

Title	The invasive duckweed Lemna minuta Kunth displays a different light utilisation strategy than native Lemna minor Linnaeus
Authors	Paolacci, Simona;Harrison, Simon;Jansen, Marcel A. K.
Publication date	2018-01-31
Original Citation	Paolacci, S., Harrison, S. and Jansen, M. A. K. (2018) 'The invasive duckweed Lemna minuta Kunth displays a different light utilisation strategy than native Lemna minor Linnaeus', Aquatic Botany, 146, pp. 8-14. doi: 10.1016/j.aquabot.2018.01.002
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://www.sciencedirect.com/science/article/pii/ S030437701730236X - 10.1016/j.aquabot.2018.01.002
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Download date	2025-06-01 07:40:29
Item downloaded from	https://hdl.handle.net/10468/5622



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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

Authors:

Simona Paolacci¹, Simon Harrison^{1,2,3} & Marcel A.K. Jansen^{1,2,4}

¹School of Biological, Earth and Environmental Sciences.

University College of Cork

Enterprise Center. Distillery Field, North Mall.

Cork, Ireland

²Environmental Research Institute

University College of Cork

Enterprise Center. Distillery Field, North Mall.

Cork, Ireland

³s.harrison@ucc.ie

⁴m.jansen@ucc.ie

- 6 **Corresponding author:** Simona Paolacci, email address: <u>spaolacci@ucc.ie</u>
- 7 telephone: +353 21 4904618

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- 9 **Date of submission:** 03/08/2017
- 10 Number figures: 7

11	Highlights
12	• <i>L. minuta</i> and <i>L. minor</i> display distinct light utilisation strategies.
13	• <i>L. minuta</i> takes advantage of high light intensities
14	• <i>L. minor</i> 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 Europe, where it competes with native congeneric Lemna minor Linnaeus. Previously, L. 17 minuta was found to grow faster than L. minor. The aim of this study was to determine 18 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 a range of different light intensities. Both physiological and morphological parameters were 21 22 determined. L. minuta showed a higher Relative Growth Rate (RGR) than L. minor when grown under medium and high intensities. Further analysis showed that, at high light 23 24 intensities, L. minuta has a higher Net Assimilation Rate (NAR), and displays more photochemical quenching (qP) and a higher quantum yield (Y(II)) than L. minor. In contrast 25 26 under low light intensities *L. minor* displayed a marginally higher RGR, due to a greater Leaf Area Ratio (LAR), and higher chlorophyll content than L. minuta. The results indicate two 27 28 distinct light utilisation strategies, and reveal that the invasive species L. minuta takes more advantage from high intensity light conditions. In turn, this may influence plant distribution, 29 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

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

47

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

ability of the two species to take advantage from high nutrients availability can be extended
to other resources. In this study we have explored the role of light in facilitating the growth of
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 can also be exploited as a model species to investigate the competition dynamics between 72 alien and native invasive aquatic plants. Lemnaceae are small, and easy to manipulate. 73 Moreover, comparisons with congeneric species are an effective method to study the 74 75 invasiveness of an alien species (Mack 1996). Closely-related species share many traits, and therefore the identification of invasiveness-related traits, not shared between the two species, 76 is possible (Mack 1985). Nevertheless, it should be appreciated that "invasiveness" traits will 77 not comprehensively explain the success of an invasive species as such success is generally 78 79 due to the interaction of multiple environmental factors with a range of intrinsic traits (Richardson and Pyšeket 2006). 80

81

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

101 photochemical and non-photochemical quenching can reveal energy transfer processes as well as energy dissipation (Maxwell and Johnson 2000). Differences in the fluorescence 102 emission can be used to identify differences in photosynthetic activity of sun and shade plants 103 (Lichtenthaler et al. 1981). Plants adapted to high light intensities can present higher rates of 104 photosynthetic light quanta conversion and a higher photosynthetic capacity on a chlorophyll 105 and chloroplast basis (Boardman 1977). On the other hand, plants adapted to low levels of 106 107 light usually present higher chlorophyll content per unit of biomass as this allows them to maximize the light harvesting (Valladares and Niinemets 2008). Therefore the analysis of 108 109 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 outcompete native species (e.g. Madsen et al. 1991). Moreover, it was observed that the light 111 saturation point, as well as the ability to grow at low light intensity, differ between duckweed 112 species (Landolt, 1986). The underlying mechanisms have not yet been identified. In the 113 114 present study we assessed the performance of L. minor and L. minuta at a range of light levels. The aim of the study was to determine whether the ability of *L. minuta* to outperform 115 116 L. minor is based on its higher ability to take advantage of intense light. Both physiological and morphological parameters, such as RGR, NAR, chlorophyll content and photosynthetic 117 efficiency, were measured and analysed. 118

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 number 5500 "Blarney". In a preliminary experiment nine different clones of L. minuta and 123 124 nine clones of L. minor were grown at high and low light and their light response was analysed in terms of RGR and chlorophyll content. The clones were collected in different 125 regions of Ireland and clones belonging to the same species showed similar behaviours. It 126 was concluded that one clone per species was representative of the Irish ecotypes. The plants 127 were cultured under sterile conditions, in glass flasks, on 100 ml of half-strength Hutner's 128 nutrient solution (Hutner 1953). Plants were kept in a growth room at a constant temperature 129 of 20°C and exposed to a light intensity of 40 µmol·m⁻²·s⁻¹, (cool-white fluorescent tubes) 130 with a light: dark cycle of 16: 8 hours. 131

132 Experimental conditions

Plants were grown in Petri dishes without a cover lid, containing 50 ml of half strength 133 Hutner's medium. The different light intensities were obtained by placing the plant at 134 different distances from a LED light source characterized by low heat emission (AP67 R-135 series, Valoya Finland). The experiment was carried out at 20°C with a light: dark cycle of 136 16: 8 hours. When necessary, distilled water was added to the Petri dishes during the 137 experiment to compensate for evaporation. L. minuta and L. minor were grown at 6, 10, 20, 138 30, 42, 93, 150, 250, 400 and 1000 μ mol·m⁻²·s⁻¹. These intensities are representative of the 139 natural range that can be measured in Lemna-habitats with different levels of canopy shade. 140 141 Each replicate started with 9 fronds $(4.62\pm0.87 \text{ mg fresh weight on average for } L. minuta and$ 11.32 ± 1.14 mg fresh weight on average for L. minor). The experiment lasted one week and 142 each treatment was replicated 4 times. Given the rapid growth of the species, after one week 143 the bulk of the L. minor fronds would have developed under the imposed experimental 144 145 conditions.

146 Measured end-points

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

- 150
- 151

152

153 Where Yi is the initial biomass or the initial number of fronds, Yf is the final biomass or final 154 number of fronds, *t* is the time in days and ln is the natural logarithm.

RGR = ln (Yf / Yi) / t

155

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

159 LAR = Leaf area per plant/ Plant weight

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

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

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

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

176
$$Y(II) = (F'm-F) / F'm$$

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

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

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

180 The terminology used in the IMAGING-PAM M-series Chlorophyll Fluorometer manual181 (Heinz Walz GmbH, 2014) was adopted.

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

187

Total Chlorophyll =
$$17.90 \cdot A647 + 8.08 \cdot A665$$

188

where A647 and A665 are, respectively, the absorbance at the wavelengths of 647 and665nm. The total chlorophyll content was normalised versus fresh biomass.

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

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

193 Data analysis

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

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

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

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

The RGR was calculated from the time dependent increase in biomass. For both species RGR increased with increasing light intensity (fig.1), with a minimum RGR at the lowest intensity of 6 μ mo·m⁻²·s⁻¹ and a maximum RGR at the highest intensity of 1000 μ mol·m⁻²·s⁻¹. There was a significant interaction effect between species and light intensity (tab.1). A comparison of the 2 species revealed that *L. minuta* had a significantly higher RGR than *L. minor* at 90, 150, 250, 400 and at 1000 μ mol·m⁻²·s⁻¹. In contrast, at 6, 10, and 20 μ mol·m⁻²·s⁻¹ the differences in the RGR of the two species were not statistically significant (fig. 1). Both species appeared to have reached light saturation, but to test for this, data were fitted in a nonrectangular hyperbola model. The applied model fitted the light response curve of *L*. *minuta* and *L. minor* very well. The nonrectangular hyperbola indicates that full light saturation was achieved by the two species at 400 μ mol·m⁻²·s⁻¹.

223

224 Changes in LAR and NAR at different light intensities

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

At low and medium light intensities the two species had a similar, low NAR (Fig.3*b*). Between 30 and 90 μ mol·m⁻²·s⁻¹ an increase in the slope of NAR versus light intensity was observed, while at intensities above 400 μ mol·m⁻²·s⁻¹ NAR appeared to have reached saturation. At the highest light intensities, *L. minuta* had a higher NAR than *L. minor*. This difference was significant at 250, 400 and at 1000 μ mol·m⁻²·s⁻¹.

238 Chlorophyll content as a function of light intensity

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

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

df	f	р

	Species	1 63.7 itensity 9 306 *light intensity 9 9.5 1 165 1 1 65 75	63.703	0.000
RGR Light intensity		9	306.703	0.000
Species*light intensity		9	9.579	0.000
	Species	1	165.871	0.000
LAR	Light intensity	1 63.703 9 306.703 9 9.579 1 165.871 9 75.554 9 8.013 1 12.982 9 120.479 9 4.799	0.000	
Species*light intensity		9	8.013	0.000
	Species	1	12.982	0.001
NAR	Light intensity	9 306.703 9 306.703 tensity 9 1 165.871 9 75.554 tensity 9 1 12.982 9 120.479 tensity 9 4.799	0.000	
	Species*light intensity	9	4.799	0.000

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

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

The quantum yield of photosystem II (Y(II)) is a good indicator of the efficiency of the 252 photosynthetic light reactions, under steady-state conditions. Y(II) depended both on the light 253 intensity during growth, as well as on the intensity of the actinic light increased . When the 254 two species were grown at a low light intensity (6, 10 and 20 μ mol·m⁻²·s⁻¹), Y(II) decreased 255 fast with increasing actinic light intensity during the actual measurements. Y(II) reached 256 saturation values close to 0 at an actinic PAR intensity of 186 μ mol· m⁻²·s⁻¹. In the case of 257 fronds of L. minor and L. minuta raised under intermediate light levels, Y(II) decreased less 258 drastically and displayed a long tail that reached saturation only at an actinic light level of 259 701 μ mol·m⁻²·s⁻¹. When the two species were raised under the highest light intensities, L. 260 minuta still displayed this tail of low Y(II) values, but this was not the case for L. minor. 261 When the plants were grown at 6 and 10 μ mol·m⁻²·s⁻¹, there was a significant interaction 262 263 between species and Photosynthetic Available Radiation (PAR) (tab.2). At these intensities, L. minuta showed a significantly higher Y(II) than L. minor at PAR=0 and 1. The interaction 264 between species and PAR was also significant when the plants were grown at 1000 265 μ mol·m²·s⁻¹ (tab.2). At this intensity, the difference between *L. minuta* and *L. minor* was not 266 267 statistically significant.

Y(II) in plants grown at 6 μ mol·m ⁻² ·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
	Sphericity Assumed	1	0.401	0.572
	Greenhouse-Geisser	1	0.401	0.572
species	Huynh-Feldt	1	0.401	0.572
	Lower-bound	1	0.401	0.572
	Sphericity Assumed	12	33.585	0.000
DAD * analias	Greenhouse-Geisser	1.033	33.585	0.009
PAR * species	Huynh-Feldt	1.083	33.585	0.008
	Lower-bound	1	33.585	0.01
Y()	II) in plants grown at 10	µmol∙m⁻	² ·s ⁻¹	•
		df	F	Sig.
	Sphericity Assumed	12	267.038	0.000
DAD	Greenhouse-Geisser	1.383	267.038	0.001
PAK	Huynh-Feldt	3.484	267.038	0.000
	Lower-bound	1	267.038	0.004
	Sphericity Assumed	1	7.764	0.108
anaaiaa	Greenhouse-Geisser	1	7.764	0.108
species	Huynh-Feldt	1	7.764	0.108
	Lower-bound	1	7.764	0.108
	Sphericity Assumed	12	171.179	0.000
DAD * cracico	Greenhouse-Geisser	1.583	171.179	0.001
PAR · species	Huynh-Feldt	6.583	171.179	0.000
	Lower-bound	1	171.179	0.006
Y(II) in plants grown at 1000) µmol∙n	n ⁻² ·s ⁻¹	
		df	F	Sig.
	Sphericity Assumed	12	49.608	0.000
DAD	Greenhouse-Geisser	1.944	49.608	0.000
PAK	Huynh-Feldt	5.469	49.608	0.000
	Lower-bound	1	49.608	0.006
	Sphericity Assumed	1	0.704	0.463
spacios	Greenhouse-Geisser	1	0.704	0.463
species	Huynh-Feldt	1	0.704	0.463
	Lower-bound	1	0.704	0.463
	Sphericity Assumed	12	19.987	0.000
PAR * species	Greenhouse-Geisser	1.091	19.987	0.017
	Huynh-Feldt	1.237	19.987	0.012
	Lower-bound	1	19.987	0.021

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

Non-photochemical quenching, qN, increased following exposure to low and intermediate levels of actinic light and then stabilized under higher actinic light levels. When the two species had been grown at high light intensities, high qN levels were already induced by relatively low levels of actinic light. However, *L. minuta* displayed a significantly lower qN than *L. minor* (overall p<0.01) when grown at 400 and 1000 μ mol·photons·m⁻²·s⁻¹. When the two species where grown at just 6, 10 or 20 μ mol·photons·m⁻²·s⁻¹ *L. minuta* had a higher qN at every actinic light level (overall p<0.01)

The curves describing the photochemical quenching qP of the two species show a decrease in qP with increasing intensity of the actinic light during the fluorescence measurements. Decreases in qP were very similar when the plants were grown at low and medium light intensities (from 6 to 250 μ mol·photons·m⁻²·s⁻¹). Only when plants were grown at 400 and 1000 μ mol·photons·m⁻²·s⁻¹, a significant interaction between species and PAR was found. At these light intensities, *L. minuta* maintained a significantly higher qP (overall p<0.01) than *L. minor* at actinic light intensities above 186 μ mol·m⁻²·s⁻¹.

qP in plants grown at 400 µmol·m ⁻² ·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
	Sphericity Assumed	1	2.849	0.19
spacios	Greenhouse-Geisser	1	2.849	0.19
species	Huynh-Feldt	1	2.849	0.19
	Lower-bound	1	2.849	0.19
	Sphericity Assumed	12	310.784	0.000
DAD * spacios	Greenhouse-Geisser	1.987	310.784	0.000
PAR * species	Huynh-Feldt	5.871	310.784	0.000
	Lower-bound	1	310.784	0.000
qP in plants grown at 1000 µmol·m ⁻² ·s ⁻¹				
	Sphericity Assumed	12	28.252	0.000
PAR	Greenhouse-Geisser	1.736	28.252	0.002
	Huynh-Feldt	3.914	28.252	0.000
	Lower-bound	1	28.252	0.013
	Sphericity Assumed	1	0.854	0.423
species	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

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

287 **DISCUSSION**

Light is a necessity for the autotrophic growth of Lemnaceae. However, the relationship 288 289 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. *minor* ranges between 300 and 600 μ mol·m⁻²·s⁻¹, depending on temperature. For *L. minuta*, 292 the only data available are those of Landolt (1986) who found that at 323 µmol·m⁻²·s⁻¹ 293 (published as 17000 lux) light saturation was not yet achieved. In the present study small 294 295 increases in RGR were found at the high light intensities tested. The model developed on the basis of the results observed (e.g. Givnish et al. 2004) indicates that full light saturation was 296 achieved by the two species at 400 μ mol·m⁻²·s⁻¹. 297

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299 The comparison of the growth of the two Lemna species suggests that L. minor is better adapted to shade conditions (Givinish 1988) while L. minuta takes more advantage from high 300 light intensities. This hypothesis is supported by the comparison of other parameters. LAR 301 and NAR are often measured to analyse variations in plant growth (Lambers et al. 1989; 302 Poorter and Remkes 1990). At high light intensities L. minuta had a higher NAR than L. 303 *minor*. This intrinsic ability to exploit high light levels was associated with a higher RGR. L. 304 305 *minor* displayed a higher LAR in shady conditions, while, under high light conditions the two species had similar values of LAR. The latter observation indicates that *L. minor* has a higher 306 307 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 is likely to contribute to the slightly higher RGR of *L. minor* in shady conditions. In several 309 310 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 other studies, NAR was the factor most closely correlated with RGR (e.g. Shipley 2002). 312 Conflicting literature might depend on several factors such as the species investigated and the 313

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

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

Chlorophyll *a* fluorometry was used to explore the mechanisms underlying differences in RGR and NAR. The photosynthetic yield (Y(II)) was measured at a range of actinic light 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 20 µmol·m⁻²·s⁻¹), Y(II) displayed a rapid initial decline with increasing actinic light during 348 the measurements. In contrast, in plants raised under intermediate and high light conditions, 349 the decline in Y(II) with increasing actinic light occurred at higher intensities and more 350 351 gradually. This suggests that plants that had acclimated to higher light levels were able to use a higher portion of the absorbed light for the photosynthetic process. The measurements of 352 the photochemical quenching qP confirmed this ability of plants grown under high light 353 intensities. The photochemical quenching is a measure of the fraction of PSII reaction centres 354 355 that are in the open state (Krause and Weis 1991). In this study the decrease of qP in plants grown at higher light intensities occurred at higher actinic levels than plant grogn at low light 356 intensities. The comparison of qP and Y(II) between the two species revealed a different 357 ability to cope with both low and high actinic light levels. The higher qP of L. minuta when 358 fronds were raised under high light intensities, suggests a higher capacity photosynthetic light 359 reactions to utilise photons at the highest light intensities. This conclusion is reinforced by a 360 slightly higher Y(II) observed in *L. minuta* grown at high light intensities. The qP data concur 361 with the higher NAR and RGR of *L. minuta* raised under high light intensities, and indicate 362 363 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 performance of *L. minor* in the shade is more dependent on morphological (higher LAR) than 365 366 on physiological (lower Y(II), qP and NAR) parameters.

Non-photochemical quenching, qN, was also analysed. This parameter refers to the portion of 367 the energy absorbed that the plant dissipates as heat (Müller et al. 2001). Both species 368 increased the extent of non-photochemical quenching when exposed to higher actinic light 369 levels, demonstrating a capability to adjust photosynthetic performance to prevailing light 370 conditions. A comparison of the two species showed that L. minor had a higher qN value than 371 372 L. minuta when the plants were grown at high light intensities. A higher qN might be a necessity for L. minor as a result of its relatively high light capture caused by high 373 374 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. minor. In contrast, L. minuta had a lower qN value, which is associated with both a higher qP 376 377 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 invasive duckweed L. minuta to outperform the native L. minor. The results show that the 379 invasive species L. minuta takes better advantage of high intensity light conditions and 380 suggest that this species can potentially out-grow L. minor in such conditions. A survey of the 381 382 literature yields further examples in which the native species copes better with shady conditions while the alien species is more competitive under high light conditions. For 383 example, Madsen et al., (1991) studied the photosynthetic rates of seven aquatic macrophytes 384 occurring in Lake George, New York at eight light intensities from 0 to 1000 µmol·m⁻²·s⁻¹. 385 The results showed that Myriophyllum spicatum (alien) exhibited a high light requirement in 386 contrast with various native species that exhibited shade-tolerance characteristics. Similarly, 387 Pattison et al., (1998) showed that invasive species in Hawaiian rainforest outgrow native 388 species at all tested light intensities, but that invasive species appear to be better suited than 389 native species to high-light environments. A pertinent question is whether the strong growth 390 performance of L. minor in the shade and of L. minuta in the light, actually leads to 391 competitive success. The data show that L. minuta is inherently more a sun-species than L. 392 minor. However, the expression of this inherent difference under field conditions will depend 393 on other parameters that govern Lemnaceae growth, such as nutrient availability, 394 395 temperature, wind and rain-exposure, and the presence of stress factors. Long-term mesocosm experiments will be required to explore how differences in light utilisation 396 397 strategy impact on competitiveness and distribution.

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

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Acknowledgements

407 We wish to thank the Irish Research Council for funding this study.

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599 Captions

Fig. 1. RGR values for *Lemna minuta* and *Lemna minor*, calculated from the increase in biomass after 7 days of growth at light intensities ranging between 6 and 1000 μ mol·m⁻² · s⁻ ¹. Values are mean of 4 replicates and error bars are standard deviations. The asterisks indicate the significance in differences between species. * means p<0.05, ** means p<0.01

Fig. 2. LAR (a) and NAR (b) and chlorophyll content (c) values for *Lemna minuta* and *Lemna minor*, calculated from the increase in biomass and area after 7 days of growth at light intensities ranging between 6 and 1000 μ mol·m⁻²·s⁻¹. Values are mean of 4 replicates and error bars are standard deviations. The asterisks indicate the significance in differences between species. * means p<0.05, ** means p<0.01

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