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Title

The invasive duckweed *Lemna minuta* Kunth displays a different light utilisation strategy than native *Lemna minor* Linnaeus

Running Head

Light utilization in *Lemna minuta* and *Lemna minor*

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Highlights

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

Abstract

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. minuta* was found to grow faster than *L. minor*. The aim of this study was to determine whether the rapid growth of invasive *L. minuta* is based on differential light utilisation. For 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 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 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 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 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, and inform management strategies.

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

INTRODUCTION

Invasive alien species pose a major threat to biodiversity and natural ecosystems worldwide (Chornesky and Randall 2003). Aquatic ecosystems are particularly at risk from alien invasive plants. These invasive aquatic plants can have substantial negative effects on freshwater communities by decreasing the biodiversity of invertebrate, fish and native plant species in aquatic systems (Zedler and Kercher 2004), and can affect water quality by altering nutrient cycling and the microclimate of the water body (D'Antonio and Vitousek 1992). Invasive plants can also negatively affect water-based recreational activities, water extraction 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 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 threat to biodiversity.

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 and Jovet - Ast 1966). Since, the species has spread widely and is now considered invasive in 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 Hungary (Lukács *et al.* 2014), in Mediterranean countries such as Italy (Conti *et al*. 2005) 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 congeneric species *Lemna minor* Linnaeus, which is native in Europe and Asia. Where *L. minuta* and *L. minor* become dominant, they form floating mats which may have a negative impact on wetland ecosystems by suppressing submerged macrophyte species (Janes *et al*. 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*. 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* populations, but the authors did not find a correlation between nutrients availability and dominance of *L. minuta*. What determines the competition advantage of one species over another is still unclear and probably the distribution pattern of the two species reflects the interaction of several environmental factors. It is reasonable to hypothesise that the different

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.

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 alien and native invasive aquatic plants. Lemnaceae are small, and easy to manipulate. Moreover, comparisons with congeneric species are an effective method to study the 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, is possible (Mack 1985). Nevertheless, it should be appreciated that "invasiveness" traits will not comprehensively explain the success of an invasive species as such success is generally due to the interaction of multiple environmental factors with a range of intrinsic traits (Richardson and Pyšek*et* 2006).

The focus of this study is to determine if the success of invasive *L. minuta* over native *L. minor* can be explained, in part, by differences in light utilisation. Light is a key-factor for 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 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 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 heliophilous species achieve the ability to thrive at a particular light level by adopting different light capture and utilisation strategies (Valladares and Niinemets 2008). For 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 smaller, but thicker leaves with more palisade and spongy mesophyll layers (Boardman 1977; Gratani & Ghia 2002; Zaragoza-Castells *et al*. 2008). In contrast, shade plants often have thin leaves with a lower weight per leaf area. Prevailing light intensities also determine the photosynthetic capacity (Boardman 1977). For example, the light intensity under which plants are grown influences pigment content and photochemical efficiency (Boardman 1977, Demmig and Björkman 1987, Valladares and Niinemets 2008). Fluorescence analysis is used to non-destructively investigate the photosynthetic efficiency of plants. Measurements of

photochemical and non-photochemical quenching can reveal energy transfer processes as well as energy dissipation (Maxwell and Johnson 2000). Differences in the fluorescence emission can be used to identify differences in photosynthetic activity of sun and shade plants (Lichtenthaler *et al*. 1981). Plants adapted to high light intensities can present higher rates of photosynthetic light quanta conversion and a higher photosynthetic capacity on a chlorophyll and chloroplast basis (Boardman 1977). On the other hand, plants adapted to low levels of 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 pigment content is another useful tool for characterisation of shade and light plants.

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 saturation point, as well as the ability to grow at low light intensity, differ between duckweed species (Landolt, 1986). The underlying mechanisms have not yet been identified. In the 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 *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 efficiency, were measured and analysed.

MATERIAL AND METHODS

Cultivation plant stocks

The strains of *L. minor* and *L. minuta* used for this experiment were collected in Blarney, Co. 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 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 regions of Ireland and clones belonging to the same species showed similar behaviours. It was concluded that one clone per species was representative of the Irish ecotypes. The plants were cultured under sterile conditions, in glass flasks, on 100 ml of half-strength Hutner's nutrient solution (Hutner 1953). Plants were kept in a growth room at a constant temperature of 20°C and exposed to a light intensity of 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$, (cool-white fluorescent tubes) with a light: dark cycle of 16: 8 hours.

Experimental conditions

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

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):

$$RGR = \ln (Yf / Yi) / t$$

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

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):

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

The NAR was calculated according to Williams (1946):

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

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

$$Y(II) = (F´m-F) / F´m$$

$$qP = (F´m-F) / (F´m- F´o)$$

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

$$qN = (Fm- F´m) / (Fm- F´o)$$

The terminology used in the IMAGING-PAM M-series Chlorophyll Fluorometer manual (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:

$$\text{Total Chlorophyll} = 17.90 \cdot A647 + 8.08 \cdot A665$$

where A647 and A665 are, respectively, the absorbance at the wavelengths of 647 and 665nm. The total chlorophyll content was normalised versus fresh biomass.

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

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

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:

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

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.

RESULTS

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 µmo·m^{-2}·s^{-1} and a maximum RGR at the highest intensity of 1000 µmol·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 µmol·m^{-2}·s^{-1}. In contrast, at 6, 10, and 20 µmol·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 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

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 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 increasing light intensity (fig. 3*a*). Both *L. minuta* and *L. minor* reached a maximum LAR at a light intensity of 6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and displayed a minimum LAR at 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At 6 $\mu mol \cdot m^{-2} \cdot s^{-1}$, *L. minor* displayed a significantly higher LAR than *L. minuta*, while, at higher light intensities the difference between the LAR of the two species decreased progressively. At the highest light intensities tested the species displayed a very similar LAR.

At low and medium light intensities the two species had a similar, low NAR (Fig.3*b*). Between 30 and 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ an increase in the slope of NAR versus light intensity was observed, while at intensities above 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

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 was 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

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).

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 photosynthetic light reactions, under steady-state conditions. Y(II) depended both on the light intensity during growth, as well as on the intensity of the actinic light increased . When the two species were grown at a low light intensity (6, 10 and 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$), Y(II) decreased fast with increasing actinic light intensity during the actual measurements. Y(II) reached saturation values close to 0 at an actinic PAR intensity of 186 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In the case of fronds of *L. minor* and *L. minuta* raised under intermediate light levels, Y(II) decreased less drastically and displayed a long tail that reached saturation only at an actinic light level of 701 $\mu mol \cdot m^{-2} \cdot s^{-1}$. When the two species were raised under the highest light intensities, *L. minuta* still displayed this tail of low Y(II) values, but this was not the case for *L. minor*. When the plants were grown at 6 and 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$, there was a significant interaction 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 between species and PAR was also significant when the plants were grown at 1000 $\mu mol \cdot m^{2} \cdot s^{-1}$ (tab.2). At this intensity, the difference between *L. minuta* and *L. minor* was not statistically significant.

Y(II) in plants grown at 6 µmol·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
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 µmol·m⁻²·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 µmol·m⁻²·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

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 µmol·photons·m^{-2}·s^{-1}. When the two species where grown at just 6, 10 or 20 µmol·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 µmol·photons·m^{-2}·s^{-1}). Only when plants were grown at 400 and 1000 µmol·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 µmol·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
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 µmol·m^{-2}·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

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

DISCUSSION

Light is a necessity for the autotrophic growth of Lemnaceae. However, the relationship between growth and light-intensity is species, and even clone, specific, while environmental factors such as temperature, nutrient and CO_2 supply can also alter this relationship (Landolt 1986). Wedge and Burris (1982) observed that the light saturation intensity for growth of *L. minor* ranges between 300 and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, depending on temperature. For *L. minuta*, the only data available are those of Landolt (1986) who found that at 323 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (published as 17000 lux) light saturation was not yet achieved. In the present study small 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 achieved by the two species at 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

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 light intensities. This hypothesis is supported by the comparison of other parameters. LAR and NAR are often measured to analyse variations in plant growth (Lambers *et al.* 1989; Poorter and Remkes 1990). At high light intensities *L. minuta* had a higher NAR than *L. minor*. This intrinsic ability to exploit high light levels was associated with a higher RGR. *L. 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 morphological plasticity in response to changing light conditions. A more extensive leaf area 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 studies LAR was recognised as the growth parameter that has the greatest impact on the RGR (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). Conflicting literature might depend on several factors such as the species investigated and the

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

To further explore the light-intensity dependency of growth, various photosynthetic 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 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 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 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 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 2008; Lewandowska and Jarvis 1977; Leverenz 1987; Thompson *et al.* 1988; Rijkers *et al.* 2000; Cao 2000). Hence, we conclude that the higher chlorophyll content in *L. minor* 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

(Maxwell and Johnson 2000). When plants were raised under low light conditions (from 6 to 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$), Y(II) displayed a rapid initial decline with increasing actinic light during the measurements. In contrast, in plants raised under intermediate and high light conditions, the decline in Y(II) with increasing actinic light occurred at higher intensities and more 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 the photochemical quenching qP confirmed this ability of plants grown under high light intensities. The photochemical quenching is a measure of the fraction of PSII reaction centres 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 intensities. The comparison of qP and Y(II) between the two species revealed a different ability to cope with both low and high actinic light levels. The higher qP of *L. minuta* when fronds were raised under high light intensities, suggests a higher capacity photosynthetic light reactions to utilise photons at the highest light intensities. This conclusion is reinforced by a slightly higher Y(II) observed in *L. minuta* grown at high light intensities. The qP data concur with the higher NAR and RGR of *L. minuta* raised under high light intensities, and indicate that at least part of the capacity for growth under high light is associated with adaptive 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 on physiological (lower Y(II), qP and NAR) parameters.

Non-photochemical quenching, qN, was also analysed. This parameter refers to the portion of the energy absorbed that the plant dissipates as heat (Müller *et al*. 2001). Both species increased the extent of non-photochemical quenching when exposed to higher actinic light levels, demonstrating a capability to adjust photosynthetic performance to prevailing light conditions. A comparison of the two species showed that *L. minor* had a higher qN value than *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 chlorophyll content. The higher portion of energy dissipated in the form of heat is generally 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 and Y(II), and therefore ultimately a higher NAR.

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 invasive *species L. minuta* takes better advantage of high intensity light conditions and suggest that this species can potentially out-grow *L. minor* in such conditions. A survey of the 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 example, Madsen *et al*., (1991) studied the photosynthetic rates of seven aquatic macrophytes occurring in Lake George, New York at eight light intensities from 0 to 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The results showed that *Myriophyllum spicatum* (alien) exhibited a high light requirement in contrast with various native species that exhibited shade-tolerance characteristics. Similarly, Pattison *et al*., (1998) showed that invasive species in Hawaiian rainforest outgrow native species at all tested light intensities, but that invasive species appear to be better suited than native species to high-light environments. A pertinent question is whether the strong growth performance of *L. minor* in the shade and of *L. minuta* in the light, actually leads to competitive success. The data show that *L. minuta* is inherently more a sun-species than *L. minor*. However, the expression of this inherent difference under field conditions will depend on other parameters that govern Lemnaceae growth, such as nutrient availability, 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 strategy impact on competitiveness and distribution.

This study details the morphological and physiological differences between *L. minuta* and *L. minor* under different light conditions. It is concluded that distinct light utilisation strategies 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 Y(II), and lower qN) to optimise carbon gain (higher NAR). In contrast, native *L. minor* can be classified as sciophilous. When grown at low light intensities, *L. minor* has a higher chlorophyll content and morphological plasticity (higher LAR) that help to limit the reduction of RGR under such growth conditions.

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References

Baars, J.-R. (2011, December). Classical Biological Control for the Management of Alien Invasive Plants in Ireland. In Biology and Environment: Proceedings of the Royal Irish Academy, Royal Irish Academy, Dulblin.

Boardman, N. (1977). Comparative photosynthesis of sun and shade plants. *Annual Review of Plant Physiology, 28*(1), 355-377.

Bramley, J. L., Reeve, J. T., Dussart, G. B., Pyšek, P., Prach, K., Rejmánek, M., & Wade, M. (1995). The distribution of *Lemna minuta* within the British Isles: identification, dispersal and niche constraints. Paper presented at the Plant invasions: general aspects and special problems. Workshop held at Kostelec nad Černými lesy, Czech Republic, 16-19 September 1993.

Cao, K.-F. (2000). Leaf anatomy and chlorophyll content of 12 woody species in contrasting light conditions in a Bornean heath forest. *Canadian Journal of Botany, 78*(10), 1245-1253.

Ceschin, S., Abati, S., Leacche, I., Iamonico, D., Iberite, M., & Zuccarello, V. (2016). Does the alien *Lemna minuta* show an invasive behavior outside its original range? Evidence of antagonism with the native *L. minor* in central Italy. *International Review of Hydrobiology*, *101*(5-6), 173-181.

Chornesky, E. A., & Randall, J. M. (2003). The threat of invasive alien species to biological diversity: setting a future course. *Annals of the Missouri Botanical Garden*, 67-76.

Connolly, J., & Wayne, P. (1996). Asymmetric competition between plant species. *Oecologia, 108*(2), 311-320.

Conti, F. Abbate, G., Alessandrini, a. & blasi, C.(eds.)(2005) An annotated checklist of the Italian vascular flora: Palombi editori, Roma.

Dai, Y., Shen, Z., Liu, Y., Wang, L., Hannaway, D., & Lu, H. (2009). Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg. *Environmental and Experimental Botany, 65*(2), 177-182.

D'Antonio, C. M., & Vitousek, P. M. (1992). Biological invasions by exotic grasses, the grass/fire cycle, and global change. *Annual Review of Ecology and Systematics, 23*, 63-87.

Demmig, B., & Björkman, O. (1987). Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O2 evolution in leaves of higher

plants. *Planta, 171*(2), 171-184.

Eilam, Y., & Klein, S. (1962). The effect of light intensity and sucrose feeding on the fine structure in chloroplasts and on the chlorophyll content of etiolated leaves. *The Journal of Cell Biology, 14*(2), 169-182.

Fang, L., Zhang, S., Zhang, G., Liu, X., Xia, X., Zhang, S., Xing, W. & Fang, X. (2015). Application of Five Light-Response Models in the Photosynthesis of *Populus euramericana* cv.'Zhonglin46'Leaves. *Applied Biochemistry and Biotechnology, 176*(1), 86-100.

Garnier, E. (1991). Resource capture, biomass allocation and growth in herbaceous plants. *Trends in Ecology & Evolution, 6*(4), 126-131.

Givnish, T. J. (1988). Adaptation to sun and shade: a whole-plant perspective. *Functional Plant Biology, 15*(2), 63-92.

Givnish, T. J., Montgomery, R. A., & Goldstein, G. (2004). Adaptive radiation of photosynthetic physiology in the Hawaiian lobeliads: light regimes, static light responses, and whole-plant compensation points. *American Journal of Botany, 91*(2), 228-246.

Gratani, L., & Ghia, E. (2002). Adaptive strategy at the leaf level of *Arbutus unedo* L. to cope with Mediterranean climate. *Flora-Morphology, Distribution, Functional Ecology of Plants, 197*(4), 275-284.

Halford, M., Heemers, L., Mathys, C., Vanderhoeven, S., & Mahy, G. (2011). Socio-economic survey on invasive ornamental plants in Belgium. *Biodiversity and Landscape Unit, University of Liège Gembloux Agro-Bio Tech*. Final report February.

Hallé, F. (1978) Architectural variation at the specific level in tropical trees. In: Tomlinson, P., B., , Zimmermann, M., H ed(s). Tropical trees as living systems . Cambridge University Press. Cambridge.

Havaux, M., & Tardy, F. (1999). Loss of chlorophyll with limited reduction of photosynthesis as an adaptive response of Syrian barley landraces to high-light and heat stress. *Functional Plant Biology, 26*(6), 569-578.

Heinz Walz GmbH (2014). IMAGING-PAM M-series Chlorophyll Fluorometer. Eichenring 6, 91090 Effeltrich, Germany. Website: www.walz.com.

Hendry, G., & Price, A. (1993). Stress indicators: chlorophylls and carotenoids. 148-152. In: (eds. Hendry G.A.F. and Grime J.P.). Methods in Comparative Plant Ecology, Chapman and Hall, London.

Hussner, A. (2012). Alien aquatic plant species in European countries. *Weed Research, 52*(4),

297-306.

Hussner, A., van de Weyer, K., Gross, E., & Hilt, S. (2010). Comments on increasing number and abundance of non-indigenous aquatic macrophyte species in Germany. *Weed Research, 50*(6), 519-526.

Hutner, S. (1953). Comparative physiology of heterotrophic growth in plants. *Growth and Differentiation in Plants.* Iowa State College Press, Ames.

Inskeep, W. P., & Bloom, P. R. (1985). Extinction coefficients of chlorophyll a and b in N, N-dimethylformamide and 80% acetone. *Plant physiology, 77*(2), 483-485.

Janes, R. A., Eaton, J. W., & Hardwick, K. (1996). The effects of floating mats of *Azolla filiculoides* Lam. and *Lemna minuta* Kunth on the growth of submerged macrophytes. *Hydrobiologia, 340*(1-3), 23-26.

Jovet, P., & Jovet-Ast, S. (1966). *Lemna valdiviana* Philippi espèce signalée pour la première fois en Europe. *Bulletin du Centre d'Etudes et de Recherches Scientifiques Biarritz, 6*, 57-64.

Krause, G., & Weis, E. (1991). Chlorophyll fluorescence and photosynthesis: the basics. *Annual Review of Plant Biology, 42*(1), 313-349.

Kura-Hotta, R., Satoh, K., & Kato, S. (1987). Chlorophyll concentration and its changes in leaves of spinach raised under different light levels. *Plant Cell Physiology, 87*, 12-19.

Laing, W. A., Greer, D. H., & Schnell, T. (1995). Photoinhibition of photosynthesis causes a reduction in vegetative growth rates of dwarf bean (*Phaseolus vulgaris*) plants. *Functional Plant Biology, 22*(4), 511-520.

Lambers, H., Freijsen, N., Poorter, H., Hirose, T., & Van der Werf, A. (1989). Analyses of growth based on net assimilation rate and nitrogen productivity. Their physiological background. In: Causes and consequences of variation in growth rate and productivity of higher plants. Instituut voor Oecologish Onderzoek. Hague, Netherlands, 1-17.

Landolt. (1986). Biosystematic investigations in the family of duckweeds (Lemnaceae). Vol. 2. Veroffent. Geobot. Inst. Eidg. Hochschule, Stift. Riubel, Zurich.

Lei, T. T., Tabuchi, R., Kitao, M., & Koike, T. (1996). Functional relationship between chlorophyll content and leaf reflectance, and light-capturing efficiency of Japanese forest species. *Physiologia Plantarum, 96*(3), 411-418.

Leverenz, J. W. (1987). Chlorophyll content and the light response curve of shade-adapted conifer needles. *Physiologia Plantarum, 71*(1), 20-29.

Lewandowska, M., & Jarvis, P. (1977). Changes in chlorophyll and carotenoid content,

specific leaf area and dry weight fraction in Sitka spruce, in response to shading and season. *New Phytologist, 79*(2), 247-256.

Lichtenthaler, H. K., Buschmann, C., Döll, M., Fietz, H. J., Bach, T., Kozel, U., Meied, D. & Rahmsdorf, U. (1981). Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. *Photosynthesis Research, 2*(2), 115-141.

Lucey, J. (2003). *Lemna minuta* kunth (least duckweed) in e. cork (vc h5). *Irish Botanical News, 13*, 5-8.

Lukács, B., Mesterházy, A., Vidéki, R., & Király, G. (2014). Alien aquatic vascular plants in Hungary (*Pannonian ecoregion*): Historical aspects, data set and trends. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, 1-8.

Lusk, C. (2004). Leaf area and growth of juvenile temperate evergreens in low light: species of contrasting shade tolerance change rank during ontogeny. *Functional Ecology, 18*(6), 820-828.

Mack, R. N. (1985). Invading plants: their potential contribution to population biology. *Studies on Plant Demography: a festschrift for John L. Harper*, 127-142.

Mack, R. N. (1996). Predicting the identity and fate of plant invaders: emergent and emerging approaches. *Biological Conservation, 78*(1), 107-121.

Madsen, J. D., Hartleb, C., & Boylen, C. (1991). Photosynthetic characteristics of *Myriophyllum spicatum* and six submersed aquatic macrophyte species native to Lake George, New York. *Freshwater Biology, 26*(2), 233-240.

Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany, 51*(345), 659-668.

Mifsud, S. (2010) First occurences of *Lemna minuta* kunth (fam. lemnaceae) in the Maltese islands. *Central Mediterranean Naturalist, 5*(2), 1-4.

Minotta, G., & Pinzauti, S. (1996). Effects of light and soil fertility on growth, leaf chlorophyll content and nutrient use efficiency of beech (*Fagus sylvatica* L.) seedlings. *Forest Ecology and Management, 86*(1), 61-71.

Müller, P., Li, X.-P., & Niyogi, K. K. (2001). Non-photochemical quenching. A response to excess light energy. *Plant Physiology, 125*(4), 1558-1566.

Njambuya, J., Stiers, I., & Triest, L. (2011). Competition between *Lemna minuta* and *Lemna minor* at different nutrient concentrations. *Aquatic Botany*,*94*(4), 158-164.

Paolacci, S., Harrison, S., & Jansen, M. A. K. (2016). A comparative study of the nutrient responses of the invasive duckweed *Lemna minuta*, and the native, co-generic species

Lemna minor. *Aquatic Botany, 134*, 47-53.

Pattison, R., Goldstein, G., & Ares, A. (1998). Growth, biomass allocation and photosynthesis of invasive and native Hawaiian rainforest species. *Oecologia, 117*(4), 449-459.

Poorter, H., & Remkes, C. (1990). Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia, 83*(4), 553-559.

Poorter, L. (1999). Growth responses of 15 rain-forest tree species to a light gradient: the relative importance of morphological and physiological traits. *Functional Ecology, 13*(3), 396-410.

Radford, P. (1967). Growth analysis formulae-their use and abuse. *Crop Science, 7*(3), 171-175.

Richardson, D. M., & Pyšek, P. (2006). Plant invasions: merging the concepts of species invasiveness and community invasibility. *Progress in Physical Geography, 30*(3), 409-431.

Rijkers, T., Pons, T., & Bongers, F. (2000). The effect of tree height and light availability on photosynthetic leaf traits of four neotropical species differing in shade tolerance. *Functional Ecology, 14*(1), 77-86.

Schreiber, U., Schliwa, U., & Bilger, W. (1986). Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynthesis Research, 10*(1-2), 51-62.

Shipley, B. (2002). Trade-offs between net assimilation rate and specific leaf area in determining relative growth rate: relationship with daily irradiance. *Functional Ecology, 16*(5), 682-689.

Shipley, B. (2006). Net assimilation rate, specific leaf area and leaf mass ratio: which is most closely correlated with relative growth rate? A meta-analysis. *Functional Ecology, 20*(4), 565-574.

Stace, C. (2010). New flora of the British Isles: Cambridge University Press.

Strauss-Debenedetti, S., & Bazzaz, F. (1991). Plasticity and acclimation to light in tropical Moraceae of different sucessional positions. *Oecologia, 87*(3), 377-387.

Thompson, W., Stocker, G., & Kriedemann, P. (1988). Growth and photosynthetic response to light and nutrients of *Flindersia brayleyana* F. Muell., a rainforest tree with broad tolerance to sun and shade. *Functional Plant Biology, 15*(2), 299-315.

Thornley, J. H. (1976). Mathematical models in plant physiology. Academic Press (Inc.)

London, Ltd..

Valladares, F., & Niinemets, Ü. (2008). Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology, Evolution, and Systematics*, 237-257.

Walters, M., Kruger, E., & Reich, P. (1993). Relative growth rate in relation to physiological and morphological traits for northern hardwood tree seedlings: species, light environment and ontogenetic considerations. *Oecologia, 96*(2), 219-231.

Wedge, R. M., & Burris, J. E. (1982). Effects of light and temperature on duckweed photosynthesis. *Aquatic Botany, 13*, 133-140.

Williams, R. (1946). The physiology of plant growth with special reference to the concept of net assimilation rate. *Annals of Botany*, 41-72.

Wójciak, H., & Urban, D. (2009). Duckweeds (Lemnaceae) and their phytocenoses in old river-beds of the Bug River valley (between Kryłów and Kostomłoty). *Woda Środowisko Obszary Wiejskie, 9*(28), 215-225.

Wright, I., & Westoby, M. (2000). Cross-species relationships between seedling relative growth rate, nitrogen productivity and root vs leaf function in 28 Australian woody species. *Functional Ecology, 14*(1), 97-107.

Zaragoza-Castells, J., Sánchez-Gómez, D., Hartley, I. P., Matesanz, S., Valladares, F., Lloyd, J., & Atkin, O. K. (2008). Climate-dependent variations in leaf respiration in a dry-land, low productivity Mediterranean forest: the importance of acclimation in both high-light and shaded habitats. *Functional Ecology, 22*(1), 172-184.

Zedler, J. B., & Kercher, S. (2004). Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. *Critical Reviews in Plant sciences, 23*(5), 431-452.

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 µmol·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 µmol·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

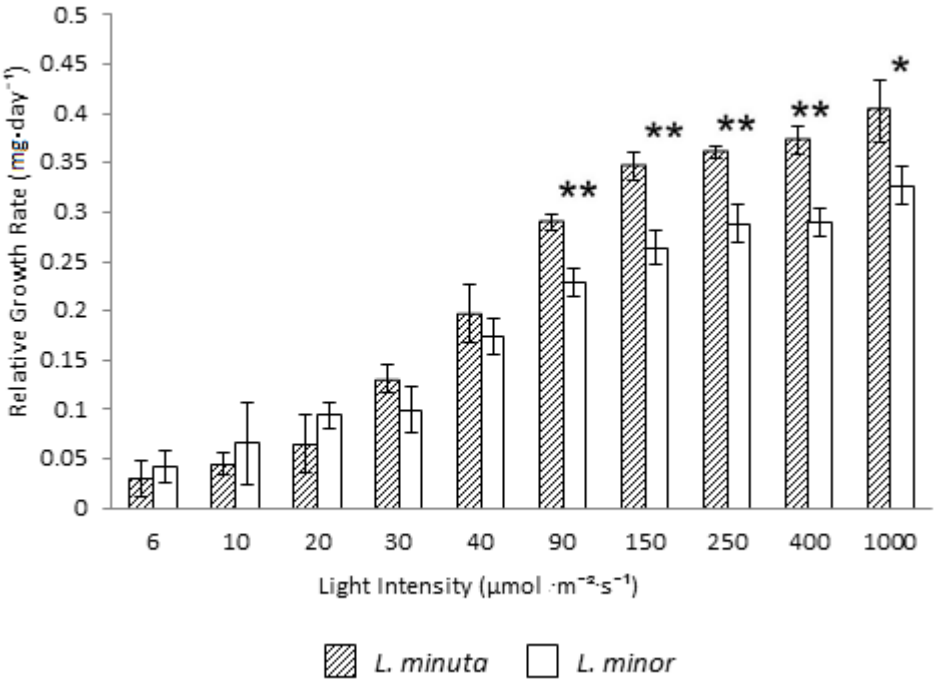
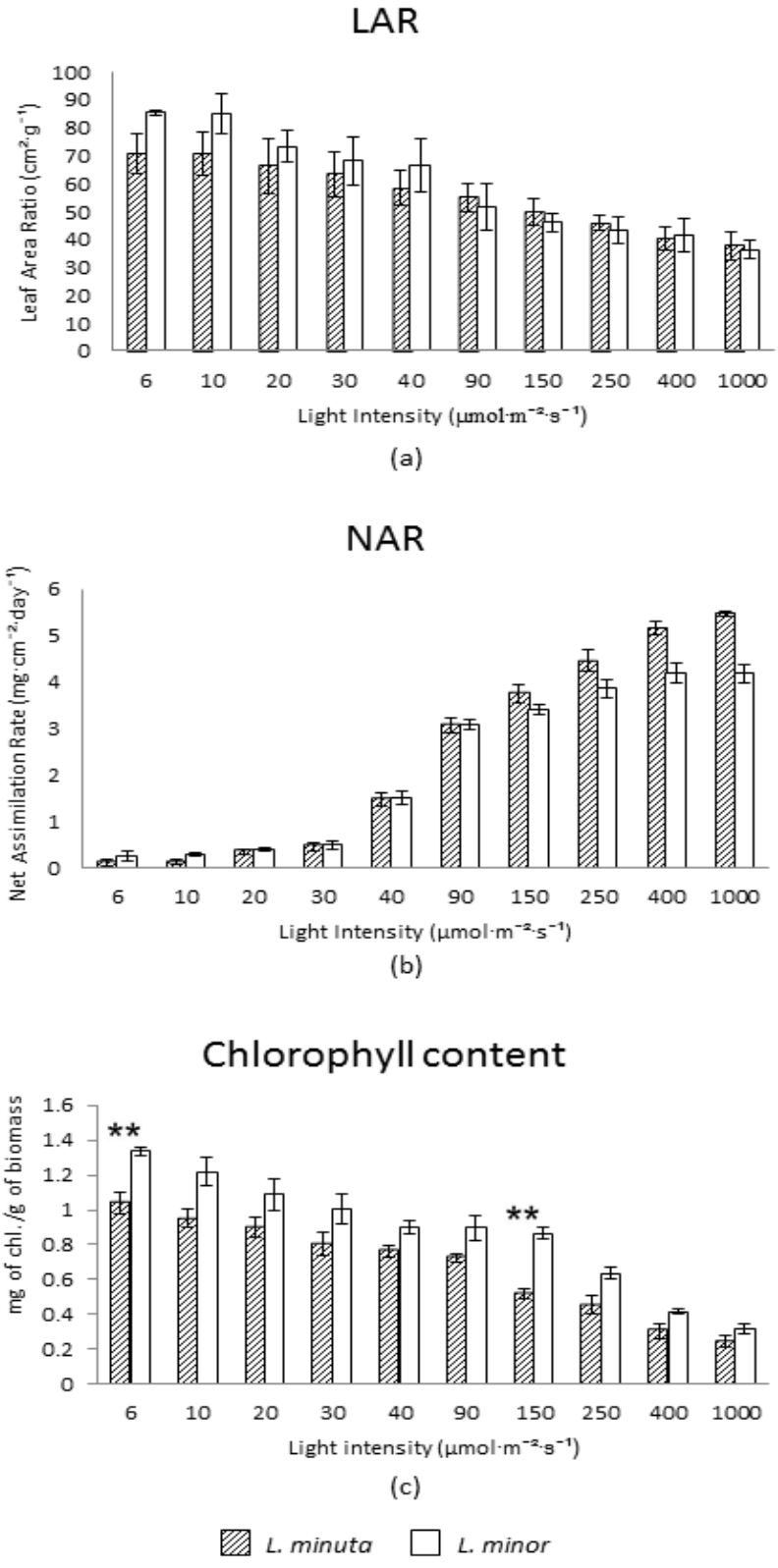


Figure 1.



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