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

Author(s): Mahdy, A., Hilt, S., Filiz, N., Beklioğlu, M., Hejzlar, J., Özkundakci, D., … Adrian, R.

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1 **Effects of water temperature on summer periphyton biomass in shallow lakes:**

2 **a pan-European mesocosm experiment**

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4 Aldoushy Mahdy[a,b,c], Sabine Hilt[a*], Nur Filiz[d], Meryem Beklioğlu[d], Josef Hejzlar[e], Deniz

5 Özkundakci[a], Eva Papastergiadou[f], Ulrike Scharfenberger[a], Michal Šorf[e,g], Kostas Stefanidis[f],

6 Lea Tuvikene[h], Priit Zingel[h], Martin Søndergaard[i], Erik Jeppesen[i,j], Rita Adrian[a,b]

7

8 [a]Leibniz Institute of Freshwater Ecology and Inland Fisheries, IGB, Berlin, Germany

9 [b]Free University Berlin, Department of Biology, Chemistry and Pharmacy, Berlin, Germany

10 [c]Department of Zoology, Faculty of Science, Al-Azhar University (Assiut Branch), Assiut

11   71524, Egypt

12 [d]Middle East Technical University, Biological Sciences Department, Limnology Laboratory,

13   Ankara, Turkey

14 [e]Biology Centre of the Academy of Sciences of the Czech Republic, Institute of

15   Hydrobiology, České Budějovice, Czech Republic

16 [f]University of Patras, Department of Biology, Patras, Greece

17 [g]Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

18 [h]Centre for Limnology, Estonian University of Life Sciences, 61117 Rannu, Tartu County,

19   Estonia

20 [i]Department of Bioscience, Aarhus University, Vejlsøvej 25, 8600 Silkeborg, Denmark

21 [j]Sino-Danish Centre for Education and Research, Beijing, China

22

23 *corresponding author: hilt@igb-berlin.de, tel.: +49 30 64181677, fax: +49 30 64181682

24   **Abstract**

25   Periphyton communities play an important role in shallow lakes and are controlled by direct

26   forces such as temperature, light, nutrients, and invertebrate grazing, but also indirectly by

27   planktivorous fish predation. We performed a pan-European lake mesocosm experiment on

28   periphyton colonization covering five countries along a north/south geographical/temperature

29   gradient (Estonia, Germany, Czech Republic, Turkey, and Greece). Periphyton biomass on

30   artificial polypropylene strips exposed at 50 cm water depth at low and high nutrient regimes

31   (with mean total phosphorus concentration of 20 and 65 µg L^{-1}, respectively) was compared

32   during mid-summer. No significant effect of nutrient loading on periphyton biomass was

33   observed as nutrient concentrations in the mesocosms were generally above limiting values.

34   Water temperature significantly enhanced summer periphyton biomass development.

35   Additionally, direct and indirect top-down control of snails and fish emerged as a significant

36   factor in periphyton biomass control.

37

38   **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

65   et al. (2002) also found grazers to benefit more than periphyton from enhanced temperatures.

66   Moreover, herbivory and omnivory among fishes increase with temperature (González-

67   Bergonzoni et al. 2012; Meerhoff et al. 2012), and many fish species (or size classes of fish)

68   feed on periphyton in subtropical and tropical lakes (Teixeira-de Mello et al. 2009).

69   Comparative studies on periphyton dynamics along latitudinal scales are scarce.

70   Bécares et al. (2008) conducted a mesocosm experiment across a European latitudinal

71   gradient from Finland to Spain and found that periphyton chlorophyll *a* concentrations were

72   overall positively related to nutrient loading. Top-down effects by fish were significant only

73   in a few sites and were assumed to be related to their contribution to the nutrient pool. Under

74   these conditions southern lakes exhibited lower periphyton densities than northern lakes

75   because of the larger phytoplankton biomass in the south and its shading effects on periphyton

76   at similar nutrient loadings. In a comparative experimental field study by Meerhoff et al.

77   (2007), a substantially lower periphyton biomass on artificial plants was found in lakes in

78   subtropical Uruguay than in temperate Denmark. Despite a much lower biomass of

79   invertebrate periphyton grazers due to high fish predation in Uruguay, the authors attributed

80   the lower periphyton biomass in the warm lakes to direct control by fish grazing. Therefore,

81   periphyton biomass might be directly or indirectly affected by nutrients, temperature, grazers,

82   and fish, but the mechanisms of the underlying processes and potential interactions are still

83   poorly understood.

84   We studied periphyton development on artificial polypropylene strips exposed in

85   mesocosms with two different nutrient loadings resembling mesotrophic and eutrophic

86   conditions and at moderate fish density in five European countries (Estonia, Germany, Czech

87   Republic, Turkey, and Greece). A latitudinal temperature gradient was expected and effects

88   on periphyton biomass were studied for a period of one month in July and August 2011. We

89   hypothesize that higher nutrient loading and warmer temperatures increase summer

90   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

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117   lake water (mesh size 500 μm) in all countries, except for Germany and the Czech Republic

118   where tap water was used because the lake water TP concentration was higher than the target

119   concentration of the low nutrient treatment (i.e. > 25 µg L^{-1}). The initial water level in each

120   mesocosms was 2 m. To ensure that naturally occurring phytoplankton, zooplankton, and

121   macroinvertebrate communities would emerge, the mesocosms were inoculated with plankton

122   and sediment samples, which were collected from five different local lakes covering a range

123   from oligotrophic to eutrophic conditions (Landkildehus et al. 2014). The mesocosm set-up

124   also included the addition of apical shoots of macrophytes (*Myriophyllum spicatum*). Six

125   adult planktivorous fish (length 2-4 cm, 3 males and 3 females to allow breeding) were

126   stocked in each enclosure at the beginning of the experiment. Three-spined sticklebacks

127   (*Gasterosteus aculeatus*) were used in all countries except of Greece where mosquito fish

128   (*Gambusia affinis*) were used. Both fish species are known to have similar diets (Offill and

129   Walton 1999; Simpson 2008). Dead fish were replaced during the experiment. The water of

130   the mesocosms was continuously circulated by using water pumps. A more detailed

131   description of the entire experimental set-up can be found in Landkildehus et al. (2014).

132

133   Variables measured

134   Periphyton growth over the experimental period was quantified based on the biomass

135   accumulation on artificial transparent polypropylene strips (2 strips, 16 x 2 cm) with a slightly

136   textured surface (IBICO®, Germany; Roberts et al. 2003). The strips were exposed at a water

137   depth of 0.5 m and kept 0.3 m away from the mesocosm walls facing south to prevent shading

138   from the walls, and the backsides of the strips were covered with adhesive tape.

139   After five weeks of colonization, the periphyton strips were gently lifted to the surface

140   to minimize disturbance and loss of periphyton mats. After removal of the adhesive tape from

141   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

168   represented well the average temperate conditions for the experimental period, established by

169   the mean air temperature. The close link between air temperature and surface water

170   temperature in shallow lakes is well established in the literature (McCombie 1959;

171   Livingstone and Lotter 1998; Mooij et al. 2008). At midday of the 24 h measurement events,

172   profiles of photosynthetically active radiation (PAR) were taken at 0.1 m intervals from top to

173   bottom. For each light profile and each concurrent light intensity measurement, an attenuation

174   coefficient K_{di} (m^{-1}) was estimated based on the Beer-Lambert law:

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

175   (1)

176   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

177   removed. K_d (m^{-1}) was then taken as the mean over all K_{di}. The attenuation coefficients from

178   July and August were subsequently averaged. Mean and maximum available PAR at 0.5 m

179   were calculated as: $I_{0.5(mean\ or\ max)} = I_{0(mean\ or\ max)} \exp(-0.5\ K_d)$, where I_0 was set to average light

180   hour PAR ($I_{0.5mean}$) or maximum PAR ($I_{0.5max}$) at the surface. Averages for the experimental

181   period were calculated from daily $I_{0.5(mean\ or\ max)}$ values. PAR was estimated from global

182   radiation as PAR = E × γ × 0.45, where E is global radiation and γ = 4.6 is the mean photon

183   flux in the wavelengths from 400 – 700 nm (Kirk 2010).

184   At the end of the mesocosm experiment in November 2011, macroinvertebrates were

185   sampled with the help of Kajak cores (diameter =52 mm) or an Ekman grab sampler.

186   Subsequent identification and enumeration of snails were carried out to genus or species level.

187   At the same time, all fish were captured and weighed. In Germany, three mesocosms (two in

188   the high nutrient and one in the low nutrient treatment) sank during heavy storm events and

189   were consequently omitted. A detailed description of the sampling procedure and processing

190   can be found in Landkildehus et al. (2014).

191

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

218   fish biomass due to their higher predation on invertebrates (cascading effect) (Liboriussen et

219   al. 2005). A weak negative effect of fish on snails was found by regression analysis between

220   fish biomass and snail abundance (b = 0.08, t(35) = 1.96, p = 0.06) and logistic regression

221   analysis between fish biomass and presence and absence of snails (b =-0.53, z(35) = -2.08, p

222   = 0.04). Therefore, two alternative ANCOVA models (using either snail abundance or fish

223   biomass as a covariate) were analyzed for both periphyton dry weight and periphyton

224   chlorophyll a. All models contained nutrient treatment (i.e. high and low) as the main factor

225   and water temperature as a covariate. Although snail abundance data were used in this

226   analysis, it should be noted that snail species composition varied between countries. The

227   ANCOVA was executed using a Type III sums of squares method to account for the

228   unbalanced design in our experiment arising from the loss of three mesocosms in Germany.

229   Where necessary, data were either log or square root transformed to improve normality of the

230   residuals and meet the assumption of homogeneity of variance.

231   Additionally, to the ANCOVA analysis, differences in periphyton dry weight and

232   periphyton chlorophyll a between nutrient treatments were tested within each country. The

233   non-parametric Mann-Whitney U-test was considered appropriate to test for statistical

234   differences between treatments owing to the small sample size (i.e. N=8) in each country.

235   This test was not conducted for Germany due to the small sample size after losing three

236   mesocosms during the experiment (see above).

237   To aid the interpretation of the ANCOVA and to potentially further isolate the effect

238   of fish on periphyton biomass, an additional regression analysis was carried out between

239   water temperature-adjusted periphyton dry weight and chlorophyll a and fish biomass.

240   Adjustment involved calculating residuals of the regression equation of periphyton dry weight

241   vs. water temperature and periphyton chlorophyll a vs. water temperature, respectively. All

242   analyses were undertaken using STATISTICA 12 (StatSoft, Inc. USA) with a significance

243   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

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

271   weight.

272   The results of the Mann-Whitney U test showed that nutrient treatment did not have a

273   significant effect on periphyton dry weight (Fig. 3b) or chlorophyll *a*, except for periphyton

274   chlorophyll *a* in Greece (Fig. 3c). Periphyton dry weight was significantly correlated with

275   water temperature (r^2=0.41, p=0.001; Fig. 5a) and periphyton chlorophyll *a* (r^2=0.28,

276   p=0.001; Fig. 5c). Fish biomass showed a weak relationship with temperature-adjusted

277   periphyton dry weight (r^2=0.1, p=0.10) (Fig. 5b) and a strongly significant relationship with

278   temperature-adjusted periphyton chlorophyll *a* (r^2=0.45, p<0.001) (Fig. 5d).

279

280   **Discussion**

281   The present pan-European lake mesocosm experiment provided evidence that increasing

282   water temperature can lead to increased development of summer periphyton biomass. Nutrient

283   enrichment had no significant effect on periphyton biomass, probably due to very low nutrient

284   limitation levels for periphyton. Indirect top-down effects of fish emerged as an important

285   factor controlling periphyton biomass and appeared to be independent of water temperature.

286   In addition, snails, when present, appeared to have a negative effect on periphyton chlorophyll

287   *a*.

288   Our results showed that periphyton biomass (measured as dry weight) was

289   significantly and positively correlated with water temperature in the 20-28°C temperature

290   range. Given the projected rise in global air and water temperatures (IPCC 2013), our results,

291   therefore, suggest that summer periphyton biomass is likely to increase in the future. Our

292   results contradict those of Hansson (1992) who found that temperature was of minor

293   importance for periphyton biomass. However, his study was conducted along a much larger

294   productivity gradient (Swedish and Antarctic lakes) ranging from extremely low (meltwater

295   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

348 depend on nutrient level (Hansson 1992; Liboriussen et al. 2005; Trochine et al. 2014), fish

349 abundance and composition (some being periphyton grazers; Gonzáles-Bergonzoni et al.

350 2012), and the strength of the cascading effects of fish on invertebrate periphyton grazers

351 (Cao et al. 2014; Meerhoff et al. 2007).

352   In conclusion, our results indicate a stimulating effect of water temperature on summer

353 periphyton biomass. Due to non-limiting nutrient levels and low differences between the

354 treatments, no significant effect of nutrient loading on periphyton biomass was observed.

355 However, apart from temperature, direct and indirect top-down control of snails and fish

356 proved to be important factors for explaining a significant amount of variation in periphyton

357 biomass.

358

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524

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

527 **Table 2:** Mid-summer (i.e. 15 July – 15 August) means (± standard deviation) of selected variables in mesocosms with different nutrient

528 treatments (high and low) in five European countries. All means are treatment means apart from mean air temperature, which is averaged

529 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	

530 **Table 3:** Summary of results from the ANCOVA models testing for the effect of nutrient

531 treatments on periphyton dry weight and periphyton chlorophyll *a*. Water temperature, snail

532 abundance, and fish biomass were used as covariates in the models. Bold *p* values denote

533 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.
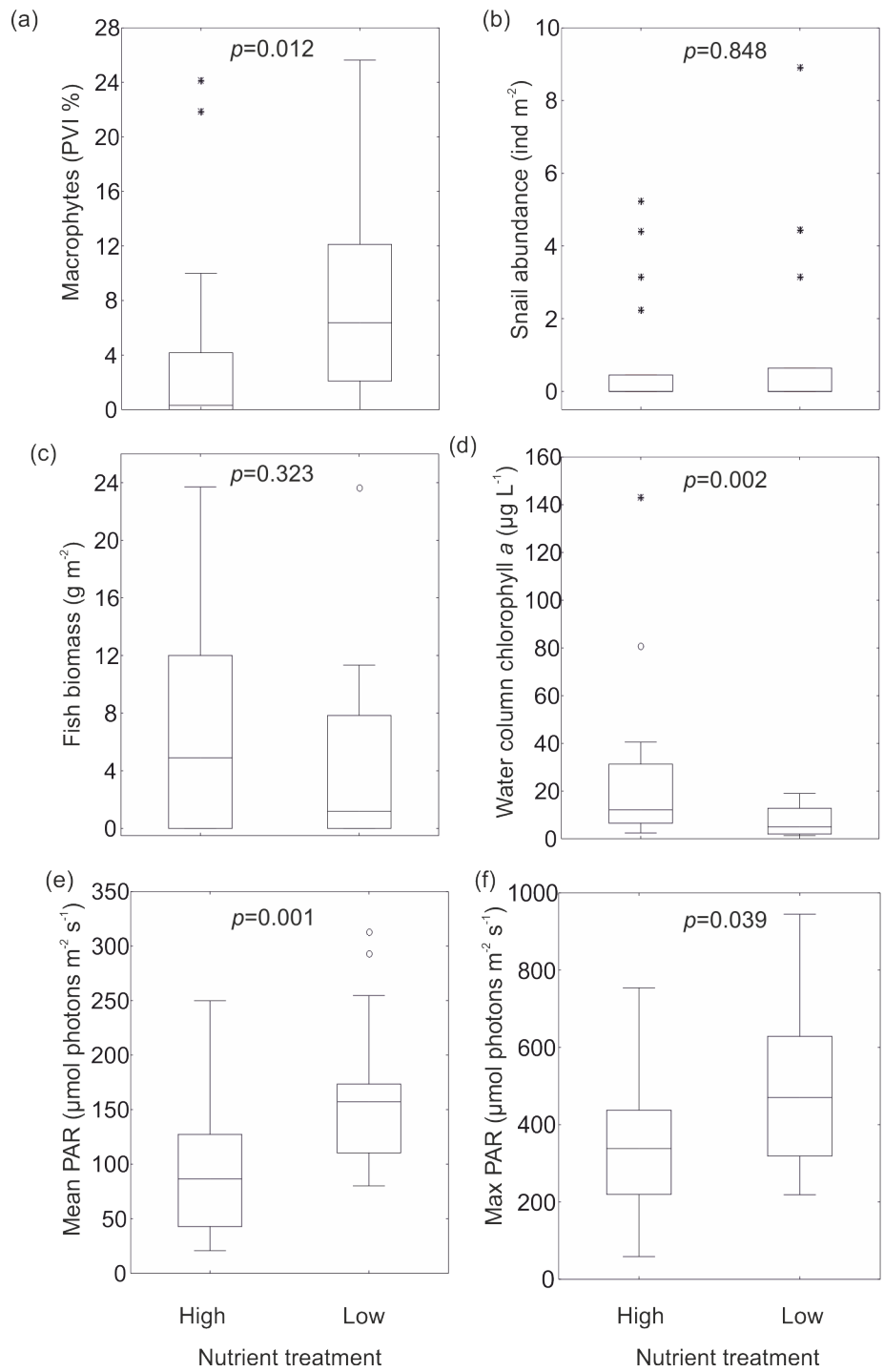
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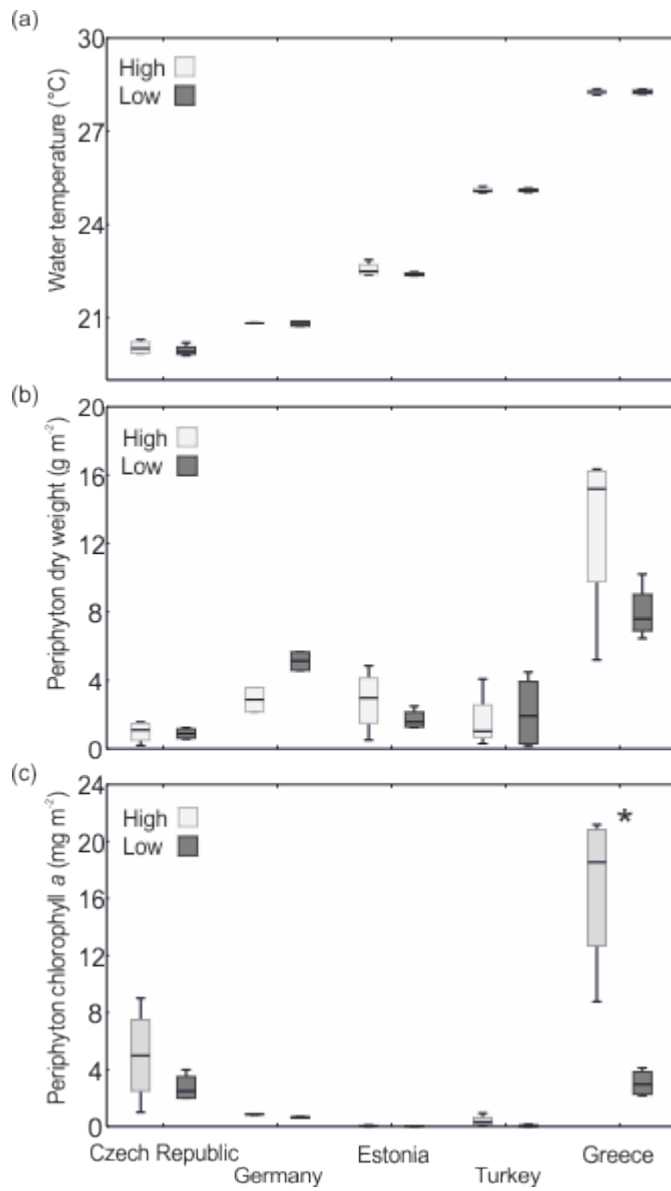
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580   Fig. 1

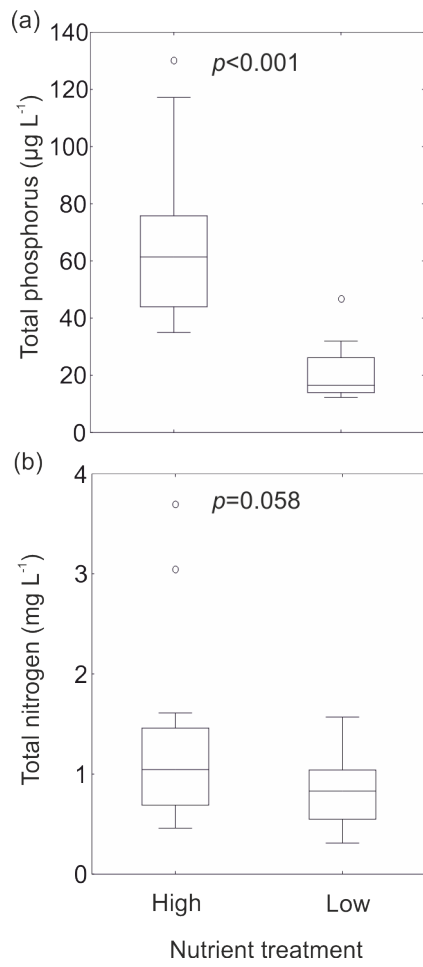581
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584   Fig. 2

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594   Fig. 3

(a)

Total phosphorus (µg L⁻¹)

p<0.001

(b)

Total nitrogen (mg L⁻¹)

p=0.058

High   Low

Nutrient treatment

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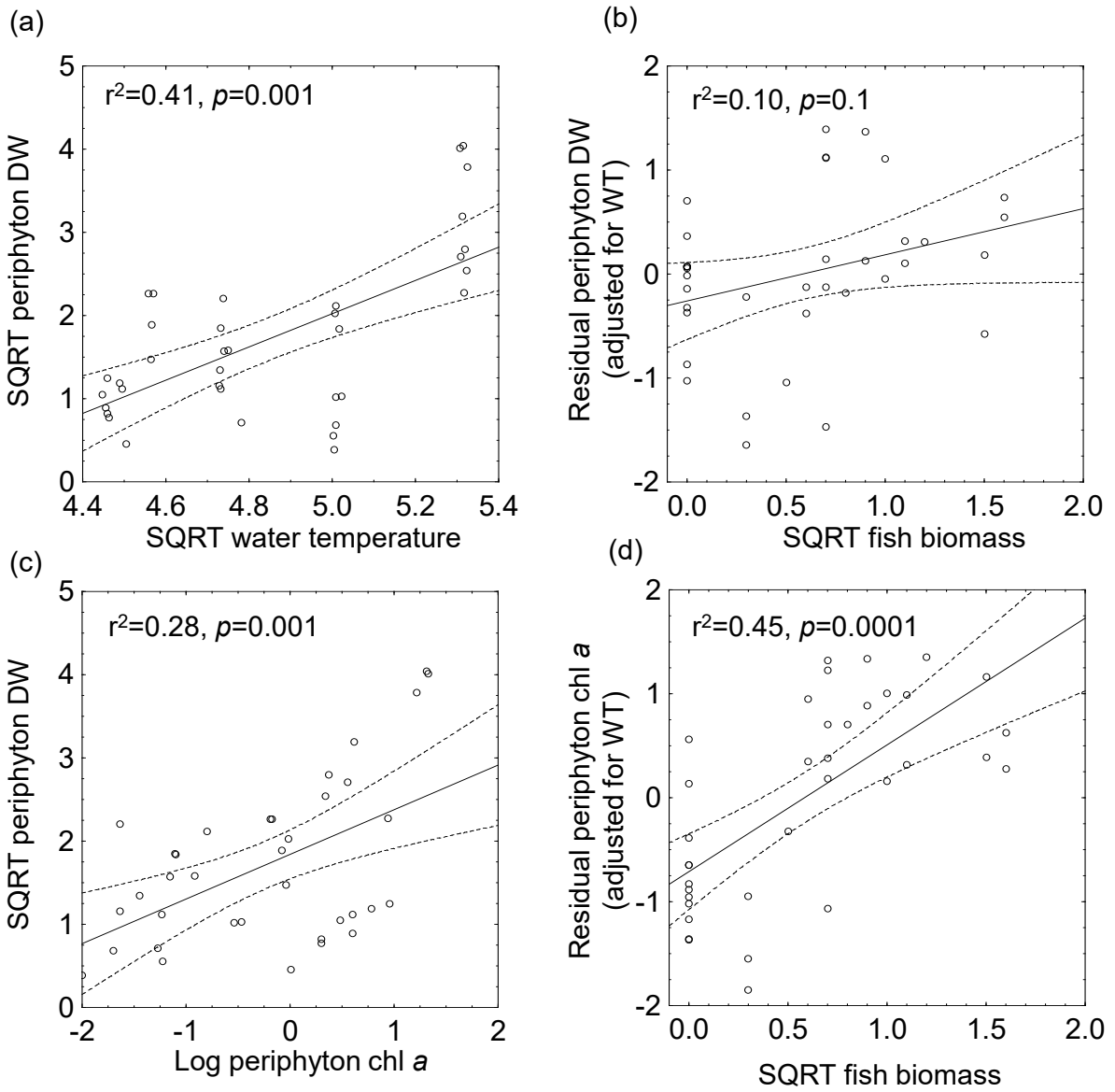605   Fig. 4

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Fig. 5

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617   **Supplementary material**
618
619   Figure S1. Comparison of mean daily air temperature values at study sites, with established

620   mean water temperatures (dashed horizontal lines) for the experimental period. Daily mean air

621   temperatures are based on hourly air temperature values. Average water temperature is based

622   on two daily mean values (24 hour averages of samples taken every two hours) measured on

623   11 July and 8 August 2011. CZ = Czech Republic, EE = Estonia, GE = Germany, GR =

624   Greece, and TR = Turkey.

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