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Primary Research Paper

Implications of climate-enforced temperature increases on freshwater pico- and nanoplankton populations studied in artificial ponds during 16 months

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Abstract

Global warming scenarios foresee increases in air temperatures of 3–5 °C in Northern European regions within the next 70 years. To evaluate the potential effects of global warming on shallow eutrophic lakes, a flow-through experiment combining three temperature scenarios and two nutrient levels was conducted in 24 outdoor mesocosms. Eight mesocosms were unheated and acted as controls, while sixteen were heated – eight according to the Intergovernmental Panel on Climate Change's (IPCC) climate scenario A2 downscaled to regional level (2.5–4.4 °C, depending on season) and eight according to scenario A2+ with an additional 50% temperature increase. Half of the mesocosms were enriched with nitrogen and phosphorus to simulate increased runoff from terrestrial sources due to the increased precipitation predicted by the A2 scenario. The other half were un-enriched and received only natural nutrient input from the groundwater that fed all the mesocosms. The abundance and development pattern of the microbial communities within the mesocosms were tracked during a 16-month period. Generally, the results showed that the abundances of picoalgae, bacteria and heterotrophic nanoflagellates changed in a similar manner over time; abundances being lower in winter than in summer. Warming in itself had no effect on abundance, albeit it significantly modified the positive effect of the nutrients. Only at ambient temperatures did the whole microbial assemblage respond positively to nutrients. In the A2 scenario, only picoalgae responded to nutrients, while in the A2+ scenario all but the heterotrophic nanoflagellates showed a response. Elevated winter temperatures seemed not to be more important for the microbial assemblage than elevated summer temperatures. Our results demonstrate that the direct effects of warming were far less important than the nutrient effect. The results furthermore reveal that warming and nutrients in combination set off complex interactions. In consequence, global warming may possibly have pronounced effects on aquatic ecosystems if accompanied by increased nutrient loading.

Introduction

Presently, there is a global warming trend and climate simulations reveal a further temperature increase of 3–5 °C in Denmark within the next 70 years, the changes being most pronounced during autumn and winter (Christensen & Christensen, 2001). Ecological effects of increased temperature on terrestrial (Forchhammer et al., 1998) and aquatic populations (Park et al., 2004;

Baulch et al., 2005) have been recorded, and the ecological consequences of a warmer climate are subject to intense debate (Scheffer et al., 2001a; Aanes et al., 2002). On the one hand, it is argued that catastrophic cascading may appear because of loss or gain of certain 'key species', on the other hand others claim that ecosystems have a buffer capacity that entails a more gradual or only insignificant change to a moderate temperature increase. Thus, the probable responses of shallow, temperate lakes in the Northern Hemisphere to global warming remain to be further elucidated (Findlay et al., 2001; Scheffer et al., 2001b; Jeppesen et al., 2003). Some of the suggested scenarios are somewhat contradictory, but the emergence of experimental tests such as those by McKee et al. (2003), Moss et al. (2003) and Baulch et al. (2005) may help elucidate the subject. McKee et al. (2003) found only moderate effects of warming, including enhanced sediment release of phosphorus, unchanged phytoplankton abundance, and enhanced dominance of invasive plant species. However, studies of contemporary time series indicate that lakes do respond to climatic variations, such as the North Atlantic Oscillation (George & Hewitt, 1998; Straile et al., 2003). However, temperature *per se* may not necessarily be the triggering force; parallel events such as human induced eutrophication may play a role as well (Brodersen & Anderson, 2002; Jeppesen et al., 2005). Climate models indicate that temperature changes in North-West Europe will entail increased precipitation. Therefore, nutrient loading to aquatic ecosystems from the surrounding terrestrial soils will expectedly increase and, in turn, enhance eutrophication (Christensen & Christensen, 2001; Moss et al., 2003).

With increased warming also the water temperature will rise, and warmer winters will lead to reduced duration of ice cover, especially in coastal lakes (Straile et al., 2003). This may affect the survival of fish and macrophytes, the key structuring elements in many shallow lakes, and lead to changes that may cascade to other trophic levels and ultimately affect lake water quality (Moss, 1990; Scheffer et al., 1993; Jeppesen et al., 1997).

Many biological processes, such as growth and production rates of microbial organisms, are positively related to temperature (e.g. Savage et al., 2004). These relationships are mostly exponential like the well established Q_{10} relationship, but they may also be linear (Montagnes et al., 2003). Thus, changes in water temperature induced by global warming may affect the microbial components of aquatic food webs in a predictable way.

Here, we report on the observed responses of the microbial component of the food web to warming and nutrient addition as observed in a mesocosm experiment focusing on changes in the abundances of picoalgae, heterotrophic bacteria and nanoflagellates. We hypothesised that these lower trophic levels would respond in a positive and additive manner to warming and nutrient enrichment. We expected that densities of the microbial assemblage would increase in the mesocosms impacted by either heating (because of higher internal nutrient loading) or nutrient addition compared to the untreated controls, the positive response being stronger at simultaneous heating and enrichment. Also, we presumed that the temperature effect on the microbial assemblage would be higher in winter than in summer due to the higher predicted and simulated warming in winter, i.e. the warming effects would be modified by season.

Materials and methods

General description of the experiment

An experiment combining three temperature scenarios and two nutrient levels in 24 outdoor mesocosms was started in August 2003 in Central Jutland, Denmark (Liboriussen et al., 2005). Each mesocosm consisted of a cylindrical stainless steel tank with a diameter of 1.9 m and a total depth of 1.5 m. Each of the mesocosms contained 0.2 m lake sediment and a 1 m water column, the latter being continuously mixed by a slowly moving paddle during ice-free periods. The mesocosms were fed with groundwater and equipped with a flow-through system, and the water residence time was approximately 2.5 months. Eight mesocosms were unheated and acted as controls, while the remaining sixteen were heated, eight according to IPCC climate scenario A2 and eight according to scenario A2+, implying an additional temperature increase of 50%. Temperature control in the heated mesocosms ran automatically, the

mesocosms being heated relative to the unheated mesocosms to the expected temperature difference between a reference period (1961–1990) and a modeled period (2071–2100) (regional model, Danish Meteorological Institute, unpublished data). The temperature difference was generally higher in August–January (scenario A2: 3.3–4.4 °C) than during the rest of the year (scenario A2: 2.5–3.0 °C). Half of the mesocosms were enriched with nitrogen and phosphorus (+NP), with a weekly dose of 538 mg N and 54 mg P per mesocosm, while the other half were un-enriched and received only natural nutrient input from groundwater (total phosphorus: 2–20 $\mu g\ l^{-1}$, total nitrogen: 51–71 $\mu g\ l^{-1}$). In the following, the six combinations of temperature and nutrient treatments will be referred to as: Control, Control + NP, A2, A2 + NP, A2 +, and A2 + + NP.

Benthic and pelagic communities representative of temperate lakes were established in the mesocosms prior to initiation of heating. Macrophytes were present in all un-enriched mesocosms, while the enriched mesocosms were dominated by phytoplankton and filamentous algae and had only sparse or no vegetation. Planktivorous fish were stocked in natural densities consistent with the nutrient treatment. For a more detailed description of the experimental set-up and functioning, see Liboriussen et al. (2005).

The microbial communities were studied in water sampled from the mesocosms using a tube water sampler integrating the whole water column. Several samples from each mesocosm were pooled into one from which further subsampling took place.

Heterotrophic and autotrophic picoplankton

Bacterial abundance was determined by flow cytometry (FACS Calibur, Becton Dickinson, New Jersey, USA) after staining 1 ml aliquots with SYBR Green I (final dilution of 10^4 of the commercial stock – Sigma-Aldrich, Missouri, USA) according to Marie et al. (1997). Fluorescent beads (Polyscience, Illinois, USA) of 0.972 μm in diameter were added for calibration by epifluorescence microscopy.

Picoalgal abundance was determined by direct counting of cells using epifluorescence microscopy (Olympus, BH-2, mounted with a HBO 103W/2 DC OSRAM light bulb) in 5 ml aliquots filtered through a 0.2 μm white polycarbonate filter. The microscope was equipped with a blue (emission wavelength 515 nm) and a green filter (590 nm) to observe the autofluorescence.

Heterotrophic nanoplankton

The abundance of heterotrophic nanoflagellates (HNF; <10 μm) was determined by direct counting of cells using epifluorescence microscopy (Olympus, BH-2, mounted with a HBO 103W/2 DC OSRAM light bulb) in 10 ml aliquots stained for 15 min with DAPI (Sigma) to a final concentration of 0.5 $\mu g\ ml^{-1}$ and subsequently filtered through a 0.8 μm black polycarbonate filter. The microscope was equipped with a UV filter (420 nm) and a blue filter (515 nm) to distinguish heterotrophs from mixo- and autotrophs. Filtration was performed within 24 h of sampling and filters were stored at −20 °C until enumeration. All HNF within 1.6 mm^2 of each filter were counted.

Data analysis

Statistical analyses were performed using StatSoft Statistica. The treatments consisted of warming (W; ambient, A2 and A2+) and nutrient addition (NP; no addition and addition of nutrients). For each of the 6 treatment combinations 4 replicates were sampled between 14 and 17 times during the 16-month experimental period. However, there were slight differences in sampling intensity, picoalge, bacteria and HNF being sampled on 16, 14 and 14 dates, respectively. All time series data for all response variables, i.e. picoalgae, bacteria and HNF, were autocorrelated.

In the time series data for HNF two outliers were removed, a value for one of the four unheated control mesocosms on 1 July 2004 and a value from one of the A2 scenario + NP mesocosms on 2 December 2004, as these were 150 and 6 times higher, respectively, than their three respective parallels.

Differences in seasonal trends of the response variables between the 6 treatment combinations were assessed by regression and were recognised as deviations from linearity when plotting the time series data from the control treatments versus the

other 5 treatments. Missing data were case-wise deleted.

Treatment effects on the mean levels of the response variables were assessed by subjecting log-transformed seasonal-means data for all replicates to a 3-way MANOVA, with warming, nutrients and season as factors, and picoalgae, bacteria and HNF as dependent response variables. Only winter (October 2003–March 2004) and summer seasons (April–September 2004) were analysed.

Theoretically, the time series dataset could have been examined by a repeated measures multivariate analysis of variance (rm-MANOVA), but despite loss of within-replicate variation we instead chose to convert the data to summer and winter means (i.e. seasonal means) for each replicate treatment combination. The reasons for this were: (1) both non-transformed and transformed time series data departed significantly from normality. This problem was minimised by using log-transformed seasonal-means data, as the ANOVA/MANOVA is fairly robust against slight departures from normality (Lindman, 1974). (2) Means and variances were highly significantly correlated in the transformed time series data, which is a severe violation of the homogeneity of variance assumption of the ANOVA/MANOVA analysis. This problem was minimised when using transformed seasonal-means data. (3) Distinction between seasons (i.e. summer and winter) was straightforward and similar for all response variables, with apparently lower values in October–March than in April–September.

Results

The abundance of bacteria, picoalgae, and HNF varied during the study period from August 2003 to December 2004, with the lowest numbers occurring in autumn and winter (October–March) and the highest during late spring and summer (April–September) in all treatment (Fig. 1).

The seasonal changes among the microbial components were relatively well correlated for all treatment combinations, especially for bacteria and picoalgae (Table 1). For these two groups the correlation was significant for most treatments (Table 1). However, for HNF, only the control + NP and A2 treatments were correlated. Season had a highly significant effect on the microbial assemblage (Table 2), bacteria, picoalgae and HNF being higher in summer 2004 than in winter 2003–2004 (*Post hoc* Tukey tests).

Nutrients also had a highly significant effect on the concentrations of bacteria, picoalgae and HNF, and although the effect of warming seemed negligible it modified the impact of nutrients (i.e. a warming $\times$ nutrient interaction); for picoalge no effect could be discerned, however (Tables 2 and 3). At ambient temperatures and in the A2 + scenario, bacteria concentrations were significantly higher at nutrient addition while HNF concentrations were higher only at ambient temperatures (*Post hoc* Tukey tests). In the treatments following the A2 temperature scenario a similar positive nutrient effect on bacteria and HNF did not appear, the opposite being true for picoalgae.

No other interactions of/between treatments occurred (Table 2), and the reaction to added nutrients and/or warming did not differ between the winter and summer season.

Discussion

The abundances of the microbial components of the food web in both the control and heated mesocosms were comparable to those observed in shallow eutrophic lakes in Denmark (e.g. Søndergaard, 1991; Jeppesen et al., 1998) and elsewhere (e.g. Kalff, 2000; Muylaert et al., 2003). Generally, seasonal abundance variations were very similar among the treatments and constituted a significant factor in the MANOVA analyses, but the underlying mechanisms are most likely related to increased nutrient loading triggered by enhanced precipitation (Christensen & Christensen, 2001; Moss et al., 2003; Jeppesen et al., 2005). However, contrary to our expectations, seasonal effects did not modify those of warming, and the observed responses to warming were neither stronger nor weaker in winter than in summer; in fact, no response to warming in itself could be traced.

For aquatic organisms, evidence is substantial that biological processes such as growth rates respond positively to higher temperature within a certain range, regardless of whether or not the response is linear or exponential (Montagnes

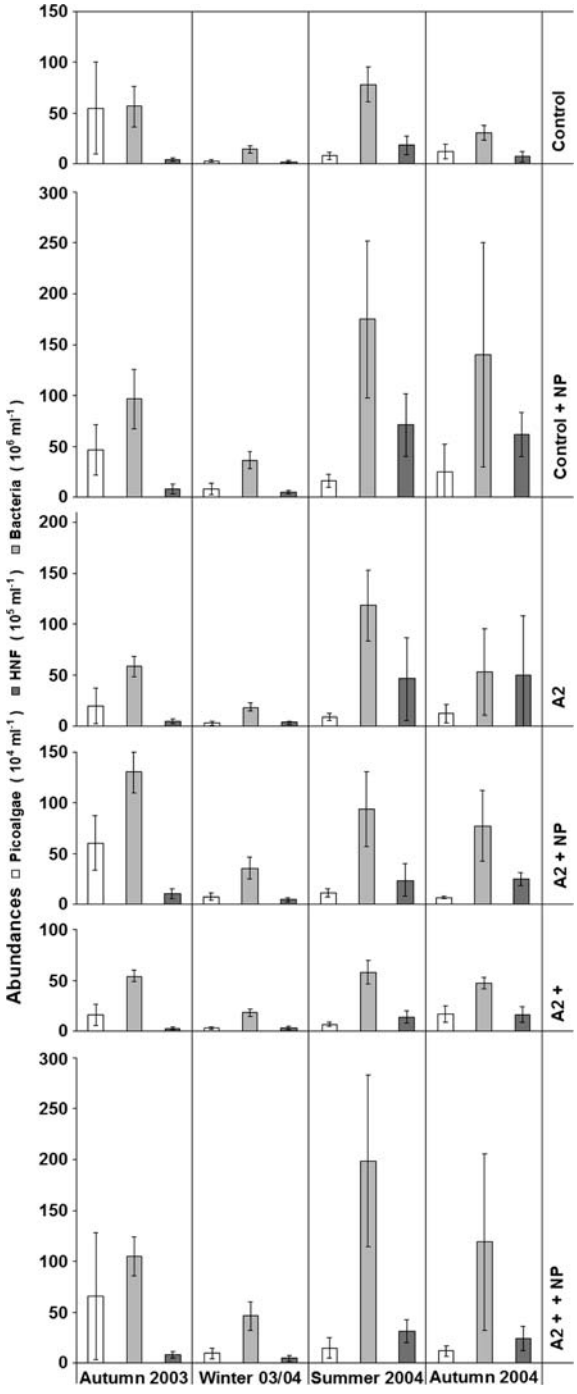

Figure 1. Abundances of picoalgae, heterotrophic nanoflagellates (HNF) and bacteria during different seasons and at different warming and nutrient treatments. Seasonal mean ± Confidence Limits of means are shown. Seasons are as follows: Autumn 2003: August–September 2003; Winter 2003/2004: October 2003–March 2004; Summer 2004: April–September 2004; Autumn 2004: September–December 2004.

et al., 2003). In accordance with this, we therefore expected all the microbial populations included in our experiment to exhibit a similar positive response to warming as the result of higher production rates facilitated by co-occurring increased the loading.

Effects of simulated global warming on microbial organisms remain though a poorly studied subject. For example, in the microcosm experiments conducted by McKee et al. (2000; 2003) and Moss et al. (2003), only components of the classical food web were included. Rae & Vincent (1998) in laboratory experiments with natural plankton communities from a lake in northern Quebec noted elevated bacterial respiratory activity but no effect on bacterial numbers when temperatures were increased from 10 to 20 °C. However, this 2-fold temperature rise increased the concentration of the <2 μm fraction of chlorophyll *a*. While the bacterial response in Rae & Vincent (1998) is in agreement with the results of the present study, their positive response of the autotrophic picoplankton is not, unless the increase in picoplanktonic chlorophyll *a* can be ascribed to more chlorophyll *a* per cell and not to more pico-sized cells.

In the present study it seems likely that warming may have affected the activity, and thus the production, of the microbial assemblage, without triggering a net increase in abundance due to an opposing effect of elevated grazing from herbivorous zooplankton. It is well-known that mechanisms such as temperature, nutrients and planktivorous fish controlling the species composition, density and feeding activity of cladocerans have indirect effects on the microbial community (e.g. Christoffersen et al., 1993, 1998).

Simulated increases in the nutrient supply had a significant effect on the microbial assemblage, and nutrient supply thus seems to be a much more important factor than warming. The response pattern, however, proved to be considerably more complex when examining in detail the effects of nutrient addition, as warming had a significant modifying effect when combined with nutrients (i.e. warming × nutrients interaction).

While picoalgae responded positively and to the same degree (2-fold) to nutrients in all three warming scenarios (i.e. no warming × nutrients interaction), the responses of the other groups

Table 1. Linear regression analyses for bacteria, picoalgae and heterotrophic nanoflagellates (HNF) (log transformed) using the full data set (the four parallels were averaged)

		N	R^2	*p*
Bacteria	Control vs. Control + NP	14	0.51	**
	Control vs. A2	14	0.66	***
	Control vs. A2 + NP	14	0.25	NS
	Control vs. A2 +	14	0.34	*
	Control vs. A2 + + NP	14	0.60	**
HNF	Control vs. Control + NP	14	0.70	***
	Control vs. A2	14	0.01	NS
	Control vs. A2 + NP	14	0.01	NS
	Control vs. A2 +	14	0.24	NS
	Control vs. A2 + + NP	14	0.54	**
	A2 vs. A2 + NP	14	0.42	**
Picoalgae	Control vs. Control + NP	16	0.21	NS
	Control vs. A2	16	0.41	*
	Control vs. A2 + NP	16	0.40	**
	Control vs. A2 +	16	0.69	***
	Control vs. A2 + + NP	16	0.07	NS

Sets with missing data were case-wise deleted. Significance is indicated as: NS, not significantly similar, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

were by no means straightforward. At ambient temperatures, all groups showed the expected positive response to nutrient addition, with 2–20-fold magnitude differences in concentrations. In contrast, only picoalgae responded when the temperature followed the A2 scenario. At even higher temperatures, in the A2 + scenario, all variables except HNF responded, with a 2–10-fold increase in concentrations.

Table 2. Statistical significance of treatment effects elucidated by the univariate results of a multivariate analysis of variance (MANOVA) on log-transformed data on picoalgae, bacteria and heterotrophic nanoflagellates (HNF)

Treatment	Picoalgae	Bacteria	HNF
Warming (W)	NS	NS	NS
Nutrients (NP)	***	***	***
Season (S)	***	***	***
W × NP	NS	**	*
W × S	NS	NS	NS
NP × S	NS	NS	NS
W × NP × S	NS	NS	NS

The analysis used mean seasonal values (winter 2003/2004 and summer 2004) for each of the four replicates of the six treatment combinations. Significance is indicated as: NS, not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

The microbial components included in the present study obviously benefited either directly or indirectly from the additional nutrient supplies, as evidenced by the differing concentrations in the Control and Control + NP treatments. Thus, the organisms were probably not limited by other resources such as carbon or light, but more likely by predator-prey interactions preventing the bacteria and HNF populations from reacting to nutrients when temperatures followed the A2 scenario and, for HNF, the A2+ scenario as well. These predatory forces must have had a lesser effect on picoalgae than on the other components of the microbial food web.

Although our study revealed no direct effects of increased temperatures on the lower trophic levels in the food web, it can be concluded that temperature changes indirectly induce changes, implying that climatic conditions are important for structuring the microbial food web. The results furthermore reveal that complex reactions occur when warming and nutrients act in combination. In consequence, global warming may possibly have pronounced effects on aquatic

Table 3. Warming × nutrient interaction elucidated by *post hoc* Tukey tests

W	NP	Bacteria			HNF		
		10^6 ml^{-1}	Δ	*p*	10^4 ml^{-1}	Δ	*p*
Ambient	−	2.9	2	*	3.0	4	***
	+	6.2			11.9		
A2	−	4.0	–	NS	5.7	–	NS
	+	4.3			6.4		
A2 +	−	3.0	2	***	4.2	–	NS
	+	7.2			7.2		

Differences between mean concentrations of the response variables in treatments with and without added nutrients. Significance of differences is indicated as: NS, not significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Significant differences are assigned an approximate value of this difference (Δ). There was no significant warming × nutrient interaction for picoalgae that increased 2-fold with the nutrient addition in all warming scenarios (from 3 to 6×10^4 ml^{-1}).

ecosystems if accompanied by increased nutrient loading.

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