer, 2004; Huppert *et al.*, 2005), grazing by much smaller rotifers and nauplii (Mayer *et al.*, 1997) or possibly parasite attacks on phytoplankton (Reynolds, 1984). Nutrient depletion seems a good candidate for causing CWPs in low productive systems. Also we did not describe the effects of other grazers in tropical systems that might be important, such as herbivorous tilapias (Fernando, 1994). The dominance of blue-green algae in tropical systems is also proposed as an important factor (Abrantes *et al.*, 2006). As this is a low quality food source for zooplankton, top-down control of cyanobacteria could be less effective. As there are many more potential explanations, we can only conclude that continuous reproduction is a possible candidate mechanism for explaining the lack of CWPs. An alternative approach would be to analyse a more complex ecosystem model that includes many more of these processes. There are many of such models published (e.g. Janse, 1997; Zhang *et al.*, 2004; Arhonditsis & Brett, 2005). Some of these describe (sub)tropical lake systems (Angelini & Petrere, 2000; Zhang *et al.*, 2003; Bruce *et al.*, 2006; Hu *et al.*, 2006; Naithani *et al.*, 2007). Disadvantage of these complex models, is not only that they can be hard to understand, but also they are usually applied to special situations. An interesting follow-up of our work would be to use a moderately complex ecosystem model to analyse effect of latitude and temperature on the system behaviour. Our analysis shows that pulsed reproduction of fish should be an important ingredient in such model for predicting CWPs.

Remarkably, our model analysis (Figure 5.2) suggests that an increase in mean temperature per-se would rather enhance the chances for a CWP to occur, though the parameter space where there occurs one spring CWP is much smaller (Figure 3). This is in line with the finding that CWPs in Dutch lakes are more common in warmer years (Scheffer

et al., 2001; but see: Jeppesen *et al.*, 2003; Van Donk *et al.*, 2003) and model studies show that the cycles become larger with increasing temperature (Norberg & DeAngelis, 1997). However, this contrasts the observation that the chances for CWPs decrease along the latitudinal gradient. Therefore, it seems unlikely that the temperature is a dominant factor that determines the latitudinal gradient of plankton dynamics. In the model, phytoplankton and zooplankton are modelled dynamically and can therefore respond to temperature by changing their abundance whereas fish only responds by increased consumption rates. Obviously, this may only be realistic for studying short-term dynamics such as the occurrence of a single spring CWP before the fish reproduction event in temperate lakes. This illustrates the importance of distinguishing between the response to long term climatic warming and the effects of single warm years on ecosystems (Mehner, 2000). Therefore, year-to-year correlations to temperature are less informative than climatic gradients for inferring the potential effects of gradual climate change.

Certainly, the functioning of trophic cascades in lakes differs widely over a latitudinal gradient. Our model analysis suggests that continuous fish reproduction is a good candidate mechanism that can explain the differences between tropical and temperate shallow lakes. Systematic experimental studies along the gradient seem the most appropriate way to test this hypothesis. The relative importance of the proposed mechanisms could be studied by creating and analyzing a more complex ecosystem model, in which most mechanisms are included. Such research is essential if we are to predict the potential implications of future climate change for lake ecosystems.

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6 The role of subtropical zooplankton as a predator of phytoplankton under different predation levels

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6.1 Summary

Large-sized zooplankton such as *Daphnia* play a fundamental role as predators in phytoplankton communities in temperate lakes. These organisms are scarce at subtropical lakes where smaller-sized cladocera or copepods take this niche. However, such smaller grazers appear to be less able to exert an effective top-down control on the phytoplankton community. We experimentally analyzed the ability of zooplankton typical of subtropical, nutrient-rich lakes, to graze effectively on the phytoplankton community. We conducted two outdoor mesocosm experiments in a hypertrophic lake, with combinations of three different zooplankton densities and three different omnivorous fish densities. In the first experiment the zooplankton community was dominated by a small-sized Cladocera (*Moina micrura,* Kurz 1874) and in the second by a Calanoid copepod (*Notodiaptomus incompositus*, Brian 1925). The phytoplankton community also differed between experiments, with dominance of larger size classes in the first experiment than in the second. Both experiments showed a strong negative effect of fish on the largest zooplankton, and revealed that a small fish quantity was sufficient to eliminate the larger species. Fish presence had positive effects on the largest phytoplankton size fraction (30-100 μm) in the first experiment. This effect was more pronounced in combination with high zooplankton biomass, indicating that nutrient recycling in these communities may have been an important mechanism promoting phytoplankton growth. None of the zooplankton communities had significant top-down effects on the phytoplankton community, and in view of the phytoplankton species that dominated the communities, inedibility, toxicity and anti-grazer defences may well explain the absence of significant effects of zooplankton grazing. Our results suggest that in subtropical nutrient-rich lakes, drastic removal of small omnivorous fish may be needed to allow an increase zooplankton biomass. In addition, our results imply that for such a change to result in effective top-down control of phytoplankton a shift in community composition is essential too, as the experimental increase in abundance of the present communities of small-sized grazers had little effect on the phytoplankton communities.

6.2 Introduction

In temperate lakes, the key role of large-sized *Daphnia* in trophic cascades is well known (Carpenter *et al.*, 1985). Thus, increasing their abundance is an important goal of the biomanipulation techniques meant to improve transparency in eutrophic lakes (Perrow *et al.*, 1997). Even though *Daphnia* is present in subtropical regions, their body size does not always resemble the size found in comparable temperate lakes (Gillooly & Dodson, 2000). Rather, typical representatives of the pelagic mesozooplankton in subtropical lakes are smaller-sized Cladocera (i.e the genera *Moina, Ceriodaphnia* and *Bosmina*), and Calanoid copepods (Crisman & Beaver, 1990; Jeppesen *et al.*, 2007).

The predation impact of zooplankton communities on phytoplankton is related to their body size as well as taxonomic composition (Cyr & Curtis, 1999). Copepods can eat larger particles than some cladocerans (Peters & Downing, 1984), and use a mixture of passive and active strategies of collection for small and large particles respectively (Vanderploeg, 1981). Small-sized cladocera and copepods feed on a narrower size range of algae and have lower grazing rates on edible algae than large-sized *Daphnia* (Cyr & Curtis, 1999). In view of these differences, the effectiveness of subtropical zooplankton as predators of the phytoplankton community is believed to be limited compared to temperate zooplankton. However, grazing in subtropical communities has also been studied much less than in temperate systems.

Fish predation is considered a major factor controlling crustacean zooplankton in subtropical lakes (Jeppesen *et al.*, 2007; Meerhoff *et al.*, 2007; Havens *et al.*, 2009; Teixeira de Mello *et al.*, 2009; Chapter 4 this thesis). Hence, it seems logical that a sufficient reduction of the predation pressure on zooplankton by removal of fish could promote top-down control of phytoplankton biomass. In principle, release from fish predation should result in increased mesozooplankton abundance in subtropical lakes, and indeed some examples confirm this possibility (Iglesias *et al.*, 2007). However, a concomitant increase in mesozooplankton body size, biomass, or shift in taxonomic composition towards large-sized *Daphnia* (>1.5 mm) is usually not observed (Crisman & Beaver, 1990).

Therefore, controlled experiments are needed to explore whether such ecological engineering methods that are being applied in temperate systems, might work in shallow (sub)tropical lakes too (Rondel *et al.*, 2008). For instance, it might be that the absence of large cladocera in (sub)tropical lakes is not only due to fish predation, but also to the presence of inedible cyanobacteria (Havens *et al.*, 2000) or physiological effects of higher water temperature (Crisman *et al.*, 1995).

Our aim in this study was to explore the effectiveness of zooplankton typical of subtropical, nutrient-rich lakes, to suppress the phytoplankton community by manipulating the level of zooplankton and fish abundance. For that we analyzed two different zooplankton communities at three density levels in two outdoor mesocosms experiments, one dominated by a small-sized Cladocera (*Moina micrura*, Kurz 1874) and the other by a Calanoid copepod (*Notodiaptomus incompositus*, Brian 1925). The underlying hypothesis is that in the absence of fish predation, high concentrations of zooplankton will reduce the phytoplankton biomass, while fish presence will hamper such phytoplankton control.

6.3 Methods

Our research was conducted in Lake Rodó a hypertrophic lake under restoration in Montevideo-Uruguay (35º55'S 56º10'W). Lake characteristics and restoration techniques applied during the period 1997-2001 are described elsewhere (Rodríguez-Gallego *et al.*, 2004; Scasso *et al.*, 2001; Kruk *et al.*, 2002). We conducted two experiments (A and B) in ca. 80-litres transparent plastic mesocosms incubated during 5 days. In both experiments bags were filled with equal amounts of lake water filtered through a 50 μm-sieve to remove zooplankton. After that we added the zooplankton organisms from a concentrate obtained after repeated 68 μm-net tows. Hereto, the density of each taxonomic group (i.e. rotifers, copepods, nauplii, and cladocerans) in the concentrate was determined. The amount of concentrate needed to obtain zooplankton as in the lake (Z1) and 10 times the concentration in the Lake (Z2) was calculated. Duplicate samples were taken from the concentrate, of which one was counted to check if the expected density was reached, and the duplicate was added to the designated enclosure. Hence, we added 3 different zooplankton densities: no zooplankton (Z0), densities similar to what was found in the lake at the moment of the experiment (Z1), and high densities of 10x those in the lake (Z2) (Table 6.1). Zooplankton densities in Z2 mimic what would be theoretically required to obtain a filtration rate high enough to control phytoplankton under our experimental conditions (Reynolds, 1984). In the case of fish we added one species (*Cnesterodon decemmaculatus*, Jenyns, 1842) in 3 densities: no fish (P0), low densities = 4 fish (P1) and high densities = 10 fish (P2). *C. decemmaculatus* is a small-bodied Poecilidae with broad distribution in South American subtropical region (Rosa & Costa, 1993). It is a visual omnivorous fish with a high preference for large-bodied zooplankton (Quintans *et al.*, 2009; Quintans, unpublished data), and can achieve extremely high abundances (Scasso *et al.*, 2001). All fish specimens were acclimated in separate bags before addition. All treatments were randomly assigned to the enclosures, and replicated three times. In each experiment (A and B) the zooplankton and phytoplankton communities exhibited different characteristics (Table 6.2, Figure 6.1).

Table 6.1 Experimental design during each experiment (A & B)

	Z0	Z1	Z2	D
P0	A&B	A&B	A&B	A
P1	A&B	A&B	A&B	A
P2	A&B	A&B	A&B	A

Experiment A. This experiment was run in spring 2000. The phytoplankton community was dominated by filamentous cyanobacteria, particularly *Aphanizomenon gracile* (maximum linear dimension, MLD=90.7 μm) (Figure 6.1). Cladocerans were the dominant grazers in the natural zooplankton community, and *Moina micrura* (average size=0.60 mm) the dominant species (Table 6.2). Since cladocera species in this experiment were small-sized, we included an extra treatment where only *Daphnia obtusa.* (D) was present (average size=1.5 mm) in order to compare with a large-sized grazer (Table 6.1). *Daphnia* specimens for this extra treatment were obtained from cultures.
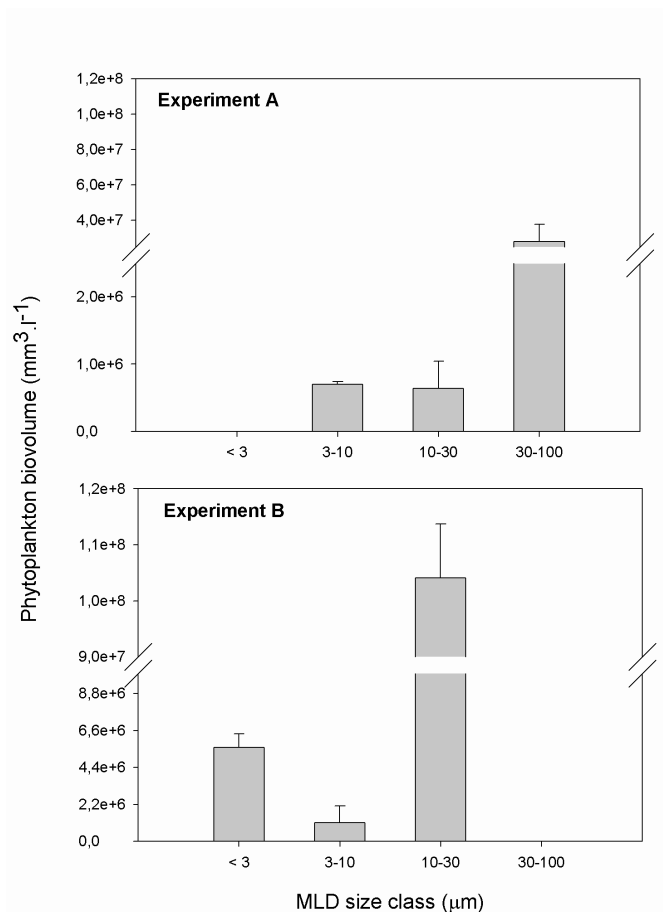

Figure 6.1 Lake. Phytoplankton biovolume divided in size classes according to the maximum linear dimension (MLD) of each species at the beginning of each experiment (A and B).

Experiment B. This experiment was run in summer 2002. Phytoplankton biovolume was lower than in the previous experiment and dominated by the chlorophyte *Monoraphidium griffithii* (MLD=28 μm) (Table 6.2, Figure 6.1). The zooplankton community biomass was also lower than experiment A, but in this case the calanoid copepod *Notodiaptomus incompositus* (average size=0.80 mm) was the dominant grazer (Table 6.2).

At the beginning and end of each experiment we measured temperature (T), dissolved oxygen (DO), conductivity (K), pH, and Secchi disk depth (SD) in the lake and inside each mesocosms. We also took water samples for soluble reactive phosphorus (P-PO$_4$) and ammonium (N-NH$_4$) concentrations. Nutrient concentrations were estimated following standard methodology (Murphy & Riley, 1962; Koroleff, 1970).

Table 6.2 Characteristics of the lake and the phytoplankton and zooplankton communities at the beginning of each experiment.

	Experiment A	**Experiment B**
	Phytoplankton	
S (n° of species)	27	27
Dominant species (MLD μm)	*Aphanizomenon gracile* (90.7)	*Monoraphidium griffithii* (28)
Total phytoplankton biovolume (mm^3.l^{-1})	29.29	110.81
Dominant size fraction (μm)	30-100	10-30
	Zooplankton	
S (n° of species)	18	-
Dominant species (mean length μm)	*Moina micrura* (600)	*Notodiaptomus incompositus* (800)
Total biomass (μgDW.l^{-1})	49.5	44.0
Dominant size fraction (biomass μgDW.l^{-1})	20.9	33.7
	Lake	
Maximum depth (m)	0.80	-
Secchi disk (m)	0.20	0.40
Temperature (°C)	19.9	20.2
Dissolved oxygen (mg l^{-1})	12.4	17.2
pH	8.13	8.13
Conductivity (mS cm^{-1})	812	-
N-NH$_4$ (μg l^{-1})	101.2	28.9
P-PO^4 (μg l^{-1})	16.69	1.24

At the end of each experiment we took water samples from each mesocosm for phytoplankton and zooplankton analysis. Phytoplankton samples were taken with a bottle and preserved in lugol solution. Samples were counted in a 1-ml Sedgewick-Rafter chamber following the methodology of Utermöhl (1958). Phytoplankton units (cells and colonies) were

enumerated in random fields. Phytoplankton biovolume was approximated according to Hillebrand *et al*. (1999). The remaining water in the mesocosms (70-80 litres) was filtered through a 50-µm sieve and preserved in 4 % neutralised formaldehyde for zooplankton analysis. All zooplankton samples were counted using 2-5 ml Sedgewick-Rafter chambers following Paggi & José De Paggi (1974) criteria.

Data analysis. Normality of all variables was tested with Kolmogorov-Smirnov test and homogeneity of variances with Levene test. We used Two-Way ANOVA with fish and zooplankton densities as fixed factors. When variables were not normally distributed, we used non-parametric Kruskall-Wallis analysis. Results were considered significant at *p<0.05*.

6.4 Results

Experiment A. In the absence of fish, manipulation of the zooplankton community had no effect on the phytoplankton biomass ($F_{3,6}$ = 0.137; *p = 0.934*) or community composition (Figure 6.2). The distribution of the different size classes was similar among treatments (*p ≥ 0.404*) and the size class 30–100 µm dominated in all treatments (Figure 6.2). This size class was comprised of cyanobacteria, mainly the filamentous *A. gracile* with an average length of 91 µm. Total biovolumes were slightly lower than at the beginning of the experiment, because the zooplankton filtration had also removed some of the cyanobacteria filaments. Zooplankton was, as expected, absent in the zooplankton-free enclosures, but showed an overall decline in the fish-free enclosures compared to the start of the experiment (Figure 6.3). Nonetheless, the effects of fish were obvious. In all treatments with fish, zooplankton biomass was lower than in the fish free enclosures and larger sized zooplankton (cladocera and copepods) were affected more by fish than the smaller sized rotifers.
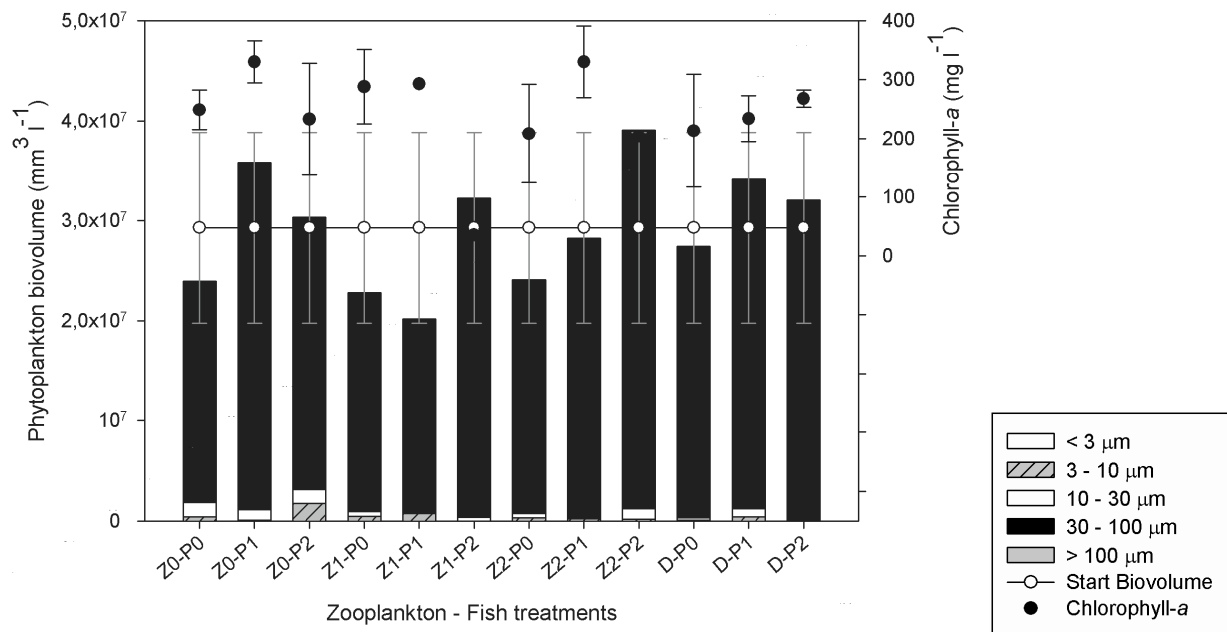
Figure 6.2 *Experiment A.* Phytoplankton biovolumes at the end (bars) and the start (open symbols) of the 1st enclosure experiment, including different size fractions and end chlorophyll-a concentrations (filled symbols) at the end of a 5-day incubation period. Z0= no zooplankton, Z1 = similar to the lake, Z2 = 10 times the lake, P0 = no fish, P1 = 4 fish, P2 = 10 fish. D=*Daphnia*.

Although fish appeared to have a strong negative effect on zooplankton biomass (Figure 6.3), the two-way ANOVA with fish and zooplankton densities as the fixed factors revealed neither a statistically significant fish effect ($F_{2,14}$ = 2.21; *p = 0.147*), nor a zooplankton effect ($F_{3,14}$ = 2.67; *p = 0.088*) or an interaction between the two factors ($F_{6,14}$ = 0.98; *p = 0.475*). However, it should be noted that loss of two Z1-P1 replicates influenced the statistical analysis. The overall trend of the impact of fish on zooplankton is more pronounced when all Z1 and Z2 treatments are used in a linear regression ($F_{1,17}$ = 4.95; *r = 0.475; p = 0.040*) against fish densities. The model: *Zooplankton biomass=33.5– 2.88*Fish density* clearly revealed the negative relation between *C. decemmaculatus* and zooplankton. More specifically, the presence of these fish had a negative effect on cladocera biomass (χ^2=10.53, *p=0.005*). In fact, at the end of the experiment no cladocera were found at all in the mesocoms with Z1 and Z2 treatments that also contained fish (Figure 6.3). In these mesocosms the negative effects were strongest on *Moina micrura* (χ^2=9.42, *p=0.009*), which was the dominant cladocera species. A strong fish predation was also observed in the mesocosm containing only *Daphnia sp.* although in this case, statistical differences were marginally significant (χ^2=5.58, *p=0.061*). Enclosures with fish had higher phytoplankton

biovolume ($\chi^2=6.68$, *p=0.035*), due to an increase in their largest size fraction (30-100 μm), composed of the filamentous cyanobacterium *A. gracile* ($\chi^2=6.80$, *p=0.033*) (Table 6.2, Figure 6.2). This effect was more pronounced in mesocosms containing either *Daphnia sp.* or high zooplankton densities (Figure 6.2).
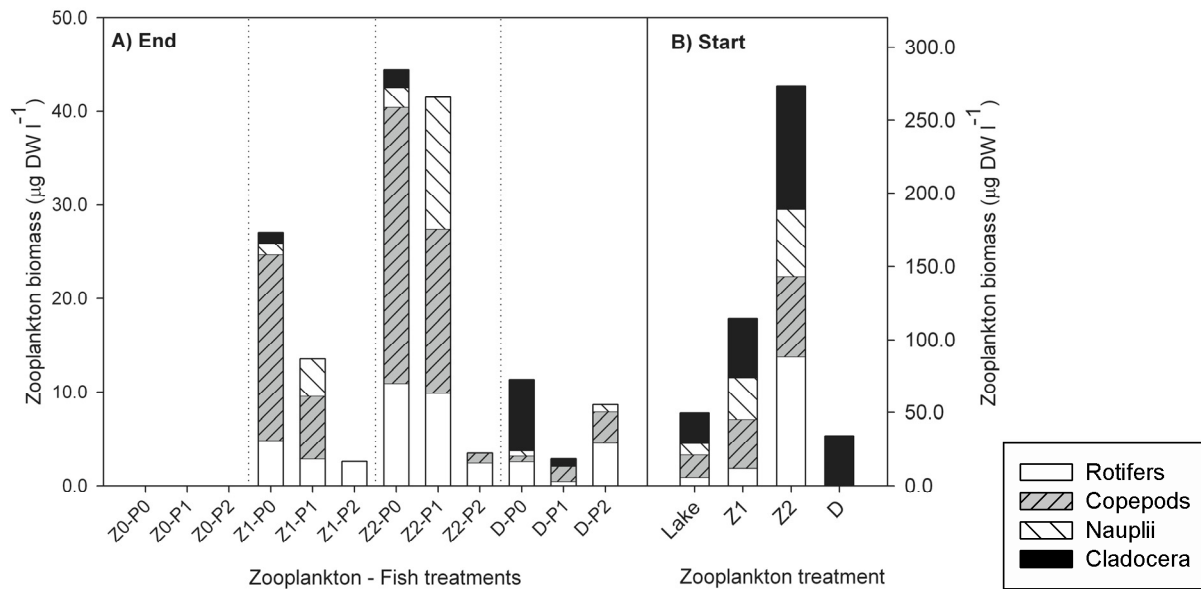

Figure 6.3 *Experiment A.* Zooplankton biomass at the end (panel A) and at the start (panel B) of the enclosure experiment.

The pH and conductivity were similar among treatments and on average 8.80 (± 0.04) and 710 (±22) μS.cm^{-1}, respectively. The chlorophyll-*a* concentrations matched the phytoplankton biovolume-estimates (Figure 6.2). Dissolved nutrients N-NO$_3$, N-NH$_4$ and P-PO$_4$ were all in the same order of magnitude (Figure 6.4). The mass N:P ratio in all enclosures was low and on average 2 to 3, which might explain the dominance of a N$_2$-fixing cyanobacterium. Phosphate concentrations tended to increase with higher fish stocking (Figure 6.4). Mesocosms with only *Daphnia sp.* tended towards higher N-NH$_4$ concentrations than the other treatments ($\chi^2=9.60$, *p=0.022*) (Figure 6.4), particularly in the presence of fish. However, differences between Z1 and Z2 treatments were less clear (Figure 6.4).
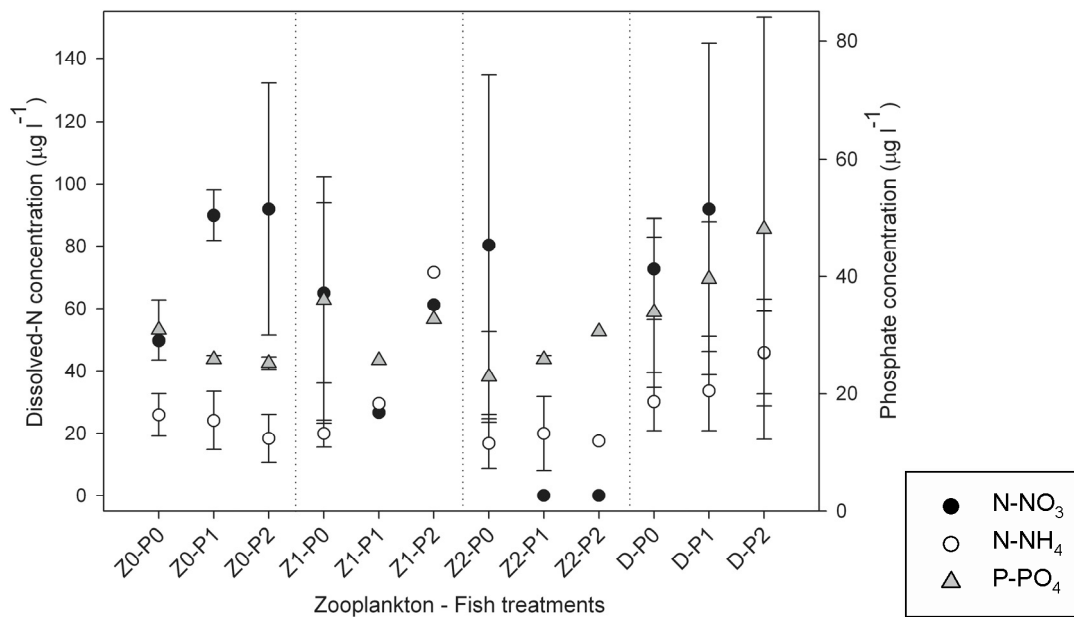
Figure 6.4 *Experiment A.* Concentrations of dissolved nutrients (N-NO$_3$, N-NH$_4$ and P-PO$_4$) in the enclosures. Z0= no zooplankton, Z1 = similar to the lake, Z2 = 10 times the lake, P0 = no fish, P1 = 4 fish, P2 = 10 fish. Error bars indicate 1 SD. D=*Daphnia*.

Experiment B. As in the first experiment, manipulation of the zooplankton community in experiment B had no effect on the phytoplankton biomass ($F_{2,5}$ = 1.08; *p = 0.407*) and community composition, even in the absence of fish (Figure 6.5). The distribution of the different size classes was similar among treatments (two-way ANOVAs; *p ≥ 0.163*) and the size class 30–100 μm dominated in all treatments (Figure 6.5). This size class was comprised mainly of *Monoraphidium griffithi*, which showed an increase in size in all treatments from *ca.* 29 μm at the start to *ca.* 48 μm at the end of the experiment. In contrast to the expectation, rotifers showed a tremendous growth in the "zooplankton-free" enclosures compared to the starting conditions (Figure 6.6). In the presence of fish, however, this growth was suppressed substantially (Figure 6.6). Copepod biomass in the high zooplankton treatments (Z2) was lower in mesocosms containing fish (χ^2=2.54, *p=0.051*), where also cladocera were diminished (Figure 6.6). Overall, there was no fish effect ($F_{2,17}$ = 1.66; *p = 0.220*) on the total zooplankton biomass.
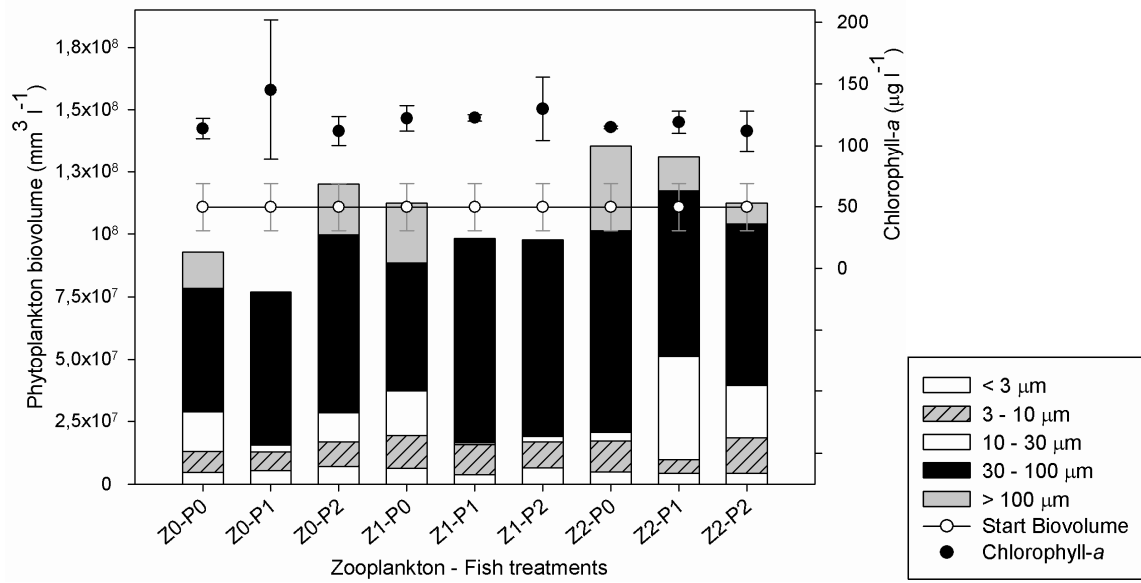
Figure 6.5 *Experiment B.* Phytoplankton biovolumes at the end (bars) and the start (open symbols) of the 2nd enclosure experiment, including different size fractions and end chlorophyll-a concentrations (filled symbols) at the end of a 5-day incubation period. Z0= no zooplankton, Z1 = similar to the lake, Z2 = 10 times the lake, P0 = no fish, P1 = 4 fish, P2 = 10 fish
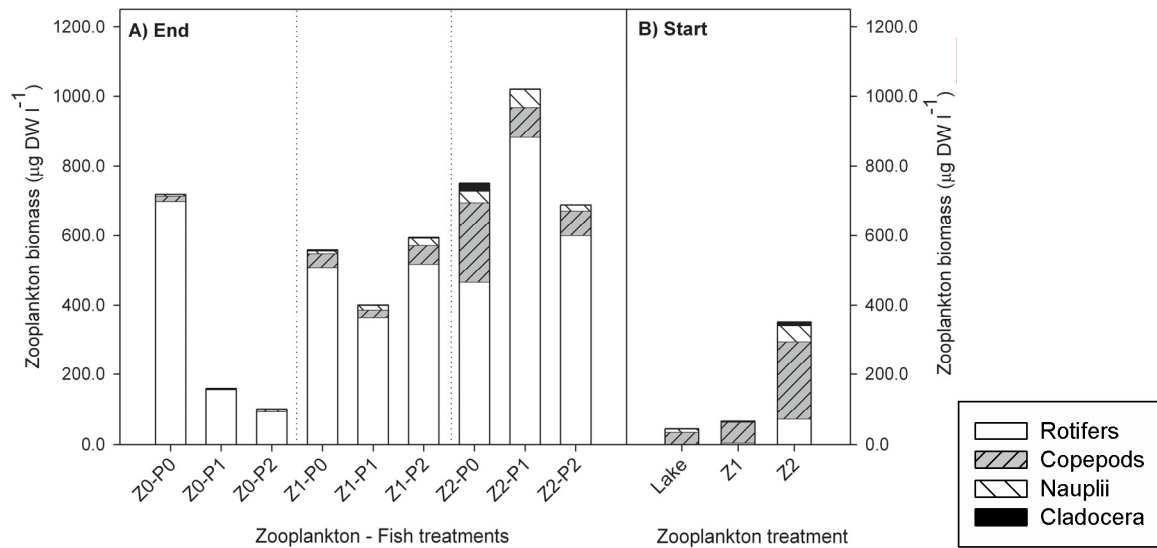

Figure 6.6 *Experiment B.* Zooplankton biomass at the end (panel A) and at the start (panel B) of the enclosure experiment. Z0= no zooplankton, Z1 = similar to the lake, Z2 = 10 times the lake, P0 = no fish, P1 = 4 fish, P2 = 10 fish.

The pH and conductivity were similar among treatments and on average 8.74 ($\pm$ 0.07) and 939 ($\pm$ 32) μS.cm^{-1}, respectively. The chlorophyll-*a* concentrations were on average 145 ($\pm$ 50) μg.l^{-1} (Figure 6.5). Dissolved nutrients N-NO$_3$, N-NH$_4$ and P-PO$_4$ showed no differences among treatments (Figure 6.7). The mass N:P ratio in all enclosures varied between 19 to 39. The oxygen concentrations in the enclosures were 17.1 ($\pm$ 1.3) mg l^{-1}.
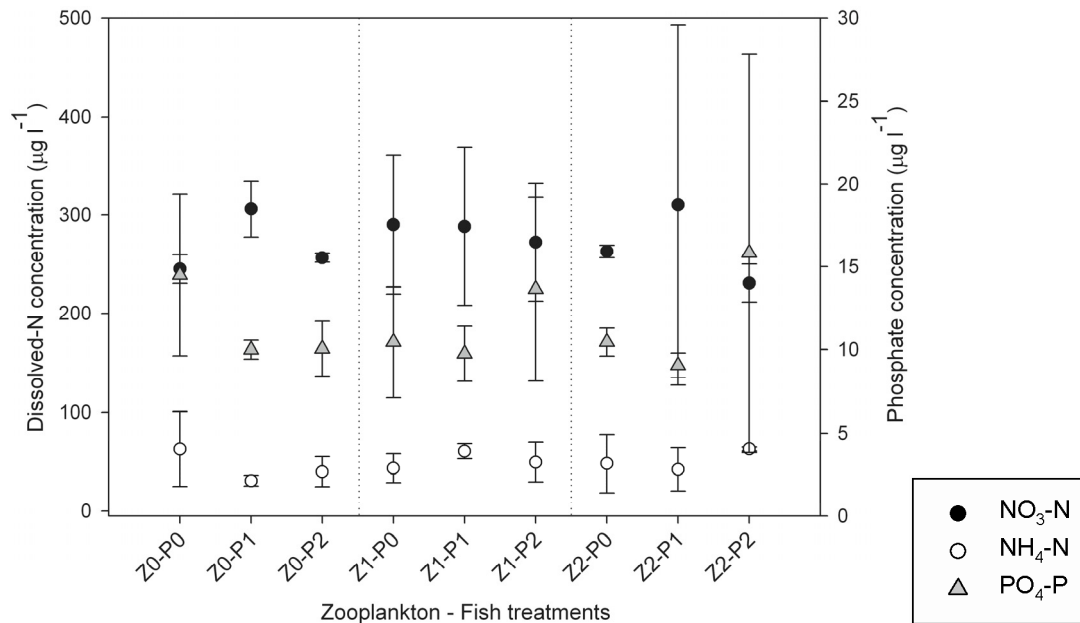

Figure 6.7 ***Experiment B.*** Concentrations of dissolved nutrients (N-NO$_3$, N-NH$_4$ and P-PO$_4$) in the enclosures. Z0= no zooplankton, Z1 = similar to the lake, Z2 = 10 times the lake, P0 = no fish, P1 = 4 fish, P2 = 10 fish. Error bars indicate 1 SD.

6.5 Discussion

Our experiments show that small-sized herbivorous zooplankton typical of subtropical, nutrient-rich lakes had limited ability to impose an effective top-down control on the phytoplankton community, even on edible phytoplankton size classes and in the absence of fish predation. Increased densities of the natural zooplankton communities did not have any effect on the phytoplankton community, although theoretically grazing rates designed for the Z2 treatments could have cleared the entire volume of the enclosures during the experiments. Such apparent uncoupling of the zooplankton-phytoplankton interaction has been described for other subtropical regions (Malthus & Mitchell, 2006; Havens, 2002; Crisman & Beaver, 1990). Our results support the variance-inedibility hypothesis (Holt & Loreau, 2002), which states that trophic cascades only occur when trophic levels are dominated by species edible to the next trophic level (Polis *et al*., 2000). Interestingly, in

experiment B the phytoplankton community was considered to be edible to the zooplankton, but no grazing down of the phytoplankton community occurred. By contrast, an increase in the size of the dominant species *M. griffithii* was observed, which could indicate an inducible defense against the explosive growth of rotifers. Such morphological changes in chlorophytes are well-known anti-predator strategies (Lürling, 2003).

Moreover, top-down control was also not found in the fish-free treatments with *Daphnia* addition included in experiment A. This might be explained by the filamentous cyanobacterial dominance (*A. gracile*) in the phytoplankton community during this experiment. Filaments can have a negative effect on the clearance rate of large-bodied grazers such as *Daphnia*, with longer filaments having a stronger effect than shorter ones (Gliwicz & Lampert, 1990; De Mott *et al.*, 2001). However, the length of *A. gracile* in experiment A was *ca*. 91 μm and earlier studies suggest that consumption of this size of filaments is possible. For example, Fulton (1988) showed that *Daphnia* consumed *Anabaena flos-aquae* filaments with a length of 111 (±18) μm, while longer filaments of *Aphanizomenon flos-aquae* (210 ±24 μm) and other *Anabaena* species (ranging from 233 to 423 μm) were not consumed. Similarly, *Planktothrix rubenscens* measuring <100 μm were preferably ingested by adult *Daphnia pulicaria* over longer filaments up to 984 μm (Oberhaus *et al.*, 2007). Although no feeding experiments have been performed, the overall negative effect on the relatively large-bodied *Daphnia* might also point to toxicity effects of this cyanobacteria species (Pereira *et al.*, 2004). Finally, and similar to observations in a tropical lake by Rondel *et al.* (2008), our results clearly demonstrate that manipulation of the fish stock, or even elimination of it, may not be enough to control a cyanobacterial bloom.

Strong size-selective predation of fish on the zooplankton community was evident in the two experiments we conducted. These results are consistent with findings from other mesocosm experiments in the region (Iglesias *et al.*, 2007; Boveri & Quirós, 2007), as well as from field data (Scasso *et al.*, 2001; Havens, *et al.*, 2002; Mazzeo *et al.*, 2003). Moreover, from similar experiments in tropical regions Okun *et al.* (2007) inferred that the mere presence (rather than particular densities) of omnivorous fish appears to guarantee a major top-down control in warm-lake food webs. In line with this idea, our results show that low fish densities are sufficient for the virtual elimination of the largest zooplankton size fraction in all zooplankton densities and community compositions tested.

Fish had a positive effect on phytoplankton during our first experiment were *Aphanizomenon gracile* was the dominant species. This effect was more pronounced when combined with high densities of natural zooplankton or *Daphnia* treatments. In this situation N-NH$_4$ concentrations were higher too, while elevated phosphate concentrations were found at the higher fish densities. Nutrient excretion by fish and zooplankton may therefore have

favoured the observed phytoplankton development. Nitrogen may have been important as a limiting nutrient as N:P ratios were less than 3.

It may well be that the absence of a significant reduction of phytoplankton biomass in experiments without fish is due to the relatively short duration of our experiments. During previous years in Lake Rodó, Scasso *et al*., (2001) observed a short increase in mesozooplankton abundance and a coincidental decrease in the phytoplankton community, resulting in higher water transparency. The mesozooplankton increment occurred immediately after fish removal procedures and was caused by an increase in small-sized cladocera (*M. micrura* and *Daphnia pulex*) (Scasso *et al.*, 2001). It should be noted, however, that the cause of the subsequent clear water phase is not unequivocal, as silica depletion may also have played a role in driving the collapse of the dominant diatom species (Kruk, C. *unpublished data*). Nonetheless, other studies have shown that relatively large cladocerans can occasionally develop and graze down phytoplankton in sub-tropical and tropical lakes in response to drastic fish removal (Boveri & Quirós, 2007).

In conclusion, while longer term absence of fish may in principle allow zooplankton communities to develop and control phytoplankton in warm lakes, our results illustrate that a mere increase in densities of the existing zooplankton community may typically not be enough to cause such a top-down effect. In practise, reduction of fish-stock as a tool to control phytoplankton may therefore be of little use as fish populations in these systems typically recuperate at very fast rates, and a situation with very low fish densities will be difficult to maintain long enough to allow the zooplankton community to truly restructure and control phytoplankton (Jeppesen *et al.*, 2007).

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7 Synthesis

The hilarious sci-fi novel "The Hitchhiker's Guide to the Galaxy" by Douglas Adams tells how a supercomputer called "Deep Thought" was built to answer the "great question of life, the universe and everything else". The answer was 42, to everybody's amazement, as they realised nobody really new what the question was. While readers around the world wonder what the number 42 meant, I just think Adams was witty enough to tell us that the important thing is to have, and ask, questions. In the chapters of this thesis I took different angles at the question how climate affects the food webs of lakes. Here I bring the bits and pieces of answers I found together, to sketch the bigger picture. Certainly that picture is incomplete, and I will highlight gaps in our understanding throughout, suggesting more questions for future research.

7.1 Why are fish smaller in warm lakes?

One of the most marked patterns revealed in this thesis is the phenomenon that small-sized fish predominate in lakes at higher temperatures (Chapters 2 and 3). The cause of this pattern remains unclear. However, one idea is that it might be associated with energy limitation under warmer conditions (Atkinson, 1994; Arim *et al.*, 2007). Would fish become smaller as the future climate becomes warmer? Although we do not understand the factors driving fish size distributions well, it is interesting that a reduction in average body size of fish in response to warmer conditions is already noted in some regions. For example, the average body size of fish communities in French rivers has decreased in the last couple of decades, together with an increase in the number of juveniles and maturation at smaller sizes (Daufresne *et al.*, 2009).

Surely basic physiological mechanisms must be involved in causing the temperature effect on fish body size. However, in Chapter 2, we note a remarkable detail in the fish size pattern that may point at the existence of a community scale feedback mechanism regulating size structure in fish communities. Rather than a gradual increase in the fraction of small fish, we see a tendency for communities to be dominated by either large fish or small fish. More precisely we show that the equilibrium fraction of small fish appears to be best described by a catastrophe fold, suggesting that a small fish dominated state and a large fish dominated situation might represent alternative attractors. Various authors have proposed potential mechanisms for such alternate body-size states (De Roos & Persson, 2002; Carpenter *et al.*,

2007; Walters & Kitchell, 2001). While the precise mechanism cannot be inferred from our data, our findings take this idea an important step further. Firstly, they suggest that the existence of alternative body-size states may be a general phenomenon in lake communities. Secondly, they imply that the probability for a lake to be in the small fish-dominated state may increase as temperatures rise. This implies the prediction that in warmer climates, pressures such as fishing for the largest individuals could change a large-fish dominated state into a small-fish dominated state more easily. Also, it suggests that tipping the system into a state dominated by larger piscivorous fish, and therefore with lower densities of small fish should be more difficult in warmer lakes than in colder lakes, where such biomanipulation is commonly used to invoke a trophic cascade controlling algal biomass. Unravelling the mechanisms behind the apparent climate effects on the community size structure of fish remains a major challenge that would most likely require controlled experiments in the laboratory as well as realistic scale field experiments along a climate gradient.

7.2 Why is fish is less predacious in warmer lakes?

In Chapter 3 the stable isotope analysis of differently sized fish points at another reason why top-down control of small fish by big ones seems less likely in the tropics. While in cold lakes large fish appear to forage higher in the food web than small fish, in warmer lakes their isotopic signature suggests that they actually forage at equal or even lower levels in the foodweb than their small counterparts. This is consistent with a range of observations from other studies. It has been found that omnivory in fish is widespread at warmer regions (Lazzaro, 1997; Winemiller, 1990) and that lakes in warm regions are often inhabited by generalist predators rather than true piscivores (Quirós, 1998).

While the field patterns are suggestive of a temperature driven functional shift towards foraging at lower trophic levels, it remains intriguing what could be the fundamental mechanism behind this change. One explanation might be that fish can assimilate plant material more easily at higher temperatures and thus rely more on it (Behrens & Lafferty, 2007), however, there is much scope for further research here. One promising line of research would be to focus on omnivorous genera that have a wide distribution in South America (i.e *Astyanax*). One may collect specimens from the same species or closely-related species at various sites and use them in feeding experiments with a range of temperatures to test the relative effect of diets based on combinations of animal and plant material. Also, the resolution of unravelling the foodweb structure in the field may be enhanced by considering other stable isotopes. For instance, detritus-based pathways may be quite important, and sulphur isotopes (^{34}S) can be used as an indicator of such pathways (Croisetière *et al.*, 2009).

7.3 Why is zooplankton smaller in warmer lakes?

Our findings in Chapter 4 show that just as in fish, body size also decreases for zooplankton from higher to lower latitudes. While it seems reasonable that physiological effects of temperature might drive this pattern we argued that in the case of zooplankton the main driver of the latitudinal pattern is a shift in predation pressure from fish. One argument is the fact that a rather large percentage of the variation in zooplankton body size could be explained from the fraction of small fish in the community despite the fact that this is obviously a rather rough indicator of fish predation. Another indicator that fish predation might be the dominant factor in driving the body size patterns of zooplankton is that the body size difference with latitude was more pronounced for species that are more sensitive to fish predation. For instance, the pattern was more pronounced for pelagic species than for species of comparable sizes but usually found among macrophytes or close to the bottom. As we argued that body size patterns in fish are driven by temperature, these results imply that the influence of temperature on zooplankton body-size differences may be a result of a cascading effect of the temperature-driven decrease in fish size.

The results of our mesocosm experiments (Chapter 6) further support the idea that fish prevents larger-sized zooplankton from becoming abundant in the water column of (sub)tropical lakes. Even low densities of a typical small omnivorous species of fish were enough to practically eliminate the larger sized zooplankton from the enclosures. Although we found strong evidence for the idea that fish predation explains the lack of larger zooplankton in warmer lakes, this does not exclude the possibility of confounding effects of other factors such as temperature and food quality on zooplankton. For example, low phosphorus content of phytoplankton (Elser *et al.*, 2000; Ferrão-Filho *et al.*, 2003) or filamentous cyanobacteria dominance (DeMott *et al.*, 2001; Gliwicz, 1990) may alter competition outcomes between large and small zooplankton, favouring smaller forms. Results from our SALGA project suggest no systematic patterns in nutrient limitation along the climate gradient (Kosten *et al.*, 2009). However, the percentage of cyanobacteria in the phytoplankton communities is higher in warmer lakes (Huszar *et al.*, unpublished), and this might be another factor pushing the balance towards smaller zooplankton. Clearly, further experimental work would be needed to unravel the effects of fish predation from the effects of food quality and from possible directs effects of temperature on zooplankton physiology.

7.4 Is a seasonal window of opportunity essential for zooplankton?

While the effect of higher temperatures is an obvious focus of research, seasonality is another possible ingredient of climate effects on foodwebs. It has been noted that while freshwater fish species in temperate systems have a seasonal pattern of reproduction,

(sub)tropical lakes fish species may spawn several times during the year (Lazzaro, 1997). The seasonal reproduction cycle in temperate lakes results in peaks in abundance of small fish biomass in early summer (Wooton, 1984). Importantly, this also implies that prior to the reproduction season there is a minimum in small fish biomass, implying a 'window of opportunity' for zooplankton in spring. It is difficult to perform experiments on realistic scales to tease the effects of temperature and seasonality on the foodweb dynamics apart. However, an alternative way to explore the potential effects of seasonality in isolation is through modelling. In Chapter 5, we use a minimal model revealing that in principle, continuous fish reproduction by itself can lead to reduction of large-sized *Daphnia* populations and a reduction of the chances of top-down control of phytoplankton. While these results suggest that not only low average temperatures, but also seasonality in fish reproduction might be essential to allow zooplankton-induced clear water phases in lakes, our modelling approach has been rather simple so far. It would be important to understand better under which conditions a synchronized annual reproduction of fish changes to a pattern of more-or-less continuous reproduction activity, and how this affects the seasonal dynamics of predation pressure on zooplankton, in relation to the variation in temperature and food dependent zooplankton growth dynamics.

7.5 Why no top-down control of phytoplankton in warmer lakes?

It is generally thought that the community of small sized zooplankton may have little potential to graze down the biomass of the typical warm-lake phytoplankton communities. However, experimental evidence for this idea has been lacking. We confirmed such 'uncoupling' unequivocally in our mesocosm experiments where we exposed the phytoplankton community of a subtropical lake to artificially altered densities of the ambient zooplankton community. Probably the lack of effects of raised zooplankton on the phytoplankton community was due to inedibility, toxicity and anti-grazer defence of phytoplankton (Chapter 6). However, our experiments cannot resolve causality here. A deeper insight might be obtained from competition experiments including cladocerans of different sizes exposed to phytoplankton of different nutritional quality.

A major limitation of this experimental work is obviously the short time scale. Perhaps time was simply insufficient for a different zooplankton community to develop that could have more effect on the phytoplankton community. Indeed, other studies have suggested that even in warm lakes, large zooplankton can flourish and control phytoplankton, provided that fish is drastically reduced for a sufficient time (Iglesias *et al*., 2007). The key issue appears to be that this situation is simply rare in warm climates. Phrased in terms of alternative stable states theory: perhaps the small fish dominated state is very resilient in warm lakes whereas a state with few small fish has a very small basin of attraction or is not stable at all. In any case, the emerging picture from this thesis and other recent studies is that

as temperatures rise, an ecosystem state with low densities of small planktivorous fish becomes increasingly unlikely, with cascading consequences for the zooplankton and phytoplankton communities.

7.6 A final thought

The results presented in this thesis and a companion thesis by Sarian Kosten hint at what might change in the communities of shallow lakes as temperatures rise due to global warming. However, our findings also illustrate the limitations of interpreting patterns in field data. Indeed, it was obvious from the very first moment of our SALGA project that while exciting patterns would emerge; numerous questions would probably be generated. Surely, we are still far from a profound understanding of what drives the structure and functioning of aquatic food webs as climate changes. The answer to that question, for the time being, remains most definitely 42.

7.7 References

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Curriculum vitae

Gissell Lacerot (1974) obtained her BSc degree in Biology (option Oceanography) at the Facultad de Ciencias-Universidad de la República in Montevideo-Uruguay. After a brief period studying the effect of pollution in estuaries at the Oceanography Department she joined the Limnology Department, where she specialised in zooplankton taxonomy and ecology and studied this community in various aquatic ecosystems: reservoirs, streams, and shallow lakes.

In 2001 she obtained a NUFFIC fellowship (The Netherlands Organization for International Cooperation in Higher Education) to follow her MSc studies at Wageningen University (WUR) in The Netherlands. There, she specialised in the ecology of shallow lakes and the effect of eutrophication on the zooplankton community. Her thesis focused on zooplankton community succession in a shallow and hypertrophic lake, during its restoration process. She graduated *cum laude* as Master in Environmental Sciences in 2003. Prof. Dr. Marten Scheffer, chair of the Aquatic Ecology and Water Quality Management Group at WUR invited her to join an upcoming project dealing with latitudinal differences in the functioning of shallow lakes. The South America Lake Gradient Analysis (SALGA) project gave her the opportunity to pursue her interest in the differences between the trophic cascades of (sub)tropical and temperate lakes. In 2004, she and Dr. Scheffer applied for and obtained a Developing Countries Fellowship from The Netherlands Organisation for Scientific Research (NWO-WOTRO division) to follow her PhD studies in the SALGA project. Her PhD addressed the question how climate affects food webs and trophic interactions in shallow lakes. Particularly, she focused on the question how climate shapes the size structure of fish and zooplankton communities, and how this affects the strength of the trophic cascade from fish to plankton.

In the upcoming future, she plans to continue working on how diversity patterns and the body size structure of aquatic communities change with climate, and how these changes modify the functioning of aquatic ecosystems.

Netherlands Research School for the
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CERTIFICATE

The Netherlands Research School for the
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(SENSE), declares that

Gissell Lacerot Silveira

Born on: *9 September 1974* at: *Montevideo, Uruguay*

has successfully fulfilled all requirements of the
Educational Programme of SENSE.

Place: *Wageningen* Date: *6 April 2010*

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The SENSE Research School declares that Ms. Gissell Lacerot Silveira has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 41 ECTS, including the following activities:

SENSE PhD courses:

° Environmental Research in Context
° Research Context Activity: " Organizing international workshop on 'Twenty Years of Limnology in Uruguay: History and Perspectives' (Montevideo, 20 November 2005"
° Life history theory

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° Writing of PhD project proposal
° PhD Competence Assessment
° The Art of Writing

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° Research and laboratory work, Federal University of Rio Grande do Norte (URGN), Brazil
° Laboratory work and Thesis data discussion, National Institute of Limnology (INALI), Argentina
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° Collaborator Organising Committee Shallow Lakes Congress: Structure and Function of World Shallow Lakes
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° South America Lake Gradient Analysis Project (SALGA) Workshop I, 3-10th January 2007, Vitoria, Brazil
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° South America Lake Gradient Analysis Project (SALGA) Workshop II, 22th November 2007, Punta del Este, Uruguay
° SENSE/EPCEM Symposium (Cutting edge of freshwater ecology), 10th October 2008, Wageningen, Netherlands
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