Top-down and bottom-up effects in shallow lake food webs with special emphasis on periphyton

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Dedication

I would like to dedicate my PhD thesis to all Egyptian martyrs of revolution, January 25, 2011 and beyond. Greeting for the loyal sons of Egypt who sacrificed their lives for our freedom.
Whether that they are Muslims, Christians,
males, females, children, adults, rich or poor.
With my thanks and appreciation
Aldoushy Mahdy
Berlin, 2014

أهداء

أود أن أهدي رسالتي لجميع شهداء الثورة المصرية، 25 يناير 2011 وما بعدها.
تحية لأبناء مصر المخلصين. الذين ضحوا بحياتهم من أجل حريتنا
سواءاً أن كانوا مسلمين، أو مسيحيين، ذكور، أو إناث،
أطفالاً أو بالغين، أغنياء أو فقراء مع شكري وتقديري
الدوشى مهدى
برلين 2014

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General Introduction

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Summary

The main objective of the current thesis was to determine the ecological relevance of top-down (predator driven) and bottom-up forces (nutrient driven) processes in shallow lake food webs with specific interest on periphyton ecology. We used three different methodological approaches: small scale laboratory microcosm experiments, lake based mesocosms experiments, and analysis of long-term field records. In the lake based mesocosm experiments we manipulated nutrient dynamics by establishing two nutrient scenarios and temperature by performing the same periphyton experiments across a temperature gradient in 5 European countries (Estonia, Germany, Czech Republic, Turkey, and Greece). In laboratory microcosms experiments we investigated the role of invertebrate grazers for periphyton removal. Finally, in long-term records we analyzed underlying mechanisms driving the abrupt changes of two coexisting copepod species in the context of regime shift- and intraguild predation theory.

Periphyton communities play important roles in shallow lakes and are controlled by direct forces, such as temperature, light, nutrients, invertebrate predation and indirectly by cascading fish predation effects. We manipulated nutrient dynamics by establishing two nutrient scenarios**;** (clear, 25µg TPL^{-1}) and (turbid, 200µg TP L^{-1}) and used the temperature gradient across the 5 European countries. All mesocosms contained a layer of sediment; (10 cm thickness); macrophytes (*Myriophyllum spicatum*), planktivorous fish, and phyto- and zooplankton species assembles, which were collected from 5 different lakes in each country. Periphyton biomass was colonized 4 weeks on artificial strips exposed at 50 cm mesocosm water depth during mid-summer (July and August 2011). The results indicated that water temperature plays an important positive role for the summer periphyton biomass development. The slope of the regression between water temperature and periphyton biomass suggests that for every degree increase in water temperature, summer periphyton biomass was stimulated positively. Even though, our nutrient loading (TP) was implemented in low and high nutrient treatments (20 µg TP L^{-1}, 65 µg TP L^{-1}, respectively), there was no significant nutrient effect on periphyton biomass. We found indirect positive top-down effects by fish on periphyton biomass (chl *a*) due to the control of periphyton scraping invertebrates grazers. The slope of the regression between fish biomass and periphyton chl *a* adjusted to water temperature suggests the top-down control of invertebrate grazers by fish.

In laboratory experiments, we compared the effect of *D. magna* on periphyton removal with a naturally associated invertebrate community dominated by chironomid larvae and chironomid larvae only. The impact of pelagic *Daphnia magna,* which migrates into the littoral zone to seek refuge from fish predation, on periphyton removal is still unknown. Periphyton biomass was significantly reduced by *D. magna*, the natural grazer community, as well as by chironomids during a two-week laboratory experiment performed at 20°C. In accordance with allometric theory, the larger chironomid larvae had higher individual periphyton removal rates than *D. magna*, whereas body mass specific periphyton removal rates of *D. magna* was 58%; higher than that of chironomids. We concluded that the role of large planktonic cladoceran such as *D. magna* for periphyton removal can be significant and deserves further attention.

We studied the long-term dynamics (30 years) of two cyclopid copepod species (*Cyclops vicinus* and *Cyclops kolensis)* along a declining gradient in trophic state in a shallow, eutrophic lake. We showed that while under high nutrient conditions and thus high algal biomass the larger *C. vicinus* is the superior competitor, it looses its advantages under medium productivity levels, because it is a less effective exploiter of algal resources. We further predicted that the release of the smaller *C. kolensis* from the competition and predation pressure by *C. vicinus* enabled its sudden and conspicuous dominance of the winter cyclopoid community. We further accessed the threshold density of the competitor necessary for this shift to happen. Underlying mechanisms of that non-linear threshold driven response of both copepod species were tested by using two scenarios of regime shift theory in the context of intraguild predation theory. Not before the larger competitor, which exhibit higher requirements for algal resources, declined below a critical threshold in abundance the coexisting smaller copepod species, exhibiting lower needs for algal prey, was able to thrive in the low algal food niche.

Finally, I provide some critical remarks on the advantages and drawbacks of the different methods applied in my thesis which ranged from small to large scale experiments to decadal long-term field studies. Small scale laboratory experiments have the advantage of full control of the tested driving forces, by lacking the inherent complexity of interactions within ecosystems. Some of that complexity was met in our enclosure experiments by including sediment, macrophytes and fish in our tanks. Long-term ecological research has the advantage that the outcome of any seen dynamic results from the complex interplay of external and internal driving forces. The challenge here is, however, to disentangle the multifarious overlapping driving forces.

Zusammenfassung

Das Ziel dieser Arbeit ist es, die ökologische Relevanz von Top-down (Räuber gesteuerte) und Bottom-up (Nährstoff gesteuerte) Prozessen in der Nahrungskette von Flachseen zu erfassen, insbesondere mit Blick auf die Ökologie des Periphytons. Dafür haben wir drei verschiedene Methoden benutzt: Mikrokosmen-Laborexperimente, Mesokosmen-Experimente im See und die Analyse von Langzeitdaten des Müggelsees. Die Mesokosmen Experimente im See wurden unter zwei Nährstoffszenarien (niedrig/hoch) und entlang eines Temperaturgradienten über fünf Europäische Staaten (Estland, Deutschland, Tschechien, die Türkei und Griechenland) durchgeführt. Mit den Mikrokosmen-Experimenten im Labor haben wir das Beweiden von Periphyton durch verschiedene Invertebraten quantifiziert. Die Zeitreihenanalyse wurde u.a. genutzt um ökologische Theorien mit empirischen Felddaten zu testen; in diesem Fall wurde die Regime Shift Theorie mit den Zeitreihenverläufen zweier Copepoden Spezies getestet.

Periphytongemeinschaften spielen eine wichtige Rolle in der Nahrungskette von Flachseen, und die Periphytonbiomasse wird direkt von Faktoren wie Temperatur, Nährstoffangebot oder Beweidung durch Invertebraten beeinflusst. Die Periphytonbiomasse wird außerdem auch indirekt durch die Anwesenheit von zooplanktivoren und benthivoren Fischen (trophische Kaskade) beeinflusst. Um den Einfluss von diesen direkten und indirekten Faktoren zu untersuchen, wurden in Mesokosmen-Experimenten je zwei Nährstoffszenarien (klar, 25µg TP L^{-1}; trüb, 200µg TP L^{-1}) entlang des Nord-Süd Europäischen Temperaturgradienten durchgeführt. Jeder Mesokosmos wurde mit einer 10 cm tiefen Lage Sediment versehen und mit Makrophyten (*Myriophyllum spicatum*), Dreistacheligen Stichlingen (*Gasterosteus aculeatus*) und lokalen Phyto- und Zooplanktongesellschaften bestückt. Die lokalen Planktongesellschaften wurden aus Populationen von je fünf verschiedenen Seen pro Land gemischt. Um den Bewuchs von Periphyton zu erfassen wurden im Sommer (Juli und August 2011) Plastikstreifen in 50 cm Tiefe in jeden Mesokosmos exponiert. Die Wassertemperatur spielte eine Hauptrolle bei der Entwicklung von Periphytonbiomasse im Sommer. Eine Regressionsanalyse zeigte, dass die Sommer Periphytonbiomasse um 42 % pro 1 °C Temperaturanstieg zunahm, während für die unterschiedlichen Nährstoffkonzentrationen kein signifikanter Effekt auf die Biomasse festgestellt werden konnte. Des Weiteren fand ich einen positiven indirekten Effekt des Fischbestandes auf die Entwicklung der Periphytonbiomasse (gemessen als Chlorophyll *a*), aufgrund der trophischen Kaskade von Stichlingen auf periphytonweidende Invertebraten. Eine

Vorhersage der Effekte von interagierenden Faktoren erweist sich als sehr schwierig, da die Effekte der Interaktionen nicht immer der Summe der Effekte der einzelnen Faktoren entsprechen. Periphytonbiomasse wird direkt durch weidende Invertebraten wie zum Beispiel Wasserflöhe (Cladocera) und/oder Larven der Zuckmücken (Chironomidae) entfernt (als Sammelbegriff für Beweidung und Turbulenz induzierte Entfernung von Biomasse). Die Rolle von pelagischen Wasserflöhen wie *Daphnia magna* (die in der Litoralzone Schutz vor Frassfeinden suchen) bei der Entfernung von Periphyton ist ungeklärt. In Laborexperimenten haben wir die Rate der Periphytonreduktion durch verschiedene Weidegänger wie *D. magna*, Chironomidenlarven und die im Feld mit Periphyton assoziierteWeidegängergesellschaft (meist dominiert von Chironomiden Larven) quantifiziert und verglichen. Nach zwei Wochen Expositionszeit und bei einer Temperatur von 20 °C hatte jeder der getesteten Weidegänger die Periphytonbiomasse signifikant reduziert. Entsprechend ihrer größeren Körpermasse hatten Chironomidenlarven individuenbasiert eine höhere Periphytonreduktionsrate als *D. magna* Körpermassenspezifisch erreichte *D. magna* jedoch 58 % höhere Periphytonreduktionsraten. Daraus lässt sich schließen, dass große, planktische Wasserflöhe wie *D. magna* eine wichtige Rolle bei der Reduktion von Periphyton spielen können.

Eine Analyse der 30 Jahre langen Verläufe von zwei Ruderfusskrebsspezies (cyclopoide Copepoden; *Cyclops vicinus* und *Cyclops kolensis*)zeigte dass die größere Art *C. vicinus* der überlegene Konkurrent unter einem hohen Nahrungsangebot war, da *C. vicinus* höhere Ansprüche an die Menge der vorhandenen Ressourcen hat. Des Weiteren konnten wir zeigen, dass die kleinere Art *C. kolensis* von der Reduktion der Konkurrenz um gemeinsame Resourzen- und des Räuberdrucks durch *C. vicinus* profitierte. Dies erklärt die plötzliche Zunahme und Dominanz von *C. kolensis* unter Bedingungen reduzierter Algenbiomasse und gleichzeitiger Abnahme von *C.* vicinus unterhalb einer kritischen Abundanzgrenze. Das nichtlineare Antwortverhalten der beiden Copepodenarten folgte der Vorhersage zweier Szenarien aus der Regimesshift Theorie. Durch Regressionsanalysen und durch die Anwendung dieser Regimeshift-Szenarien im Kontext der Intraguilt-Predation-Theorie konnten wesentliche zugrundeliegende Treiber der gegenläufigen Entwicklung der beiden Copepodenarten quantifiziert werden.

Thesis Outline

The thesis presented here is a cumulative work and is based on two parts, the first part deals with questions revolving around the role of bottom-up and top-down forces on periphyton biomass in shallow lakes (Chapters II–IV), the second part deals with the effects of changes in food availability and of intraguild predation on two copepod species (*Cyclops vicinus* and *Cyclops kolensis*) in a shallow lake (Ch. V). The thesis is presented in a cumulative way of one published paper (Ch. V), one submitted manuscript (Ch. IV), and two manuscripts are in preparation (Chs. II and III). These articles are presented separately as independent chapters (Chs. II-V). Each chapter includes its own introduction, materials and methods, results, discussion and reference parts (Chs. II–VI). The thesis also contains a general introduction (Ch. I) and final discussion that connect my findings to previous works and provides an outlook on questions that remain to be investigated (Ch. VI). All manuscripts have been reprinted with kind permission of the respective publishing house. However, the layout of the published or currently under-review manuscript was modified to be fit with the thesis layout.

This dissertation is based on the following articles:

Chapter II:

Frank Landkildehus, Martin Søndergaard, Meryem Beklioğlu, Rita Adrian, David G. Angeler, Josef Hejzlar’, Eva Papastergiadou, Priit Zingel, Ayşe İdil Çakiroğlu, Ulrike Scharfenberger, Stina Drakare, Tiina Nõges, Michal Šorf, Konstantinos Stefanidis, Ü. Nihan Tavşanoğlu, Cristina Trigal, **Aldoushy Mahdy**, Christina Papadaki, Lea Tuvikene, Martin Kernan and Erik Jeppesen. Climate change effects on shallow lakes: Design and preliminary results of a cross-European climate gradient mesocosm experiment. In preparation

Author contributions

This manuscript is a methodology for the lake mesocosm experiment in REFRESH project (6 countries) and the purpose of including it here is solely to provide a comprehensive description of the design of the cross-European mesocosm experiment executed by all REFRESH partners. The co-authors are from the six countries involved in the REFRESH mesocosm experiments as well as the coordinators. **Aldoushy Mahdy**, U.S. and R.A. are the German partners in REFRESH; they conducted the experiments in Müggelsee (Berlin, Germany), analyzed German data, and contributed in writing of the manuscript.

Chapter III:

Aldoushy Mahdy, Ulrike Scharfenberger, Deniz Özkundakci, Sabine Hilt, Lea Tuvikene, Priit Zingelc, Josef Hejzlar, Michal Šorf, Nur Filiz, Meryem Beklioğlu, Kostas Stefanidis, Eva Papastergiadou, Martin Søndergaard , Erik Jeppesen, Rita Adrian. Effects of water temperature on summer periphyton biomass in shallow lakes: a pan-European mesocosm experiment. In preparation

Author contributions

Aldoushy Mahdy designed and conducted the experiments, analyzed the data, and compiled the manuscript. U.S. co-designed the study, co-conducted the experiments. D.Ö. co-analyzed the data and contributed to the text. S.H. co-designed the study, co-analysed the data and contributed to the text. R.A. co-designed the study and contributed to writing the manuscript. The other co-authors designed the study in the other 4 European countries.

Chapter IV:

Aldoushy Mahdy, Ulrike Scharfenberger, Rita Adrian and Sabine Hilt. (Submitted, 2013). Experimental comparison of periphyton removal by chironomid larvae and *Daphnia magna*. Freshwater Science

Author contributions

Aldoushy Mahdy designed the study, conducted the experiments, analysed the data, and wrote the manuscript. U.S. co-designed the study and contributed to the data analysis. RA co-designed the study and co-wrote the manuscript. S.H. co-designed the study, co-analysed the data and helped to compile the paper.

Chapter V:

Ulrike Scharfenberger, **Aldoushy Mahdy**, and Rita Adrian. 2013. Threshold-driven shifts in two copepod species: Testing ecological theory with observational data. Limnology and Oceanography 58:741-752.

Author contributions

U.S. co-designed the study, analysed the data and compiled the manuscript. **Aldoushy Mahdy** co-designed the study, co-analysed the data and contributed to the text. R.A. co-designed the study and co-wrote the manuscript.

Chapter I: General Introduction

Part one

I.I. Shallow lake periphyton

Freshwater ecosystems comprise a minor fraction of the total available water in the biosphere, have been critical in sustaining life and establishing civilizations throughout history. Humans rely on freshwater systems not only for drinking water, but also for agriculture, transportation, energy production, industrial processes, waste disposal, and the extraction of fish and other products. As a result of this dependence, freshwater ecosystems are vital for terrestrial life and fundamental to host rich biodiversity, thanks to their littoral zone (Wezel, 2001). However, misuse and overexploitation have led to deterioration of these vulnerable ecosystems, especially after the industrial revolution. Over the past couple of decades, the scientific interest in shallow lakes was accelerated worldwide (Meerhof, 2010). Shallow lakes, especially the small ones with extensive littoral area, have a high potential for periphyton growth (Wetzel, 2001).

Periphyton definition—A survey on the relevant literature revealed that the terminology describing attached algal communities is highly confusing and inconsistent. Many different and potentially synonymous terms have been in use for several decades such as “benthos”, “attached algae”, “biofilms”, “microlayers”, “Aufwuchs”, “epiphyton” and “periphyton”. The term “benthos” includes the entire assemblage of organisms associated with the hard substrate interface in aquatic ecosystems (Wetzel, 2001). In practice the term “periphyton”, tends to be applied when special attention is paid to the attached algae in the community, while the term “biofilm” is more frequently used in engineering applications primarily it refers to heterotrophic components (Wetzel, 2001). Lampert and Sommer (1999) classified the attached algae in relation to the type of substratum colonized: macrophytes (epiphyton), wood (epixylon), stone (epilithon), sandy sediments (episammon) and muddy sediments (epipelon). Throughout my thesis I used the term periphyton as defined by Wetzel (1983) who identified it as “a complex community of microbiota (algae, bacteria, fungi, animals) which are attached to substrata. The substrata are inorganic or organic detritus, living or dead.”

It is not unusual to find submerged vegetation and other underwater surfaces covered by periphyton densities of several hundreds micrograms of algal chlorophyll per square meter. Moss

(1980) stated that all submerged surfaces receiving enough light become colonized by periphyton. Periphyton plays an important role in aquatic food webs as a food resource because of their high productivity and turnover rate (McIntire et al., 1996). Periphyton communities have received relatively little attention in lake ecosystems. Vadeboncoeur et al. (2002) established database search during the period from 1990 to 1999. She discovered that limnologists measured the periphyton productivity 10 times less often than phytoplankton productivity during the same period. Like other primary producer communities, periphyton may be controlled by biotic growth limiting factors like resources (bottom-up forces), temperature, or by consumption (top-down forces).

I.II. Factors affecting periphyton growth

I.II.I. Bottom-up forces (nutrient dynamics)

When considering periphyton mediated effect on freshwater ecosystem, attention is usually drawn to nutrient dynamics.Within the context of decades with eutrophication of many lakes, there has been a natural interest focused on how aquatic primary production is related to the available nutrient pool. Enhancing nutrient input tend to favour algae (phytoplankton and periphyton) over macrophytes because algae obtain nutrients primarily from the water column. In addition, microalgae have more rapid rate of nutrient uptake and growth (Enriquez et al., 1996), and their compensation irradiance is generally low (1-10 μmol photons m^{-2} s^{-1}) compared to that of macrophyte (10-100 μmol photons m^{-2} s^{-1}) (Duarte, 1995).

Periphyton growth can be light-limited (Quinn et al., 1997; Kiffney and Bull, 2000) or nutrient limited (Perrin and Richardson, 1997; McCormick and Stevenson, 1998; Cascallar et al., 2003), or a combination of both. In addition, periphyton can serve as a major regular of the nutrient dynamics in shallow lake ecosystems (Wetzel 1990, Wetzel, 2001) and can improve shallow lakes to retain externally loaded nutrients by reducing internal loading of phosphorus (Wetzel, 1999). However, it also directly enriches a relatively nutrient-poor water column by exporting dissolved or particulate organic matter originally sequestered from the substrata. Laboratory studies by Hansson (1990) demonstrated that periphyton growing on the sediment surface reduce nutrient availability in the overlying water. Consequently, periphyton may competitively reduce growth of phytoplankton. Indirectly the pelagic nutrient pool may be affected by photosynthetic active periphyton on the sediment, because the produced oxygen may immobilize several dissolved elements and reduce the chemical fluxes at the sediment-water interface (Carlton and Wetzel, 1988; Hansson, 1989; Woodruff et al., 1999). The effect of nutrient quality and availability on periphyton biomass varies with several factors like nutrient enrichment level, grazing pressure, algal composition, and substrate type (Makarevich et al., 1993; Azim et al., 2002). In a European mesocosm experiment Bécares et al. (2008) found that periphyton biomass increases with increasing nutrients. In a meta-analysis of 85 experiments with periphyton communities, Hillebrand (2002) found that both the effects of nutrient addition and grazer's removal were positive and highly consistent. He concluded that the strength of the biotic interaction acting on the periphyton may be relatively similar across different aquatic environment, and that the observed variation in the outcome of single experiments is not a result of general large-scale difference between environments. Most of studies on the effect of nutrients on periphyton biomass were done in lotic ecosystems or come from laboratory experiments. Little is known about the effect of nutrients loading status on periphyton biomass development along a longitudinal scale in the context of climate change (Bécares et al., 2008).

Role of periphyton in the switch between stable states—Shallow lakes tend to exist in one of two conditions: a macrophyte dominated state with high water transparency or a turbid phytoplankton dominated state without submerged macrophytes (Scheffer et al., 1993). Phillips et al. (1978) suggested that periphyton may play an important role in mediating the switch from a macrophyte-dominated clear-water state to a phytoplankton-dominated turbid state. In sediment cores of lakes that had switched from a macrophyte-dominated clear state to a phytoplankton-dominated turbid state they found that periphyton species increased faster than planktonic species, a succession that was confirmed in laboratory experiments. This led them to propose a model that takes periphyton into account as the first step leading to the decline of submerged macrophytes in lakes with increasing nutrient loading. This general mechanism is supported by evidence from Daldorph and Thomas (1995) who found that nutrient increase lead to periphyton increase and macrophyte decline, a process that could be ameliorated by introducing sufficient numbers of snails to control periphyton biomass. It is conceivable that, with increasing nutrient loading, periphyton simply out-competes phytoplankton for nutrients until sufficient excess of nutrients is present; at that point phytoplankton (no longer nutrient-limited) out-competes periphyton for light. Evidence for the latter explanation came from Blumenshine et al. (1997) who found that periphyton was more responsive to increased nutrient loading than phytoplankton, even without adding effects of allelopathic suppression of phytoplankton by macrophytes. Allelopathic effects cannot, however, be entirely ignored, as direct experiments confirmed that some macrophytes do indeed secrete substances that suppress phytoplankton growth (Gross et al., 1996; Körner and Nicklisch, 2002).

I.II.II. Top-down forces (grazing vs. fish)

The vital importance of top-down effects by grazers on lentic periphyton is well documented (Steinman, 1996; Hillebrand, 2002). Grazing is usually assessed by measuring the reduction in producer biomass at community level, individual level and/or body mass specific dry weight level after experimental enclosure or exclosure of grazers, and is thus measured as the net effect of grazing. More correctly it should be defined as ’removal rate’ to indicate that such producer biomass reduction comprises both losses by grazer ingestion and losses of material due to grazer activity within or on the surface of the periphyton mat. However, to allow consensus with most research papers I will use the term removal rates instead of grazing to describe all possible effects of grazing and mechanical removal of periphyton by locomotion of grazers.

In a review of the literature, Cattaneo and Mousseau (1995) found that periphyton removal rates increased with grazer body mass and with food availability and decreased with grazer abundance. Additionally, they found that removal rates, corrected for body size, were similar among all grazer taxa except from amphibians, which tended to have lower rates. Traditionally, most studies have considered either the importance of resource-mediated or consumer-mediated regulation (Lamberti, 1996) but several investigations also pointed out that bottom-up and top-down forces may act simultaneously on primary producer communities (Carpenter et al., 1987; Hansson, 1992; Rosemond et al., 1993). One approach to explore the conditions under which top-down forces operate and interact is to experimentally manipulate resources and consumers separately and simultaneously in laboratories experimental set-ups. Rosemond et al. (1993) used this approach to study effects of nutrients and snail grazing on periphyton. Grazer effects on periphyton include decrease (or sometimes even increase) of periphyton biomass through direct consumption or through dislocation, alteration of community composition, and nutrient mobilization. Hillebrand (2002) concluded in his review that, grazers significantly reduced periphyton biomass. Grazers may occasionally increase the biomass and stimulate local production by shifting the taxonomic composition of algal community toward more productive species or by improving the nutrient supply to individual algal cells (Steinman, 1996). Our knowledge on the grazer-periphyton relationship is limited as most studies have been conducted in lotic ecosystems (Feminella and Hawkins, 1995). However, little information is available for lentic ecosystem (Francis and Kane, 1995; Chaloner and Wotton, 1996). Most of these studies on shallow lake were done on the periphyton removal by sessile organisms like chironomids (Hillebrand and kahlert, 2001) or snails (Brönmark and Weisner, 1992; Brönmark, 1994).

The importance of grazing by herbivorous zooplankton on the development of phytoplankton populations became recognized by many studies. In many lentic ecosystems, Cladocera are the dominant group of zooplankton (Lampert, 1978; Sommer et al., 1986; Lampert and Sommer et al., 2007). Due to their high filtration capacity, they are able to control the abundance of phytoplankton (Lampert, 1978; Sommer et al., 2012). The cladoceran *Daphnia magna*, a well-known filter-feeder, is very efficient at filtering the seston in the pelagic zone (Geller and Müller, 1981; Hadas et al., 1982; Flössner, 2000). However, as far as we know, few studies have examined the role of periphyton as a food source for *Daphnia*. Some stable isotope analysis suggested that a number of *Daphnia* species in shallow lakes can use periphyton as a food source (*D. magna*; Jones and Waldron, 2003 and *D. middendorfiana*; Rautio and Vincent, 2006). Recently, Siehoff et al. (2009) examined the ability of *D. magna* to establish a stable population when fed on periphyton as the only food source. However, little is known about the impact of mobile grazers such as cladoceran zooplankton on periphyton biomass despite their high number and biomass production in the littoral zone. One of the knowledge gaps in this field, the current work tries to fill in the role of periphyton as food source for (motile) grazers such as *Daphnia magna* and (sessile) grazers such as chironomids (for more detail, see Chapter IV). As far as we know, this is the first comparable work focusing on chironomids and *D. magna* using periphyton as a food source. The comparison will be on community level, individual level, and on body mass specific dry weight level.

The top-down control by fish stimulates periphyton by increasing available nutrients via nutrients recycling or by indirect cascading effect via a negative impact on the grazer's community. This result recorded thought experiments involving three trophic levels (fish-grazers-periphyton) are known (Mazumdlr et al., 1989; McCormick and Stevenson, 1989). Generally, fish are a driving force in controlling food webs in shallow lakes by nutrient enrichment, by excretion, and predation on zooplankton (Timms and Moss, 1984; Beklioğlu and Moss, 1996). In a classical experiment on fish-snail periphyton interactions Brönmark et al. (1992) found that fish reduced the snail biomass and thus indirectly stimulated periphyton. Liboriussen et al. (2005) manipulated the effect of fish on phytoplankton, periphyton and periphyton grazer in a mesocsom experiment and found that top-down effect by fish regulates the invertebrate grazer communities associated to periphyton and indirectly increases periphyton biomass. To explore the interaction of fish-mediated top-down regulation and bottom-up regulation of strips periphyton and the associated macro-grazer community, we conducted mesocosm experiments in 5 European lakes (Estonia to Greece) in midsummer during the periphyton growing season (July-August).

I.II.III. Effects of temperature on periphyton biomass

Other abiotic and biotic factors (e.g. bottom-up and top-down) are evidently also important in determining seasonal succession patterns in periphyton communities. Temperature is one of the most important abiotic factors influencing periphyton growth and seems to play a key role on periphyton development. Periphyton can generally tolerate a wide range of temperatures from 0-30 °C (DeNicola, 1996), although the specific thermal range tolerated by individual species varies greatly. Generally, temperature caused more significant differences in periphyton growth rate and algal taxonomic composition than irradiance (Vermaat and Hootsmans, 1994). The strong positive relationship between water temperature and periphyton biomass was documented in lotic (Rosa et al., 2013) and lentic ecosystems (Tarkowska-Kukuryk and Mieczan, 2012). Moore (1978) demonstrated that the seasonal change of periphyton in Lake Erik occurs above 10 °C and was primarily determined by water temperature. The effect of temperature gradients on periphyton biomass need more work as little is known (Bécares et al., 2008). The increase of water temperature and nutrient concentrations, are known to stimulate periphyton metabolism (Rosa et al., 2013) and increase algal respiration. The observed stimulation of periphyton respiration promoted by increasing temperature and nutrient concentrations has been done in stream habitats (Rosa et al., 2013). It can lead to a change in lake carbon budgets. The positive global warming feedback on lakes periphyton might be also leads to more CO_2 released to the atmosphere (Rosa et al., 2013). We hypothesized that increase water temperature will enhance periphyton biomass in the Mediterranean region directly and indirectly via increase in nutrients concentration mediated by low precipitation, low runoff, low water level, and high evaporation (Beklioğlu et al., 2007).

Part two

I.III. Threshold-driven shifts in two copepod species

There is an apparent gap between testing of ecological theory and observed data (Scheffer et al., 2001; Scheffer and Carpenter, 2003; Dakos et al., 2008). The nutrient status of a lake together with water temperature largely determines the timing, quality and quantity of food availability for many zooplankton species. Thereby they also affect zooplankton life history traits and their timing (development times, productivity, as well as the occurrence and timing of resting stages) (Hansen, 1992; Santer and Lampert, 1995; Seebens et al., 2009). In addition many studies have demonstrated the effects of changes in climate and trophic status of a lake either on single species level or on the community structure level (Adrian, 1997; Adrian et al., 2006; Seebens et al., 2009). Intraguild predation (IGP) is a concept governing the intrinsic dynamics of the competition among predators, eating and killing between species that use similar resources (Polis et al., 1989). Intraguild predation has mainly been studied in theoretical deterministic models (Polis, 1989; Holt, 1997; Mylius, 2001; Holt, 2007). Contrary to traditional linear food chains, simple IGP models, consisting of an intraguild predator, another predator and a resource, predict the out-competition of the consumer by the intraguild predator at high productive environments, while at intermediate productivities coexistence is possible. In addition, potentially two alternative stable states exist at this intermediate productivity level (Holt, 1997; Mylius, 2001). As a precondition for such dynamics, the consumer must be superior at exploitative competition for the common resource. Extend the model with an invulnerable state for the consumer, makes coexistence even more likely, while keeping the possibility for the alternative stable equilibria (Scharfenberger et al., 2013).

In Müggelsee, under constant reduction of the nutrient load since the 1990s (Köhler et al., 2005), there was a sudden and dramatic shift in the winter cyclopoid community from a *Cyclops vicinus* dominance to a *Cyclops kolensis* dominance. We were interested in the population dynamics of the two cyclopoid copepod species, which have been monitored for over 30 years. The two species are linked in an intraguild relationship as *C. kolensis* can predate on the larval stages of *C. vicinus* and vice versa. Slight differences seem to exist between the two species. For instance, *C. vicinus* has a much more flexible phenology. Under high nutrient conditions it tends to be present in the pelagic all year round, while it enters into summer diapause under lower nutrient conditions (Santer and Lampert, 1995), with close timing to the spring bloom to ensure optimal food conditions for the nauplii (Seebens et al., 2009). In contrast, *C. kolensis* life cycle is relatively uniform and it seems always to enter into diapause during summer. Another distinctive feature of *C. vicinus* is ability to live and develop on a purely herbivorous diet (Santer and Bosch, 1994). In addition *C. vicinus* has been shown to be very sensitive to food shortage, but can react fast to improve nutrient conditions due to short development times under abundant food (Hansen and Santer, 1995). *C. vicinus* stronger dependence on high nutrient conditions is further affirmed through its high ingestion rates. In particular *a C. vicinus* ingestion rate (both for phytoplankton and zooplankton) is higher than that for *C. kolensis*, but the two species reduce their ingestion rates for algae in the presence of other prey (Adrian, 1991). This observation is in line with reports of declining *C. vicinus* populations in lakes undergoing re-oligotrophication (Seebens et al., 2009), or strong dominance in lakes with increasing nutrient loading (Adrian, 1997). One of my aims is to clarify the regime shift scenario between the two cyclopoid copepods. The abrupt change scenario happened between the two species, when *C. kolensis* changing status from a rare species to a dominant species, as available algal prey declined and *C. vicinus* changing status from a dominant species to a rare species in Müggelsee, Germany. We tested observational data on plankton community with regime shift theory by Andersen et al. (2009) in the context of IGP theory.

I.IV. Aims of the thesis

This thesis consists of two parts, the first one deals with questions revolving around the role of bottom-up and top-down forces on periphyton in shallow lakes, the second one deals with food and intraguild predation based changes of two copepod species in a shallow lake.

Part (1): Shallow lake periphyton

The results from the experimental work on bottom-up and top-down forces on periphyton growth will contribute to a better understanding of shallow lake periphyton biomass development (measured as dry weight, ash free dry weight and periphyton chlorophyll *a*). Here, I present the results of the laboratory microcosm and field mesocosm experiments on periphyton biomass development.

Chapter II:

The purpose of this chapter is to describe the design of a cross-European mesocosm experiment to study the effects of climate change on structure, function and metabolism at two nutrient levels and water depths along a climatic gradient ranging from Sweden to Turkey to describe the experimental design and set-up. An experimental, 2 × 2 × 4 factorial, fiber glass mesocosm was designed and used in 6 European lakes. A key objective was to implement highly standardized procedures across countries to optimize the comparison of results and reduce the risk of bias.

Chapter III:

The goal of this chapter was to evaluate the effect of nutrient enrichment on periphyton biomass development across a longitudinal European gradient. As a member of the European project REFRESH, I used the five country mesocosm experiment results. I tested whether nutrient enrichment, water temperature and top-down effect by fish influenced the periphyton biomass. To assess the effects of nutrient enrichment in more controlled conditions, I also conducted a mesocosm experiments (midsummer experiment), using different nutrient enrichment levels (25, clear and 200 μg TP L^{-1}, i.e. low and high nutrient).

Chapter IV:

This chapter deals with testing the grazing effect by a natural invertebrate community (i.e. chironomids and cladocerans), as well as chironomids and *Daphnia magna* separately on periphyton biomass development. I compared the effect of the grazer identity on different levels: community level, individual level and body mass specific dry weight level. The biomass of periphyton grazers was assessed before and after the laboratory experimental period to test the effect of grazer biomass on periphyton biomass. The laboratory experiment lasted two weeks in controlled laboratory conditions

Part (2): Threshold-driven shifts in two copepod species

Chapter V:

The main goal of this chapter was to determine the mechanisms leading to a shift in two coexisting copepod species in a shallow lake. We embedded our findings into the framework of intraguild predation theory and theoretical scenarios of threshold-driven regime shifts. We hypothesized that the abrupt increase in *Cyclops kolensis* depends on the decrease of algal prey, for which it was the superior competitor compared to *Cyclops vicinus*. To test our hypothesis, we analysed the long-time series of about 30 years in eutrophic lake, Müggelsee, Germany.

Chapter II:

Climate change effects on shallow lakes: Design and preliminary results of a cross-European climate gradient mesocosm experiment

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Abstract

Climate change is expected to profoundly affect not only temperature but also net precipitation in some parts of the world and, in consequence, lake water levels and the overall ecological status of lakes. In this paper we describe the design of a cross-European (six countries from Sweden to Turkey) mesocosm experiment, which was conducted simultaneously and identically to elucidate the effects of climate change on shallow lake community structure, functioning and metabolism at low and high nutrient levels crossed with contrasting depths. We used cylindrical fibreglass mesocosms with a one- or two-meter water column and a 10 cm sediment layer. Nitrogen and phosphorus were dosed in monthly intervals and water was circulated continuously in the mesocosms from spring to autumn. We inoculated the mesocosms with a mixed sample of sediment and plankton from lakes in each country with contrasting nutrient concentrations and added macrophytes and planktivorous fish. Sediment was pre-equilibrated to the experimental nutrient concentration. During the experiment, the water level decreased with increasing temperature, and in the Mediterranean mesocosms the water level was reduced by 90 cm; in contrast, conductivity was more than doubled. The average chlorophyll *a* concentration in the deep mesocosms with high nutrients increased along the temperature gradient but peaked at intermediate temperatures in the shallow mesocosms. Average macrophyte PVI (% plant volume inhabited) increased with rising temperature in the shallow mesocosms, while oxygen saturation data suggest that net primary production peaks at intermediate temperature and is lowest in warm lakes, and overall lower in deep than in shallow mesocosms. We conclude that our experimental design has a great potential for tracking the interacting effects of global warming and eutrophication in shallow lakes.

Introduction

Climate change is expected to significantly change temperature regimes and precipitation patterns across the world (IPCC, 2007; Bates *et al*., 2008), with implications for the nutrient status of lakes. In the northern temperate zone, loadings of phosphorus (P) and nitrogen (N) are expected to increase due to elevated precipitation and soil decomposition levels, leading to higher nutrient loss from land to lakes (Schindler & Vallentyne, 2008; Adrian *et al*., 2009; Jeppesen *et al.*, 2009, 2011). Moreover, internal nutrient loading tends to increase in eutrophic lakes due to higher temperature and increased mineralisation and as an indirect warming effect of prolonged or temporary thermal stratification (Jensen & Andersen, 1992; Søndergaard *et al.*, 2003; Mooji *et al.*, 2005; Wilhelm & Adrian, 2008). These changes favour the outbreak of algal blooms, often toxic cyanobacteria (Huisman *et al.*, 2004; Wagner & Adrian, 2009). However, there are examples of shallow lakes showing lower nutrient concentrations in warm years due to higher water levels. For example, in Estonian lakes higher winter NAO (North Atlantic Oscillation) years, which are characteristic of warmer and wetter winters, are associated with higher water temperatures but also with higher lake water levels (Nõges, 2004). In shallow Lake Võrtsjärv, high water level years are characterised by lower P concentration due to weaker resuspension and leakage from bottom sediments, while the N concentration is higher because of lower denitrification rates in the deeper water column, resulting in a higher N:P ratio and less favourable conditions for N-fixing cyanobacteria (Nõges *et al*., 2003).

In Europe global climate change is predicted to result in an approximately 25-30% decrease in precipitation and enhanced evaporation in the Mediterranean region. Pronounced interannual variations can be expected due to increasing frequencies and magnitudes of extreme drought events (Giorgi, 2006; Giorgi & Lionello, 2008). Droughts may reduce runoff, cause lower external nutrient loading and potentially increase water clarity. However, such effects may be context dependent. For instance, in eutrophic lakes higher evaporation, higher internal nutrient loading and, possibly, reduced nitrification under low-oxygen conditions can lead to higher nutrient concentrations and lower water clarity instead (Jeppesen *et al.*, 2009, 2011; Özen *et al.*, 2010; Papastergiadou *et al.*, 2010). Climate warming may also affect trophic structure and dynamics. In warm lakes, top-down control by fish is strong due to dominance of small and abundant planktivorous and benthivorous fish (Jeppesen *et al.*, 2010a; 2012), enhancing zooplankton predator control and reducing grazing on phytoplankton (Gyllström *et al.*, 2005; Meerhoff *et al.*, 2007; Stefanidis & Papastergiadou, 2010). This may have an adverse influence on submerged macrophytes due to the diminished light conditions. Conversely, a reduced water level can also improve the light climate for macrophyte growth (Blindow, 1992; Nõges & Nõges, 1999; Coops *et al*., 2003, Beklioğlu *et al*., 2006), depending on lake morphology (Beklioğlu *et al*., 2006). A mesocosm study in a Turkish shallow lake showed that macrophytes resisted increased nutrient loading, perhaps as a result of an unintentional, but major evaporation-triggered water level reduction, overriding the deleterious effect of periphyton- and phytoplankton-induced turbidity (Özkan *et al*., 2010). A similar outcome was observed in a recent experiment where water level and the presence and absence of fish were manipulated in a warm eutrophic shallow lake (Central Anatolia, Ankara) (Bucak *et al.,* 2012). Results from both studies suggest that reduced evaporation during summer in this region may help maintain the growth of submerged macrophytes in eutrophic shallow lakes despite the reduction in water clarity caused by fish predation. Bucak *et al.* (2012) concluded that the adverse effects of climate-driven eutrophication on water clarity may be counteracted by reduced water levels, provided that physical disturbance is not severe in the shallow margins of the lake. Climate change induced alterations in water levels may therefore be critical, both in shallow lakes in the Mediterranean and the continental part of the north temperate region where summer may become more dry in the future. Consequently, water level effects need to be considered more explicitly in the analyses of climate change impacts on shallow lakes. Space-for-time substitution, although not unproblematic, is one of the most widely used approaches in ecological and climate change research. It accounts for key differences between ecosystems resulting from their location along broad latitudinal and altitudinal gradients and therefore allows assessment of how otherwise similar factors influencing ecosystem structure and function vary along these gradients (Meerhoff *et al.*, 2012, Jeppesen *et al.*, 2014). This approach has been used to analyse a series of lake surveys in Europe (Moss *et al.*, 2003; Gyllström *et al.*, 2005, Declerck *et al.*, 2005; DeMeester *et al.*, 2006; Brucet *et al.*, 2012), South America (Kosten *et al.*, 2009, 2011) and in cross-continental studies (Jeppesen *et al.*, 2007; Meerhoff *et al.*, 2007, Kosten *et al.*, 2012). The strength of this approach is that the biological assemblages *per se* have had time to evolve and adapt to the climate in which they live. The weaknesses are that biogeographical issues may be of importance and that correlative studies do not necessarily provide causal relationships. Furthermore, the influence of co-variates of temperature and latitudinal variation in other climate-related variables such as seasonality and the length of growing seasons may go undetected, and confounding factors that cannot be controlled may swamp the climate signal. Thus, alternative approaches are also needed to elucidate climate change effects more mechanistically, despite the challenging nature of the macroecological scales of interest. A useful alternative is to combine correlative space-for-time substitution approaches with controlled experiments. The cross-European mesocosm experiment conducted in 1998 and 1999 (under the auspices of the SWALE project) in six lakes provides an example of such an approach. In this experiment lakes were distributed from Finland to southern Spain, and the effects of fish addition and nutrient loading on shallow lakes could be studied using a common protocol (Moss *et al.*, 2004; Stephen *et al.*, 2004). In these experiments, and contrary to the findings from latitude gradient studies, no differences in the degree of top-down control along the latitudinal gradient were observed, perhaps because of fixed fish densities. However, the variability of the experimental outcome from one year to the next, increased with latitude, reflecting the greater variation in weather at the onset of the experiments (Moss *et al.*, 2004). The SWALE study was restricted to a single depth and differed in starting level of nutrient levels. Moreover, lake metabolism was not included.

This paper describes a refined design of a cross-European mesocosm experiment that accounts for the importance of water level fluctuation as an important driver of ecological processes in lakes resulting from global change. It aimed at studying the overarching effects of climate change on trophic structure and dynamics as well as lake metabolism at two nutrient levels and water depths (1 m and 2 m) along a climatic gradient in six countries from Sweden to Turkey. The aim of this paper is to describe the highly standardised designed experimental and sampling procedures, which was a key objective in order to optimise the comparison of results and reduce the risk of bias. We present the design and some physico-chemical and biological data from the experiment in brief and discuss the strengths and weaknesses of the approach and design. This paper will be followed by a number of more specific papers on the various physico-chemical variables studied.

Methods

Experimental lakes and study period—For the experiment we selected a shallow, alkaline, clear freshwater lake in each of the six participating countries (Table 1).

Table 1 Basic information on the study sites. Precipitation and air temperature cover the period May 1 – Nov 1 2011.

Experimental site	**Coordinates**	**Climate**	**Altitude (m a.s.l)**	**Total precipitation (mm)**	**Air temperature (^{0}C)**
Sweden , Erken	59°49'59"N 18°33'55"E	Boreal	11	385	14.6
Estonia, Võrtsjärv	58°12'17" N 26°06'16" E	Boreal	35	298	14.9
Germany, Müggelsee	52°26'0" N 13°39'0" E	Transient maritime/continental	32.4	431	16.9
Czech Republic, Vodňany	49°09'14" N, 14°10'11"E	Transient maritime/continental	395	401	15.3
Turkey, ODTU-DSI Golet	39°52'38″ N 32°46'32″ E	Transient /continental Mediterranean	998	223	18.7
Greece, Lysimachia	38°33'40″ N 21°22'10″ E	Mediterranean	16	252	23.4

The lakes had: i) a mean depth < =4 m, allowing us to cover natural patterns of temperature seasonality that characterise shallow lakes, ii) low nutrient concentrations as we wanted to use natural lake water (where possible) and to run the experiment at both low and high nutrient concentrations, iii) total alkalinity between 1 and 4 meq/l, iv) low salinity (<1‰) and colour <50 mg Pt/l. These conditions reflect natural abiotic settings of lakes across Europe, increasing the generality of our study. The experiments ran for six months in 2011 from spring (May) to the end of autumn (October/November), thus avoiding the ice-covered period at the northern sites. In all countries the experiments were started on 9th May (= day 1) to enable use of a synchronised sampling protocol.

Experimental set-up—The experimental set-up in each country encompassed two nutrient levels crossed with two water levels, each represented by four replicates (16 mesocosms in total). A pontoon bridge with eight mesocosms (Fig. 2) arranged in two rows divided by a boardwalk, allowing easy sampling, was established in each lake (Fig. 3). The treatment position was organised randomly but followed the same protocol in all countries (Fig. 1).

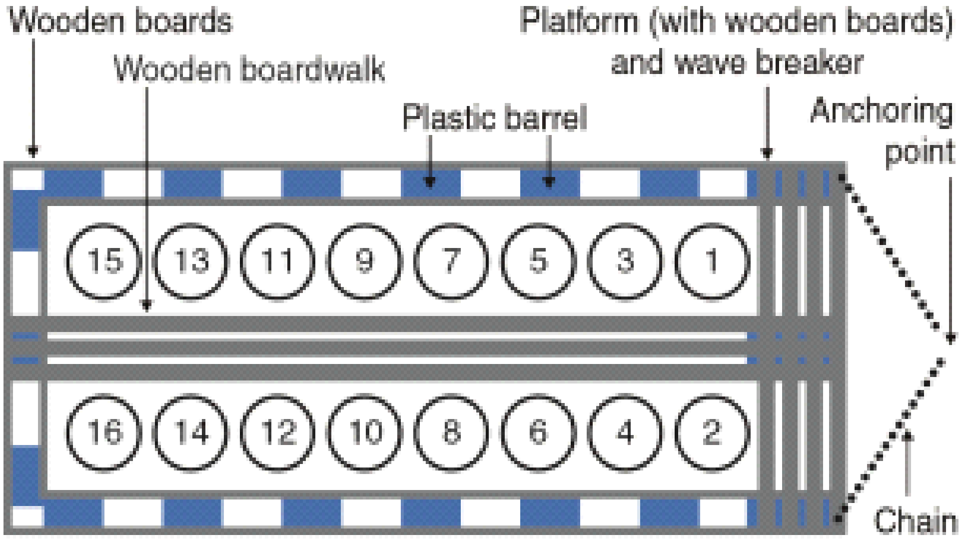

Fig. 1. Schematic representation of the floating pontoon bridge established at sites with wind exposure. A platform, functioning as landing platform and wave breaker, is placed on the right side, which also serves as the anchoring end of the bridge. Randomly selected enclosure numbers are inserted.

The pontoon bridges were constructed from wooden or plastic boards and floating devices consisting of thirty-two 120-l plastic barrels (or similar) or, in one case, the bridge was anchored at the bottom (the Czech Republic). At one end of the pontoon bridge a small working platform was established. For the free-floating systems (five countries) the pontoon was anchored only at the platform end, which, irrespective of wind direction, functioned as wave breaker and boat landing place. To prevent birds from landing on, or foraging in, the mesocosms, fruit nets (thin net with, for example, 5x5 cm or 10x10 cm mesh sizes) were fixed above the mesocosms with a minimum height of 50 cm. All mesocosms were constructed by the same manufacturer (Fig. 2) and consisted of cylindrical (D = 1.2 m) fibreglass (4 mm) tanks with two heights, 1.2 and 2.2 m. Their robustness not only minimises the risk of losing sites or treatments and offer the potential for reuse but also prevents diffusion of O_2 and CO_2, thereby avoiding artefacts in the calculation of mass balances on oxygen and carbon. The upper edges of all mesocosms were attached to the pontoon bridges 20 cm above the water surface to avoid spill-over from the lake during windy periods. The water level was 1 and 2 m in the 1.2 and 2.2 m deep mesocosms, respectively.

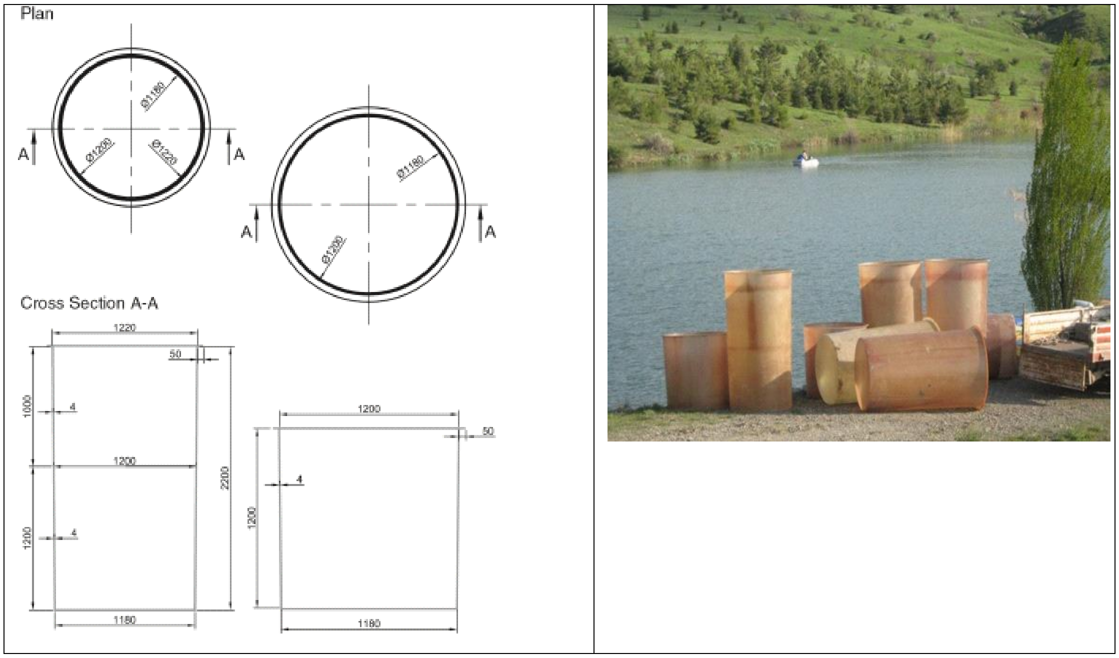

Fig. 2. Dimensions and photograph of the enclosures produced by Armaplast Composites and Plastics in Turkey.

After establishment, a 10 cm deep sediment layer was added to each mesocosm. The sediment consisted of 90% (by volume) washed sand (grain size < 1 mm) and 10% sediment from 5 lakes situated nearby the experimental site in each country. Large particles were removed (e.g. plant fragments, mussels, stones, debris, etc.) by sieving through a 10 mm mesh. Before the sediment was added, it was equilibrated to the two experimental P treatment levels (25 and 200 µg TP L^{-1}).

This avoids transient states in sediment equilibration (resulting from different nutrient loading patterns in the original lakes) during the experiment observed in earlier experiments (E. Jeppesen and M. Søndergaard, personal observations). The pre-equilibration (done country-wise) commenced during late autumn and winter of the previous year and ran until the start of the experiment.

Fig. 3. Photographs of the experimental set-up in the six countries.

Two tanks were filled with a 10 cm sediment layer, which were collected from a local oligotrophic lake, (ca. 0.2 m^3 sediment in each tank), and 20-50 cm water with low (25 µg L^{-1}) or high (200 µg L^{-1}) TP concentrations, respectively. This sediment:water ratio ensured proper exchange of nutrients between the water and the sediment. Following water addition, the sediment and water were mixed with a rake. TP was measured periodically (at least biweekly) and the water was replaced with fresh nutrient-high or nutrient-low water whenever the TP differed from the initial concentrations of 25 and 200 µg TP L^{-1}, respectively. This procedure was repeated until the TP concentrations in the water were at the desired levels (±25%). Following the addition of sediment, the mesocosms were filled with 500 µm filtered nutrient-poor water from neighbouring lakes with P concentrations < 25 µg TP L^{-1}. If this was not possible, water from another oligotrophic lake was transported to the enclosures or, less preferably, tap water was used (e.g. Germany). The mesocosms were left undisturbed for four days to allow the suspended matters to settle. To simulate a shallow, fully mixed lake, the water in the mesocosms was continuously circulated during the experiment using water pumps. Standard 2 to 5 W aquarium pumps were adequate to pump ca. 300 l h^{-1}. To establish comparable mixing, pumps running at half power were used for the shallow (1 m) mesocosms. The pumps were placed with their inlets (via a PVC tube) in the middle of the mesocosm, ca. 10 cm above the sediment, and their outlets 10 cm below the surface. Power for the pumps was obtained either by: i) a power cord from the shore (Czech Republic, Estonia, Sweden), ii) a solar panel (placed horizontally to ensure similar irradiance irrespective of the direction of the floating pontoon bridge) in Greece (240 W, in combination with 12 V 250 Ah batteries) or solar panels from a nearby lake station (Germany) or 3) a power supply with six parallel connected 120 Ah batteries exchanged three times a week (Turkey).

Inoculations—standardise starting conditions and enable the potential for developing a diverse flora and fauna, the mesocosms were inoculated with a mixed sample of sediment and plankton from five different local lakes in each country covering a nutrient gradient of 25-200 µg TP L^{-1}. The inoculum was added on day 4 after setting up the experiment and when the initial disturbance effect of adding water and sediment had diminished. For the inocula of plankton, five vertical net hauls (using a plankton net with a diameter of 20 cm and 50 µm mesh), covering the entire water column (bottom to the surface) without disturbing the sediment, were taken in each of the five selected lakes and pooled (per lake). Some water was kept in the net to avoid damaging the plankton when removing it from the water. The five samples were kept separately in 5-litre barrels, which were filled with lake water from the sample lake. Plankton samples were kept cool prior to adding to the enclosures and were not stored longer than 24 h. The contents of the five 5-litre barrels from the five lakes were mixed and a 1-litre subsample was added to each mesocosm. Five litres of sediment were also collected from each of the five lakes to add biota and/or resting stages of biota. The sediment was collected at a depth approximating the mean depth of the lake. To avoid introducing fish and large mussels (e.g. *Anadonta* spp. and *Dreissena* spp.), the sediment was sieved through a 10 mm mesh. Care was also taken to remove fish eggs.

The inoculum sediment from the five lakes was mixed thoroughly, and one litre was added on day 4 to each enclosure, being dispersed evenly on top of the TP equilibrated sediment-sand layer. Submerged macrophytes and fish were added to the mesocosms three days after the sediment and plankton additions (day 7). Eight 5-10 cm long apical shoots of water-milfoil (*Myriophyllum spicatum*) collected from the experimental lake (or another lake in the region) were planted (evenly distributed) into each mesocosm. Water-milfoil was selected as it was present in lakes in all countries. In Germany the plants were collected in autumn of the previous year to ensure their availability at the start of the experiment. These plants were kept cool in aquaria in the laboratory until use. Before introducing plants to the mesocosms, the plants were placed in soda mineral water (carbonated) for 15 minutes to remove snails and invertebrates. To ensure that shoots would sink into and remain in the sediment until root development (approx. 2 weeks), ca. 5 g pebble stones were attached to the shoots by duct tape.

The original intention was to stock a fish species present in all countries and three-spined stickleback, *Gasterosteus aculeatus* L., size 2-4 cm, was selected. However, it was not possible to catch sticklebacks in Greece, Turkey and Sweden prior to the experiment, either due to low population numbers or ethical issues (legislation prohibiting the transfer of populations between sites). Thus, sticklebacks were imported from Germany and acclimatised for the Turkish experiment. Underyearling roach (*Rutilus rutilus* L.) and western mosquito fish (*Gambusia affinis* Baird and Girard) obtained from the study lakes were used in Sweden and Greece, respectively. Between 4 and 20 g of fish biomass (equating to six sticklebacks or mosquito fish or two roach) were added to each enclosure irrespective of nutrient level. We aimed at attaining a male:female ratio of 1:1, allowing breeding during the experiment. Before addition, the fish were measured to nearest millimetre. Fish were caught at least 1-2 weeks before the experiment was initiated to ensure that they would survive handling (Table 3). Length-weight relationships were established from the remaining pool (20 fish used). Dead fish were replaced from a stock of reserve fish when necessary. Fish density, length and weight were determined at the end of the experiment.

Nutrient loading—The experiment included two levels of nutrient concentrations in the enclosures with four replicates at each of the two depths. To adjust and maintain the concentrations, P and N were dosed to all mesocosms (Table 2) using Na_2HPO_4 and $Ca(NO_3)_2$ as the P and N source, respectively. The stock solution consisted of 1.00 g PO_4-P or 4.60 g Na_2HPO_4 per litre tap water and 20.0 g NO_3-N or 117.2 g $Ca(NO_3)_2$ per litre distilled tap water, respectively. P and N were added at a ratio (by molecular weight) of 1:20. The initial nutrient addition was conducted on day 4 after the addition of the sediment and plankton inoculum.

Table 2. Nutrient dosage to the mesocosms four days after establishment (initial) and thereafter monthly. The ratio between phosphorus (P) and nitrogen (N) addition is 1:20 (by molecular weight).

Mesocosm type	Initial P (mg mesocosm^{-1})	Monthly P (mg mesocosm^{-1})	Initial N (mg N mesocosm^{-1})	Monthly N (mg N mesocosm^{-1})
Shallow (1m) - low NP	0	5.1	0	102
Shallow (1m) - high NP	179*	81.6	1575***	1632
Deep (2 m) - low NP	0	10.8	0	216
Deep (2 m) - high NP	376**	172	3225****	3440

*) = 1020 L * 175 µg P L^{-1}, **) = 2150 L * 175 µg P L^{-1}

) = 1020 L * 1.5 mg N L-1, *) = 2150 L * 1.5 mg N L^{-1}

At this stage only high nutrient mesocosms were dosed with the objective of increasing the P concentration by 175 µg P L^{-1} and the N concentration by 1.5 mg N L^{-1}. Thereafter, all mesocosms received monthly dosing of N and P (Table 2). The dosing levels were determined from the experience obtained in previous experiments (Gonzales Sagrario *et al.*, 2005; Jeppesen *et al.*, 2007). In addition, the tanks received input via precipitation; in the Czech Republic measured to be, on average, 3.4 mg TP and 76 mg TN per mesocosm and month, which are low compared to the added TP and TN in the high nutrient mesocosms (Table 2), but as much as 68% and 75% of the added TP and TN, respectively, in the low-dosed shallow mesocosms (Table 2). The addition of nutrients took place after sampling and ecosystem metabolism measurements had been concluded.

Sampling procedures—Sampling was initiated on day 7 following the addition of fish and macrophytes. Thereafter, samples were taken at monthly intervals; on an optional basis biweekly samples were also taken in some countries, but in the comparative studies only the shared monthly samples were used. The entire water column, from the surface to approximately 5 cm above the sediment, was sampled randomly with a tube sampler (diameter 7 cm) 10, 30 and 60 cm apart from the mesocosm wall and pooled. For phytoplankton, chlorophyll *a* and water chemistry analyses, the sample was taken outside the macrophyte stands. For zooplankton, samples were collected irrespective of whether plants were present or not at the sampling point as zooplankton tend to hide in the daytime among the plants (Timms & Moss, 1984; Burks *et al.*, 2002). If water depth in the enclosure was low, three to six extra samples were taken (10, 30 and

60 cm from the enclosure wall, respectively, but at different points than the first sample) to obtain sufficient water. For chemical analyses a 500 ml sample was taken and kept dark and cool (5^oC) until reaching the laboratory where a 100 ml subsample was filtered. The filtered and unfiltered samples were stored frozen until analysis. For phytoplankton, 50 ml glass bottles were filled with unfiltered water, 0.5 ml Lugol's solution was added and the bottles were kept dark until analysis. As for zooplankton, 5 litres of the well-mixed pooled water were filtered through a 20 µm mesh, poured into a 50 ml bottle and preserved with 2.5 ml Lugol's solution. After sampling, the remaining water was returned to the mesocosm. Salinity, conductivity, temperature, dissolved oxygen and pH were measured *in situ* in the centre of the mesocosms using a multi-probe, and water depth averaged from measurements at four points. On each sampling date and for each mesocosm, a species list of dominant and subdominant submerged macrophytes (not including filamentous algae) was elaborated. Percent Plant Volume Inhabited (PVI) was calculated monthly by visually estimating the percentage coverage and measuring macrophyte average plant height using the formula: PVI (%) = %coverage × average height / water depth. To determine macrophyte coverage, the enclosures were divided into quarters to allow estimation of area. Percentage coverage was classified as follows: 0: no plants, 1: 0-5% coverage, 2: 5-25% coverage, 3: 25-50% coverage, 4: 25-75% coverage, 5: 75-95% coverage and 6: 95-100% coverage. If present, filamentous algae were included in the estimate of total macrophyte coverage. Following the last PVI% estimate and at the end of the experiment, the macrophytes were harvested by cutting the stands close to the sediment surface. Surplus water was removed from the harvested material and wet and dry weights (drying at 60 ^oC for 24 hrs) were measured. Also at the end of the experiment, benthic invertebrates were sampled by taking three separate Kajak cores (Plexiglass cores, ø = 52 mm and core length = approx. 10 cm) per mesocosm or an Ekman grab. Samples were pooled, rinsed and filtered through a 500 µm mesh in the field before being preserved in 96% ethanol in a 500 ml plastic beaker with a wide opening (one beaker per mesocosm).

From 15 July to 15 August a periphyton growth experiment was undertaken in all deep mesocosms. Artificial transparent polypropylene strips (21 mm X 297 mm each) with a slightly textured surface (IBICO®, Germany) were placed 30 cm from the mesocosm wall, 50 cm below the water surface (Köhler *et al*., 2010). A small weight was attached to the middle of each strip to ensure that they remained fixed at the required depth, even during severe water table losses. After removing the strips in August, they were kept cool and dark in a plastic container and frozen in the laboratory until analysis.

Processing of water chemistry and chlorophyll a samples—Total phosphorus, soluble reactive phosphorus, total nitrogen, ammonia, nitrate + nitrite, alkalinity and chlorophyll *a* were determined using comparable standard procedures within the different laboratories.

Processing of plankton samples—At least two phytoplankton and two zooplankton samples from each mesocosm were counted: one sample representing starting conditions and one sample representing an integrated sample of the whole experimental period. The latter consisted of a mixed sample of subsamples (25% of the original sample volumes) from each of the monthly samples. The remaining 75% of the original samples was stored separately to allow studying of seasonal dynamics.

Ecosystem metabolism—Metabolism in each mesocosm was estimated on a monthly basis. Oxygen, pH and temperature were measured (upper 20 cm of the fully mixed water column) at least every second hour for a 24-hour period using a multi-parameter probe. Light attenuation was measured once monthly at 1 p.m. (every 10 cm down to < 1% of the surface light) using a light meter. At the same time samples for alkalinity were taken. The gas exchange coefficient was estimated in late October or early November when temperatures were low and respiration therefore low. The oxygen concentration was lowered to ca. 30% of saturation in the late evening by adding N_2 immediately. Oxygen recovery was then followed during the night and the exchange rate calculated taking into account also respiration (for details about the method see Liboriussen *et al*., 2011).

Statistical analysis—In the present paper we used averages for the entire experiment for all variables except for submerged macrophyte PVI% which covered the period. We analysed these data with mixed effects models with nutrient dosing and depth as class variables and water temperature as random factor using Proc mixed in SAS and the REML estimation method. All variables except temperature were log-transformed.

Results

Average water temperature during the experiment varied between 15.5 and 25.1 (Fig. 4) and air temperature between 14.6 and 23.4 ^{o}C, but did not fully follow the latitude gradient (Table 1). While limited water level changes occurred in Sweden and the Czech Republic (ranging from -3 cm to +12 cm), profound reductions were observed in Turkey and Greece (averaging 48 and 93 cm, respectively) (Fig. 4, significant temperature effect, but no significant depth effect (Table 3). This coincided with the high summer temperatures and low precipitation (223 and 252 mm, respectively, compared to 298-431 mm at the more northern sites), mimicking the typically low summer water level in the Mediterranean region. Conductivity tended to be higher in the mesocosms at these two southern sites, but also in the mesocosms in Germany, likely due to the use of tap water. The mixed effect model showed a significant temperature effect (Table 3).

Table 3. Mixed model test of significance using mean values for the study period for each mesocosm with temp as random variable. Significant variables are in bold. Marginally significant values are in parenthesis.

	Water level change	Total nitrogen	Total Phosphorus	Chloro-phyll a	PVI% Macrophytes	Conductivity	O_2
Depth	0.84	0.25	**(0.055)**	0.18	**(0.056)**	0.81	0.74
Nutrients	0.99	0.27	**0.0012**	0.49	0.59	0.65	**0.0001**
Depth x Nutrients	0.99	0.52	0.37	0.27	0.43	0.94	0.83
Temp	**<0.0001**	**0.03**	0.35	0.97	**<0.0001**	**<0.0001**	**0.0001**
Temp x Depth	0.90	0.16	0.10	0.17	0.60	0.87	0.80
Temp x Nutrients	0.97	0.73	0.64	**0.040**	0.64	0.86	**0.003**
Temp x Depth x Nutrients	0.99	0.38	0.57	0.15	0.67	0.96	0.95

Low and high nutrient levels were maintained throughout the experiment (Fig. 4, Table 3). Average TP in the shallow and deep low nutrient mesocosms was 35 µg P L^{-1} and 24 µg P l^{-1}, respectively, and 103 and 90 µg P L^{-1} in the shallow and deep high nutrient mesocosms, respectively. Nutrient dosing and marginal depth, but not temperature, had a significant effect in the mixed models (Table 3). Total nitrogen averaged 0.96 and 0.81 mg N L^{-1} in the shallow and deep mesocosms with low nutrient addition, respectively. The corresponding figures were 1.65 (shallow) and 1.70 (deep) mg L^{-1} in the high nutrient mesocosms. The mixed models showed a temperature effect only, but no effect of dosing (Table 3). Chlorophyll *a* averaged 17.3 (shallow) and 8.8 (deep) µg L^{-1} in the low nutrient mesocosms and 33 (shallow) and 47 (deep) µg L^{-1} in the high nutrient mesocosms (Fig. 4). While the concentration in the deep mesocosms demonstrated an increasing trend from cold to warm lakes, as expected, chlorophyll *a* tended to peak at intermediate temperatures in the shallow mesocosms. The mixed effect model showed that the interaction between temperature and nutrients was significant, but no depth effect was detected (Table 3). Submerged macrophyte PVI% in summer showed an increasing trend with temperature, not least in the shallow lakes. The mixed effect model revealed temperature to be significant and depth to be marginally significant, whereas no nutrient effect was observed (Fig. 5, Table 3). The northern lakes were generally supersaturated with oxygen during the study period and saturation tended to peak at intermediate temperatures during the experimental period, while saturation in the mesocosms in Greece and Turkey was negative (Fig 4). The mixed effect model showed saturation to be significantly affected by depth, temperature and interactions between nutrient and temperature (Table 3).

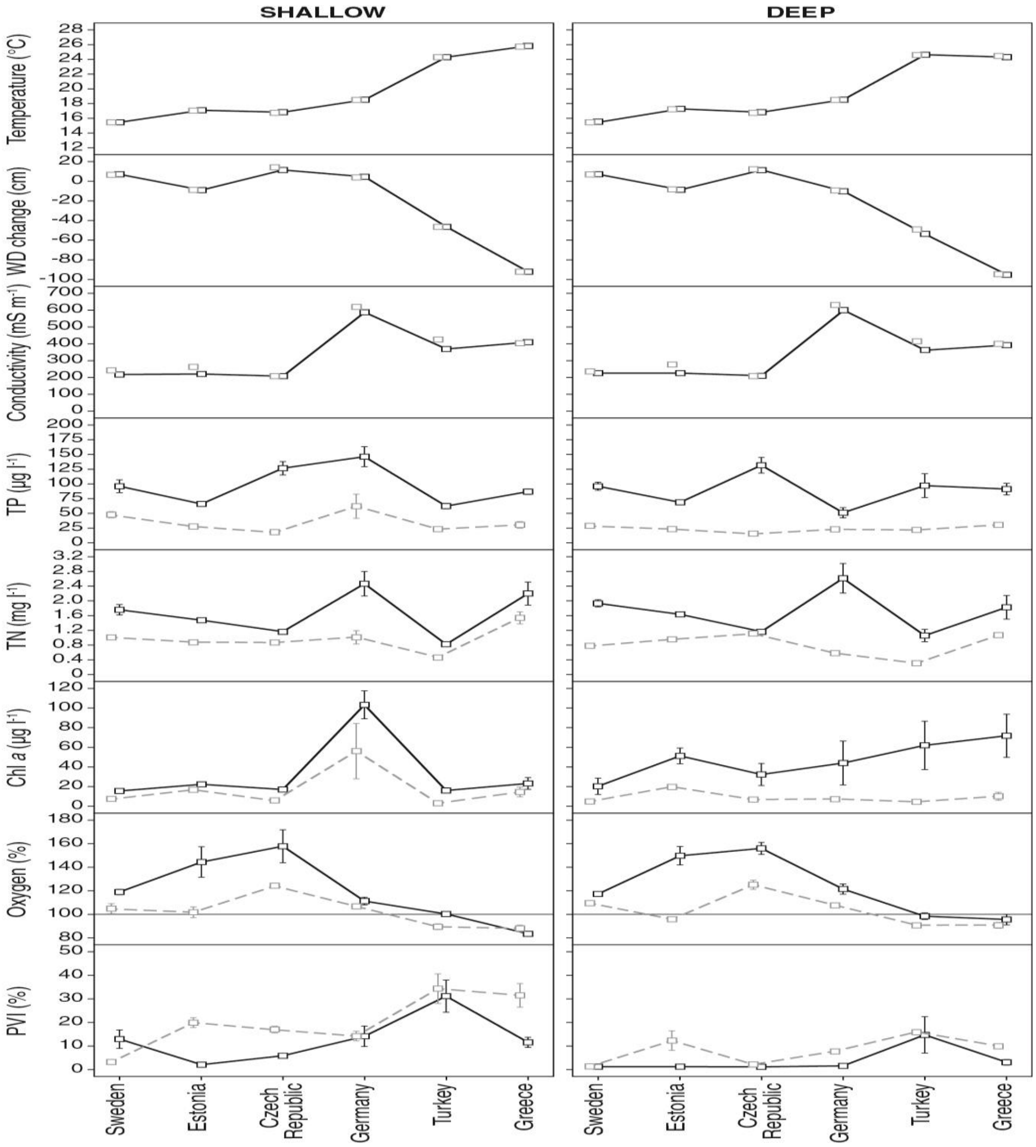

Fig. 4. Selected physical and chemical variables ordered country-wise according to an increasing mean water temperature during the experiment. Means of water temperature, conductivity, total phosphorus, total nitrogen, chlorophyll and maximum change in water level during the experiment.

Discussion

Our experiment illustrates how a careful design, preparation and sampling procedure can be used to optimise the results from cross-national mesocosms experiments. The more detailed results from the experiment still remain to be published, but some overall conclusions can already be made. As expected, we observed a strong gradient in temperature (air temperature 14.6-24.3 ^{o}C, water temperature 15.5-25.1^{o}C) as an average for the experimental period and an accompanied large change in water level due to variation in net precipitation. While no significant difference was found in the water level in the mesocosms from Sweden to the Czech Republic, it declined 48 to 93 cm in Turkey and Greece, respectively, with particularly strong implications for the shallow mesocosms. The temperature gradient did not follow the latitude. The Czech and Turkish sites were located at higher altitude and were therefore exposed to cooler climatic conditions relative to their latitudinal pairs (Germany, Greece). The phosphorus levels differed significantly among high and low nutrient treatments as we aimed for. We attribute this to both the thorough pre-equilibration of the sediment to the experimental P concentrations over a period of several months prior to the start of the experiment using a standardised protocol, and to standardisation of the sediment composition (1:10 ratio of sediment to sand). Although some minimal variation still occurred, our procedure allowed maintenance of relatively similar P concentrations across sites.

Experience from a Danish long-term mesocosm study has shown that the same sand-sediment combination as in the present study, but without pre-equilibration, results in prolonged high internal loading (not least of phosphorus) in the systems under low nutrient concentrations but in P retention under high nutrient concentrations (Søndergaard *et al.*, unpublished results). Ideally, the same type of sediment should have been used in all countries, but this was not possible for practical reasons. Nitrogen also tended to be higher in the mesocosms receiving high nutrient doses, although temperature rather than nitrogen differed significantly in the experiment. Nitrogen concentration increased with temperature, possibly resulting from reduced nitrogen retention under warmer condition as carbon can be limiting under high assimilation rates (Kosten *et al.*, 2012). By dosing differently in the deep and shallow mesocosms (Table 2), we managed to get rather similar concentrations at the contrasting depths as we planned for. While this provides good opportunities for comparing shallow lakes with similar N and P concentrations but contrasting depths, the drawback is that the biota has more total nutrients available in the deep tanks receiving higher input, which somehow makes the comparison of deep and shallow tanks difficult, not least when discussing the nutrient balances.

For chlorophyll *a* we found the interaction between nutrients and temperature to be significant, with nutrients enhancing the effect of temperature. This coincides with several recent studies based on space-for-time analysis of large datasets (Jeppesen *et al.*, 2007, 2010b; Moss *et al.*, 2011), time series (Wilhelm & Adrian, 2008; Wagner & Adrian, 2009), some experiments (see review in Stewart *et al.*, 2013) and modelling (Mooij *et al.*, 2005; Trolle *et al.*, 2011); for a review, see Jeppesen *et al.* (2014). We found an overall increasing PVI% of macrophytes with increasing temperature, not least in the shallow mesocosms. Concurrently, Bucak *et al.* (2012) found that water level reductions in the shallow mesocosm experiment in Turkey led to fast growth of macrophytes due to improved light conditions for the plants. The water level reduction in the deep mesocosms of Bucak *et al.* (2012) was, however, not accompanied by increasing macrophyte coverage. Macrophyte growth and colonisation depth are in general positively affected by temperature (Rooney & Kalff, 2000), but whether this growth potential is realised depends on a number of factors, including the trophic structure, periphyton and plant grazer abundance and winter climate (Jeppesen *et al.*, 2014), which largely were standardised in our study. We further aimed at describing system production and respiration from diurnal variation in oxygen and CO_2 exchange based on alkalinity measurements and diurnal variation in pH. Generally, there is a good relationship between oxygen saturation and net production in fully mixed lakes and mesocosms (Nielsen *et al.*, 2013). Our results indicate occurrence of higher net production for the study period in the mesocosms in the northernmost four countries, peaking in mid Europe, than in Greece and Turkey. This concurs with other findings suggesting net production to decrease from cold to warm lakes (Kosten *et al.*, 2010).

In conclusion, the experimental design described is suitable for studying how changes in nutrients affect the trophic dynamics and metabolism in shallow lakes at contrasting water levels in different climate zones. We used considerable effort to standardise the experiments and to ensure low risk of failure. During the course of a year we discussed and prepared a detailed sampling protocol and considered the types of mesocosms to be used. Detailed protocols are particularly needed when groups with very different backgrounds and experiences are running a joint experiment and cannot be underestimated. Our design also has some drawbacks. 1) For budget reasons our mesocosms were relatively small, and scale is of considerable importance (Schindler, 1998). We had a simple fish structure, with no piscivores. At such a small scale, adequate conditions for piscivory cannot be created. It is evident from a number of recent studies that the proportion of piscivores decreases with decreasing latitude (Jeppesen *et al.*, 2010a,b; Meerhoff *et al.*, 2012), and the experiment cannot account for this. In larger (and more costly) systems, such problems could be minimised and stocking of piscivorous fish would be possible, enabling natural development of planktivorous fish. By ensuring full mixing of the water column as in natural shallow lakes that are typically polymictic, scale was, in part, compensated for. 2) We lost a few mesocosms, either because they sank during heavy storm events or leaked water (due to shooting!). In total, 6 of the 96 mesocosms were lost using strict criteria. The storm effect could have been avoided by running the experiment on land (perhaps in buried tanks), but such tanks tend to get warmer than those placed in water. Despite these drawbacks we find that the system presented here offers great potential to study the effects of global warming and eutrophication on in-lake processes and dynamics in shallow lakes. The extremely thorough preparatory phase encompassing the design of the experiment and protocol writing has been essential for the success of this multi-national, multi-cultural experiment.

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Chapter III:

Effects of water temperature on summer periphyton biomass in shallow lakes: a pan-European mesocosm experiment

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Abstract

Periphyton communities play an important role in shallow lakes and are controlled by direct forces such as temperature, light, nutrients and invertebrate predation, but also indirectly by fish predation. In REFRESH we performed a pan-European lakes mesocosm experiment on periphyton colonization covering 5 counties along a north/south geographical/temperature gradient (Estonia, Germany, Czech Republic, Turkey, and Greece). Periphyton biomass was colonized on artificial strips exposed at 50 cm mesocosm water depth under low (25 µg TP L^{-1}) and high (200 µg TP L^{-1}) nutrient regimes during mid-summer. Even though our nutrient loading (TP) was implemented, there was no significant effect on periphyton biomass. Water temperature played an important positive role on summer periphyton biomass development. The slope of the regression between water temperature and periphyton biomass suggested that, for every degree increase in water temperature, summer periphyton biomass was stimulated by 42%. A positive top-down effect by fish on periphyton biomass (chl *a*) was detected due to a control of periphyton scraping invertebrates grazers. The slope of the regression between fish biomass and periphyton

chl *a* adjusted to water temperature suggested that, for every degree increase in water temperature and its equivalent top-down control of invertebrate grazers by fish, summer periphyton biomass (chl *a*) was stimulated by 45%. The present study suggested that care should be taken when predicting the combined effect of changes in multiple factors from their individual effects.

Introduction

Shallow lakes tend to exist in one of two stable states, a macrophyte-dominated state with high water transparency or a turbid, phytoplankton-dominated state without submerged macrophytes (Scheffer et al., 1993). Phillips et al. (1978) found that lakes that had switched from a macrophyte-dominated clear state to a phytoplankton-dominated turbid state during eutrophication showed an increase in periphyton biomass before plankton development. Jones and Sayer (2003) supported these findings of increased periphyton shading as the first step leading to the decline of submerged macrophytes in eutrophic lakes, but they suggested that a top-down cascade from fish via scraping invertebrates would be responsible for periphyton control rather than nutrient concentrations. Liboriussen et al. (2005) also found a strong role of top-down control on periphyton biomass in a mesocosm experiment in Denmark. The relative importance of bottom-up and top-down control on periphyton biomass, however, may vary widely across spatial (i.e. between lakes) and temporal (i.e. between years) scales (Jeppesen et al., 1997).

Climate regimes are likely to affect lake biota communities (IPCC, 2013) and differences in factors controlling periphyton growth can be expected among lakes of different latitudes. For example, high annual temperatures influence fish growth and predation rates on zooplankton (Bachmann et al., 1996). Periphyton biomass has been shown to be strongly directly related to water temperature in shallow lakes (Tarkowska-Kukuryk and Mieczan, 2012) and can generally tolerate a wide range of temperatures (DeNicola, 1996). In productive lakes, climate warming acts as an acceleration of the lake trophic state (Mooij et al., 2005; Adrian et al., 2009) and consequently affecting light conditions. Subsequent responses on plankton communities have been studies extensively (Adrian et al., 2006; Seebens et al., 2009; Wagner and Adrian, 2009). Comparative studies on periphyton dynamics along latitudinal scales, however, are scarce.

Bécares et al. (2008) conducted a mesocosm experiment across a European latitudinal gradient and found that periphyton chlorophyll *a* (chl *a*) concentrations were overall significantly related to nutrient loading. Top-down effects by fish were significant only in a few sites and related to their contribution to the nutrient pool. Under these conditions, southern lakes achieved lower periphyton densities than northern lakes because of the larger phytoplankton biomass and its shading effects on periphyton.

In this study we hypothesized that increased water temperatures increase summer periphyton biomass when nutrients and light are not limiting and fish exert top-down control on periphyton grazers. We studied periphyton development on artificial strips exposed in mesocosms with two different nutrient loadings and a moderate fish density in five European countries (Estonia, Germany; Czech Republic; Turkey and Greece) for a one month period in July/August.

Materials and methods

Study sites and experimental set-up—We conducted a lake mesocosms experiment in five lakes across Europe representing a latitudinal gradient from the north (58° N 26° E) to the south (38° N 21° E). The lakes were Võrtsjärv (Estonia), Müggelsee (Germany); Vodňany (Czech Republic); ODTU-DSI Golet (Turkey) and Lysimachia (Greece) (Table 4).

Table 4. Lake mesocosm experiment - Basic information, modified from Landkildehus et al., in preparation).

Experimental site	**Coordinates**	**Climate**	**Altitude** (m a.s.l)	**No. meso-cosm**	**Total preci-pitation** (mm)	**Mean air temperature** (^{0}C)
Czech Republic, Vodňany	49°09'14"N, 14°10'11"E	Transient maritime/ Continental	395	8	401	15.3
Germany, Müggelsee	52°26'0" N 13°39'0" E	Transient maritime/ Continental	32.4	5	431	16.9
Estonia, Võrtsjärv	58°12'17" N 26°06'16" E	Boreal	35	8	298	14.4
Turkey, ODTU-DSI Golet	39°52'38″ N 32°46'32″ E	Transient /continental Mediterranean	998	8	223	18.8
Greece, Lysimachia	38°33'40″ N 21°22'10″ E	Mediterranean	16	8	252	23.9

These lakes are apart of the REFRESH project. This project is a series of lake experiments along a north-south gradient across continental Europe with the aim to investigate the effects of water level fluctuation accompanied by changes in salinity under high and low nutrient conditions on shallow lake ecology. The entire experimental duration of the REFRESH project was 7 months between May and November 2011. Specific details on the experimental setup for different aspects of the project are described in (Landkildehus et al., in preparation). While the entire project encompassed various aspects of the trophic cascade (macrophyte, phytoplankton, periphyton, zooplankton and fish), we focused collecting data that were deemed necessary to test our main hypothesis for which the methodology is described below.

The mesocosm design consisted of cylindrical enclosures (2 m depth and 1.2 m diameter) made of fiberglass; the design was identical across countries. The duration of our experiment was four weeks between 15 July to15 August 2011 and consisted of 8 mesocosms in each country. The period was chosen to reflect the growing season for periphyton during midsummer.

Nutrient treatments—To test the effect of nutrient loading on periphyton growth, two levels of phosphorus concentrations, with four replicates for each treatment, was added to the enclosures representing low and high nutrient status i.e. 25 and 200 µg TP L^{-1}, respectively). Thereafter, all mesocosms received monthly dosing a ratio of 1:20 (molecular weight) of N and P (Table 2, Landkildehus et al., in preparation).

Periphyton growth over the experimental duration was quantified by the biomass accumulation on artificial polypropylene, transparent strips (2 strips, 16 x 2 cm) with a slightly textured surface (IBICO®, Germany; Roberts et al., 2003). The strips were suspended at a water depth of 50 cm in each mesocosms in the five lakes. Periphyton strips were kept 30 cm away from tank walls facing south to ensure sufficient light exposure from this direction. The backsides (i.e. facing north) of the strips were covered with adhesive tape to prevent periphyton growth on this sides. The mesocosms were filled with sieved waters (mesh size 500 µm lake water in all countries, except in Germany tap water was used because the TP concentration in the lake water was higher than the starting conditions of the low nutrient treatment (i.e. > 25 µg L^{-1}). As part of the REFRESH project, all mesocosms contained a layer of sediment (thickness 10 cm); macrophytes (*Myriophyllum spicatum*), stickleback fish (*Gasterosteus aculeatus*), and phyto- and zooplankton species assembles which were collected from 5 different lakes in each country (see Landkildehus et al., in preparation).

Fish treatments—Planktivorous fish were added to each enclosure. We used three-spined stickleback, L., size 2-4 cm, between 4 and 20 g of fish biomass, 3 male and 3 female. The aim was to attain a male: female ratio of 1:1 to allow breeding during the experiment. In Greece, mosquito fish *Gambusia affinis* were used. All fish were measured to nearest millimeter before they were added to the mesocosms. Length-weight relationships were established (20 fish used). Any dead fish during the experiment were replaced.

Variables measured—At the end of the exposure period, periphyton strips were gently lifted to the surface, in order to minimize disturbance and loss of periphyton mat. After removal of the adhesive tape from the back side of the strips, they were immediately placed in round plastic tubes and transported to the laboratory in a portable cooler box containing tap water to provide 100% humidity during transportation. The metrics for periphyton used here were, periphyton dry weight (DW), ash free dry weight (AFDW, weight of organic material), and periphyton chlorophyll *a* (chl *a*). At the time of periphyton harvest, water samples were taken for subsequent analysis of total phosphorus (TP), total nitrogen (TN), soluble reactive phosphorus (SRP), and phytoplankton chlorophyll *a* (chl *a*). Additionally, measurements of light intensity at 0.5 m depth (LI), and fish length and weight were determined at the start (May) and the end of the experiment (November). Macrophyte percent plant volume inhabited (PVI %) was calculated by visually estimating the percentage coverage and measuring macrophyte average plant height using the formula: PVI (%) = % coverage × average height / water depth. Water temperature (WT) was calculated as an average of July and August from two 24-hour measurement periods (hourly intervals) between these two months.

Laboratories analysis—Upon return to the laboratory, periphyton was scrubbed from the strips using a soft toothbrush and suspended in a defined amount of filtered mesocosm water (we used, two cellulose acetate filters, CAF, diameter 50 mm, pore size 0.24 and 0.8 μm). Invertebrate grazers (mostly, cladocera and chironomids) were removed from the strips using carbonate water (3-5 Min.). After homogenization, an aliquot sub-sample of each suspension were filtered onto two pre-weighed and pre-washed (GF/C, diameter 25 mm, pore size 0.7 μm) glass-fiber filters and dried at 105 °C for 12 h to analyze dry weight (DW). After drying, filters were used for combustion analysis (500 °C, 5 h) to determine ash free dry weight (AFDW). Chlorophyll *a* pigments (in periphyton, chl *a* and phytoplankton, chl *a*), TP, SRP, TN, LI were determined using standardized methods (for more detail see Landkildehus et al., in preparation). In Germany, three mesocosms (two in the high nutrient and one in the low nutrient treatments) sank during heavy storm events and were omitted as they could not fulfill the strict criteria established. To somehow account for the loss of mesocosms we used pseudo replicate by adding additional strips in the remaining mesocosms. At last we ended up with having the same number of periphyton strips.

Data analyses—We analyzed the data using analysis of covariance (ANCOVA) to test for significant differences of two aspects of periphyton growth (i.e. dry weight and chlorophyll *a* content) between the two nutrient treatments (i.e. low and high nutrient status). We did not test for the effect of nutrient treatment of AFDW because this variable was closely correlated to DW (r^2= 0.91, p= 0001), and thus, we regarded both measurements to represent the same aspects of periphyton growth. Because of our hypothesized effects of temperature and grazing on periphyton growth, WT and fish biomass were included as covariates in the analysis. Fish biomass was used in this analysis as a surrogate for grazing pressure on periphyton, assuming a cascading effect of fish on periphyton invertebrate grazers (Liboriussen et al., 2005). To test the appropriateness of WT and fish biomass as covariates in the ANCOVA model, a one way analysis of variance was carried out to test significant differences of these variables between the two nutrient treatments. The ANCOVA was carried out using a Type III sums of squares method to account for the unbalanced design in our experiment, due to the loss of three mesocosms in Germany. Where necessary, data were either log or square root transformed to improve normality of the residuals and meet the assumption of homogeneity of variance. A Student t-test was performed to test for significant differences between nutrient concentrations of the two nutrient treatments. Where appropriate, additional univariate correlation analysis was carried out to aid the interpretation of the measured variables. All analyses were carried out using STATISTICA 12 (StatSoft, Inc. USA).

Results

Water temperature—Our assumption that latitude is correlated with water temperature failed in the European summer in 2011, as water temperature did not follow a clear latitudinal gradient. Between July and August 2011, average water temperature in Estonia (58°12'17" N, 26°06'16" E) was 22.5 °C, in Czech republic (49°09'14"N, 14°10'11"E) 20.0 °C, and 20.8 °C in Germany (52°26'0" N, 13°39'0" E). To account for this discrepancy between water temperature and latitude, countries were arranged by water temperature for the following analyses.

Our hypothesis was tested under two nutrient treatments. The intention was to achieve in all countries TP concentrations of 25 and 200 µg L^{-1} for the low and high nutrient treatment, respectively. While the measured average TP concentrations in the low treatment was close to the target concentration (i.e. 20.1±6.9 µg L^{-1}), the measured concentration in the high nutrient treatment was lower than expected (i.e. 65.4±27.8 µg L^{-1}) (Table 6). However, the TP concentration between the two treatments was significantly different (Student t-test, *p*=0.001, Fig. 5A).

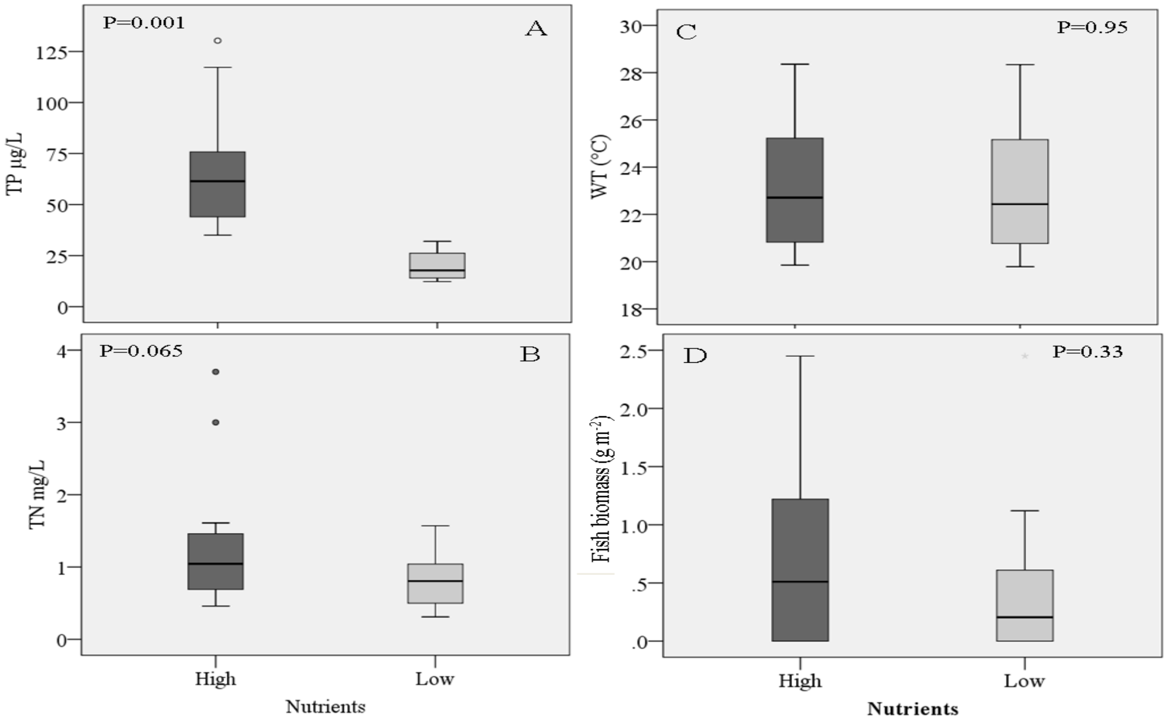

Fig. 5. (A) Total phosphorus (TP), (B) total nitrogen (TN), (C) water temperature (WT), and (D) fish biomass (gm^{-2}) for two nutrient treatments (i.e. high and low).

No significant difference of TN concentrations was detected between high versus low nutrient treatments (t-test, *p*= 0.065, Fig. 5B) with mean TN concentrations of 1.2 ±0.8 mg L^{-1} in 'high' and 0.8±0.4 mg L^{-1} in 'low' nutrients, respectively (Table 6, Fig. 5B).

We tested the response of two aspects of periphyton growth using two different nutrient treatments with an ANCOVA model. There was no significant difference between the two nutrient treatments for both periphyton biomass indicators (i.e. DW, F=0.17, *p*=0.68 and chl *a*, F=1.81, *p*=0.19; Table 6). Nutrient loading had no significant effect either covariate (ANOVA, WT: F=0.004, *p*=0.95 and fish biomass: F=0.98, *p*=0.33) (Fig. 5C and D). We found a significant relationship between periphyton DW as dependant variable and WT as a covariate (F=20.3, *p*=0.0001), but no significant relationship with fish biomass as a covariate (F=3.4, *p*=0.07) (Table 5A). In the case of periphyton chl *a* (Table 5B) both covariates were significant (WT: F=6.3, *p*=0.006 and fish biomass: F=28.1, *p*=0.0001). To further interpret the relationship between periphyton biomass, WT and fish biomass, we perfumed a univariate correlation analysis between these variables. Periphyton DW significantly increased (r^2=0.42, *p*=0.001) with water temperature (Fig. 6B, 3A).

Table 5. ANCOVA model for the effect of water temperature (WT) and fish biomass on, (A) periphyton dry weight (DW) and (B) periphyton chlorophyll *a* (chl *a*). Data were square root (SQET) transformed to improve normality of the residuals and meet the assumption of homogeneity of variance.

A- Periphyton DW				
Effect	Df	SS	F	*P*
SQRT WT	1	11.2	20.3	0.000
SQRT Fish biomass	1	1.9	3.4	0.07
Nutrients	1	0.02	0.03	0.85
B- Periphyton chl *a*				
SQRT WT	1	3.1	6.3	0.006
SQRT Fish biomass	1	14.1	28.1	0.000
Nutrients	1	0.39	0.77	0.39

Periphyton chl *a* increased (r^2=0.23, *p*=0.001) with periphyton DW (Fig. 7C). The effect of fish on periphyton biomass was discovered by adjusting the periphyton DW and chl *a* to water temperature and plot the residual with fish biomass. The result show a weak relation with periphyton DW (r^2=0.1, *p*=0.10) (Fig. 7B), and a strongly significant relation with periphyton chl *a* (r^2=0.45, *p*=0.0001) (Fig. 7D).

Non significant difference was detected between periphyton chl *a* in high and low nutrient treatments (t-test, *p*=0.2) (Table 6). Mean SRP concentrations were very low in all mesocosms, especially in the low nutrient treatments, and SRP concentrations were significantly different (t-test, *p*=0.001) between high and low nutrient treatments (Table 6).

Table 6. Means and standard deviation of selected variables from the evaluation of the effects of nutrient treatments (High and Low) on periphyton biomass in five European countries.

Countries	Czech Republic		Germany		Estonia		Turkey		Greece		All countries	
Variables/Treatments	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
Periphyton AFDW (g m^{-2})	0.5±0.3	0.5±0.2	1.5±0.2	1.7±0.2	2.2±1.6	1.0±0.2	0.9±0.8	0.3±0.3	9.2±4.1	6.0±1.6	3.0±3.9	1.9±2.4ns
Periphyton chl *a* (mg m^{-2})	5.0±3.4	2.8±1.0	0.9±0.1	0.7±0.0	0.1±0.0	0.04±0.0	0.4±0.4	0.1±0.1	16.8±5.7	3.1±0.9	5.0±7.3	1.4±1.5ns
TP (µg L^{-1})	84.6±31.3	15.2±3.2	40.0±5.7	25.0±8.5	45.0±9.6	14.0±0.8	65.7±35.9	19.9±4.6	79.0±17.2	29.0±2.9	65.4±27.8	20.1±6.9**
TN (mg L^{-1})	0.8±0.3	0.9±0.31	3.4±0.5	0.6±0.1	1.4±0.2	0.8±0.0	0.7±0.1	0.4±0.1	1.1±0.4	1.4±0.2	1.2±0.8	0.8±0.4 ns
SRP (µg L^{-1})	31.5±29.3	1.7±0.3	0.7±0.2	0.9±0.3	8.3±1.3	0.7±1.0	6.7±1.4	1.3±1.0	17.3±3.6	5.7±1.5	14.2±16.4	2.2±2.2**
Fish biomass (g m^{-2})	1.3±0.2	0.9±0.2	1.4±0.5	0.9±0.2	0.0±0.0	0.0±0.0	0.3±0.4	0.2±0.2	0.7±0.1	0.7±0.7	0.7±0.6	0.5±0.5
Phytoplankton chl *a* (µg L^{-1})	5.4±2.1	5.7±1.3	72.5±11.0	6.9±1.6	20.4±12.4	13.4±3.6	9.8±3.4	2.2±1.1	52.5±61.7	7.0±8.1	27.6±36.0	7.0±5.5**
Light intensity µmol (m^{-2} s^{-1})	263±57	129±75	764±206	1163±319	179±40	685±262	484±147	631±3	104±74	871±190	313±232	644±365**
Macrophyte (PVI %)	0.0±0.0	1.0±1.0	0.0±0.0	6.6±0.4	11.9±12.9	10.2±10.3	4.8±5.6	8.2±3.3	2.4±2.1	10.8±2.1	4.2±7.5	7.4±6.0 *

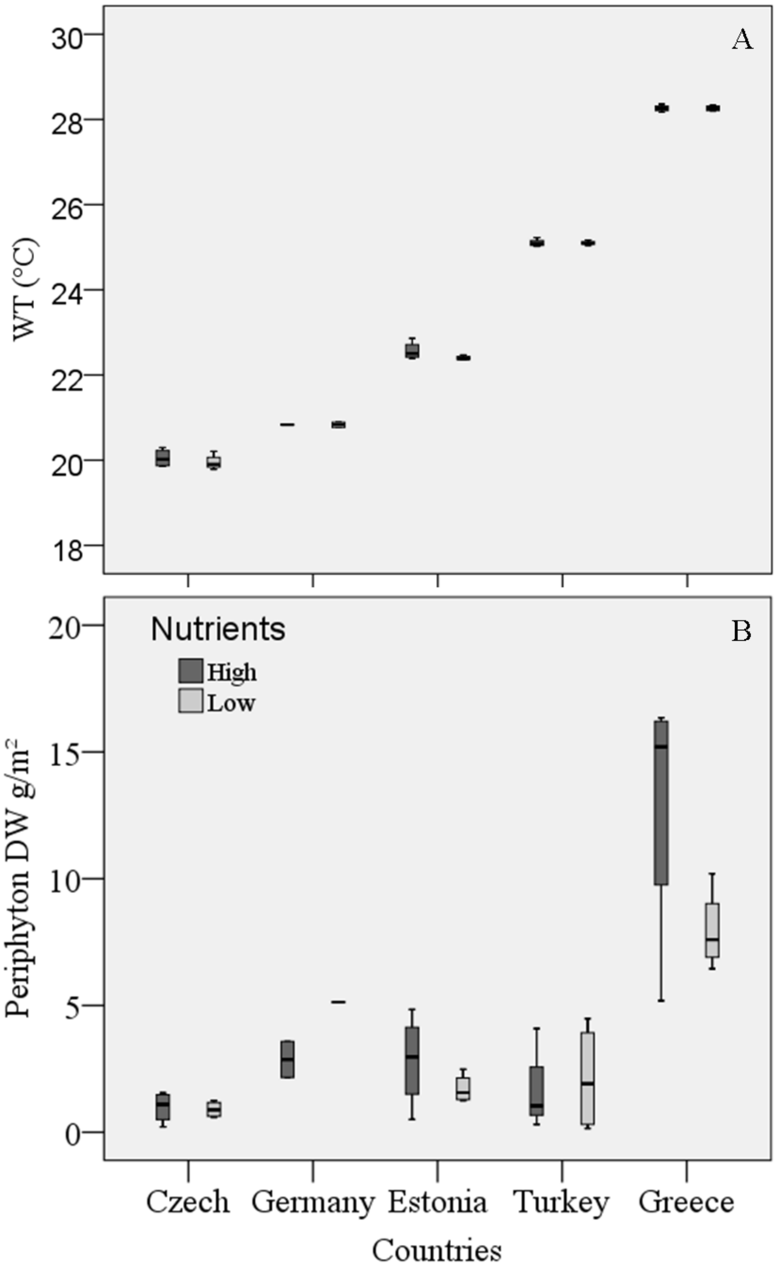

Fig. 6. Mean (A) water temperature (WT) and (B) periphyton dry weight (DW) in mesocosm experiments in five European countries with two nutrient concentrations (high and low).

No correlation (*r*= 0.22) was detected between phytoplankton chl *a* and periphyton chl *a*, while phytoplankton chl *a* was significantly different between the two nutrient treatments (t-test, *p*= 0.007) (Table 6). Light intensity did not drop below 104 µmol m^{-2} s^{-1} in all countries and significant differences were detected between treatments (t-test, *p*= 0.005) (Table 6). In all five countries, macrophytes had a lower PVI or in some cases were absent in the high nutrient treatments. Macrophyte PVIs was significantly different between the two nutrient treatments (t-test, *p*= 0.02, Table 6).

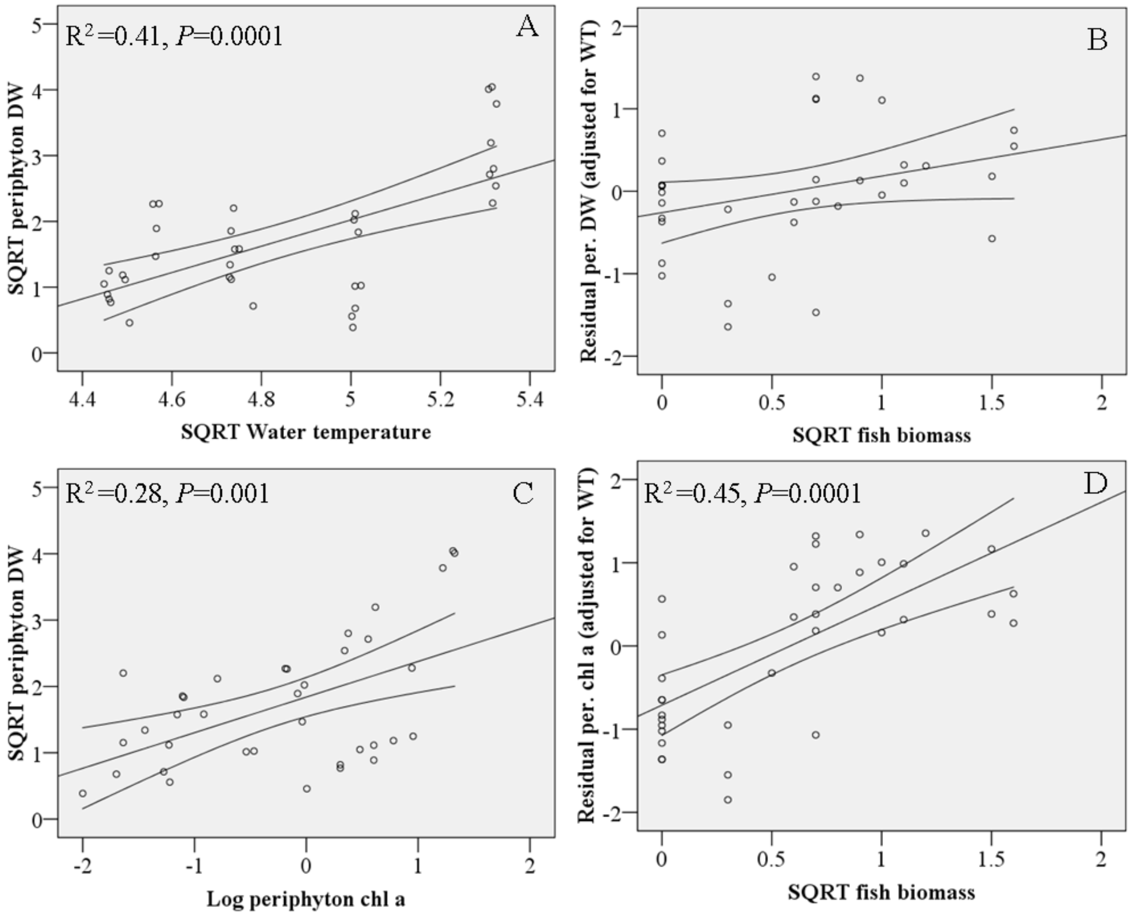

Fig. 7. Relation between (A) periphyton dry weight (DW) and water temperature (WT), (B) residuals of periphyton dry weight (DW) adjusted for water temperature and fish biomass (g m^{-2}), (c) periphyton chlorophyll *a* (chl *a*) and periphyton dry weight (DW), and (D) residuals of periphyton chlorophyll *a* (chl *a*) adjusted for water temperature (WT) and fish biomass (g m^{-2}) in the mesocosm study in five European countries.

Discussion

This pan-European lake mesocosm experiment provides compelling evidence that increasing water temperature can lead to increase summer periphyton biomass development. Nutrient enrichment had no significant effect on periphyton biomass in our experiment, which allows excluding indirect effects of water temperature on this bottom-up control factor of periphyton. In contrast, indirect top-down effects of fish appeared an important factor controlling periphyton biomass, especially in the warmest mesocosms in Greece.

Effects of water temperature on periphyton biomass—Our results showed that periphyton biomass (DW) increased significantly with rising water temperatures in the range from 20-28°C (see Figs. 2A, B and Fig. 7A). The slope of the regression between water temperature and periphyton biomass suggests that for every degree increase in water temperature, summer periphyton biomass was stimulated by 42%. While we do not intent to use this correlation for prediction by any means, the implications of this relationship is highly relevant for projecting the potential effects of climate change on primary productivity. Because global air temperature is expected to increase by 2100 even under the most optimistic scenario by the IPCC (IPCC, 2013), water temperature will also increase (IPCC, 2013). Therefore, we expect that periphyton biomass is also likely to increase in the future. The effect of temperature on freshwater periphyton can be considered in terms of capacity adaptation (Schlesinger and Shuter, 1981) and in growth curves (Vermaat and Hootsmans, 1994).

Our results are in contradiction with Hansson (1992), who found that temperature was of minor importance for periphyton biomass in a study along a productivity gradient of Swedish and Antarctic lakes ranging from extremely low productivity meltwater to highly productive lakes. The considered temperatures in his study (2-10°C) were most likely in a limiting range where light may be the more important driver. Our results are also different from those obtained in a similar pan-European study by Bécares et al. (2008), who considered a temperature range of 17.7-29°C. They showed that periphyton chl *a* achieved in northern lakes was higher than that in southern lakes. These results were explained by a stronger shading effect of phytoplankton on periphyton in southern lakes, phytoplankton chl *a* concentrations in this study were much higher (40-564 µg chl *a* L^{-1}) than that in our study (2-53 µg chl *a* L^{-1}). Maximum periphyton biomass was also much higher (84 mg chl *a*/m^2) as compared to our study (16 mg chl *a*/m^2). In general, nutrients had a greater effect on periphyton chl *a* than fish in the study of Bécares et al. (2008), which was also opposite in our study. We thus assume that our results are valid in eutrophic lakes with a prevailing top-down control of periphyton biomass by a fish-scraping invertebrates cascade as suggested by Jones and Sayer (2003).

Effects of nutrients on periphyton biomass—Even though the measured TP concentrations in our treatments were significantly different (mean 20 and 65 µg TP L^{-1} at low and high nutrients, respectively); there was no significant effect on periphyton biomass (see Table 6; Fig. 6B). Generally, the current study indicated that the mean nutrient concentrations were saturating for periphyton growth (Lalonde and Dowining, 1991: 39 µg TP L^{-1}; Liboriussen and Jeppesen, 2005: 60-200 µg TP L^{-1}).

In our study, SRP concentrations (mean 2.2-14.2 µg L^{-1} in low and high nutrient treatments, respectively) were very low in comparison with result obtained by Bothwell (1988). He found that periphyton reaches a saturating response at concentrations of around 28 µg SRP L^{-1}.

Effects of light on periphyton biomass—The interaction between phytoplankton and periphyton biomass plays an important role to control each other when aquatic lake productivity is high. When nutrients are not limiting, the factor limiting the growth of periphyton is most often light as phytoplankton growth may shade periphyton (Stevenson, 1985; Hansson, 1992). In our study light limitation was unlikely a significant factor affecting periphyton growth as light levels were always above the limit given by Sand-Jensen and Borum (1991). They give values in the range of 1-10 μmol m^{-2} s^{-1} for the minimum light requirement for growth of microalgae, while light conditions in our mesocosms range between 104 and 1163 μmol m^{-2} s^{-1}.

Effect of fish on periphyton biomass—We found a positive top-down effect of fish on periphyton biomass (chl *a*) probably due to a control of periphyton scraping invertebrates as suggested by Jones and Sayer (2003) and Danger et al. (2008). The slope of the regression between fish biomass and periphyton chl *a* adjusted to water temperature suggested that, for every degree increase in water temperature and its equivalent indirect top-down control by fish, summer periphyton biomass (chl *a*) was stimulated by 45%. In our experiment we used *Gasterosteus aculeatus*, a bottom-feeder that feeds basically on plankton and benthic prey (Sánchez-Gonzáles et al., 2001). Periphyton biomass was highest, however, in Greece, were a different fish species (*Gambusia affinis*) were used, which may have affected the recorded interactions. Mosquitofish fed mainly on the same food zooplankton and chironomids like stickleback (Gkenas et al., 2012).

The complexity between high temperatures food web variability most likely benefited the periphyton biomass development in the southern European countries. In addition, warmer climate benefited omnivorous and benthivorous fish due to more frequent spawning and the absence of piscivores, which may otherwise exert a strong negative effect on the periphyton grazers. This may lead to dominance of bottom-up control of periphyton biomass due to decreased top-down control (Fernández-Aláez, 2004; Romo, 2004). Mediterranean shallow lakes can remain in a turbid state with a weak control of periphyton biomass by grazers due to more intense fish predation than this is the case in temperate lakes, for a given nutrient level (Beklioğlu et al., 2007). Moss et al. (2004) found that the ratio between prey and predatory fish: zooplankton biomass increased from northern to southern Europe, while the zooplankton: phytoplankton biomass ratio decreased in the same direction.

In conclusion our results indicated a strong positive temperature on periphyton growth. However, synergistic interactions between bottom-up and top-down factors might occur, suggesting that care should be taken when predicting the combined effect of changes in multiple factors from their individual effects.

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Chapter IV:

Experimental comparison of periphyton removal by chironomid larvae and *Daphnia magna*

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Abstract

Periphyton is an important component of aquatic ecosystems and is often controlled by invertebrate grazers such as snails or chironomid larvae. However, the impact of pelagic cladocerans, such as *Daphnia magna*, that migrate into the littoral zone to seek refuge from fish predation on periphyton removal is unknown. In laboratory experiments, we compared the effect of *D. magna* on periphyton removal and algal composition with that of a naturally associated invertebrate community dominated by chironomid larvae and chironomid larvae only. Periphyton biomass was significantly reduced by *D. magna* (38%), the natural grazer community (33%), as well as chironomids (37-62%) during a two-week laboratory exposure at 20°C. Periphyton removal rates of all tested grazers were comparable at the community level (360 -540 mg dry weight (DW) m^{-2} d^{-1}). In accordance with allometric theory, the larger chironomid larvae had higher individual periphyton removal rates (0.11-0.17 mg DW $individual^{-1}$ d^{-1}) than *D. magna* (0.06 mg DW $individual^{-1}$ d^{-1}), whereas body mass specific periphyton removal rates of *D. magna* (0.87 mg DW mg grazer DW^{-1} d^{-1}) were 58% higher than that of chironomids. We conclude that the role of large planktonic cladocerans such as *D. magna* for periphyton removal and its algal composition can be significant and deserves further attention.

Introduction

Food webs of shallow lakes are complex heterogeneous environments with a variety of primary producers. Periphyton can contribute more than 80% to total primary production in shallow lakes and can thus be a significant energy source for higher trophic levels (e.g., Vadeboncoeur et al. 2003). In addition, periphyton affects nutrient turnover and the transfer of nutrients between the benthic and pelagic zones (Vadeboncoeur et al. 2003). There is a considerable body of evidence for top-down effects by different invertebrate grazers on periphyton biomass, productivity, species composition, diversity, and physiology (e.g., Feminella and Hawkins 1995). Top-down control of periphyton by a fish-macroinvertebrate cascade has been suggested to be responsible for changes in the abundance of submerged plants in shallow lakes (Brönmark et al. 1992, Jones and Sayer 2003). As submerged macrophytes are pivotal for the stabilization of clear-water conditions (Scheffer et al. 1993), their loss has dramatic consequences for shallow ecosystems (Hilt et al. 2013) and their functions, e.g. for regional carbon budgets (Brothers et al. 2013).

Epiphytic chironomid larvae are an important invertebrate group in streams (e.g., Pinder 1992, Tall et al. 2006) and shallow lakes (e.g., Tarkowska-Kukuryk 2013). They are often a dominant group of plant-associated macroinvertebrates (Körner et al. 2002, Boll et al. 2012). Their grazing can have marked effects on periphyton standing crop (e.g., Mason and Bryant 1975, Cattaneo 1983) due to their high grazing rate and quick growth (Lalonde and Downing 1991, Pinder 1992). Plant-associated cladocerans such as *Sida crystallina* (O. F. Müller), *Chydorus sphaericus* (O. F. Müller), and *Alona affinis* (Leydig) have also been shown to use periphyton. Their periphyton ingestion rates were related to periphyton concentration, concentration of phytoplankton as alternate food, and body size (Downing 1981). Only few studies, however, have examined the use of periphyton as a food source for herbivorous pelagic zooplankton. *Daphnia* and other important pelagic filter feeders of phytoplankton often undergo diel horizontal migration (DHM) into macrophytes or other structures in the littoral zone of shallow lakes (Burks et al. 2002). Scarcity of phytoplankton in the littoral due to direct and indirect negative effects of macrophytes on phytoplankton (e.g., Schriver et al. 1995, Hilt and Gross 2008), however, may force them to use periphyton as alternate food source. Stable isotope analyses indeed revealed that some daphnids use periphyton as a food source (Jones and Waldron 2003). Recently, Siehoff et al. (2009) showed the ability of *Daphnia magna* (Straus) to establish a stable population when fed solely on periphyton and switch to periphyton when phytoplankton availability was low. In the same study, *D. magna* reduced the thickness of the periphyton layer, but did not remove understory periphyton and was thus classified as a gatherer or shredder rather than a scraper or rasper (Siehoff et al. 2009). However, it has not yet been shown whether this feeding activity of *D. magna* has also a significant impact on the removal of periphyton.

In this study we tested whether *D. magna* could significantly remove periphyton, and compared its effects on periphyton with both that of a naturally associated invertebrate community and that of chironomid larvae only. Specifically, we compared community, individual and body mass specific periphyton removal rates.

Materials and Methods

Periphyton—One-month-old periphyton samples were collected from artificial polypropylene, transparent strips (16 x 2 cm) with a slightly textured surface (IBICO®, Germany) exposed to 50-cm water of mesocosms floating in Lake Müggelsee (Germany, 52º 26´N, 13º 39´E) in August, September and October 2011 (Table 7). Mesocosms (four cylindrical fibreglass tanks, 2 m deep, 1.2 m diameter) contained tap water, 10 cm sediment, macrophytes, sticklebacks, and phyto- and zooplankton collected from five different German lakes. Nutrient levels were kept at 200 µg/L phosphate and 2 mg/L nitrate, by means of monthly nutrient dosing of 80 µg/L phosphate (monosodium phosphate dihydrate) and 1.6 mg/L nitrate (calcium nitrate tetrahydrate) (Landkildehus et al. in preparation). Periphyton strips were kept 30 cm away from northern tank walls. Two strips of each mesocosm were used to determine the initial dry weight (DW), ash-free dry weight (AFDW), chlorophyll a (chl *a*), C:N ratio and total phosphorus (TP) content of periphyton (methods see below). TP, total nitrogen (TN), and dissolved silica (DSi) of the mesocosm water were measured using standard methods (Anonymous 2005).

Periphyton removal experiments—Three subsequent laboratory experiments were performed testing the periphyton removal capacity of the natural grazer community (NGC) present in the mesocosms (exp. 1), two different size classes of chironomid larvae (exp. 2, 3) and *Daphnia magna* (exp. 3) (Table 7). For all experiments, periphyton strips were harvested from the mesocosm and cut in two halves, so that one half could always serve as the control (4 replicates per treatment). The 8 x 2 cm stripes were then horizontally placed in 0.5 L glass beakers filled with GF/F filtered mesocosm water under a light: dark cycle of 12:12 h and a constant temperature of 20 °C for two weeks. The beakers were distributed randomly under saturating light conditions (166 µE m^{-2} s^{-1}). Evaporated water was replenished with GF/F filtered mesocosm water. Five mL of nutrient solution (described in Körner and Nicklisch 2002) was added to all treatments every other day to prevent nutrient limitation.

For exp. 1, the control halves of the strips were treated with carbonated water for 3 min to remove all grazers. The collected grazers were fixed in formaldehyde for identification and biomass determination (see below). The other half of the strips with the NGC remained untreated. For exp. 2 and 3, all strips were treated with carbonated water for 3 min to eliminate grazers. In exp. 2, each of the strips from the treatment half were stocked with 5 chironomid

Table 7. Exposure periods, initial and final grazer and periphyton biomass and composition and mesocosm water quality (±1 SE). Asterisks indicate significant differences between control and treatment at $p \leq 0.05$ (t-tests).

Exp. No	Exposure (mesocosm, experiment)	Treatments		Grazer						Mesocosm water		
				DW (µg DW/cm²)	DW (g/m^2)	AFDW (g/m^2)	chl *a* (mg/m^2)	TP (mg/m^2)	C:N ratio	TP (µg/L)	TN (mg/L)	DSi (mg/L)
Exp.1	15.7.-16.8.11	Initial	control	No	2.9±0.9	1.5±0.5	0.9±0.2	8.5±0.8	8.0±0.0	44.0±4.0	3.0±1.5	2.0±0.1
			NGC: Chironomids	1.1±0.26								
			NGC: *C. sphaericus*	0.25±0.08								
	16.-30.8.11	Final	Control	No	21.3±0.8	13.6±0.8	13.1±1.8	63.0±6.5	8.4±0.33			
			NGC: Chironomids	17.6±5	14.2±1.7*	9.6±0.8*	9.0±1.4	49.0±6	8.6.0±0.53			
			NGC: *C. sphaericus*	12.1±1.6								
Exp.2	16.8.-13.9.11	Initial	Control	No	1.3±0.4	0.9±0.3	0.6±0.3	4.2±0.05	8.0±0.0	50.0±11.0	3.0±0.5	3.0±0.05
			Chironomids	20±1.4								
	13.-28.9.11	Final	Control	No	12.5±0.8	8.6±1.0	5.3±0.1	38.0±5.3	8.9±0.2			
			Chironomids	150±1.7	4.9±0.2*	2.7±0.3*	2.5±0.1*	32.0±2.0	8.1±0.5			
Exp.3	14.9.-12.10.11	Initial	Initial	No	1.0±0.5	0.7±0.2	0.5±0.3	3.5±0.6	7.0±0.0	57.0±18.0	4.0±1.0	3.0±0.1
			Chironomids	10.9± 1.1								
			D. magna	14.4±0.2								
	12.-26.10.11	Final	Control	No	13.7±0.5	8.6±0.6	7.6±0.7	68.0±3.5	7.1±0.27			
			Chironomids	112±0.2	8.4±0.6*	3.7±0.4*	3.3±0.4*	47.0±7.0	6.15±0.12*			
			D. magna	63.3± 1.9	8.6±0.4*	4.0±0.6*	3.4±0.6*	49.0±10.0	6.45±0.05			

larvae (mean length: 5.1 ± 0.1 mm, 3 - 5 days old) whereas the control was kept free of chironomids. Exp. 3 was similar, but the chironomid larvae were smaller (mean length: 4.0 ± 0.1 mm, 3 - 5 days old) and an additional treatment contained 10 adult *D. magna* (Table 7). After two weeks, all strips were treated with carbonated water for 3 min and collected grazers were fixed in formaldehyde. Chironomids used in exp. 2 and 3 were collected from the mesocosms and adapted to laboratory conditions three days before the experiments started. Two-day old *D. magna* were collected from a laboratory culture and adapted to experiment condition for 5 days. The laboratory culture of *D. magna* originated from a single female isolated from Müggelsee. The culture was held at 20 ± 2 °C, and a light: dark cycle of 14:10 h. It was fed daily with Scenedesmus sp. cultured in aerated columns in Z8 medium (Kotai 1972) and a light: dark cycle of 14:10 h before experiments started.

Biomass, nutrient and chl a content of periphyton—After grazer removal, periphyton was scrubbed from the strips using a toothbrush and suspended in a defined amount of GF/F-filtered mesocosm water. After homogenization, sub-samples were filtered onto two pre-weighed and pre-washed GF/C glass-fibre filters and dried at 105 °C for 12 h to analyze DW. One filter was combusted (500 °C, 5 h) to determine AFDW. The second filter was used to determine the contents of organic carbon (C) and nitrogen (N) using a Vario EL analyser (Elementar) after removing the carbonates by addition of 0.5 M HCl until no more foaming occurred and subsequent drying at 36 °C. TP concentrations in suspensions were measured using standard methods (Anonymous 2005).

For chl a determination, sub-sample of the suspension were filtered (GF/F) and frozen at -80°C until further processing. Chlorophyll *a* was extracted by vibration shaking (2000 rpm, 1.5 h) with dimethylformamide and glass beads (0.75–1.0 mm diameter). After centrifugation for 20 min (2500x g, 4°C), chl a was separated, identified and quantified with a HPLC system (Waters, USA) as described by Fietz and Nicklisch (2004).

Grazer species identification, biomass and growth rates—Chironomid larvae are difficult to determine and were thus identified after breeding out of larvae in exp. 1. Chironomid larvae in all experiments were a mixture of Chironomidae (*Glyptotendipes pallens* (Meigen), *Paratanytarsus* spp., *Parachironomus* spp. (subfamily: Chironominae), and *Cricotopus reversus* (Hirvenoja) (subfamily: Orthocladinae)). These chironomids are free-living scrapers or grazers (*Paratanytarsus*, *Parachironomus*, *C. reversus*) or collector gatherer, eating fine deposits (*G. pallens*). The cladocerans in exp. 1 were initially dominated by *Chydorus* *sphaericus*, while at the end some *Diaphanosoma brachyurum* (Lievin) and *Bosmina longirostris* (O.F. Müller) were found in addition, which contributed less than 0.4 % to the total cladoceran biomass and were thus neglected as grazers. Initial and final grazer DW were calculated using length-dry weight regressions of *D. magna*, *D. brachyurum*, and *C. sphaericus* (Bottrell et al. 1976), *B. longirostris* (Michaloudi 2005), and chironomids (Mährlein, M., unpublished data). Length measurements were performed using a Nikon (SMZ1500) stereomicroscope with Nikon digital camera and NIS-Elements D 3.1 software. To establish body-mass specific periphyton removal rates mean biomass per day for chironomids and *D. magna* in exp. 2 and 3 needed to be estimated. This was done based on a population growth model, whose integrated form yields the logistic function:

$$P(t) = \frac{KP_o e^{n}}{K + P_o(e^{n} - 1)} \qquad (1)$$

Where P = the population size, t = time, P_o the initial population size at t = 0, K = carrying capacity and r = growth rate.

The choice of the functional form was based on population growth experiments for chironomids and daphnids (Armitage et al. 1995, Larsson and Weider 1995). To stabilize the model fit the following assumptions for a third data point were made. Chironomids enter the pupa phase after 2-3 weeks in summer. We thus assumed, based on the final biomass, that a hypothetical 18th day of the experiment, would have been reached with an increase in biomass of 2% (exp. 2) and 4% (exp. 3). The lower growth for exp. 2 assumes a lower ability to gain further weight for the already very heavy animals at day 14. Daphnids are known to have a low growth under limited food supply (Siehoff et al. 2009); we thus assumed that until day 18 the reduced food resources might have supported a limited weight gain of around 4%. Best-fit curves were found by minimizing a sum of square error function (non-linear minimization function (nlm), R Development Core Team 2012). The mean value theorem for integrals was then used to calculate the mean biomasses per day.

Statistical analyses—All experimental data were tested for normal distribution using Kolmogorov-Smirnov tests and log(x+1) transformed if needed. In the cases of two-sample comparisons the Students t-test was used. In cases of more than two factor levels one-way analysis of variance (ANOVA) with subsequent Tukeys HSD post-hoc test was applied. All analyses were carried out using the statistical package SPSS 17 (SPSS Inc., Chicago, IL, USA).

Results

The quality of periphyton used for the removal experiments was slightly different in exp. 1 (NGC) with higher initial periphyton DW, AFDW and TP than that in periphyton used for exp. 2 and 3, but was very similar in exp. 2 and 3 (Table 7). Differences in initial chl a concentrations and C:N ratios were less pronounced or absent between experiments (Table 7). Final TP concentrations in periphyton were higher than that initial one in all experiments, but did not differ between controls and grazer treatments (Table 7). Initial and final C:N ratios were not significantly different. Grazing slightly reduced C:N ratios in exp. 3 (Table 7). Periphyton accumulation rates were 1.47 ± 0.1 (SE), 0.87 ± 0.08 and 0.96 ± 0.03 g DW m-2 d-1 (0.92 ± 0.12, 0.37 ± 0.007 and 0.53 ± 0.06 mg chl a m-2 d-1) in controls without grazers of exp. 1, 2 and 3, respectively.

In the presence of the natural grazer communities, which consisted mainly of chironomids (78% of total initial grazer biomass) and the cladoceran *C. sphaericus*, periphyton DW was significantly reduced by 33% in two weeks. Larger chironomid larvae in exp. 2 grazed about 62% and smaller chironomid larvae in exp. 3 grazed about 37% of the initial periphyton DW (Table 7). Similar values were reached by *D. magna*, which removed 38% of the initial periphyton DW in exp. 3 (Table 7). AFDW of the initial periphyton was reduced by 29%, 69%, 57% and 53% and initial chl a was reduced by 31%, 52%, 57% and 55% by the NGC in exp. 1, chironomid larvae in exp. 2 and chironomids and *D. magna* in exp. 3, respectively (Table 7). Total periphyton removal was 7.1 ± 1.7, 7.6 ± 0.2, 5.1 ± 0.4 and 5.3 ± 0.6 g DW/m2 (4.0 ± 1.4, 2.8 ± 0.1, 4.2 ± 0.6 and 4.3 ± 0.4 mg chl a/m2) for NGC, large and small chironomid larvae and *D. magna*, respectively.

Mean chironomid larvae biomass were calculated at 980 mg DW/m^2 and 660 mg DW/m^2 in exp. 2 and 3, respectively, and mean biomass of *D. magna* in exp. 3 was 430 mg DW/m^2 (Fig. 8). Community periphyton removal rates of NGC and large chironomid larvae as well as small chironomid larvae and *D. magna* were in the same order of magnitude, for DW, AFDW and chl a (Fig. 9A,B, one-way ANOVA, $p > 0.05$, data for AFDW not shown). Individual periphyton removal rates decreased significantly with decreasing animal size for DW and AFDW (one-way ANOVA, DW: F = 63.5, $p < 0.001$, Fig. 9C; AFDW: F = 18.5, p = 0.001), whereas data for chl *a* indicated similar values for large chironomid larvae in exp. 2 and *D. magna* (one-way ANOVA, F = 8.7, p = 0.008; Fig. 9D). Body mass specific periphyton removal rates of *D. magna* were significantly higher than those of chironomid larvae in exp. 2 and 3 for DW and AFDW (one-way ANOVA, DW: F = 9.0, p = 0.007; Fig. 9E; AFDW: F = 13.10, *p* = 0.002) as well as for chl a (one-way ANOVA, F = 22.9, $p < 0.001$; Fig. 9F).

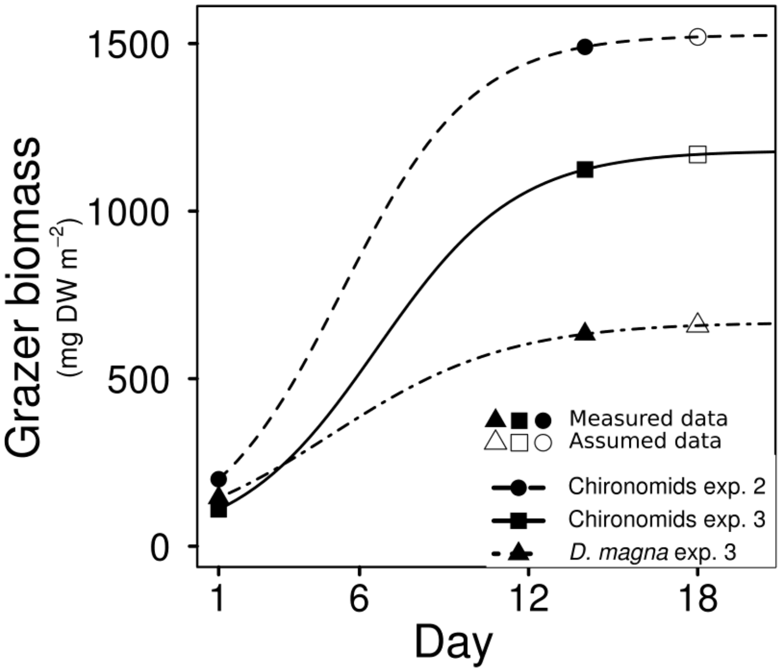

Fig. 8. Logistic growth function used to calculate mean grazer biomass of chironomid larvae and *Daphnia magna* during periphyton removal experiments 2 and 3.

Discussion

Our experiments revealed that *D. magna* could significantly remove periphyton from artificial substrates at a similar community removal rate compared to chironomid larvae. Body mass specific periphyton removal rates of *D. magna* were 58% higher than that of chironomids. Periphyton removal rates of *D. magna* at 20°C reached about 41% of periphyton accumulation rates indicating their potential importance for periphyton standing crop.

Periphyton removal rates measured under laboratory conditions at 20°C showed a striking similarity between NGC and chironomid larvae at the community level (Fig. 9A,B) in exp. 1 and 2 despite differences in initial grazer biomass of about one order of magnitude. The NGC potentially reached similar removal rates at lower initial biomass due to higher initial periphyton biomass (Table 7) as shown by Cattaneo and Mousseau (1995) or due to a higher food quality of the periphyton in exp. 1, e.g. lower C:P ratios (Table 7, Burks et al. 2002). Overcrowding effects as suggested for many laboratory grazing experiments by Hillebrand (2009) can also reduce the effect size of grazers on periphyton, but our grazer densities were lower or comparable to natural densities (see below). In exp. 3, chironomid larvae and *D. magna* where tested at the same periphyton and showed similar periphyton removal rates at the community level (Fig. 9A). Both, chironomid larvae (0.3 individuals/cm^2, 660-980 mg DW/m^2) and *D. magna* (0.6 individuals/cm^2, 20 individuals/L, 1.4 mg DW/L) were tested at naturally occurring densities. Even higher densities of chironomid larvae (Mason and Bryant (1975): 0.7/cm^2 on Typha stems, Menzie (1981): 4.5/cm^2 on *Myriophyllum* leaves, Cattaneo (1983): up to 6/cm^2 on artificial plants, Gresens and Lowe (1994): 1.04/cm^2 on *Potamogeton leaves*) and of *D. magna* (Lauridsen and Buenk (1996): 40/L in macrophyte stands, Östmann (2011): up to 100/L in rock pools) have been reported. This indicates that our data are ecologically relevant although we excluded interspecific competition.

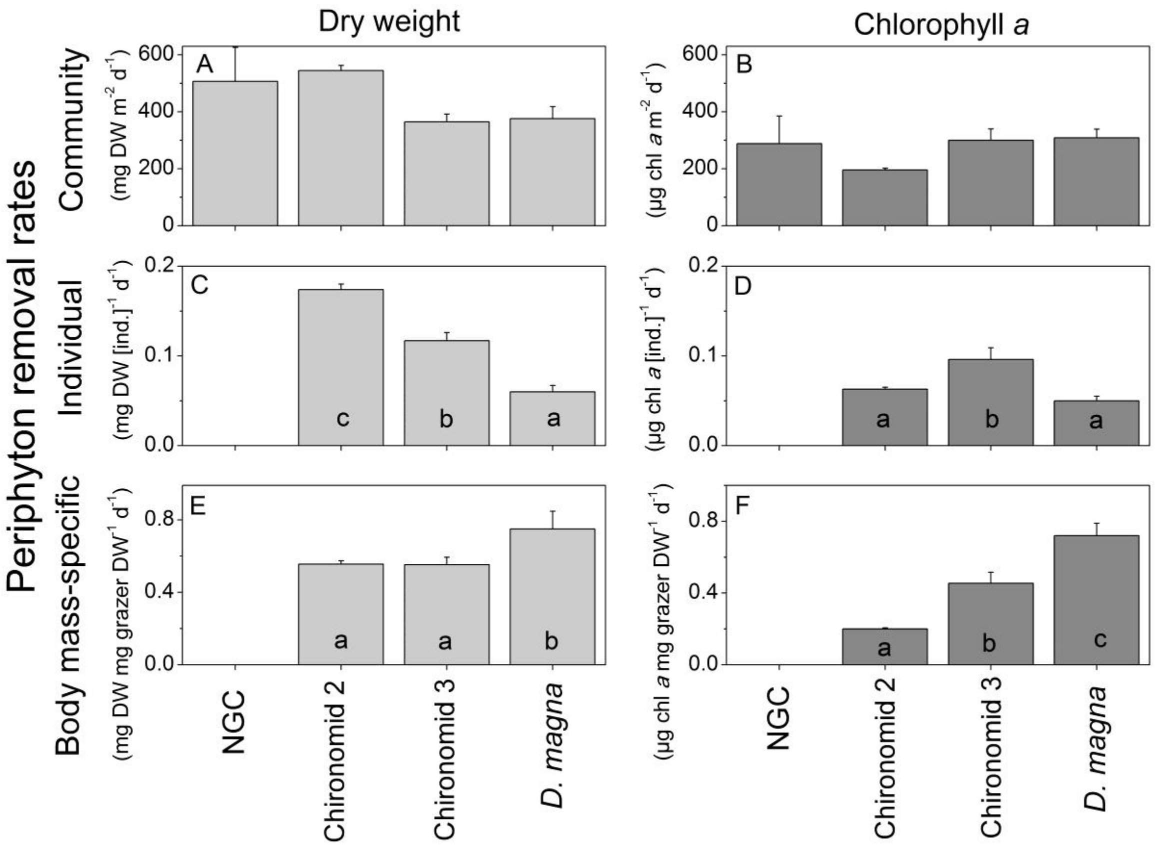

Fig. 9. Periphyton removal rates (+ 1 SE, based on dry weight (DW) and chlorophyll *a*) at the level of the community (A, B), the individual (C, D) and body mass specific (E, F) of a natural grazer community (NGC), chironomid larvae of exp. 2 (chironomid 2) and exp. 3 (chironomid 3) and *Daphnia magna* after two weeks incubation in the laboratory. Different letters indicate significant differences between treatments (one-way ANOVA, $p < 0.05$).

Our periphyton removal rates of chironomid larvae were higher than those reported from field studies. Mason and Bryant (1975) measured periphyton removal rates by chironomids of 74 mg DW m^{-2} d^{-1} on Typha stems from April to November and Kesler (1981) found maximum periphyton removal by chironomids of 107 mg DW m^{-2} d^{-1} in June. Periphyton removal rates of chironomids at the level of the individual level, however, were in the order of those predicted by

Cattaneo and Mousseau (1995) for grazers of a body mass of 200-300 µg. Their multiple regression on periphyton removal rate data by grazers showed that individual rates were positively correlated with grazer body mass, while crowding and food availability only explained a minor part of the variation (Cattaneo and Mousseau 1995). In their study, chironomids and oligochaetes were the smallest grazers tested. Mean individual periphyton ingestion rates of the plant-associated cladocerans *S. cristallina*, *C. sphaericus* and *A. affinis* (18.4, 7.6 and 8.5 µg DW individual^{-1} d^{-1}, respectively) were smaller than those determined for *D. magna* in our study, but variability seems high as maximum values up to 923 µg DW individual^{-1} d^{-1} were reported for the largest species *S. cristallina* (Downing 1981). In accordance with allometric theory (Cyr and Pace 1993), the larger chironomid larvae had higher individual periphyton removal rates than *D. magna* (Fig. 9C), whereas body mass specific periphyton removal rates (DW) of *D. magna* were 58% higher than that of chironomids (Fig. 9E).

The diet composition of periphyton grazers is size-class-specific and may affect the community composition of periphyton (Cattaneo and Kalff 1986, Tarkowska-Kukuryk 2013), which was not subjected of our study. However, we observed that *D. magna* as a filter feeder with a highly developed filtering apparatus detached small periphyton particles by turbulence and subsequently accumulated them. A similar behaviour was previously reported for Chydoridae (Fryer 1968) and *D. magna* (Siehoff et al. 2009) using the first trunk limb to remove small particles. Such physical damage of periphyton due to locomotion and other activities have already been reported for chironomids and explained the fact that grazing rates were up to 6 times lower than removal rates (Cattaneo and Mousseau 1995).

We concluded that the planktonic cladoceran *D. magna* which is known to conduct a strong control on seston components (e.g., Gliwicz 1990) can also significantly affect periphyton standing crop by grazing. Previous laboratory studies indicated that periphyton is only used below certain threshold levels (0.05 mg C/L) of phytoplankton densities (Siehoff et al. 2009). Such low phytoplankton concentrations have been reported, e.g. inside of macrophyte beds with a plant volume infested (PVI) above 15-20% (Schriver et al. 1995) or in allelopathically active macrophyte stands (Jasser 1995). We thus argue that effects of large planktonic cladocerans on periphyton have been underestimated. Top-down control of fish on zooplankton (Jeppesen et al. 1997) would then not only affect phytoplankton abundance but also directly contribute to the periphyton - macrophyte cascade suggested by Jones and Sayer (2003). To confirm the ecological relevance of significant periphyton removal by *D. magna* and other larger daphnids, as suggested by our results, in situ tests considering phytoplankton as alternate food are needed.

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Chapter V:

Threshold-driven shifts in two copepod species: Testing ecological theory with observational data

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Abstract

We used an observed abrupt shift in the dominance pattern of two coexisting copepod species in Müggelsee, a shallow eutrophic lake in Germany, to investigate mechanisms leading to this shift, by embedding our findings into the framework of intraguild predation theory and theoretical scenarios of threshold-driven regime shifts. We proposed that the abrupt increase in *Cyclops kolensis*, changing its status from a rare to the dominant species as available algal prey declined in the lake, was due to its superior exploitative competition for commonly consumed algal prey. However, *C. kolensis* was only able to thrive in the emerging low food niche when abundances of competing larger *Cyclops vicinus*, a predator of *C. kolensis* juveniles, fell below a critical threshold. This is consistent with the ‘‘state threshold scenario’’ of regime shift theory, for which a response variable exhibits an abrupt shift, here *C. kolensis*, after the driver (*C. vicinus*) crosses a threshold. We confirmed the nonlinear relationship between the two copepod species by excluding potentially matching abrupt changes in other abiotic and biotic driving variables, and successfully classifying *C. kolensis* abundance probability on the basis of *C. vicinus* abundances using logistic generalized linear modeling. *C. vicinus* decline followed the ‘‘driver threshold scenario’’ of regime shift theory, whereby an abrupt change in a driver (cryptophytes) causes a sudden shift in a response variable (*C. vicinus*). We illustrated how observational data on plankton communities match predictions derived from ecological theory.

Introduction

In freshwater ecosystems, relative community composition of zooplankton ensembles constantly fluctuates over the years. This is because the communities are embedded in a highly dynamic and complex system governed by a huge range of extrinsic and intrinsic forces, where the latter often mediate the effects of the former (Sommer et al. 2012).

Thus, changing conditions can favor different species, depending on their ecological niches. The resulting changes in abundance can thereby be gradual or abrupt with elapsing time.

Abrupt changes constitute regime shifts and may be the result of different mechanisms. The regime shift might arise from an equally sudden change in a driving variable, or it may be a drastic answer to a gradual changing driving variable surpassing a threshold. Thereby the drastic response may or may not be the result of a jump between alternative stable states (Andersen et al. 2009; Scheffer 2009; Bestelmeyer et al. 2011). In recent years, drastic responses in ecosystem dynamics have gained increasing attention in ecology because of their seeming unpredictability and potentially large effect on an ecosystem. Hence, if a regime shift occurs, it is desirable to first differentiate whether potential drivers exhibit matching sudden changes or if their change is gradual. If the latter is the case, important insight into ecological systems can be gained by quantifying potential thresholds in those drivers.

Extrinsic changes driven by climate and trophic states, which many freshwater systems have been subjected to over the past decades (Mooij et al. 2005; Adrian et al. 2009), have a large effect either at the single species level (Gyllström et al. 2005; Seebens et al. 2009) or on plankton communities as a whole (Adrian et al. 2006; Elliot and May 2008; Wagner and Adrian 2009). General concepts governing the intrinsic dynamics of a community include competition, predation, and intraguild predation (IGP); the latter being a special case describing the ‘‘killing and eating of species that use similar, often limiting, resources and are thus potential competitors’’ (Polis et al. 1989). In contrast to linear food chains, simple IGP models, comprising a predator, a consumer, and a resource, predicts the consumer’s competitive exclusion by an omnivorous predator in highly productive environments, while coexistence becomes possible at intermediate productivities. Potentially two alternative stable states can occur at this intermediate productivity level (Holt and Polis 1997; Mylius et al. 2001; Verdy and Amarasekare 2010). A precondition for these dynamics is the consumers’ superiority in exploitative competition for the common resource and the predators’ significant fitness benefit from the consumption of the consumer. However, Holt and Huxel (2007) showed that both prerequisites can be relaxed, if alternative resources are added to the simple IGP food-web model.

There is an apparent gap between theoretical frameworks in terms of driving forces of regime shifts and their applicability to the abrupt shifts observed in nature, i.e., testing ecological theory with observational data (but see Scheffer et al. 2001; Scheffer and Carpenter 2003; Dakos et al. 2008). Our present study contributes to filling this gap by exploring the driving forces for a sudden shift in the relative abundances of two coexisting freshwater cyclopoid copepod species. *Cyclops vicinus* gradually declined in abundance, whereas *Cyclops kolensis* populations abruptly increased between 1980 and 2010 in the Müggelsee (Berlin, Germany).

This lake has experienced an improvement in its trophic state (Köhler et al. 2005). At the same time water temperatures have been increasing over the past three decades (Adrian et al. 2006; Wilhelm et al. 2006; Huber et al. 2008). Hence, we were able to test the combined effects of changes in the availability of prey and enhanced water temperature in the context of IGP interactions between these two copepod species, since they share the same prey spectrum and prey on each other’s juvenile stages. We then integrated the observed interlinked system dynamics into scenarios of regime shift theory as proposed by Andersen et al. (2009).

C. kolensis is a winter species of northern European lakes (Rivier 1996) known for its strict life cycle, with a diapause in summer, and a pelagic existence in winter until early spring. *C. vicinus* is a common species in eutrophic lakes. In less productive lakes, it usually develops two population maxima in spring and autumn, interrupted by a diapause during summer (Santer and Lampert 1995). In highly eutrophic lakes, it tends to be present all year round in the pelagic zone (Hansen and Jeppesen 1992; Hansen 1996). Both species are omnivorous (Tóth and Zánkai 1985; Tóth et al. 1987; Adrian 1991) and have similar diets including juvenile stages of their own genus (Adrian 1991; Krylov et al. 1996). However, the larger species *C. vicinus* has higher ingestion rates for both algal and zooplankton prey (Adrian 1991; Krylov et al. 1996) and is known for its high food demand (Santer and Lampert 1995; Hopp and Maier 2005). This is in line with observed declining populations of *C. vicinus* in lakes undergoing reoligotrophication (Seebens et al. 2009), or their prominence in highly eutrophic lakes (Adrian and Deneke 1996; Adrian 1997; Maier 1998).

On the basis of long-term observational records (1980 to 2010) we explored the nature of the driving forces underlying the abrupt increase of *C. kolensis* in terms of (1) intraguild competition for prey between *C. vicinus* and *C. kolensis*, (2) threshold-driven release of *C. kolensis* from predation pressure by the larger *C. vicinus*, and (3) the role of warming trends. We postulate that *C. vicinus*, due to its higher food demands, was negatively affected by the observed reduction in the trophic state of the lake, which resulted in less available herbivorous prey. By contrast, *C. kolensis*, with its lower resource demands, profited in two ways: through release from competition for shared food resources, and through release from predation pressure on its juvenile stages, i.e., by the larger but declining population of *C. vicinus*. We first quantified critical thresholds for prey availability, separating high and low food niches, and second *C. vicinus* densities, separating high vs. low abundance probabilities of *C. kolensis*.

Our observed data are in line with the IGP model (Polis et al. 1989) such that the two species are able to coexist under conditions of intermediate productivity. As for the nature of the observed shifts in driver and response variables, our data exhibited an intriguing match with scenarios proposed by regime shift theory (Andersen et al. 2009).

Methods

Study site—The Müggelsee classifies as a shallow, polymictic, and eutrophic lake, situated in the temperate climate zone at the transition from maritime to continental climate, southeast of suburban Berlin (52° 26' N, 13° 39' E). The lake covers an area of 7.3 km^2, has a mean depth of 4.9 m, and a maximum depth of 7.9 m. The trophic state has changed from hypereutrophic to eutrophic, with a transitional period in the mid-1990s (Köhler et al. 2005; Huber et al. 2008). In addition, the Müggelsee has been subjected to seasonal warming trends in water temperature in the range of 1°C to 2.4°C over the last three decades (Adrian et al., 2006). For a comprehensive limnological characterization of the lake see Driescher et al. (1993).

Data collection and data processing—Between 1980 and 2010 surface water temperature was recorded daily between 08:00 h and 09:00 h at a depth of around 0.5 m. At times of ice cover duration, surface temperatures were assumed to be 0°C (Gerten and Adrian 2000). Monthly means were calculated from the daily water temperatures. Four remaining gaps, with a maximal length of 1 month, were linearly interpolated. Subsequently, September to May means were calculated, when the time window of the pelagic phases of *C. vicinus* and *C. kolensis* populations overlapped. Comparing this water temperature series against local air temperatures showed the expected relationship (data not shown). Unless otherwise stated, all statistical analyses were performed from September to May of the following year, depicted here as yearly means.

Sampling of phytoplankton and zooplankton was carried out weekly and in winter months every 2 weeks, using standard limnological techniques. A detailed description of sampling and sample processing is given in Driescher et al. (1993) and in Gerten and Adrian (2000). To avoid overestimating abundances due to missing values and differences in sampling intervals between winter months (fortnightly) and the growing season (weekly), we first calculated monthly means and linearly interpolated remaining missing values on the monthly scale. In total, 15 gaps in the zooplankton time series were interpolated, all occurring between 1980 and 1987. For phytoplankton, only one missing value had to be interpolated. From these time series, unless stated otherwise, means from September until May were calculated for each winter–spring period.

Abundances of *C. kolensis* and *C. vicinus*, the two dominant species during winter–spring, refer to adult females and males. Before gaining dominance in 1995, *C. kolensis* was a rare species, resulting in random effects and zero inflation in the first half of the data set. *Cyclops strenuus* also present between September and May, was not considered in this study, since its long-term dynamics lay outside the influential time window for *C. kolensis*.

We tested for abiotic (water temperature) and biotic (prey availability) forces, as well as intraguild predatory interactions between *C. kole*nsis and *C. vicinus* as potential drivers for the observed strong shift in their relative abundance, by using statistical modeling and statistically based classification. We considered total algal biomass, cryptophyte biomass (important food source for nauplii (Santer and van de Bosch 1994; Hansen and Santer 1995; Santer and Lampert 1995), and total rotifer abundances as potential prey for the two studied cyclopoid copepod species, both known to be omnivorous (see Introduction). In the observed phosphate and nitrogen ranges in Müggelsee, both total algal and cryptophyte biomass responded linearly to declining nutrients in the lake (data not shown). Before analysis, all variables were standardized by mean centering and standard deviation scaling. All data manipulation, analysis, and graphical visualization were conducted using the R language environment for statistical computing (R Core Team 2012) and associated library extensions. Although predation by fish is an important factor in top-down control of zooplankton communities, this was, due to the lack of fish data, not considered in this study.

Statistical analyses—Our analytical approach is closely related to the road map proposed by Bestelmeyer et al. (2011) (Fig. 10). The analysis of monotonic and abrupt trends in response and potential driving variables were followed by correlation and regression analysis between response and driving variables. The analysis was then completed by investigating the response mechanisms, i.e., food niche separation and the quantification of thresholds.

Analysis of abrupt and monotonic trends—The observed abrupt change over time in *C. kolensis* abundances could have arisen via three different mechanisms: it could have been (1) the result of a linearly mediated response to a matching abrupt change in one of the driving variables; (2) a drastic response to a driving variable surpassing a threshold; or (3) the result of a jump between alternative stable states, caused by either an external driver passing a bifurcation point or a perturbation of the state variables (Andersen et al. 2009; Scheffer 2009). To distinguish between the first two possibilities, we tested all potential drivers for monotonic and abrupt trends. A differentiation between the latter two mechanisms was not possible with the available data. Regime shift is not a well-defined term. So, here, we use the term for single-system levels in the sense defined by Andersen et al. (2009).

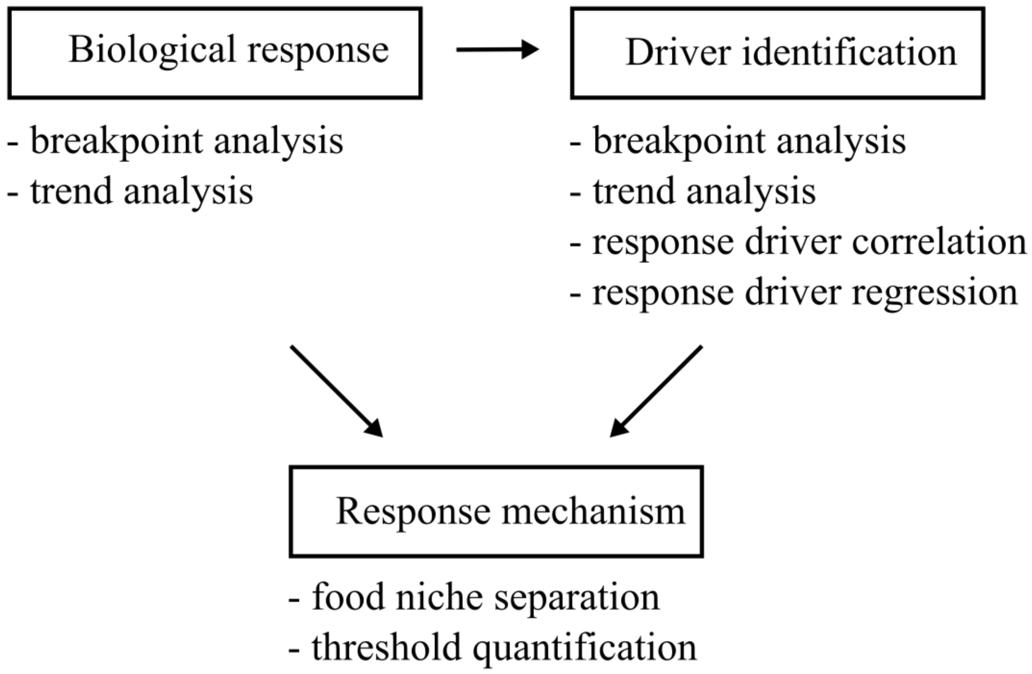

Fig. 10. Flow chart of statistical analysis performed in this study

Monotonic trends—To access monotonic linear and nonlinear trends for the entire time interval we used the procedure described in Yue et al. (2002). This iterative procedure is based on the nonparametric Mann–Kendall trend test (MKT) and the nonparametric robust Theil–Sen estimator (TSE) for slopes. The approach of Yue et al. (2002) has the advantage of accounting for the potential mutual influence between autocorrelation (AR (1)) and linear trends in time series data. To evaluate the monotonic trends, as well as the TSE, the R package ‘‘zyp’’ (Bronaugh and Werner 2009) was used.

Abrupt trends—Before the break-point analysis, all variables were standardized and significant linear trends were removed using TSE. For *C. kolensis* and rotifer abundances, no linear trend components needed to be removed. Chronological clustering was conducted using nonparametric univariate regression trees with time as the only explanatory variable (Borcard et al. 2011). For breakpoint analyses, we used the R packages ‘‘mvpart’’ (De'ath 2012) and ‘‘rpart’’ (Therneau et al. 2012), which follow the classification and regression trees of Breiman et al. (1984). During model building, the groups are formed such that the sums of squares about the group means are minimized, without changing the chronological order. This leads to detecting changes over time in the mean of the response variable. The resulting tree estimates the response variable through the group means found for the leaf nodes, i.e., through a piecewise constant function, which we refer to here as the break-point model. To avoid overfragmentation, only groups with at least 15 data points were split further. Additionally, the tree size was controlled by using the so-called 1 SE rule for selecting the final tree model.

Relation of C. kolensis and C. vicinus to abiotic and biotic drivers—To assess differences in the monotonic relation of *C. kolensis* and *C. vicinus* to the abiotic and biotic drivers, as well as to each other, we used the nonparametric Spearman correlation coefficient. The relationships of *C. vicinus* could be analyzed using the entire time series, whereas for *C. kolensis* only data from 1995 onward were included, due to rare species random effects and zero inflation for the first half of the data set. Since all variables exhibited strong linear or nonlinear long-term dynamics, there is on the one hand the problem of detecting spurious correlation, whereas on the other hand rigorous nonlinear detrending might weaken potential signals beyond detection, since biological signals tend to have small signal-to-noise ratios. Furthermore, common trends most likely carry biological meaning. We therefore performed the analysis for both detrended and trendaffected data. We obtained nonlinear detrended data as the residuals from a locally weighted regression smoother (LOESS) of polynomial degree 2 against time, with a window width that spanned 95% of the data. Detrending of water temperature was performed on log(x) transformed data. Detrending of all other variables was performed on log(x+1) transformed data. Furthermore, since all signals for *C. vicinus* using detrended data became rather weak, we additionally analyzed *C. vicinus* peak abundances in the form of April to May means. Sequential regression was used to model the relationship between *C. vicinus*, water temperature, and food variables

Sequential regression was chosen to deal with the comparatively high colinearity between the explanatory variables, which makes interpretation of regression coefficients difficult since they are no longer partial. Furthermore, weak biological signals are often not detected at all due to the inflation of variance of the regression parameters (Zuur et al. 2010). For sequential regression, one needs to establish a hierarchy for the explanatory variables. Such hierarchy can then be used to decorrelate the variables, i.e., make them orthogonal through subtraction of common variation from the less important variables (Graham 2003; Dormann et al. 2012). On the basis of biological reasoning, we established the following hierarchy: cryptophyte biomass (bottleneck for nauplii development), total algal biomass, rotifer abundances (omnivorous feeding ecology of *C. vicinus* and *C. kolensis*), and water temperature. To assess the robustness of our results, we exchanged cryptophyte biomass with total algae biomass in subsequent analyses, and tested for water temperature leading the hierarchy. For this analysis only *C. vicinus* peak abundances (April to May means) were considered. To minimize the influence of extreme values, we performed a logarithmic transformation to all the variables before analysis. If the preliminary single variable generalized additive models (GAMs) suggested nonlinear relationships, we included higher-order terms of the corresponding variable in the model. GAMs were calculated using the R package ‘‘mgcv’’ (Wood 2012).

Violation of independence was accounted for by an autoregressive model for the residuals, and violation of homoscedasticity was corrected by an exponential variance structure for the residuals within the framework of generalized least-square modeling (R package ‘‘nlme’’ (Pinheiro et al. 2012). Model selection was based on the importance and significance of the regression coefficients, as well as on ANOVA comparison and the Akaike information criterion. If necessary, the hierarchical variables were updated accordingly.

Food niche separation and quantification of thresholds— High discriminative power for the relative dominance status of *C. kolensis* and *C. vicinus* in one of the driving variables is an indicator for a niche apportionment between the two species with regard to this variable. Furthermore, the decision border between the different states quantifies respective thresholds. We therefore calculated a binary response variable, where ≥50% share of *C. kolensis* on the overall abundance of *C. kolensis* and *C. vicinus* was coded as 1 and 0 otherwise. This new response variable was used in a classifier based on a logistic generalized linear model and a receiver operating characteristic (ROC). To optimize the classifier the sum of sensitivity and specificity was maximized. Whereas sensitivity measures the probability of the classifier detecting a >50% share of *C. kolensis*, when it is really the case, specificity measures the probability of detecting a <50% share when this is truly the case. The point of maximization (the sum of sensitivity and specificity) was found as the minimal ROC distance. The cutoff value connected with this maximum was taken as the decision border of the classifier, and with that as the threshold value. The area under the curve (AUC) of the ROC is used to access the general ability of the model to discriminate between the binary states of the response variable. The AUC takes values between 0.5 and 1, where a value of 0.5 equals pure guessing and 1 is perfect discrimination. For evaluating the ROC, the R package ‘‘DiagnosisMed’’ (Brasil 2010) was used.

Quantifying thresholds for intraguild competition and predation—To find the threshold at which *C. kolensis* was able to overcome the competition and predation of *C. vicinus*, by degree we varied the cutoff value for the definition of high and low *C. kolensis* abundance. Low abundance was then coded as 0, high as 1. For each coding scheme, an optimal classifier was calculated as described above, and the optimal thresholds for the dependent and independent variables were again found on the basis of the AUC.

Results

Copepod long-term trends and phenology—The cyclopoid copepod winter community in the Müggelsee was dominated by *C. kolensis* and *C. vicinus*. Comparing the results from trend and regression tree analyses revealed that whereas *C. vicinus* abruptly decreased in abundance in 1988 and 1993, its overall dynamics were dominated by a significant gradual decline over the entire study period (Figs. 2a, 3a; Table 8). By contrast, *C. kolensis* underwent one abrupt abundance increase in 1995 (Figs. 2a, 3b). Between 1980 and 1995—before this abrupt change—*C. kolensis* comprised, on average, 2.5% (0%, 12%, and 2% in autumn, winter, and spring, respectively) of the cyclopoid copepod winter community, and became by far the most dominant species in 1995 to 2010 (6%, 86%, and 57% in autumn, winter, and spring, respectively; Fig. 11a).

Table 8. Trend of key drivers and response variables. The analysis follows the iterative procedure suggested by Yue et al. (2002). Estimated slopes of the trends and their corresponding confidence intervals (CI) are based on the non-parametric robust Theil-Sen estimator. Significant levels (*p*-value) of the monotonic trends are based on the non-parametric Mann-Kendall trend test.

	Slope	CI	*P* value
Water temperature (°C)	0.03	-0.01 to 0.06	0.04
Total algal biomass (mg L^{-1})	-0.05	-0.09 to -0.03	0.03
Cryptophytes (mg L^{-1})	-0.09	-0.12 to -0.06	0.00
Rotifers (ind. L^{-1})	-0.02	-0.05 to 0.02	0.46
Cyclops vicinus (ind. L^{-1})	-0.09	-0.12 to -0.06	0.00
Cyclops kolensis (ind. L^{-1})	0.05	0.01 to 0.09	0.16

C. kolensis developed its pelagic population between September and May (Fig. 11c). The phenology of *C. kolensis* remained unchanged over the entire investigation period. *C. vicinus* was usually present throughout the year, with population peaks in spring and autumn. A 1month phenology shift toward later in autumn was observed for *C. vicinus* in the second investigation period (1995 to 2010; Fig. 11b).

Trends in prey availability and water temperature—Total algal and cryptophyte biomass declined abruptly in 1991and 1993, dropping by 46% and 31%, respectively, from the 5 yr period before the abrupt change to the following one (Fig. 12d,e). Rotifers exhibited an abrupt decline in 1993, where they lost 40% in mean abundance (5 yr comparison as above), but increased again in 2002 (Fig. 12f). Rotifer abundances never decreased below 245 individuals (ind.) L^{-1} during the entire study period (Fig. 13e,f).

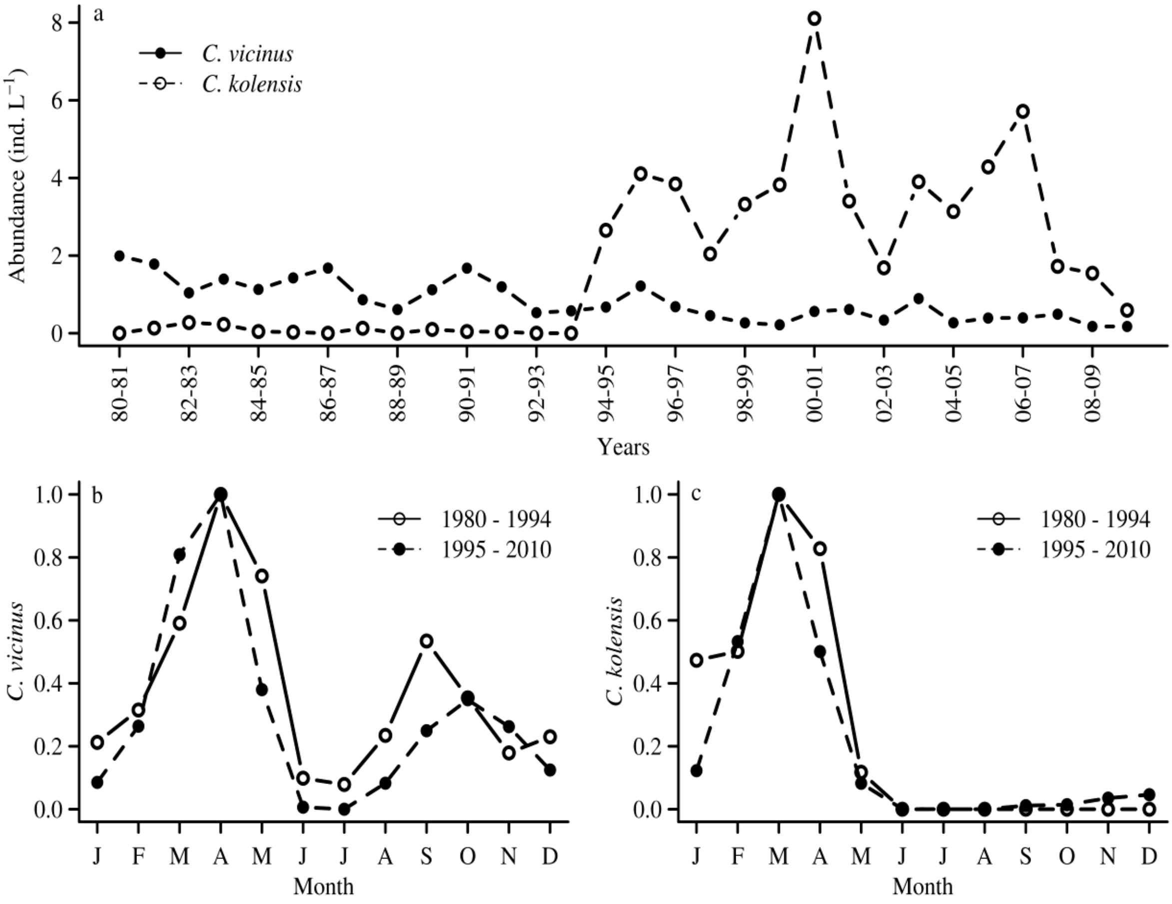

Fig. 11. Copepod long-term trends and phenology. (a) September to May mean abundances of *Cyclops kolensis* (open circles) and *Cyclops vicinus* (closed circles). (b) Phenology of *C. vicinus* and (c) *C. kolensis*, depicted as monthly mean abundances for the periods 1980 to 1994 (open circles) and 1995 to 2010 (closed circles). Mean abundances for each period were standardized with the maximal abundance of the respective species and period.

In addition to the abrupt changes, significant monotonic trends for water temperature, as well as cryptophyte and total algal biomass, were found. Water temperature, with an average rise of 0.9°C over the entire study period, was the only variable with an increasing trend (Fig. 12c; Table 8).

Abiotic and biotic drivers—Following the Spearman correlation coefficient, we found a significant relation between *C. kolensis* and *C. vicinus* abundances as well as rotifer abundances (Table 9). Spearman correlation coefficients between key drivers and *C. vicinus* using trendaffected data were high for total algal biomass, cryptophyte biomass, and water temperature. We found no strong relationships between *C. vicinus* abundance and detrended time series apart from a moderate association with cryptophytes (Table 9).

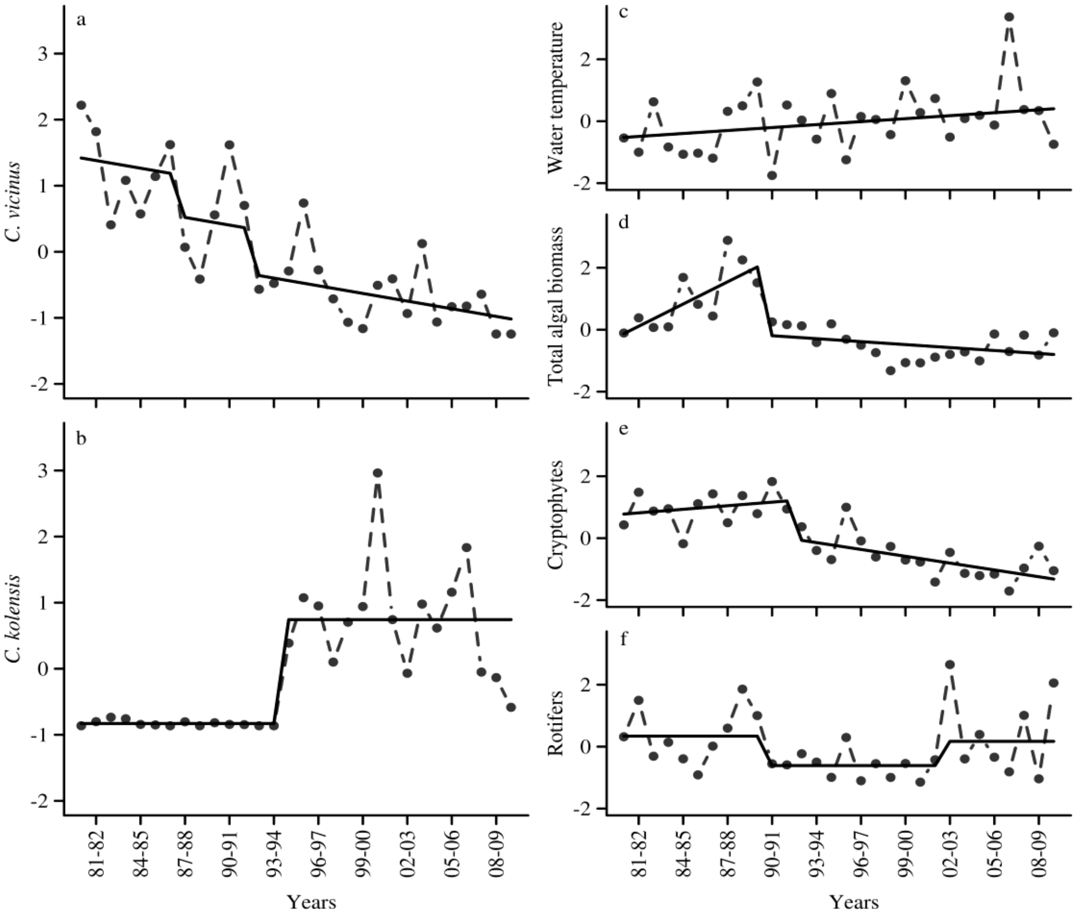

Fig. 12. Trends in prey availability and water temperature. Abrupt and linear trend dynamics in yearly means. Solid lines depict robust regression models combining the largest abrupt changes in the mean from regression tree analysis for each variable, together with significant trends from monotonic trend analysis if these exist for the respective variable. (a) *C. vicinus*, (d) total algal biomass, and (e) cryptophyte biomass were dominated by both abrupt changes and gradual linear trends in the mean. (b) *C. kolensis,* and (f) rotifers were purely breakpoint driven, while (c) water temperature was solely marked by a linear trend. Data were standardized with the maximum value of the respective variables.

Table 9. Monotonic relationships between *C. kolensis* or *C. vicinus* and abiotic and biotic key drivers. The analysis was based on Spearman's rank correlation coefficient. Spearmans's rho is given first followed by the respective *p*-value in brackets. The coefficients for *C. kolensis* were based on data ranging from 1995 to 2010, whereas those for *C. vicinus* were based on data from 1980 to 2010.

	*C. kolensis**	*C. kolensis***	*C. vicinus**	*C. vicinus***	*C. vicinus****
Water temperature	0.16 (0.54)	0.32 (0.22)	-0.4 (0.02)	-0.26 (0.16)	-0.19 (0.32)
Total algal biomass	-0.14 (0.62)	-0.18 (0.51)	0.63 (0)	0.16 (0.39)	0.05 (0.77)
Cryptophytes	-0.23 (0.39)	0.06 (0.84)	0.72 (0)	0.13 (0.5)	0.26 (0.17)
Rotifers	-0.36 (0.17)	-0.63 (0.01)	0.1 (0.6)	-0.04 (0.83)	0.1 (0.61)
C. vicinus	0.50 (0.05)	0.34 (0.20)			

* Results from trend-affected data, ** results from nonlinear detrended data, *** results from April to May means of non-linear detrended data

However, the importance of cryptophyte biomass and water temperature for *C. vicinus* abundances, as well as the direction of the relationship, was further confirmed in the sequential regression analysis. For both detrended and trend-affected data, cryptophyte biomass was significantly positively related to *C. vicinus* abundance, irrespective of whether cryptophyte biomass or total algal biomass led the sequential regression hierarchy. This result was also robust against including a linear trend on top of the hierarchy. If water temperature led the hierarchy, cryptophyte biomass and water temperature were both significant for trend-affected data, and again robust against explicit inclusion of a linear trend into the model. However, in the case of detrended data, only water temperature was significant when leading the hierarchy. As a primary abiotic driver, water temperature did not account for >50% *C. kolensis* dominance (Table 10). This was confirmed by the low power of the logistic regression-based classifier to distinguish between *C. kolensis* or *C. vicinus* dominance, on the basis of water temperature (Table 10).

Niche separation for high abundance probability of C. kolensis—We found a clear segmentation for high vs. low abundances of *C. vicinus* and *C. kolensis* for total algal and cryptophyte biomass (Fig. 13a,b,c,d). *C. kolensis* occupied the low food niche, whereas *C. vicinus* was dominant when herbivorous food availability was high. The threshold partitioning the two food niches lay at 6.2 mg L^{-1} for total algal mass and at 0.6 mg L^{-1} for cryptophyte biomass (Table 10). With only a few exceptions, both thresholds were surpassed from 1994 onward. A food niche separation was not obvious for rotifer prey, which were present in abundances of >245 ind. L^{-1} throughout the period of investigation (Fig. 13e,f).

Table 10. Classification analysis based on the basis of abiotic and biotic key drivers for *C. kolensis* dominance (≥50%) relative to *C. vicinus*. The values of the classifiers' decision border are given, which can be interpreted as the threshold for a 50% dominance of *C. kolensis*. The area under the curve (AUC) and its corresponding confidence intervals (CI) give a measure for the goodness of-fit of the classification with respect to each driver. Whereas an AUC of 0.5 equals pure guessing, an AUC of 1 equals a perfect classification. High discriminative power in one of the driving variables is an indicator for a niche apportionment between the two copepods with regard to this variable.

	Threshold	AUC	CI
Water temperature	7.02 °C	0.67	0.46 to 0.88
Total algal biomass	6.15 mg L^{-1}	0.95	0.88 to 1.02
Cryptophytes	0.57 mg L^{-1}	0.94	0.86 to 1.03
Rotifers	419.91 ind. L^{-1}	0.65	0.44 to 0.85

Thresholds for C. vicinus competitive and predation superiority—Inspecting the direct relationship between *C. kolensis* and *C. vicinus* abundance, a threshold of 0.7 ind. L^{-1} (AUC 5 0.9) of *C. vicinus* separated high from low abundance probability of *C. kolensis* (Fig. 14).

Discussion

Abrupt changes in ecosystem responses to climate and environmental change can have potentially large effects on ecosystem functioning. However, the detailed nature of change is difficult to predict and data-driven proof of model outputs is rare (Carpenter et al. 2011). We investigated mechanisms behind the abrupt increase of an omnivorous copepod species (*C. kolensis*), changing from a rare to the dominant species, while coexisting *C. vicinus* concurrently decreased in abundance. These two species are linked in a consumer-structured IGP food web such that their coexistence, sharing the same resources, is more likely under intermediate productivity conditions in their lake habitat. In our model system, *C. kolensis* occupies the position of the consumer with lower prey requirements, whereas *C. vicinus* acts as its predator. This particular position of *C. kolensis* enabled it to profit from the improved trophic state and subsequent gradual decline of *C. vicinus*, releasing it from predation on its larval stages and competing for commonly shared algal prey, while not being negatively affected by the decline in prey availability itself. Our analyses shed light on the mechanisms underlying the abrupt change in *C. kolensis* abundance in the context of the IGP hypothesis, and we discuss the nature of the driving forces in the context of regime shift theory.

Gradual decline in C. vicinus—The decline of *C. vicinus* closely mirrored the decline in total algal and cryptophyte biomass (Fig. 12). The significant and robust relationship between *C. vicinus* and cryptophytes, confirmed by both coinciding break points and regression analysis, highlights them as the main driver, followed by total algal biomass. These findings agree with results from grazing experiments where algae constituted 66% of the daily ingested dry mass of *C. vicinus* (Adrian and Frost 1992). It is also known that *C. vicinus* nauplii rely on phytoflagellates (cryptophytes), with limiting concentrations being between 0.2 and 0.5 mg C L^{-1} (Santer and van de Bosch 1994; Hansen and Santer 1995). Assuming a conversion factor between cryptophyte fresh weight and carbon of 0.2 pg C μm^{-3} (Rocha and Duncan 1985), cryptophytes were most likely close to limiting concentrations for *C. vicinus* nauplii over the entire study period, but particularly in the second period (from 1980 to 1994 and 1995 to 2010, cryptophyte carbon declined on average from 0.2 to 0.1 mg C L^{-1}).

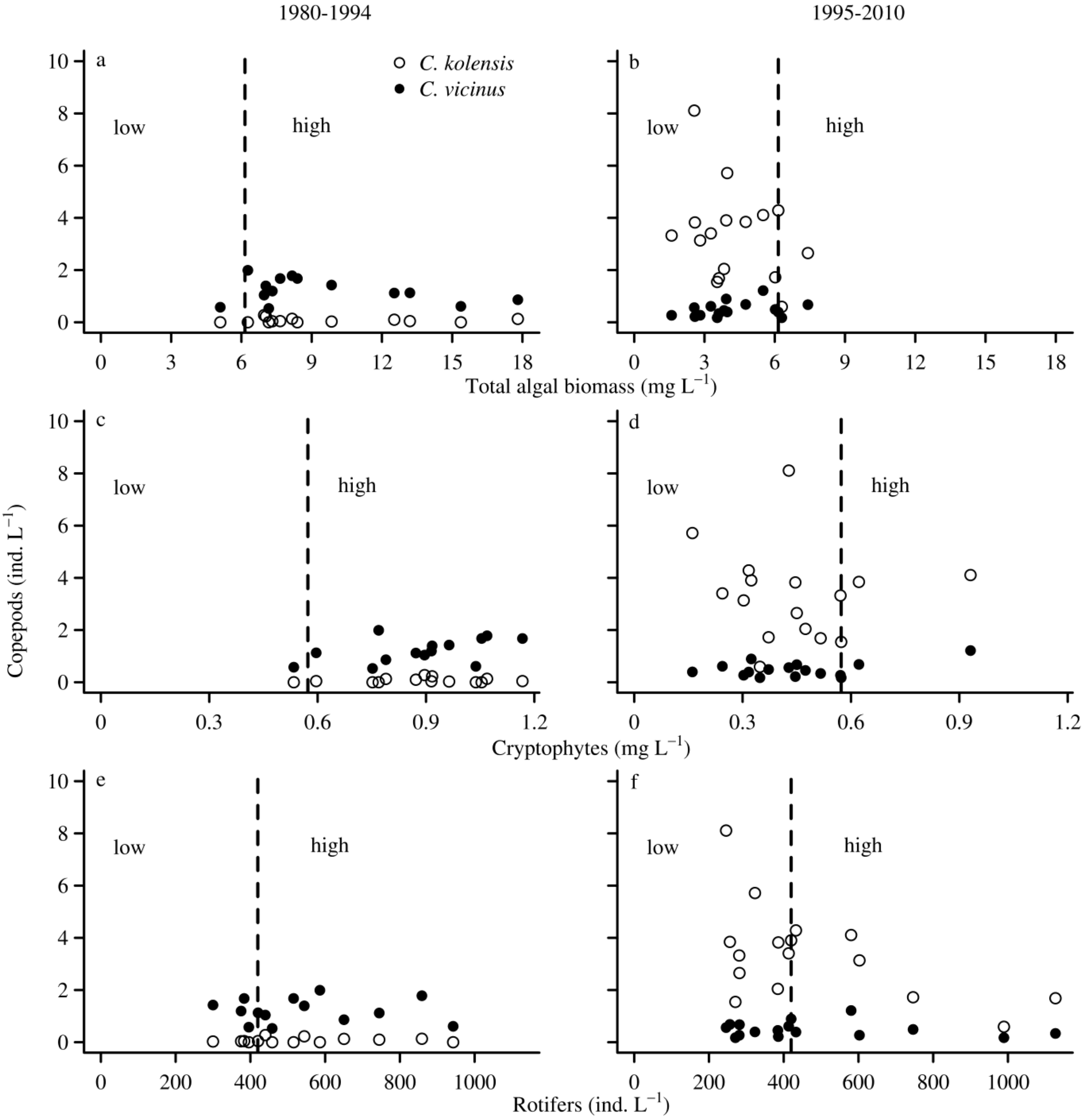

Fig. 13. Food niche separation for high abundance probability of *C. kolensis. C. kolensis* (open circles) and *C. vicinus* (closed circles) abundance for the periods before 1980 to 1994 (left) and after 1995 to 2010 (right) the shift in *C. kolensis* abundance with respect to (a, b) total algal biomass, (c, d) cryptophytes, and (e, f) rotifers. Dashed lines depict the thresholds estimated by the classification summarized in Table 10.

Since nauplii are considered the bottleneck in a copepod life cycle, an insufficient supply of cryptophytes has a large effect on their ability to develop into adults (Santer and van de Bosch 1994; Hansen and Santer 1995; Santer and Lampert 1995).

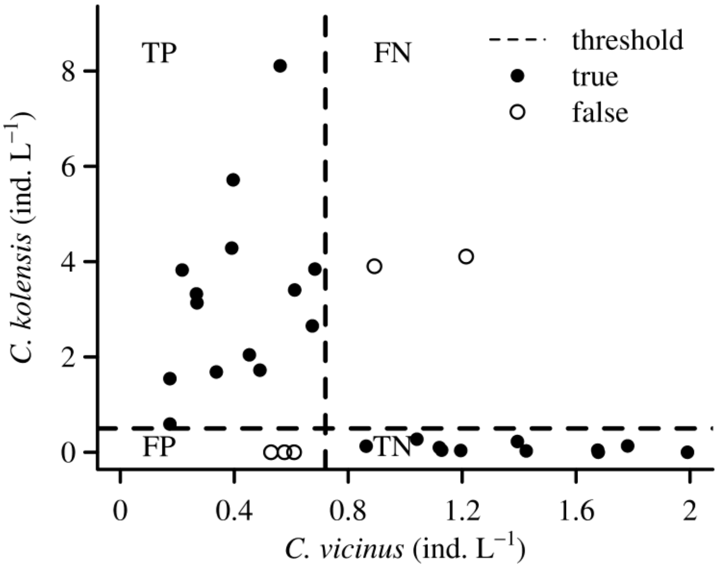

Fig. 14. Thresholds for intraguild competition and predation superiority. Dashed lines correspond to thresholds found by the optimal classifier to separate high and low *C. kolensis* abundance (horizontal line) based on the abundance of *C. vicinus* (vertical line). Closed circles correspond to correct classification results (TP = true positives, TN = true negatives), open circles to false ones (FP = false positive, FN = false negative) based on the estimated thresholds.

Furthermore, the high herbivorous food demand of *C. vicinus* is also reflected by its presence, and often dominance, in eutrophic lakes (Hansen and Jeppesen 1992; Santer and Lampert 1995; Adrian 1997) aided by its disproportional ability to profit from high prey availability through shorter development times (Hansen and Santer 1995). On the basis of the break-point component in the long-term dynamics of *C. vicinus* and cryptophytes, the relationship between them matches the driver threshold scenario hypothesized in regime shift theory (Andersen et al. 2009). In this regime shift scenario, an abrupt change in a driving force (here cryptophytes) causes an equally sudden change in a response variable (*C. vicinus* in our case), such that the thresholds are only present in the time series (Fig. 15).

Abrupt increase in C. kolensis—The only significant direct connection between C. *kolensis* and any of its prey was found for rotifer abundance. The strong negative correlation most likely arose from a top-down effect of *C. kolensis* on its prey, marking rotifers as an important food source. However, *C. kolensis* abundance increased at a time of general decrease in all food categories. Thus, the species was unlikely to have been affected by deprivation of prey. Rather, *C. kolensis* indirectly benefited from algal prey decline, due to the decline in *C. vicinus* releasing it from competition.

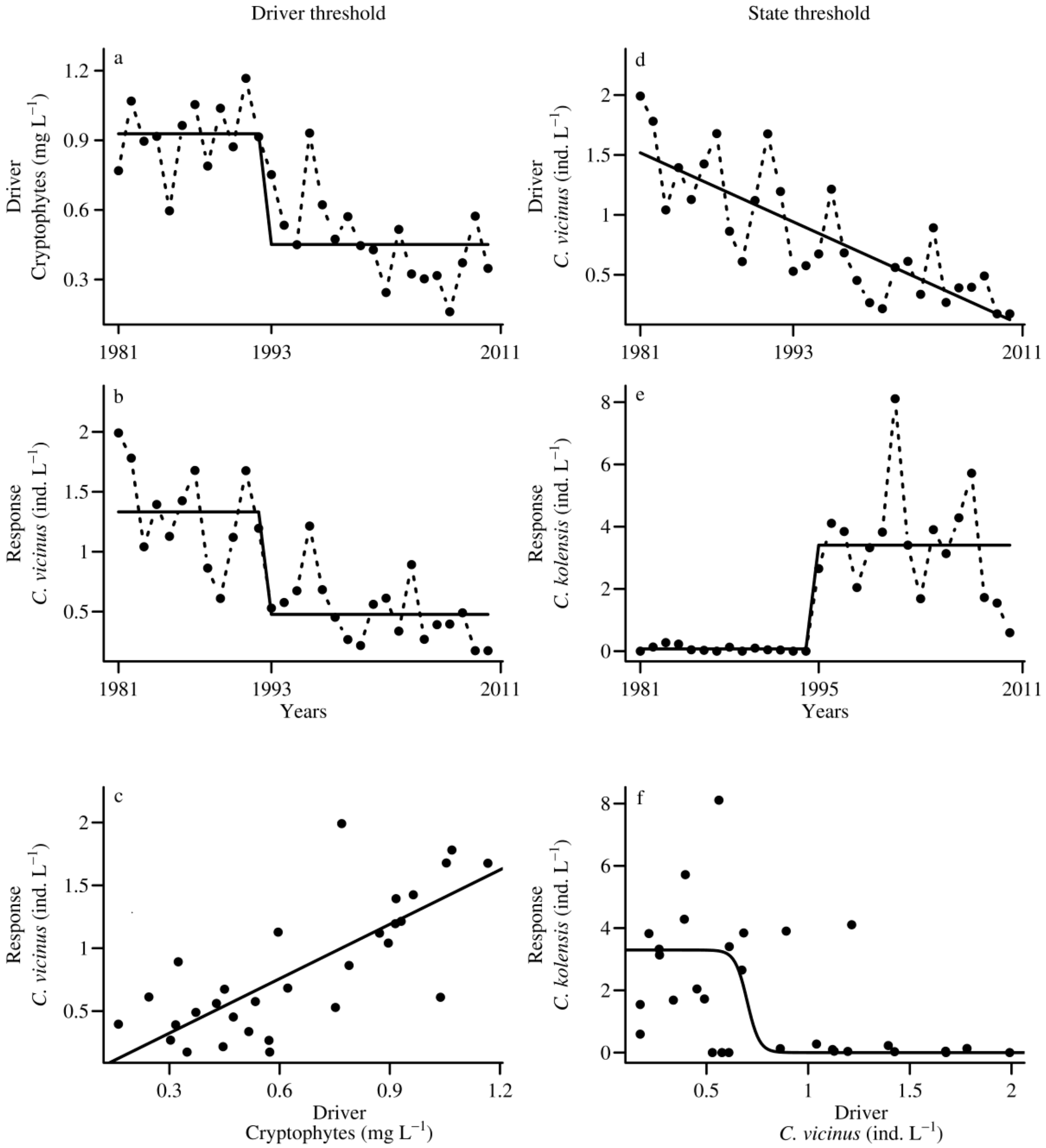

Fig. 15. 'Driver threshold' and 'state threshold' regime shift scenarios. Analyses on the basis on fig. 1 in Andersen et al. 2009. (a, b, c) The driver threshold scenario refers to the existence of a threshold in the driver (cryptophytes), which is linearly mediated to a response (*C. vicinus*). (a) The regime shift in cryptophyte biomass in 1993 was immediately followed by (b) a regime shift in *C. vicinus* abundances, resulting in (c) a linear driver (cryptophytes) response (*C. vicinus*) scatter plot. The solid lines in driver and response variables (panels a, and b) stress the breakpoint components in the dynamics of the two variables. (d, e, f) The state threshold scenario refers to the existence of a threshold in the response (*C. kolensis*), but not in the driver (*C. vicinus*). (e) *C. kolensis* abundances underwent a regime shift (d) after *C. vicinus* crossed a threshold, such that an abrupt change appears in the time series of *C. kolensis* and (f) a threshold appears in the scatter plot between the two variables. The line in panel d solely depicts the linear trend component of *C. vicinus'* abundance, since only this part of the dynamics is relevant in this regime shift scenario.

The opposing effects of herbivorous food decrease on the two species are explained by apportionment of the food niches. Whereas *C. vicinus* was dominant under plentiful algal and cryptophyte biomass, *C. kolensis* seemed particularly able to thrive under low food conditions, to occupy the low food niche (Fig. 13). These findings are in line with lower algal prey ingestion rates for *C. kolensis* (5.5 μg dm ind.$^{-1}$ d^{-1}) than those observed for *C. vicinus* (7.3 μg dm ind.$^{-1}$ d^{-1}) when offered the same natural lake plankton mixture of algal and invertebrate prey (Adrian and Frost 1992). Ingestion rates of invertebrates were within the same range of 4.4 and 4.2 μg dm ind.$^{-1}$ d^{-1} for both species

(Adrian and Frost 1992). This is consistent with the lack of any evidence, from either correlation or regression analysis, or food niche partitioning, that the two species might have been limited by carnivorous prey (Tables 8, 9; Fig. 13).

The food niche separation marks C. *kolensis* as the superior exploitative competitor, and explains why it was unaffected by the general decline in algal biomass, much in line with the IGP hypothesis. This is supported by the fact that the larger *C. vicinus* exhibited higher ingestion rates for algal prey than *C. kolensis* when offering the same prey assemblages (Adrian and Frost 1992). Additionally, the strong relation between *C. kolensis* and rotifer abundances (Table 9) underlines slight differences in the diet of *C. kolensis* when compared with *C. vicinus*. From IGP models embedded in a richer food web, the existence of such alternative food resources is known to relax the assumption of the consumers' superiority in exploitative competition and still enables coexistence at intermediate productivity (Holt and Huxel 2007). However, this does not necessarily account for the abrupt nature of *C. kolensis*' success. Rather, the dynamics governing its interaction in the IGP food web must be highly sensitive to a critical abundance threshold, matching the state threshold scenario proposed by Andersen et al. 2009 (Fig. 15). In this scenario the response variable abruptly shifts (here *C. kolensis*) after the driver crosses a threshold (here *C. vicinus*), putting the focus on the monotonic trend component in *C. vicinus'* long-term dynamics (Fig. 12). This is in line with the high discriminative power of the logistic classifier using *C. vicinus* abundance to distinguish between high and low *C. kolensis* presence (Fig. 14). The critical threshold of *C. vicinus* density that enables *C. kolensis* to overcome the inhibiting effects of competition for prey and predation pressure in our system was 0.7 *C. vicinus* L^{-1}.The shift in *C. kolensis* abundance could have also been caused by a transition between alternative stable states, since IGP theory predicts the existence of such states for a consumer with a superior ability to exploit competition at intermediate productivity levels (Holt and Polis 1997; Mylius et al. 2001; Verdy and Amarasekare 2010). However, we were not able to differentiate the state threshold further into an explicit transition between alternative stable states, or driver-state hysteresis scenario as coined by Andersen et al. 2009.

Our view of *C. vicinus* being the main predator of *C. kolensis* juveniles, although both copepod species prey on nauplii (Adrian 1991; Krylov et al. 1996), stems from the phenology of both species. The abundance peaks of adult, reproducing *C. kolensis* occurred in March, whereas on average those of *C. vicinus* were in April (Fig. 11b,c), coinciding with the new batch of *C. kolensis* offspring, which are highly susceptible to predation. The phenology shift of the second population peak of *C. vicinus* from September to October most likely benefited *C. kolensis* (Fig. 11). The 1 month delay in maximum *C. vicinus* population might as well have diminished predation on emerging *C. kolensis* copepodites in September. Within a month late copepodites easily develop into the adult stage, which are less susceptible to predation by adult *C. vicinus*, giving the *C. kolensis* population a head start. Water temperature, although an important driver of copepod life cycles and phenology (Adrian et al. 1999; Gerten and Adrian 2002; Seebens et al. 2009), correlated weakly with C. *kolensis* abundance, and it was not possible to adequately distinguish between high and low *C. kolensis* abundance on the basis of water temperature. This excluded water temperature as a major direct driver of the dominance shift in *C. kolensis*. Nevertheless, *C. kolensis* might have indirectly benefited from warmer water temperatures, not only due to the earlier spring phytoplankton bloom (Gerten and Adrian 2000), giving its nauplii an earlier resource, but also temperature-driven shorter developmental times (Hansen and Jeppesen 1992). Furthermore, water temperature might have played another indirect role through its negative relationship to *C. vicinus* abundance (Table 9), possibly further promoting its decline. The negative relationship between water temperature and *C. vicinus* is not easily explainable, since both copepod species should profit in terms of shorter generation times due to higher water temperatures.

To conclude, we have presented an example of an intriguing match between observational long-term records and ecological food web and regime shift theory. The two coexisting copepod species are linked in an IGP food web, with *C. vicinus* as the predator and C. *kolensis* as the consumer. The latter proved superior in exploitative competition through its ability to occupy a low food niche at times when the lake’s trophic state had improved (Köhler et al. 2005; Huber et al. 2008). Consistent with our observed results, theory predicts that coexistence in such a food web is only possible at intermediate levels of resource productivity, in combination with the consumer being better at exploiting the resource (Polis et al. 1989; Holt and Polis 1997) or has additional resources (Holt and Huxel 2007). Our results were also in line with results from deterministic models (Mylius et al. 2001; Verdy and Amarasekare 2010), as well as laboratory experiments (Morin 1999; Diehl and Feißel 2000) investigating the effect of food enrichment within IGP food webs. As for the nature of the complex interaction between driving forces and copepod regime shifts, we were able to distinguish between true threshold-driven dynamics underlying the abrupt increase in *C. kolensis* and a mere, linearly mediated decline in *C. vicinus*, responding to a matching abrupt change in a driving variable (Fig. 15). The most likely initiator for the abrupt increase of *C. kolensis* in 1995 was the monotonic decline of *C. vicinus* crossing a critical threshold. Once *C. vicinus* declined to a critical abundance of 0.7 ind. L^{-1}, *C. kolensis* was able to thrive in the low food niche that had already opened up between 1991 (total algal mass) and 1993 (cryptophytes).

Overall, we have presented a successful case of how observed changes in nature on the basis of long-term observational data match results from ecosystem theory. In combination, they provide powerful tools in understanding the complex interactions in ecosystems and help to generate hypotheses for a better understanding of the remaining unexplained variability. Since modeling is a prerequisite to project responses, e.g., toward global environmental changes, testing model outputs with long-term observational data or experimental studies is desirable. We have presented a plausible case study on how threshold-driven regime shifts at single system levels in a lake have the potential to change relative species composition within plankton communities.

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Chapter VI: General Discussion

Freshwater periphyton plays many important roles in aquatic ecosystems; it acts as an important primary producer and behaves as chemical modulators as it transforms many inorganic chemicals into organic forms (Stevenson, 1996). Moreover, the benthic algae stabilize substrata and serve as important habitats for many other organisms. In recent years, periphyton has been intensively studied. Relevant and important data on various aspects of periphyton ecology and its role in whole ecosystem processes have been obtained. Littoral periphyton communities, for example have been recognized to contribute significantly to whole ecosystem primary production. In addition, they play a key role in nutrient cycling and food webs interaction (Feminella and Hawkins, 1995; Steinman, 1996; Vadeboncoeur et al., 2003).

In chapter III of this thesis, I focused on the effects of climate change on periphyton biomass with respect to clear and turbid European shallow lakes (Estonia, Germany, Czech Republic, Turkey, and Greece). I found a positive relationship between water temperature and periphyton biomass to mediate the effect of nutrient loading. In chapter IV, periphyton removal rates were determined for the mobile grazer *Daphnia magna* and for sessile chironomid larvae. The larger chironomid larvae had higher individual periphyton removal rates than *D. magna*, whereas body mass specific periphyton removal rates by *D. magna* were higher than that of chironomids. I concluded that the role of *D. magna* (large planktonic cladocerans) in removing periphyton biomass was significant. Moreover, top-down control of invertebrate predators by fish seemed to play an important role in enhancing periphyton biomass (chl *a*) especially in the southern Mediterranean experimental sites (Ch. III). Finally, in a study aside from the periphyton ecology we tested observational data on plankton communities with scenarios of regime shift theory in the context of intraguild predation theory as shown in chapter V.

In the current study, we used a standardized protocol of artificial plastic strips in all 5 countries because it facilitates comparison of periphyton biomass development across countries and it is less variable than natural surfaces (Morin and Cattaneo, 1992). To date this is the first study to use artificial substrates successfully to determine three important periphyton biomass measurements (DW, AFDW and chl *a*) in a pan-European mesocosm experiment. Previous pan-European mesocosm experiment attempts had focused only on periphyton chl *a* as a periphyton biomass measure (Bécares et al., 2008). In our study, periphyton DW and periphyton chl *a* revealed, however, different responses to the indirect top-down control by fish, when periphyton chl *a* responded positively, while periphyton DW showed weak responses. In Figure 16 the factors that affect periphyton biomass based on the studies in my thesis are presented.

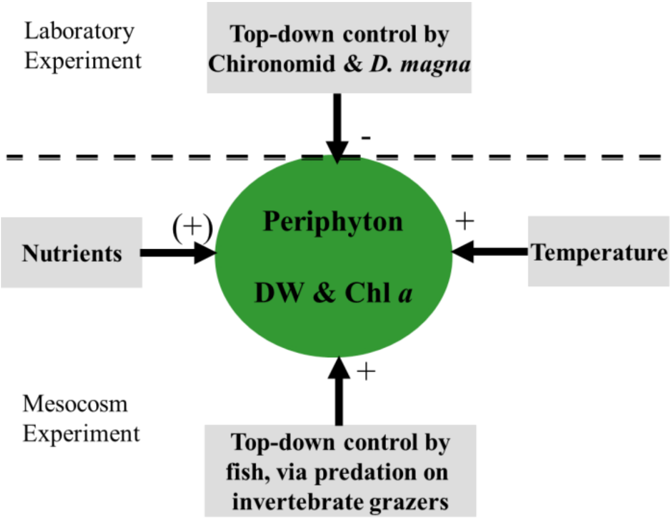

Fig.16. Factors influencing periphyton biomass based on laboratory grazing and mesocosm experiments.

VI.I Effects of water temperature on periphyton biomass

In this work, water temperature was an important factor which positively affected periphyton biomass development in a pan-European mesocosm experiment (see a detailed explanation in Ch. III and in Figs. 16 and 17).

In freshwater lake ecosystems, a positive effect of temperature on periphyton growth is well known (Francoeur et al., 1999; Morin et al., 1999; Weckström and Korhola, 2001). Freshwater periphyton has been shown to grow in a wide temperature range from 0 to 75 °C (Fogg, 1969), and from 0 to 30 °C (DeNicola, 1996). Our temperature range was within these published values (20-28 °C) and thus, a strong positive correlation between temperature and periphyton growth was expected- with higher effects in the southern countries as compared to the northern countries. In our study a one degree rise in water temperature was equivalent to a 42 % increase in periphyton dry weight. Moore (1978) demonstrated that the seasonal change of coexisting periphyton species in Lake Erie were primarily determined by temperature. These results are in agreement with our predictions that periphyton biomass in natural environments depends on ambient water temperatures. Our results contradicted, however, the results of Bécares et al., (2008). In their pan-European mesocosm experiment they found periphyton biomass (chl *a*) was more related to nutrient loading than to temperature. In addition, they found that in southern European lakes periphyton was negatively affected by phytoplankton shading. This was not the case in our study, where light conditions never fell below the limiting level- in both low and high nutrient treatments.

The Mediterranean area is projected to be a potentially vulnerable region to climatic change (Sánchez et al., 2004). As changes in air temperature are reflected closely in water temperature of shallow lakes they are particularly sensitive to climatic change. Moreover, eutrophication may be intensified by climate warming due to changes in thermal regime and subsequent deterioration of oxygen, which may result in an increase in internal nutrient loading, in the reduction of submerged plants, predatory fish and waterfowl. This will lead to a deterioration of water clarity and a loss of ecological and conservation values of shallow lakes (Scheffer et al., 1993; Jeppesen et al., 2007). Southern European lakes will most likely experience an increase in nutrients concentrations due to low precipitation, low runoff, low water level, and high evaporation (Beklioğlu et al., 2007).

VI.II. Effects of nutrient on periphyton biomass

Nutrient enrichment is a widespread problem throughout the world, as it affects the quality of domestic, industrial, agricultural and recreational water resources (see Figs. 16 and 17). Increased nutrient loading has generally been found to increase total algal biomass and chlorophyll content of periphyton, especially in temperate shallow lakes (Jensen et al., 1994; Scheffer et al., 1997; Jeppesen et al., 2000). The quantity of nutrients plays a strong role in determining the quantity and quality of periphyton. Positive periphyton biomass responses to enhanced nutrient loading have been shown by Hillebrand (2002) and Bécares et al., (2008). However, when nutrients are abundant, periphyton is light-limited rather than nutrient-limited, due to the subsequent proliferation of phytoplankton (Hansson, 1992). In the current experiment (see Ch. III) our nutrient (TP) loading was implemented with clear significant separation between high and low nutrient treatments, but with weak effects on periphyton biomass in the tested nutrient ranges of (20-65 μg TP L^{-1}).

VI.III. Effects of direct and indirect top-down control on periphyton biomass

So far grazing effects on periphyton biomass was mainly related to grazing by macroinvertebrates such as chironomids (Hillebrand and kahlert, 2001; Tarkowska-Kukuryk, 2013) or by snails (Brönmark and Weisner, 1992; Brönmark, 1994). The results of chapter IV showed that *Daphnia magna* removed periphyton even at higher rates than chironomids based on body mass specific removal rates. Our results go beyond previous work by Jones and Waldron (2003), who revealed that daphnia used periphyton as a food source and by Siehoff et al. (2009) who showed that *D. magna* used periphyton as a sole food source when phytoplankton food is scare. In addition, we concluded that periphyton supported growth of the studied invertebrate grazer's community, chironomid larvae, and *D. magna*.

In chapter III, we revealed that the role of indirect top-down control by fish may be underestimated. Fish may indirectly promote periphyton growth by preying upon periphyton-associated grazers (Jones and Sayer, 2003), as shown in chapter III. This pattern is consistent with the results of a previous enclosure experiment which showed that the presence of fish had directly or indirectly a positive effect on the periphyton biomass (Liboriussen et al., 2005).

VI.IV. Interactions between periphyton and its limiting factors

The occurrence and abundance of periphyton is affected by many factors that can control their biomass and community composition (e.g., temperature, bottom-up and top-down control). In the following we document our finding in the context of the chapters III and IV – as depicted in figure 17. This model describes periphyton development under two contrasting top-down fish scenarios- low versus high. Basically periphyton development profited from high fish biomass conditions – while periphyton development was at a disadvantage under low fish densities. The underlying mechanisms are, however, complex and involve positive and negative feedback loops including nutrient and light conditions, temperature and indirect effects by the number of planktivorous and benthivorous fish (Fig. 17). The conditions that characterize periphyton increase were high abundances of planktivorous and benthivorous fish (see Ch. III, IV). Fish is known as an important factor for maintenance of periphyton dominance (Neely, 1994; Jones and Sayer, 2003; Liboriussen et al., 2005). In contrast, in the case of lower planktivorous and benthivorous fish, invertebrate grazers decrease periphyton biomass (chl *a*) (see, Fig. 17). In the present study, the relation between water temperature and periphyton biomass was positive. In addition, fish biomass was playing an important role to increase periphyton biomass in the Mediterranean lakes with increasing temperature (Table 5, Ch. III). In addition, high periphyton biomass affects pelagic phytoplankton development in a negative way because of competition for common resources such as light and nutrient. Contrarily, low periphyton leads to high nutrient available for the pelagic phytoplankton.

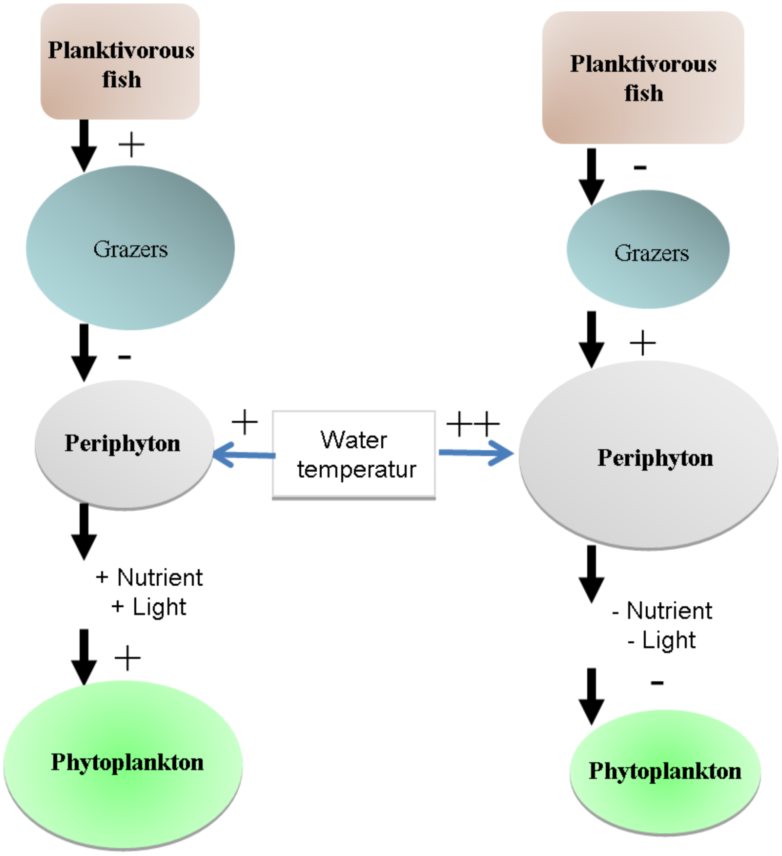

Fig. 17. General model depicting top-down and bottom-up forces affecting periphyton biomass development in shallow lakes – as derived from large scale mesocosm and small scale microcosm experiments.

VI.V Remarks on applied methodology

In a project aside to the pan-European mesocosm experiment we investigated the sudden dominance of *C. kolensis* in the winter cyclopoid community in Müggelsee based on several decadal records (Scharfenberger et al., 2013; Ch. IV). As the two projects (periphyton ecology/copepod ecology) concern very different topics – I like to put some attention on the value of using different methodology in order to understand major driving forces of dynamics observed in ecosystems. I used small scale laboratory experiments, large scale mesocosm experiments and long-term field data. In the single year mesocosm experiments we manipulated nutrient dynamics by establishing two nutrient scenarios and temperature by performing the same periphyton experiments across a temperature gradient across European countries. By using decadal records of a lake which underwent substantial changes in external nutrient loading and was affected by global warming we were able to determine the most important drivers of the opposing development of two coexisting copepods – by applying two models of ecological theories.

There is a long history of using large scale mesocosm experiments all the way to whole lake experiments to actually understand mechanisms driving the structure and function of ecosystems with respect to e.g. eutrophication (Schindler, 1998) or trophic cascades in food webs (Carpenter et al., 1987; Jones and Sayer, 2003; Liboriussen et al., 2005). There is also a long history of small scale laboratory grazing experiments, which substantially improved our understanding of the autecology of single species. This knowledge derived from more or less standardized experiments help us to understand e.g. the long-term dynamics of aquatic communities or is provide baseline information for ecosystem modelling.

Microcosm experiments have been conducted since long, providing basic information on e.g. the autecology of organisms, trophic interactions, resource acquisition and production (Lampert and Sommer, 2007). Information derived from controlled laboratory experiments have substantially contributed to our understanding of the dynamics in natural ecosystems (Kassen et al., 2000) and to understand and predict the effect of single factor on one trophic level (Fig. 2 in Stewart et al., 2013). In the current study (Ch. IV) we performed laboratory experiments to understand predator prey relationship under control conditions. The advantages of laboratory microcosm experiment are manifold: the results derive from controlled conditions (temperature, food availability, grazer's biomass, shorter time). The experimental time is usually short in order to work under more or less unchanged conditions (autecology). In our grazing studies the grazer's biomass changed in the course of the period. In order to account for that change we applied a logistic growth model and corrected the actual grazer number in the course of the experimental period (Fig. 8, Ch. IV). The disadvantage of microcosm experiments is their simplicity – and as such it is hard to judge whether results from such experiments apply to whole ecosystems

Mesocosm experiments were used in experimental ecology since the early twentieth century (e.g. see review by Benton et al., 2007). They play an important role in increasing our understanding of different aquatic ecosystems (Stewart et al., 2013). Recently, the combined space-for-time surveys with mesocosm approaches were increasingly used to examine ecosystem responses to factors such as climate change, nutrient addition, and trophic cascades (phytoplankton-periphyton-macrophyte-fish) (Moss et al., 2004; Bécares et al., 2008). In addition, the relative importance of top-down and bottom-up forces in shallow lakes has been widely investigated using mesocosms under different environmental conditions, including environmental warming (e.g. McQueen et al., 1986). The disadvantage of mesocosms experiments is that they were never conceived to mimic the full complexity of nature (Lawton, 1996), or attain high levels of control, so they do not necessarily imply a focus on constant conditions. Limited replicates provide problems given the high variation often observed between mesocosms. Additionally, different starting communities in different countries / lakes also lead to high variation. One of theses variations between mesocosm experiments in our study was that different fish species were used. While most countries used three-spined stickleback, *Gasterosteus aculeatus*, Greece used *Gambusia affinis* (Ch. II). Moreover, it takes time to establish the desired nutrient treatment. In our case it took about 6 weeks. Also, biota constitute across countries should not be different. The advantage of large scale mesocosm experiment: semi-natural conditions can be achieved, for example we used mud, sediment, natural biota, fish and macrophyte. In the current study we used standardized protocol for all experimental lake countries.

The strength of long-term ecological studies is that the observed outcome incorporates the entire natural complexity of directly and indirectly operating driving forces. Based on time series analysis and advanced statistical modeling the identification of major driving forces of change has been very successful. Long-term ecological research has been e.g. successful in disentangling combined effects of changes in the climate and nutrient status for lake plankton based on various approaches such as detrending (Seebens et al., 2007), mechanistic (Elliot and May, 2008; Huber et al., 2008) or simulation modelling (Schalau et al., 2008). A disadvantage of long-term studies is that they are very cost intensive.

The combinations between the three methodological approaches are important in the future. They can be an important tool in filling gaps of our mechanistic understanding of the structure and functioning of ecosystems. Every microcosm, mesocosm, or long-term study should be designed to test a specific hypothesis using information gained in previous steps of the hypothesis testing. Combining all three methods in research is also an interesting way to find out how easily (or not) results from highly controlled but simple microcosm experiments can be extrapolated to a) larger and more complex systems (mesocosms or even lakes) and b) how results of short-term microcosm experiments relate to outcomes of long-term field records.

VI.VI Conclusion

The main objective of this work, (I) to demonstrate the relative influences of top-down and bottom-up forces on periphyton growth in a pan-European mesocosm and laboratory experiment. (II) to test our observational data about the regime shift of two cyclopoid copepod species with ecological theory. We conclude that the experimental design described is suitable for studying how changes in nutrients affect the trophic dynamics and metabolism in shallow lakes at contrasting water levels in different climate zones (more details in Ch. II). Nevertheless, we believe that the system described offers a great potential for the study of the effects of global warming and eutrophication on in-lake processes and dynamics in shallow lakes. The extremely thorough preparatory phase for the project was essential for the successes of this multi-national, multi-cultural experiment. Our results indicate that a strong positive temperature on periphyton growth. Moreover, top-down control by fish enhances periphyton biomass in warmer lake by decreasing invertebrate grazers. However, synergistic interactions between bottom-up and top-down factors might occur, suggesting that care should be taken when predicting the combined effect of changes in multiple factors from their individual effects. Results in chapter IV showed that, planktonic cladoceran *D. magna* which is known to conduct a strong control on seston components can also significantly affect periphyton standing crop by removing periphyton biomass. Thus we argue that effects of large planktonic cladocerans on periphyton have been underestimated. Top-down control of fish on zooplankton would then not only affect phytoplankton abundance but also directly contribute to the periphyton - macrophyte cascade suggested. To confirm the ecological relevance of significant periphyton removal by *D. magna* and other larger daphnids, as suggested by our results, in situ tests considering phytoplankton as alternate food are needed.

Finally, we have presented an example of an intriguing match between observational long-term records and ecological food web and regime shift theory (as in Ch. V). The two coexisting copepod species are linked in an IGP food web, with *C. vicinus* as the predator and *C. kolensis* as the consumer. As for the nature of the complex interaction between driving forces and copepod regime shifts, we were able to distinguish between true threshold-driven dynamics underlying the abrupt increase in C. *kolensis* and a mere, linearly mediated decline in *C. vicinus*, responding to a matching abrupt change in a driving variable. The most likely initiator for the abrupt increase of *C. kolensis* in 1995 was the monotonic decline of *C. vicinus* crossing a critical threshold. Once *C. vicinus* declined to a critical abundance of 0.7 ind. L^{-1}, *C. kolensis* was able to thrive in the low food niche that had already opened up between 1991 (total algal mass) and 1993 (cryptophytes). Overall, we have presented a successful case of how observed changes in nature on the basis of long-term observational data match results from ecosystem theory. In combination, they provide powerful tools in understanding the complex interactions in ecosystems and help to generate hypotheses for a better understanding of the remaining unexplained variability. Since modeling is a prerequisite to project responses, e.g., toward global environmental changes, testing model outputs with long-term observational data or experimental studies is desirable. We have presented a plausible case study on how threshold-driven regime shifts at single system levels in a lake have the potential to change relative species composition within plankton communities.

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Statement of academic integrity

I hereby certify that the submitted thesis “Top-down and bottom-up effects in shallow lake food webs with special emphasis on periphyton” is my own work, and that all published or other sources of material consulted in its preparation have been indicated. Where any collaboration has taken place with other researchers, I have clearly stated my own personal share in the investigation (see Thesis outline). I confirm that this work, in the same or a similar form, has not been submitted to any other university or examining body for a comparable academic award.

Berlin, .01.2014

Aldoushy Mahdy

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الملخص العربي

يلعب البريفيتون دورا محوريا وهاما بالبحيرات الضحله حيث أنه يقوم على امتصاص المغذيات من الماء ويعتبر أيضا كغذاء ومأوى لكثير من الكائنات ويتأثر البريفيتون بكثير من العوامل البيئيه التي تحد من وجوده مثل درجة الحراره والمغذيات والضوء والافتراس (عن طريق اللافقاريات) ويتأثر بشكل غير مباشر بالأسماك (آكلة اللافقاريات).

الهدف من الدراسة الحاليه هوتحديد أهمية المفترسات آكله البريفيتون periphyton (مجموعه الطحالب واللا فقاريات المتعايشه سويا) والمغذيات في الشبكه الغذائيه للبحيرات الضحله مع التركيز على دراسة بيئة البريفيتون. تم في هذه الدراسه استخدام ثلاث تجارب مختلفه: أولا تجارب معمليه تقوم على استخدام أواني زجاجيه صغيره نصف لتر. ثانيا تجارب حقليه باستخدام أواني ارتفاعها مترين تم وضعها بخمس بحيرات أوروبيه مختلفه. ثالثا تحليل إحصائي لدراسة طويله المدى (30 عام) لنتائج سابقهأ لأحد البحيرات ببرلين ألمانيا (Müggelsee). ولدراسة العوامل البيئيه كالمغذيات ودرجة الحرارة المؤثره على البريفيتون تم فى الدراسه الحاليه تعريض أواني (8 فى كل بحيرة) بنوعين مختلفين من المغذيات عالي التركيز (200 ميكرون فوسفات/لتر) وقليل التركيز (25 ميكرون فوسفات/لتر) بخمس بحيرات أوروبيه متباينة درجة الحراره وهذه الدول هي من شمال الى جنوب أوروبا (استونيا ,ألمانيا ,جمهورية التشيك ,تركيا ,اليونان). اعتمدت الدراسه على اتباع برتوكول موحد بكل الدول من حيث أن كل إناء يحتوى على 10 سم رواسب تم جمعها ومعاملتها قبل بداية التجربه. يحتوى أيضا كل نوع من الأواني على نوع من النباتات المغموره (الميكروفايت|macrophyte) *Myriophyllum spicatum* ونوع واحد من الأسماك (ثلاثي الزعنفه الظهريه) آكلة الهوائم والافقاريات القاعية three-spined stickleback, *Gasterosteus aculeatus*. ويحتوي كل إناء على مجموعه من الكائنات الهائمه النباتيه والحيوانيه والتي جمعت من خمس بحيرات مختلفه من كل بلد. تم تجميع البريفيتون لمدة شهر من يوليو إإلى أغسطس سنة 2011 على رقائق بلاستيكيه تماثل أسطحها النباتات المغموره ووضعت هذه الرقائق على عمق نصف متر من السطح لكل إناء وعلى بعد 30 سم من حافة الإناء. أسفرت التجربه عن أن درجة الحراره لعبت دورا هاما ومحوريا لنمو البريفيتون بفصل الصيف. ودلت العلاقه الطرديه بين درجة الحراره والبريفيتون على أن زيادة درجة حراره يقابلها زيادة فى البريفيتون. وعلى الرغم من أن المغذيات في هذه الدراسه (الفوسفات) كانت مختلفه إحصائيا بين التركيزين ولكن لم يكن هناك تاثيرا إحصائيا للمغذيات على البريفيتون في التركيزين. دلت الدراسة أيضا أن الأسماك المستخدمه في التجربه لعبت دورا غير مباشر على زيادة البريفيتون نظرا لافتراسها اللافقاريات آكلة البريفيتون.

ثانيا تم دراسة تاثير اللافقاريات على ازالة البريفيتون عن طريق الأكل او الازالة الميكانيكية عن طريق الحركة. النوعين محل الدراسة برغوث الماء (الدافنيا *Daphnia*) *Daphnia magna* ويرقات الحشرات (chironomid larvae). تمت الدراسه تحت ظروف معمليه خاصه (درجه حراره ثابته 20° مئوية إضافة محلول مغذى وإضائة مشبعة). وذلك على ثلاث مراحل الأولى بالنظر فى تأثير الكائنات محل الدراسه سويا بجلبهم من الأواني بعد تعريض الرقائق البلاستيكيه لمدة شهر بالأواني وتبين من النتائج أن الافقاريات عملت على إزالة البريفيتون. التجربه الثانية والثالثة تم بها تقييم تأثير الكائئنات محل الدراسة بدون اختلاط. وكانت النتائج على النحو التالي حيث أن كلا الكائنين قد عملا على إزالة البريفيتون بنسبه متساويه طبقا لحجم الكائن ولكن عند مقارنة النتائج بالنسبه إلى المليجرام من حجم الجسم إلى واحد ملجرام من البريفيتون وجد أن برغوث الماء يزيل حوالي 58% بريفيتون أكثر من يرقات الحشرات. حتى الآن تعد هذه الدراسه أول دراسه تم بها رصد كون برغوث الماء يستطيع العيش على البريفيتون كغذاء وحيد مما يدل على أن برغوث الماء قد يعمل على تناقص البريفيتون من الشبكة الغذائية بالبحيرات الضحله عند نقص كمية الكائنات الهائمة النباتيه بها.

الدراسة الثالثه تم من خلالها عمل دراسه إحصائيه للكائنات الهائمه الحيوانية بفصل الشتاء ببحيرة Müggelsee لمدة ثلاثين عاما من 1980 الى 2010م لنوعين من أنواع الهائمات الحيوانيه وهما *Cyclops vicinus* و *Cyclops kolensis*. دلت النتائج على أن *Cyclops vicinus* تناقص بصورة مضطردة فى الفترة محل الدراسه بصورة مفاجئة عام 1995م حيث أنه في الوقت الذي كانت فيه كمية المغذيات كبيره كان هو النوع الأكثر تنافسا فى النصف الأول. أما النوع الثانى *Cyclops kolensis* فقد زاد فى نفس الفترة بعد عام 1995 حيث أنه كان النوع الاقل تنافسا فى النصف الأول. وتواكب مع انخفاض *Cyclops vicinus* انخفاض الطحالب والكريبتوفايت (Total algal biomass and cryptophyte) وتواكب مع زيادة *Cyclops kolensis* زيادة العجليات (Rotifer). وقد وجد أن النوعين يقعان في شبكة افتراس واحدة (Intraguild predation) حيث أن الحيوان البالغ يأكل يرقات النوع الآخر. وبينت الدراسه أيضا أن وجود *Cyclops vicinus* بنسبة 0,7 كائن لكل لتر يؤثر على وجود *Cyclops kolensis* بالسلب.

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Curriculum Vitae

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