1 Effects of warming on *Potamogeton crispus* growth and tissue stoichiometry in
2 the growing season

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16 **Abstract**

17 Increased water temperature due to climate change may affect macrophyte
18 phenology and nutrient content. In experimentally heated mesocosms the
19 emergence and growth of *Potamogeton crispus* shoots under ambient and
20 increased temperatures (+4.5 °C) were tracked over 55 days. At the end of the
21 experiment we measured the C, N and P content of the *P. crispus* leaves. The
22 results indicate that warming advanced the emergence of *P. crispus* shoots by
23 approximately 10 days, whereas the final number of shoots and plant biomass
24 were similar in ambient and heated tanks. Furthermore, warming influenced the
25 ecological stoichiometry of this plant significantly. Leaf C and N content were
26 both less in the heated tanks resulting in an increase in C:N ratios, whereas P
27 content and C:P and N:P ratios were not affected.

28 **Keywords:** climate change, ecological stoichiometry, growth, macrophytes,
29 phenology, shallow lakes

30 **1. Introduction**

31 During the period 1905-2001, the temperature rise in China was 0.5-0.8 °C,
32 which is slightly higher than the global warming range of 0.6 ± 0.2 °C in the
33 same period (Ding et al., 2007). It is likely that the global temperature will
34 increase by 1.1-6.4 °C in the 21st century compared to 1980-1999 on top of the
35 warming in the last century (IPCC, 2007). Warming affects a myriad of
36 biological processes, including individual metabolic rates and growth rates
37 (Yvon-Durocher et al., 2010), life history traits and phenology, which may
38 induce trophic mismatches between consumer demands and prey availability
39 (Winder and Schindler, 2004).

40 Shallow lakes, with their low depth and large surface : volume ratio, are
41 sensitive to warming (Coops et al., 2003; Meerhoff et al., 2012). Macrophytes
42 are key components in the ecological functioning of shallow lake ecosystems
43 and they are affected by warming in various ways (Kosten et al., 2009). At the
44 level of the plant individual, warming may affect plant physiology (Madsen
45 and Brix, 1997; Rooney and Kalff, 2000), growth (Haag and Gorham, 1977;
46 Short and Neckles, 1999) and reproduction (Van Vierssen et al., 1984; Mckee et
47 al., 2002). Furthermore, warming may result in alterations of trophic
48 interactions (O'Connor, 2009), which may have cascading effects throughout
49 the food web (Shurin et al., 2012) due to a mismatch in timing (Winder and
50 Schindler, 2004) or change in food quality. Plant nutrient concentrations and
51 the C:nutrient stoichiometry have been shown to be strong predictors of plant

52 consumption rates across ecosystems (Elser et al., 2000a; Sterner and Elser,
53 2002). High C:N and C:P ratios of primary producers indicate poor food, as N
54 and P are needed by organisms to produce proteins and RNA (Elser et al.,
55 2000b). The temperature-plant physiology hypothesis (Reich and Oleksyn,
56 2004) predicts that plant N and P content should decline with increasing
57 temperatures and indeed leaf N and P concentration of terrestrial plants decline
58 towards lower latitudes as the average temperature goes up, but few studies
59 have focused on aquatic plants. In aquatic plants at lower latitudes, temperature
60 optima may be surpassed and growing season length may become limiting
61 plant presence due to a shortening of their life cycle (Santamaría and van
62 Vierssen, 1997).

63 In the present study, we test how warming influences the growth and
64 stoichiometry of a submerged aquatic macrophyte. We hypothesize that
65 warming (1) advances the start of macrophyte growth and (2) increases
66 macrophyte biomass. Furthermore, we hypothesize that (3) if the
67 temperature-plant physiological hypothesis also applies to aquatic plants, the
68 tissue concentrations of C, N and P will decrease as the temperature goes up
69 resulting in (4) increasing C: nutrient ratios of the plant with warming. We used
70 the widespread submerged macrophyte *Potamogeton crispus* L. (Curlyleaf
71 pondweed) as a model species and grew it under ambient and experimentally
72 heated conditions (+ 4.5 °C higher than ambient).

73 **2. Materials and Methods**

74 *2.1. Study species*

75 *P. crispus* has a wide geographic distribution (Rogers and Breen, 1980;
76 Catling and Dobson, 1985), and occurs in many freshwater ecosystems
77 throughout China (Wu et al., 2009). It is most common in nutrient rich hard
78 waters (Bolduan et al., 1994), and considered a pioneer species in the
79 restoration of eutrophic lakes (Wu et al., 2009). Turions of *P. crispus* germinate
80 in the fall when the temperature goes down (Catling and Dobson, 1985;
81 Nichols and Shaw, 1986), then overwinter with little growth (Tobiessen and
82 Snow, 1984). *P. crispus* grow fast in early spring while the water temperature is
83 still low (10 - 15 °C). Then during the late spring to the summer, *P. crispus*
84 forms turions, flowers and seeds, and after that, senescence and decomposition
85 of *P. crispus* progress rapidly (Tobiessen and Snow, 1984; Hongda, 1985).
86 Temperature has a significant influence on the germination and growth of *P.*
87 *crispus*. With warming from 15 °C to 30 °C, germination of turions decreases
88 (Rogers and Breen, 1980), whereas increased temperatures increase the number
89 of turions formed (Sastroutomo, 1980). From 5 °C to 10 °C, a significant
90 increase in biomass was found with increasing temperature (Tobiessen and
91 Snow, 1984). The optimum temperature range for *P. crispus* appears to be 10 -
92 20 °C, above and below which growth is impaired (Ren et al., 1997).

93

94 *2.2. Heating system*

95 The experimental system was located at Wuhan (30°28'N, 114°21'E), in the
96 center of China. Ten fiberglass tanks (1.6 m height and 1.5 m diameter)
97 wrapped with insulation materials were used to simulate climate warming,
98 equipped with a heating rod in the center of the water and a pump to promote
99 the mixture of the water, connected to a computer control system. There were
100 two temperature probes placed at two sides of each mesocosm which read one
101 value every ten seconds. Temperature values of five ambient tanks were
102 averaged and returned to the computer, and the computer determined how to
103 heat the other five mesocosms for the heated treatment. According to the
104 prediction models provided by IPCC, the annual mean temperature in the
105 middle and lower reaches of Yangtze River would be increased by 4.5 °C at the
106 end of this century (Xu et al., 2004). Therefore, we selected the heated
107 treatment to be 4.5 °C higher than the control exposed to ambient temperatures.
108 All tanks were placed randomly to eliminate small spatial differences and
109 evenly covered with 10 cm deep sediment (TN $5.3 \pm 0.3 \text{ mg} \cdot \text{g}^{-1}$ dry weight, TP
110 $0.41 \pm 0.04 \text{ mg} \cdot \text{g}^{-1}$ dry weight) dredged from Lake Liangzi. The lake is located
111 in Hubei, China (30°5' - 30°18'N, 114°21' - 114°39'E), with a surface area of
112 304 km^2 and an average depth of 4.2 m (Wang and Dou, 1998). We dredged the
113 sediment (location: 30°10'55"N, 114°37'40"E, with water depth 1.6 ± 0.3 (SD)
114 m, secchi disk 1.5 ± 0.3 m, pH 8.8 ± 0.6 , and conductivity $178 \pm 3 \text{ } \mu\text{S} \cdot \text{cm}^{-1}$),
115 where *P. crispus* was a dominate species. Then we filled each tank with tap
116 water to yield 1m water depth. The heating system began to operate on 6 April

117 2013 before the emergence of *P. crispus*, and our first camera record was on 24
118 April when *P. crispus* could be seen in most of the tanks. The experiment
119 ended on 31 May. The submerged macrophytes *Najas minor*, *Hydrilla*
120 *verticillata* and *Ceratophyllum demersum* emerged in some of the tanks during
121 the experiment, but *P. crispus* was the absolute dominant species during the
122 entire experiment. We weekly monitored water quality in the tanks, which in
123 the ambient tanks ranged from: NH_4^+ : 0.30 ~ 0.74 $\text{mg}\cdot\text{L}^{-1}$, NO_3^- : 0.11 ~ 0.96
124 $\text{mg}\cdot\text{L}^{-1}$, PO_4^- : 1 ~ 26 $\mu\text{g}\cdot\text{L}^{-1}$, Chl-a: 0.54 ~ 2.1 $\mu\text{g}\cdot\text{L}^{-1}$, and in the heated
125 mesocosms: NH_4^+ : 0.36 ~ 1.53 $\text{mg}\cdot\text{L}^{-1}$, NO_3^- : 0.08 ~ 0.76 $\text{mg}\cdot\text{L}^{-1}$, PO_4^- : 2 ~ 33
126 $\mu\text{g}\cdot\text{L}^{-1}$, Chl-a: 0.91 ~ 3.29 $\mu\text{g}\cdot\text{L}^{-1}$ (see Fig. S1 in the Supplementary Material).

127

128 2.3. Camera record

129 We used a waterproof camera (Nikon COOLPIX AW100s) to record the
130 growth of *P. crispus* every week. The camera was fixed in a steel framework
131 with a long rod. There was a steel ruler (range 20 cm) fixed 30 cm from the
132 camera lens. Recording in each tank followed a standard procedure: (1) record
133 the label; (2) put the camera under the water just above the sediment, make the
134 rod vertical and tract 360-degrees around; (3) with the back of the camera
135 attached to the wall of the tank, walk along the side to record 360-degrees
136 above the sediment; (4) if plants could be seen by eye, we placed the camera
137 with the ruler attached to it on the sediment and tracked the shoots vertically
138 from the bottom to the top; (5) as plants nearly reached the water surface, we

139 used a long ruler to measure the height directly. All videos were analyzed in the
140 computer, to read all recorded plant heights.

141

142 2.4. Sampling and Detecting

143 Attached filamentous algae and non-target floating-leaved macrophytes
144 (especially *Trapa natans* and *Nymphoides peltata*) were removed from the
145 mesocosms during the experiment. At the end of the experiment, we collected 3
146 to 20 individuals (according to the total amount of individuals in each tank) in
147 each mesocosm of *P. crispus* with a height range from several to more than 100
148 centimeters. Attached filamentous algae and other debris were cleaned off the
149 plants and the total above-ground height of each shoot was measured. Wet
150 weight of each plant was measured, and then the plants were put in a plastic
151 bag and brought to laboratory to dry. After complete drying in the oven at 60 °C
152 for 48 h, we collected the leaves from each shoot and used a vibration grinding
153 machine (MiniBeadbeater-16) to grind the leaves to powder with two steel balls
154 vibrating randomly in a special plastic tube. About 2~3 mg dry powder was
155 used to detect the carbon and nitrogen content using the CN element analyzer
156 (Flash EA 1112, CE Instruments, Italy). Phosphorus content of the leaves was
157 measured by the ammonium molybdate ascorbic acid method (Sparks et al.,
158 1996; Li et al., 2013).

159

160 2.5. Data analysis

161 We used the wet weight biomass and height of the plants collected at the
162 end of the experiment to determine the relationship between plant height and
163 wet weight biomass, which could be described by a power equation (Niklas and
164 Enquist, 2001; Brown et al., 2004). We used the equation $Y = 0.0535 \cdot X^{0.9602}$
165 ($F_{1,152} = 682$, $p < 0.001$, $r^2 = 0.82$), where Y is plant biomass in g wet weight
166 and X is plant height in cm, to calculate plant wet weight biomass from the
167 height measurements taken during the experiment both in ambient, and heated
168 mesocosms. We used a Repeated measures ANOVA to test the effect of
169 warming on shoot number, number of newly emerged shoots and biomass of *P.*
170 *crispus* plants during the experiment. Where significant interactions were found
171 between time of measurement and warming, the effect of warming was tested
172 for each date using independent t-tests. To determine whether there was a
173 difference in phenology between the warming treatments, we calculated the
174 date at which half of all shoots had emerged in each tank. This was done by
175 extrapolating between the nearest two dates on which the number of shoots was
176 counted, assuming a linear increase in number of shoots between these two
177 dates. The effect of warming on the day at which half of all shoots were present
178 was tested with an independent t-test. The effect of warming on plant C, N and
179 P concentration and C:N, C:P and N:P ratios ($\text{g} \cdot \text{g}^{-1}$) was tested with a nested
180 ANOVA, with warming treatment as a fixed and experimental tank as a random
181 factor nested in warming treatment. Normality of the data distribution was
182 tested with a Kolmogorov-Smirnov test. Spread versus level plots and residual

plots were used to control other parametric assumptions. These indicated that no data transformations were necessary. The analyses were performed in SPSS 22.0 (IBM Corp. 2013) and Statistica 12 (StatSoft Inc. 2014) .

3. Results

3.1. Temperature comparison

During our experiment, the average temperature in the heated mesocosms was 4.44 ± 0.28 °C higher than in the control (daily average in heated tanks minus average in ambient tanks, averaged over all days of the experiment). The daily average ambient water temperature varied from 13.9 °C to 27.1 °C (Fig. 1).

3.2. Population growth

The number of shoots in the tanks increased significantly over time in both the ambient and heated tanks (Fig. 2a; Repeated measures ANOVA: time in experiment $F_{5,40} = 41.6$, $p < 0.001$). There was no significant effect of heating treatment ($F_{1,8} = 0.08$, $p = 0.78$), whereas there was a marginal interaction between time and heating treatment ($F_{5,40} = 2.56$, $p = 0.042$), but further testing revealed no significant differences among heating treatments per date (independent t-tests, $p > 0.1$). The day at which 50% of the individuals had emerged was significantly affected by temperature treatment (independent t-test: $t_8 = 4.27$, $p = 0.003$). In the heated treatment 50% of the individuals was

204 on average present on May 3 (day 123 ± 1 SE), whereas under the ambient
 205 temperatures 50% of the individuals was present ten days later on May 13 (day
 206 133 ± 2 SE). The number of newly emerging shoots was not directly affected
 207 by the heating treatment ($F_{1,8} = 1.34$, $p = 0.28$), but there was a strong
 208 interaction between heating treatment and time in the experiment for the
 209 emergence of new shoots (interaction heating $\times$ time: $F_{4,32} = 7.02$, $p < 0.001$;
 210 effect of time: $F_{4,32} = 3.65$, $p = 0.015$). Only at the last day of measurement
 211 there was a significant difference among heating treatments: there were more
 212 new shoots in the ambient compared to the heated treatment (independent t-test:
 213 $t_8 = 2.94$, $p = 0.019$). On the other dates there were no significant differences
 214 among heating treatments (independent t-tests, $p > 0.1$) (Fig. 2b). The wet
 215 biomass of *P. crispus* strongly increased over time (Fig. 2c; Repeated measures
 216 ANOVA: effect of time $F_{5,40} = 32.25$, $p < 0.001$). There was no effect of the
 217 heating treatment on plant biomass during the experiment (effect of heating:
 218 $F_{1,8} = 0.30$, $p = 0.60$, time $\times$ heating: $F_{5,40} = 0.82$, $p = 0.54$).

219

220 3.3. Stoichiometric properties

221 Heating significantly affected the nutrient concentrations and ratios in the *P.*
 222 *crispus* leaves (Table 1). In total 109 individuals were measured, 54 for
 223 ambient tanks, and 55 for the heated tanks. Heating decreased the carbon and
 224 nitrogen concentration in the leaves, whereas there was no significant effect on
 225 phosphorus concentrations (Table 1). As a result the C:N ratio in the leaves was

226 higher when heated, whereas there were no differences in C:P or N:P ratio
227 (Table 1). There was considerable variation between the tanks, but the warming
228 effect was significant when the variation among tanks was taken into account.

229 **4. Discussion**

230 *4.1. Warming effects on growth*

231 Warming significantly advanced the emergence of *P. crispus* shoots by ten
232 days in our study. Hansson et al. (2012) found that a 3 °C warming will advance
233 both phytoplankton and zooplankton spring peak abundances about two weeks.
234 Previous studies also found that warming will advance the growth of aquatic
235 plants (Haag and Gorham, 1977; Rooney and Kalff, 2000). Although some
236 studies demonstrated that warming may increase total aquatic plant biomass
237 (Barko and Smart, 1981; Rooney and Kalff, 2000; Feuchtmayr et al., 2009),
238 others found no effect (McKee et al., 2002; McKee et al., 2003) or a decreasing
239 total plant biomass (Barko and Smart, 1981). Our results are in line with the
240 studies that did not observe a difference in total plant biomass in response to
241 warming. The contrasting results of the impact of warming on aquatic plant
242 biomass may be related to the optimal temperature range of the plant species
243 studied. Within the optimal range, warming may enhance the growth of the
244 plant, but if the elevated temperature exceeds the optimal range, then high
245 temperatures may hinder the growth of the plant. At temperatures above 24 °C,
246 the growth of *P. crispus* is inhibited, and when temperature surpasses 30 °C, the

247 plant will start to senescence (Ren et al., 1997). During our experiment, the
248 ambient water temperature ranged from 13.9 °C to 27.1 °C, so the growth of
249 plants was probably inhibited more in the heated tanks during the second half
250 of the experiment, as their temperatures were 4.5 °C higher than the tanks
251 exposed to the ambient temperatures. Therefore we conclude that warming will
252 more likely advance the life cycle of *P. crispus* earlier into the season, than
253 increase its biomass in the subtropical middle and lower reaches of Yangtze
254 River.

255

256 4.2. *P. crispus* stoichiometry

257 The nutrient concentration and stoichiometry of *P. crispus* leaves was
258 significantly influenced by warming in the mesocosms. The concentration of C
259 and N decreased in the warmed tanks but there was no significant effect on P
260 concentration. This supports the temperature plant physiological hypothesis,
261 that as the temperature goes up, plants invest less nutrients to produce proteins
262 to sustain biochemistry reactions (Oleksyn et al., 1998; Tjoelker et al., 1999;
263 Reich and Oleksyn, 2004; Xia et al., 2014). The C:N ratio in the plants
264 increased in the heated mesocosms, reflecting a lower N-based biomass per
265 unit C in the plants. This indicates that warming increases the nutrient use
266 efficiency of this aquatic plant. A similar temperature-driven increase in
267 nutrient use efficiency was observed for other angiosperms (Reich and Oleksyn,
268 2004), and in phytoplankton communities (Domis et al., 2014). In our study,

269 the highest total plant biomass in the heated tanks was similar to the biomass in
270 the ambient ones. As the N concentration of each plant decreased with warming,
271 as a consequence, the total amount of N in the plant population declined with
272 warming. The lack of a significant response of P concentration to warming may
273 be partly due to the larger variation in plant P concentration among replicates,
274 as there was a tendency to lower P concentrations with warming. Alternatively,
275 P concentration may be less responsive to warming.

276 The reasons for the considerable variation between tanks may be that the
277 initial numbers of *P. crispus* propagules in each tank were small and may have
278 differed, also, the turion sprouting time can vary significantly (Jian et al., 2003),
279 and finally turion age may be a factor (Heuschele and Gleason, 2014). As our
280 turion were buried in the sediment, we could not establish their age. These may
281 have been sources of variation in sprouting among the tanks.

282

283 4.3. Consequences for aquatic ecosystems

284 The response of *P. crispus* to warming can have several consequences for
285 the aquatic ecosystem. With warming, fast growth of a *P. crispus* population
286 could inhibit phytoplankton abundance in early spring resulting in clear water
287 conditions. However, when the temperature further increases beyond the
288 optimal range for *P. crispus* growth, the onset of senescence may also be earlier.
289 During decomposition of this plant material, nutrients will be released to the
290 water column (Bolduan et al., 1994), and, when no other submerged

291 macrophytes dominate, a phytoplankton bloom may follow (Sayer et al., 2010).
292 As we only tested one macrophyte species, the response of other aquatic plants
293 with different life styles and nutrient acquisition strategies need to be tested, to
294 allow generalisation of these results.

295 Higher C:N ratios of *P. crispus* with warming reflect lower quality and
296 palatability of food for herbivores (Mattson Jr, 1980; Demment and Van Soest,
297 1985; Elser et al., 2000b; Dorenbosch and Bakker, 2011). This may have a
298 negative effect on the higher trophic levels and influence the whole aquatic
299 food web. We conclude that warming alters the timing of *P. crispus* emergence
300 and the nutrient composition of the plant. Therefore, we speculate that warming
301 can affect the timing and duration of the clear water phase and the strength of
302 aquatic food web interactions through its effect on freshwater macrophytes.

303

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430

431 Table 1. Effects of heating treatment on plant nutrient concentrations and
 432 stoichiometry. Results of nested ANOVA with heating treatment as fixed factor
 433 and tank nested in heating treatment as a random factor. Values are Mean $\pm$
 434 standard error (SE).

Parameter	Ambient	Heated	Heating treatment		Tank	
			F _{1,99}	P	F _{8,99}	P
C (mg·g ⁻¹)	419 $\pm$ 4.7	407 $\pm$ 6.6	13.70	<0.001	3.59	0.001
N (mg·g ⁻¹)	41.4 $\pm$ 1.7	36.9 $\pm$ 1.9	19.99	<0.001	2.33	0.025
P (mg·g ⁻¹)	3.20 $\pm$ 0.48	2.75 $\pm$ 0.37	2.25	0.14	3.41	0.002
C:N (g·g ⁻¹)	10.2 $\pm$ 0.42	11.2 $\pm$ 0.47	15.83	<0.001	3.24	0.003
C:P (g·g ⁻¹)	145 $\pm$ 21	162 $\pm$ 23	1.06	0.31	4.03	<0.001
N:P (g·g ⁻¹)	14.0 $\pm$ 1.6	14.4 $\pm$ 1.6	0.05	0.83	4.49	<0.001

435

436 Figure captions

437

438 Figure 1 Mean daily temperature in the ambient and heated tanks (n=5 per
439 treatment). Data are means $\pm$ SD.

440

441 Figure 2 The average number of *P. crispus* shoots during the growing season
442 (a), vertical lines represent emerging time of 50% of the total number of shoots.
443 The average newly emerging number of shoots of *P. crispus* (b). The average
444 wet weight biomass of *P. crispus* in each treatment (c). Error bars indicate $\pm$
445 standard error (SE).

446

Fig. 1

Fig. 2a, b, c

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