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PRIMARY RESEARCH PAPER

Responses of submerged macrophytes and periphyton to warming under two nitrogen scenarios: A microcosm study

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Abstract Warming and higher nitrogen loading induced by increasing precipitation are expected scenarios in north temperate regions as consequence of global climate change, with potential effects on the functional traits of submerged macrophytes and periphyton. Using an experimental heating facility we investigated the responses of three-week growth of two submerged macrophytes (*Potamogeton crispus* Linn. and *Elodea canadensis* Michx.), and periphyton on these plants and their artificial mimics. Analysis

was based on IPCC climate scenarios A2 (ca. + 3°C) and A2 + 50% (called A3 in our study) relative to ambient conditions, across warming in spring and early summer (summer showed higher nitrogen loading). Some functional traits of plants showed species-specific responses to warming: A3 promoted the growth of *E. canadensis* in both seasons, while for *P. crispus* warming reduced the leaf number in spring but enhanced the turion production in early summer. Periphyton biomass was lower in A3 in early summer, but not in spring. Our results further show that the growth of *E. canadensis* and the asexual reproduction

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of *P. crispus* might increase in a warmer future. Moreover, we found a complex response of periphyton to the temperature increase and substrate type, varying with season and nutrient state.

Introduction

Submerged macrophytes and periphyton are key primary producers in shallow lakes and valuable ecological indicators of water quality (Vadeboncoeur & Steinman, 2002; Liboriussen & Jeppesen, 2009; Søndergaard et al., 2010). The responses of clear water shallow lakes to increased nutrient loading is highly dependent on the interactions between periphyton and submerged macrophytes (Phillips et al., 1978; Sand-Jensen & Borum, 1991; Phillips et al., 2016) as periphyton growing on plant surfaces can result in plant losses inducing a shift to phytoplankton-dominated states (Phillips et al., 2016). In recent decades, increasing anthropogenic pressures have led to a global decline in submerged macrophyte abundance in lakes (Zhang et al., 2017).

The global surface air temperature is expected to increase between 1.1 and 6.4°C by 2090–2099 (Solomon et al., 2007). Warming potentially increases the biomass and distribution of submerged macrophytes in low-nutrient clear-water lakes (Rooney & Kalff, 2000), while in eutrophic lakes warming can intensify eutrophication and lead to the disappearance of submerged macrophytes (Jeppesen et al., 2010; Xu et al., 2020). Warming affects plant traits related to plant growth, reproduction and survival; for example, a mesocosm study conducted in a long-term heating experimental facility showed that the plant height of the submerged species *Potamogeton crispus* Linn. responded negatively to warming, while the adventitious roots of *Elodea canadensis* Michx. were promoted by warming under low nitrogen (N) and phosphorus (P) conditions (Cao et al., 2015). Moreover, Cao et al. (2015) showed that heatwaves enhanced the asexual reproduction of *P. crispus* by increasing the abundance of small turions. Global warming may also increase the frequency of extreme events, and these events can affect the reproduction of

submerged macrophytes (e.g. reducing the flower number of *Myriophyllum spicatum* L. as reported in Li et al. (2017)). The response of plant traits to warming also varies with the season; for instance, Silveira & Thiébaut (2017) found that elevated temperature (3°C) had a significantly greater impact on plant growth (e.g. promoted relative growth rate and branch number) of *E. canadensis* in spring than in summer and winter under low nutrient conditions.

Besides being impacted by warming, eutrophication also threatens the growth of submerged macrophytes. Nutrient loading may increase in summer in north temperate and tropical lakes due to episodes of extreme rain, intensifying the eutrophication (Bouraoui et al., 2004; Jeppesen et al., 2009). Control of the P input is known to mitigate eutrophication of freshwater lake ecosystems (Schindler 1977; James et al., 2005). However, N is also important, especially in shallow lakes (Søndergaard et al., 2017), as also revealed by an analysis of data from 573 lakes where N control or N and P co-control was found to be effective at mitigating eutrophication in shallow lakes (Qin et al., 2020). Although Özkan et al. (2010) did not find adverse effects of N on plant growth at moderately low TP concentrations (< 100 μg L^{-1}), high N levels reduced the macrophyte abundance and increased periphyton biomass by a factor of 2–3 over the summer at moderate P concentrations (Barker et al., 2008; Olsen et al., 2015). Furthermore, N contributes to reducing the chances of maintaining a clear-water macrophyte-dominated state at high phosphorus concentrations (González Sagrario et al. 2005; Moss et al., 2013; Puche et al., 2018). However, more studies are needed about the role of increased nitrogen levels and warming on macrophyte growth in shallow lakes, especially at high P levels (Chen et al., 2013; Scheffer & van Nes, 2007).

Both warming and eutrophication are expected to affect the production and species composition of periphyton in lakes (Jeppesen et al., 2009; Jeppesen et al., 2011; Rühland et al., 2015). How warming affects periphyton growth is debatable as ambiguous results have been obtained so far. Some studies have shown an increase in periphyton biomass with a temperature increase (Patrick et al., 2012; Tarkowska-Kukuryk & Mieczan, 2012). Others found higher biomass in colder lakes, in a comparative analysis of lakes from contrasting latitudes (Meerhoff et al., 2007), latitudinal gradient experiments (Bécares et al.,

2008) and a global data meta-analysis (Meerhoff et al., 2012). Similar results were obtained in experimental studies over 16 months in Canada (Shurin et al., 2012). That periphyton biomass is higher in temperate than in warmer climates at comparable nutrient levels can to a large extent be explained by increasing consumption by fish in warmer lakes (Meerhoff et al., 2007; 2012). Moreover, the respiration of autotrophic organisms is higher than in temperate systems, leading to lower net production and biomass accumulation (O'Connor et al., 2009). Warming may also reduce the biomass of periphyton by enhancing the growth and reproduction of snails, thereby increasing grazing pressure (Jones & Sayer, 2003; McKee et al., 2003; Cao et al., 2014). In addition to warming, periphyton growth in freshwaters is also affected by eutrophication (Havens et al., 1999; Cao et al., 2017), and in a mesocosm study, Hao et al. (2020) showed an interaction effect of temperature and nutrients on periphyton biomass, which was significantly higher in the unheated treatments with nutrient addition than that in the warming treatments without nutrient addition.

The substrate type also influences periphyton growth. Higher macrophyte complexity promotes periphyton growth (Hao et al., 2017; Casartelli & Ferragut, 2018). In a one-year mesocosms study Hao et al. (2020) found that plants and their plastic mimics with more complex morphological structure promotes higher periphyton biomass.

Periphyton biomass may be affected by complex interacting processes created by climate change, particularly warming, and high N loading triggered by increased summer precipitation in shallow eutrophic lakes. Furthermore, macrophyte species may show different plant trait responses to warming and excessive nutrient loading, entailing a shift in the species composition of macrophyte communities. We conducted two experiments to investigate the effects of warming on the growth of two macrophyte species (*Potamogeton crispus* and *Elodea canadensis*) and periphyton in eutrophic experimental microcosms inside mesocosms with high P concentrations – one experiment in spring with low and one experiment in summer with high N loading. We hypothesised that at high P concentrations, the plant traits of the two macrophyte species would be affected by warming differently since warming can promote growth traits differentially depending on their respective growing season. Therefore, different responses of growth traits

(e.g. plant biomass and height) and reproduction traits (e.g. flower and turion production) were expected. We further expected a negative response of periphyton biomass to warming and a positive response to increasing complexity of the substrate, since complex morphological structure of plants can hold more periphyton and warming can increase grazing pressure of snails. Thus, periphyton biomass could decrease with warming while increase with the complexity of the substrate.

Material and methods

We performed two experiments in microcosms inside mesocosm facilities mimicking shallow lakes in two seasons in Central Jutland, Denmark (56° 14′ N, 9° 31′ E) to analyse the responses of submerged plants and periphyton to warming. The climate change mesocosms facilities consisted of 24 tanks (12 eutrophic and 12 low-nutrient) 1 m-depth and a diameter of 1.9 m. From these, we chose the 12 eutrophic tanks with four replicates at 3 temperature scenarios: ambient (Amb), A2 (ca. +3°C) and A3 (A2 + 50%, ca. + 4.5°C) according to the Intergovernmental Panel on Climate Change scenarios (IPCC) (Solomon et al., 2007) (Fig. 1). Heating elements are placed above the sediment and monitoring devices that are remotely supervised. These mesocosms have been running continuously since August 2003 and are the world's longest-running heated lake mesocosm facility (further details in Liboriussen et al., 2005). The tanks are weekly enriched with 7 mg P m^{-2} day^{-1} and 27.1 mg N m^{-2} day^{-1}, with $Ca(NO_3)_2$ and Na_2HPO_4 as detailed in Liboriussen et al. (2005). The N addition was ceased in June 2018, i.e. one year before this study, to elucidate the role of N on the lake ecosystem at contrasting temperatures. During this year the mesocosms only received N loading via groundwater with relatively low N concentrations Liboriussen et al. (2005). Our first experiment was performed under low N conditions from 20th May to 10th June 2019 (spring) in Central Jutland, Denmark (56° 14′ N, 9° 31′ E) following one year without N addition (after 15 years with N addition). We ran the second experiment under high N conditions from 5th July to 26th July (early summer) 2019 in these same mesocosms after resuming the weekly N addition (27.1 mg N m^{-2} day^{-1}) from 11th June 2019.
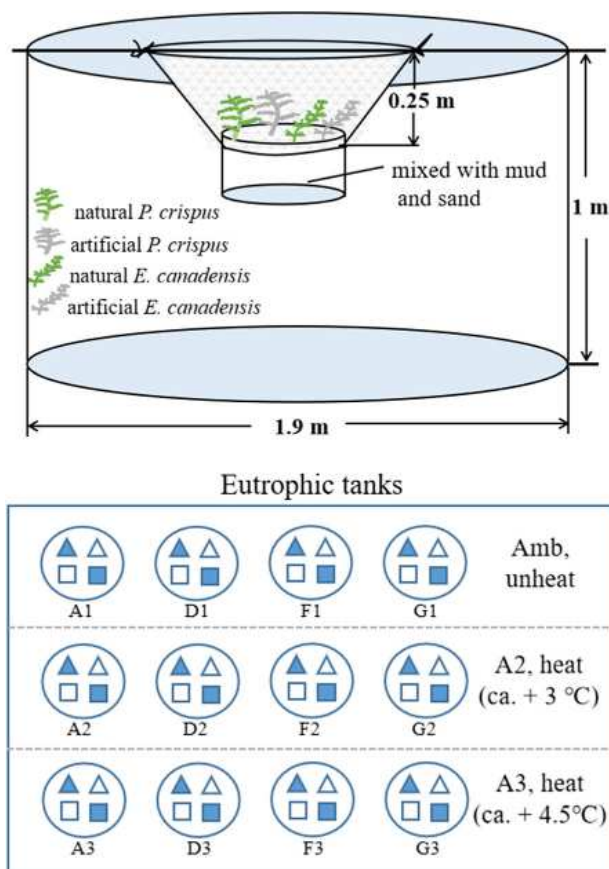
Eutrophic tanks

Fig. 1 Experimental design with natural *P. crispus* (white triangles) and *E. canadensis* (white squares) and their artificial mimics (in blue). Each treatment had four replicates: A, D, F and G replicates (referring to tank codes). Temperature scenarios: Amb = ambient unheated, A2 = heating of + 3°C and A3 = heating of + 4.5°C

In these mesocosms, we placed microcosms with two species of submerged plants *Elodea canadensis* and *Potamogeton crispus,* and artificial plastic substrates mimicking these plants. *Elodea canadensis* and *Potamogeton crispus* with different growth forms are common in northern Europe, and are both perennial plants that grow well during late spring and summer. The two species were initially cultivated in all mesocosms but are today only present in a few. We used two differently structured types of substrates 'Natural' *P. crispus* and *E. canadensis* (termed as Pota_Real and Elod_Real) and their mimics 'Artificial' (termed as Pota_Arti and Elod_Arti) (Fig. 1) to elucidate the effect of different substrates on periphyton considering the potential effect of allelochemicals released by the macrophytes (Nakai et al., 1999; Pakdel et al., 2013).

We collected top shoots (20 cm long) of the two species from the low-nutrient mesocosms in ambient conditions and carefully washed them with a soft brush and clean water to remove previous periphyton. We planted one shoot of each species (approximately 5 cm of the shoot inside the sediment) together with their artificial mimics, in plastic pots (diameter 23 cm, height 29 cm) filled with sediment, inside of the mesocosms for three weeks to allow periphyton growth. Afterwards, we placed the plastic pot in the centre of each mesocosm at 25 cm depth to avoid shading from the tank edge. The pots were wrapped with a plastic net (mesh size 0.5 cm) to avoid entry of large invertebrates and fish grazing. These microcosms was imbedded in the mesocosms, and were only separated by the fish/macroinvertebrate net. Therefore, the microcosm had the same environmental conditions as the mesocosms, including the same temperature, as the mesocosms were continuously fully mixed with paddles.

The temperature in the mesocosms was measured by a PR electronics temperature sensor with a registration frequency of 30 minutes. Water samples were collected weekly for phytoplankton biomass and water chemistry from the tanks (i.e. mesocosms). During the experiment, we measured total nitrogen (TN) and total phosphorus (TP) that were digested by $K_2S_2O_8$ (Ebina et al., 1983). We determined alkalinity twice, each per experiment, by Gran's titration with 0.1 mM HCl (Gran, 1952).

After three weeks, we collected the natural and artificial macrophytes from each pot, taking care not to remove the periphyton or create sediment resuspension. All above-ground parts of the macrophytes were stored at 4°C in a cooling box and taken to the laboratory. In the laboratory, we carefully rinsed off the periphyton from the artificial and natural plants using a soft toothbrush, after which it was pooled in 500 ml of tap water. Of this, we took subsamples for biomass estimation as Chl_a and ash-free dry weight (AFDW).

For chlorophyll a (Chl_a) estimation of phytoplankton and periphyton, we filtered the samples through Whatman GF/C filters followed by extraction in 95% cold ethanol and measurement using a spectrophotometer (Lorenzen 1967; Jespersen & Christoffersen, 1987). We acidified and re-measured the samples with 0.1 mM HCl for pheophytin calculation. This is a standard method to correct the

Chlorophyll_a data and to check the quality of these data in case of the overestimation of Chlorophyll_a derived from the accumulation of death material. Periphyton Chl_a and AFDW were expressed in μg cm^{-2}.

Small snails were found within wash-offs from the determination of periphyton and carefully collected. Snail density and biomass were calculated per plant leaf area with the unit of ind m^{-2} and g m^{-2}, respectively.

These methods were applied for both spring and early summer independent experiments.

Plant traits

The height of macrophytes was measured and the leaf area estimated by scanning the plants in a known area using ImageJ (Rasband, 2009; Levi et al., 2015). Leaf size was calculated by dividing total leaf area by total leaf number. We estimated Damaged Leaf Ratio (DLR) of *P. crispus* as the ratio between damaged to total leaf number, "damaged" being defined as leaves with broken edges or holes caused, presumably, by snail grazing (Cao et al., 2019) and plant biomass as the dry weight after drying the plants for 48 hours at 80°C. We divided plant traits into growth traits, including above-ground biomass, height and leaf traits, and reproductive traits, including turion number and flower number.

Statistical analysis

To analyse the effects of temperature on plant biomass and traits, we performed one-way ANOVA. Additionally, two-way ANOVA analysis was conducted using substrate type and temperature as the two main factors as indicators of snail and periphyton.

Normality and homogeneity of variance were checked by visual inspection of residuals. To identify the differences, we performed a *post hoc* Tukey's test. In these analyses, we assumed a significance level of 0.05.

The statistical analyses were performed using R software (version 3.5.4).

Results

The water temperature ranged between 12–25°C in spring and 19–30°C in early summer (Fig. 2). The concentration of TN was ca. 1 mg L^{-1} during spring in Exp. I and reached ca. 2 mg L^{-1} in early summer after N addition, except for A3 where the mean concentration was still lower than 1 mg L^{-1} (Fig. 3). The concentration of TP fluctuated from ca. 0.15 to 0.30 mg L^{-1} during the two experiments. Similar to the TN concentration, phytoplankton biomass (Chl_a) ranged between ca. 20 and 70 μg L^{-1} in spring, early summer average values being higher with ca. 220 μg L^{-1} in Amb and A2 and ca. 70 μg L^{-1} in A3.

Experiment I

Total nutrients (TN and TP) and alkalinity did not differ among the three temperature treatments (for all three indicators: $F < 2.59$, $P > 0.05$, Fig. 3). However, phytoplankton biomass was lower in A3 than in Amb and A2 ($F = 8.211$, $P < 0.01$; Fig. 3).

For *P. crispus*, the leaf number and leaf area were significantly larger under ambient conditions (Amb) than in the heated mesocosms (A2 and A3) (Table 1, Fig. 4). The Damaged Leaf Ratio (DLR) was significantly higher in A2 than in Amb and A3, and < 50% of the leaves were intact in A2. We found no differences in other plant traits, e.g. above-ground
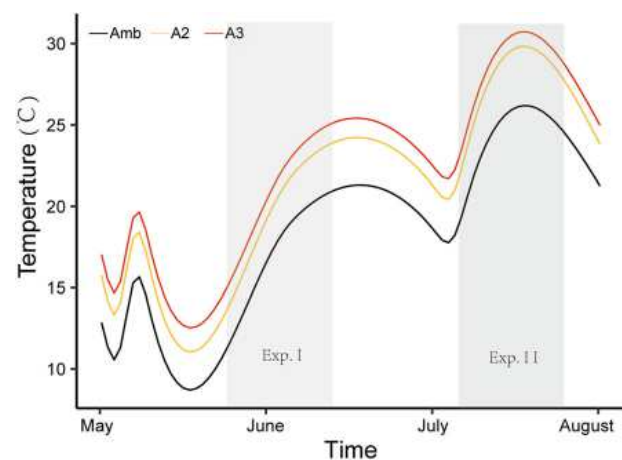

Fig. 2 Water temperature from 30th April to 31st July covering the two three-week experiments (20th May to 10th June and 5th July to 26th July). Amb, A2 and A3 refer to the three temperature treatments. Amb = temperature under ambient conditions, A2 = heated according to IPCC climate scenario A2 (ca. + 3°C), A3 = heated according to A2 + 50% (ca. + 4.5°C) relative to Amb
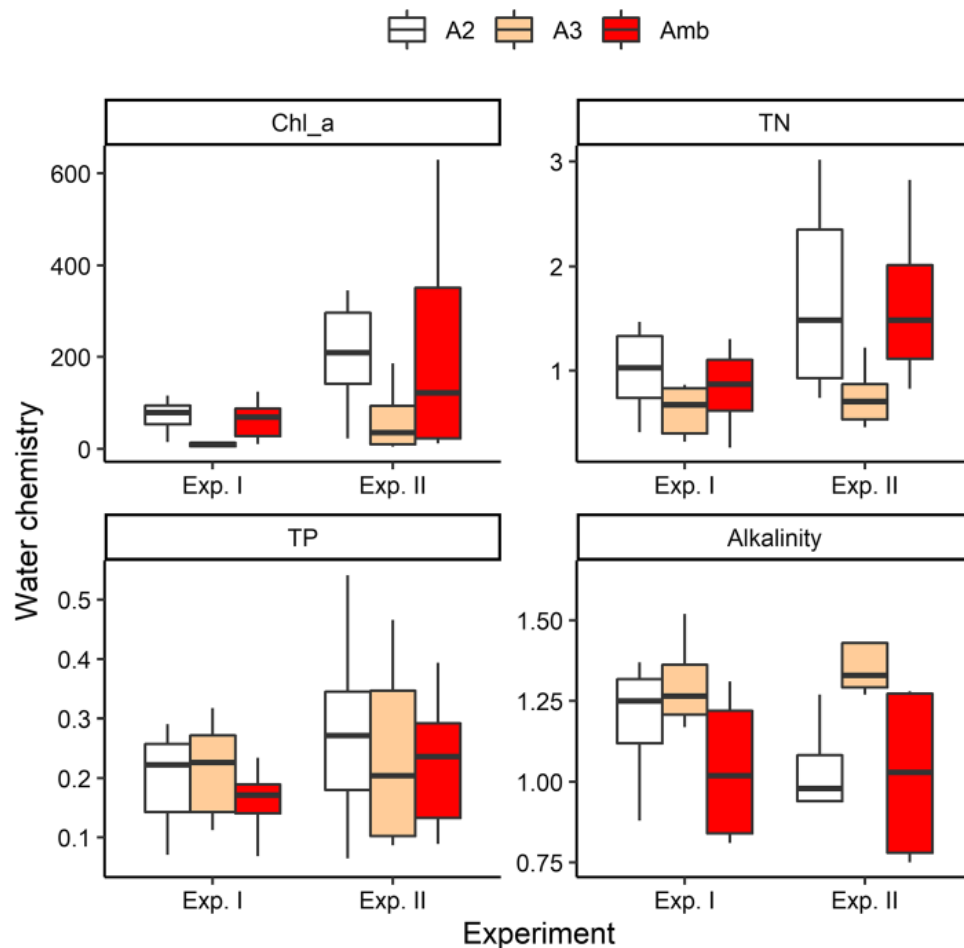
Fig. 3 Physico-chemical water variables including phytoplankton chlorophyll a (Chl_a, µg L^{-1}), total phosphorus (TP, mg L^{-1}), total nitrogen (TN, mg L^{-1}) and alkalinity (mmol L^{-1}) in the two experiments ($n = 4$). Boxplots show median, inner-quartile range, minimum, and maximum values. The middle line of the box plot represents the median of the data; the upper and lower bars represent the maximum and minimum values of the results

biomass, plant height and branch number, between the three temperature treatments. The flower number of *P. crispus* was ca. 1 per individual and did not differ among the three temperature treatments.

For *E. canadensis*, branch number was significantly promoted by warming, being larger in A2 and A3 than in Amb (Table 1, Fig. 5). Leaf number and above-ground biomass were larger at the highest temperature than in Amb, while smaller leaf size was found in the two elevated temperature treatments than in Amb.

Periphyton AFDW did not respond to warming, but it was affected by substrate type, with higher AFDW on Elod_Arti than on Elod_Real (Fig. 6 and Table 2). Moreover, snail density was higher in Elod_Real (mostly newly hatched small snails) than in Pota_Arti and Pota_Real. Snail biomass was significantly larger in A2 than in Amb and A3 (Table 2). Chl_a did not respond significantly to the heating treatments.

Experiment II

In Exp. II, under recovered high N conditions, TN was significantly lower in A3 than in Amb and A2 ($F = 8.032$, $P < 0.01$, Fig. 3). There were no differences in phytoplankton Chl_a, TP and alkalinity among the three temperature treatments (for the three indicators: $F < 3.259$, $P > 0.05$, Fig. 3).

Turion number and DLR of *P. crispus* were promoted by warming, with more turions in A3 than in Amb and higher DLR in A2 and A3 than in Amb (Table 1, Fig. 4). The above-ground biomass of *P. crispus* in Exp. II was 25% ∼ 50% of those cultivated

Table 1 Plant traits in experiment I and experiment II

	Experiment I				Experiment II			
Species	P. crispus		E. canadensis		P. crispus		E. canadensis	
Plant trait	F, Sig.	Post hoc test	F, Sig.	Post hoc test	F, Sig.	Post hoc test	F, Sig.	Post hoc test
Above-ground biomass	0.184, n.s.		4.78*	Amb < A3	3.294, n.s.		5.714*	Amb < A3
Plant height	4.126, n.s.		0.103, n.s.		0.281, n.s.		0.167, n.s.	
Branch number	1, n.s.		7.61*	Amb < A2, A3	2.333, n.s.		2.512, n.s.	
Leaf area	8.46**	Amb > A2, A3	3.964, n.s.		0.734, n.s.		4.906*	Amb < A3
Leaf number	12.27**	Amb > A2, A3	4.789*	Amb < A3	0.123, n.s.		3.763, n.s.	
Average leaf size	0.813, n.s.		9.473**	Amb > A2, A3	0.193, n.s.		0.648, n.s.	
Damaged leaf ratio	14.21**	Amb, A3 < A2			5.63*	Amb < A2, A3		
Turion number	2.66, n.s.				7.426*	Amb < A3		
Flower number	0.913, n.s.				–			

Temperature treatments are Amb, A2 and A3. Tukey's post hoc test. *P. crispus* flowers were absent in experiment II, and thus no ANOVA analysis was conducted for flower number ($n = 4$)

Significance levels: $P > 0.05$ = n.s.; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

in Exp. I, and *P. crispus* had new branches but no flowers.

The above-ground biomass and leaf area of *E. canadensis* were higher in A3 than in Amb (Table 1, Fig. 5). However, plant height, leaf number and leaf size did not change significantly in the two heating treatments compared with Amb. Abundant newly grown small leaves were observed in July, reaching an average of ca. 500 per individual for all treatments.

Snail density and biomass did not differ among treatments (warming or substrates) in Exp. II (Table 2). Periphyton was negatively affected by warming, with higher AFDW in Amb than in heating treatments as well as higher Chl_a in Amb than in A3. None of the two indicators of periphyton biomass differ between the two substrate types. Following Exp. I, we found no interaction between substrates and temperature treatments for any of the indicators of periphyton biomass (for all indicators $F < 2.396$, $P > 0.05$).

Discussion

Consistent with our first hypothesis, we found that warming affected the growth and reproduction traits of the two macrophyte species differently. The growth of *Elodea canadensis* Michx. was promoted in the highest temperature treatment (A3) in both seasons, whereas the growth of *Potamogeton crispus* Linn. was reduced in spring in A3. While we found no responses to warming in periphyton biomass in spring, periphyton decreased with warming in early summer under higher N loading.

Most of the plant growth traits of *P. crispus*, such as plant height, did not respond to warming either in Exp. I in spring or in Exp. II in early summer. Concurrently, an earlier study conducted in the same low-nutrient mesocosm revealed no significant warming effects on the growth of *P. crispus* in late autumn (Zhang et al., 2015). Meanwhile, a short-term mesocosm study conducted in subtropical China indicated that a 3 or 4.5°C warming did not affect the biomass of *P. crispus*
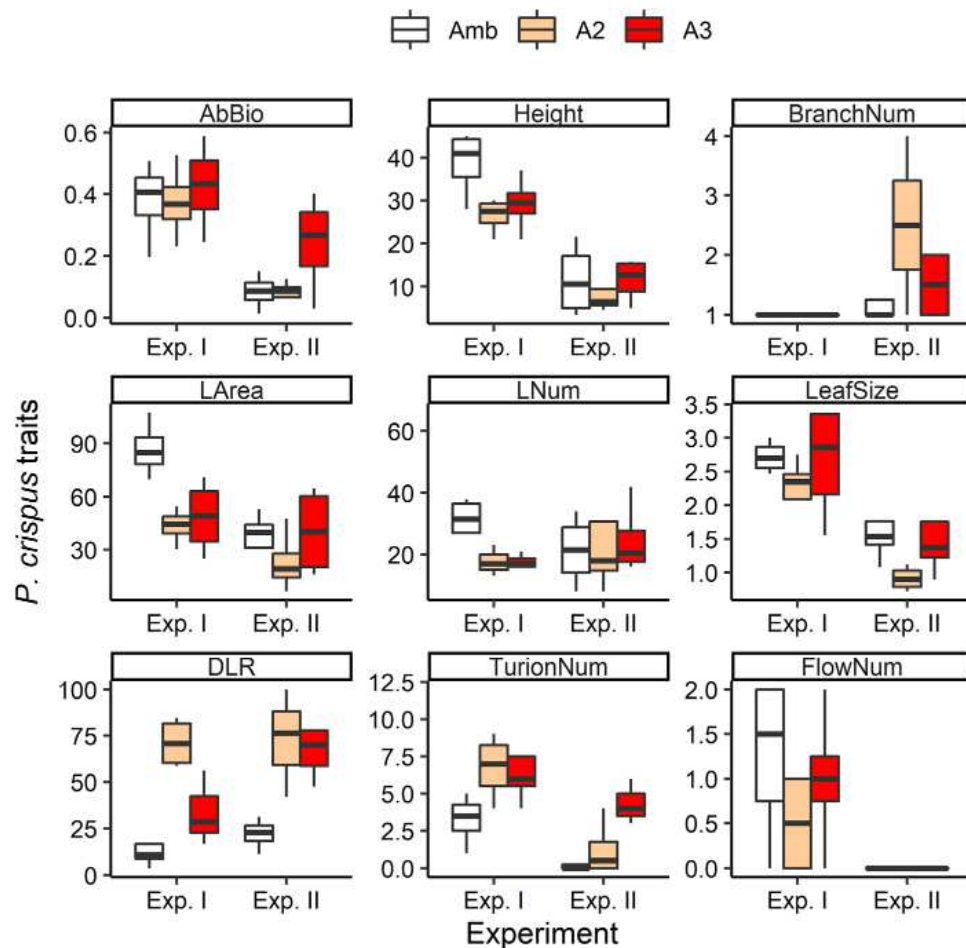
Fig. 4 Plant traits of *P. crispus* in experiment I and experiment II in the three different temperature treatments, including above-ground biomass (AbBio, g DW ind^{-1}), height (unit: cm), branch number (BranchNum, ind), leaf area (LArea, cm^2), leaf number (LNum, ind), average leaf size (LeafSize, cm^2 ind^{-1}), damaged leaf ratio (DLR), turion number (TurionNum, ind) and flower number (FlowNum, ind), DLR = damaged leaf number/leaf number × 100 ($n = 4$). The statistical description of the box-whisker graph is explained in Fig. 3

in either spring or summer (Cao et al., 2014; Zhang et al., 2016). However, at temperature increase by 4.5°C starting in December, the plant biomass of *P. crispus* in May was significantly reduced in warming treatments in another subtropical mesocosm study (Hao et al., 2018b). Thus, the effects of warming on *P. crispus* are season dependent with windows of growth in the early and late seasons with cooler temperature, leading to either insignificant or negative effects of warming on plant growth.

As for the reproduction traits of *P. crispus*, we found that turion production was higher in A3 than in ambient conditions (Amb) in early summer. A previous study conducted in low-nutrient mesocosms at the same experimental facility with *P. crispus* cultured for one month under warmer conditions revealed that the

number of turions for the individuals collected in the higher temperature treatments (A2 and A3) in late spring, after a 10-year adaptation period, was higher than for those collected in Amb (Cao et al., 2015). In addition, heatwaves enhanced the vegetative reproduction of *P. crispus* by increasing the abundance of small turions (Cao et al., 2015). A study conducted between spring and summer (March- August) in subtropical China found advanced flowering under warming conditions (within the range + 4°C and + 8°C) but no significant temperature effects on seed biomass (Xu et al., 2020). Hence, the reproduction traits (sexual vs asexual) of *P. crispus* may show different responses to warming.

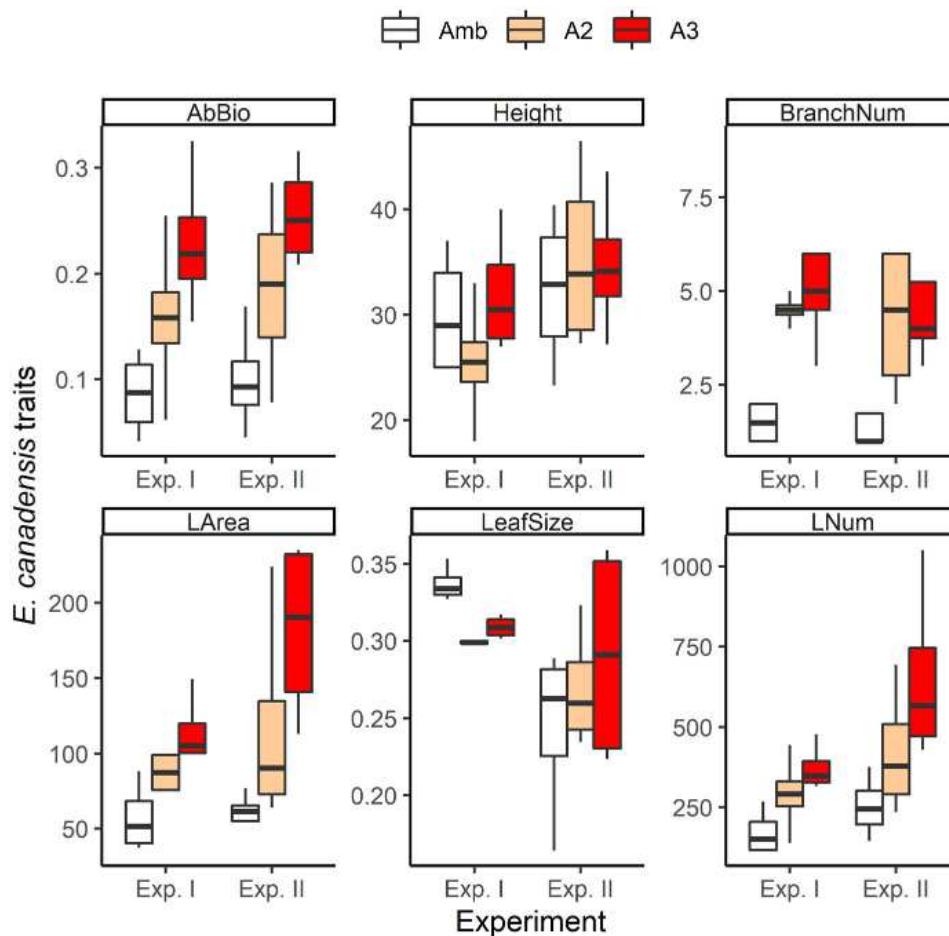In our experiment, run at high phosphorus levels, the plant height of *E. canadensis* did not respond

Fig. 5 Plant traits of *E. canadensis* in experiment I and experiment II in the three temperature treatments, including above-ground biomass (AbBio, g DW ind^{-1}), height (cm), branch number (BranchNum, ind^{-1}), leaf area (LArea, cm^2), leaf size (LeafSize, cm^2 ind^{-1}) and leaf number (LNum, ind) ($n = 4$). The statistical description of the box-whisker graph is explained in Fig. 3

significantly to warming in spring. In early summer, the above-ground biomass (growth trait) of *E. canadensis* was larger in A3 than in Amb, implying that the plants responded to the highest temperature (and higher N) by growing more leaves and not by elongation of stems. Cao et al. (2015) found that *E. canadensis* adapted to the warmer temperatures under low nutrient conditions by developing adventitious roots and longer stems, and they argued that these responses were conducive to nutrient uptake for macrophyte growth. Thus, the nutrient supply in the water column affects the response of macrophyte growth to warming conditions.

For *E. canadensis*, significant plant trait responses to the elevated temperatures were mainly found in the treatment with a temperature increase of 4.5°C, while for *P. crispus* significant responses were generally found in both elevated temperature treatments, suggesting different responses of plant traits of the two species to warming as also seen in other studies (Barko et al., 1982; Mckee et al., 2002). For example, in a study by McKee et al. (2002), flowering occurred earlier and floating leaf size became larger for *Potamogeton natans* L. under warming (3°C) but did not significantly influence the growth of *Elodea nuttallii* (Planch.) H.St.John. Another low-nutrient microcosm study found that a temperature increase of 5°C and 10°C significantly promoted the growth of *Potamogeton lucens* L., *E. nuttallii* and *Vallisneria spiralis* L., as indicated by increases in plant biomass and relative growth rate, both during autumn and winter (Zhang et al., 2019).

In spring, *P. crispus* had a higher proportion of damaged leaves in A2, coinciding with a higher snail
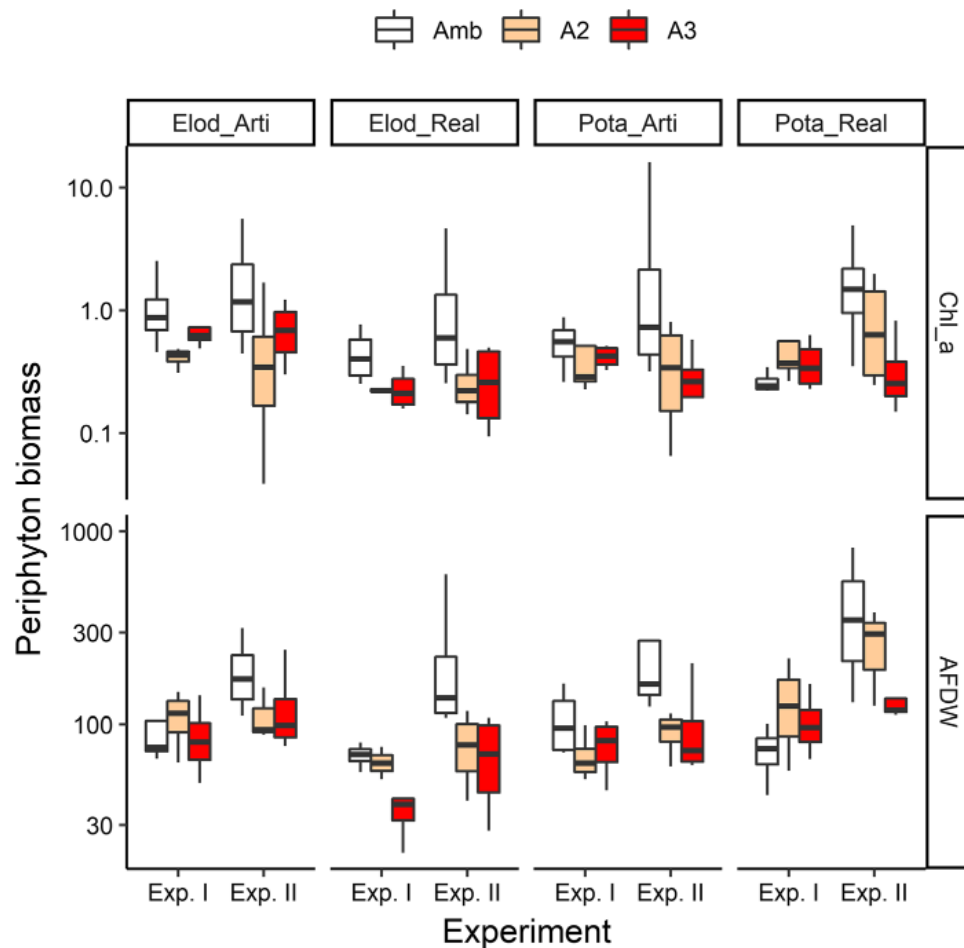
Fig. 6 Indicators of periphyton on different substrate types in experiment I and experiment II in the three temperature treatments. Periphyton indicators: periphyton Chl_a (Chl_a, $\mu g\,cm^{-2}$), periphyton AFDW (AFDW, $\mu g\,cm^{-2}$). Four substrate treatments: real *E. canadensis* (Elod_Arti) and *E. canadensis* (Elod_Real) and artificial *P. crispus* (Pota_Arti) and *P. crispus* (Pota_Real) ($n = 4$). The statistical description of the box-whisker graph is explained in Fig. 3

biomass than in Amb and A3, indicating that snails contributed to the damage of the plant leaves, as also suggested in experiments by Cao et al. (2019). Though no differences were observed in snail biomass or abundance among the three temperature treatments in early summer, the proportion of damaged leaves of *P. crispus* was significantly higher in the two elevated temperature treatments, likely reflecting a more advanced senescence state of this species under warming conditions (Rooney & Kalff, 2000; Hao et al., 2018a; Xu et al., 2020) and perhaps also higher specific snail grazing rates at the elevated temperatures (McAskill & Douglass, 2017). A slight caveat in our experiment is that the two plant species were grown in the same pots and therefore might have interacted through various allelophatic reactions, as well as with the plants and filamentous algae outside in

the main mescoscosm, though dominated by the same plant species.

Warming negatively affected periphyton biomass exclusively in early summer under conditions of high N loadings, but not in spring with low external N loading; meanwhile, in a previous study performed in the mesocosms, Hao et al. (2020) also found that periphyton biomass was higher in the ambient than in the heated treatments in summer but not in the three other seasons, both at low and high nutrient loadings. According to Cao et al. (2014), McKee et al. (2003) and O'Connor et al. (2009), three main processes were suggested to explain lower net periphyton production at elevated temperatures. The first one was higher increase in respiration than in production in response to warming, reflecting that warming increases respiration more than production for primary producers,

Table 2 Snail and periphyton indicators relative to temperature treatment and substrate type

| Treatment | Experiment I | | | | Experiment II | | | |
| | Temperature | | Substrate | | Temperature | | Substrate | |
Indicator	F, Sig.	Post hoc test	F, Sig.	Post hoc test	F, Sig.	Post hoc test	F, Sig.	Post hoc test
Snail density	0.388, n.s.		4.861**	Pota_Arti, Pota_Real < Elod_Real	1.219, n.s.		0.655, n.s.	
Snail biomass	8.928***	Amb, A3 < A2	0.975, n.s.		0.12, n.s.		0.649, n.s.	
Periphyton Chl_a	0.508, n.s.		1.481, n.s.		3.956*	Amb > A3	0.377, n.s.	
Periphyton AFDW	0.702, n.s.		3.137*	Elod_Real < Elod_Arti	6.073**	Amb > A2, A3	1.562, n.s.	

Temperature: Amb, A2 and A3. Substrate types: real *P. crispus* (Pota_Real), real *E. canadensis* (Elod_Real), artificial *P. crispus* (Pota_Arti) and artificial *E. canadensis* (Elod_Arti). Tukey's post hoc test ($n = 4$)

Significance levels: $P > 0.05$ = n.s.; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

leading to lower overall production of periphyton (Allen et al., 2005; O'Connor et al. 2009; Scharfenberger et al., 2019). If this was of key importance, we should also have seen a reduction not only in A3 but also in A2, which we did not. Moreover, lower net periphyton production at elevated temperatures could be explained by increasing herbivory with increasing temperature. Several studies indicate that warming increases the consumption of basal resources (Cao et al., 2014; Feuchtmayr et al., 2007; McKee et al., 2003), strengthening the top-down effects on periphyton (O'Connor et al., 2009; Hoekman, 2010). Supporting these results, Cao et al. (2014) found positive effects of temperature on periphyton biomass only in the absence of snails. In our experiment, however, we attempted to reduce the grazing impact by fish and large macroinvertebrates, including snails. Nevertheless, small (newly hatched) snails were found on the plants, so we cannot fully discard snail grazing as a factor affecting periphyton biomass. However, the biomass or abundance of the snails did not differ between treatments as expected, suggesting that differences in snail biomass cannot explain the lower periphyton biomass under the warming conditions, but grazing rates could have been higher due to the higher temperature. Stronger N limitation of growth at higher temperatures due to increases in denitrification could also be of importance (Pinay et al., 2007, Herrman et al., 2008; Moss et al., 2013). Indeed, we found lower TN concentrations in A3—but not in A2. Furthermore,

an experiment conducted in the same mesocosm facility showed that N did not limit periphyton growth (J. Pacheco et al., unpublished data). In addition to the three main processes, shading by phytoplankton could also be important; however, this effect cannot explain the low periphyton biomass in A3 as phytoplankton biomass (Chl_a) was also low here.

Contrary to what we expected from our second hypothesis, no significant difference in periphyton biomass between real and artificial plants (except for *E. canadensis* in spring) or between the two types of leaf structural complexity (the leaf structure of *E. canadensis* being more complex than that of *P. crispus*) in both seasons. In spring, a higher density of small snails or leaf expansion may be part of the reasons for the lower periphyton biomass on the real *E. canadensis* (compared with artificial ones), while in summer, the effects of substrate type and leaf structural complexity might potentially have been weakened by the abundant nutrient supply at high N loading (Guariento et al., 2009; Tarkowska-Kukuryk & Mieczan, 2012; Cao et al., 2014). However, in our experiment we cannot fully disentangle the effect of these factors due to the covariation of seasonality and nutrient loading and the fact that we could not fully avoid grazing by snails.

In summary, our findings revealed that the plant traits of *P. crispus* and *E. canadensis* responded differently to warming and that the growth of *E. canadensis* and asexual reproduction of *P. crispus*

increased with warming in both seasons. Our results indicate that *E. canadensis* growth will be promoted in a warmer future while *P. crispus* will produce more turions. In addition, warming negatively affected periphyton biomass in early summer under high N loading. However, we did not find an effect of plant leaf complexity on periphyton biomass, whereas the other factors – substrate type (natural vs artificial), nutrient supply and temperature –played partial roles in regulating periphyton growth.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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