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

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Abstract

Periphyton communities play an important role in shallow lakes and are controlled by direct forces such as temperature, light, nutrients, and invertebrate grazing, but also indirectly by planktivorous fish predation. We performed a pan-European lake mesocosm experiment on periphyton colonization covering five countries along a north/south geographical/temperature gradient (Estonia, Germany, Czech Republic, Turkey, and Greece). Periphyton biomass on artificial polypropylene strips exposed at 50 cm water depth at low and high nutrient regimes (with mean total phosphorus concentration of 20 and 65 μg L^{-1}, respectively) was compared during mid-summer. No significant effect of nutrient loading on periphyton biomass was observed as nutrient concentrations in the mesocosms were generally above limiting values. Water temperature significantly enhanced summer periphyton biomass development. Additionally, direct and indirect top-down control of snails and fish emerged as a significant factor in periphyton biomass control.

Keywords: climate change, epiphyton, eutrophication, grazing, top-down control

Introduction

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

Climate regimes are likely to affect lake biota communities (IPCC 2013). In productive lakes, climate warming accelerates a shift in trophic state (Mooij et al. 2005; Adrian et al. 2009) and consequently affects light conditions. The subsequent responses by plankton communities have been studied extensively (Adrian et al. 2006; Seebens et al. 2009; Wagner and Adrian 2009). How periphyton growth is affected by warming is debated and the results obtained so far are ambiguous. Some studies have shown an increase in periphyton biomass with increasing water temperature (Tarkowska-Kukuryk and Mieczan 2012; Patrick et al. 2012), while Shurin et al. (2012) in a mesocosm study demonstrated that periphyton chlorophyll *a* declined with elevated temperatures (3 ºC above ambient). Such differences may be attributed to variations in the grazing pressure by invertebrates and fish. In microcosm experiments, Cao et al. (2014) observed as response to increased temperatures an increase in periphyton biomass when snails were absent but no effect when snails were present. McKee

et al. (2002) also found grazers to benefit more than periphyton from enhanced temperatures. Moreover, herbivory and omnivory among fishes increase with temperature (González-Bergonzoni et al. 2012; Meerhoff et al. 2012), and many fish species (or size classes of fish) feed on periphyton in subtropical and tropical lakes (Teixeira-de Mello et al. 2009).

Comparative studies on periphyton dynamics along latitudinal scales are scarce. Bécares et al. (2008) conducted a mesocosm experiment across a European latitudinal gradient from Finland to Spain and found that periphyton chlorophyll *a* concentrations were overall positively related to nutrient loading. Top-down effects by fish were significant only in a few sites and were assumed to be related to their contribution to the nutrient pool. Under these conditions southern lakes exhibited lower periphyton densities than northern lakes because of the larger phytoplankton biomass in the south and its shading effects on periphyton at similar nutrient loadings. In a comparative experimental field study by Meerhoff et al. (2007), a substantially lower periphyton biomass on artificial plants was found in lakes in subtropical Uruguay than in temperate Denmark. Despite a much lower biomass of invertebrate periphyton grazers due to high fish predation in Uruguay, the authors attributed the lower periphyton biomass in the warm lakes to direct control by fish grazing. Therefore, periphyton biomass might be directly or indirectly affected by nutrients, temperature, grazers, and fish, but the mechanisms of the underlying processes and potential interactions are still poorly understood.

We studied periphyton development on artificial polypropylene strips exposed in mesocosms with two different nutrient loadings resembling mesotrophic and eutrophic conditions and at moderate fish density in five European countries (Estonia, Germany, Czech Republic, Turkey, and Greece). A latitudinal temperature gradient was expected and effects on periphyton biomass were studied for a period of one month in July and August 2011. We hypothesize that higher nutrient loading and warmer temperatures increase summer periphyton biomass under these conditions.

Materials and methods

Study sites and experimental set-up

We conducted a mesocosm experiment in five countries across Europe covering a climate gradient from Estonia (58° N 26° E) to Greece (38° N 21° E) (Fig. 1). The mesocosms were set up in the lakes listed in Table 1. The mesocosms were closed systems (i.e. no direct connection with the lake water column or bottom sediments) but were exposed to the same climatic forcing as the lakes. The periphyton experiment presented here is part of a comprehensive study of the effects of climate change on shallow lake ecosystems, which started in May 2011 and continued until the end of October of the same year. Set-up and sampling were standardized by a common protocol to ensure comparability between the countries (Landkildehus et al. 2014). The present study lasted four weeks between 15 July and 15 August 2011, thus reflecting mid-summer growth conditions. The mesocosms included in this experiment (8 in each country) consisted of 2.2 m deep cylindrical enclosures made of fiberglass with a diameter of 1.2 m. The experimental treatment design comprised two nutrient levels, resembling mesotrophic and eutrophic conditions. Manipulation of nutrient levels was carried out using inorganic phosphate (P) [Na_2HPO_4] and nitrogen (N) [$Ca(NO_3)_2$] at an N:P mass ratio of 1:20. Nutrients were added to the mesocosms at the beginning of the experiment to get starting P and N concentrations of 25 µg L^{-1} and 0.5 mg L^{-1} in the mesotrophic (low loading) treatment and 200 µg L^{-1} and 2 mg L^{-1} in the eutrophic (high loading) treatment, respectively. Later, during the course of the experiment, monthly nutrient additions amounted to 10.8 mg of P and 216 mg of N per mesocosm at low loading and 172 mg of P and 3440 mg of N at high loading (Landkildehus et al., 2014). These nutrient additions took place after monthly sampling of the mesocosms. For each nutrient treatment, four replicates were implemented in each country. During the mesocosm set-up in May 2011, a 10 cm layer of sediments was added to all mesocosms (90% washed sand and 10% natural sediment from oligotrophic local lakes). Subsequently, the mesocosms were filled with sieved

lake water (mesh size 500 µm) in all countries, except for Germany and the Czech Republic where tap water was used because the lake water TP concentration was higher than the target concentration of the low nutrient treatment (i.e. > 25 µg L^{-1}). The initial water level in each mesocosms was 2 m. To ensure that naturally occurring phytoplankton, zooplankton, and macroinvertebrate communities would emerge, the mesocosms were inoculated with plankton and sediment samples, which were collected from five different local lakes covering a range from oligotrophic to eutrophic conditions (Landkildehus et al. 2014). The mesocosm set-up also included the addition of apical shoots of macrophytes (*Myriophyllum spicatum*). Six adult planktivorous fish (length 2-4 cm, 3 males and 3 females to allow breeding) were stocked in each enclosure at the beginning of the experiment. Three-spined sticklebacks (*Gasterosteus aculeatus*) were used in all countries except of Greece where mosquito fish (*Gambusia affinis*) were used. Both fish species are known to have similar diets (Offill and Walton 1999; Simpson 2008). Dead fish were replaced during the experiment. The water of the mesocosms was continuously circulated by using water pumps. A more detailed description of the entire experimental set-up can be found in Landkildehus et al. (2014).

Variables measured

Periphyton growth over the experimental period was quantified based on the biomass accumulation on artificial transparent polypropylene strips (2 strips, 16 x 2 cm) with a slightly textured surface (IBICO®, Germany; Roberts et al. 2003). The strips were exposed at a water depth of 0.5 m and kept 0.3 m away from the mesocosm walls facing south to prevent shading from the walls, and the backsides of the strips were covered with adhesive tape.

After five weeks of colonization, the periphyton strips were gently lifted to the surface to minimize disturbance and loss of periphyton mats. After removal of the adhesive tape from the backside of the strips, these were immediately placed in round plastic tubes and

142 transported to the laboratory in a portable cooler box containing tap water to prevent the
143 samples from drying out.
144 For periphyton dry weight and chlorophyll *a* analysis, periphyton was scrubbed from
145 the strips using a soft toothbrush and suspended in a defined amount of filtered mesocosm
146 water (two cellulose acetate filters, diameter 50 mm, pore sizes 0.24 and 0.8 μm). Before
147 scrubbing, invertebrate grazers (mostly cladocerans and chironomids) were removed from the
148 strips using carbonated water (3-5 min exposure). After homogenization, aliquot subsamples
149 of each suspension were filtered onto two pre-weighed and pre-washed glassfiber filters
150 (Whatman GF/C, diameter 25 mm, pore size 0.7 μm) and dried at 105 °C for 12 h to analyze
151 periphyton dry weight. Ash-free dry weight was determined after combustion at 500 °C for 5
152 h. For chlorophyll *a* analyses, aliquot samples were filtered through glassfiber filters
153 (Whatman GF/F; 25 mm). Concomitantly with the periphyton harvest, water samples were
154 taken to determine concentrations of total phosphorus and total nitrogen, and phytoplankton
155 chlorophyll *a*. In each country, chlorophyll *a* (periphyton and phytoplankton), total
156 phosphorus, and total nitrogen concentrations were determined using the procedures
157 described in Landkildehus et al. (2014). Macrophyte plant volume inhabited (PVI %) was
158 calculated using the formula: PVI (%) = % coverage × average height / water depth, and
159 percent coverage and average height were visually estimated.
160 Mean air temperature for the experimental period was calculated from daily mean air
161 temperature data (hourly values). Air temperature and global radiation data were provided by
162 the Centre for Limnology of the Estonian University of Life Sciences, Leibniz-Institute of
163 Freshwater Ecology and Inland Fisheries (IGB), Czech Hydrometeorological Institute,
164 Turkish State Meteorology Service, and Hellenic National Meteorological Service.
165 Daily mean water temperature (24 hour averages of samples taken every 2 hours) was
166 measured on two occasions in July and August (11 July 2011 and 8 August 2011). The July–
167 August average water temperature values were used in the analysis (Table 2) as they

represented well the average temperate conditions for the experimental period, established by the mean air temperature. The close link between air temperature and surface water temperature in shallow lakes is well established in the literature (McCombie 1959; Livingstone and Lotter 1998; Mooij et al. 2008). At midday of the 24 h measurement events, profiles of photosynthetically active radiation (PAR) were taken at 0.1 m intervals from top to bottom. For each light profile and each concurrent light intensity measurement, an attenuation coefficient K_{di} (m^{-1}) was estimated based on the Beer-Lambert law:

$$K_{di} = \frac{\ln\left(\frac{I_i}{I_{i+1}}\right)}{z_{i+1} - z_i} \quad (1)$$

where I_i and I_{i+1} are PAR values at depth z_i and z_{i+1}, respectively. Values with $I_{i+1} > I_i$ were removed. K_d (m^{-1}) was then taken as the mean over all K_{di}. The attenuation coefficients from July and August were subsequently averaged. Mean and maximum available PAR at 0.5 m were calculated as: $I_{0.5(\text{mean or max})} = I_{0(\text{mean or max})} \exp(-0.5\ K_d)$, where I_0 was set to average light hour PAR ($I_{0.5\text{mean}}$) or maximum PAR ($I_{0.5\text{max}}$) at the surface. Averages for the experimental period were calculated from daily $I_{0.5(\text{mean or max})}$ values. PAR was estimated from global radiation as PAR = E × γ × 0.45, where E is global radiation and γ = 4.6 is the mean photon flux in the wavelengths from 400 – 700 nm (Kirk 2010).

At the end of the mesocosm experiment in November 2011, macroinvertebrates were sampled with the help of Kajak cores (diameter =52 mm) or an Ekman grab sampler. Subsequent identification and enumeration of snails were carried out to genus or species level. At the same time, all fish were captured and weighed. In Germany, three mesocosms (two in the high nutrient and one in the low nutrient treatment) sank during heavy storm events and were consequently omitted. A detailed description of the sampling procedure and processing can be found in Landkildehus et al. (2014).

192 Data analyses

193 We analyzed the data using analysis of covariance (ANCOVA) to test for significant
194 differences in periphyton dry weight and chlorophyll *a* between the two nutrient treatments.
195 We did not test for the effect of nutrient treatment on periphyton ash free dry weight because
196 this variable was closely correlated with periphyton dry weight (r^2= 0.91, p= 0001).
197 ANCOVA was chosen because of the use of two discrete nutrient treatments (low and high)
198 in the experimental mesocosm set-up, these being considered factors in the analysis.
199 However, since the measured nutrient concentrations in the mesocosms showed considerable
200 variation within and among treatments (i.e. low and high), we confirmed the suitability of
201 applying ANCOVA by testing for significant differences in TP and TN concentrations
202 between treatments using one-way ANOVA.

203 The appropriateness of including potential covariates in the model was tested prior to
204 conducting the ANCOVA analysis. The candidate covariates were submerged macrophyte
205 PVI, snail abundance, fish biomass, phytoplankton chlorophyll *a* concentrations, mean PAR
206 at 0.5 m, and maximum PAR at 0.5 m. We used one-way ANOVA to test for significant
207 differences for each variable between the two nutrient treatments. The appropriateness of
208 including covariates was rejected if the factor nutrient treatment significantly affected a
209 particular variable. Furthermore, pairwise Pearson product moment correlation coefficients
210 were calculated between candidate covariates to ensure that selected covariates were not
211 strongly correlated (not reported). Based on the analysis above, water temperature, snail
212 abundances, and fish biomass were selected as covariates in the ANCOVA models. Because
213 submerged macrophyte PVI, phytoplankton chlorophyll *a* concentrations, mean PAR at 0.5
214 m, and maximum PAR at 0.5 m showed significant differences between nutrient treatments,
215 these variables were not included as covariates (see Fig. 2a-f). Snail abundance and fish
216 biomass were presumed to reflect the grazing pressure on periphyton. We assumed higher
217 periphyton grazing with higher snail abundance and lower periphyton grazing with increasing

fish biomass due to their higher predation on invertebrates (cascading effect) (Liboriussen et al. 2005). A weak negative effect of fish on snails was found by regression analysis between fish biomass and snail abundance (b = 0.08, t(35) = 1.96, $p = 0.06$) and logistic regression analysis between fish biomass and presence and absence of snails (b =-0.53, z(35) = -2.08, $p = 0.04$). Therefore, two alternative ANCOVA models (using either snail abundance or fish biomass as a covariate) were analyzed for both periphyton dry weight and periphyton chlorophyll *a*. All models contained nutrient treatment (i.e. high and low) as the main factor and water temperature as a covariate. Although snail abundance data were used in this analysis, it should be noted that snail species composition varied between countries. The ANCOVA was executed using a Type III sums of squares method to account for the unbalanced design in our experiment arising from the loss of three mesocosms in Germany. Where necessary, data were either log or square root transformed to improve normality of the residuals and meet the assumption of homogeneity of variance.

Additionally, to the ANCOVA analysis, differences in periphyton dry weight and periphyton chlorophyll *a* between nutrient treatments were tested within each country. The non-parametric Mann-Whitney U-test was considered appropriate to test for statistical differences between treatments owing to the small sample size (i.e. N=8) in each country. This test was not conducted for Germany due to the small sample size after losing three mesocosms during the experiment (see above).

To aid the interpretation of the ANCOVA and to potentially further isolate the effect of fish on periphyton biomass, an additional regression analysis was carried out between water temperature-adjusted periphyton dry weight and chlorophyll *a* and fish biomass. Adjustment involved calculating residuals of the regression equation of periphyton dry weight vs. water temperature and periphyton chlorophyll *a* vs. water temperature, respectively. All analyses were undertaken using STATISTICA 12 (StatSoft, Inc. USA) with a significance threshold for all tests of $p \leq 0.05$.

244 **Results**

245 A clear temperature gradient was obtained by deploying mesocosms in five countries across
246 Europe simultaneously (Fig. 3a). Average air temperatures over the study period ranged from
247 17.0 °C in the Czech Republic to 27.3 °C in Greece. Established water temperatures were
248 strongly correlated with average air temperature ($R=0.97$, $p<0.001$) and were either equal or
249 slightly warmer than average air temperatures, which is consistent with a high heat storage
250 capacity of large water bodies (Table 2, Fig. S1). The measured average TP concentrations in
251 the low nutrient treatment were 20.1 ± 6.9 µg L^{-1}, while the measured TP concentrations in
252 the high nutrient treatment were 65.4 ± 27.8 µg L^{-1} (Table 2). The mean TP concentration
253 difference between the two treatments was significant (ANOVA, $F_{1,36}= 81.57$, $p< 0.001$; Fig.
254 4a). Total nitrogen concentrations did not differ between the high and low nutrient treatments
255 ($F_{1,36}= 3.813$, $p= 0.059$, average of 1.46 ± 1.05 mg L^{-1} and 0.82 ± 0.36 mg L^{-1} for the high and
256 low nutrient treatment, respectively; Fig. 4b).

257 The summary statistics of all potential candidate covariates for the ANCOVA model
258 are presented in Table 2. Nutrient treatment had no significant effect on the candidate
259 covariates snail abundance and fish biomass (ANOVA, $F_{1,36}=0.042$, $p=0.839$; $F_{1,36}=0.11$,
260 $p=0.742$; $F_{1,36}=1.005$, $p=0.323$, respectively), but significant effects on macrophytes, water
261 column chlorophyll *a*, and mean and the maximum PAR were observed (Fig. 2a-g). Snails
262 were present in mesocosms in Estonia (*Valvata piscinalis*), the Czech Republic (*Lymnaea*
263 *stagnalis*), and Turkey (members of Planorbidae, Physidae, Lymnaeidae) but absent in
264 Germany and Greece.

265 The results of the ANCOVA analysis are summarized in Table 3. Overall, nutrient
266 treatment had no significant effect on either periphyton dry weight or chlorophyll *a*. Water
267 temperature was a significant covariate in all models, except for periphyton chlorophyll *a*
268 when snail abundance was included as a second covariate. Snail abundance was a significant
269 covariate in both models, for periphyton dry weight and chlorophyll *a*, respectively. Fish

biomass was a significant covariate for periphyton chlorophyll *a* but not for periphyton dry weight.

The results of the Mann-Whitney U test showed that nutrient treatment did not have a significant effect on periphyton dry weight (Fig. 3b) or chlorophyll *a*, except for periphyton chlorophyll *a* in Greece (Fig. 3c). Periphyton dry weight was significantly correlated with water temperature (r^2=0.41, p=0.001; Fig. 5a) and periphyton chlorophyll *a* (r^2=0.28, p=0.001; Fig. 5c). Fish biomass showed a weak relationship with temperature-adjusted periphyton dry weight (r^2=0.1, p=0.10) (Fig. 5b) and a strongly significant relationship with temperature-adjusted periphyton chlorophyll *a* (r^2=0.45, p<0.001) (Fig. 5d).

Discussion

The present pan-European lake mesocosm experiment provided evidence that increasing water temperature can lead to increased development of summer periphyton biomass. Nutrient enrichment had no significant effect on periphyton biomass, probably due to very low nutrient limitation levels for periphyton. Indirect top-down effects of fish emerged as an important factor controlling periphyton biomass and appeared to be independent of water temperature. In addition, snails, when present, appeared to have a negative effect on periphyton chlorophyll *a*.

Our results showed that periphyton biomass (measured as dry weight) was significantly and positively correlated with water temperature in the 20-28°C temperature range. Given the projected rise in global air and water temperatures (IPCC 2013), our results, therefore, suggest that summer periphyton biomass is likely to increase in the future. Our results contradict those of Hansson (1992) who found that temperature was of minor importance for periphyton biomass. However, his study was conducted along a much larger productivity gradient (Swedish and Antarctic lakes) ranging from extremely low (meltwater lakes) to highly productive lakes. In our study, total phosphorus concentrations covered a

296 smaller range (20-65 µg L^{-1}) and had no significant effect on periphyton biomass. Others have
297 found a unimodal relationship between periphyton biomass and total phosphorus, peaking at
298 39 µg L^{-1} (Lalonde and Downing 1991) or between 60-200 µg L^{-1} (Liboriussen and Jeppesen
299 2006). The concentrations of dissolved reactive silicon were mostly above limiting levels (0.5
300 mg L^{-1}) in the low and high nutrient treatments of the Czech Republic (0.9 ± 0.1 mg L^{-1} and
301 0.3 ± 0.1 mg L^{-1}) and Germany (1.8 ± 1.5 mg L^{-1} and 1.0 ± 1.2 mg L^{-1}). However, data are
302 lacking for the other countries. Light levels ranged on average between 39 and 408 µmol
303 photons m^{-2} s^{-1} and were always above the minimum light requirement for growth of
304 microalgae (1-10 µmol photons m^{-2} s^{-1}) given by Sand-Jensen & Borum (1991). Therefore,
305 light limitation was unlikely in our study.
306 Furthermore, our results are different from those obtained in a pan-European study by
307 Bécares et al. (2008), covering a temperature range of 17.7-29°C. They found higher
308 periphyton chlorophyll *a* in northern lakes than in southern lakes and explained this by a
309 stronger shading effect by phytoplankton on periphyton in southern lakes. The phytoplankton
310 chlorophyll *a* concentrations in their study were generally higher (40-564 µg L^{-1}) than in our
311 study (2-53 µg L^{-1}), even if age of periphyton is comparable. The same applies for the
312 maximum periphyton chlorophyll a concentration (84 mg m^{-2}), which with was five times
313 higher than in our study (16 mg m^{-2}). Furthermore, they found nutrient concentrations to be an
314 important driver (tested at six levels of NO_3^--N and PO_4^{3-}-P up to 100 mg L^{-1} and 10 mg L^{-1},
315 respectively). In contrast, a positive top-down effect of fish on periphyton biomass was found
316 in our study, which was indicated by the significant positive relation recorded between fish
317 biomass and periphyton chlorophyll a in both ANCOVA and regression analysis. This result
318 was probably due to the prevailing top-down control by fish of periphyton-scraping non-snail
319 invertebrates, as suggested by Jones and Sayer (2003) and Danger et al. (2008). Körner and
320 Dugdale (2003) showed a switch of fish to periphyton-scraping invertebrates at low
321 zooplankton abundance. In our experiment, in all countries except Greece, we used

sticklebacks, a bottom-feeder that essentially feeds on plankton and benthic prey (Sánchez-Gonzáles et al. 2001). However, periphyton biomass was highest in Greece where mosquitofish were used instead of sticklebacks. Although both species feed mainly on the same food items (planktonic and littoral zooplankton, chironomid larvae) (Offill and Walton 1999; Simpson 2008), we cannot rule out the potential occurrence of confounding factors in the cascading effects of different fish species in the mesocosms.

Snail abundance had a significant effect on periphyton biomass in our study, but snails were absent in Germany and Greece. Snails may have directly scraped periphyton as known from various other studies (e.g. Brönmark 1989) and thus contributed to the low periphyton biomass observed in the Czech Republic, Estonia, and Turkey. Nutrient recycling from snail faeces and excreta might also have increased nutrient availability for periphyton in the low nutrient treatments and contributed to the lack of differences in periphyton biomass compared to the high nutrient treatments in these countries (Liess and Haglund 2007). Periphyton biomass was, however, also low in Germany without the presence of snails. Given the size of sticklebacks, top-down effects of fish on snails (Brönmark et al. 1992) seem unlikely (snail size: 5 mm – 7 cm). Yet, snail abundance tended to be lower if fish were present.

In general, in Mediterranean shallow lakes fish seem to exert strong trophic cascading effects due to dominance by frequently spawning omnivores and benthivores and absence of efficient piscivores (Beklioğlu et al. 2007; Papastergiadou et al. 2010). Gyllström et al. (2005) found that the ratio between prey and predators and fish:zooplankton biomass increased from northern to southern Europe, while the zooplankton:phytoplankton biomass ratio decreased. The absence of large-bodied zooplankton due to strong fish predation seems to be the reason for lack of phytoplankton control, and a similar mechanism may explain the lack of top-down control of periphyton by scraping invertebrates in Greece. In contrast, fish biomass in the Turkish mesocosms was low, which might explain the low periphyton biomass despite warmer conditions. Therefore, the overall effect of temperature on periphyton seems to

depend on nutrient level (Hansson 1992; Liboriussen et al. 2005; Trochine et al. 2014), fish abundance and composition (some being periphyton grazers; Gonzáles-Bergonzoni et al. 2012), and the strength of the cascading effects of fish on invertebrate periphyton grazers (Cao et al. 2014; Meerhoff et al. 2007).

In conclusion, our results indicate a stimulating effect of water temperature on summer periphyton biomass. Due to non-limiting nutrient levels and low differences between the treatments, no significant effect of nutrient loading on periphyton biomass was observed. However, apart from temperature, direct and indirect top-down control of snails and fish proved to be important factors for explaining a significant amount of variation in periphyton biomass.

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525 **Table 1:** Lake mesocosm experiment – basic information about sites and mean conditions during the entire experimental period from May to

526 October (modified from Landkildehus et al. 2014).

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

Table 2: Mid-summer (i.e. 15 July – 15 August) means (± standard deviation) of selected variables in mesocosms with different nutrient treatments (high and low) in five European countries. All means are treatment means apart from mean air temperature, which is averaged over daily mean values.

Countries	**Czech Republic**		**Germany**		**Estonia**		**Turkey**		**Greece**	
Nutrient treatments	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**
Periphyton AFDW (g m^{-2})	0.5±0.3	0.5±0.2	1.5±0.2	1.7±0.2	2.2±1.6	1.0±0.2	0.9±0.8	0.3±0.3	9.2±4.1	6.0±1.6
Periphyton chl *a* (mg m^{-2})	5.0±3.4	2.8±1.0	0.9±0.1	0.7±0.0	0.1±0.0	0.04±0.0	0.4±0.4	0.1±0.1	16.8±5.7	3.1±0.9
TP (µg L^{-1})	84.6±31.3	15.2±3.2	40.0±5.7	25.0±8.5	45.0±9.6	14.0±0.8	65.7±35.9	19.9±4.6	79.0±17.2	29.0±2.9
TN (mg L^{-1})	0.8±0.3	0.9±0.31	3.4±0.5	0.6±0.1	1.4±0.2	0.8±0.0	0.7±0.1	0.4±0.1	1.1±0.4	1.4±0.2
Fish biomass (g m^{-2})	1.3±0.2	0.9±0.2	1.4±0.5	0.9±0.2	0.0±0.0	0.0±0.0	0.3±0.4	0.2±0.2	0.7±0.1	0.7±0.7
Phytoplankton chl *a* (µg L^{-1})	5.4±2.1	5.7±1.3	72.5±11.0	6.9±1.6	20.4±12.4	13.4±3.6	9.8±3.4	2.2±1.1	52.5±61.7	7.0±8.1
Mean PAR at 0.5 m (µmol photons m^{-2} s^{-1})	146.3±17.7	178.8± 21.6	125.7±30.4	119.7±13.8	140.9±13.1	178.4±21.6	304.4±68.5	408.4 ± 55.5	38.6 ±11.7	218.6± 53.3
Max PAR at 0.5 m (µmol photons m^{-2} s^{-1})	447.3±54.1	546.6±66.0	265.0±64.0	252.3±29.1	313.2±29.1	396.4±48.0	614.0±138.2	823.9±111.9	70.9±21.4	401.9±98.0
Submerged macrophytes (% plant volume inhabited)	0.0±0.0	1.0±1.0	0.0±0.0	6.6±0.4	11.9±12.9	10.2±10.3	4.8±5.6	8.2±3.3	2.4±2.1	10.8±2.1
Snail abundance (individuals m^{-2})	2.5±2.7	0.08±0.2	0.0±0.0	0.0±0.0	1.4±1.6	5.2±2.5	0.3±0.2	0.5±0.2	0.0±0.0	0.0±0.0
Mean water temperature (°C)	20.0 ±0.2	20.0 ±0.2	20.8 ±0.0	20.8 ±0.1	22.6 ±0.2	22.4 ±0.1	25.1 ±0.1	25.1 ±0.1	28.3 ±0.1	28.3 ±0.1
Mean air temperature (°C)	17.0 ± 2.4		18.0 ±1.9		19.2 ±3.2		25.8 ±2.5		27.3 ±1.4	

Table 3: Summary of results from the ANCOVA models testing for the effect of nutrient treatments on periphyton dry weight and periphyton chlorophyll *a*. Water temperature, snail abundance, and fish biomass were used as covariates in the models. Bold *p* values denote significant effects.

Dependent variable	Effect	SS	df	F	*p*
Periphyton dry weight	Intercept	6.561	1	13.386	**<0.001**
	Water temperature	9.415	1	19.211	**<0.001**
	Snail abundance	4.782	1	9.756	**0.003**
	Nutrient treatment	0.020	1	0.041	0.84
	Error	16.173	33		
Periphyton dry weight	Intercept	10.369	1	17.983	**<0.001**
	Water temperature	14.803	1	25.674	**<0.001**
	Fish biomass	1.927	1	3.342	0.076
	Nutrient treatment	0.003	1	0.005	0.942
	Error	19.028	33		
Periphyton chlorophyll *a*	Intercept	0.214	1	0.304	0.585
	Water temperature	0.045	1	0.065	0.801
	Snail abundance	7.089	1	10.096	**0.003**
	Nutrient treatment	1.343	1	1.912	0.176
	Error	23.173	33		
Periphyton chlorophyll *a*	Intercept	4.179	1	8.543	**0.006**
	Water temperature	2.94	1	6.011	**0.019**
	Fish biomass	14.121	1	28.869	**<0.001**
	Nutrient treatment	0.279	1	0.57	0.456
	Error	16.142	33		

534 **Figure captions**

535 **Fig. 1.** Map of Europe showing the five experimental locations: Estonia (Võrtsjärv), Germany
536 (Müggelsee), Czech Republic (Vodňany), Turkey (ODTÜ-DSİ Gölet), and Greece
537 (Lysimachia).

538

539 **Fig. 2.** Box and whisker plots representing the median values of (a) macrophyte plant volume
540 inhabited (PVI), (b) snail abundance, (c) fish biomass, (d) water column chlorophyll *a*
541 concentrations, (e) mean PAR measurements between July and August, and (f) maximum
542 PAR measurements between July and August for each nutrient treatment (high and low) in the
543 mesocosm experiments conducted in five European countries. Horizontal lines denote the
544 medians, boxes denote the 25th and 75th percentile, whiskers denote non-outlier range, circles
545 are outliers, and the asterisks are extreme values. *P* values were derived from a one-way
546 ANOVA to test for significant differences between nutrient treatments.

547

548 **Fig. 3.** Box and whisker plots representing the median values of (a) water temperature, (b)
549 periphyton dry weight, and (c) periphyton chlorophyll *a* content for each nutrient treatment
550 (high and low) in five European countries. Horizontal lines denote the medians, boxes denote
551 the 25th and 75th percentile, and whiskers denote non-outlier range. Asterisk indicates
552 significant differences between nutrient treatments based on Mann-Whitney U test at $p \leq 0.05$.

553

554 **Fig. 4.** Box and whisker plots representing the median values of (a) total phosphorus
555 concentrations and (b) total nitrogen concentrations for each nutrient treatment (high and low)
556 in the mesocosm experiments conducted in five European countries. Horizontal lines denote
557 the medians, boxes denote the 25th and 75th percentile, the whiskers denote non-outlier
558 range, circles are outliers. *P* values were derived from a one-way ANOVA to test for
559 significant differences between nutrient treatments.

560

561 **Fig. 5.** Relationship between (a) periphyton dry weight (DW) and water temperature (WT),
562 (b) periphyton dry weight (DW) adjusted for water temperature and fish biomass (g m^{-2}), (c)
563 periphyton chlorophyll *a* (chl *a*) and periphyton dry weight (DW), and (d) periphyton
564 chlorophyll *a* (chl *a*) adjusted for water temperature (WT) and fish biomass (g m^{-2}) in
565 mesocosm experiments in five European countries. Only significant *p*-values were included.

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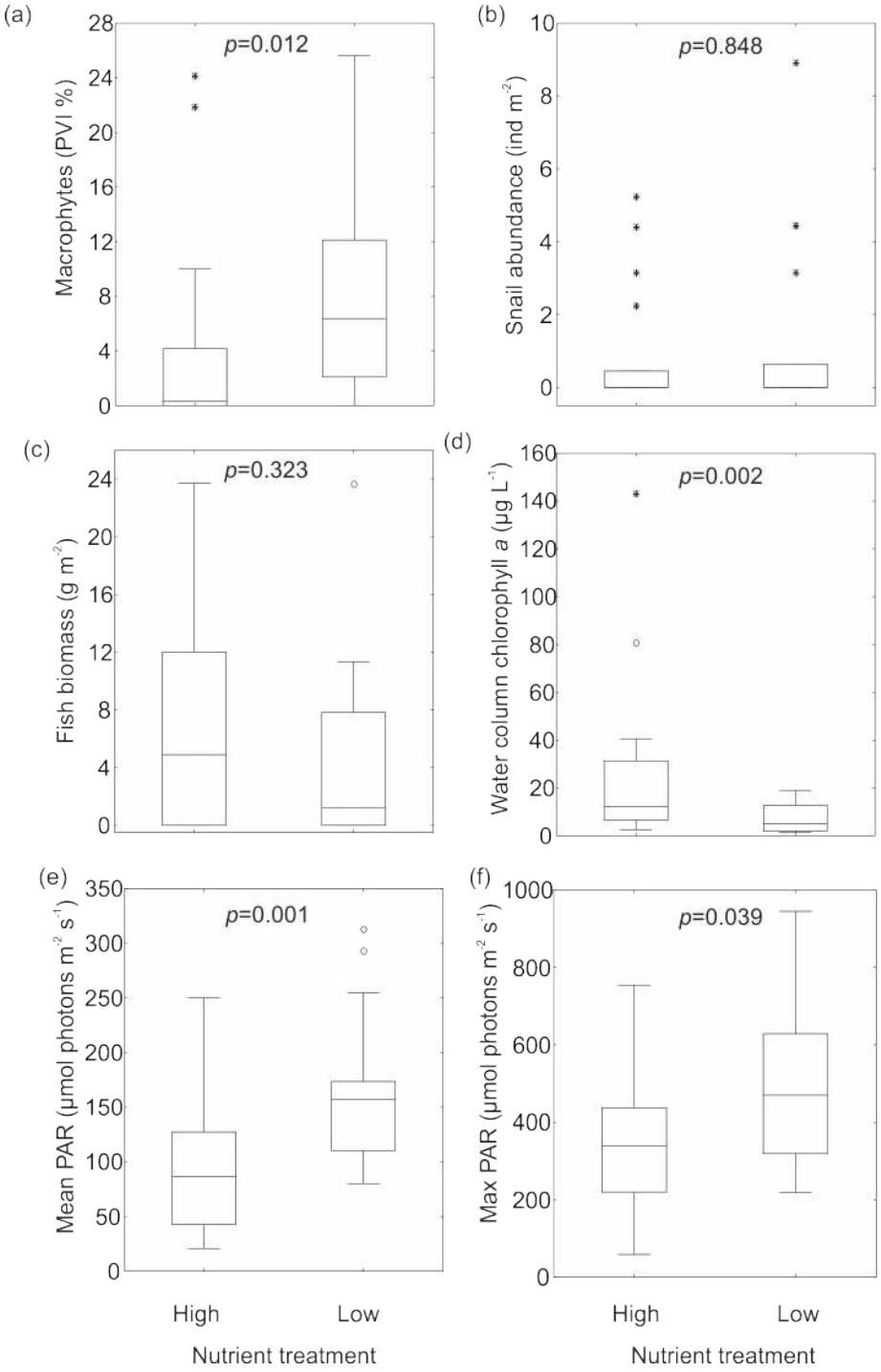
580 Fig. 1



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584 Fig. 2

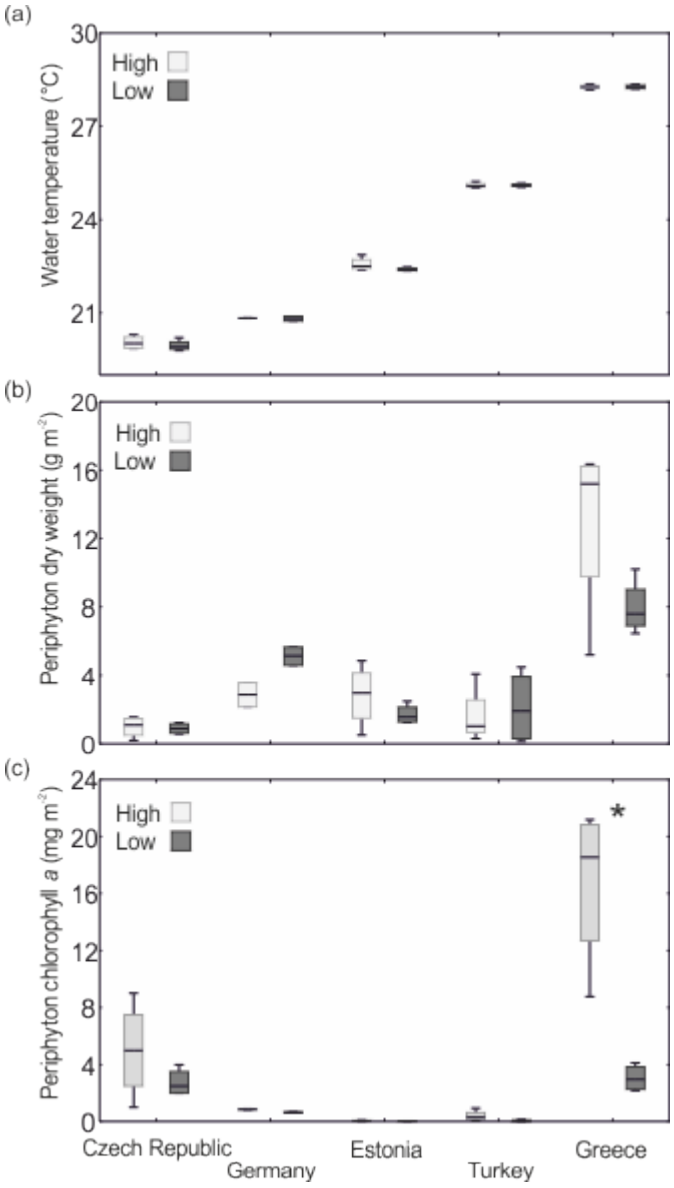
(a)
Water temperature (°C)
30
27
24
21
High
Low
(b)
Periphyton dry weight (g m-2)
20
16
12
8
4
0
High
Low
(c)
Periphyton chlorophyll a (mg m-2)
24
20
16
12
8
4
0
High
Low
*
Czech Republic
Germany
Estonia
Turkey
Greece

Fig. 3

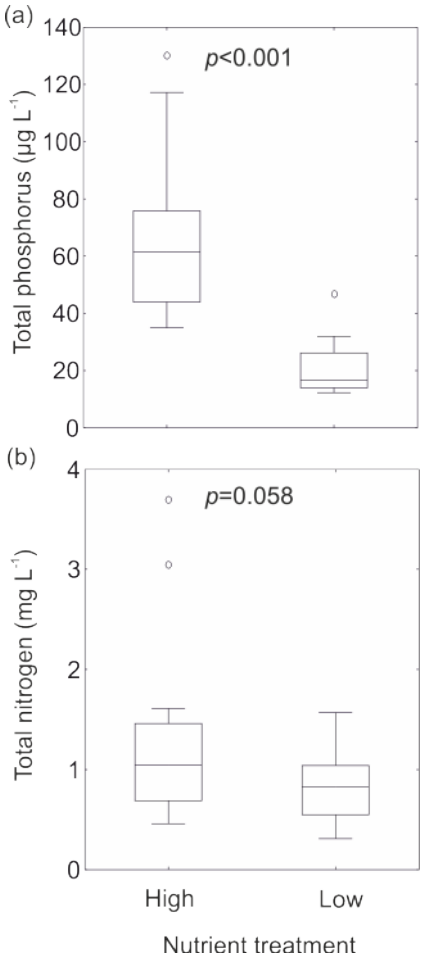
(a)
140
120
100
80
60
40
20
0
p<0.001
Total phosphorus (µg L-1)
(b)
4
3
2
1
0
p=0.058
Total nitrogen (mg L-1)
High
Low
Nutrient treatment

Fig. 4

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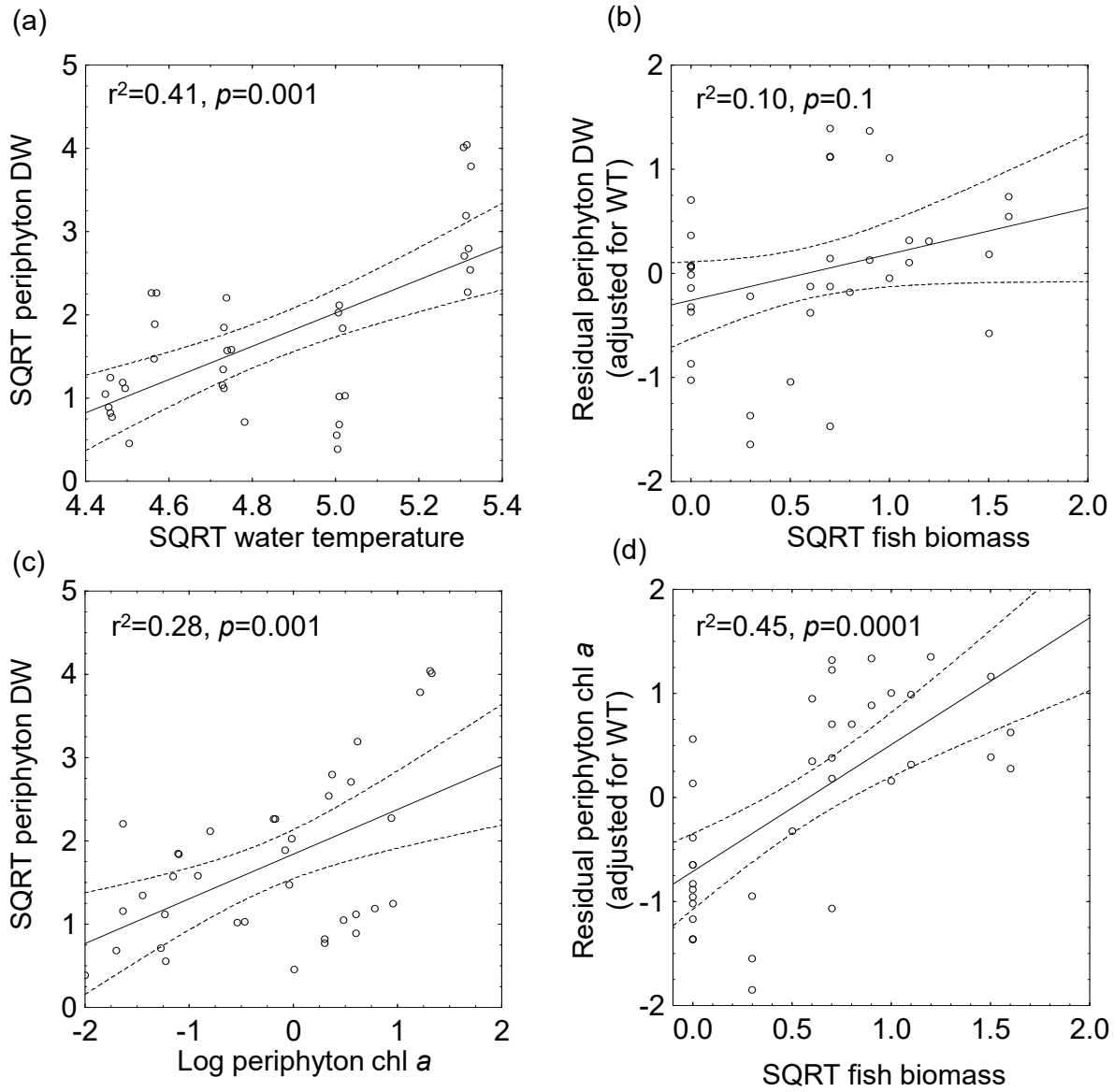

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615 Fig. 5

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Supplementary material

Figure S1. Comparison of mean daily air temperature values at study sites, with established mean water temperatures (dashed horizontal lines) for the experimental period. Daily mean air temperatures are based on hourly air temperature values. Average water temperature is based on two daily mean values (24 hour averages of samples taken every two hours) measured on 11 July and 8 August 2011. CZ = Czech Republic, EE = Estonia, GE = Germany, GR = Greece, and TR = Turkey.