

RESEARCH PAPER

Phenotypic plasticity as a clue for invasion success of the submerged aquatic plant *Elodea nuttallii*

S. Szabó¹, E. T. H. M. Peeters², G. Várbíró^{3,4}, G. Borics^{3,4} & B. A. Lukács³ 

¹ Department of Biology, University of Nyíregyháza, Nyíregyháza, Hungary

² Department of Environmental Sciences, Aquatic Ecology and Water Quality Group, Wageningen University, Wageningen, the Netherlands

³ Department of Tisza River Research, MTA Centre for Ecological Research DRI, Debrecen, Hungary

⁴ GINOP Sustainable Ecosystems Group, MTA Centre for Ecological Research, Tihany, Hungary

Keywords

Alien; aquatic plant; competition; light; macrophyte; nitrogen.

Correspondence

B. A. Lukács, Department of Tisza River Research, MTA Centre for Ecological Research-DRI, Bem tér 18/C. H-4026, Debrecen, Hungary.

E-mail: lukacs.balazs@okologia.mta.hu

Editor

J. T. M. Elzenga

Received: 5 April 2018;

Accepted: 19 September 2018

doi:10.1111/plb.12918

ABSTRACT

- Two closely related alien submerged aquatic plants were introduced into Europe. The new invader (*Elodea nuttallii*) gradually displaced *E. canadensis* even at sites where the latter was well established. The aim of the study was to evaluate the combined effects of environmental factors on several phenotypic characteristics of the two *Elodea* species, and to relate these phenotypic characteristics to the invasion success of *E. nuttallii* over *E. canadensis*.
- In a factorial design, *Elodea* plants were grown in aquaria containing five different nitrogen concentrations and incubated at five different light intensities. We used six functional traits (apical shoot RGR), total shoot RGR, relative elongation, root length, lateral spread, branching degree) to measure the environmental response of the species. We calculated plasticity indices to express the phenotypic differences between species.
- Light and nitrogen jointly triggered the development of phenotypic characteristics that make *E. nuttallii* a more successful invader in eutrophic waters than *E. canadensis*. The stronger invader showed a wider range of phenotypic plasticity. The apical elongation was the main difference between the two species, with *E. nuttallii* being more than two times longer than *E. canadensis*. *E. canadensis* formed dense side shoots even under high shade and low nitrogen levels, whereas *E. nuttallii* required higher light and nitrogen levels.
- We found that under more eutrophic conditions, *E. nuttallii* reach the water surface sooner than *E. canadensis* and through intensive branching outcompetes all other plants including *E. canadensis*. Our findings support the theory that more successful invaders have wider phenotypic plasticity.

INTRODUCTION

Invasions of non-indigenous species are recognised as one of the major environmental problems worldwide. Invasions may have many ecological impacts (Peterson 2003) such as a drastic reduction in biodiversity (Williamson 1999). Furthermore, by jeopardising ecosystem services, species invasions can have high economic costs (Pimentel 2005). Several hypotheses have been developed to explain the mechanisms of establishment and spread of invasive species. According to Davis *et al.* (2000), a plant community becomes more susceptible to invasions when there is an increase in the amount of unused resources such as light, nutrients or water. It has been argued that global environmental changes may also accelerate species invasion (Mormul *et al.* 2012) partly due to increases in available resources, since higher temperature accelerates decomposition leading to higher availability of nutrients (Dukes & Mooney 1999; Hellmann *et al.* 2008; Netten *et al.* 2010). In aquatic ecosystems, eutrophication caused by excessive anthropogenic nutrient loading (N, P) often triggers instability of the ecosystems (Scheffer *et al.* 2003), and these altered systems can easily be invaded by several, globally invasive, submerged weeds.

Characteristics identified as important determinants of plant invasiveness are rapid growth, high offspring production and potential long-distance dispersal of propagules (Rejmanek & Richardson 1996; Goodwin *et al.* 1999). Successful invaders have also been suggested to have fewer enemies (Keane & Crawley 2002) and to be better competitors than native species (Vilà & Weiner 2004; Lukács *et al.* 2017).

Phenotypic plasticity, the capacity of a given genotype to express different phenotypes in different environments (Sultan 2000), is the primary adaptive mechanism for plants to spread into a range of habitats. Under various environmental conditions such as light, nutrient or temperature, plants are able to rapidly change their phenotypic characters (apical elongation, chlorophyll concentration in leaves, branching; Larson 2007; Riis *et al.* 2010; Molnár *et al.* 2015). Using such acclimation mechanisms submerged aquatic plants can optimise light and nutrient utilisation in their natural habitats, and these acclimations enable them to outcompete other aquatic plants (Chambers 1987; Chambers & Kalff 1987).

Several aquatic plant species are considered as invasive aliens and have important impacts on the invaded communities. Among them, being introduced from North America in the

middle of the 19th century, *Elodea canadensis* was recently integrated into most Western European freshwater systems (Simpson 1984; Cook & Urmi-König 1985). The subsequent introduction of its congeneric species pair *E. nuttallii* (Plach) St. John from North America in the middle of the 20th century resulted in a gradual displacement of *E. canadensis* by this new invader, even at sites where the latter was well established (Cook & Urmi-König 1985; Simpson 1990; Greulich & Trémolières 2006). This displacement took place over a period of 1 or 2 years, as documented in the British Isles (Lund 1979; Simpson 1990) and in Western Europe (Barrat-Segretain 2001). The new invader plays a key role in structuring aquatic plant communities since it often forms monospecific vegetation types in hypertrophic waters throughout Western Europe (van Zuidam & Peeters 2013). Since the beginning of the 21st century *E. nuttallii* has appeared in the Central European countries Poland (Kolada & Kutya 2016), Slovakia (Ot'ahel'ová & Valachovič 2002), Hungary (Király *et al.* 2007; Lukács *et al.* 2016), Slovenia (Király *et al.* 2007; Kuhar *et al.* 2007) and Croatia (Grudnik & Germ 2013; Kočić *et al.* 2014). Therefore, it is possible that *E. canadensis* will be displaced in these areas.

The mechanisms resulting in the successful invasion by *E. nuttallii* into habitat previously occupied by *E. canadensis* are unclear. Some hypotheses suggest differences in ecophysiological characteristics, such as biomass growth rate (Barrat-Segretain & Elger 2004), photosynthesis and respiration (James *et al.* 1999), nitrogen and phosphorus accumulation (Robach *et al.* 1995; James *et al.* 2006), life history traits (Barrat-Segretain *et al.* 2002; James *et al.* 2006; Barrat-Segretain & Lemoine 2007). Competition experiments between the two species revealed that growth of *E. canadensis* was negatively influenced by intra- and interspecific neighbours, but in the case of *E. nuttallii* this impact was not significant (Barrat-Segretain & Elger 2004). Shading induced by the formation of a canopy may be a key factor in explaining the success of *E. nuttallii* (Simpson 1990; Barrat-Segretain 2004, 2005). This confirmed Thompson's (1991) theory that invaders must have such advantageous traits that are not shared by pre-existing species.

The question arises: what environmental factors trigger the phenotypic response of *E. nuttallii* in canopy formation? The aim of this study was to evaluate in a laboratory experiment the combined effects of environmental variables (light exposure and nitrogen concentrations) on several phenotypic characteristics of the two *Elodea* species; and to relate these phenotypic characteristics to the invasion success of *E. nuttallii* over *E. canadensis*.

Since canopy formation of submerged macrophytes takes place along an increasing light gradient (from the shady bottom to the water surface), we therefore hypothesised that light intensity might play a key role. Nitrogen concentration of the water could be another potential key factor in canopy formation, because large-scale analyses of presence-absence and abundance data of aquatic plants from The Netherlands (Knoben & Peeters 1997; van Zuidam & Peeters 2013) and from Central European waters (Király *et al.* 2007; Grudnik *et al.* 2014) suggested that the replacement of *E. canadensis* by *E. nuttallii* was especially pronounced in ditches in agricultural areas, where total nitrogen input was much higher. Consequently, we hypothesised that: (i) *E. nuttallii* has a wider range of phenotypic plasticity than *E. canadensis* along the light intensity and nitrogen concentration gradients; (ii) increasing

nitrogen concentrations stimulate the relative elongation of *E. nuttallii* better than that of *E. canadensis*; and (iii) high light level together with high nitrogen concentration stimulate branching degree of *E. nuttallii*.

Both *Elodea* species may strongly modify light conditions if they are grown in co-cultures (Barrat-Segretain & Elger 2004; Barrat-Segretain 2005). In order to exclude these effects both species were cultivated separately.

MATERIAL AND METHODS

Plant collection, pre-incubation

Elodea canadensis plants were collected from the River Bodrog (48.172491°N, 21.363358°E) and *E. nuttallii* from the Eastern Principal Channel in the surroundings of Hajdunánás (47.860911°N, 21.382270°E) in Hungary. Genetic variability among the selected shoots was minimal because only vegetative reproduction takes place among the European *Elodea* species (Barrat-Segretain *et al.* 2002). In addition, the shoots were selected from the same colony (polycormon), consequently the selected shoots were genetically similar (*i.e.* ramets). Pre-incubation under experimental conditions lasted for 18 days. Apical *Elodea* shoots were placed in five 20-l aquaria containing growth medium, modified from Barko & Smart (1985) by adding NH_4NO_3 to a final nitrogen concentration of 0.05, 0.25, 0.5, 2.5 or 5.00 $\text{mg}\cdot\text{N}\cdot\text{l}^{-1}$ among treatments. Phosphorus was added as K_2HPO_4 to a final concentration of 0.2 $\text{mg}\cdot\text{P}\cdot\text{l}^{-1}$ and a supply of micronutrients was ensured by adding TROPICA micronutrient solution (TROPICA, Egå, Hungary) with a 10,000-fold dilution. The solution was renewed every second day. After the pre-incubation period, length of the shoots was reduced to 65 mm, thus the shoot had no lateral branches or adventitious roots (Madsen *et al.* 1998).

Laboratory experiment

Three shoots were placed in an upright position on a plastic mesh and placed in 2-l aquaria with 12 cm depth containing 2-l solutions described above. Based on the water temperature profile (May–July) of small lakes in the Carpathian Basin (Borics *et al.* 2015) and on the optimal temperature range for shoot growth (Barko *et al.* 1982; Mormul *et al.* 2012), all aquaria were set to a controlled temperature (23–25 °C) in a water bath. For both *Elodea* species, all the five different N treatments were incubated at five different light intensities from 0, 10, 28, 80 to 180 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PAR on the water surface (cosine irradiance) in a 16-h:8-h light:dark regime, with renewing of the medium every second day. Illumination was achieved with Philips 400 W metal halogen lamps and by using green plastic gauze with different mesh sizes between the light source and the aquaria. Each treatment was replicated four times, meaning that 200 aquaria were used. In order to keep the initial light levels more or less constant, as well as to avoid self-shading of the plants, the duration of the experiment was only 12 days before the plants were harvested (Madsen *et al.* 1998; Forchhammer 1999).

Plant trait measurement

We measured five traits (shoot dry weight, root dry weight, shoot length, root length and number of branches), which were

used to calculate five additional traits [total shoot relative growth rate (RGR), apical shoot RGR, relative elongation, lateral spread and plasticity index]. All traits were measured and calculated at the individual level in all aquaria; however, for statistical analysis only the mean trait values of the three plants of each aquarium were considered. Shoot and root dry weight were measured after samples had been dried at 105 °C for 24 h in a forced-oven until constant weight was achieved. Total shoot length refers to the length of the apical and lateral (side) shoots, while apical shoot length represented the main (longest) shoot of the plant. Total shoot length data were used in the calculation of RGR, branching degree, relative elongation and lateral spread. Total shoot RGR was calculated as $RGR = (\ln l_t - \ln l_0)/t$, where l_0 represents the initial and l_t the final total shoot length of the plants and t was the incubation time in days. Apical shoot RGR was calculated as $RGR = (\ln l_t - \ln l_0)/t$ where l_0 represents the initial and l_t the apical shoot length of the plants and t was the incubation time in days. Relative elongation of the plants was calculated as shoot length formed from a unit biomass ($\text{mm} \cdot \text{mg}^{-1}$ DW). Lateral spread was calculated as side shoot/apical shoot length. Branching degree (*i.e.* branching architecture) was the number of living ramifications per unit of shoot length (Pérez-Harguindeguy *et al.* 2013); it was calculated as the number of branches/length of shoot.

We calculated the plasticity index for both *Elodea* species for nitrogen (PI_N) and for light (PI_L) according to Valladares *et al.* (2002, 2005) as: $PI = (\text{maximum mean} - \text{minimum mean}) / \text{maximum mean}$. Plasticity index for nitrogen (PI_N) was calculated under L_{max} light intensities, plasticity index for light (PI_L) was calculated under N_{max} nitrogen concentration, where the variable showed its highest value (*i.e.* root formation L_{max} $180 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, N_{max} $0.5 \text{ mg} \cdot \text{l}^{-1}$). The index ranges from 0 (no plasticity) to 1 (maximum plasticity).

Statistical analysis

A principal components analysis (PCA) was performed with all measured traits as independent variables to determine the co-variation between traits. Normality of the variables was checked with a Kolmogorov-Smirnov test. Total shoot RGR, apical shoot RGR, relative elongation and root length were all normally distributed ($P > 0.05$). The branching degree of the shoots were normally distributed only between $10\text{--}180 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. A generalised linear model (GLM) was used to test the significance of the factors (light, nitrogen, species identity) and their interactions on the variables. Residuals were checked for normality and homogeneity of variances evaluated with Levene's test. Depending on the homogeneity of the variances, Tukey and Dunnett T3 *post-hoc* tests were used to evaluate which treatments differed from each other. Pair-wise comparisons were used to test the variables for significant differences between species where mean differences (MD) \pm SD were indicated. All analyses were done in SPSS 16.0 (SPSS, Chicago, IL, USA).

RESULTS

Ordination with PCA

The results of PCA ordination revealed that *E. canadensis* and *E. nuttallii* were separated along the studied phenotypic

characters, especially along the first PCA axis. The first two axes of PCA ordination explained 94.5% of total variability. *E. nuttallii* showed higher apical shoot RGR and higher relative elongation, while *E. canadensis* showed higher rates of branching degree, root formation and lateral spread (Fig. 1).

Differences in phenotypic plasticity

Along the examined light and nitrogen gradient the two *Elodea* species showed characteristic differences in their phenotypic plasticity (Table 1). Regarding the overall phenotypic characteristics, *E. nuttallii* showed a higher plasticity than *E. canadensis*. *E. canadensis* showed higher (by 0.1) plasticity for light regarding relative elongation. *E. nuttallii* showed higher (by 0.1) plasticity for nitrogen in apical shoot RGR, root formation, lateral spread and branching degree for light in apical shoot RGR and branching degree. Branching degree PI_N values showed the most characteristic difference between the two *Elodea* species (*E. canadensis* 0.13, *E. nuttallii* 1.00).

Relative growth rate

Total shoot RGR and apical shoot RGR were significantly influenced by species identity, nitrogen concentration, light intensity and their interactions (Table S1). Increase in nitrogen concentration caused significantly ($P < 0.001$ Tukey test) higher total shoot RGR for both species, and this effect was far less pronounced at low light level ($10 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) than in well illuminated conditions ($80\text{--}180 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; Fig. 2). Total shoot RGR of both *Elodea* species increased toward higher nitrogen concentrations, up to $2.5 \text{ mg} \cdot \text{l}^{-1}$. Under high nitrogen concentrations ($2.5\text{--}5.0 \text{ mg} \cdot \text{l}^{-1}$) both *Elodea* species showed significantly ($P < 0.001$ Tukey test) increased total shoot RGR with increasing light intensity until $80 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Fig. 2). Pair-wise comparisons indicated that the total shoot RGR of *E. nuttallii* (0.086 ± 0.002) was not significantly ($P = 0.052$) lower than that of *E. canadensis* (0.090 ± 0.002). Apical shoot RGR of *E. nuttallii*

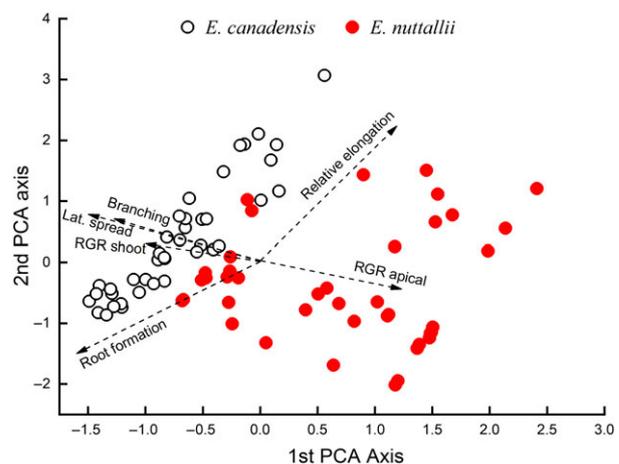


Fig. 1. PCA ordination of the studied phenotypic characters of *E. canadensis* and *E. nuttallii*. Data were square root-transformed RGR apical: relative growth rate of apical shoot; Branching: branching degree, Lat. spread, lateral spread.

Table 1. Plasticity index of *E. canadensis* and *E. nuttallii* for nitrogen (PI_N) and light (PI_L). PI_N were calculated under L_{max} light intensities, PI_L were calculated under N_{max} nitrogen concentration. L_{max} and N_{max} are nitrogen and light levels, respectively, where the variable showed its highest value. Higher PI values are underlined if they differ by 0.1 between the two *Elodea* species.

variable	PI_N		PI_L		L_{max} ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	N_{max} ($\text{mg}\cdot\text{l}^{-1}$)
	<i>E. canadensis</i>	<i>E. nuttallii</i>	<i>E. canadensis</i>	<i>E. nuttallii</i>		
RGR shoot	0.67	0.70	0.71	0.69	80	2.5
RGR apical shoot	0.53	<u>0.64</u>	0.36	<u>0.63</u>	28	2.5
Relative elongation	0.17	0.20	<u>0.57</u>	0.45	10	5
Root formation	0.34	<u>0.76</u>	1.00	1.00	180	0.5
Lateral spread	0.83	<u>1.00</u>	0.95	1.00	180	5
Branching degree	0.13	<u>1.00</u>	0.64	<u>1.00</u>	180	5

(0.073 ± 0.001) was significantly higher ($P < 0.001$) than that of *E. canadensis* (0.031 ± 0.001). In general, *E. nuttallii* had twice as high apical shoot RGR as *E. canadensis* and also had the highest apical shoot growth under relatively low light ($28 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; see Fig. 3, S1).

Relative elongation

Relative elongation, *i.e.* the shoot length formed from a unit of biomass, was significantly influenced by light intensity, nitrogen concentration, species identity and their interactions (Table S1). Relative elongation of the shoots was highest in media with high nitrogen concentration ($2.5\text{--}5.0 \text{ mg}\cdot\text{N}\cdot\text{l}^{-1}$) at low light intensity ($10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for both *Elodea* species (Fig. 3). With increasing light intensity from $10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the relative elongation of the plants was significantly lowered ($P < 0.001$ Dunnett T3 test). With increasing nitrogen concentration, the relative elongation of the plants significantly increased ($P < 0.001$ Tukey test). Under the highest light level ($180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) *E. nuttallii* (4.75 ± 0.166) showed significantly higher ($P < 0.001$, pair-wise comparisons) relative elongation than *E. canadensis* (3.50 ± 0.166 ; Fig. 4).

Root length

Since only apical shoots were planted, all roots were formed during the experiment. Growth stimulation by light was reflected in increased root length, which was highest at the higher light intensities in cultures growing at $0.5 \text{ mg}\cdot\text{N}\cdot\text{l}^{-1}$. Root length was significantly influenced by light intensity, nitrogen concentration, species identity and their interactions (Table S1). *E. canadensis* (0.461 ± 0.008) produced significantly longer ($P < 0.001$, pair-wise comparisons) root than *E. nuttallii* (0.270 ± 0.008 ; Fig. 4).

Lateral spread

For both *Elodea* species, lateral spread (*i.e.* side shoot length per apical shoot length) of the shoots increased with increasing nitrogen concentration and with increasing light intensity (Figs 3 and 5). However, lateral spread seemed to be saturated above $10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for *E. canadensis*. Under illumination ($10\text{--}180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) *E. canadensis* (1.49 ± 0.05) had significantly higher ($P < 0.001$, pair-wise comparisons) lateral spread than *E. nuttallii* (0.242 ± 0.050).

Branching degree

Branching degree (number of branches per meter shoot length) was significantly influenced by light and species identity (Table S1). Nitrogen had a significant ($df = 4$, $F = 31.633$, $P < 0.001$, ANOVA) impact on branching degree of *E. nuttallii* but not on *E. canadensis* ($df = 4$, $F = 0.792$, $P = 0.534$, ANOVA). The branching degree of *E. canadensis* was very high ($>10 \text{ m}^{-1}$) under any light level and it remained independent of the applied nitrogen concentration (see Figs 3, 6 and S2). *E. nuttallii* on the other hand, did not show intensive branching under low light intensity and under low nitrogen concentrations; it started to produce some branches only at higher light levels ($80\text{--}180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) combined with higher nitrogen concentration ($2.5\text{--}5.0 \text{ mg}\cdot\text{N}\cdot\text{l}^{-1}$; Figs 6, S2). Under illuminated conditions ($10\text{--}180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) *E. canadensis* (16.037 ± 0.245) had a significantly higher ($P < 0.001$, pair-wise comparisons) rate of branching degree than *E. nuttallii* (5.112 ± 0.245).

DISCUSSION

Interactive effects of light and nitrogen

Previous studies found that the distribution of *E. canadensis* and *E. nuttallii* was basically determined by nutrient, temperature and light variables. Among the two congeneric species, *E. nuttallii* was found to be more resistant to nutrient enrichment and temperature increases (Greulich & Trémolières 2006). *E. canadensis* was found to be more sensitive to eutrophication than *E. nuttallii* (Kolada & Kutya 2016). Moreover, light availability had a stronger effect on growth rate and plant morphology of *E. canadensis* than temperature (Riis *et al.* 2010). In our study we investigated the environmental-induced phenotypic plasticity of both species to nutrient and light variables in more detail. We found that light and nitrogen limited the growth of both *Elodea* species simultaneously. The two factors had an interactive effect on many of the measured traits: under high nitrogen concentrations (2.5 and $5.0 \text{ mg}\cdot\text{l}^{-1}$), higher light levels stimulated growth of the shoots twice as much as those at low N levels ($0.05\text{--}0.25 \text{ mg}\cdot\text{l}^{-1}$). These results revealed that in order to sustain the same growth, under decreasing light intensity, the plants need more nitrogen; and *vice versa*, under lowered nitrogen level they need more light. Interactive effects of various factors on plant growth have previously been observed more often for light and inorganic

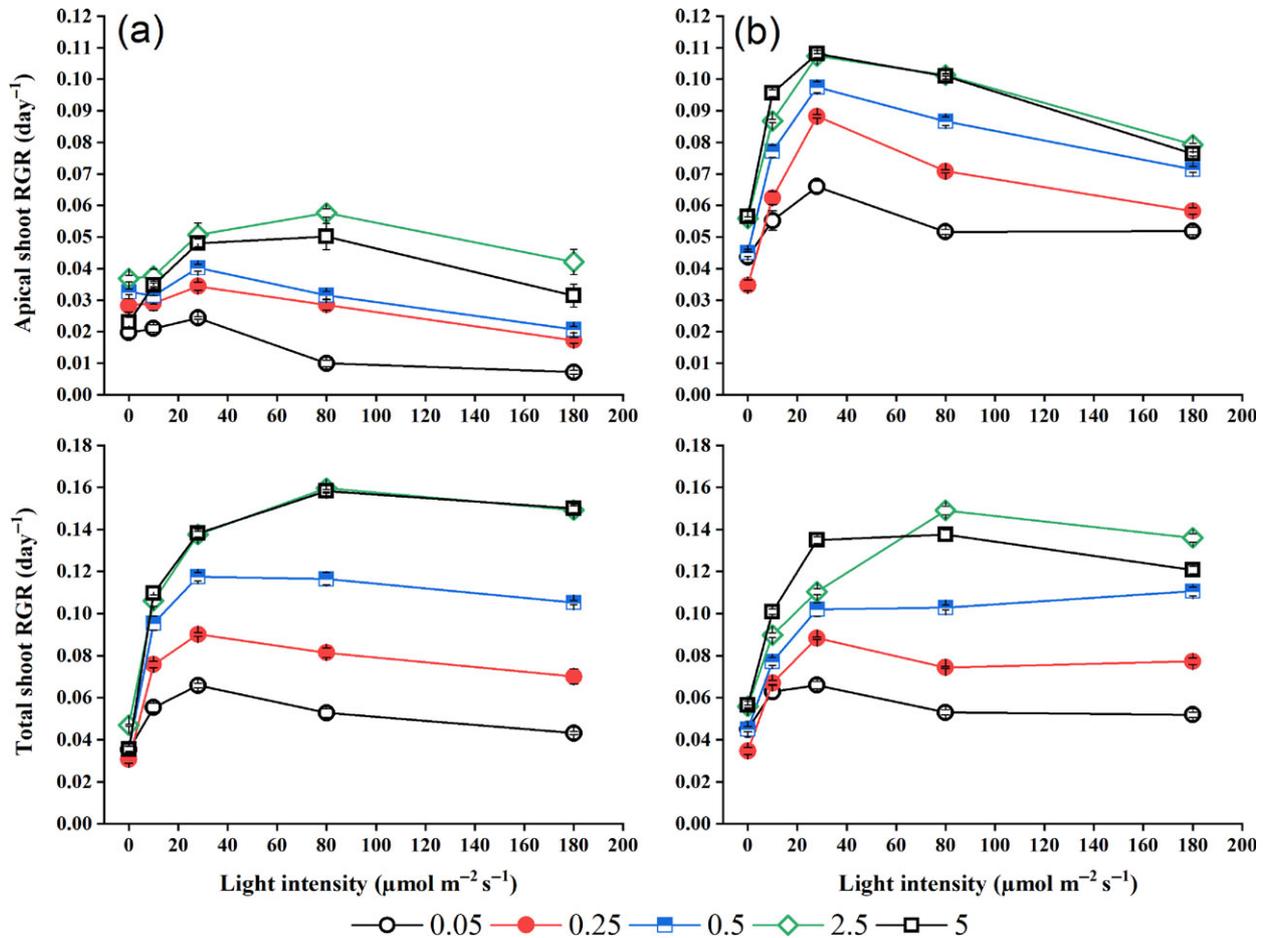


Fig. 2. Apical shoot RGR and total shoot RGR of *E. canadensis* (a) and *E. nuttallii* (b) at different nitrogen and light levels. Error bars indicate \pm SE.

carbon (Madsen & Sand-Jensen 1994), light and temperature (Barko *et al.* 1982), temperature and phosphorus (Peeters *et al.* 2013) and nitrogen and phosphorus (Cao *et al.* 2011).

Different resource acquisition strategies

Elodea canadensis and *E. nuttallii* showed high ability to balance the need and capacity to acquire light and nutrients. The two studied species had many similarities and differences in response to different light and nutrient exposure. Under maximum applied light intensity (which reflects the light conditions in the upper water body) and under low nitrogen levels both species showed increased root formation and reduced plant elongation, as also found by James *et al.* (2006). This clearly indicated that plants invested more in nutrient uptake than in photon capture, since more belowground biomass was necessary for nutrient uptake in nitrogen-poor water (Madsen & Cedergreen 2002). The differences between the two species were that, on one hand, *E. canadensis* invested much more in root formation and branching than *E. nuttallii*, while *E. nuttallii* invested relatively more in apical shoot elongation than *E. canadensis*. On the other hand, at low light levels (which reflect the bottom growing conditions), both species redirected resources towards more efficient photon capture rather than nutrient uptake, as indicated by the increased shoot elongation

per unit biomass, reduced allocation to root formation and drop in lateral spread and branching. In this study, changes in phenotypic traits were mainly related to environmental changes but not to changes in genotype, since the selected shoots were genetically similar.

Similar morphological changes in shoot length (Barko *et al.* 1982; Rodrigues & Thomaz 2010), lateral spread and branching degree (Riis *et al.* 2012; Zefferman 2014) and root biomass allocation (Sand-Jensen & Madsen 1991) have been found for several submerged species, including *Egeria densa*, *E. canadensis*, *E. nuttallii* and *Lagarosiphon major*. In our study, both the increase in elongation and the trends in the various morphological acclimations (branching degree, root length, length specific weight and shoot length) were similar to those observed in response to decreased light intensities through shading of floating vegetation found in submerged aquatic plants (Larson 2007; Lu *et al.* 2013). The observed changes are in line with the foraging concept, where plants develop their traits (longer stems, less root biomass, less branches) under low light conditions to improve resource acquisition and reduce respiratory costs (de Kroon & Hutchings 1995).

We found that under optimal light (80–180 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and nitrogen (2.5–5.0 $\text{mg}\cdot\text{N}\cdot\text{l}^{-1}$) conditions *E. canadensis* had much the same total shoot growth rate as *E. nuttallii*, which confirms the result of Barrat-Segretain & Elger (2004).

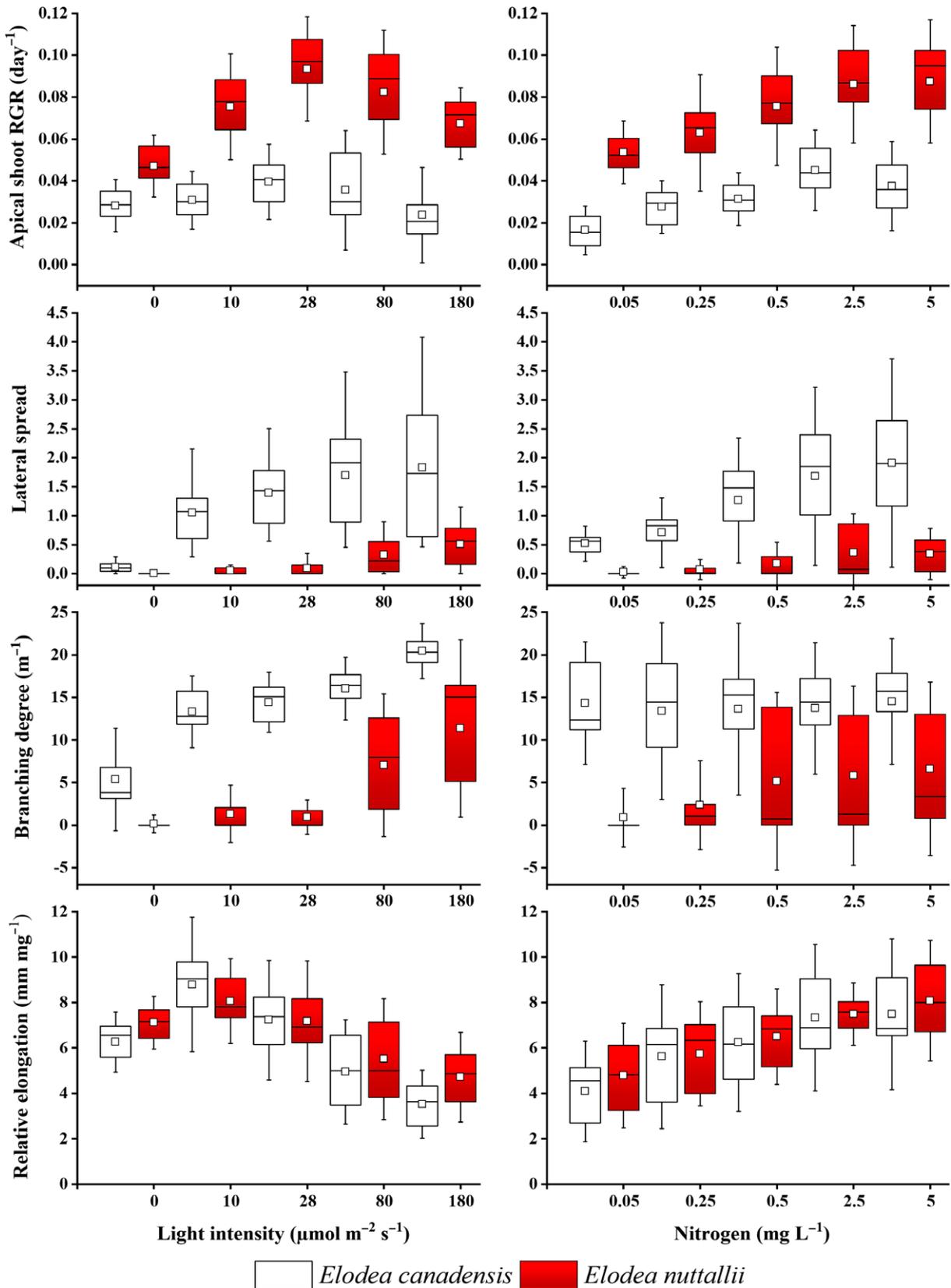


Fig. 3. Boxplots of apical shoot RGR, lateral spread and branching degree and relative elongation of *E. canadensis* and *E. nuttallii* under different light levels and different nitrogen concentrations. Each boxplot represents data of five nitrogen concentrations in the left panel, or data of five light intensity in the right panel. Boxes: +25–75% percentiles; whiskers: \pm SD; \square : median, $n = 20$.

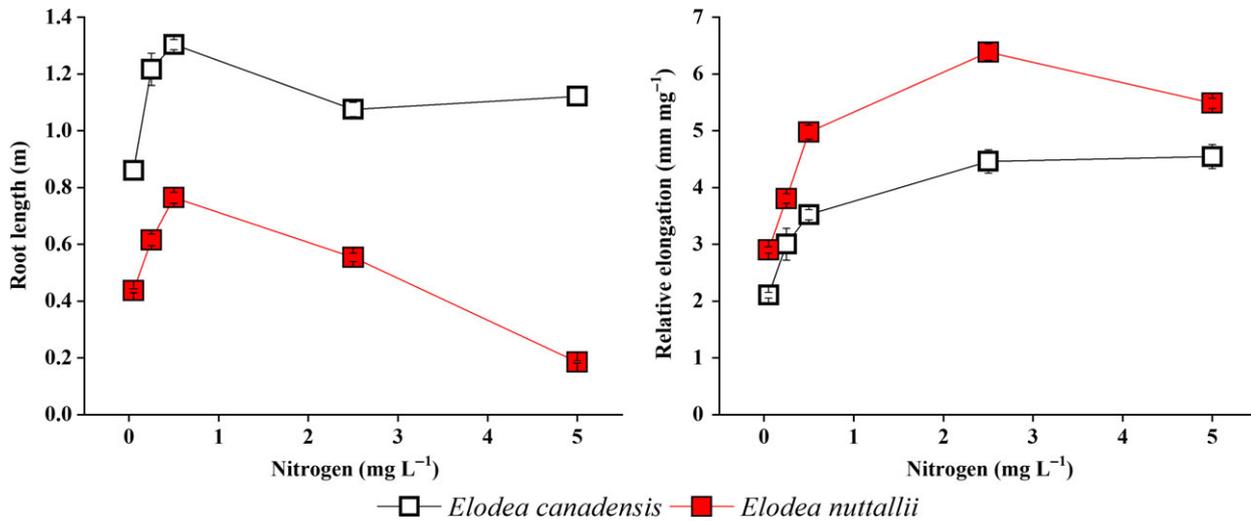


Fig. 4. Root length (m) and relative elongation (length of shoot per unit biomass) of the studied *Elodea* species at different nitrogen concentrations. Cultures were grown under maximum light intensity (180 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Error bars indicate $\pm\text{SD}$.

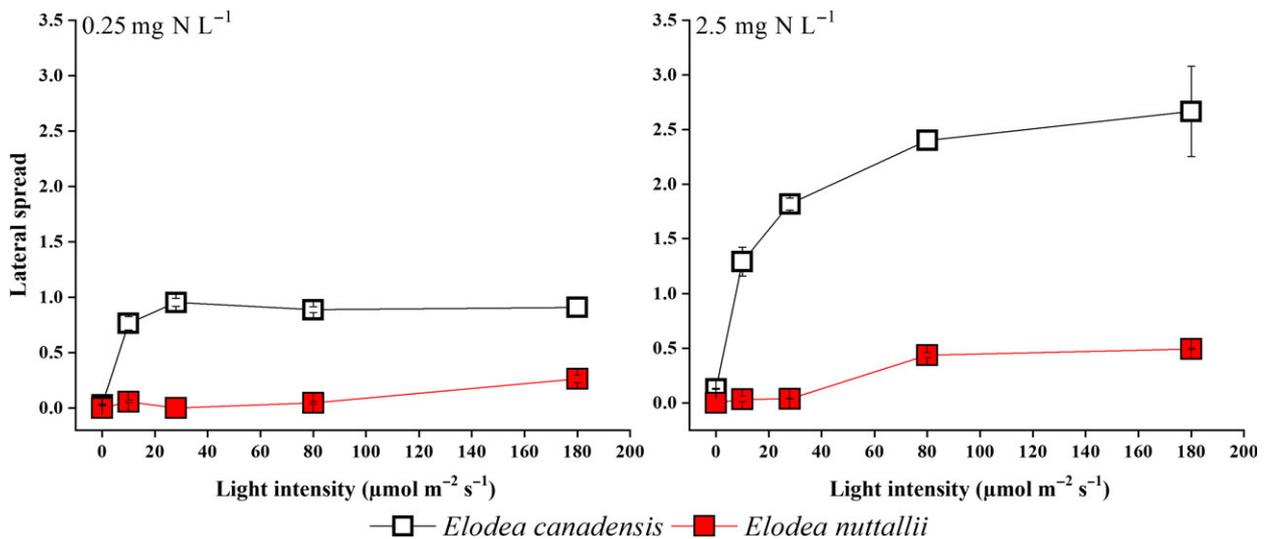


Fig. 5. Lateral spread (lateral shoot length per apical shoot length) of *Elodea* species at different light levels under low (0.5 mg N L⁻¹) and high (2.5 mg N L⁻¹) trophic level. Error bars indicate $\pm\text{SD}$.

However, our results highlighted a few remarkably different acclimation strategies between *E. nuttallii* and *E. canadensis* under a wide range of nitrogen concentrations and light levels. In accordance with our first hypothesis, we found that the phenotypic plasticity of *E. canadensis* for branching and for apical growth was much lower than that of *E. nuttallii*. This specifically means that *E. canadensis* has less chance to adjust its morphology to altering nutrient and light conditions. Our results clearly showed that *E. canadensis* tends to produce dense branches and put more effort into lateral spread even under low light intensity (10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and low nitrogen level (see Figs 5 and 6). Therefore, *E. canadensis* forms dense stands on the bottom of the water body and shows less apical growth, which might be a disadvantage in the competition for light with *E. nuttallii*. On the other hand, *E. nuttallii* showed much higher phenotypic plasticity than *E. canadensis* for branching

degree along an increasing nitrogen concentration gradient combined with an increasing light gradient, which is in line with our first hypothesis. Under shaded conditions, *E. nuttallii* did not form side shoots but *E. canadensis* did (Fig. 6). Therefore, under low light levels *E. nuttallii* was able to elongate much faster from the same biomass due to the higher plant elongation, lower branching degree and low lateral spread ability, which is in line with our second and third hypotheses. Furthermore *E. nuttallii* stems are more slender, meaning that biomass formation contributes more to length extension in comparison with that of *E. canadensis*. (Barrat-Segretain & Elger 2004). Rapid stem elongation is an essential trait for reaching the surface and forming canopies. In addition, at high trophic levels *E. nuttallii* has much less periphytic algal biomass than *E. canadensis* (James *et al.* 2006), thus the new invader may have an even greater advantage for light capture compared

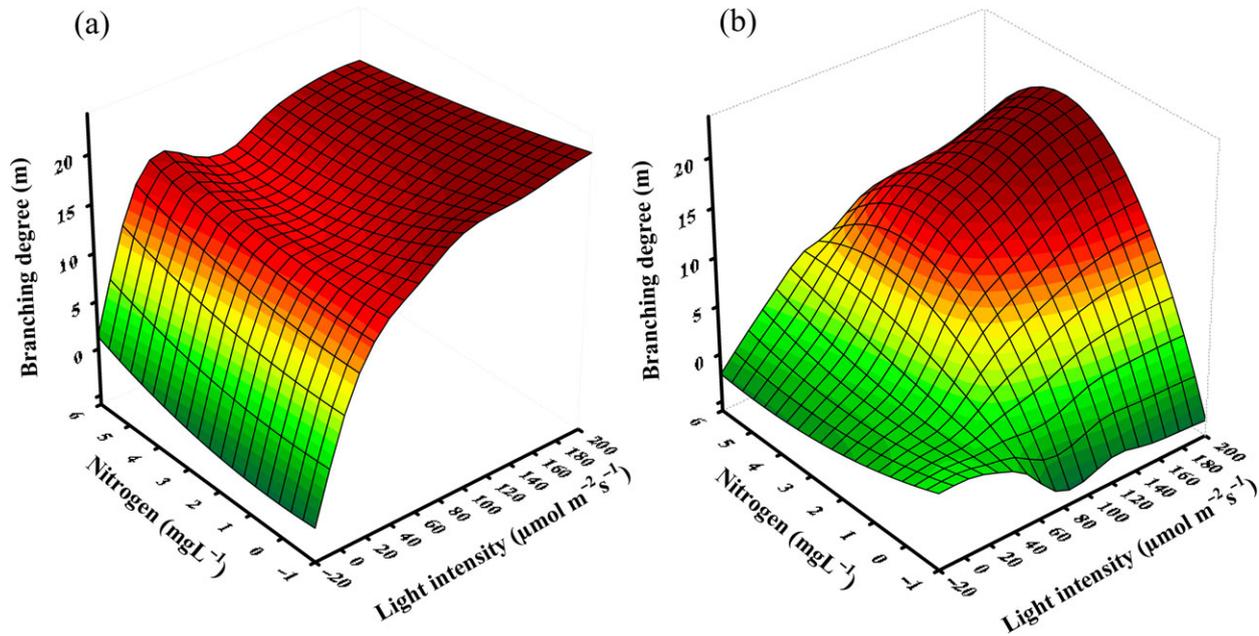


Fig. 6. Branching degree (number of branches per meter shoot length) of *E. canadensis* (a) and *E. nuttallii* (b) grown at different nitrogen and light levels.

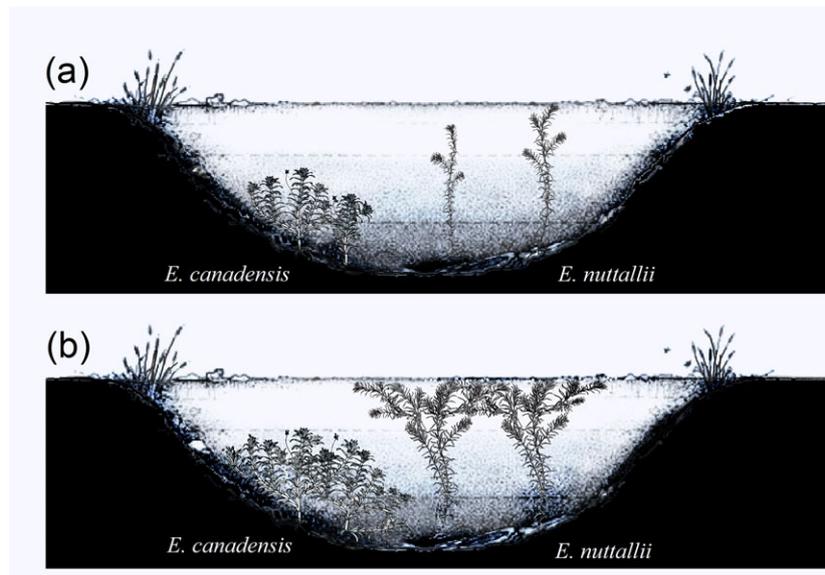


Fig. 7. Phenotypic characters of *E. canadensis* and *E. nuttallii* grown in water bodies under low (a) and high (b) nitrogen concentrations. The drawing is based on data of apical shoot RGR and branching degree.

to *E. canadensis*. Thus, shoots of *E. nuttallii* are able to reach the optimal light levels at the water surface much faster. Under eutrophic and hypertrophic conditions, once shoots reach the layer of high light intensity, they can form a dense canopy close to the surface (Kuni 1984) due to their increased lateral spread and branching degree, resulting in strong shading of other submerged plants (Barrat-Segretain 2005). Consequently, light and nitrogen jointly trigger the development of those phenotypic traits that makes *E. nuttallii* successful in light competition *via* canopy formation (Fig. 7), which is in line with our third hypothesis. Due to this strategy, *E. nuttallii* is a better

competitor against other submerged plants and algae, and due to its strong nutrient uptake, it is also a better competitor against floating plants (*Lemna*; Szabó *et al.* 2010). Our results provide a mechanistic explanation as to why *E. nuttallii* has a great advantage in occupying the surface of eutrophic waters in those areas where other submerged plants form only dispersed stands (Barrat-Segretain 2005).

Our results corroborate other studies which found that higher phenotypic plasticity promotes species survival and reproduction in heterogeneous and temporarily highly variable environments (Kaplan 2002; Dorken & Barret 2004).

Moreover, our findings are in line with the fluctuating resource availability hypotheses (Davis *et al.* 2000), since successful invasion of *E. nuttallii* more frequently occurs under eutrophic and hypertrophic conditions (van Zuidam & Peeters 2013). Our results are also in line with the empty niche hypothesis (Elton 1958), with the difference that resource use efficiency appears not just between alien and native species but between congeneric alien species as well. Since the apical shoot RGR and branching degree were found to have higher plasticity for *E. nuttallii*, therefore the later invader may use resources more efficiently than the former one (*E. canadensis*).

CONCLUSIONS

The results of any invasion depend not only on the characteristics of invasive species and the species of the target community (Tilman 1997), but also on the interactions between the invasive and target species (Alpert *et al.* 2000). Our results clearly support the view that successful invaders have wider phenotypic plasticity that permits a better species–environment response, maximising its fitness in variable environmental conditions (Agrawal 2001; Davidson *et al.* 2011). However, the advantage of this higher phenotypic plasticity mainly depends on the kind of species with which they interact. Shading *via* canopy formation is a general mechanism of interspecific competition between aquatic plants. This mechanism determines the interaction between native–native, native–alien as well as alien–alien interactions. Our study revealed how this mechanism can determine the co-occurrence of two congeneric alien species. We conclude that *Elodea nuttallii* and *E. canadensis* possess different growth form strategies, determined by light and nutrient variables. They have marked differences in their environmental-induced phenotypic plasticity, which might

determine their competitive success against other native or alien species.

ACKNOWLEDGEMENTS

The authors would like to thank Joan Mattia for improving the language of the manuscript and Tamás Havasi for drawings of *Elodea* species (Fig. 7). GV and GB was supported by the GINOP-2.3.2-15-2016-00019 project. BAL was supported by National Research, Development and Innovation Office – NKFIH, OTKA grants (PD120775, KH129520, FK127939), New National Excellence Program of the Ministry of Human Capacities (UNKP Bolyai+) and by the Bolyai János Research Scholarship of the Hungarian Academy of Sciences.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Analysis of variance of the relative growth rates (RGR) of the shoots, relative growth rates (RGR) of the apical shoots, relative elongation, root length, and branching degree of *Elodea* species (*E. canadensis*, *E. nuttallii*) grown in aquaria under different nitrogen concentrations in the water combined with different light intensities.

Figure S1. Relative growth rate (RGR) of the apical shoot of *Elodea canadensis* and *E. nuttallii* at different light levels under low (0.25 mg·N·l⁻¹) and high (2.5 mg·N·l⁻¹) trophic levels. Error bars indicate the standard error of the data.

Figure S2. Branching degree of *E. canadensis* (a) and *E. nuttallii* (b) at different light levels under low (0.25 mg·N·l⁻¹) and high (2.5 mg·N·l⁻¹) trophic levels. Error bars indicate the standard error of the data.

REFERENCES

- Agrawal A.A. (2001) Phenotypic plasticity in the interactions and evolution of species. *Science*, **294**, 321–326.
- Alpert P., Bone E., Holzapfel C. (2000) Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. *Perspectives in Plant Ecology, Evolution and Systematics*, **3**, 52–66.
- Barko J.W., Smart R.M. (1985) Laboratory culture of submerged freshwater macrophytes on natural sediments. *Aquatic Botany*, **21**, 251–263.
- Barko J.W., Hardin D.G., Matthews M.S. (1982) Growth and morphology of submersed freshwater macrophytes in relation to light and temperature. *Canadian Journal of Botany*, **60**, 877–887.
- Barrat-Segretain M.-H. (2001) Invasive species in the Rhône River floodplain (France): replacement of *Elodea canadensis* Michaux by *E. nuttallii* St. John in two former river channels. *Archiv für Hydrobiologie*, **152**, 237–251.
- Barrat-Segretain M.-H. (2004) Growth of *Elodea canadensis* and *Elodea nuttallii* in monocultures and mixture under different light and nutrient conditions. *Archiv für Hydrobiologie*, **161**, 133–144.
- Barrat-Segretain M.-H. (2005) Competition between invasive and indigenous species, impact of spatial pattern and developmental stage. *Plant Ecology*, **180**, 153–160.
- Barrat-Segretain M.-H., Elger A. (2004) Experiments on growth interactions between two invasive macrophyte species. *Journal of Vegetation Science*, **15**, 109–114.
- Barrat-Segretain M.-H., Lemoine D.G. (2007) Can snail herbivory influence the outcome of competition between *Elodea* species? *Aquatic Botany*, **86**, 157–162.
- Barrat-Segretain M.-H., Elger A., Sagnes P., Puijalón S. (2002) Comparison of three life-history traits of invasive *Elodea canadensis* Michx. and *Elodea nuttallii* (Planch.) H. St. John. *Aquatic Botany*, **74**, 299–313.
- Borics G., Abonyi A., Várbíró G., Padisák J., T-Krasznai E. (2015) Lake stratification in the Carpathian basin and its interesting biological consequences. *Inland Waters*, **5**, 173–186.
- Cao T., Ni L., Xie P., Xu J., Zhang M. (2011) Effects of moderate ammonium enrichment on three submersed macrophytes under contrasting light availability. *Freshwater Biology*, **56**, 1620–1629.
- Chambers P.A. (1987) Light and nutrients in the control of aquatic plant community structure. II. In situ observation. *Journal of Ecology*, **75**, 621–628.
- Chambers P.A., Kalff J. (1987) Light and nutrients in the control of aquatic plant community structure. I. In situ experiments. *Journal of Ecology*, **75**, 611–619.
- Cook C.D.K., Urmi-König K. (1985) A revision of the genus *Elodea* (Hydrocharitaceae). *Aquatic Botany*, **21**, 111–156.
- Davidson A.M., Jennions M., Nicotra A.B. (2011) Do invasive species show higher phenotypic plasticity than native species and if so, is it adaptive? A meta-analysis. *Ecology Letters*, **14**, 419–431.
- Davis M.A., Grime J.P., Thompson K. (2000) Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology*, **88**, 528–534.
- Dorken M.E., Barret S.C.H. (2004) Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae): a clonal aquatic plant. *Journal of Ecology*, **92**, 32–44.
- Dukes J.S., Mooney H.A. (1999) Does global change increase the success of biological invaders? *Trends in Ecology & Evolution*, **14**, 135–139.
- Elton C.S. (1958) *The ecology of invasion by animals and plants*. Chapman & Hall, London, UK, pp 181.
- Forchhammer N.C. (1999) Production potential of aquatic plants in systems mixing floating and submersed macrophytes. *Freshwater Biology*, **41**, 183–191.
- Goodwin B.J., McAllister A.J., Fahrig L. (1999) Predicting invasiveness of plant species based on biological information. *Conservation Biology*, **13**, 422–426.
- Greulich S., Trémolières M. (2006) Present distribution of the genus *Elodea* in the Alsatian Upper Rhine floodplain (France) with a special focus on the expansion of *Elodea nuttallii* St. John during recent decades. *Hydrobiologia*, **570**, 249–255.
- Grudnik Z.M., Germ M. (2013) Spatial pattern of native species *Myriophyllum spicatum* and invasive alien species *Elodea nuttallii* after introduction of the latter one into the Drava River (Slovenia). *Biologia*, **68**, 202–209.

- Grudnik Z.M., Jelenko I., Germ M. (2014) Influence of abiotic factors on invasive behaviour of alien species *Elodea nuttallii* in the Drava River (Slovenia). *International Journal of Limnology*, **50**, 1–8.
- Hellmann J.J., Byers J.E., Bierwagen B.G., Duker J.S. (2008) Five potential consequences of climate change for invasive species. *Conservation Biology*, **22**, 534–543.
- James C.S., Eaton J.W., Hardwick K. (1999) Competition between three submerged macrophytes, *Elodea canadensis* Michx. *Elodea nuttallii* (Planch.) St John and *Lagarosiphon major* (Ridl.) Moss. *Hydrobiologia*, **415**, 35–40.
- James C.S., Eaton J.W., Hardwick K. (2006) Responses of three invasive aquatic macrophytes to nutrient enrichment do not explain their observed field displacements. *Aquatic Botany*, **84**, 347–353.
- Kaplan Z. (2002) Phenotypic plasticity in *Potamogeton* (Potamogetonaceae). *Folia Geobotanica*, **37**, 141–170.
- Keane R.M., Crawley M.J. (2002) Exotic plant invasion and the enemy release hypotheses. *Trends in Ecology & Evolution*, **17**, 164–170.
- Király G., Steták D., Bányász Á. (2007) Spread of invasive macrophytes in Hungary. *Neobiota*, **7**, 123–130.
- Knoben R.A.E., Peeters E.T.H.M. (1997) *Eco-atlas van waterorganismen. Deel II: fytoplankton en macrofyten*. STOWA (rapport 97-38), Utrecht, the Netherlands, pp 292.
- Kočić A., Horvatić J., Jelaska D. (2014) Distribution and morphological variations of invasive macrophytes *Elodea nuttallii* (Planch.) H. St. John and *Elodea canadensis* Michx in Croatia. *Acta Botanica Croatica*, **73**, 437–446.
- Kolada A., Kutya S. (2016) *Elodea canadensis* (Michx.) in Polish lakes: a non-aggressive addition to native flora. *Biological Invasions*, **18**, 3251–3264.
- de Kroon H., Hutchings M.J. (1995) Morphological plasticity in clonal plants: the foraging concept reconsidered. *Journal of Ecology*, **83**, 143–152.
- Kuhar U., Gregorc T., Renčelj M., Šraj-Kržič N., Gabersčik A. (2007) Distribution of macrophytes and condition of the physical environment of streams flowing through agricultural landscape in north-eastern Slovenia. *Limnologia*, **37**, 146–154.
- Kuni H. (1984) Seasonal growth and profile structure development of *Elodea nuttallii* (Planch.) St. John in pond Ojaka-Ike, Japan. *Aquatic Botany*, **18**, 239–247.
- Larson D. (2007) Growth of three submerged plants below different densities of *Nymphaeoides peltata* (S. G. Gmel.) Kuntze. *Aquatic Botany*, **86**, 280–284.
- Lu J., Wang Z., Xing W., Liu G. (2013) Effects of substrate and shading on the growth of two submerged macrophytes. *Hydrobiologia*, **700**, 157–167.
- Lukács B.A., Mesterházy A., Vidéki R., Király G. (2016) Alien aquatic vascular plants in Hungary (Pannonian ecoregion): historical aspects, data set and trends. *Plant Biosystems*, **150**, 388–395.
- Lukács B.A., Vojtkó A.E., Mesterházy A., Molnár V.A., Süveges K., Végvári Z., Brusa G., Cerabolini B.E.L. (2017) Growth-form and spatiality driving the functional difference of native and alien aquatic plants in Europe. *Ecology and Evolution*, **7**, 950–963.
- Lund J.W.G. (1979) The mystery of *Elodea* Michx. *Watsonia*, **12**, 388.
- Madsen T.V., Cedergreen N. (2002) Sources of nutrients to rooted submerged macrophytes growing in a nutrient-rich stream. *Freshwater Biology*, **47**, 283–291.
- Madsen T.V., Sand-Jensen K. (1994) The interactive effects of light and inorganic carbon on aquatic plant growth. *Plant, Cell and Environment*, **17**, 955–962.
- Madsen T.V., Halm P., Johansen J. (1998) Effects of inorganic carbon supply on the nitrogen requirement of two submerged macrophytes, *Elodea canadensis* and *Callitriche cophocarpa*. *Aquatic Botany*, **62**, 95–106.
- Molnár V.A., Tóth J.P., Sramkó G., Horváth O., Popiela A., Mesterházy A., Lukács B.A. (2015) Flood induced phenotypic plasticity in amphibious genus *Elatine* (Elatinaceae). *PeerJ*, **3**, e1473.
- Mormul R.P., Ahlgren J., Ekvall M.K., Hansson L.A., Brönmark C. (2012) Water brownification may increase the invasibility of a submerged non-native macrophyte. *Biological Invasions*, **14**, 2091–2099.
- Netten J.J.C., Arts G.H.P., Gylstra R., van Nes E.H., Scheffer M., Roijackers R.M.M. (2010) Effects of temperature and nutrients on the competition between free-floating *Salvinia natans* and submerged *Elodea nuttallii* in mesocosms. *Fundamental and Applied Limnology*, **177**, 125–132.
- Ot'ahel'ová H., Valachovič M. (2002) Effects of the Gabčíkovo hydroelectric-station on aquatic vegetation of the Danube river (Slovakia). *Preslia*, **74**, 323–331.
- Peeters E.T.H.M., Van Zuidam J.P., Van Zuidam B.G., Van Nes E.H., Kosten S., Heuts P.G.M., Roijackers R.M.M., Netten J.J.C., Scheffer M. (2013) Changing weather conditions and floating plants in temperate drainage ditches. *Journal of Applied Ecology*, **50**, 585–593.
- Pérez-Harguindeguy N., Díaz S., Garnier E., Lavorel S., Poorter H., Jaureguierry P., Bret-Harte M.S., Cornwell W.K., Craine J.M., Gurvich D.E., Urcelay C., Veneklaas E.J., Reich P.B., Poorter L., Wright I.J., Ray P., Enrico L., Pausas J.G., de Vos A.C., Buchmann N., Funes G., Quétier F., Hodgson J.G., Thompson K., Morgan H.D., ter Steege H., van der Heijden M.G.A., Sack L., Blonder B., Poschlod P., Vaieretti M.V., Conti G., Staver A.C., Aquino S., Cornelissen J.H.C. (2013) New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, **64**, 715–716.
- Peterson A.T. (2003) Predicting the geography of species' invasions via ecological niche modeling. *Quarterly Review of Biology*, **78**, 419–433.
- Pimentel D. (2005) Aquatic nuisance species in the New York State Canal and Hudson River systems and the Great Lakes Basin: an economic and environmental assessment. *Environmental Management*, **35**, 692–701.
- Rejmanek M., Richardson D.M. (1996) What attributes make some plants species more invasive? *Ecology*, **77**, 1655–1661.
- Riis T., Lambertini C., Olesen B., Clayton J.S., Brix H., Sorrel B.K. (2010) Invasion strategies in clonal aquatic plants: are phenotypic differences caused by phenotypic plasticity or local adaptation? *Annals of Botany*, **106**, 813–822.
- Riis T., Olesen B., Clayton J.S., Lambertini C., Brix H., Sorrel B.K. (2012) Growth and morphology in relation to temperature and light availability during the establishment of three invasive aquatic plant species. *Aquatic Botany*, **102**, 56–64.
- Robach F., Thiébaud G., Tremolières M. (1995) Phosphorus sources for aquatic macrophytes in running waters: water or sediment? *Acta Botanica Gallica*, **142**, 719–731.
- Rodrigues R.B., Thomaz S.M. (2010) Photosynthetic and growth responses of *Egeria densa* to photosynthetic active radiation. *Aquatic Botany*, **92**, 281–284.
- Sand-Jensen K., Madsen T.V. (1991) Minimum light requirements of submerged freshwater macrophytes in laboratory growth experiments. *Journal of Ecology*, **79**, 749–764.
- Scheffer M., Szabó S., Gagnani A., van Nes E., Rinaldi S., Kautsky N., Norberg J., Roijackers R.M.M., Franken R.J.M. (2003) Floating plant dominance as a stable state. *Proceedings of the National Academy of Sciences USA*, **100**, 4040–4045.
- Simpson D.A. (1984) A short history of the introduction and spread of *Elodea* Michx in the British Isles. *Watsonia*, **15**, 1–9.
- Simpson D.A. (1990) Displacement of *Elodea canadensis* Michx by *Elodea nuttallii* (Planch.) H. St John in the British Isles. *Watsonia*, **18**, 173–177.
- Sultan S.E. (2000) Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science*, **5**, 537–542.
- Szabó S., Scheffer M., Roijackers R., Waluto B., Braun M., Nagy P.T., Borics G., Zambrano L. (2010) Strong growth limitation of a floating plant (*Lemna gibba*) by submerged macrophyte (*Elodea nuttallii*) under laboratory conditions. *Freshwater Biology*, **55**, 681–690.
- Thompson J.D. (1991) The biology of an invasive plant. *BioScience*, **41**, 393–401.
- Tilman D. (1997) Community invasibility, recruitment limitation and grassland biodiversity. *Ecology*, **78**, 81–92.
- Valladares F., Chico J.M., Aranda I., Balaguer L., Dizengremel P., Manrique E., Dreyer E. (2002) Greater high light seedling tolerance of *Quercus robur* over *Fagus sylvatica* is linked to a greater physiological plasticity. *Trees*, **16**, 395–403.
- Valladares F., Arrieta S., Aranda I., Lorenzo D., Tena D., Sánchez-Gómez D., Suarez F., Pardos J.A. (2005) Shade tolerance, photoinhibition sensitivity and phenotypic plasticity of *Ilex aquifolium* in continental-Mediterranean sites. *Tree Physiology*, **25**, 1041–1052.
- Vilá M., Weiner J. (2004) Are invasive plants species better competitors than native plant species? Evidence from pair-wise experiments. *Oikos*, **105**, 229–238.
- Williamson M. (1999) Invasions. *Ecography*, **22**, 5–12.
- Zefferman E. (2014) Increasing canopy shading reduces growth but not establishment of *Elodea nuttallii* and *Myriophyllum spicatum* in stream channels. *Hydrobiologia*, **734**, 159–170.
- van Zuidam J.P., Peeters E.T.H.M. (2013) Occurrence of macrophyte monocultures in drainage ditches relates to phosphorus in both sediment and water. *Springer Plus*, **2**, 564.