Response diversity of free-floating plants to nutrient stoichiometry and temperature: Growth and resting body formation

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Free-floating plants, like most groups of aquatic primary producers, can become nuisance vegetation under certain conditions. On the other hand, there is substantial optimism for the applied uses of free-floating plants, such as wastewater treatment, biofuel production, and aquaculture. Therefore, understanding the species-specific responses of floating plants to abiotic conditions will inform both management decisions and the beneficial applications of these plants. I measured the responses of three floating plant species common in the northeast United States (*Lemna minor, Spirodela polyrhiza,* and *Wolffia brasiliensis*) to nutrient stoichiometry (nitrogen and phosphorus) and temperature in the laboratory. I also used survey data to determine the pattern of species richness of floating plants in the field and its relationship with the dominance of this group. Floating plant species exhibited unique responses to nutrient stoichiometry and temperature in the laboratory, especially under low temperatures (18°C) and low nutrient conditions (0.5 mg N L^{-1}, 0.083 mg P L^{-1}). The three species displayed an apparent tradeoff with different strategies of growth or dormancy. In the field, water bodies with three or more species of floating plants were not more frequently dominated by this group. The response diversity observed in the lab may not be associated with the dominance of this group in the field because it is masked by environmental variability, has a weak effect, or is only important during transient circumstances. Future research to develop applied uses of floating plants should examine response diversity across a greater range of species or clones and environmental conditions.

1 **Title:** Response diversity of free-floating plants to nutrient stoichiometry and temperature:

2 Growth and resting body formation

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9 **Abstract**

10 Free-floating plants, like most groups of aquatic primary producers, can become nuisance

11 vegetation under certain conditions. On the other hand, there is substantial optimism for the

12 applied uses of free-floating plants, such as wastewater treatment, biofuel production, and

13 aquaculture. Therefore, understanding the species-specific responses of floating plants to abiotic

14 conditions will inform both management decisions and the beneficial applications of these

15 plants. I measured the responses of three floating plant species common in the northeast United

16 States (*Lemna minor, Spirodela polyrhiza,* and *Wolffia brasiliensis*) to nutrient stoichiometry

17 (nitrogen and phosphorus) and temperature in the laboratory. I also used survey data to

18 determine the pattern of species richness of floating plants in the field and its relationship with

19 the dominance of this group. Floating plant species exhibited unique responses to nutrient

20 stoichiometry and temperature in the laboratory, especially under low temperatures (18°C) and

21 low nutrient conditions (0.5 mg N L^{-1}, 0.083 mg P L^{-1}). The three species displayed an apparent

22 tradeoff with different strategies of growth or dormancy. In the field, water bodies with three or

23 more species of floating plants were not more frequently dominated by this group. The response

24 diversity observed in the lab may not be associated with the dominance of this group in the field

25 because it is masked by environmental variability, has a weak effect, or is only important during

26 transient circumstances. Future research to develop applied uses of floating plants should

27 examine response diversity across a greater range of species or clones and environmental

28 conditions.

29 **Introduction**

30 Free-floating plants (or simply "floating plants"), like most groups of aquatic primary producers,

31 can become nuisance vegetation under certain conditions (Portielje and Roijackers 1995, Janse

32 and Van Puijenbroek 1998, Scheffer et al. 2003, Smith 2012). Shallow lakes and ponds,

33 agricultural ditches, and tropical lakes can be dominated by thick mats of floating plants, altering

34 abiotic conditions and reducing biotic diversity (Morris and Barker 1977, Janes et al. 1996,

35 Morris et al. 2003, Verdonschot and Verdonschot 2013). The dominance of this functional group

36 is driven by nutrient enrichment (both nitrogen and phosphorus), and low levels of either of these

37 nutrients can limit floating plant growth (Portielje and Roijackers 1995, Kufel et al. 2010, Smith

38 2014). In addition to eutrophication, increased temperatures due to climate change may also

39 favor the dominance of this group over other primary producers (Netten et al. 2011, Peeters et al.

40 2013). On the other hand, there is substantial optimism for the applied uses of free-floating

41 plants, such as wastewater treatment, biofuel production, ecotoxicological assessment, and

42 aquaculture (e.g., Greenberg et al. 1992, Skillicorn et al. 1993, Ge et al. 2012, Xu et al. 2012,

43 Verma and Suthar 2014). Therefore, understanding the species-specific responses of floating

44 plants to nutrients and temperature will have both management implications and beneficial

45 applications (Ziegler et al. 2015). For example, if floating plant species exhibit response diversity

46 (i.e., unique response to abiotic conditions) (Elmqvist et al. 2003), then a more diverse

47 assemblage of floating plants may be more resilient or dominant (Naeem and Wright 2003), and

48 thus, harder to manage. Furthermore, if particular species have unique responses, than those with

49 a desirable suite of traits may be identified for applied uses.

50

51   Although both nitrogen and phosphorus are important drivers of floating-plant dominance, the

52   ratio of both nutrients may have important consequences, especially in multi-species contexts

53   (Smith 2014). Depending on the conditions of the growth medium, species and clones of floating

54   plants differ in their N:P content (reviewed by Landolt and Kandler 1987). For example, Karpati

55   and Pomogyi (1979) reported N:P tissue content ranging from 2.65 for *Lemna trisulca* to 10.53

56   for *Lemna minor* in naturally growing plants. Docauer (1983) reported N:P content of 8.12 for

57   *Spirodela polyrhiza ,*10.38 for *L. minor,* and 3.46 and 6.54 for two species of *Wolffia* (*W.*

58   *borealis* and *W. columbiana,* respectively) when the plants were growing at half of their

59   maximum growth rate. Depending on the nutrient content of the growth medium, tissue N:P in

60   *Lemna gibba* can range from approximately 3 to nearly 40 (Fulton et al. 2010). These species-

61   specific and context-dependent stoichiometric differences are important because nutrient

62   stoichiometry will differ depending on the source of nutrient loading and various other factors,

63   resulting in wide variation in nutrient stoichiometry of different water bodies (Downing and

64   McCauley 1992). If floating plant species are constrained in their nutrient stoichiometry, then

65   this may affect the outcome of competition among floating plants or with other primary producer

66   groups (Sterner and Elser 2002), although nitrogen alone explained most of the outcome of

67   competition among floating plants in the early stages of a field mesocosm experiment (Smith

68   2014).

69

70   I used laboratory experiments to determine the response diversity (i.e., unique response to abiotic

71   conditions) among floating plant species. To address this question, I performed two laboratory

72   experiments to examine the growth and turion-formation of floating plant species in response to

73   nitrogen, phosphorus, and temperature. The experiments were conducted with three of the most

74   common floating plant species in the northeast United States: *Lemna minor* L*., Spirodela*

75   *polyrhiza* (L.) Schleid*.,* and *Wolffia brasiliensis* Wedd. To understand whether the response

76   diversity in the laboratory corresponds to increased dominance in the field, I also analyzed a

77   dataset of over 200 freshwater lakes and ponds to determine the association between floating

78   plant species composition and richness and the occurrence of floating plant dominance. If

79   substantial differences exist among floating plant species in the lab, then a more diverse

80   assemblage may be more likely to be dominant over a broader range of conditions. In this case,

81   it is expected that water bodies with greater floating plant richness will be more frequently found

82   in a floating plant state.

83

84   **Materials & Methods**

85   *Laboratory conditions*

86   Although conducting a single, large experiment to determine the response to all environmental

87   conditions of interest would have some advantages, I conducted two separate experiments to

88   allow for a manageable amount of effort and maintain adequate sample sizes in each experiment.

89   For all experiments, plants were collected from Setauket Mill Pond, East Setauket, New York,

90   USA (40.946061°, -73.115613°) and acclimated under experimental conditions prior to the start

91   of the experiments. Plants were collected in mid-August or mid-June for experiments I and II,

92   respectively. Species were identified according to Crow and Hellquist (2000). Modified Barko-

93   Smart media (Smart and Barko 1985; Szabó et al. 2005) with phosphorus supplied as potassium

94   dihydrogen phosphate and nitrogen supplied as a 1:1 ratio of nitrogen from nitrate and

95   ammonium (potassium nitrate and ammonium chloride) was used as the nutrient media.

96   Micronutrients were supplied to the media by Tropica Aquacare Plant Nutrition Liquid at a

97   concentration of 0.1 mL L^{-1} (Szabó et al. 2003, 2010). Plants were grown in plastic, multiwell

98   plates with individual well diameters of 22.75 mm containing 4 mL of media. Each well housed

99   a single replicate of an experimental treatment. Multiwell plates were cleaned in 10%

100   hydrochloric acid for at least 1 hour, and then rinsed thoroughly with deionized water prior to

101   using. Living plants (i.e., green) were moved to clean multiwell plates with fresh media every

102   two to four days, depending on the experiment. Any dead fronds (i.e., entirely white or brown)

103   were removed. Light was supplied at an intensity of 130-150 μE m^{-2} s^{-1} and a 14:10 hr light:dark

104   photoperiod, which is within the range of many previous studies (reviewed by Landolt and

105   Kandeler 1987). Temperature-controlled walk-in chambers were used to achieve the target

106   temperatures. Nutrient treatments and species were systematically assigned to wells to ensure

107   that replicates were dispersed across plates and not in adjacent wells. A systematic assignment of

108   treatments, rather than completely randomized, was used to reduce the likelihood that the wrong

109   nutrient medium or plant species would be placed in a well during each nutrient media change.

110   Because of the large number of replicates and the small spatial scale of the experimental setup, it

111   is unlikely that the systematic assignment of treatments correlated with an unknown,

112   confounding variable that varied along the same systematic spatial pattern of treatment

113   assignments. Initial plant area (20 mm^2 or 5% of the total well area available for growth) was

114   approximately equal for all species within an experiment. Frond number differed because of the

115   size differences among species. In order to prevent crowding, experiments were ended when

116   plants in some treatments filled approximately two-thirds of the well area.

117

118   Raw data from both experiments are available in Supplemental Information 1 and 2.

119

120   *Experiment I: Response to nutrients and temperature*

121   I measured the responses of floating plant species to nutrients and temperature by measuring

122   their growth (in terms of surface area) and turion formation at three nutrient levels (low: 0.5 mg

123   N L^{-1} and 0.083 mg P L^{-1}; medium: 5 mg N L^{-1} and 0.83 mg P L^{-1}; or high: 10 mg N L^{-1} and 1.66

124   mg P L^{-1}) fully-crossed with three temperatures (18, 24, and 30 °C), for a total of nine treatment

125   combinations. The three nutrient treatments are all at a N:P mass ratio of ~6 and correspond to

126   experimental treatments used in previous studies (e.g., Scheffer et al. 2003; Szabó et al. 2010).

127   At the lowest nutrient level, nutrients were expected to be limiting to growth (Lüönd 1983,

128   Szabó et al. 2010), but for the two highest nutrient levels, nutrients may be saturated (Szabó et al.

129   2010). Although this is not an exhaustive combination of treatments, these levels sample some of

130   the possible environmental conditions encountered by floating plants in nature, and potentially in

131   engineered applications (e.g., wastewater treatment). Eight replicates of each treatment

132   combination for each species were grown for 12 days. Plants were transferred to new nutrient

133   media 3, 5, 7, and 10 days after the start of the experiment.

134

135   *Experiment II: Response to nutrient stoichiometry*

136   In a second experiment, I measured the responses of floating plant species to nutrient

137   stoichiometry by measuring their growth and turion formation at all nine combinations of three

138   nitrogen (0.5, 5, and 10 mg N L^{-1}) and three phosphorus levels (0.083, 0.83, and 1.66 mg P L^{-1}),

139   producing a variety of N:P mass ratios, ranging from 0.30 to 120.48 (Table 1). At the lowest

140   treatment level of each nutrient, that nutrient may limit plant growth. Six replicates of each plant

141   species at each of the nine nutrient treatments were grown for 17 days. A smaller number of

142   replicates per each treatment combination (6 vs. 8 replicates) were used in this experiment

143   because of the relatively low variance observed in Experiment I. I transferred plants to new

144   nutrient media 3, 7, 10, and 14 days after the start of the experiment. Plants were grown at

145   approximately 30°C, which had similar growth rates as the 24 °C treatment and resulted in the

146   maximum growth rates in Experiment I. This temperature would ensure that only nutrient

147   stoichiometry would limit plant growth in this experiment.

148

149   *Growth and turion production*

150   To quantify growth, plants in each replicate of each treatment were photographed (Nikon

151   Coolpix 5700 Digital Camera) with backlighting from a light box (Laboratory Supply Company,

152   60 Watts) on days that nutrient media were changed. The two-dimensional plant area on the

153   water surface was measured with ImageJ, version 1.47 (Rasband 2014), using the threshold

154   function on an 8-bit grayscale photo, after dead fronds had been removed by hand (see above).

155   Relative growth rate (RGR) was calculated between each measurement. RGR was calculated as

156     $[\ln(A_2) - \ln(A_1)] / (t_2 - t_1)$, where *A* is the area of plants in mm^2, *t* is time in days, and subscripts *2*

157   and *1* indicate two sequential time points in the experiment. Plant thickness or mass was not

158   measured during these experiments, but the overheard surface area is a commonly used measure

159   in many experiments (reviewed by Landolt and Kandeler 1987). Turions were distinguished as

160   plants that had sunk to the bottom of the experimental vessel, and they typically differed in size,

161   texture, or color from plants on the surface. The number of turions (i.e., asexual resting bodies)

162   produced were counted for each experimental replicate after the live plants had been moved to

163   fresh media. The number of turions was converted to area with equations developed in another

164   study (Supplemental Information 3). In some replicates, all plants in a replicate died (i.e.,

165   bleached white) during the experiment, and were re-started with new plants, assuming that the

166   failed growth was due to damage to the plant when handling. These replicates were excluded

167   from analysis if they were not grown for at least 10 days.

168

169   *Statistical analysis*

170   For all ANOVAs, data were tested for normally distributed residuals with a Shapiro-Wilk test

171   and equal variance among treatment groups with Levene's test. If the data did not meet these

172   assumptions of ANOVA, they were power transformed to ensure that these criteria were met. I

173   performed all statistical analyses in R version 3.0.2 (R Development Core Team 2013).

174

175   The goal of these experiments was to test whether floating plant species differed in their

176   response to environmental conditions and under what conditions they differed. Therefore,

177   analyses tested for differences between species under particular combinations of environmental

178   conditions (i.e., treatment levels). For both experiments, I performed a one-way ANOVA for

179   each treatment combination to test for an effect of species on the average RGR. I used a Dunn-

180   Šidák correction to adjust p-values for multiple comparisons. When significant treatment effects

181   were found, Tukey's HSD was used to detect differences among species. An alternative

182   approach to analyzing these data is to use factorial ANOVAs to test for the main and interactive

183   effects of species and experimental conditions on growth rates (Supplemental Information 4).

184   Since the number of possible pairwise, posthoc comparisons in each experiment is large (351)

185   and species differences under identical conditions (i.e., response diversity) was the focus of this

186   study, this statistical approach is not reported in the main text.

187

188   In the first experiment (the effect of nutrients and temperature) only *W. brasiliensis* formed

189   turions. I analyzed the effect of nutrients and temperature on turion area produced (mm^2 day^{-1})

190   by *W. brasiliensis* with a two-way ANOVA. When significant treatment effects were found, I

191   used Tukey's HSD to detect differences among treatment levels. In the second experiment (i.e.,

192   the effect of nitrogen and phosphorus), *W. brasiliensis* formed turions under all treatment levels

193   and *S. polyrhiza* under some treatment levels. To detect differences in turion production rate

194   between species at particular nutrient levels, I used one-way ANOVAs at each nutrient level

195   where both *W. brasiliensis* and *S. polyrhiza* formed turions. I used a Dunn-Šidák correction to

196   adjust p-values for multiple comparisons.

197

198   *Floating plant richness and abundance in natural water bodies*

199   I examined the occurrence and dominance of floating plant species in lakes and ponds with a

200   dataset of 205 freshwater water bodies in Connecticut and Long Island, NY (Supplemental

201   Information 5). The data came from two sources: 1) 184 surveys by the Connecticut Agricultural

202   Experiment Station (CAES) in 2005 to 2013, and 2) 21 surveys that I conducted in Long Island,

203   New York and Connecticut, USA in 2011 to 2013. This data set spanned a range of perennial,

204   freshwater lakes and ponds and included the list of floating plant species present in the water

205   body and the maximum floating plant cover (percent of water body covered as quantified

206   through visual observation and mapping) during the late summer (late July to September). See

207   Capers et al. (2007) for a description of the survey methods used for the CAES data. For the

208   Long Island surveys, plant cover was estimated through visual observation similar to methods

209   used in previous studies (Driever et al. 2005, Smith 2012). In this study, I use high floating plant

210   cover as a surrogate for dominance by floating plants, while acknowledging that a consideration

211   of other primary producers (e.g., phytoplankton, submerged vegetation) and covariates is

212   necessary for a rigorous demonstration of complete floating-plant dominance.

213

214   I used a goodness of fit test (G-test, based on a chi-square) to test if all floating plant species

215   richness levels were equally likely to occur (i.e., random), excluding water bodies without

216   floating plants. I used a second G-test to determine if floating plant dominance ($\geq$ 66.67% cover)

217   was equally likely to occur under different levels of floating plant richness. The expected value

218   for each richness level in water bodies dominated by floating plants was based on the observed

219   frequency of each floating plant richness level across all water bodies (both dominated and non-

220   dominated). Floating plant richness was categorized as 1, 2, or $\geq$ 3 species to ensure adequate

221   sample sizes in each level.

222

223   **Results**

224   *Experiment I: Response to nutrients and temperature*

225   Average relative growth rate (RGR) was different among species at six of the nine combinations

226   of nutrients and temperature (Table 2, Figure 1). Species growth rates were equal when nutrients

227   and temperatures were high (10 mg N L^{-1} and 1.66 mg P L^{-1} and 24 or 30 °C) or at 18°C and

228   medium nutrients (5 mg N L^{-1} and 0.8 mg P L^{-1}). Typically, *Lemna minor* and *Spirodela*

229   *polyrhiza* growth rates were equal to each other and both were greater than the growth rate of

230   *Wolffia brasiliensis* (Figure 1). Only *W. brasiliensis* formed turions in this experiment. There

231   was a significant effect of nutrients ($F_{2,64} = 4.770$, $p = 0.012$), temperature ($F_{2,64} = 38.706$, p <

232   0.001), and significant interaction ($F_{4,64} = 4.089$, $p = 0.005$) on the turion production rate of *W.*

233 *brasiliensis* (Figure 2). At both 18 and 30 °C *W. brasiliensis* decreased turion production at the

234 highest nutrient level, but at 24 °C turion production increased with nutrient level (Figure 2).

235

236 *Experiment II: Response to nutrient stoichiometry*

237 Average RGR differed among species in four of the nine nutrient combinations (Table 3, Figure

238 3). Species differences were found whenever nitrogen was low (0.5 mg N L^{-1}) or when

239 phosphorus was low and nitrogen was medium (0.08 mg P L^{-1} and 5 mg N L^{-1}). Both *S.*

240 *polyrhiza* and *W. brasiliensis* formed turions in this experiment. *W. brasiliensis* formed turions at

241 all combinations of nitrogen and phosphorus, whereas *S. polyrhiza* only formed turions at low

242 nitrogen and medium and high phosphorus or low phosphorus and medium and high nitrogen

243 (Figure 4). At low nitrogen and medium phosphorus (ANOVA, $F_{1,9} = 51.62$, p <0.001), low

244 nitrogen and high phosphorus (ANOVA, $F_{1,10} = 49.91$, p < 0.001), and medium nitrogen and low

245 phosphorus (ANOVA, $F_{1,10} = 49.82$, p <0.001), *W. brasiliensis* had a greater turion production

246 rate than *S. polyrhiza* (Figure 4). Only at low phosphorus and high nitrogen both species had

247 equal turion production rates (ANOVA, $F_{1,6} = 6.64$, p = 0.042) (Dunn-Šidák adjusted critical p-

248 value 0.013)

249

250 *Floating plant richness and abundance in natural water bodies*

251 Most freshwater lakes and ponds in Connecticut and Long Island, NY did not have any floating

252 plants present (106 of 205, Table 4). Across all water bodies with floating plants present, a total

253 of seven taxa were found. *L. minor, S. polyrhiza*, and *Wolffia* spp. were the most common taxa,

254 occurring in 82, 47, and 42 of the 205 water bodies, respectively. The next most common

255 species, *L. trisulca,* only occurred in 4 lakes and ponds. Among water bodies with floating

256   plants present, the occurrence of different levels of species richness levels was non-random

257   (Table 4, Figure 5a, G-test, G = 6.909, df = 2, p < 0.031). Monocultures were more common than

258   expected and three- and four-species polycultures were less common than expected (Table 4,

259   Figure 5a).

260

261   Only twenty water bodies had floating plant cover greater than 66.67%. Among these water

262   bodies, there was no significant association between floating plant richness categories and the

263   frequency of occurrence (Table 4, Figure 5b, G-test, G = 2.430, df = 2, p = 0.7). Although not

264   statistically significant, water bodies dominated by floating plants tended to have three or more

265   species of floating plants; whereas, water bodies not dominated by floating plants tended to have

266   one or two species (Figure 5b). The results of these analyses did not change if a higher threshold

267   for floating plant dominance (e.g., 80% cover) was used or if the analysis was limited to small

268   water bodies (< 5 ha surface area) or water bodies with higher nutrients (total phosphorus > 0.02

269   mg P L^{-1}).

270

271   **Discussion**

272   In general, the floating plant species in this study exhibited differences (i.e., response diversity)

273   in their average growth rates across nitrogen, phosphorus, and temperature conditions.

274   Differences among species were typically seen under less favorable conditions (i.e., low

275   nutrients and low temperatures), whereas, species typically had similar growth rates under

276   conditions expected to be most favorable for their growth (i.e., high nutrients and high

277   temperature). When differences were detected, *Lemna minor* and *Spirodela polyrhiza* typically

278   grew at rates that were equal to each other and higher than *Wolffia brasiliensis*. On the other

279   hand, *W. brasiliensis* produced more resting bodies across most experimental conditions,

280   whereas *S. polyrhiza* only occasionally produced turions. *L. minor* never produced turions in

281   these experiments. This suggests a tradeoff between producing floating or sinking biomass under

282   these experimental conditions and may explain the lower relative growth rate of *W. brasiliensis*.

283   In the field, a floating plant-dominated state did not occur more frequently in water bodies with

284   higher floating plant richness, opposite to the expectation if response diversity is important for

285   formation of floating plant dominance.

286

287   The apparent tradeoff between growth and resting body production among floating plant species

288   may have important consequences for this functional group. The different strategies among

289   species can allow the floating plant functional group as a whole to have both rapid growth on the

290   water surface and insurance against perturbation via their resting bodies. Therefore, floating

291   plant polycultures may have a combination of strategies that may not be achievable by a single

292   species. For example, the floating plant functional group in a water body with both *L. minor* and

293   *W. brasiliensis* could have both faster growth at low nutrients or temperatures (due to the traits of

294   *L. minor*) and a greater number of resting bodies to re-colonize the water body at the start of a

295   growing season (due to the traits of *W. brasiliensis*). A polyculture of floating plant species and

296   their unique responses to environmental conditions may allow the functional group to attain

297   higher biomass or persist in a water body over a broader range of conditions.

298

299   Although, response diversity in the lab did not translate to a correlation between floating plant-

300   dominance and higher floating plant richness in the field, the response diversity may be relevant

301   for applied uses of these plants (e.g., wastewater treatment, biofuel production, and aquaculture).

302   While the optimal species or combination of species will depend on the particular application,

303   some general patterns exist. For example, if maximization of floating plant surface growth is the

304   sole objective (e.g., a highly controlled application where biomass is harvested), then either *L.*

305   *minor* or *S. polyrhiza* would be a better choice than *W. brasiliensis* because of their higher

306   growth rates. Future experiments would need to determine whether a combination of these two

307   species or a monoculture would be optimal. On the other hand, if formation of resting bodies was

308   a desirable property in the application (e.g., perennial, outdoor uses), then including *S. polyrhiza,*

309   *W. brasiliensis,* or another species that produces turions would be recommended. For some

310   applied uses of floating plants, other types of experiments need to be conducted. For example,

311   use in combined aquaculture systems with fish would require an understanding of the preference

312   and nutritional attributes of plant species with regards to the fish species.

313

314   The lack of a relationship between response diversity of this functional group and its ability to

315   become dominant may be due to a variety of factors. In the field, environmental variability may

316   outweigh the relationship between species richness and the formation of the floating plant state.

317   In addition to response diversity, other factors will influence the occurrence of the floating plant

318   state in the field. For example, water bodies in the northeast United States above a size threshold

319   ($\sim$ 5 ha) are rarely dominated by floating plants (McCann, *personal observation*). It is also

320   possible that the floating plant response diversity observed in the laboratory only has a small

321   effect on dominance in the field. Although species differences are quantifiable in the laboratory,

322   their magnitude may not be large enough to be detectable in the field. Furthermore, since

323   floating plant dominance is relative uncommon in this region (<10% of water bodies), there may

324   be low statistical power to detect a relationship between species richness and floating plant

325   dominance, especially if effect sizes are small.

326

327   It is also possible that response diversity is only important during transient circumstances or

328   conditions rarely encountered in these surveys. Therefore, the response diversity exhibited by

329   floating plant species in the lab will not determine whether this functional group is currently in a

330   dominant state in the field. As a result, species-rich water bodies could lose floating plant species

331   due to local extinction and still maintain floating plant dominance. Response diversity may also

332   help this functional group form a dominant state in other geographic regions where low

333   temperatures and low nutrients are more common (e.g., the Upper Midwest United States).

334   Rather than allowing floating plants to achieve dominance, the response diversity observed may

335   help this functional group persist in a water body, despite unfavorable conditions. Interestingly,

336   *L. minor,* which did not produce resting bodies in the lab but typically had the fastest growth rate

337   (along with *S. polyrhiza*), was the most common floating plant species in this region (present in

338   82 of 205 water bodies). Despite differences in growth rates and resting body production, *S.*

339   *polyrhiza* and *W. brasiliensis* occurred in a similar number of water bodies (47 and 42,

340   respectively).

341

342   The lower growth rates observed in Experiment II (Figure 3) relative to Experiment I (Figure 1)

343   may be due to the fact that the nutrient media was changed less frequently (every 3-4 days

344   compared to 2-3 days) and nutrient levels likely decreased to a greater extent between media

345   changes in Experiment II. Also, *S. polyrhiza* only produced turions in Experiment II. The

346   difference in media change frequency may have caused the difference in turion production

347   between experiments, or there may be differences based on the timing when plants were

348   collected from the field (Mid-August and mid-June for Experiments I and II, respectively).

349   Therefore, strict comparison of the growth rates or turion production between experiments

350   should not be done without consideration of the differences in experimental conditions.

351

352   There are few others studies of the response diversity of the floating plant functional group to

353   temperature, nutrients, or other environmental variables. Lüönd (1983) measured the response of

354   *L. minor, S. polyrhiza,* and two other species of *Lemna* to nitrogen and phosphorus at 25°C. All

355   species increased their growth rates in response to increases of both nutrients, as in this study,

356   and all species decreased their growth rate at extremely high nutrient levels (e.g., 1.75 g N L^{-1},

357   1.36 g P L^{-1}) (Lüönd 1983). The presence, but not the rate, of turion production was reported for

358   *S. polyrhiza.* Unfortunately, no statistical comparisons were made to determine if species had

359   unique responses under particular conditions (Lüönd 1983). Lemon et al. (2001) examined the

360   growth of *L. minor, S. polyrhiza*, and *W. borealis,* a cogener of *W. brasiliensis* at 24°C and very

361   high nutrients (33% v/v Hutner's medium, ~31 mg N L^{-1}, ~23 mg P L^{-1}), and found that *W.*

362   *borealis* has the highest growth rate, while *S. polyrhiza* has the lowest (in terms of frond number,

363   not area growth rate). Results of turion production were not reported (Lemon et al. 2001). Some

364   studies have examined response diversity to variables not included in this study. Floating plants

365   appear to have response diversity to pH (Hicks 1932, McClay 1976). *Lemna minor, Spirodela*

366   *oligorrhiza,* and *Wolffia arrhiza* all have a similar pH range (pH ~3 to 10), but their optimal pH

367   differs, from mildly acidic (*W. arrihiza,* pH 5.0 or *L. minor* pH 6.2) to neutral (*S. oligorrhiza,* pH

368   7.0) when grown in the lab at 25°C at very high nutrients (~241 mg N L^{-1} and ~32 mg P L^{-1})

369   (McClay 1976).

370

371   While this study was only able to examine a subset of all floating plant species under particular

372   combinations of environmental conditions, it found some conditions where species have

373   response diversity and others where species are redundant. Further studies, including a greater

374   number of species or clones and environmental variables, as well as determining tradeoffs

375   between responses (e.g., growth or resting bodies), are necessary to determine the full breadth of

376   response diversity of this functional group. For example, previous work on floating plant

377   performance under low temperature conditions (~10 °C) shows that species differ in their

378   minimum temperature (Landolt and Kandeler 1987), which may have important consequences

379   for growth of this functional group at the beginning and end of a growing season. While Ziegler

380   et al. (2015) examined the maximum relative growth rate of 39 clones of 13 species in all five

381   floating plant genera under a single set of nutrient temperature conditions, future research efforts

382   should systematically examine floating plant response diversity in a larger number of clones and

383   environmental conditions. Future work should also consider variability in environmental

384   conditions and species composition through space and time. Floating plants are expected to be

385   easily dispersed by waterfowl and other vectors (Barrat-Segretain 1996); therefore, species

386   composition in a waterbody may change through time, with possible consequences for floating

387   plant dominance.

388

389   **Conclusions**

390   This study has identified differences in three floating plant species common to the northeast

391   United States. Although species differences existed in the laboratory, there was no statistical

392   support that the species richness of floating plants increases their dominance in the field.

393 Although free-floating plants can be viewed as both a nuisance and an opportunity for applied

394 uses, understanding the species-specific responses of these plants to abiotic conditions is

395 essential for both management and applications.

396

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403

404 **Supplemental Information**

405 **Supplemental Information 1.** Raw data from experiment I.

406 **Supplemental Information 2.** Raw data from experiment II.

407 **Supplemental Information 3.** Converting from turion number to turion area

408 **Supplemental Information 4.** Analysis of growth rates using factorial ANOVAs.

409 **Supplemental Information 5.** Properties of 205 surveyed water bodies.

410

411

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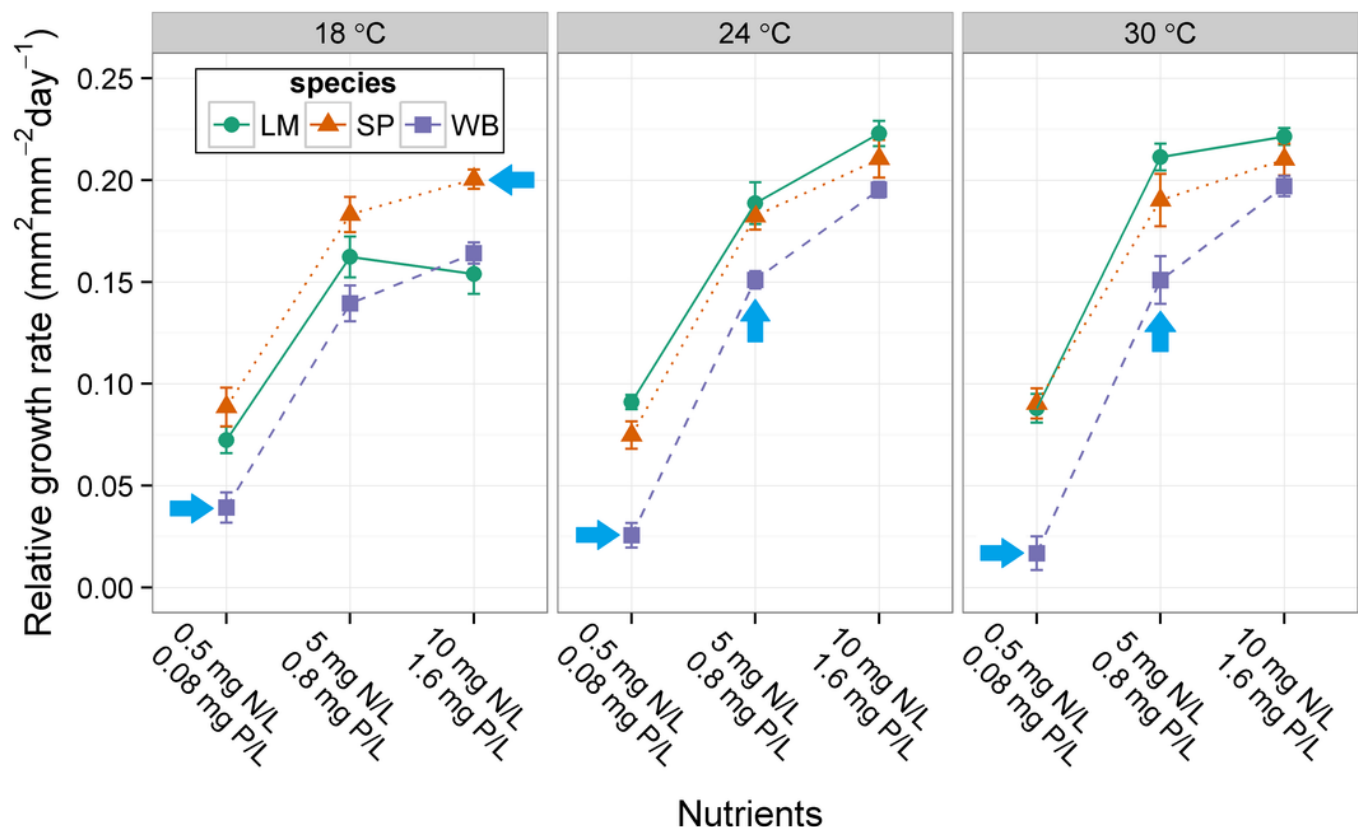
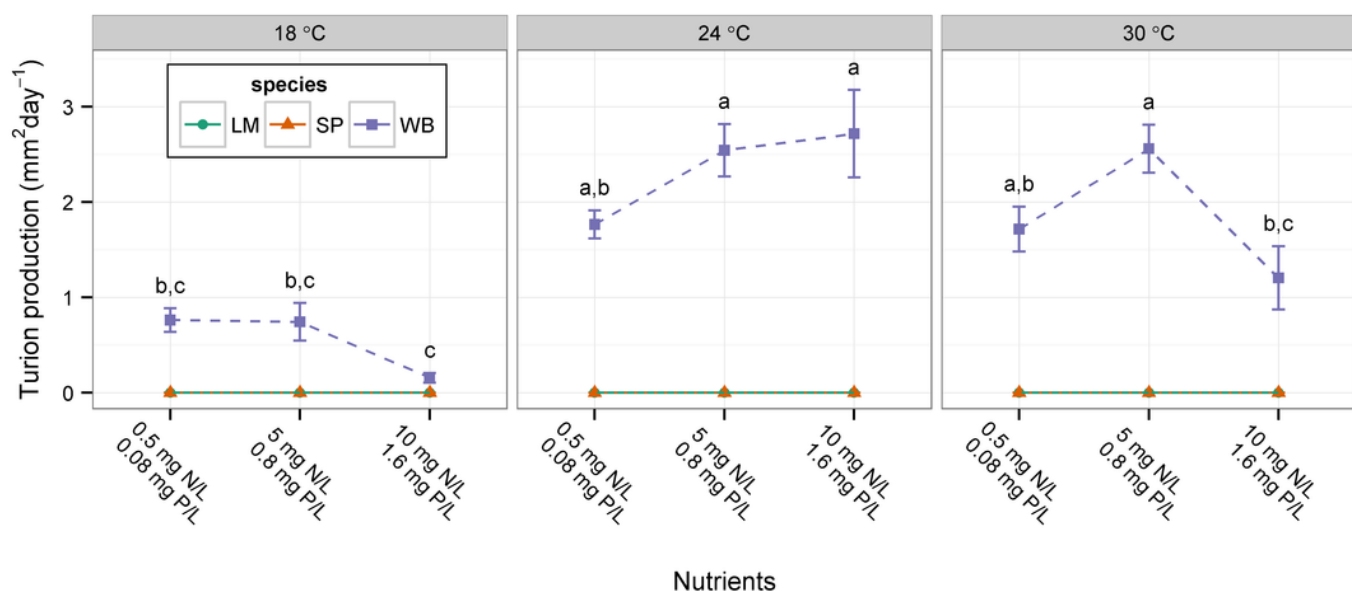
509

1

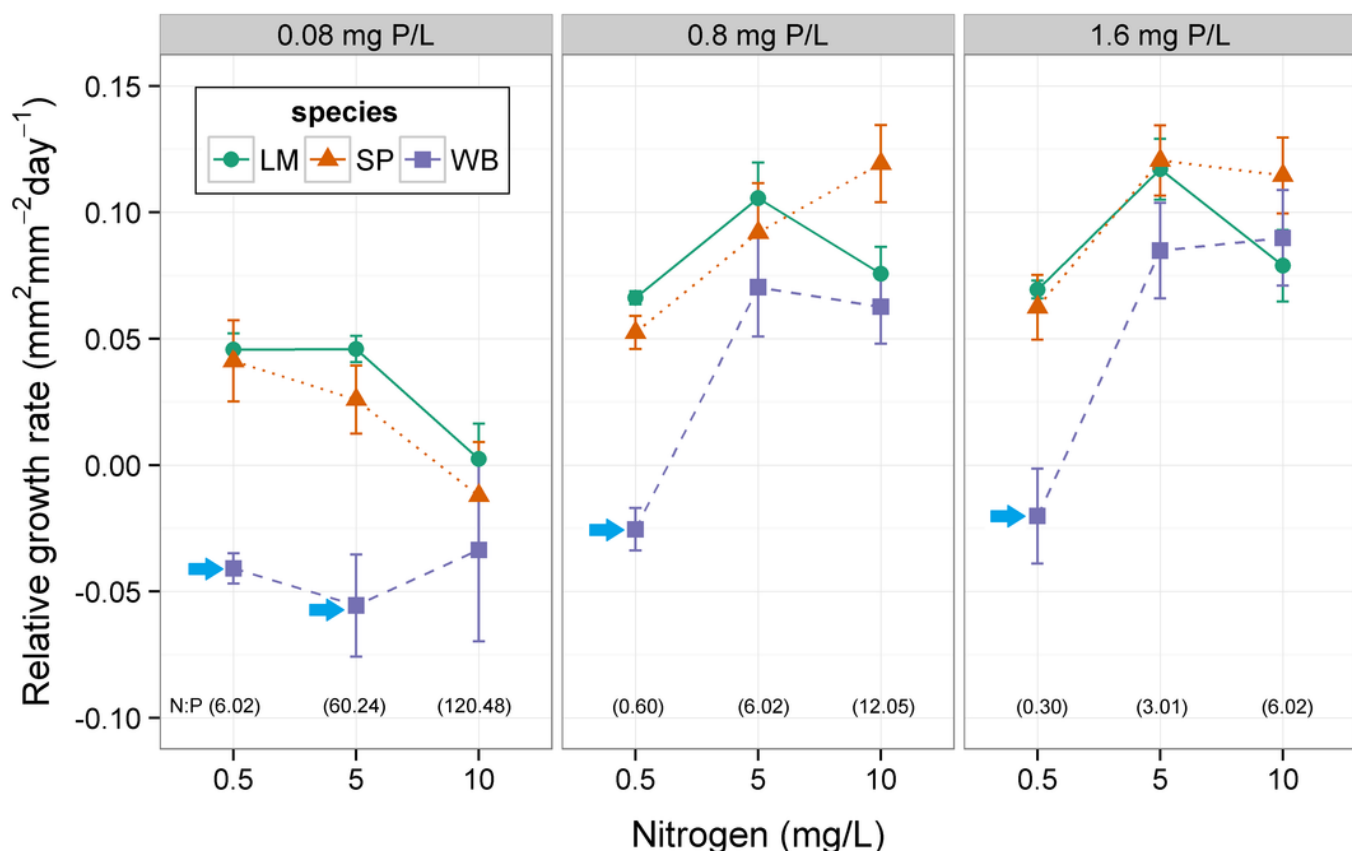
Effect of nutrients and temperature on relative growth rate (RGR) of three species of floating plants.

Error bars are standard errors. Post-hoc comparisons among species are for each response variable at each level of nutrients and temperature. Arrows indicate a species that is statistically different (Tukey's HSD p > 0.05) at a given nutrient and temperature level. LM = *Lemna minor*, SP = *Spirodela polyrhiza,* and WB = *Wolffia brasiliensis*

2

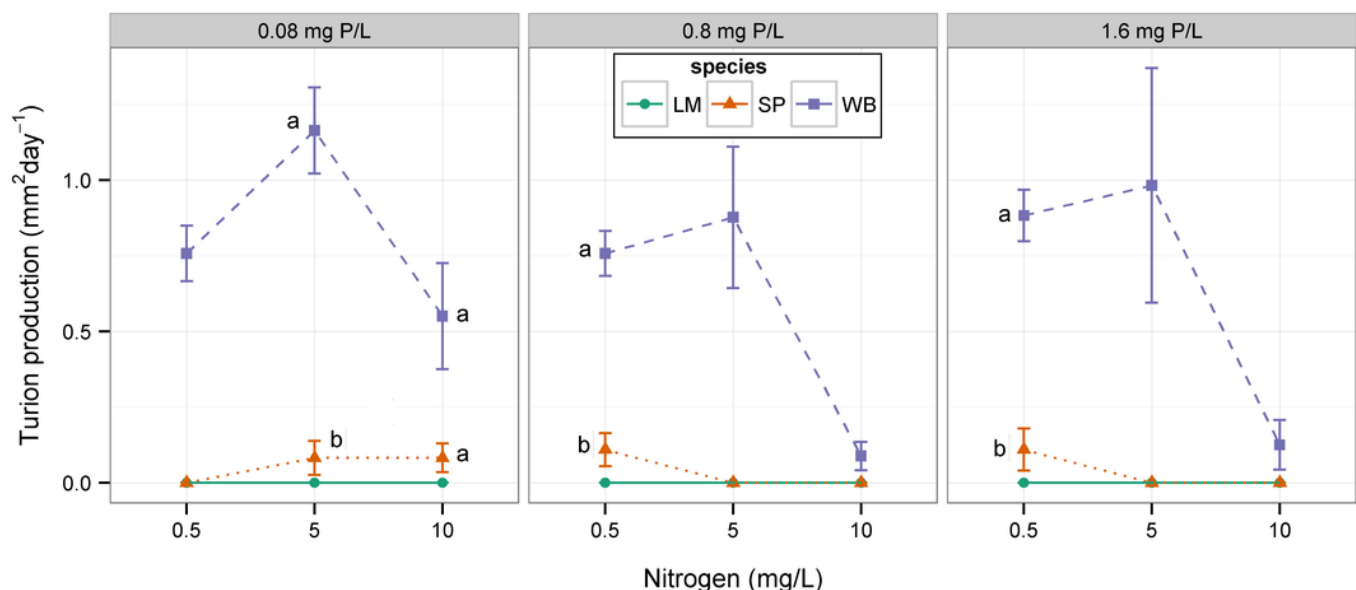
Effect of nutrients and temperature on resting body formation.

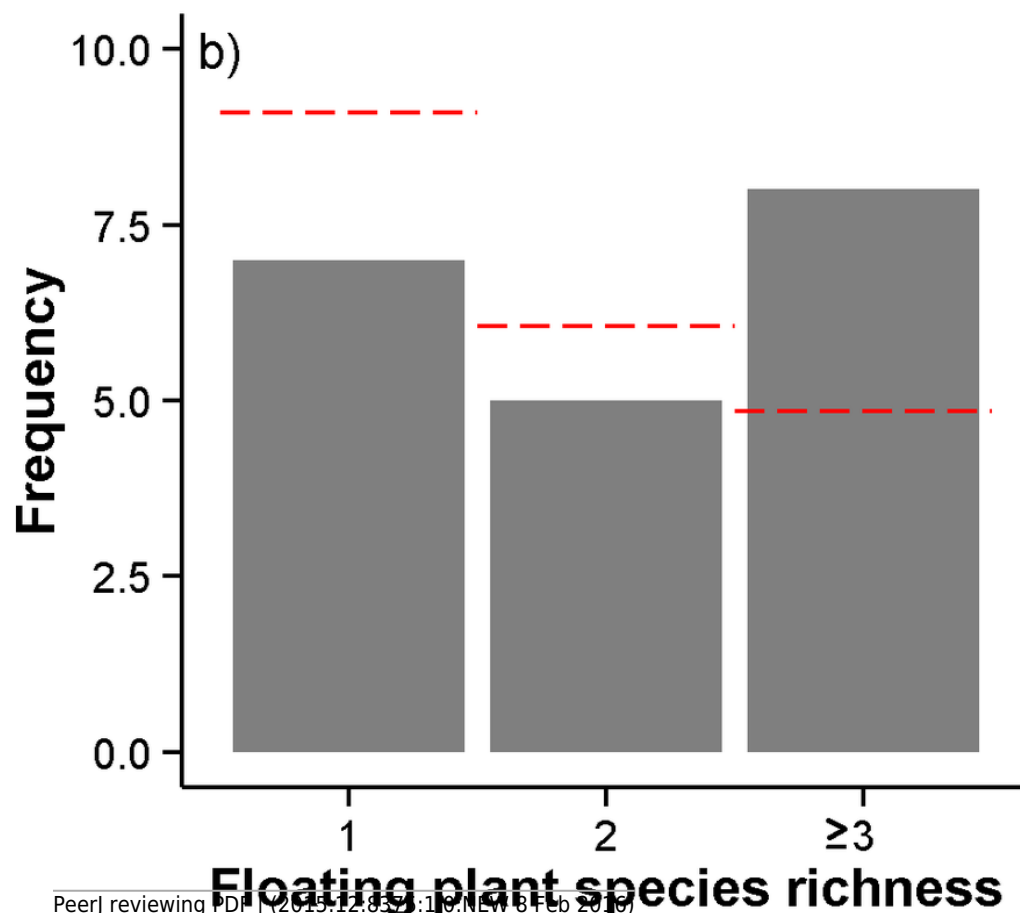
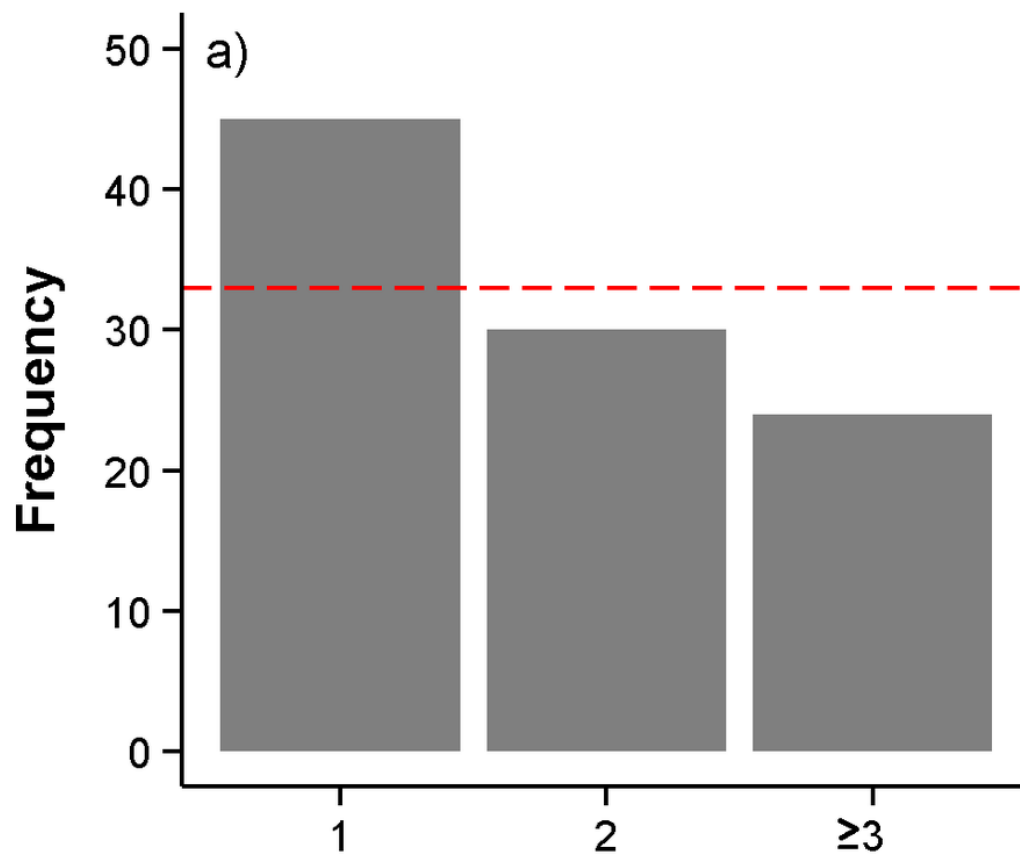
Error bars are standard errors. Shared letters indicate no difference between treatment levels (Tukey's HSD p > 0.05) for *W. brasiliensis* turion production. LM = *Lemna minor*, SP = *Spirodela polyrhiza*, and WB = *Wolffia brasiliensis*

3

Effect of nitrogen and phosphorus on relative growth rate (RGR) of three species of floating plants at 30 °C.

Error bars are standard errors. Post-hoc comparisons among species are for each response variable at each level of nitrogen and phosphorus. Arrows indicate a species that is statistically different (Tukey's HSD p > 0.05) at a given nutrient and temperature level. N:P ratios are indicated in parentheses above the horizontal axis. LM = *Lemna minor*, SP = *Spirodela polyrhiza*, and WB = *Wolffia brasiliensis*

4

Effect of nitrogen and phosphorus on turion formation of three species of floating plants.

When more both *L. minor* and *S. polyrhiza* produced turions at a particular treatment level, significant differences between those species are indicated by unique letters (Tukey's HSD, p>0.05). Error bars are standard errors. LM = *Lemna minor,* SP = *Spirodela polyrhiza,* and WB = *Wolffia brasiliensis*.

Floating plant species richness in lakes and ponds in Connecticut and Long Island, NY USA.

a) All water bodies with floating plants present (n = 99), and **b)** water bodies with floating plant cover >66.67% of the water surface (n = 20). Dashed lines indicate expected value if random

N:P mass ratios produced by nine combinations of nitrogen and phosphorus at 30 °C in Experiment II.

1   **Table 1.** N:P mass ratios produced by nine combinations of nitrogen and phosphorus at 30 °C in

2   Experiment II.

		Nitrogen (mg L^{-1})		
		0.5	5	10
Phosphorus (mg L^{-1})	0.083	6.02	60.24	120.48
	0.83	0.60	6.02	12.05
	1.66	0.30	3.01	6.02

One-way ANOVAs for the effect of species on the average relative growth rate (RGR) of floating plants at nine combinations of nutrients and temperature.

1 **Table 2.** One-way ANOVAs for the effect of species on the average relative growth rate (RGR)

2 of floating plants at nine combinations of nutrients and temperature.

Treatment		Average RGR	
Nutrients	Temperature (°C)	F-statistic	p-value
low	18	11.403	<0.001
	24	39.83	<0.001
	30	30.14	<0.001
medium	18	5.703	0.011
	24	7.172	0.004
	30	8.136	0.002
high	18	12.44	<0.001
	24	4.106	0.031
	30	4.325	0.027

4 Note: Degrees of freedom for all ANOVAs were 2 and 21, except for at low nutrients and 30°C,

5 where df = 2, 20. Dunn-Šidák adjusted critical p-value is 0.0057. Nutrient levels are low = 0.5

6 mg N L^{-1} and 0.083 mg P L^{-1}, medium = 5 mg N L^{-1} and 0.83 mg P L^{-1}, or high = 10 mg N L^{-1}

7 and 1.66 mg P L^{-1}.

One-way ANOVAs for the effect of species on the average relative growth rate (RGR) of floating plants at nine combinations of nitrogen and phosphorus at 30 °C.

1 **Table 3.** One-way ANOVAs for the effect of species on the average relative growth rate (RGR)

2 of floating plants at nine combinations of nitrogen and phosphorus at 30 °C.

Treatment		Average RGR	
Nitrogen	Phosphorus	F-statistic	p-value
low	low	21.24	<0.001
	medium	60.61	<0.001
	high	14.1	<0.001
medium	low	14.08	<0.001
	medium	0.985	0.396
	high	1.666	0.222
high	low	0.506	0.613
	medium	4.727	0.026
	high	1.283	0.306

4 Note: Degrees of freedom for all ANOVAs were 2 and 15. Dunn-Šidák adjusted critical p-value

5 is 0.005. Nitrogen levels are low = 0.5 mg N L^{-1}, medium = 5 mg N L^{-1}, and high = 10 mg N L^{-1}.

6 Phosphorus levels are low = 0.083 mg P L^{-1}, medium = 0.83 mg P L^{-1}, and high = 1.66 mg P L^{-1}.

The frequency of floating plant species compositions and the frequency of floating plant cover exceeding two-thirds of the surface area of freshwater lakes and ponds in Connecticut and Long Island, NY.

1   **Table 4.**The frequency of floating plant species compositions and the frequency of floating plant

2   cover exceeding two-thirds of the surface area of freshwater lakes and ponds in Connecticut and

3   Long Island, NY.

Floating plant species richness	Species composition	Frequency of occurrence	Frequency floating plant cover >66.67%
4	A, **LM**, **SP**, **W**	1	1
	LM, LV, **SP**, **W**	1	0
	LM, R, **SP**, **W**	1	0
3	**LM**, LT, **SP**	2	0
	LM, **SP**, **W**	18	7
	LT, **SP**, **W**	1	0
	All ≥3 species polycultures	24	8
2	A, **W**	1	1
	LM, **SP**	14	2
	LM, **W**	13	2
	SP, **W**	2	0
	All 2 species polycultures	30	5
1	A	1	1
	LM	32	4
	LT	1	0
	SP	7	0
	W	4	2
	All monocultures	45	7
0	None	106	0
	TOTAL	205	20

5   Note: A = *Azolla* sp., **LM** = *Lemna minor*, LT = *L. trisulca*, LV = *L. valdiviana*, R = *Riccia sp.*,

6   **SP** = *Spirodela polyrhiza*, **W** = *Wolffia* sp. Taxa used in the laboratory experiments are

7   indicated by bold letters.