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- 1 **Title**
- 2 **The invasive duckweed *Lemna minuta* Kunth displays a different light utilisation**
3 **strategy than native *Lemna minor* Linnaeus**
- 4 **Running Head**
- 5 **Light utilization in *Lemna minuta* and *Lemna minor***

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11

Highlights

- 12 • *L. minuta* and *L. minor* display distinct light utilisation strategies.
- 13 • *L. minuta* takes advantage of high light intensities
- 14 • *L. minor* limits the reduction of growth in shady conditions.

15

Abstract

16 *Lemna minuta* Kunth is an invasive, alien duckweed that is present throughout much of
17 Europe, where it competes with native congeneric *Lemna minor* Linnaeus. Previously, *L.*
18 *minuta* was found to grow faster than *L. minor*. The aim of this study was to determine
19 whether the rapid growth of invasive *L. minuta* is based on differential light utilisation. For
20 this purpose, the growth performance of *L. minuta* was compared with that of *L. minor* under
21 a range of different light intensities. Both physiological and morphological parameters were
22 determined. *L. minuta* showed a higher Relative Growth Rate (RGR) than *L. minor* when
23 grown under medium and high intensities. Further analysis showed that, at high light
24 intensities, *L. minuta* has a higher Net Assimilation Rate (NAR), and displays more
25 photochemical quenching (qP) and a higher quantum yield (Y(II)) than *L. minor*. In contrast
26 under low light intensities *L. minor* displayed a marginally higher RGR, due to a greater Leaf
27 Area Ratio (LAR), and higher chlorophyll content than *L. minuta*. The results indicate two
28 distinct light utilisation strategies, and reveal that the invasive species *L. minuta* takes more
29 advantage from high intensity light conditions. In turn, this may influence plant distribution,
30 and inform management strategies.

31

32 **Key words:** *Lemna minuta*; *Lemna minor*; invasiveness; growth rate; photosynthesis
33 efficiency; morphological adaptations; light utilisation strategies

34 INTRODUCTION

35 Invasive alien species pose a major threat to biodiversity and natural ecosystems worldwide
36 (Chornesky and Randall 2003). Aquatic ecosystems are particularly at risk from alien
37 invasive plants. These invasive aquatic plants can have substantial negative effects on
38 freshwater communities by decreasing the biodiversity of invertebrate, fish and native plant
39 species in aquatic systems (Zedler and Kercher 2004), and can affect water quality by altering
40 nutrient cycling and the microclimate of the water body (D'Antonio and Vitousek 1992).
41 Invasive plants can also negatively affect water-based recreational activities, water extraction
42 and shipping (Hussner 2012), and governments spend a considerable amount of money on
43 aquatic invasive species removal (Baars *et al.* 2011). Improved understanding of the
44 environmental conditions that facilitate excessive growth of invasive species may help focus
45 management on those ecosystems where a particular invasive species poses the most serious
46 threat to biodiversity.

47

48 *Lemna minuta* Kunth is native in temperate areas of North and South America (Stace 2010),
49 but alien in much of Europe. In Europe, *L. minuta* was first recorded in 1965 in France (Jovet
50 and Jovet - Ast 1966). Since, the species has spread widely and is now considered invasive in
51 northern European countries such as Belgium (Halford *et al.* 2011), and Germany (Hussner *et*
52 *al.* 2010), in eastern European countries such as Poland (Wójciak and Urban 2009) and
53 Hungary (Lukács *et al.* 2014), in Mediterranean countries such as Italy (Conti *et al.* 2005)
54 and Malta (Misfud 2010), and in western European countries such as Britain (Bramley *et al.*
55 1995) and Ireland (Lucey 2003). In Europe, *L. minuta* commonly co-occurs with the
56 congeneric species *Lemna minor* Linnaeus, which is native in Europe and Asia. Where *L.*
57 *minuta* and *L. minor* become dominant, they form floating mats which may have a negative
58 impact on wetland ecosystems by suppressing submerged macrophyte species (Janes *et al.*
59 1996). Experiments carried out in fully controlled conditions highlighted the ability of *L.*
60 *minuta* to outgrow *L. minor* in conditions of high nutrients availability (Njambuya *et al.*
61 2011, Paolacci *et al.* 2016). However, in a study carried out in Central Italy, Ceschin *et al.*
62 (2016) found that *L. minuta* was more abundant than *L. minor*, and dominant in mixed *Lemna*
63 populations, but the authors did not find a correlation between nutrients availability and
64 dominance of *L. minuta*. What determines the competition advantage of one species over
65 another is still unclear and probably the distribution pattern of the two species reflects the
66 interaction of several environmental factors. It is reasonable to hypothesise that the different

67 ability of the two species to take advantage from high nutrients availability can be extended
68 to other resources. In this study we have explored the role of light in facilitating the growth of
69 these two free floating freshwater species belonging to the family of Lemnaceae.

70

71 Irrespective of the ecological impacts of *L. minuta* on European water bodies, these species
72 can also be exploited as a model species to investigate the competition dynamics between
73 alien and native invasive aquatic plants. Lemnaceae are small, and easy to manipulate.
74 Moreover, comparisons with congeneric species are an effective method to study the
75 invasiveness of an alien species (Mack 1996). Closely-related species share many traits, and
76 therefore the identification of invasiveness-related traits, not shared between the two species,
77 is possible (Mack 1985). Nevertheless, it should be appreciated that “invasiveness” traits will
78 not comprehensively explain the success of an invasive species as such success is generally
79 due to the interaction of multiple environmental factors with a range of intrinsic traits
80 (Richardson and Pyšek *et al.* 2006).

81

82 The focus of this study is to determine if the success of invasive *L. minuta* over native *L.*
83 *minor* can be explained, in part, by differences in light utilisation. Light is a key-factor for
84 plant growth, and its capture and utilisation plays an important role in determining the
85 relative success of one species over another. Different species have evolved different
86 adaptations to optimise growth and photosynthesis in environments with, for example, low or
87 high light availability. In general, plants more adapted to high levels of direct sunlight are
88 called heliophilous, while plants that thrive at low light levels are called sciophilous. Plants
89 that are adapted to intermediate light levels are called mesic (Hallé 1978). Sciophilous and
90 heliophilous species achieve the ability to thrive at a particular light level by adopting
91 different light capture and utilisation strategies (Valladares and Niinemets 2008). For
92 example, plants grown at high light intensities typically have a different leaf morphology than
93 plants grown at low light intensities (Boardman 1977). Heliophilous plants have usually
94 smaller, but thicker leaves with more palisade and spongy mesophyll layers (Boardman 1977;
95 Gratani & Ghia 2002; Zaragoza-Castells *et al.* 2008). In contrast, shade plants often have thin
96 leaves with a lower weight per leaf area. Prevailing light intensities also determine the
97 photosynthetic capacity (Boardman 1977). For example, the light intensity under which
98 plants are grown influences pigment content and photochemical efficiency (Boardman 1977,
99 Demmig and Björkman 1987, Valladares and Niinemets 2008). Fluorescence analysis is used
100 to non-destructively investigate the photosynthetic efficiency of plants. Measurements of

101 photochemical and non-photochemical quenching can reveal energy transfer processes as
102 well as energy dissipation (Maxwell and Johnson 2000). Differences in the fluorescence
103 emission can be used to identify differences in photosynthetic activity of sun and shade plants
104 (Lichtenthaler *et al.* 1981). Plants adapted to high light intensities can present higher rates of
105 photosynthetic light quanta conversion and a higher photosynthetic capacity on a chlorophyll
106 and chloroplast basis (Boardman 1977). On the other hand, plants adapted to low levels of
107 light usually present higher chlorophyll content per unit of biomass as this allows them to
108 maximize the light harvesting (Valladares and Niinemets 2008). Therefore the analysis of
109 pigment content is another useful tool for characterisation of shade and light plants.

110 Previous studies demonstrated that light can impact on the ability of invasive species to
111 outcompete native species (e.g. Madsen *et al.* 1991). Moreover, it was observed that the light
112 saturation point, as well as the ability to grow at low light intensity, differ between duckweed
113 species (Landolt, 1986). The underlying mechanisms have not yet been identified. In the
114 present study we assessed the performance of *L. minor* and *L. minuta* at a range of light
115 levels. **The aim of the study was to determine whether the ability of *L. minuta* to outperform**
116 ***L. minor* is based on its higher ability to take advantage of intense light.** Both physiological
117 and morphological parameters, such as RGR, NAR, chlorophyll content and photosynthetic
118 efficiency, were measured and analysed.

119 MATERIAL AND METHODS

120 Cultivation plant stocks

121 The strains of *L. minor* and *L. minuta* used for this experiment were collected in Blarney, Co.
122 Cork, Ireland. The *L. minor* strain has since been registered in the RDSC database as strain
123 number 5500 “Blarney”. In a preliminary experiment nine different clones of *L. minuta* and
124 nine clones of *L. minor* were grown at high and low light and their light response was
125 analysed in terms of RGR and chlorophyll content. The clones were collected in different
126 regions of Ireland and clones belonging to the same species showed similar behaviours. It
127 was concluded that one clone per species was representative of the Irish ecotypes. The plants
128 were cultured under sterile conditions, in glass flasks, on 100 ml of half-strength Hutner's
129 nutrient solution (Hutner 1953). Plants were kept in a growth room at a constant temperature
130 of 20°C and exposed to a light intensity of 40 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, (cool-white fluorescent tubes)
131 with a light: dark cycle of 16: 8 hours.

132 **Experimental conditions**

133 Plants were grown in Petri dishes without a cover lid, containing 50 ml of half strength
134 Hutner's medium. The different light intensities were obtained by placing the plant at
135 different distances from a LED light source characterized by low heat emission (AP67 R-
136 series, Valoya Finland). The experiment was carried out at 20°C with a light: dark cycle of
137 16: 8 hours. When necessary, distilled water was added to the Petri dishes during the
138 experiment to compensate for evaporation. *L. minuta* and *L. minor* were grown at 6, 10, 20,
139 30, 42, 93, 150, 250, 400 and 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. These intensities are representative of the
140 natural range that can be measured in Lemna-habitats with different levels of canopy shade.
141 Each replicate started with 9 fronds (4.62 ± 0.87 mg fresh weight on average for *L. minuta* and
142 11.32 ± 1.14 mg fresh weight on average for *L. minor*). The experiment lasted one week and
143 each treatment was replicated 4 times. Given the rapid growth of the species, after one week
144 the bulk of the *L. minor* fronds would have developed under the imposed experimental
145 conditions.

146 **Measured end-points**

147 After one week of growth, plants were harvested and the biomass and frond area were
148 measured. The relative growth rate (RGR) based on fresh biomass was calculated using the
149 formula by Connolly and Wayne (1996):

150

$$151 \text{RGR} = \ln(Y_f / Y_i) / t$$

152

153 Where Y_i is the initial biomass or the initial number of fronds, Y_f is the final biomass or final
154 number of fronds, t is the time in days and \ln is the natural logarithm.

155

156 Frond area was measured using the Image-J software and the Leaf Area Ratio (LAR) and Net
157 Assimilation Rate (NAR) were calculated. The LAR was calculated according to Radford
158 (1967):

$$159 \text{LAR} = \text{Leaf area per plant} / \text{Plant weight}$$

160 The NAR was calculated according to Williams (1946):

$$161 \text{NAR} = [(W_2 - W_1) / T] \cdot [(\ln A_2 - \ln A_1) / A_2 - A_1]$$

162 Where W_2 is the final biomass, W_1 is the initial biomass, T is the time in days, A_2 is the final
163 area and A_1 is the initial area.

164 Before determination of the biomass, photosynthetic characteristics of fronds grown at
165 different light intensity were analysed using pulse amplitude modulated chlorophyll *a*
166 fluorometry (Schreiber *et al.* 1986) (WALZ Imaging fluorometer, Effeltrich, Germany).
167 Chlorophyll *a* fluorescence analysis was carried out on plants dark adapted for 15 minutes.
168 Three colonies were analysed for each of the 4 replicates. In each colony three different
169 fronds were randomly chosen for analysis. The three values measured for each colony were
170 averaged and, considered as one replicate (% variance of measurements within the same plant
171 never exceeded 1%). The steady state yield (Y(II)), photochemical quenching (qP) and non-
172 photochemical quenching (qN) were measured following exposure to different actinic light
173 intensities, ranging between 0 and 701 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Plants were exposed to each actinic light
174 intensity for 40 seconds. The fluorescence parameters were calculated using the following
175 formula (Maxwell and Johnson 2000):

176
$$Y(\text{II}) = (F'm - F) / F'm$$

177
$$qP = (F'm - F) / (F'm - F'o)$$

178
$$Fv/Fm = (Fm - Fo) / Fm$$

179
$$qN = (Fm - F'm) / (Fm - F'o)$$

180 The terminology used in the IMAGING-PAM M-series Chlorophyll Fluorometer manual
181 (Heinz Walz GmbH, 2014) was adopted.

182 The chlorophyll content of fronds was also determined at the end of the experiment,
183 according to the method of Inskip and Bloom (1985). In short, the biomass was suspended
184 in N,N-dimethylformamide, the absorbance was measured using a spectrophotometer
185 Thermo, model Genesys 10-S and the total chlorophyll content was calculated using the
186 formula:

187
$$\text{Total Chlorophyll} = 17.90 \cdot A_{647} + 8.08 \cdot A_{665}$$

188

189 where A_{647} and A_{665} are, respectively, the absorbance at the wavelengths of 647 and
190 665nm. The total chlorophyll content was normalised versus fresh biomass.

191 Chlorophyll *a* and chlorophyll *b* ratio was also calculated using the formula:

$$192 \text{ Chl.}a/\text{Chl.}b = \frac{(12.70 \cdot A665) - (2.79 \cdot A647)}{(20.70 \cdot A647) - (4.62 \cdot A665)}$$

193 Data analysis

194 The statistical analysis was conducted using IBM- SPSS statistic data editor. A two-way
195 ANOVA was conducted in order to examine the differences between the two species on
196 RGR, LAR, NAR and chlorophyll content when grown at different light intensities. The
197 differences in Y(II), qP and qN at different actinic light and between species were analysed
198 using a 2-way repeated measures ANOVA. When a statistically significant interaction
199 between species and treatments was found, an analysis of simple main effects was performed
200 with statistical significance receiving a Bonferroni adjustment.

201 In order to study the light saturation, a nonrectangular hyperbola was fitted to model the light
202 response of RGR using R software (R i386 3.3.3). The expression of the model used
203 (Thornley 1976; Fang *et al.* 2015) was:

$$204 \text{ RGR}(I) = \frac{\alpha I + \text{RGRmax} - \sqrt{(\alpha I + \text{RGRmax})^2 - 4I\alpha\theta \text{RGRmax}}}{2\theta} - R_d$$

205 Where α is the initial quantum efficiency, RGRmax is the light-saturated relative growth rate,
206 θ is the convexity (curvilinear angle) of the nonrectangular hyperbola, R_d is the dark
207 respiration rate, and I is the light intensity. The RGR values calculated on the basis of the
208 biomass were used to fit the light-response curve under different light intensities. α , θ , R_d ,
209 and RGRmax were determined by the trend of the measured light-response curve.

210 RESULTS

211 Relative Growth Rate (RGR) as a function of light intensity

212 The RGR was calculated from the time dependent increase in biomass. For both species RGR
213 increased with increasing light intensity (fig.1), with a minimum RGR at the lowest intensity
214 of $6 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a maximum RGR at the highest intensity of $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. There
215 was a significant interaction effect between species and light intensity (tab.1). A comparison
216 of the 2 species revealed that *L. minuta* had a significantly higher RGR than *L. minor* at 90,
217 150, 250, 400 and at $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. In contrast, at 6, 10, and $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ the
218 differences in the RGR of the two species were not statistically significant (fig. 1).

219 Both species appeared to have reached light saturation, but to test for this, data were fitted in
220 a nonrectangular hyperbola model. The applied model fitted the light response curve of *L.*
221 *minuta* and *L. minor* very well. **The nonrectangular hyperbola indicates that full light**
222 **saturation was achieved by the two species at 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.**
223

224 **Changes in LAR and NAR at different light intensities**

225 The total frond area was measured in order to calculate the LAR and NAR of the two species
226 at all the light intensities tested. There was a significant interaction between species and light
227 intensity in determining both LAR and NAR (tab.1). In general, the LAR decreased with
228 increasing light intensity (fig. 3a). Both *L. minuta* and *L. minor* reached a maximum LAR at a
229 light intensity of 6 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and displayed a minimum LAR at 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. At 6
230 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, *L. minor* displayed a significantly higher LAR than *L. minuta*, while, at higher
231 light intensities the difference between the LAR of the two species decreased progressively.
232 At the highest light intensities tested the species displayed a very similar LAR.

233 At low and medium light intensities the two species had a similar, low NAR (Fig.3b).
234 Between 30 and 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ an increase in the slope of NAR versus light intensity was
235 observed, while at intensities above 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ NAR appeared to have reached
236 saturation. At the highest light intensities, *L. minuta* had a higher NAR than *L. minor*. This
237 difference was significant at 250, 400 and at 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

238 **Chlorophyll content as a function of light intensity**

239 The analysis of the total chlorophyll content per unit of biomass showed a decrease of the
240 plant pigment content with increasing light intensity in both species (fig. 4). The maximum
241 chlorophyll content was reached at the lowest light intensity and the minimum content was
242 observed at the highest intensity. There was not a significant interaction between light
243 intensity and chlorophyll content. *L. minor* had a higher chlorophyll content than *L. minuta* at
244 every light intensity tested ($p < 0.01$ for the overall difference). The results of the pairwise
245 comparison are shown in figure 4.

246 **The chl.a/chl.b ratio did not change significantly at different light intensities and there was**
247 **not a significant difference between the two species (data not shown).**

	df	f	p
--	----	---	---

RGR	Species	1	63.703	0.000
	Light intensity	9	306.703	0.000
	Species*light intensity	9	9.579	0.000
LAR	Species	1	165.871	0.000
	Light intensity	9	75.554	0.000
	Species*light intensity	9	8.013	0.000
NAR	Species	1	12.982	0.001
	Light intensity	9	120.479	0.000
	Species*light intensity	9	4.799	0.000

248 Table 1. Summary of 2-way ANOVAs for effects of species, light intensity and their
249 interaction, on Relative growth Rate (RGR), Leaf Area Ratio (LAR) and Net Assimilation
250 Rate (NAR).

251 Chlorophyll *a* fluorescence of plants raised under different light intensities

252 The quantum yield of photosystem II (Y(II)) is a good indicator of the efficiency of the
253 photosynthetic light reactions, under steady-state conditions. Y(II) depended both on the light
254 intensity during growth, as well as on the intensity of the actinic light increased . When the
255 two species were grown at a low light intensity (6, 10 and 20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), Y(II) decreased
256 fast with increasing actinic light intensity during the actual measurements. Y(II) reached
257 saturation values close to 0 at an actinic PAR intensity of 186 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. In the case of
258 fronds of *L. minor* and *L. minuta* raised under intermediate light levels, Y(II) decreased less
259 drastically and displayed a long tail that reached saturation only at an actinic light level of
260 701 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. When the two species were raised under the highest light intensities, *L.*
261 *minuta* still displayed this tail of low Y(II) values, but this was not the case for *L. minor*.
262 When the plants were grown at 6 and 10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, there was a significant interaction
263 between species and Photosynthetic Available Radiation (PAR) (tab.2). At these intensities,
264 *L. minuta* showed a significantly higher Y(II) than *L. minor* at PAR=0 and 1. The interaction
265 between species and PAR was also significant when the plants were grown at 1000
266 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (tab.2). At this intensity, the difference between *L. minuta* and *L. minor* was not
267 statistically significant.

Y(II) in plants grown at 6 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$				
		df	F	Sig.
PAR	Sphericity Assumed	12	119.452	0.000
	Greenhouse-Geisser	1.182	119.452	0.001
	Huynh-Feldt	1.501	119.452	0.000
	Lower-bound	1	119.452	0.002
species	Sphericity Assumed	1	0.401	0.572
	Greenhouse-Geisser	1	0.401	0.572
	Huynh-Feldt	1	0.401	0.572
	Lower-bound	1	0.401	0.572
PAR * species	Sphericity Assumed	12	33.585	0.000
	Greenhouse-Geisser	1.033	33.585	0.009
	Huynh-Feldt	1.083	33.585	0.008
	Lower-bound	1	33.585	0.01
Y(II) in plants grown at 10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$				
		df	F	Sig.
PAR	Sphericity Assumed	12	267.038	0.000
	Greenhouse-Geisser	1.383	267.038	0.001
	Huynh-Feldt	3.484	267.038	0.000
	Lower-bound	1	267.038	0.004
species	Sphericity Assumed	1	7.764	0.108
	Greenhouse-Geisser	1	7.764	0.108
	Huynh-Feldt	1	7.764	0.108
	Lower-bound	1	7.764	0.108
PAR * species	Sphericity Assumed	12	171.179	0.000
	Greenhouse-Geisser	1.583	171.179	0.001
	Huynh-Feldt	6.583	171.179	0.000
	Lower-bound	1	171.179	0.006
Y(II) in plants grown at 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$				
		df	F	Sig.
PAR	Sphericity Assumed	12	49.608	0.000
	Greenhouse-Geisser	1.944	49.608	0.000
	Huynh-Feldt	5.469	49.608	0.000
	Lower-bound	1	49.608	0.006
species	Sphericity Assumed	1	0.704	0.463
	Greenhouse-Geisser	1	0.704	0.463
	Huynh-Feldt	1	0.704	0.463
	Lower-bound	1	0.704	0.463
PAR * species	Sphericity Assumed	12	19.987	0.000
	Greenhouse-Geisser	1.091	19.987	0.017
	Huynh-Feldt	1.237	19.987	0.012
	Lower-bound	1	19.987	0.021

269 **Table 2. Summary of 2-way repeated ANOVAs for effects of species, Photosynthetic Actinic**
 270 **Radiation (PAR) and their interaction, on quantum yield (Y(II)).**

271 Non-photochemical quenching, qN, increased following exposure to low and intermediate
 272 levels of actinic light and then stabilized under higher actinic light levels. When the two
 273 species had been grown at high light intensities, high qN levels were already induced by
 274 relatively low levels of actinic light. However, *L. minuta* displayed a significantly lower qN
 275 than *L. minor* (overall $p < 0.01$) when grown at 400 and 1000 $\mu\text{mol}\cdot\text{photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. When the
 276 two species were grown at just 6, 10 or 20 $\mu\text{mol}\cdot\text{photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *L. minuta* had a higher qN
 277 at every actinic light level (overall $p < 0.01$)

278 The curves describing the photochemical quenching qP of the two species show a decrease in
 279 qP with increasing intensity of the actinic light during the fluorescence measurements.
 280 Decreases in qP were very similar when the plants were grown at low and medium light
 281 intensities (from 6 to 250 $\mu\text{mol}\cdot\text{photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Only when plants were grown at 400 and
 282 1000 $\mu\text{mol}\cdot\text{photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, a significant interaction between species and PAR was found. At
 283 these light intensities, *L. minuta* maintained a significantly higher qP (overall $p < 0.01$) than *L.*
 284 *minor* at actinic light intensities above 186 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

qP in plants grown at 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$				
		df	F	Sig.
PAR	Sphericity Assumed	12	349.378	0.000
	Greenhouse-Geisser	2.281	349.378	0.000
	Huynh-Feldt	9.91	349.378	0.000
	Lower-bound	1	349.378	0.000
species	Sphericity Assumed	1	2.849	0.19
	Greenhouse-Geisser	1	2.849	0.19
	Huynh-Feldt	1	2.849	0.19
	Lower-bound	1	2.849	0.19
PAR * species	Sphericity Assumed	12	310.784	0.000
	Greenhouse-Geisser	1.987	310.784	0.000
	Huynh-Feldt	5.871	310.784	0.000
	Lower-bound	1	310.784	0.000
qP in plants grown at 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$				
PAR	Sphericity Assumed	12	28.252	0.000
	Greenhouse-Geisser	1.736	28.252	0.002
	Huynh-Feldt	3.914	28.252	0.000
	Lower-bound	1	28.252	0.013
species	Sphericity Assumed	1	0.854	0.423
	Greenhouse-Geisser	1	0.854	0.423
	Huynh-Feldt	1	0.854	0.423

	Lower-bound	1	0.854	0.423
PAR * species	Sphericity Assumed	12	8.842	0.000
	Greenhouse-Geisser	1.137	8.842	0.049
	Huynh-Feldt	1.369	8.842	0.036
	Lower-bound	1	8.842	0.059

285 **Table 3. Summary of 2-way repeated ANOVAs for effects of species, Photosynthetic Actinic**
286 **Radiation (PAR) and their interaction, on quantum photochemical quencing (qP).**

287 **DISCUSSION**

288 Light is a necessity for the autotrophic growth of Lemnaceae. However, the relationship
289 between growth and light-intensity is species, and even clone, specific, while environmental
290 factors such as temperature, nutrient and CO₂ supply can also alter this relationship (Landolt
291 1986). Wedge and Burris (1982) observed that the light saturation intensity for growth of *L.*
292 *minor* ranges between 300 and 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, depending on temperature. For *L. minuta*,
293 the only data available are those of Landolt (1986) who found that at 323 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$
294 (published as 17000 lux) light saturation was not yet achieved. In the present study small
295 increases in RGR were found at the high light intensities tested. The model developed on the
296 basis of the results observed (e.g. Givnish *et al.* 2004) indicates that full light saturation was
297 achieved by the two species at 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

298

299 The comparison of the growth of the two *Lemna* species suggests that *L. minor* is better
300 adapted to shade conditions (Givnish 1988) while *L. minuta* takes more advantage from high
301 light intensities. This hypothesis is supported by the comparison of other parameters. LAR
302 and NAR are often measured to analyse variations in plant growth (Lambers *et al.* 1989;
303 Poorter and Remkes 1990). At high light intensities *L. minuta* had a higher NAR than *L.*
304 *minor*. This intrinsic ability to exploit high light levels was associated with a higher RGR. *L.*
305 *minor* displayed a higher LAR in shady conditions, while, under high light conditions the two
306 species had similar values of LAR. The latter observation indicates that *L. minor* has a higher
307 morphological plasticity in response to changing light conditions. A more extensive leaf area
308 represents an advantage at low light (Lusk 2004), thus the observed morphological plasticity
309 is likely to contribute to the slightly higher RGR of *L. minor* in shady conditions. In several
310 studies LAR was recognised as the growth parameter that has the greatest impact on the RGR
311 (e.g. Poorter and Remkes 1990; Walters *et al.* 1993; Wright and Westoby 2000), although, in
312 other studies, NAR was the factor most closely correlated with RGR (e.g. Shipley 2002).
313 Conflicting literature might depend on several factors such as the species investigated and the

314 experimental conditions. For example Garnier (1991) found that there is a difference in the
315 extent to which NAR impacts on the RGR between monocotyledonous and dicots. Another
316 hypothesis proposed by Poorter (1999) is that LAR and NAR affect the RGR to different
317 extents depending on the light intensity at which the experiment is carried out. In particular,
318 the author hypothesized that, at low light, the scope for variation in photosynthetic activity
319 between species is diminished and therefore LAR plays a relatively important role in
320 determining the RGR, as it was observed in this study. Vice versa, at high light intensities,
321 NAR has a relatively greater impact on the plant growth, as it was demonstrated in this study
322 by the observed high values for NAR and RGR for *L. minuta*. This explanation is also
323 confirmed by Shipley (2006). The author reviewed 37 studies on 614 different species finding
324 that NAR was the best predictor of variation in RGR in herbaceous species. However, for
325 determining RGR, the importance of NAR decreased with decreasing daily quantum input.
326 Thus, the data in this paper reveal distinct light utilisation strategies for *L. minuta* and *L.*
327 *minor*, with the latter species performing better at low light, due to its higher LAR, while the
328 former species performs better at high light intensities due a higher NAR.

329

330 To further explore the light-intensity dependency of growth, various photosynthetic
331 parameters were measured. This study showed an inverse correlation between light and
332 chlorophyll content. A similar correlation has been observed in numerous studies using a
333 broad range of species (e.g. Eilam and Klein 1962; Minotta and Pinzauti 1996; Cao 2000; Dai
334 *et al.* 2009). Indeed, plant responses to varying light intensities are commonly reported as
335 changes in chlorophyll concentration (Strauss-Debenedetti and Bazzaz 1991). At high light
336 intensities the reduction in chlorophyll content is considered an acclimation to avoid light
337 damage due to over-excitation (Havaux and Tardy 1999), and specifically photo-oxidation
338 (Hendry and Price 1993). Conversely, at low light intensities, the increase in chlorophyll
339 content helps maximise light capture (Kura-Hotta *et al.* 1987, Lei *et al.* 1996). Higher
340 chlorophyll content is usually associated with shade-tolerance (Valladares and Niinemets
341 2008; Lewandowska and Jarvis 1977; Leverenz 1987; Thompson *et al.* 1988; Rijkers *et al.*
342 2000; Cao 2000). Hence, we conclude that the higher chlorophyll content in *L. minor*
343 confirms its adaptation to more shady conditions.

344 Chlorophyll *a* fluorometry was used to explore the mechanisms underlying differences in
345 RGR and NAR. The photosynthetic yield (Y(II)) was measured at a range of actinic light
346 intensities and provides an indication of the photochemical efficiency of photosystem II

347 (Maxwell and Johnson 2000). When plants were raised under low light conditions (from 6 to
348 $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), Y(II) displayed a rapid initial decline with increasing actinic light during
349 the measurements. In contrast, in plants raised under intermediate and high light conditions,
350 the decline in Y(II) with increasing actinic light occurred at higher intensities and more
351 gradually. This suggests that plants that had acclimated to higher light levels were able to use
352 a higher portion of the absorbed light for the photosynthetic process. The measurements of
353 the photochemical quenching qP confirmed this ability of plants grown under high light
354 intensities. The photochemical quenching is a measure of the fraction of PSII reaction centres
355 that are in the open state (Krause and Weis 1991). In this study the decrease of qP in plants
356 grown at higher light intensities occurred at higher actinic levels than plant grown at low light
357 intensities. The comparison of qP and Y(II) between the two species revealed a different
358 ability to cope with both low and high actinic light levels. The higher qP of *L. minuta* when
359 fronds were raised under high light intensities, suggests a higher capacity photosynthetic light
360 reactions to utilise photons at the highest light intensities. This conclusion is reinforced by a
361 slightly higher Y(II) observed in *L. minuta* grown at high light intensities. The qP data concur
362 with the higher NAR and RGR of *L. minuta* raised under high light intensities, and indicate
363 that at least part of the capacity for growth under high light is associated with adaptive
364 responses at the level of the photosynthetic machinery. Conversely, the data suggest that the
365 performance of *L. minor* in the shade is more dependent on morphological (higher LAR) than
366 on physiological (lower Y(II), qP and NAR) parameters.

367 Non-photochemical quenching, qN, was also analysed. This parameter refers to the portion of
368 the energy absorbed that the plant dissipates as heat (Müller *et al.* 2001). Both species
369 increased the extent of non-photochemical quenching when exposed to higher actinic light
370 levels, demonstrating a capability to adjust photosynthetic performance to prevailing light
371 conditions. A comparison of the two species showed that *L. minor* had a higher qN value than
372 *L. minuta* when the plants were grown at high light intensities. A higher qN might be a
373 necessity for *L. minor* as a result of its relatively high light capture caused by high
374 chlorophyll content. The higher portion of energy dissipated in the form of heat is generally
375 expected to be associated with decreased RGR (Laing *et al.*, 1995), as was observed for *L.*
376 *minor*. In contrast, *L. minuta* had a lower qN value, which is associated with both a higher qP
377 and Y(II), and therefore ultimately a higher NAR.

378 The aim of this study was to determine whether light intensity is a factor enabling the
379 invasive duckweed *L. minuta* to outperform the native *L. minor*. The results show that the
380 invasive species *L. minuta* takes better advantage of high intensity light conditions and
381 suggest that this species can potentially out-grow *L. minor* in such conditions. A survey of the
382 literature yields further examples in which the native species copes better with shady
383 conditions while the alien species is more competitive under high light conditions. For
384 example, Madsen *et al.*, (1991) studied the photosynthetic rates of seven aquatic macrophytes
385 occurring in Lake George, New York at eight light intensities from 0 to 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.
386 The results showed that *Myriophyllum spicatum* (alien) exhibited a high light requirement in
387 contrast with various native species that exhibited shade-tolerance characteristics. Similarly,
388 Pattison *et al.*, (1998) showed that invasive species in Hawaiian rainforest outgrow native
389 species at all tested light intensities, but that invasive species appear to be better suited than
390 native species to high-light environments. A pertinent question is whether the strong growth
391 performance of *L. minor* in the shade and of *L. minuta* in the light, actually leads to
392 competitive success. The data show that *L. minuta* is inherently more a sun-species than *L.*
393 *minor*. However, the expression of this inherent difference under field conditions will depend
394 on other parameters that govern Lemnaceae growth, such as nutrient availability,
395 temperature, wind and rain-exposure, and the presence of stress factors. Long-term
396 mesocosm experiments will be required to explore how differences in light utilisation
397 strategy impact on competitiveness and distribution.

398 This study details the morphological and physiological differences between *L. minuta* and *L.*
399 *minor* under different light conditions. It is concluded that distinct light utilisation strategies
400 are adopted by the two species. *L. minuta* is a heliophile species which, when grown at high
401 light intensities, maximises its RGR by using a large portion of available light (higher qP and
402 Y(II), and lower qN) to optimise carbon gain (higher NAR). In contrast, native *L. minor* can
403 be classified as sciophilous. When grown at low light intensities, *L. minor* has a higher
404 chlorophyll content and morphological plasticity (higher LAR) that help to limit the reduction
405 of RGR under such growth conditions.

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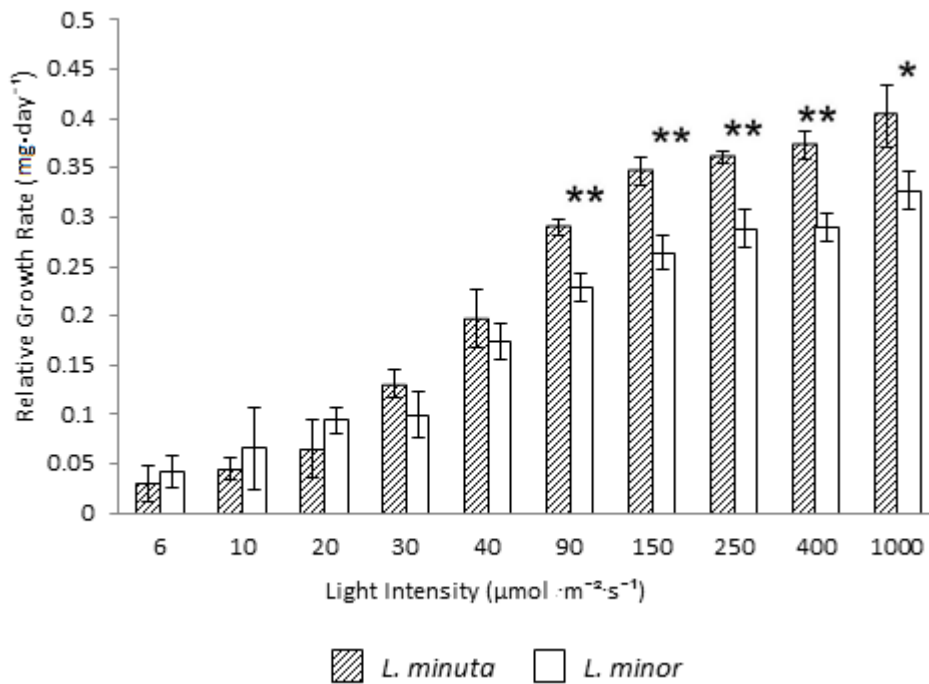
599 **Captions**

600 **Fig. 1.** RGR values for *Lemna minuta* and *Lemna minor*, calculated from the increase in
601 biomass after 7 days of growth at light intensities ranging between 6 and 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.
602 Values are mean of 4 replicates and error bars are standard deviations. The asterisks
603 indicate the significance in differences between species. * means $p<0.05$, ** means $p<0.01$

604 **Fig. 2.** LAR (a) and NAR (b) and chlorophyll content (c) values for *Lemna minuta* and
605 *Lemna minor*, calculated from the increase in biomass and area after 7 days of growth at light
606 intensities ranging between 6 and 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Values are mean of 4 replicates and
607 error bars are standard deviations. The asterisks indicate the significance in differences
608 between species. * means $p<0.05$, ** means $p<0.01$

609

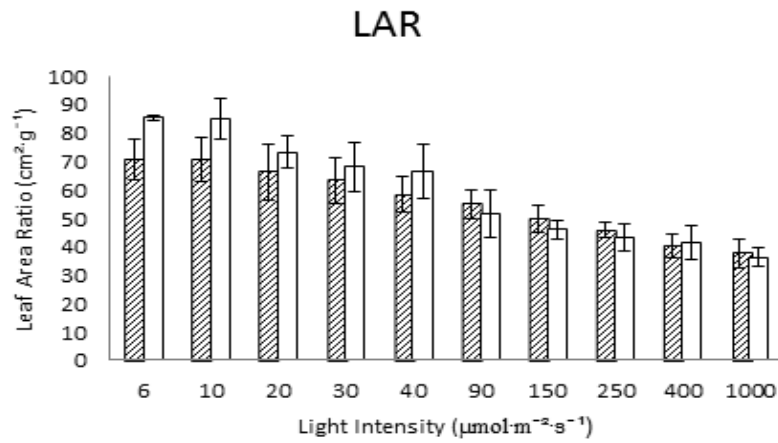
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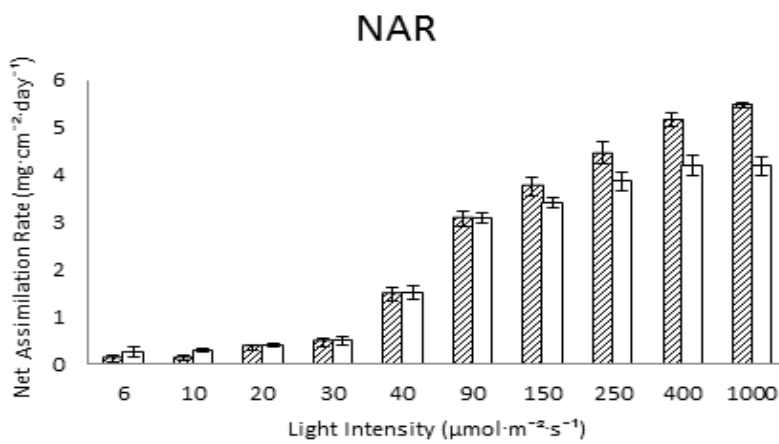
611

612 Figure 1.

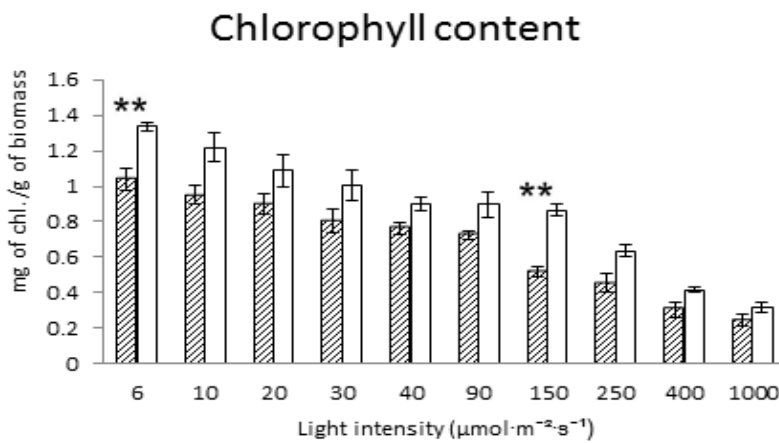
613



(a)



(b)



(c)

L. minuta
 L. minor

614

615 Figure 2.