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Long-term effects of warming and nutrients on microbes and other plankton in mesocosms

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SUMMARY

1. We followed microbial and other planktonic communities during a 4-month period (February–May) in 12 outdoor flow-through mesocosms designed to elucidate the effect of global warming and nutrient enrichment. The mesocosms were established in 2003.
2. Warming had a smaller effect than nutrients on the biomass of the microbial and planktonic communities, and warming and nutrients together exhibited complex interactions.
3. We did not find direct effects of warming on the biomass of bacterioplankton or ciliates; however, warming significantly added to the positive effect of nutrients on these organisms and on heterotrophic nanoflagellates (HNF). No warming effects on any of the other planktonic groups analysed were detected.
4. The zooplankton: phytoplankton biomass ratio was lowest, and the HNF: bacteria and rotifer: bacteria biomass ratios highest in the heated, nutrient-rich mesocosms. We attribute this to higher fish predation on large-bodied zooplankton, releasing the predation on HNF and competition for rotifers.
5. The proportion of phytoplankton to the total plankton biomass increased with nutrients, but decreased with warming. The opposite pattern was observed for the proportion of phytoplankton to the total microbial biomass.
6. As climate warming may lead to eutrophication, major changes may occur in the pelagic food web and the microbial community due to changes in trophic state and in combination with warming.

Keywords: eutrophication, food web, global warming, microbial loop, nutrients

Introduction

The world is steadily warming, and temperature is predicted to increase 2–4 °C within the next century in temperate regions (IPCC, 2007). Shallow lakes are likely to be particularly susceptible to global warming (Mooij *et al*., 2005; Kundzewicz *et al*., 2008; Jeppesen *et al*., 2009, 2011). Climate models also predict that precipitation, and accordingly nutrient loading to lakes, will increase in Northern Europe. Combined with major changes in trophic structure, eutrophication is expected to intensify (Moss *et al*., 2003; Jeppesen *et al*., 2009, 2010; de Senerpont Domis *et al*., 2013). Among the effects on phytoplankton are increases in total biomass, shifts in the timing and

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magnitude of spring blooms and higher dominance of cyanobacteria (Huber, Adrian & Gerten, 2008; Johnk *et al*., 2008; Jeppesen *et al*., 2009), while for zooplankton, shifts in seasonal phenology and size structure are to be expected (Gerten & Adrian, 2002; Gyllström *et al*., 2005; Jeppesen *et al*., 2010).

How global warming will affect microbial communities is under debate. Many biological processes, such as the growth of microbes, are positively related to temperature (e.g. Savage *et al*., 2004), but stimulation of growth may not necessarily increase abundance due to counteracting effects, such as elevated predation (Rae & Vincent, 1998; Christoffersen *et al*., 2006). Global warming may also affect microbial communities through warming-induced eutrophication (Jeppesen *et al*., 2009, 2010), as this community is strongly affected by changes in trophic state (Carrick *et al*., 1991; Nixdorf & Arndt, 1993; Gaedke & Straile, 1994; Mathes & Arndt, 1994). Moreover, a shift in fish community structure towards smaller and more abundant plankti-benthivorous fish may enhance predatory control of zooplankton (Jeppesen *et al*., 2009), with cascading effects on bacteria, protozoans and small-bodied zooplankton (Porter & Mcdonough, 1984; Nõges *et al*., 1998; Jürgens & Jeppesen, 2000).

To elucidate the effect of warming on the microbial community at contrasting nutrient levels, we followed microbes and other plankton during a 4-month period (February–May, 2010) in 12 outdoor flow-through mesocosms (Liboriussen *et al*., 2005). The mesocosms were established in 2003 (7 years before the present investigation) and have been running since then. Christoffersen *et al*. (2006) followed the microbial loop in these mesocosms during the first 16-month period (2003–2004). Their results, obtained in an early transient phase, indicated that warming in itself had no effect on the abundance of bacterioplankton, heterotrophic nanoflagellates (HNF) or ciliates, whereas interactive effects of nutrients and warming occurred. They concluded that the direct effects of warming were far less important than those of nutrients; thus, warming and nutrients in combination can trigger complex interactions that may have pronounced effects on aquatic ecosystems if global warming is accompanied by increased nutrient loading (Christoffersen *et al*., 2006).

We tested the effects of nutrient enrichment and warming during winter (mesocosms covered by ice) to spring on the structure of the microbial and planktonic food web. Eutrophication levels in the nutrient-enriched mesocosms were further intensified after the initiation of the first study of the microbial community by increasing the loading of nitrogen (to a 4 times higher level) in December 2004 and allowing breeding of fish since July 2006 (by the addition of female sticklebacks, *Gasterosteus aculeatus* L.). To gain further insight into the dynamics of the microbial community, our study also encompassed zooplankton and ciliates which were not included in the study by Christoffersen *et al*. (2006). We expected (i) bacterioplankton production to increase with both warming and nutrients and (ii) stronger effects of nutrient-warming interactions on the microbial food-web structure than observed in the study by Christoffersen *et al*. (2006) due to enhanced top–down control by fish on zooplankton and higher nitrogen loading.

Methods

Mesocosms used for the experiment

The mesocosm experiment was initiated in August 2003 in Lemming, Central Jutland, Denmark. It is now the longest running lake mesocosm experiment in the world. A detailed description of the mesocosms and the experimental set-up can be found in Liboriussen *et al*. (2005). It includes 24 fully mixed outdoor flow-through (tap water added several times daily, retention time ca. 2.5 month) mesocosms combining three temperature scenarios (simulating the unheated IPCC A2 scenario (Houghton *et al*., 2001) and A2 + 50%) and two nutrient levels with four replications (Liboriussen *et al*., 2005). A 10-cm layer of washed sand was initially added to each mesocosm with a 10-cm layer of sediment collected from a nearby nutrient-rich freshwater pond on top. To remove large fragments of vegetation and avoid uncontrolled introduction of vertebrates such as fish or amphibians, the sediment was flushed through a net (mesh size: 1×2 cm) and drained of excess water before being placed in the mesocosms. In 2003 (first year of the study), nutrients were added weekly as Na_2HPO_4 and $Ca(NO_3)_2$ solutions with a constant loading of 54 mg P and 538 mg N per mesocosm each week. Depending on the results from the first year, the loading was adjusted later in the experiment between 2003 and 2010. Nutrients were added weekly to half of the mesocosms (dose: 2.7 mg P m^{-2} day^{-1} and 27.1 mg N m^{-2} day^{-1}), while the rest of the mesocosms remained unenriched in the present study. Macrophytes (mainly *Elodea canadensis* Michx and *Potamogeton crispus* Linnaeus, 1753) are present in all low nutrient mesocosms, while the enriched mesocosms are dominated by phytoplankton and filamentous algae and have sparse or no vegetation. In 2003, planktivorous fish (male three-spined sticklebacks) were stocked in natural densities consistent with

the nutrient treatment (Liboriussen *et al*., 2005), being 1 in the nutrient-poor and 12 fish in the nutrient-rich mesocosms. Since summer 2006, fish were allowed to breed in the high-nutrient tanks by replacing some males with females.

Experimental set-up of the current study

Not all available mesocosms were used in the present study. We randomly selected three of four replicates of the two nutrient treatments (enriched and unenriched) and two of the temperature scenarios: unheated ambient and heated, according to the IPCC climate scenario A2 scaled to local conditions in the region (average over five 25×25 km grid cells using a regional model [pers. comm. O. Bøssing Christensen, Danish Meteorological Institute]. Climate scenario A2 models actually predict air temperatures, but since the temperature of shallow lakes closely follows that of the air, we chose to use the modelled air temperatures as a surrogate for water temperatures. Warming was calculated as the mean air temperature increase in a particular month relative to a 30-year reference period (1961–1990), and the modelled temperatures for the same month from 2071 to 2100 (Liboriussen *et al*., 2005). The difference between the ambient and modelled temperature for the A2 scenario is generally higher in August to January (max: 4.4 °C in September) than during the rest of the year (min: 2.5 °C in June). Hereafter, the treatments are termed as follows: ambient temperature, unenriched (A); ambient temperature, nutrient-enriched (A+NP); heated, unenriched (H) and heated, nutrient-enriched (H+NP), respectively. A randomised block design was used for statistical analysis.

Sampling

All parameters were estimated monthly between February and May 2010. An 8-L water sample to determine microbial communities, including bacteria, HNF, ciliates and chlorophyll-a (Chl-*a*), was collected from the mesocosms using a 1-m-long tube water sampler integrating the whole water column. We took care not to touch the plants to avoid contamination of the sample with epiphytic material. An extra sample of 8 L pooled water was taken for zooplankton analysis using the same tube sampler. In ice-covered periods (February and part of March), samples were taken through a hole drilled through the ice in the middle of the mesocosms. From the bulk water sample, we took a 50 mL subsample for bacteria and HNF analyses, a 100 mL subsample for ciliates and a 1L subsample for Chl-*a* analyses. The 8 L subsample of the pooled zooplankton sample was filtered through a 50-μm mesh and dispersed into a 100-mL bottle containing 2 mL acid Lugol (4% Lugol's iodine (v/v)) solution for preservation. Before identification, each sample bottle was washed with distilled water to avoid the browning effect of Lugol.

Nutrients were determined monthly, and the water was frozen prior to the analysis of total phosphorus (TP) and ortho-phosphate (PO_4-P) (Grasshoff, Ehrhardt & Kremling, 1983), total nitrogen (TN) (Solorzano & Sharp, 1980) and nitrate+nitrite (NO_3-N) using a cadmium reduction method (Grasshoff *et al*., 1983).

Bacteria and HNF

Samples for enumeration of bacteria and HNF were fixed immediately after collection by adding glutaraldehyde (Sigma, Taufkirchen, Germany) to a final concentration of 2% (v/v). Subsamples for bacteria and HNF analyses were stained for 10 min with 4'6-diamidino-2-phenylindole (DAPI; Sigma, Taufkirchen, Germany) at a final concentration of 10 μg DAPI mL^{-1} (Porter & Feig, 1980). A Whatman GF/C glass microfibre filter with a pore size of 1.2 μm as a pad was used to obtain a uniform distribution of cells under low pressure (<0.2 bar). Within 2 h following sampling, we filtered the subsamples to count bacteria (2 mL) and HNF (15 mL) onto 0.2- and 0.8-μm pore-size black Nuclepore filters, respectively. Filters were stored at −20 °C until enumeration. The abundances of bacteria and HNF were determined by direct counting of cells using epifiuorescence microscopy (Leica, DMLB; mounted with a HBO 103W/2 DC OSRAM light bulb, Wetzlar, Germany) at 1500× magnification. At least 400 bacteria cells from different fields were counted for each sample with a UV filter (420 nm). All specimens of HNF found within 1.6 mm^2 of each filter were counted. The microscope was equipped with a UV (420 nm) and a blue (515 nm) filter to distinguish heterotrophs from mixo- and autotrophs for HNF counting. Conversion to carbon biomass was made using a factor of 0.22 pg C μm^{-3} for bacteria and HNF (Bratback & Dundas, 1984; Borsheim & Bratback, 1987).

Measurement of bacterial production

Bacterial production was estimated monthly during the study period by measuring the incorporation of [^{3}H]-thymidine into bacterial DNA (Fuhrman & Azam, 1982). We incubated 20 mL subsamples in duplicates with two 50% TCA-killed controls for 45–60 min (depending on the season) at the experimental treatment temperatures (control or A2 scenario) in the dark with thymidine.

Incubation was stopped by adding 2 mL 50% TCA. After incubation, the samples (between 10 and 20 mL) were filtered in the laboratory onto mixed cellulose ester filters (MFS 0.2 μm, 25 mm filter diameter) and rinsed seven times with 5% TCA for 5 min. Then, we transferred the filters to plastic vials and added 7 mL scintillation liquid. The next day, we measured bacterial production in a liquid scintillation analyser (Packard, Tricarb 1900 TR).

Ciliates

Ciliates were fixed with acidic Lugol [4% Lugol's iodine (v/v)]. Counting was performed in sedimentation chambers following Utermöhl (1958). Ciliates were counted under an inverted microscope with 500× magnification (Leitz Labovert). At least 200 ciliate cells or the entire chamber was counted and identified to genus or species level according to Foissner & Berger (1996) and Foissner, Berger & Schaumburg (1999). Biovolumes of ciliates were calculated from the measurements of lengths and width dimensions of animals with approximations to an appropriate geometric shape. Conversion to carbon biomass was calculated using the factor 0.14 pg C μm^{-3} (Putt & Stoecker, 1989).

Chlorophyll-a (phytoplankton)

For Chl-*a* concentration, 100–1000 mL of the water samples was filtered through Whatman GF/C filters (47 mm in diameter, England). Chl-*a* was determined spectrophotometrically after ethanol extraction (Jespersen & Christoffersen, 1987). Phytoplankton biomass was estimated using a carbon Chl-*a* ratio of 30 (Reynolds, 1984).

Zooplankton

Counting of the preserved samples was performed on a 100 mL subsample at 63× magnification (cladocerans and copepods) using a stereomicroscope (Leica MZ12, Wetzlar, Germany). Rotifers were counted at 400× magnification (Leitz Labovert). The studies of Ruttner-Kolisko (1974), Koste (1978), Smirnov (1996), Rivier (1998), Ueda & Reid (2003) and Petrusek, Bastiansen & Schwenk (2005) were used to identify zooplankton. Biomass of rotifers was calculated using standard dry weights from Bottrell *et al.* (1976) and Dumont, Van de Velde & Dumont (1975). Cladoceran biomass was calculated based on length–weight relationships from Bottrell *et al.* (1976), Dumont *et al.* (1975), Culver *et al.* (1985) and Luokkanen (1995). Carbon content of zooplankton was calculated using a conversion factor of 0.48 μg C per μg dry weight (Andersen & Hessen, 1991).

Statistical data analyses

To test for the effects of nutrient enrichment and warming over time (months), we used repeated measures ANOVA (RM-ANOVA) by applying SAS 9.13 software (SAS Institute Inc, Cary, NC). The full data set was used for all biological variables. Data were log-transformed before analysis to reduce skewness and to approximate to normal distribution.

Results

Nutrients

During the experimental period, the average (±SD) TP concentrations were 11.8 ± 4.2 mg P L^{-1} in ambient mesocosms and 73.3 ± 11.4 mg P L^{-1} in ambient enriched mesocosms, 8.5 ± 3.5 mg P L^{-1} in heated mesocosms and 67.3 ± 46.8 mg P L^{-1} in heated enriched mesocosms (Fig. 1). While PO_4-P and TP were low in all months in the unenriched mesocosms, TP was high throughout the period in the enriched mesocosms, exhibiting an increasing trend with time in the heated mesocosms, while PO_4-P declined to low levels as the season progressed.

The average TN concentrations were 0.34 ± 0.32 mg N L^{-1} in ambient mesocosms, 7 ± 3.1 mg N L^{-1} in ambient enriched mesocosms, 0.18 ± 0.16 mg N L^{-1} in heated mesocosms and 4.8 ± 1.4 mg N L^{-1} in heated enriched mesocosms (Fig. 1). NO_3-N and TN were low in the unenriched mesocosms throughout the experiment, and both variables were high, but declined in the enriched mesocosms as the season progressed.

Biological variables

Biomasses of bacteria, ciliates, phytoplankton and zooplankton varied during the season as expected, with the lowest biomass occurring during the ice-covered period in winter (February and March) and the highest in spring (April and May) in all treatments (Table 1, Fig. 2). Accordingly, the time effect (season) in the RM-ANOVA was significant for all the response variables studied (data not shown).

Bacteria biomass and bacterial production (BP). Bacterial biomass ranged between 17 and 282 μg C L^{-1} (Fig. 2a). RM-ANOVA showed no direct significant warming effect on bacterial biomass, whereas an interactive positive nutrient-warming effect was recorded (Table 1).

Bacterial production (BP) increased from 22 to 616 μg C L^{-1} h^{-1} during the study period. RM-ANOVA

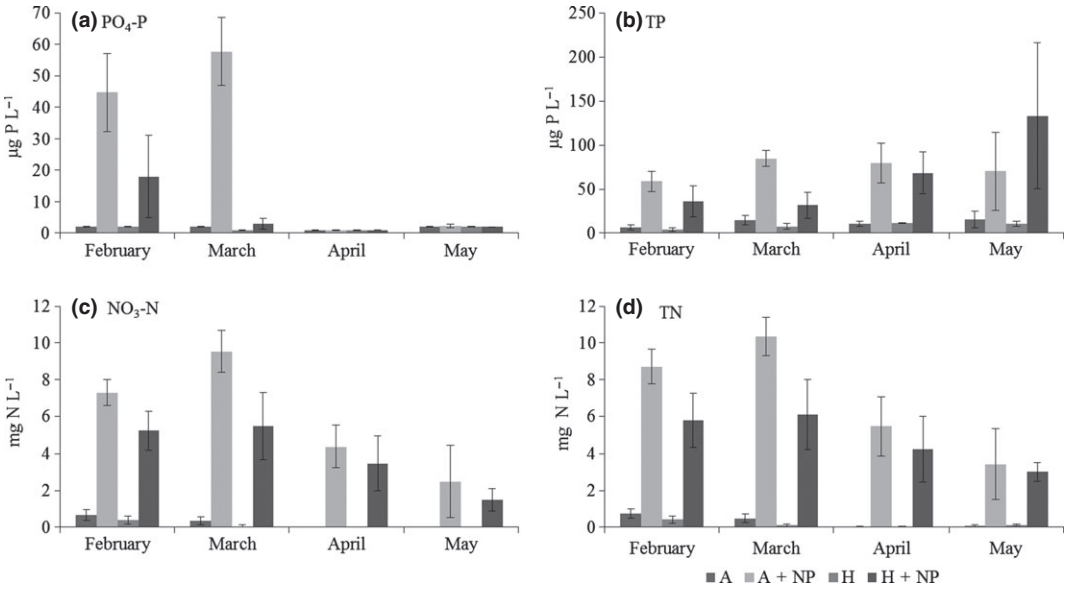

Fig. 1 Monthly mean concentrations (± 1 SD) of (a) orthophosphate (PO_4-P), (b) total phosphorus (TP), (c) nitrate+nitrite (NO_3-N) and (d) total nitrogen (TN) in Ambient (A), Ambient+NP (A+NP), Heated (H) and Heated+NP (H+NP) mesocosms.

revealed a significant effect of nutrient enrichment, while the effect of warming was not significant (Table 1).

Heterotrophic nanofiagellates. Heterotrophic nanofiagellates biomass ranged between 46 and 770 µg C L^{-1}, and a significant positive nutrient-warming interaction was observed (Fig. 2 and Table 1). The biomass of HNF was higher during the ice-covered period for H+NP and peaked in March, while for A+NP and A treatments, HNF biomass peaked in April (Fig. 2 and Table 1). The effect of the nutrient-warming interaction was significant and positive for the HNF:bacteria ratio (Table 1).

Ciliates. Ciliate biomass ranged between 0.3 and 13.8 µg C L^{-1} with maximum in spring (Fig. 2d). For the ambient, unenriched (A) treatment, ciliates peaked in March and showed a hump-shaped pattern. Oligotrichida dominated in most mesocosms and included the genera *Strobilidium*, *Strombidium* and *Halteria*. The nutrient-warming interaction had a significant positive effect on ciliate biomass and the ciliate:bacteria biomass ratio, while no effect was found on the ciliate:HNF biomass ratio (Table 1).

Chlorophyll-a (phytoplankton). Phytoplankton biomass ranged between 44 and 5936 µg C L^{-1} (Fig. 2e). Only nutrient enrichment contributed significantly to the variation in chlorophyll-a throughout the whole study period. The nutrient-warming interaction effect on the bacteria:phytoplankton ratio was significant and negative (Table 1). No effect of nutrients or warming was observed for the HNF:phytoplankton or ciliate:phytoplankton biomass ratios (Table 1).

Zooplankton. Total zooplankton biomass varied between 0.2 and 174 µg C L^{-1} with a maximum in May for all treatments (Fig. 2f). Nutrients positively affected total zooplankton biomass. Following ice-out, total zooplankton biomass increased in all mesocosms, and the effect of nutrient enrichment became apparent (Fig. 2f). The nutrient-warming interaction had a significant negative effect on the zooplankton:phytoplankton biomass ratio and the zooplankton:HNF ratio, while no treatment effects were found on the zooplankton:ciliate biomass ratio (Table 1). Cladoceran biomass ranged from 0 to 9.1 µg C L^{-1} in the monthly samples (Fig. 2g). None of the treatments significantly affected cladoceran biomass. Regardless of temperature, cladocerans dominated in the non-nutrient-enriched mesocosms where *Chydorus sphaericus* (O.F. Müller) and *Bosmina longirostris* (O.F. Müller) were the most abundant species (Fig. 2g). We found a significant negative effect of nutrients on the Cladocera:phytoplankton, Cladocera:HNF and Cladocera:bacteria ratios.

Generally, copepod biomass was low, varying between 0 to 0.91 µg C L^{-1} in the monthly samples (Fig. 2h). We found a significant effect of nutrients on copepod biomass. The highest biomass of copepods (cyclopoids) occurred in the ambient mesocosms (Fig. 2h). The Copepoda:bacteria, Copepoda:HNF and Copepoda:phytoplankton ratios decreased significantly with increasing nutrient levels.

Table 1 Summary of the univariate repeated measures of two-way ANOVA testing the effect of warming and nutrient enrichment on biomass of microbes and other plankton

	Warming (W)	Nutrient Enrichment (NE)	WXNE
Bacteria	NS	***	***↑
BP	NS	*↑	NS
Heterotrophic nanofiagellates (HNF)	**	***	*↑
Ciliate	NS	**	**↑
T.Microbial Community	**	***	*↑
% T.Microbial Community	*↑	***↓	NS
Pliytoplankton	NS	***↑	NS
% Pliytoplankton	*↓	***↑	NS
Zooplankton	NS	**↑	NS
% Zooplankton	NS	NS	NS
Cladocera	NS	NS	NS
Copepoda	NS	**↓	NS
Rotifera	NS	**↑	NS
All Community	NS	***↑	NS
HNF:Bacteria	*	*	*↑
Ciliate:Bacteria	NS	NS	*↑
Ciliate:HNF	NS	NS	NS
Rotifera: Bacteria	NS	*↑	NS
Rotifera:HNF	NS	NS	**
Rotifera:Ciliate	NS	NS	NS
Copepoda: Bacteria	NS	**↓	NS
Copepoda:HNF	NS	**↓	NS
Copepoda:Ciliate	NS	NS	NS
C opepoda: Phytoplankton	NS	**↓	NS
C la docera: Ba ctena	NS	*↓	NS
Cladocera:HNF	NS	*↓	NS
C la docera:Ciliate	NS	NS	NS
C la docera: Pliytoplankton	NS	*↓	NS
C la docera:Ciliate	NS	NS	NS
Zooplankton :Bact.eria	NS	NS	NS
Zooplankton:FINF	***	***	***↓
Zooplankton:Ciliate	NS	NS	NS
Zooplankton :Phytoplauktou	**	**	***↓
Bacteria: Pliytoplankton	NS	**	*↓
HNF: Pliytoplankton	NS	NS	NS
Ciliate: Pliytoplankton	NS	NS	NS

Arrows show the direction of the treatment effect on the organisms and ratios. Significance is indicated as $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, NS, not significant.

Total rotifer biomass ranged from 0.21 to 173 μg C L^{-1} in the monthly samples (Fig. 2i). The dominant rotifer species were *Asplancha* sp. (A mesocosms), *Brachionus angularis* Gosse (A+NP), *Lepadella patella* (O.F.Müller) (H) and *Notholca squamula* (O.F. Müller) (H+NP) in February and March, whereas *Keratella quadrata* (Müller) became the dominant rotifer species in all mesocosms after ice-out. We found a significant effect of nutrients on rotifer biomass. Rotifers were the dominant zooplankter in the A+NP and H+NP mesocosms (Fig. 2i). Following ice-out, mean rotifer biomass markedly increased in all mesocosms, and the effect of nutrients was significant throughout the ice-free period. We found a direct relationship between nutrients and the Rotifera:bacteria ratio and a significant positive interactive nutrient-warming effect on the Rotifera:HNF ratio. Consequently, among the mesozooplankton groups, only Rotifera:HNF ratio was positively affected by warming.

Proportion of zooplankton, phytoplankton and microbial biomass. The estimated contribution of phytoplankton to total plankton biomass increased at high nutrient levels, but decreased with warming, while the opposite trend was observed for the contribution to total microbial biomass (Fig. 3, Table 1). Finally, no treatment differences were found for the contribution of zooplankton (Table 1).

Discussion

As expected, major seasonal changes occurred in microbial and other planktonic biomasses from the ice-covered period (February–March) to the ice-free period (mid-March–May), with many-fold increases in most variables in all treatments accompanied by an increase in TP and a decrease in orthophosphate, nitrate and TN as are typical for shallow lakes during this season (Søndergaard, Jensen & Jeppesen, 2005).

As in the study by Christoffersen *et al.* (2006), we found that warming had a smaller effect than nutrients on the biomass of the microbial community and that combined warming and nutrients exhibited complex interactions. Mesocosm-warming experiments in England, involving nutrient enrichment, also showed nutrients to have a far greater impact than temperature on the plankton food web, zooplankton and phytoplankton (McKee *et al.*, 2002, 2003; Moss *et al.*, 2003; Feuchtmayr *et al.*, 2007).

We did not find a direct effect of warming on the biomass of bacterioplankton or ciliates, although warming significantly added to the positive effect of nutrients on these organisms. A similar observation was made for HNF in a previously published study of the mesocosms (Christoffersen *et al.*, 2006). No warming effect was revealed for chlorophyll-a and the zooplankton groups analysed, whereas chlorophyll-a and total zooplankton biomass as expected were higher in nutrient-enriched mesocosms. The contribution of rotifers to total zooplankton biomass was higher at the highest nutrient level, while the contribution of copepods was lower. These nutrient effects concur with other studies (Mathes & Arndt, 1994; Jeppesen *et al.*, 2000; Burns & Galbraith, 2007). The contribution of phytoplankton to total plankton biomass increased with rising nutrient concentrations, and the

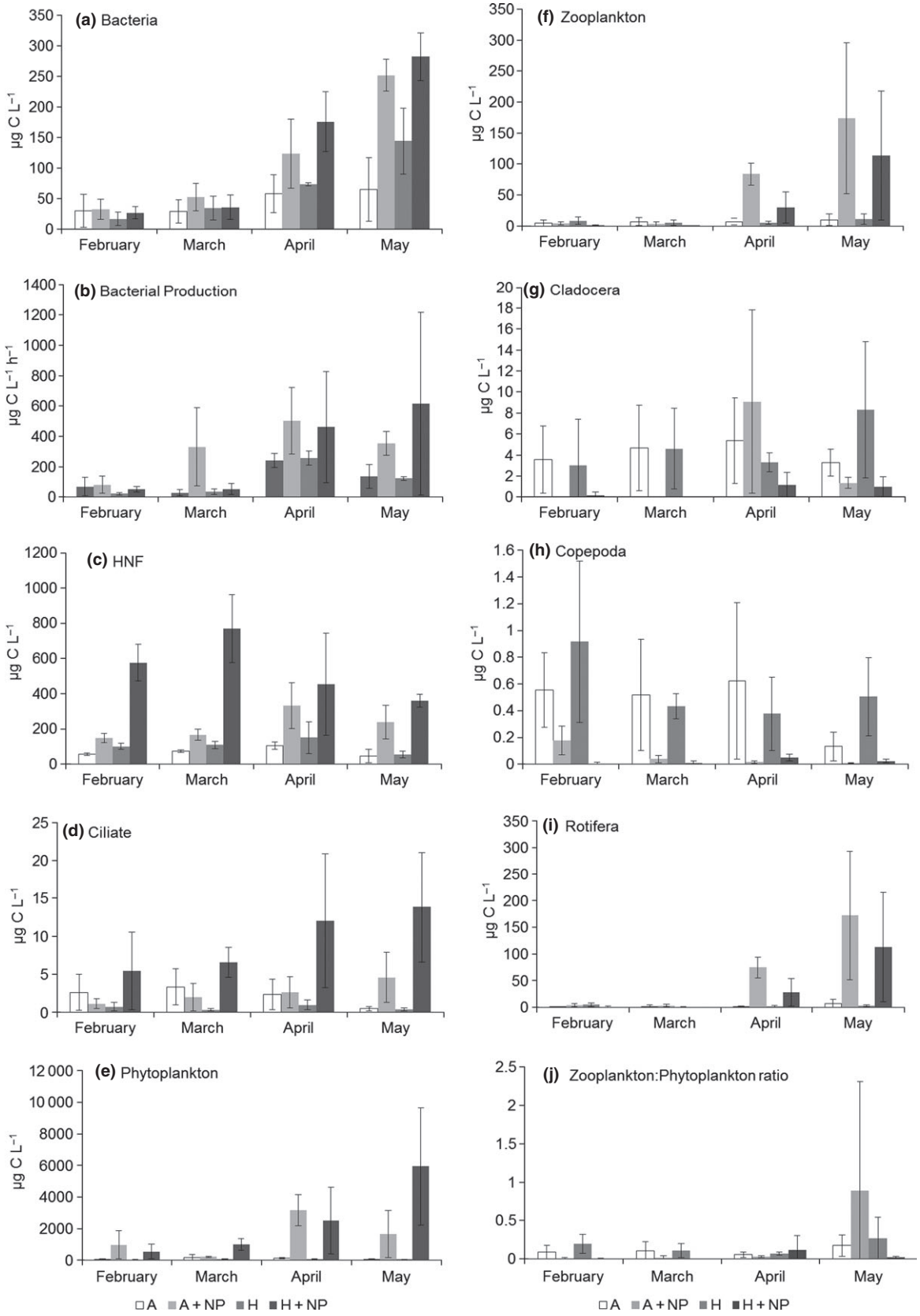

Fig. 2 Monthly biomasses (± 1 SD) of (a) bacteria, (c) HNF, (d) ciliates, (e) phytoplankton, (f) total zooplankton, (g) Cladocera, (h) Copepoda, (i) Rotifera and (j) zooplankton:phytoplankton ratio and (b) bacterial production in Ambient (A), Ambient+NP (A+NP), Heated (H) and Heated+NP (H+NP) mesocosms.

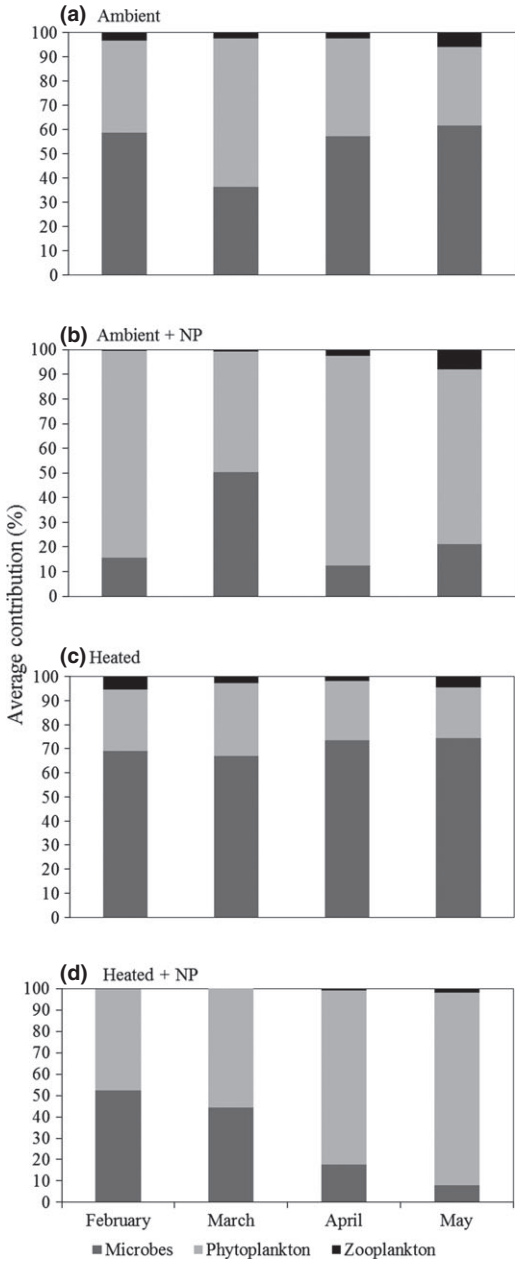

Fig. 3 Average contribution (%) of zooplankton, phytoplankton and microbes (the sum of HNF, ciliates and bacterioplankton) to total plankton biomass in Ambient, Ambient+NP, Heated and Heated+NP mesocosms (NP: nitrogen and phosphorous).

contribution of microbial biomass decreased as observed in other studies of eutrophication (Mathes & Arndt, 1994).

We found indications of synergistic effects of nutrients and warming on food-web dynamics as judged from changes in selected ratios. For example, the lowest zooplankton:phytoplankton biomass ratio occurred in the warm nutrient-rich (H+NP) mesocosms. It is well established that this ratio decreases with increasing eutrophication (e.g. Jeppesen *et al*., 2000, 2003), but our results indicate that the effect will be stronger when lakes get warmer. This may be attributed to higher fish predation on zooplankton in warm systems, resulting in lower grazing control of phytoplankton (Jeppesen *et al*., 2009, 2010). At high fish predation in warm lakes, the zooplankton is dominated by small-bodied species (Meerhoff *et al*., 2007; Havens *et al*., 2011; Iglesias *et al*., 2011), and the abundance of rotifers (not observed in our study), ciliates (Crisman & Beaver, 1990; Havens *et al*., 2011) and HNF tend to be higher, as in our study.

The bacterioplankton community is notably affected by grazing (Pace, McManus & Findlay, 1990), and heterotrophic flagellates tend to be the major bacterivores in fresh waters, followed by ciliates, rotifers and cladocerans (Jürgens & Jeppesen, 2000; Zöllner *et al*., 2003). However, rotifer grazing on bacteria may sometimes be far more important than that of protozoans (Starkweather, Gilbert & Frost, 1979; Bogdan, Gilbert & Starkweather, 1980; Boon & Shiel, 1990; Arndt, 1993). We found the highest HNF:bacteria biomass ratio as well as the highest Rotifera:bacteria biomass ratios in the warm nutrient-rich mesocosms (H+NP), which indicates high predation on bacterioplankton. Rotifers have been found to be more important grazers of bacteria in the nutrient-rich warm lakes (Conty, Garcia-Criado & Becares, 2007), likely as a result of higher fish predation on large-bodied zooplankton in such warm lakes (Gyllström *et al*., 2005). Accordingly, the bacteria:phytoplankton ratio was lowest in the nutrient-rich warm mesocosms, also suggesting grazer control of bacterioplankton. Several studies have demonstrated the bacteria:phytoplankton ratio to be lowest in eutrophic lakes where the importance of microzooplankton and protozoans are highest (Biddanda, Ogdahl & Cotner, 2001; Cotner & Biddanda, 2002; Auer, Elzer & Arndt, 2004).

Higher grazer control of bacterioplankton in warm mesocosms may also explain why bacterioplankton production, contrary to our expectations, did not increase with warming, but was affected only by nutrient addition. Supporting our results, Roland *et al*. (2010) found the ratio of bacterioplankton to phytoplankton abundance (Chl-*a*) to be lower in tropical than in temperate lakes, which they attributed to dominance of microzooplankton and protozoans in tropical lakes.

Christoffersen *et al*. (2006) found higher biomasses of bacteria and HNF in late spring and summer (April-September) than in autumn and winter (October–March). Likewise, we found higher biomasses of bacteria and HNF in the ice-free period (April and May) than in the ice-covered period (February and March), but only HNF biomass was lower in ice-free period in the warm mesocosms at high nutrient levels (H+NP). This might

be due to higher ciliate grazing in these mesocosms. With the expected decrease in ice cover in the future in north temperate lakes, the importance of the microbial community may therefore decline relative to phytoplankton (and fish), particularly in systems with high nutrient levels.

Although the results of the study by Christoffersen *et al*. (2006) partly concur with ours in highlighting the stronger effect of nutrients compared to temperature, some differences are also evident. As in our study, Christoffersen *et al*. (2006) found warming by itself to have no effect on the abundance of bacterioplankton and HNF. They showed, however, that warming significantly modified the positive effect of the nutrients and that only at ambient temperatures did the whole microbial assemblage respond positively to nutrients. By contrast, we found positive warming–nutrient interactions in the microbial community. Whether these differences reflect that the mesocosms have been running for a longer time is uncertain as the nitrogen loading and fish abundance also have changed in the meantime. We believe, however, that our study was run under more realistic conditions, as the mesocosms were severely nitrogen-limited during the early phase of the experiment (2003–2004) and because allowing fish breeding (since 2006) led to more natural fish densities and size variation than during the previous investigation. Moreover, after 7 years, the mesocosms have passed the early transient phase that typically characterises such experimental systems. Our results strongly support that nutrient and warming together have a stronger effect on the pelagic communities than either of them alone. In conclusion, we found that when warming and nutrient enrichment act in combination, the microbial food-web structure is affected more notably than when warming and nutrient enrichment act alone. Consequently, the effects of warming may be strongest in nutrient-enriched systems. Warming may reinforce eutrophication (Jeppesen *et al*., 2009, 2011) and thereby further stimulate changes in the microbial as well as the classical aquatic food web and their interactions.

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