



Seasonal plasticity of *Ludwigia grandiflora* under light and water depth gradients: An outdoor mesocosm experiment



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ABSTRACT

Abiotic factors in the introduced area can limit the establishment of exotic species. Adaptation to new abiotic parameters through plastic responses is important for their establishment and distribution pattern. Survival and growth of exotic hydrophytes are related to their plasticity and their ability to adapt to local conditions. Light and water depth are two of the predominant factors determining survival, growth, distribution and abundance of aquatic plants. Although many studies have been carried out on the effect of water level and light on plant performance, their potential interactions are poorly understood. Here, we carried out an outdoor mesocosm experiment, in order to test the role of water depth, light intensity and their potential interactions on the growth and vigour of the invasive macrophyte species *Ludwigia grandiflora*. Three light intensities (100%, 70% and 50% of sunlight) and three water depths (30, 50 and 80 cm) were tested over a 6-week period, in spring, summer and autumn 2011. We showed that the mortality, biomass, apical and lateral growth of *L. grandiflora*, as well as its biomass allocation, varied according to the season and responded to both light intensity and water depth. Interactions between water depth and light intensity can have an amplifying effect on the growth of *L. grandiflora*. Similarities between shade and deep water adaptation responses were observed. A higher biomass and a high morphological plasticity in response to these factors were found particularly at the beginning of the life cycle. Although the invasive amphibious *L. grandiflora* shows a high tolerance to different water levels and light intensities, the optimal growth conditions for *L. grandiflora* in our experiment seem to be under full sunlight and in 30 cm of water. Colonization of light or low shade aquatic habitats, as well as shallow environments, can occur due to the high plasticity of *L. grandiflora*. These results may be important relative to increasing level of water bodies under climatic changes.

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Introduction

Distribution and abundance of aquatic plants are influenced by variations in environmental factors such as light, temperature, nutrient availability and hydrology (Lacoul and Freedman, 2006). Spreading aquatic plants may respond to environmental gradients thanks to their morphological plasticity, which is a characteristic feature of these plants. Fluctuation of water level or trophic status often provoke dramatic responses in their vegetative traits and biomass allocation (Dorken and Barrett, 2004; Garbey et al., 2006; Gerber and Les, 1994).

In order to capture more light, macrophytes allocate their biomass to aboveground parts, thus increasing leaf area and stem

length (Strand and Weisner, 2001; Vretare et al., 2001) in order to reach the water surface to achieve photosynthesis (Garbey et al., 2006). For example, the amphibious *Nasturtium officinale* increases its leaf area and canopy surface area under shade conditions (Going et al., 2008). The submersed *Egeria densa*, *Hydrilla verticillata*, *Myriophyllum spicatum* (Barko and Smart, 1981) and *Potamogeton tricarlinatus* (Cenzato and Ganf, 2001) increase shoot elongation and canopy formation, and *Potamogeton perfoliatus* increases its stem length and its chlorophyll a concentration (Goldsborough and Kemp, 1988; Twilley and Barko, 1990) in shady environments. Water depth also directly affects plants through changed stem length and number of branches (Garbey et al., 2006). The growth of *Hydrocotyle ranunculoides* is higher under waterlogged conditions (water levels 5 cm above the soil surface) than under semi-drained and drained conditions (Hussner and Meyer, 2009). In deep water, *Myriophyllum spicatum* (Budd et al., 1995; Strand and Weisner, 2001) is taller with fewer and longer shoots.

Although many studies have been carried out on the effect of water level and of light on plant performance, the combined

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effects of water depth and light are poorly understood (Battaglia and Sharitz, 2006; Mommer et al., 2005), in particular in the context of invasive macrophytes. Abiotic factors in the introduced area act as a “filter” to exotic plant invasions and could limit the establishment of exotic species. Water depth and light account for 74% of the sampling variability of total biomass of macrophytes (Hudon et al., 2000). They also simultaneously affect plant growth and its vigour. Plasticity in biomass allocation and morphology is especially important for the survival and performance of aquatic plants, which may reflect their ability to occupy habitats in nature. In the case of introduced plants, phenotypic plasticity and local adaptation improve the survival and the dispersal of invasive species (Riis et al., 2010). The effect of abiotic factors on the growth of exotic plants during the invasion process has been little studied during the establishment phase. Propagules of exotic species can be introduced at any season into new ecosystems, and responses can vary according to the stages of growth, life cycle or season. Studying the adaptation to new abiotic parameters through responses or the evolution of specific life-history traits depending on the season is important when evaluating the establishment success of an exotic plant in a new ecosystem and to approaches to manage this.

Here, we tested the role of water depth and light and their potential interactions on the growth and vigour of an invasive macrophyte species, *Ludwigia grandiflora* subsp. *hexapetala*, the water primrose. According to Hussner (2010), the water level affects the growth rate of *Ludwigia peploides*, whereas *Ludwigia grandiflora* and *L. peploides* are also able to grow on partly flooded and drained soil (Hussner, 2010). To this end, the seasonal responses of *L. grandiflora* to three light intensities and to three water depths were analyzed in an outdoor mesocosm experiment. Growth performance and biomass allocation patterns were investigated to test the following hypotheses. (1) Both high water level and shade reduce the growth of *L. grandiflora*. (2) The responses due to a wide morphological plasticity vary according to the season.

Materials and methods

Biological material

Of the invasive amphibious plant species present worldwide, the water primrose, *Ludwigia grandiflora* (Michaux) Greuter and Burdet subsp. *hexapetala* (Hook. & Arn.) Nesom & Kartesz (2000)/Onagraceae, native to South America, is considered to be a water pest. Its amphibious character and its high morphological plasticity have allowed it to tolerate a broad range of environmental conditions, and to efficiently colonize water bodies and many types of environments (Lambert et al., 2010) of many countries in Europe (Dutartre et al., 2007; Hussner, 2009). *Ludwigia* species preferentially colonize stagnant waters (Pieterse and Murphy, 1990) and the flow velocity constrains its development (Dutartre et al., 2007). *L. grandiflora* possesses a rapid growth rate and an efficient vegetative reproduction capacity (Okada et al., 2009). This species prefers high nutrient substrates (Hussner, 2010) and becomes dominant in nutrient-rich conditions (Rejmánková, 1992). It is tolerant to a wide range of fluctuations in habitat, including water level (Hussner, 2010), soil moisture (Riaux, 2008), and salinity (Thouvenot et al., 2012).

Experimental design

The experiment was carried out in 2011, in the INRA Aquatic Ecology and Ecotoxicology Experimental Research Unit in Rennes (Brittany, France). Nine outdoor mesocosms (6 m in length × 4 m in wide × 1.20 m in depth; 18 m³) of the experimental platform

were selected. Each mesocosm was lined with a green plastic film which limited light reflection and was then filled with 15 m³ of tap water.

The impacts of light intensity and water depth on the growth of *L. grandiflora* were tested during the vegetative period (spring, summer and autumn). Three light intensities (100%, 70% and 50% of sunlight) and three water depths (30, 50 and 80 cm) were tested. Light intensities were created using green polyethylene shade canvases (95 g m⁻²), with an anti U.V. treatment (BOUILLION S.A.S. Innovations, France). For example, for one summer day, at a water depth of 50 cm, under natural light intensity (100%), we measured 25.03 ± 6.77 PAR (mean ± sd; μmol cm² s⁻¹), 18.58 ± 4.97 PAR for 70% of sunlight and 10.36 ± 3.07 PAR for 50%. Breeze-blocks were used to increase the height of the plants and shade canvases were arranged above the mesocosms and fixed to the ground, without preventing air circulation. The experiment was a randomized split-plot design. Three blocks with three mesocosms were defined. Each light intensity was represented in each block, with one light intensity per mesocosm (light intensity = whole-plot) and three replicates per intensity. Each mesocosm was divided into three sections to create three random water levels (water depth = subplot) with 9 replicates at the whole-plot level (9 mesocosms). 18 plants were transplanted into each mesocosm (six plants per water depth).

162 water primrose individuals (approximately 15 cm long shoot sections with an apex and without buds and roots) were collected in spring, in summer and in autumn 2011 from a pond called Apigné (01°44'25.2" O, 48°05'41.4" N), in Brittany (France). The macrophytes were washed and dry-blotted carefully before being weighed fresh. Five cm of each individual of *L. grandiflora* were planted in separate plastic pots, each 15 cm in diameter and 20 cm high, containing 12 cm of organic substrate and 3 cm of sand. The organic substrate (Bio Substrats 693, Klasmann, Germany, pH ≈ 6.3; conductivity: 40 mS cm⁻¹, with fertilizer NF U 42-001 (Regulations CE n°834/2007 and 889/2008)), consisted of frozen black *Sphagnum* peat, white sod peat, white peat and coco fibres, which corresponded to 40% of dry matter using gross product mass, or 85% of organic matter using dry product mass, and with a water retention capacity of 70%. Sand was used to stabilize the substrate and to avoid turbidity. The experiment was carried out over a period of 6 weeks, because *L. grandiflora* biomass can double in 15–20 days in slow flowing waters and in 70 days in rivers (Eppo, 2011), which can lead to competition between plants. During the experiment, tap water was added to maintain a constant water depth, and physico-chemical parameters were recorded at the beginning of each experiment in each mesocosm (Table 1). Dissolved oxygen concentration, pH and conductivity were measured using portable apparatuses (HACH HQ10-HQ30; WTW Sentis Plus 41 and WTW Tetracon 325, respectively). Concentrations of nutrients (NO₂⁻, NO₃⁻, NH₄⁺, PO₄³⁻) were performed using spectrophotometric techniques (WTW kit and Photolab S12). Water temperature was monitored every 30 min in three mesocosms: one mesocosm per light intensity, using an HOBO Pendant Temp/Light Data Logger (64K, UA-002-64).

Morphological traits

At the end of each experiment, nine morphological parameters were measured on each individual. The ability to characterize the growth of macrophytes is based on the Relative Growth Rate (RGR) of the plants, and its measurement was adapted from Hunt (1990): $RGR = (\ln B1 - \ln B0) / (T1 - T0)$, where *B0* and *B1* refer to total fresh biomass at times 0 and 1. The apical growth (main stem length, mean length of stem internodes, perimeter of the main stem (measured at stem base), the lateral growth (number and mean length of lateral shoots) and biomass allocation were measured. Biomass

Table 1
Climatic conditions and physico-chemical composition of water (mean \pm SE; $n=9$) at each season.

	Environmental conditions			Physico-chemical composition of water						
	Precipitation (mm)	Air temperature ($^{\circ}$ C)	Water temperature ($^{\circ}$ C)	Dissolved oxygen concentration (mg/l)	pH	Conductivity (μ S/cm)	NH ₄ ⁺ (mg/l)	NO ₂ ⁻ (mg/l)	NO ₃ ⁻ (mg/l)	PO ₄ ³⁻ (mg/l)
Spring	30.35	15.6 \pm 1.2	21.03 \pm 0.06	11.95 \pm 0.60	8.66 \pm 0.15	386.00 \pm 12.61	0.11 \pm 0.02	0.26 \pm 0.03	22.36 \pm 0.54	0.02 \pm 0.002
Summer	81.05	17.7 \pm 0.7	21.27 \pm 0.04	10.97 \pm 0.42	8.86 \pm 0.04	479.00 \pm 6.51	0.10 \pm 0.02	0.20 \pm 0.02	22.57 \pm 0.51	0.02 \pm 0.002
Autumn	25.05	15.7 \pm 2.6	17.14 \pm 0.05	11.21 \pm 0.44	8.38 \pm 0.09	430.66 \pm 7.38	0.08 \pm 0.05	0.15 \pm 0.01	20.03 \pm 0.55	0.008 \pm 0.004

allocation was determined using the root/shoot ratio, the weight of pneumatophores, as well as the length and width of leaves. The root/shoot ratio was determined from the fresh weight of below-ground and above-ground parts after rinsing to remove sand. Nine leaves were taken randomly from each plant in order to measure their length and width using Scion Image software.

Statistical analysis

Survival of individuals was analyzed using a generalized linear model. The results of the mesocosm experiment were analyzed using split-plot analysis of variance with season, light and water depth as factors. ANOVA assumptions regarding normality were not met for all traits (using the Shapiro–Wilk W -test; Zar, 1984). Therefore, transformations were used to meet ANOVA assumptions regarding homoscedasticity and normality and applying Tukey's HSD tests. Statistical tests were performed using statistical RTM 2.13.0 software. Untransformed means and standard errors are presented for ease of interpretation.

Results

Survival rate

The rate of mortality of *L. grandiflora* differed according to season (Table 2). The mortality of propagules was impacted by the interaction "season-light intensity-water depth" ($Df=8$; $F=403.16$; $p=0.03$). The mortality of *L. grandiflora* was higher in summer and lower in autumn than in spring ($Df=2$; $F=548.94$; $p<0.0001$; Table 3). Less than 50% of the individuals survived at water depths of 50 and 80 cm ($Df=2$; $F=563.99$; $p<0.000$; Table 2). However, the survival of *L. grandiflora* increased in the shade (50% of light intensity; $Df=2$; $F=602.29$; $p<0.0001$; Table 2). Season, light intensity, water depth and their interactions had an impact on growth (apical and lateral) as well as the biomass allocation of the water primrose.

Table 2
Rate of mortality (%) of *Ludwigia grandiflora* at each season according to both light intensity and water depth.

	Treatment	Rate of mortality of propagules (%)		
		Spring	Summer	Autumn
100% sunlight	30 cm	23.5	44.4	38.9
	50 cm	38.9	100	66.7
	80 cm	55.6	94.4	83.3
70% of sunlight	30 cm	16.7	44.4	44.4
	50 cm	11.1	83.3	94.4
	80 cm	55.6	100	94.4
50% of sunlight	30 cm	0	22.2	5.6
	50 cm	5.6	44.4	11.1
	80 cm	50	55.6	16.7

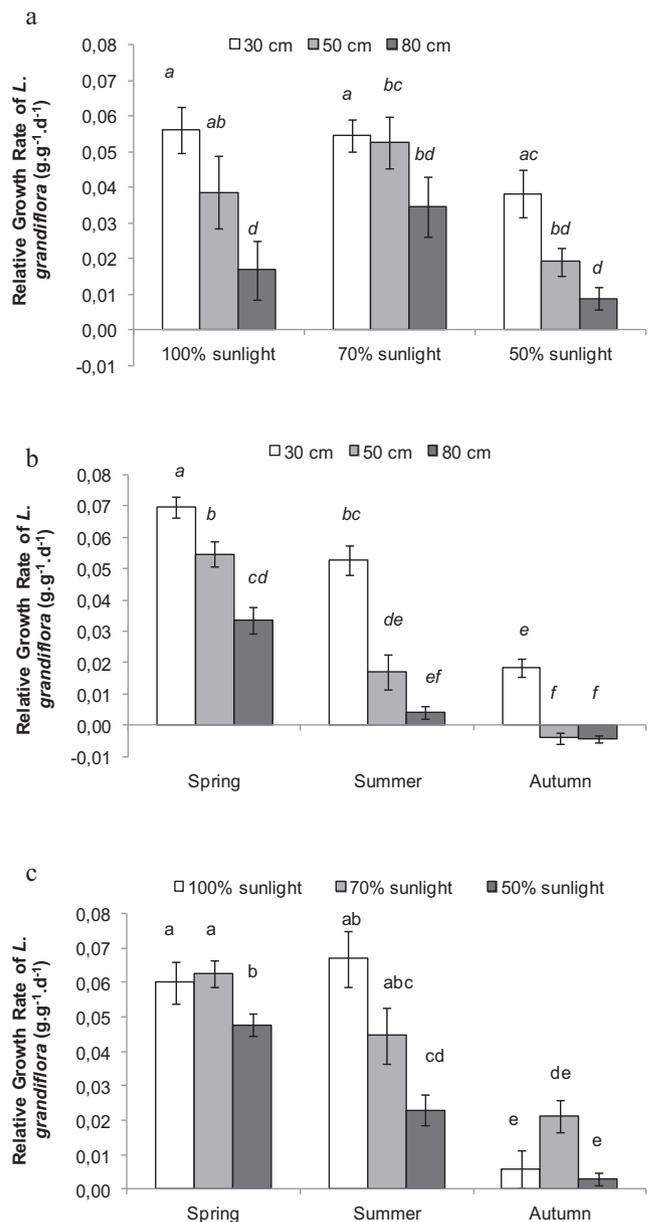


Fig. 1. Influence of the combined action of light intensity and water depth (a), season and water depth (b) and season and light intensity (c) on the Relative Growth Rate ($g\ g^{-1}\ d^{-1}$; mean \pm SE) of *L. grandiflora*. Different small letters above columns indicate significant differences between treatments. Tendencies are in italics.

Biomass

The RGR depended on the interactions between "light intensity and water depth", "light intensity and water depth" and "season-light intensity". The biomass tended to decrease with an increase in water depth and shade ($Df=4$; $F=2.26$; $p=0.06$; Fig. 1a;

Table 3 Summary of split-plot ANOVA on morphological traits of *Ludwigia grandiflora* (with *p* and *F*-values for each factor, Season (S): Df= 2; Block (B): Df= 2; Light (L): Df= 2; Interaction (B) × (S): Df= 8; Depth (D): Df= 2; Interaction (L) × (D): Df= 4; Interaction (S) × (D): Df= 4; Interaction (S) × (L) × (D): Df= 8). Significant results are in bold type, and tendencies are in italic.

	First step split-plot ANOVA						Second step split-plot ANOVA											
	Season (S)		Block (B)		Light (L)		B × S		Depth (D)		L × D		S × D		S × L		S × L × D	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
RGR	83.81	<0.0001	10.68	<0.0001	28.68	<0.0001	1.07	0.37	52.92	0.06	2.01	0.09	4.21	0.003	0.62	0.71		
Apical growth																		
Stem length	81.65	<0.0001	5.60	0.0009	7.16	0.0009	2.43	0.05	8.17	0.41	8.52	<0.0001	0.79	0.53	0.96	0.45		
Internode length	9.99	<0.0001	0.59	0.0002	8.94	0.0002	0.65	0.63	29.32	0.004	9.88	<0.0001	3.05	0.02	3.01	0.01		
Stem perimeter	19.30	<0.0001	1.59	<0.0001	31.41	<0.0001	2.15	0.07	31.97	0.005	0.21	0.93	6.17	0.0001	1.79	0.10		
Lateral growth																		
Lateral shoot	20.75	<0.0001	11.43	<0.0001	21.92	<0.0001	3.15	0.015	68.78	0.008	0.76	0.55	6.27	<0.0001	1.95	0.07		
Shoot length	38.94	<0.0001	1.50	<0.0001	24.64	<0.0001	1.68	0.16	14.68	<0.0001	13.82	<0.0001	1.25	0.29	4.28	0.001		
Biomass allocation																		
Root/shoot ratio	64.98	<0.0001	7.11	0.001	36.26	0.001	3.19	0.01	52.06	0.32	2.14	0.08	0.98	0.42	0.55	0.77		
Aerenchyma roots	22.87	<0.0001	7.30	0.0008	16.46	0.0008	0.13	0.97	92.81	0.01	6.55	<0.0001	2.34	0.06	0.74	0.61		
IDMC	44.61	<0.0001	19.54	<0.0001	42.89	<0.0001	7.31	<0.0001	67.99	0.002	3.34	0.01	4.40	0.02	2.36	0.03		
Leaf length	35.74	<0.0001	2.23	0.11	12.11	<0.0001	3.75	0.005	34.80	<0.0001	10.18	<0.0001	8.17	<0.0001	3.15	0.008		
Leaf width	12.01	<0.0001	3.72	0.02	40.15	<0.0001	2.03	0.09	54.06	0.05	11.68	<0.0001	2.74	0.03	3.62	0.003		

Table 3). Medium and high water levels (50 and 80 cm) led to a decrease in biomass of *L. grandiflora*, especially in summer and autumn (Df=4; *F*=2.01; *p*=0.09; Fig. 1b; Table 3) compared with the high biomass obtained at water depths of 30 cm in spring. Furthermore, the RGR of *L. grandiflora* decreased strongly with increasing shade in autumn (Df=4; *F*=4.21; *p*=0.003; Fig. 1c; Table 3).

Apical growth

Stem perimeter decreased as sunlight decreased and water depth increased. At 50% of the light intensity, at water depths of 50 and 80 cm, and under full and 70% of the sunlight at water depths of 80 cm, it was lower than at water depths of 30 cm in full sunlight (Df=4; *F*=3.80; *p*=0.005, Table 3). Internode length and stem perimeter of *L. grandiflora* were related to “light intensity and water depth”. Internode length was higher at water depths of 80 cm and increased with shade (Df=4; *F*=3.92; *p*=0.004; Fig. 2a). Stem length, as well as internode stem length were also explained by the “season-water depth” interaction: the length of stem (Df=4; *F*=8.52; *p*<0.0001; Table 3) and internode length (Df=4; *F*=9.88; *p*<0.0001; Fig. 2b) were lower in summer and autumn at all water depths than in spring, especially at water depths of 80 cm. Furthermore, the “season-light intensity” interaction induced changes in internode length and stem perimeter of *L. grandiflora*: the stem perimeter was higher in spring and summer than in autumn whatever the light intensities, especially under full sunlight conditions (Df=4; *F*=6.17; *p*=0.0001, Table 3). Conversely, internode length increased with the shade and was optimal at the beginning of the year: it was the highest at 50% of sunlight in spring (Df=4; *F*=3.05; *p*=0.02; Fig. 2c).

Lateral growth

The production of lateral shoots decreased with increased water depth and shade: *L. grandiflora* produced more lateral shoots at water depths of 30 cm under full sunlight and 70% of sunlight (Df=4; *F*=3.56; *p*=0.008; Fig. 3a; Table 3) and lateral shoots were smaller at 50% of light intensity whatever the water depth, compared to water depths of 30 cm in full sunlight and 70% of sunlight (Df=4; *F*=7.13; *p*<0.0001; Table 3). There was no significant effect of the “season-water depth” interaction on the number of lateral shoots (Fig. 3b; Table 3) but they were longer at water depths of 30 and 50 cm in spring, as well as at water depths of 30 cm in summer, than at all water depths in autumn (Df=4; *F*=13.82; *p*<0.0001, Table 3). Furthermore, the number of lateral shoots produced was higher whatever the light intensities in spring and summer under full sunlight and 70% of sunlight, than in autumn (Df=4; *F*=6.27; *p*<0.0001, Fig. 3c; Table 3).

Biomass allocation

The root/shoot ratio of *L. grandiflora* decreased significantly with an increase in water depth (Df=2; *F*=52.06; *p*<0.0001; Fig. 4a), and was the lowest at 50% of sunlight, whereas the root/shoot ratio was also higher under 70% of sunlight than under full sunlight conditions (Df=2; *F*=36.26; *p*<0.0001; Fig. 4b). Furthermore, it was the lowest in autumn and lower to a lesser extent in summer than in spring (Df=2; *F*=64.98; *p*<0.0001; Fig. 4c). Pneumatophore biomass was higher at water depths of 30 cm under full sunlight and 70% of sunlight than at 50 and 80 cm at all light intensities (Df=4; *F*=3.40; *p*=0.01; Table 3). Additionally, pneumatophore biomass was higher at water depths of 30 cm in spring and summer than in autumn and at 50 and 80 cm whatever the season (Df=4; *F*=6.55; *p*<0.0001; Table 3). In spring and summer under full sunlight and 70% of sunlight, the quantity of pneumatophores tended to be

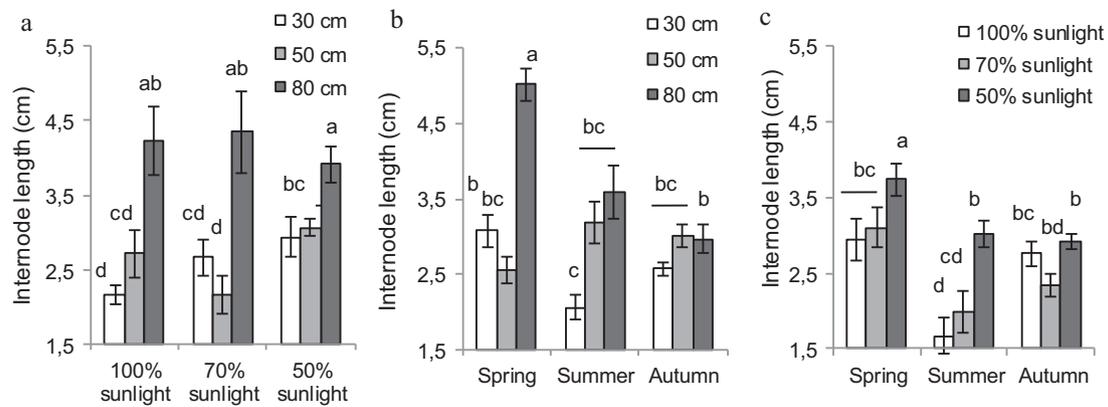


Fig. 2. Influence of the combined action of light intensity and water depth (a), season and water depth (b) and season and light intensity (c) on the internode stem length (cm; mean \pm SE) of *L. grandiflora*. Different small letters above columns indicate significant differences between treatments.

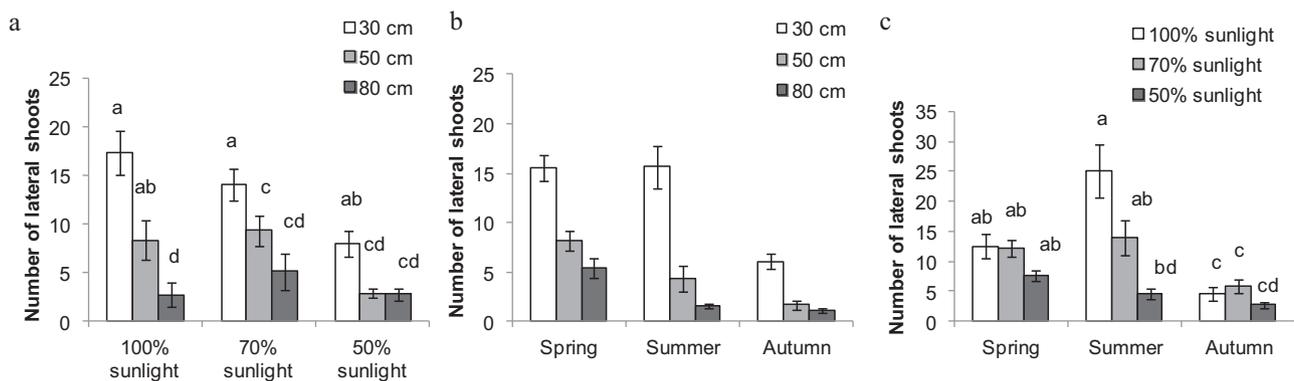


Fig. 3. Influence of the combined action of light intensity and water depth (a), season and water depth (b) and season and light intensity (c) on the number of lateral shoots (mean \pm SE) produced by *L. grandiflora*. Different small letters above columns indicate significant differences between treatments.

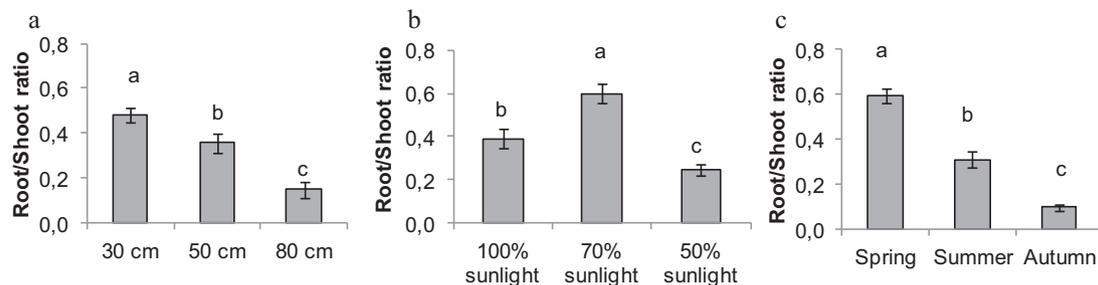


Fig. 4. Influence of water depth (a), light intensity (b) and season (c) (mean \pm SE) on the root/shoot ratio of *L. grandiflora*. Different small letters above columns indicate significant differences between treatments.

higher than at 50% of sunlight and in autumn at all light intensities (Df=4; $F=2.34$; $p=0.06$, Table 3).

On the other hand, the leaf morphology varied according to the “season-light intensity-water depth” interaction (Table 3). Leaf length (Df=4; $F=7.67$; $p<0.001$; Fig. 5a, Table 3) and leaf width (Df=4; $F=2.43$; $p=0.05$, Table 3) of *L. grandiflora* decreased strongly with increased water depth under full sunlight, whereas the decrease with water depth was lowest under 70% and 50% of sunlight. Our results showed that *L. grandiflora* increased its leaf area (leaf length: Df=4; $F=10.18$; $p<0.001$; Fig. 5b, Table 3 and leaf width: Df=4; $F=11.68$; $p<0.0001$, Table 3) more in spring and summer at water depths of 30 cm than at 80 cm in spring and summer, and whatever the water depths in autumn. Furthermore, leaf length was higher in spring at 70% of sunlight and in summer at all light intensities than under full sunlight and 70% of sunlight in autumn (Df=4; $F=8.17$; $p<0.001$; Fig. 5c, Table 3). Leaf width was higher in

spring at all light intensities and in summer under full sunlight than in autumn under full sunlight (Df=4; $F=2.74$; $p=0.03$; Table 3).

Discussion

Responses to light intensity and to water level

Survival, vigour and growth (apical and lateral) of *Ludwigia grandiflora*, as well as its biomass allocation varied according to season and responding to both light intensity and water depth. Although the influence of 70% of sunlight resulted in only small differences of the measured parameters as compared with the full sunlight intensity, the root/shoot ratios, stem lengths and leaf areas were stimulated under reduced light (70% of sunlight intensity). These results indicate that more biomass (and hence also energy) was allocated to the stem to facilitate its elongation, and to leaves

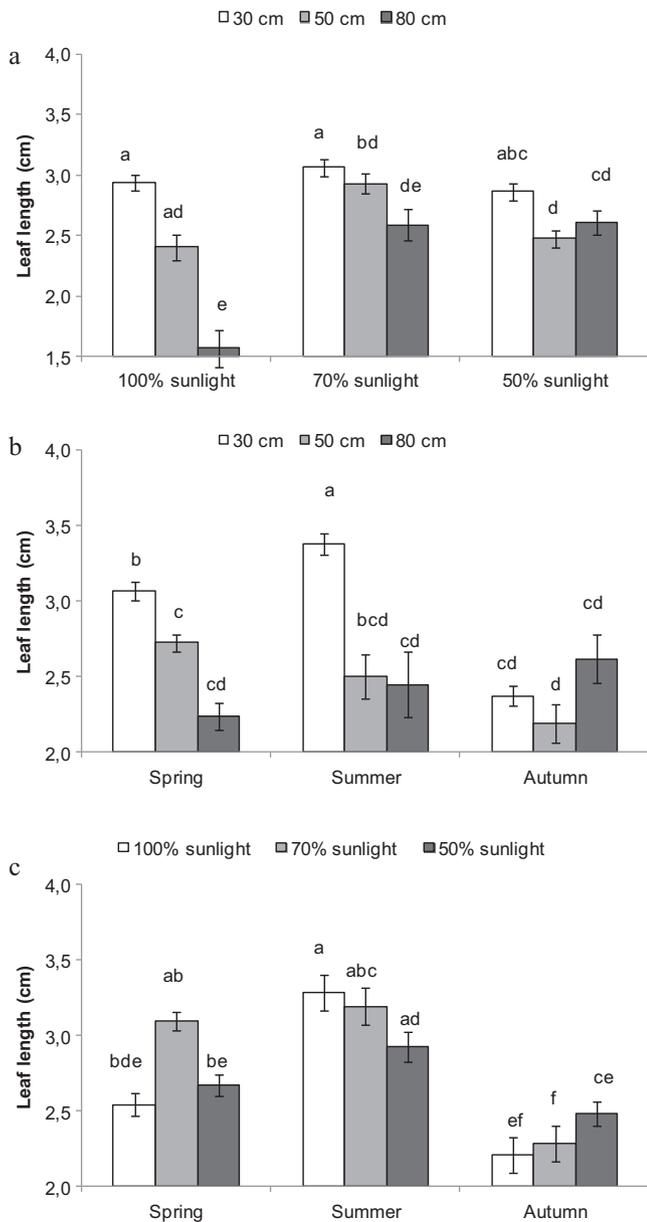


Fig. 5. Influence of the combined action of light intensity and water depth (a), season and water depth (b) and season and light intensity (c) on the leaf length (cm; mean \pm SE) of *L. grandiflora*. Different small letters above columns indicate significant differences between treatments.

in order to capture light and to grow out of the shade. A switch from below- to above-ground allocation, with an increase in shoot length, decrease in branch number (Strand and Weisner, 2001), changes in canopy structure and of leaf area are common adaptations of many aquatic plants under reduced light (Blanch et al., 1998; Cenzato and Ganf, 2001; Goldsborough and Kemp, 1988) in order to concentrate photosynthetic tissues close to the water surface (Barko and Smart, 1981). *Potamogeton amplifolius* (Cronin and Lodge, 2003) and *Myriophyllum spicatum* (Abernethy et al., 1996) allocate more biomass to shoots than roots in response to shade. 25% of light reduced the belowground biomass, increased stem length, and reduced branching and lateral spread of the three exotic in Europe species *Elodea canadensis*, *Egeria densa* and *Lagarosiphon major* (Ris et al., 2012). Low light (50% of sunlight) significantly reduced the biomass and lateral growth as well as the root/shoot ratio and the pneumatophore biomass of *L. grandiflora*. Our results

suggest that *L. grandiflora* responds to shade by stem elongation, and by reducing its branching and its root biomass similarly like other species. *L. grandiflora* did not tolerate strong shade conditions (50% of sunlight), whereas the plant could resist reduced light conditions (70% of sunlight). *L. grandiflora* obviously prefers high light intensity: its leaves reach maximum photosynthetic rates of up to $2200 \mu\text{mol CO}_2 \text{ h}^{-1} \text{ g}^{-1}$ dry weight (Hussner, 2010). This species may endure less favourable environmental conditions for a certain length of time, instead of responding immediately by allocating its energy morphogenetically investing into costly plastic responses in order to avoid the shade effect.

The amphibious species *L. grandiflora* shows similarities between shade and deep water adaptation responses. The survival and the growth responses of *L. grandiflora* are dependent on the water depth. At the deepest water levels, the root/shoot ratio and the biomass were strongly reduced. Water depth and light intensity induced the same morphological changes and responses in *L. grandiflora*. These two environmental factors are strongly linked (Lacoul and Freedman, 2006). Stems and internodes were the longest in the deepest water. Stem lengths were similar at water depths of 30 and 80 cm, which could be due to its widespread canopy formation in shallow water, capturing light near the water surface and colonizing the available water column. Thus, deep water reduced the amount and allocation of biomass, stem perimeter, and lateral growth of *L. grandiflora*. The reduction of stem diameter increases its flexibility, reducing mechanical damage from high water velocity (Boeger and Poulson, 2003).

Reduction of biomass, stem length and leaf area were also found as responses to deep water by *Ipomaea aquatica* (Lin et al., 2012), *Eleocharis sphacelata* (Asaeda et al., 2006), *Vallisneria spiralis* (Xiao et al., 2007), and *Myriophyllum aquaticum* (Wersal and Madsen, 2011). Furthermore, the reduction of root biomass in deep water could increase the risk of becoming uprooted, which may favour dispersal capacity.

In this study, pneumatophores (or aerenchymatous roots) were well developed in 30 cm of water. The increase of aerenchymatous tissue in shoots and roots is the most common and important adaptation to survive long-term flooding (Jackson and Armstrong, 1999) and the quantity of pneumatophores seemed to be linked to the canopy biomass of *L. grandiflora*. Most wetland and amphibious species have roots containing highly porous tissues (Blom, 1999). These aerenchymatous roots potentially aerate the submersed shoots (Ellmore, 1981a,b). For example, the root porosity of *Myriophyllum aquaticum* increases with increasing water level (Hussner et al., 2009). The morphological responses of *L. grandiflora* to water depth suggested that this plant is better adapted to low and medium water levels, than to deep water.

When plants were stressed with high water levels and shade simultaneously, their growth was not only influenced by each of these two factors independently, but also by the effects that different water depths had on the resulting light intensity. Thus, the interaction of these two factors could lead to 3 types of effect. Firstly, interaction could have an amplifying effect, corresponding to a total effect on plants which is stronger than that expected on the basis of both effects occurring separately (Lenssen et al., 2003). Secondly, the two factors may also affect plant growth independently or interact in such a way that one factor reduces the impact of additional factors (Lenssen et al., 2003). Thirdly, the interaction could result in a reduced effect in cases when one stress is so strong that additional limitations hardly depress plant growth any further (Chapin et al., 1987; Lenssen et al., 2003). Only stem length and root/shoot ratio are not related to the interaction of water depth and light intensity, whereas most of the measured traits could be explained by this interaction. The combination of a high shading effect and deep water has an amplified effect on morphological traits of *L. grandiflora*. Biomasses, apical and lateral

growth, as well as pneumatophore biomass were all reduced as a result of a combination of both effects. However, the leaf area decreased strongly with increasing water depth under full sunlight conditions, but the reduction of leaf area was lower under 70% and 50% of sunlight than under full sunlight conditions. This result was also found on emergent wetland species, *Eupatorium cannabinum*, *Mentha aquatica* and *Myosotis scorpioides* (Lenssen et al., 2003).

Responses according to season

The response of plants to environmental factors might be different according to the stage in their life cycle (Battaglia et al., 2000; Zedler et al., 1990). In *L. grandiflora* a significant temporal effect was found for most of the investigated traits, suggesting that the responses to either water depth or light intensity changed seasonally. The survival of *L. grandiflora* in relation to environmental factors was higher at the beginning (spring) and at the end (autumn) of its life cycle. Morphological traits of *L. grandiflora* were more impacted by these factors in autumn than in spring and summer. High biomass and canopy area were produced during the spring and summer with a gradual decline in autumn. Senescence occurred during winter, and the development of new shoots during spring.

The growing phase of *L. grandiflora* begins in spring with the emergence and rapid development of new shoots from buds of the rhizomes. To obtain the nutrients necessary for growth, many plants invest more energy in the production of root biomass than in shoots during the early stage of growth (Going et al., 2008), and plasticity in biomass allocation patterns may be partially constrained by the seasonal cycle of species (McConnaughay and Coleman, 1999). The production of aerenchymatous roots by *L. grandiflora* was higher during this period under low water depth as well as under natural, full, and under 70% of sunlight.

In late spring and in early summer, apical growth and branching were observed. The stems elongated horizontally and branched, extensively colonizing a maximum surface area. According to Boeger and Poulson (2003), the increase in stem diameter and length of internodes of the amphibious *Veronica anagallis-aquatica* leads to the development of temporary aerial branches in spring and summer. The development of temporary aerial branches of *L. grandiflora* could permit sexual reproduction through the development of flowers and fruits. However, the summer period, with unpredictable variations in water level is a critical stage in the successful establishment of *L. grandiflora* (Ruaux, 2008). The emergence of the reproductive organs is related to the erect stems which are strongly linked to water level: no reproductive organs are formed if stems of *L. grandiflora* are erected less than 10 cm above the water (Ruaux, 2008).

In autumn, degeneration of mats, defoliation and a decrease in the number and total length of branches appeared, which could explain the lower biomass and morphological response to water depth or shade in autumn during our experiment.

Invasiveness of *L. grandiflora* and implications for its management

The morphological plasticity of *Ludwigia grandiflora* is an important feature which explains the invasive success of this species, and its establishment in new habitats. *L. grandiflora* is a plastic species which shows a high tolerance to different water levels and to different light intensities which confers an adaptive advantage to this plant. However, many factors have not been taken into account in this study, such as the influence of substrate or water velocity, on plant growth. In our experiment, full sunlight and water depths

of 30 cm seemed to be the optimal growth conditions for *L. grandiflora*, with a higher vigour in spring and summer during its growing phase. Light intensity and water depth could play an important role in determining the success of the establishment of *L. grandiflora* into new habitats. *L. grandiflora* could colonize a wide range of habitats with the colonization of light-exposed or moderately shaded aquatic habitats, as well as shallow water environments and their terrestrial margins. Our findings have some implications for the management of *L. grandiflora*. Increased water depth and shade in the environment could limit invasion by *L. grandiflora* and might reduce their invasion dynamics. The presence of helophytes or well-developed riparian vegetation could reduce the light available for *L. grandiflora* (emerged and submerged growth forms). A restoration strategy (light manipulation techniques) could consist of the development of a closed and dense canopy cover, through plantation, to limit the colonization of water bodies by water primrose. However, increased canopy cover can decrease the growth of some native aquatic species. In order to evaluate such a restoration strategy, knowledge about effects of light availability on the growth and survival of both native macrophytes and invasive species is required. Similarly, the maintenance of high water levels could reduce the growth of *L. grandiflora*, by avoiding the production of erect stems, thus limiting its colonization. Furthermore, the previous establishment of native helophytes which are better adapted to the given conditions (Dutartre et al., 2007) could outcompete *L. grandiflora*.

A major challenge is to limit the expansion of this invasive species in the context of global changes. Climate changes will potentially favour the invasiveness of some exotic species, but other currently invasive species could become greater or lesser threats (Thuiller et al., 2007). They will impact inland aquatic ecosystems. Water levels are expected to increase in lakes at high latitudes, where climate models indicate increased precipitation, while water levels at mid- and low latitudes are projected to decline (Bates et al., 2008). Aquatic non-indigenous plants could benefit from increasing seasonality and more marked wet and dry cycles. Fewer winter frosts and the decrease of water levels at mid-latitudes may cause the expansion of colonization areas of tropical species, such as *Ludwigia grandiflora*, whereas deep water levels and colder winter could limit the growth of this species at high latitudes.

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