


Temperature effects on periphyton, epiphyton and epipelon under a nitrogen pulse in low-nutrient experimental freshwater lakes

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Abstract The ongoing global climate change involves not only increased temperatures but may also produce more frequent extreme events, such as severe rainfall that could trigger a pulse of nutrients to lakes. In shallow lakes, this may affect primary producers through a number of direct and indirect mechanisms. We conducted a six-month mesocosm experiment to elucidate how periphyton (on inert substrata), epiphyton and epipelon biomass responded to a nitrogen (N) pulse, an approximately tenfold enrichment of the NO_3 -pool, under three contrasting warming scenarios: ambient temperature and ca. $+3^\circ\text{C}$ and ca. $+4.5^\circ\text{C}$ elevated

temperatures (hereafter T1, T2 and T3). After the N pulse, we found a higher periphyton biomass at elevated than at ambient temperatures but no change in epiphyton biomass. Epipelon biomass was lower in T3 than in T1. Both periphyton and epiphyton biomasses correlated negatively with snail biomass, while epiphyton biomass correlated positively with light. Different responses to higher temperatures under short-term extreme nutrient loading conditions may be attributed to differences in the access to nutrient sources and light. Our data suggest that the biomass of periphyton in oligotrophic clear-water lakes will increase significantly under conditions exhibiting short-term extreme nutrient loading in a warmer climate.

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Introduction

The global mean surface air temperature has been predicted to increase between 1.1 and 6.4°C by 2090–2099 relative to 1980–1990 temperatures, with an average increase of ca. 3°C according to the A2 scenario reported by the Intergovernmental Panel on Climate Change (IPCC) (Solomon et al., 2007). This will significantly influence the primary production in lakes (Schindler et al., 1996). In addition to phytoplankton and macrophytes, periphytic algae (including epipelton and epiphyton) are important contributors to whole-lake primary production in oligotrophic lakes (Cattaneo & Kalff, 1980; McCormick et al., 1997; Liboriussen & Jeppesen, 2003). Several studies have focused on the direct and indirect effects of warming on the relationships between fish, invertebrates, zooplankton and phytoplankton in shallow lakes with or without submerged macrophytes (McKee et al., 2002, 2003; Meerhoff et al., 2007; Kosten et al., 2011; Yvon-Durocher et al., 2011; Cao et al., 2014). Few studies have, however, dealt with the effects on periphyton (here specified as periphytic algae on inert substrata), epiphyton and especially epipelton, and the results obtained so far are ambiguous. Thus, higher experimental temperatures have led to either an increase or a decrease in biomass or have strongly shaped the species composition of three types of experimental algae depending on the set-up used (Hickman, 1974; Baulch et al., 2005; Shurin et al., 2012; Cao et al., 2014). In consequence, it remains unclear how these primary producers will change with climate warming.

Eutrophication is known to cause loss of macrophytes from shallow lakes and to increase phytoplankton abundance, thereby reducing the biomass of epipelton and periphyton (Scheffer et al., 1993; Vadeboncoeur et al., 2003). Whether warming has similar serious effects is currently debated (McKee et al., 2003; Shurin et al., 2012). It is evident that warming often increases phytoplankton biomass and turbidity, thereby exacerbating lake eutrophication (Jeppesen et al., 2009; Moss et al., 2011) and perhaps reducing the growth of epipelton and periphyton due to increased light limitation (Moss et al., 2011). Yet, some studies have shown that warming enhances the

capacity of snails, when abundant, to reduce the biomass of epiphyton in eutrophic systems and thus promote the stability of a clear-water state, at least when fish predation on snails is low (McKee et al., 2003; Cao et al., 2014). Fish predation may also increase with warming with potential cascading effects all the way down to the level of primary producers (Jeppesen et al., 2012). In addition, the effects of eutrophication and climate warming are often very similar, and perhaps synergistic, and therefore rather difficult to fully disentangle from empirical data (Doyle et al., 2005; Liu et al., 2011).

The IPCC 2014 report states that extreme weather events, including extreme rainfall, will increase in frequency during the twenty-first century (Field et al., 2014). This could lead to a rise in nutrient loading due to pulse events, potentially affecting primary producers in lakes. Thus, a pulse of nutrients following severe rainfall may increase the phytoplankton and periphyton production (López-Figueroa & Rüdiger, 1991). Evidence consistent with this prediction was recently obtained in a mesocosm study in China by Zhang et al. (2016).

Periphyton, epiphyton and epipelton access nutrients and light in different ways (Liboriussen & Jeppesen, 2003; Roberts et al., 2003) and occupy different niches; accordingly, their responses to warming and extreme events, such as nutrient pulses, may differ. To study the effects of warming on periphyton, epiphyton and epipelton under extreme events, we conducted a 6-month N pulse experiment with three different temperatures in a mesocosm facility running uninterruptedly for 11 years. We expected that following the N pulse, (1) the biomasses of periphyton and epiphyton would be higher in the high-temperature mesocosms than in the ambient temperature mesocosms (synergistic response, Jeppesen et al., 2009; Moss et al., 2011), while epipelton biomass would decrease at higher temperatures due to greater shading in the water column; and (2) the responses of the epiphyton, periphyton and epipelton to different temperatures might be modulated by biotic interactions, particularly grazers.

Materials and methods

Study site

The experiment was run at three different temperatures in twelve fully mixed (by paddles), flow-through

experimental outdoor mesocosms (four replicates) located in Lemming, Denmark (56°14'N, 9°31'E). The mesocosms consisted of cylindrical stainless steel tanks with a diameter of 1.9 m and a water depth of ca. 1 m with 2800 l capacity. Wires were strung over the tops of the mesocosms to prevent interference from large animals and birds. The temperature treatments were ambient, ca. +3°C (A2 scenario, Solomon et al., 2007) and ca. +4.5°C (A2 + 50% scenario), hereinafter T1, T2 and T3. The mesocosms were fed by groundwater with low-nutrient concentrations. The water retention time was approximately 75 days. The mesocosms held a macrophyte community consisting of the two commonly found species *Potamogeton crispus* and *Elodea canadensis*. The system has been running uninterruptedly since August 2003, and the typical initial transient development is therefore of minor importance. The different temperature treatments were achieved by a triangle-shaped heating bar installed near the bottom of the mesocosms and controlled by software setting the heating on/off several times per minute based on the temperature recorded in the ambient mesocosms. More details about the set-up can be found in Liboriussen et al. (2005). We simulated an N pulse by adding 5.8 g KNO₃, representing a ca. 10 times enrichment of initial levels (ca. 0.2 mg l⁻¹) on 10 June 2013.

Sampling frequency

Two samples were taken on 30 May and 6 June in 2013 prior to the N pulse, and from 11 June to 24 Oct thirteen samples were taken on day 1 (the day of N addition) as well as 3, 8, 10, 15, 22, 31, 59, 79, 105 and 135 days after the addition, i.e. the sampling frequency changed from very frequent (twice a week) to less frequent (monthly) based on the assumption that the effects of the N pulse would abate with time.

Water sampling

Depth-integrated water samples were collected from three randomly chosen places in each mesocosm with a core sampler and subsequently pooled for analysis. Turbidity was measured using turbidity meters from AQUALYTIC® Company. The concentration of phytoplankton chlorophyll-*a* (Chl-*a*) was measured by filtering water through Whatman GF/C filters followed by spectrophotometric analysis via ethanol extraction

(Jespersen & Christoffersen, 1987). Total nitrogen (TN) and total phosphorus (TP) were measured spectrophotometrically after K₂S₂O₈ digestion (Ebina et al., 1983).

Periphyton and epiphyton sampling

For periphyton sampling, we used metal strips (width 3.6 cm, length 60 cm) vertically fixed on the inner side of the mesocosm walls one year prior to our study. The metal strips were carefully removed from the water, and a sharp scraper was used to collect the periphyton. The strips were scraped five times to remove the attached matter, and the collected periphyton was flushed into a plastic bottle using tap water. To avoid sampling the same area twice, we collected periphyton samples from strips placed 20–50 cm below the water surface. At each sampling event, we removed periphyton (7.2 cm², 2 cm strip length) at the same water depth in all mesocosms to allow comparison of results. On each occasion, samples of periphyton were collected and divided into two sub-samples to determine Chl-*a* using ethanol extraction (Jespersen & Christoffersen, 1987) and dry weight (DW). Based on field observations, the growth of epiphyton was similar among individuals of the same species, and to minimize the effects of sampling a single macrophyte individual (aboveground part) was chosen and carefully collected with scissors and put into a plastic bag. In the lab, the sample was shaken vigorously for 1 min and gently brushed to dislodge the epiphyton from the plant after adding tap water to the bag; then, the epiphyton was divided into two sub-samples for Chl-*a* (Jespersen & Christoffersen, 1987) and DW analyses after filtration through GF/C filters, respectively. Periphyton biomass was calculated per unit of sampling area, while epiphyton biomass was calculated per unit of biomass of the host plant.

Epipelon sampling

A sediment core (diameter 5.2 cm², depth 2 cm) sample was taken according to the method in Blumenshine et al. (1997), avoiding sediment near the mesocosm walls and plant roots. Each sample site was chosen at random and immediately marked in order not to sample the same location twice. A sub-sample was taken to determine epipellic Chl-*a* after freeze-drying and ethanol extraction, expressed as per unit DW of sediment.

Plant and snail observations

Plant volume inhabited (PVI) by macrophytes was determined by measuring total plant coverage (%) and plant height (e.g. Lauridsen et al., 2003). Cover by filamentous algae (CFA) was expressed as percentage cover in the mesocosm following the same principle as for plant coverage. The dominant taxa of filamentous algae were *Cladophora* sp. and *Oedogonium* spp.

Snails were present on the mesocosm walls, macrophytes, and the sediment surface. The snails on the walls were used as an indicator of snail densities in the mesocosms as they were easy to detect and classify to species level and to count. We counted the number of snails on the whole wall area (5.97 m²) by visual inspection. If the number was large (>300), a random part of the wall was selected for counting. Several individuals of each species were collected throughout the experiment with a small net (pore size 500 µm) and frozen for species classification. Five individuals of each species were weighed and the average weight (tissue and shell) was used to calculate snail biomass. The species included *Lymnaea stagnalis* (dominant in most mesocosms), *Bithynia leachii*, *Potamopyrgus jenkinsi*, *Gyraulus albus*, and *Radix balthica*.

The light conditions for the three algal types

Data were obtained from the Danish Meteorological Institute, representing the accumulated ground surface radiation within a 20 × 20 km grid covering our system at the experimental site per day, and the radiation data within each month were summed to derive the initial radiation accumulation (MJ m⁻²) from May to October.

To evaluate the role of light for algal growth, light availability was calculated at relevant depths for each algal group using the initial radiation accumulation and shading coefficients in the water column for each mesocosm, defined as the monthly radiation accumulation (MRA) for each algae type. The shading coefficients consist of (i) the light attenuation calculated from the turbidity (mainly due to phytoplankton) and (ii) shading from filamentous algae and submerged macrophytes. The light attenuation coefficients in the water column showed that light diminished almost exponentially with increasing depth, and the attenuation of light by turbidity was

calculated according to Kirk (1977). Shading from filamentous algae and submerged macrophytes was estimated by multiplying the summed coverage proportion of the water surface of the two plant groups with the incoming radiation. The depth used was determined by the distance between the bottom and water surface for epipelton (ca. 95 cm), the distance between half of the plant height to the surface for epiphyton (ca. 45 cm) and the distance between the centre of the sampling area to the water surface for periphyton (within the 20–50 cm range). These specific values of the depth used in the calculation were based on our recordings at each sampling event.

Statistical analysis

We ran three statistical analyses. Firstly, in an attempt to analyse the effects of different temperatures on the investigated variables (see below), we conducted separate analyses for the two phases ‘pre-N pulse’ (using ANOVA) and the phase ‘post-N pulse’ (using linear mixed models). Secondly, we investigated the effects of the N pulse on the biomass of three types of algae by using paired t-tests to compare pre- and post-pulse biomass. Finally, a linear mixed model was used to identify which factors (nutrients, snails or light) influenced the biomass of the three types of algae.

To elucidate the effects of the temperature treatments on the investigated variables (including periphyton, epiphyton and epipelton biomass (as indicated by Chl-*a* or DW), snail biomass, turbidity, phytoplankton Chl-*a*, PVI, cover by filamentous algae in addition to TN and TP in the water), data were separated into two groups: pre- and post-N pulse.

For the pre-N pulse phase, one-way ANOVA was used to compare each variable on two sampling dates to identify the effects of warming.

For the post-N pulse phase, we used linear mixed models with the R package ‘nlme’ and the function ‘lme’ to analyse all the variables (as above), setting the temperature treatment as the sole fixed effect (Zuur et al., 2009). We used time-weighted average data for each month (due to the uneven sampling schedule) and log transformation (in the form of $\log(x + 1)$) to reduce heterogeneity of variance and to better approximate normal distribution. The parameter ‘month’, indicating the sampling month after compiling the data, was chosen as the random factor after the likelihood ratio tests with the null model. Residual

plots were used to check for normality and homogeneity of variance by visual inspection. Post hoc Tukey tests among the temperature treatments were conducted using the package ‘multcomp’ in R.

In addition, to explore the effects of the N pulse on the biomass of the three algal types, we conducted paired t-tests to compare the biomasses before and after the N pulse by permuting all the possible combinations of the data from the different sampling events (all data representing time-weighted averages, thus allowing individual comparisons to be made between the one sample prior to the N pulse with each of the five samples after the N pulse).

To identify the factors influencing the three types of algae, a linear mixed model was used including the fixed factors snail biomass, nutrient concentrations (TN and TP) and light conditions and one random factor, month of sampling. Analysis of the pre-pulse dataset was not possible due to the small size of the dataset. Model selection was performed using backward selection and likelihood ratios to identify the least significant term at each step applying the ‘lme4’ package (Zuur et al., 2009). Residual plots were used as above. All statistical analyses were performed using R software (version 3.0.1) (R Development Core Team, 2014).

Results

Periphyton, epiphyton and epipelton biomass

Prior to the N pulse, periphyton biomass (Chl-*a* and DW) did not differ among the three temperature

treatments (Table 1). After the N pulse, periphyton Chl-*a* remained around $1 \mu\text{g cm}^{-2}$ in T1, which was significantly lower than in two heated treatments (Table 1; Fig. 1). Periphyton DW showed a different trend as it was higher in T3 than in T1 after the N pulse, but neither T1 nor T3 differed significantly from T2.

The epiphytic Chl-*a* was $<200 \mu\text{g g}^{-1}$ plant DW in all temperature treatments prior to the N pulse, but afterwards it peaked around $300\text{--}400 \mu\text{g g}^{-1}$ plant DW in July in most mesocosms (Fig. 1). Epiphyton DW followed a similar pattern as for epiphyton Chl-*a*, being $<0.5 \mu\text{g g}^{-1}$ DW in all the treatments prior to the N pulse and peaking at $0.7\text{--}1 \mu\text{g g}^{-1}$ DW in July. Epiphyton Chl-*a* and DW exhibited no significant differences between the temperature treatments throughout the experiment (Table 1).

The epipelton biomass ranged from ca. $34\text{--}364 \mu\text{g Chl-}a \text{ g}^{-1}$ sediment DW during the entire experiment (Fig. 1). Epipelton biomass did not differ significantly among the three temperature treatments prior to the N pulse but was significantly higher in T1 than T3 afterwards; however, neither T1 nor T3 differed from T2 (Table 1).

Four main explanatory factors

Weighted monthly average snail biomass fluctuated around 100 g (fresh weight) per mesocosm in T2 and was higher in T2 than in T1 and T3 before and after the N pulse (Table 2; Fig. 2). TN was low prior to the N pulse but quickly rose to $>1 \text{ mg l}^{-1}$ immediately after the N pulse, after which it decreased and remained at ca. 0.5 mg l^{-1} or less. TN did not differ among the

Table 1 Statistical summary of epiphyton, periphyton and epipelton biomass before and after the nitrogen (N) pulse (pre- and post-N pulse) under three different temperature treatments

| Variables | Pre-N pulse (one-way ANOVA) | | Post-N pulse (linear mixed model) | | |
|--------------------------|-----------------------------|----------------|-----------------------------------|----------------|---------------|
| | Temperature treatment | <i>F</i> value | Temperature treatment | <i>F</i> value | Post hoc test |
| Periphyton Chl- <i>a</i> | NS | 1.020 | *** | 9.682 | T1 < T2, T3 |
| Periphyton DW | NS | 0.321 | ** | 6.319 | T1 < T3 |
| Epiphyton Chl- <i>a</i> | NS | 2.610 | NS | 0.294 | |
| Epiphyton DW | NS | 0.174 | NS | 0.029 | |
| Epipelton Chl- <i>a</i> | NS | 0.474 | ** | 6.730 | T1 > T3 |

NS not significant

** $P < 0.01$; *** $P < 0.001$

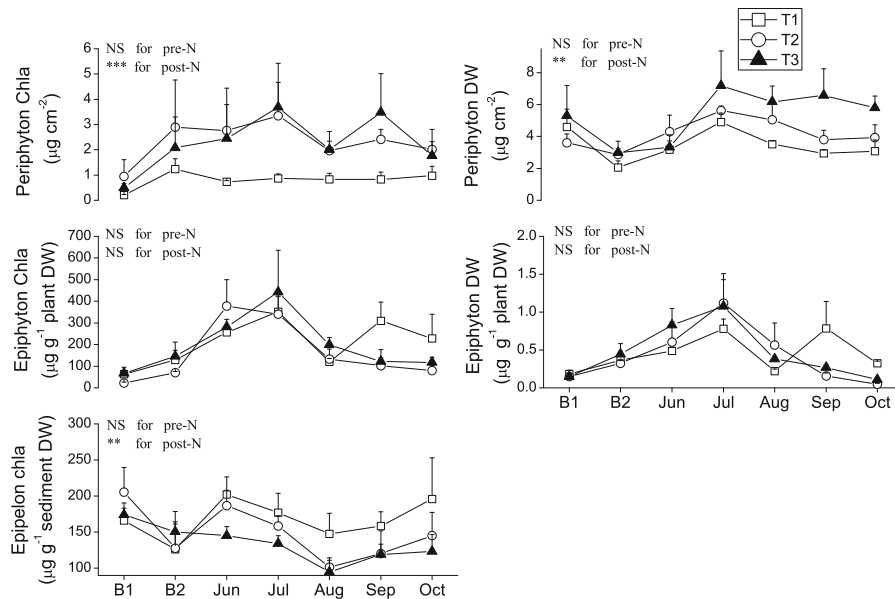


Fig. 1 Mean (+SE) biomass (shown as Chl-*a* and dry weight (DW)) of epiphyton, epipelton and periphyton at three experimental temperatures. 'B1' and 'B2' denote two sampling dates

before N addition, 30 May and 6 June, respectively. Pre- and post-N indicate two phases prior to and post the N pulse. NS not significant; ** $P < 0.01$; *** $P < 0.001$

Table 2 Statistical summary of total phosphorus (TP), total nitrogen (TN), snail biomass and monthly radiation accumulation (MRA) for periphyton, epiphyton and epipelton, turbidity, phytoplankton chlorophyll-*a* (Chl-*a*), cover by filamentous

algae (CFA) and plant volume inhabited (PVI) before and after the nitrogen (N) pulses (pre- and post-N pulse) under three different temperature treatments

| Variables | Pre-N pulse (one-way ANOVA) | | | Post-N pulse (linear mixed model) | | |
|---------------------------------|-----------------------------|----------------|---------------|-----------------------------------|----------------|---------------|
| | Temperature treatment | <i>F</i> value | Post hoc test | Temperature treatment | <i>F</i> value | Post hoc test |
| TP | NS | 2.63 | | *** | 13.433 | T1, T2 < T3 |
| TN | NS | 0.86 | | NS | 1.240 | |
| Snail biomass | * | 4.426 | T2 > T3 | *** | 8.199 | T2 > T3 |
| MRA for periphyton ^a | NS | 1.537 | | ** | 7.642 | T1, T2 > T3 |
| MRA for epiphyton ^b | NS | 1.524 | | *** | 8.624 | T1, T2 > T3 |
| MRA for epipelton ^c | NS | 0.675 | | NS | 0.824 | |
| Phytoplankton Chl- <i>a</i> | NS | 3.53 | | *** | 9.315 | T1, T2 < T3 |
| Turbidity | NS | 1.53 | | *** | 8.785 | T1, T2 < T3 |
| CFA | NS | 0.1 | | * | 3.223 | T1 < T2 |
| PVI | NS | 0.42 | | NS | 1.620 | |

One-way ANOVA was used for the data before the N pulse and a linear mixed model was used for the data after the N pulse
NS not significant

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

^{a,b,c} MRA of May was analysed as before the N pulse and the other five months as after the N pulse

three treatments during the experiment (Table 2). TP was consistently low ($<0.04 \text{ mg l}^{-1}$); before the N pulse, it did not differ significantly among the three temperature treatments, whereas it was pronouncedly

higher in T3 than in the other two treatments afterwards (Table 2).

Monthly accumulated ground surface radiation (MRA) decreased from July (ca. 700 MJ m^{-2}) to

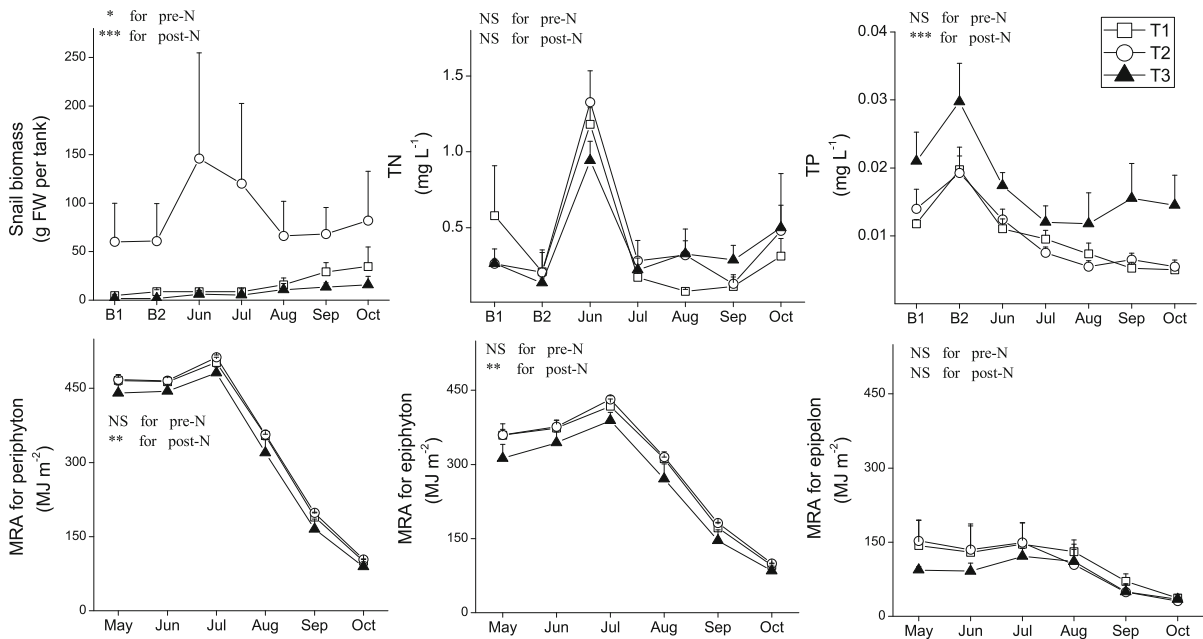


Fig. 2 Mean (+SE) snail biomass, total nitrogen, total phosphorus and monthly radiation accumulation (MRA) for periphyton, epiphyton and epipelon under three experimental temperature treatments. ‘B1’ and ‘B2’ denote the two sampling

dates before N addition, 30 May and 6 June, respectively. Pre- and post-N indicate two phases prior to and post the N pulse. NS not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

the end of our study (lower than 200 MJ m^{-2}). Before the N pulse, MRA levels reaching the surface of periphyton, epiphyton and epipelon were ca. 450 MJ m^{-2} for periphyton, $300\text{--}400 \text{ MJ m}^{-2}$ for epiphyton and $150\text{--}200 \text{ MJ m}^{-2}$ or lower for epipelon (Fig. 2). MRA for periphyton and epiphyton did not differ among the three temperature treatments before the N pulse but was greater in T1 and T2 than in T3 afterwards (Table 2). The MRA for epipelon did not differ between the three temperature treatments throughout the experiment.

Other related variables

Turbidity, phytoplankton Chl-*a*, cover by filamentous algae and PVI did not differ significantly between the three temperature treatments before the N pulse (Table 2; Fig. 3). Turbidity and phytoplankton Chl-*a* reached ca. 10 NTU and $50 \mu\text{g Chl-}a \text{ l}^{-1}$ from August to October, being notably greater in the T3 treatment than in T1 and T2 after the N pulse. Meanwhile, per cent coverage of filamentous algae was greater in T2 than in T1, whereas PVI did not differ among the three treatments.

Paired *t* test of periphyton, epiphyton and epipelon biomass before and after the N pulse

A significant short-term increase in periphyton Chl-*a* in July and in periphyton DW in July and August occurred relative to the periphyton biomass before the N pulse according to the results of the paired *t*-test (Table 3; Fig. 1). In contrast to periphyton biomass, epiphyton biomass increased in June, immediately after the N pulse, but the biomass did not differ between the initial dates and the dates after September for epiphyton Chl-*a* or between the initial dates and the dates after August for epiphyton DW. Epipelon Chl-*a* did not vary noticeably after the N pulse, apart from the lower values recorded in August.

Correlation analyses between periphyton, epiphyton and epipelon biomass and four main explanatory factors after the N pulse

The linear mixed model revealed that both periphyton Chl-*a* and DW were weakly (negatively) correlated with snail biomass but not with other factors after the

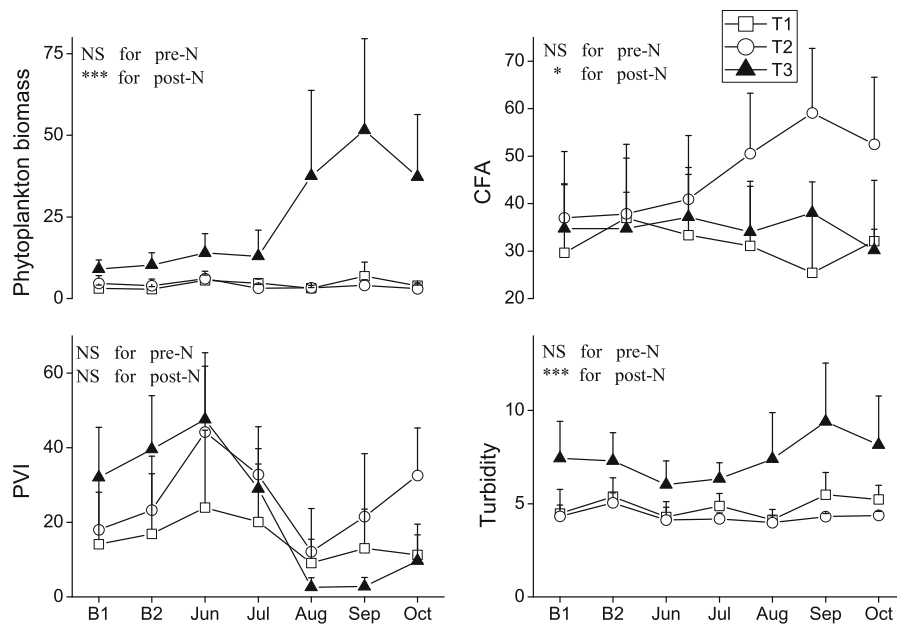


Fig. 3 Mean (+SE) phytoplankton chlorophyll-a (Chl-*a*), coverage by filamentous algae (CFA), plant volume inhabited (PVI) and turbidity under three experimental temperature treatments. ‘B1’ and ‘B2’ denote the two sampling dates before

N addition, 30 May and 6 June, respectively. Pre- and post-N indicate two phases prior to and post the N pulse. *NS* not significant; **P* < 0.05; ****P* < 0.001

Table 3 Paired *t* test of periphyton, epiphyton and epipelon biomass (chlorophyll-*a* (Chl-*a*) and dry weight (DW)) prior to and after the N pulse

| Combination | Paired <i>t</i> test (<i>t</i> values and significance) | | | | |
|-------------|--|---------------|-------------------------|--------------|------------------------|
| | Periphyton Chl- <i>a</i> | Periphyton DW | Epiphyton Chl- <i>a</i> | Epiphyton DW | Epipelon Chl- <i>a</i> |
| Init–June | –2.166 (NS) | –0.038 (NS) | –4.083 (**) | –3.717 (**) | –1.44 (NS) |
| Init–July | –2.679 (*) | –3.273 (**) | –3.474 (*) | –4.338 (**) | 0.117 (NS) |
| Init–Aug | –0.611 (NS) | –2.593 (*) | –4.532 (**) | –2.255 (NS) | 2.903 (*) |
| Init–Sep | –1.598 (NS) | –1.561 (NS) | –2.110 (NS) | –1.398 (NS) | 1.496 (NS) |
| Init–Oct | –0.510 (NS) | –1.118 (NS) | –1.933 (NS) | 0.418 (NS) | 0.134 (NS) |

Combinations of the paired *t* test include one sample prior to the N pulse (Init) and five samples after the N pulse (5 months: June, July, Aug, Sep and Oct)

NS not significant

* *P* < 0.05; ** *P* < 0.01

N pulse (Table 4). Both epiphyton Chl-*a* and DW were significantly related to snail biomass, indicating a negative relationship. In addition, epiphyton biomass showed a positive correlation with MRA. Neither periphyton, epiphyton nor epipelon biomasses were related to TN, but epipelon biomass was negatively correlated with TP.

Discussion

Climate change is likely to affect freshwaters in various ways, one being that more frequent extreme events such as storms and flooding may lead to nutrient pulses to lakes. Consistently with our first hypothesis, following an N pulse to oligotrophic

Table 4 Correlation analysis between periphyton, epiphyton and epipelon biomass (chlorophyll-*a* (Chl-*a*) and dry weight (DW)) and four main factors after the N pulse using a linear mixed model. MRA is monthly radiation accumulation for periphyton, epiphyton and epipelon

| Correlation | TN | TP | MRA | Snail biomass |
|--------------------------|----|-----|-----|-----------------|
| Periphyton Chl- <i>a</i> | NS | NS | NS | NS ^a |
| Periphyton DW | NS | NS | NS | NS ^b |
| Epiphyton Chl- <i>a</i> | NS | NS | +** | -** |
| Epiphyton DW | NS | NS | +** | -*** |
| Epipelon Chl- <i>a</i> | NS | -** | NS | NS |

NS not significant

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; “+” positive relationship; “-” negative relationship

^{a,b} The significance value was marginally greater than 0.05–0.0531 and 0.0581, respectively—and both were negatively correlated with snail biomass

shallow lake mesocosms, we found greater periphyton biomass at elevated temperatures than at the ambient temperature and lower epipelon biomass at the highest temperature. Opposed to our hypothesis of higher biomass at warmer temperatures, we found no temperature effect for epiphyton biomass. As for our second hypothesis, different relationships between snails and three algal types were observed.

Periphyton Chl-*a* did not differ among the three temperatures prior to the N pulse but was greater at both ca. +3°C and +4.5°C than at ambient temperature during the post-N pulse (Fig. 1). A study undertaken in the same system in 2008–2009 revealed that nutrient limitation of periphyton growth varied seasonally, periphyton biomass potentially being N limited in late summer when TN reached <0.5 mg l⁻¹, following P limitation in spring (Trochine et al., 2014). Therefore, low TN concentrations (<0.5 mg l⁻¹) at elevated temperatures prior to the N addition may indicate N limitation of periphyton, possibly diminishing (but not eliminating, see Fig. 1) the positive effects of the temperature increase on the periphyton biomass. In accordance with our results from the post-N phase, Cao et al. (2014) experimentally showed that algal growth on artificial substrata increased under a ca. +3°C temperature rise at low grazing pressure from snails. The mixed model also displayed a marginally significant negative effect of the snail biomass on periphyton Chl-*a*. The effects of higher temperature on periphyton could be reduced at

large snail densities (Cao et al., 2014) or become negative at high invertebrate grazing pressure (Shurin et al., 2012). The snail density in our study was slightly lower (with the exception of a temporary large abundance of small-sized species in some mesocosms) than in other studies demonstrating obvious grazing effects on periphyton (McCollum et al., 1998; Cao et al., 2014), which may explain the relatively weak correlation observed between periphyton Chl-*a* and snail biomass.

Periphyton DW and Chl-*a* reached peak values in different periods of the experiment (Fig. 1). Whereas Chl-*a* is a measure of periphyton algal biomass, periphyton DW includes, among others, bacteria, fungi and protists whose abundances might also be influenced by higher temperatures (Patrick et al., 2012). However, mature periphyton consists mainly of algae (Aizaki, 1980). Our experiment showed that periphytic algae and the whole periphytic biofilm (represented by Chl-*a* and DW in our study) exhibited somewhat different responses to warming. Light was apparently not a limiting factor for periphyton growth as no significant relationship with light availability and nutrient resources emerged after the N pulse.

Unlike periphyton, the biomass of epiphyton (including Chl-*a* and DW) was small and did not differ among the three temperatures throughout the experiment; instead it appeared to be positively correlated with light availability. A short-term mesocosm study conducted in subtropical China (Cao et al., 2014) showed that higher temperature did not affect the epiphyton biomass on the submerged macrophyte *Vallisneria spinulosa* in the presence of snails or the biomass of *P. crispus* whether or not snails were present. The authors attributed this to augmented grazing by snails (*V. spinulosa*) and decay of macrophytes (*P. crispus*), leading to nutrient release, as also observed by Guariento et al. (2009). As described above, snail density was low in our experimental mesocosms, so a possible scenario is that the grazing pressure from snails counteracted the potential positive effects of higher temperatures on the epiphyton but not strongly enough to negatively affect epiphyton biomass. Correspondingly, epiphyton biomass did not differ among the three temperatures after the N pulse.

The different roles played by snails in regulating epiphyton and periphyton Chl-*a* can perhaps be ascribed to the different properties of the substrata (Cattaneo & Amireault, 1992; van Dijk, 1993). The

epiphyton on the macrophytes or the macrophytes themselves might attract snails, though this is a topic subject to debate (Lodge, 1985; Iwan Jones et al., 2000; Li et al., 2009; Mormul et al., 2010). If snails are attracted, this could lead to a greater grazing pressure on epiphyton and thereby a stronger relationship between epiphyton and snails, as revealed in our experiment. Apart from snail grazing, the availability of nutrient resources and light is also considered an important regulatory factor of epiphyton biomass (Lalonde & Downing, 1991; Liboriussen & Jeppesen, 2006). The mixed model showed that epiphyton biomass was positively correlated with light, but no significant relationship between epiphyton biomass and TN and TP could be traced (Table 4). In the warmest mesocosms, lower irradiance accumulation was recorded for both epiphyton and periphyton; yet, the effects of light conditions on periphyton and epiphyton differed. This may reflect that the periphyton light conditions were measured near the surface, while the light conditions for epiphyton were measured at middle macrophyte height, the latter having stronger light attenuation and therefore being potentially light limited. During the experiment, *Potamogeton crispus* started to grow in early spring; senescence occurred in mid-summer, and re-growth started again in late summer. In contrast, *Elodea canadensis* grew actively from June, and the macrophytes exhibited slightly different growing statuses at different temperatures (personal observation; Fig. 3). This makes it difficult to discern the effects of nutrients on epiphyton as they vary with species and growing status (Guariento et al., 2009; Tarkowska-Kukuryk & Mieczan, 2012; Cao et al., 2014).

There are few studies of the effects of higher temperatures on epipelton biomass, but those that exist show effects on the species composition of benthic algae (Hickman, 1974; Watermann et al., 1999). We found that after the N pulse the biomass of epipelton was higher in the ambient temperature than in the warmed mesocosms (ca. +4.5°C), whereas it did not differ significantly from the biomass recorded at ca. +3°C (Table 1). A high density of benthic snails may control the epipelton biomass (Connor et al., 1982); however, *Lymnaea stagnalis*, the dominant snail species in our mesocosms, is a pulmonate species, which can spend much less time in benthic habitats and whose grazing influence on epipelton biomass might therefore be lower than that of the benthic

species *Ilyanassa obsoleta* observed in the study by Connor et al. study. Epipelton grew on the nutrient-rich sediment and is therefore considered not to be limited by nitrogen or phosphorus in the water column (Liboriussen & Jeppesen, 2003). Light limitation is important for epipelton growth in eutrophic lakes (Jenkerson & Hickman, 1986; Liboriussen & Jeppesen 2003; Casco et al., 2009), but, in contrast to our prediction, epipelton biomass did not decrease in any of the treatments when irradiance accumulation declined drastically during the experiment. This suggests that the light reaching the sediment was sufficient to maintain epipelton growth in this clear-water, low-nutrient system. In correspondence with this, we found no correlation between light availability and epipelton biomass. Thus, there is no straightforward explanation for the smaller epipellic biomass recorded at ca. 4.5°C and the negative relationship observed between epipellic biomass and total phosphorus.

Apart from the evident effects of higher temperature, we found different responses in the biomasses of the three algal types when comparing the pre- and post-N pulse results (Table 3). Periphyton and epipelton showed a short-term but delayed increase after the N pulse, which may indicate seasonal variation rather than a direct effect of the N pulse. In contrast, epiphyton biomass increased immediately after the N pulse, suggesting an effect of the N addition. Effects of seasonal variation cannot, however, be fully separated from those of the N pulse in our system. From September, the biomasses of the three algal types did not differ from those recorded at the initial conditions, which point to that the effects of the N pulse, if any, were only temporary or less important than those of seasonal variations in the clear-water state.

In summary, the differences in access to nutrients and light and/or biotic interactions, for instance grazing by snails, might explain the different responses to temperature increases found for the biomasses of periphyton, epiphyton and epipelton. Our study also indicates that greater phytoplankton and periphyton biomass in clear-water lakes may be expected under conditions of extreme nutrient loading and higher temperatures, which adds more evidence to the suggested effects of extreme climate events and warming on freshwater ecosystem. Besides, we found that after an extreme N loading event, light might become an important limiting factor for epiphyton

growth but is not of critical importance for periphyton growth in the surface water and epipelton growth on the bottom in clear, low-attenuation lakes. As we did not have a true control for the simulated nitrogen pulse due to the limitations of the experimental system, we cannot fully rule correct for seasonality effects, so our results should be interpreted with caution. As for biotic interactions, snails may be important modulators regulating the growth of periphyton and epiphyton but likely not epipellic growth in low-nutrient lakes subjected to nutrient pulses.

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