



# Warming induced shade tolerance to become a key trait in invasion success of free-floating plant *Pistia stratiotes* over the native *Hydrocharis morsus-ranae*

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## ABSTRACT

Changes in nutrient concentration, temperature and light intensity due to climate change can alter the species composition of aquatic ecosystems, since global climate change can intensify the process of eutrophication. Eutrophication can enhance the biological invasion and the distribution of alien aquatic plants. Here we investigated the competition ability of alien *Pistia stratiotes* and native *Hydrocharis morsus-ranae* and the effect of different light intensities, temperatures and nutrient concentrations on the functional traits of the two species. In short term (8 days) monoculture experiment, we applied low (0.5 mg L<sup>-1</sup> N; 0.05 mg L<sup>-1</sup> P) and high nutrient concentrations (2 mg L<sup>-1</sup> N; 0.2 mg L<sup>-1</sup> P), four different light intensities (25–295 μmol m<sup>-2</sup> s<sup>-1</sup>) as well as cold and warm (21.5; 27.5 ± 0.5°C) water treatments in full factorial design. In mixed cultures we cultivated the plants for 28 days with various biomass ratio, in shaded and well illuminated conditions, at a high nutrient concentration (4 mg L<sup>-1</sup> N 1 mg L<sup>-1</sup> P). In monocultures, the relative growth rate of *P. stratiotes* in warm water was significantly higher than that of *H. morsus-ranae*, however, this difference was not significant in colder water. In the co-culture experiment, *P. stratiotes* had significantly higher growth rate compared to *H. morsus-ranae* regardless of initial plant biomass ratio. Under shaded (65 ± 5 μmol m<sup>-2</sup> s<sup>-1</sup>) conditions, *P. stratiotes* out-competed *H. morsus-ranae*, resulting in its decay. Experimental results imply that with elevated temperature, the spread of alien *P. stratiotes* can be expected. Furthermore, under shaded conditions, *P. stratiotes* has a higher chance of occupying the water surface over the native plant *H. morsus-ranae*.

## 1. Introduction

The invasion of alien species (sensu Richardson et al., 2000) is recognized as a significant threat to the diversity of natural ecosystems (Getsinger et al., 2014; Brundu, 2015). Invasive alien aquatic plants may threaten natural ecosystems due to their intense growth, wide distribution, efficient dispersal mechanisms, high phenotypic plasticity and flexible use of environmental resources (Thiébaud, 2007; Szabó et al.,

2019; 2020).

Invasive alien species (thereafter IAS) species can cause a lot of complications in the new habitats causing economic and ecological damage (Getsinger et al., 2014; Brundu, 2015). Ecological impacts include altering environmental factors such as light (Rommens et al., 2003), pH, amount of dissolved oxygen and carbon dioxide (James et al., 1999; Vilas et al., 2017), or nutrient concentration (Szabó et al., 2020). These factors greatly influence the viability of aquatic macrophytes.

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Furthermore, IAS may affect the composition of macroinvertebrate fauna (Stiers et al., 2011), modify the food web structure (Villamagna and Murphy, 2010), and could cause regime shifts between aquatic vegetation (Netten et al., 2010). The economic effects of alien aquatic species invasion are also complex. IAS can form dense mats of biomass, barricading streams, canals, blocking the flow of water and trade routes for ships (Day, 2012; May et al., 2021). Invasion by free-floating aquatic plants may have additional effects, such as anoxia caused by shading the entire water surface, thereby, inhibiting photosynthesis and enhancing decomposition, which leads to further reduction in oxygen concentration, promoting the local disappearance of fish and the process of eutrophication (Mallya, 1999). Furthermore, these changes largely contribute to the decline of ecosystem services, such as sport activities (e.g. surfing, swimming, bathing) and reduce the water availability in irrigation systems (Verhofstad and Bakker, 2019). In addition, IAS may decrease, or they could completely change the biodiversity of native macrophytes (Ramey, 2009; Brunel et al., 2010). Therefore, it is important to prevent their spread to conserve the natural plant communities that characterize a specific region.

*Pistia stratiotes* L. (Araceae) or water lettuce, is a free-floating perennial aquatic plant, originating in South America (Chamier et al., 2012), that can reproduce by both vegetative shoots or seeds (Williams and Hecky, 2005). It is one of the most common aquatic weeds in almost all tropical and subtropical regions (Labrada and Fornasari, 2002). *P. stratiotes* is present in more than 15 European countries according to the newest literature (EPP0, 2017). *P. stratiotes* has been used for medical purposes, fodder for cattle (Sculthorpe, 1967) human food, stock food (Holm et al., 1977), and phytoremediation (Piyush et al., 2012). Despite its invasiveness and potential harmful effects on aquatic ecosystems, it became a popular ornamental plant in aquaria and garden ponds (Holm et al., 1977; Brunel et al., 2010). Accidental release of *P. stratiotes* is often noticed (Šajna et al., 2007; Brundu et al., 2012). In Europe, escape of water lettuce was reported from the Netherlands in 1973 (Mennema, 1977), but winter survival has only been reported in Austria (Hartl et al., 1992), Hungary (Simon, 2000), Slovenia (Šajna et al., 2007), Germany (Hussner et al., 2014) and Italy (Ercolini, 2008). In recent years, there has been a significant invasion of the species in Hungary and neighboring countries (Pliszko and Górecki, 2021; Šajna et al., 2023). In the oxbow lake with a thermal spring of the Sava River (SE Slovenia), the distribution of *P. stratiotes* resulted the gradual disappearance of six native submerged species (Jaklič et al., 2020). Huang et al. (2019) showed that the impacts of eutrophication are highly favorable for the spread of *P. stratiotes* in comparison to native free-floating plants in China.

*Hydrocharis morsus-ranae* L. (Hydrocharitaceae), or European frog-bit, is a perennial, free-floating aquatic plant, native to Europe but invasive in North America (Zhu et al., 2018). It reproduces via turions, which are easily dispersed vegetative propagules that can remain viable for at least two years in dormant state (Catling et al., 2003). Since frog-bit and water lettuce have similar growth- and life forms, they are often observed in the same habitats in Europe (Lukács et al., 2014). Theoretically, similar species commonly use resources in similar ways therefore expected to experience high niche overlap (i.e. limiting similarity) in nature.

Until now, no studies have investigated experimentally the interaction between *P. stratiotes* and *H. morsus-ranae*, and how they respond to altered abiotic factors. We hypothesized that small differences in functional traits between *P. stratiotes* and *H. morsus-ranae* become more pronounced with higher temperature and nutrient concentration along a light gradient (H1), and this may contribute to the invasion success of *P. stratiotes* over *H. morsus-ranae* (H2). The present study aims to evaluate these hypotheses by investigating the combined effects of temperature, light and nutrient concentration on the functional traits (relative growth rate, specific leaf area, leaf dry matter content) of the two species in a monoculture experiment. Furthermore, we investigated competitive interactions between the two species in mixed cultures under two levels

of light intensity (low and high) at a range of planting ratios. Both experiments conducted are aimed at investigating the immediate (in the short and very short term) responses of species.

## 2. Method

### 2.1. Plant collection, preincubation

*H. morsus-ranae* was collected from an oxbow lake near Rakamaz, NE Hungary (N 48.120367; E 21.457485). *P. stratiotes* plants for the experimental work were purchased from a commercial source. For monoculture experiment, the plants were cultivated separately in a general-purpose culture solution medium detailed by Barko and Smart (1985) at high (2 mg L<sup>-1</sup> N; 0.2 mg L<sup>-1</sup> P) and low (0.5 mg L<sup>-1</sup> N; 0.05 mg L<sup>-1</sup> P) nutrient concentrations detailed by Koleszár et al. (2022). Preincubation of the plants for the density-dependent interactions experiment between *P. stratiotes* and *H. morsus-ranae* were cultivated in a general-purpose culture solution (Barko and Smart, 1985) at higher nutrient concentration (4 mg L<sup>-1</sup> N and 1 mg L<sup>-1</sup> P) in order to avoid nutrient limitation. Plants were preincubated at 160–180 μmol m<sup>-2</sup> s<sup>-1</sup> PAR photon flux density under moderately warm water 24 ± 0.5 °C for 14 days. Before starting the experiments, we removed water from the surface of the plants using a centrifuge (600 RPM, 10 sec). We measured subsamples of each species from all nutrient concentrations for fresh weight (FW<sub>0</sub>) and dry weight (DW<sub>0</sub>).

### 2.2. Effects of environmental factors on plant traits in monocultures

In order to achieve approximately the same initial biomass per experimental unit, we placed three *H. morsus-ranae* and one *P. stratiotes* plants separately in 2-L aquaria (height: 11.5 cm, width: 11.5 cm, length: 18 cm) containing the culture media described above. The sides of the aquaria were covered with black foil to avoid light penetration from the sides. For both species, two different nutrient treatments (0.5 mg N L<sup>-1</sup>, 0.05 mg P L<sup>-1</sup>; 2 mg N L<sup>-1</sup>, 0.2 mg P L<sup>-1</sup>) were applied at four different light intensities varying from strongly shaded to well-illuminated conditions: 22–28 (L1), 52–82 (L2), 170–290 (L3) and 260–330 (L4) μmol m<sup>-2</sup> s<sup>-1</sup> PAR photon flux density detailed by Koleszár et al. (2022). The plants were grown under moderately cold and warm water (21.5 and 27.5 ± 0.5 °C) using a controlled temperature water bath. Each treatment (2x2x2x4=32) was replicated three times meaning that we used 96 aquaria. We renewed the culture medium on day 2 and 6 day. We finished the experiment after 8 days in order to avoid overcrowding of the plants as well as to keep the initial light levels more or less constant.

We measured leaf area of three leaves from each aquarium (96x3=288) using a LI-COR LI-3000 Leaf area meter + LI-3050 C Transparent Belt Conveyor Accessory (LI-COR Biosciences GmbH, Germany). We selected fully developed fresh leaves from each of the three *H. morsus-ranae* plants and from the single *P. stratiotes* specimen. The total amount of plants per aquarium and three cut leaves were used for fresh weight and for dry weight determination. We dried samples at 80 °C for 48 hours.

The RGR of the plants was calculated as  $RGR = (\ln W_t - \ln W_0)/t$  (day<sup>-1</sup>), where  $W_0$  represents the initial and  $W_t$  the final dry weight of the plants in each aquarium, and  $t$  is the cultivation time in days. Specific leaf area (SLA) was calculated as  $SLA = (LA/W_t \text{ mm}^2 \text{ mg}^{-1})$  where  $LA$  represents the leaf area and  $W_t$  the dry weight of the leaves. We calculated the SLA for each leaves, however we used the mean SLA data of the 3 leaves per aquaria for further statistical analyses. Leaf dry matter content (LDMC) was calculated as  $LDMC = (W_t/W_0 \text{ mg g}^{-1})$ , where  $W_0$  represents the initial (water saturated) and  $W_t$  the final dry weight of the leaves (Koleszár et al., 2022).

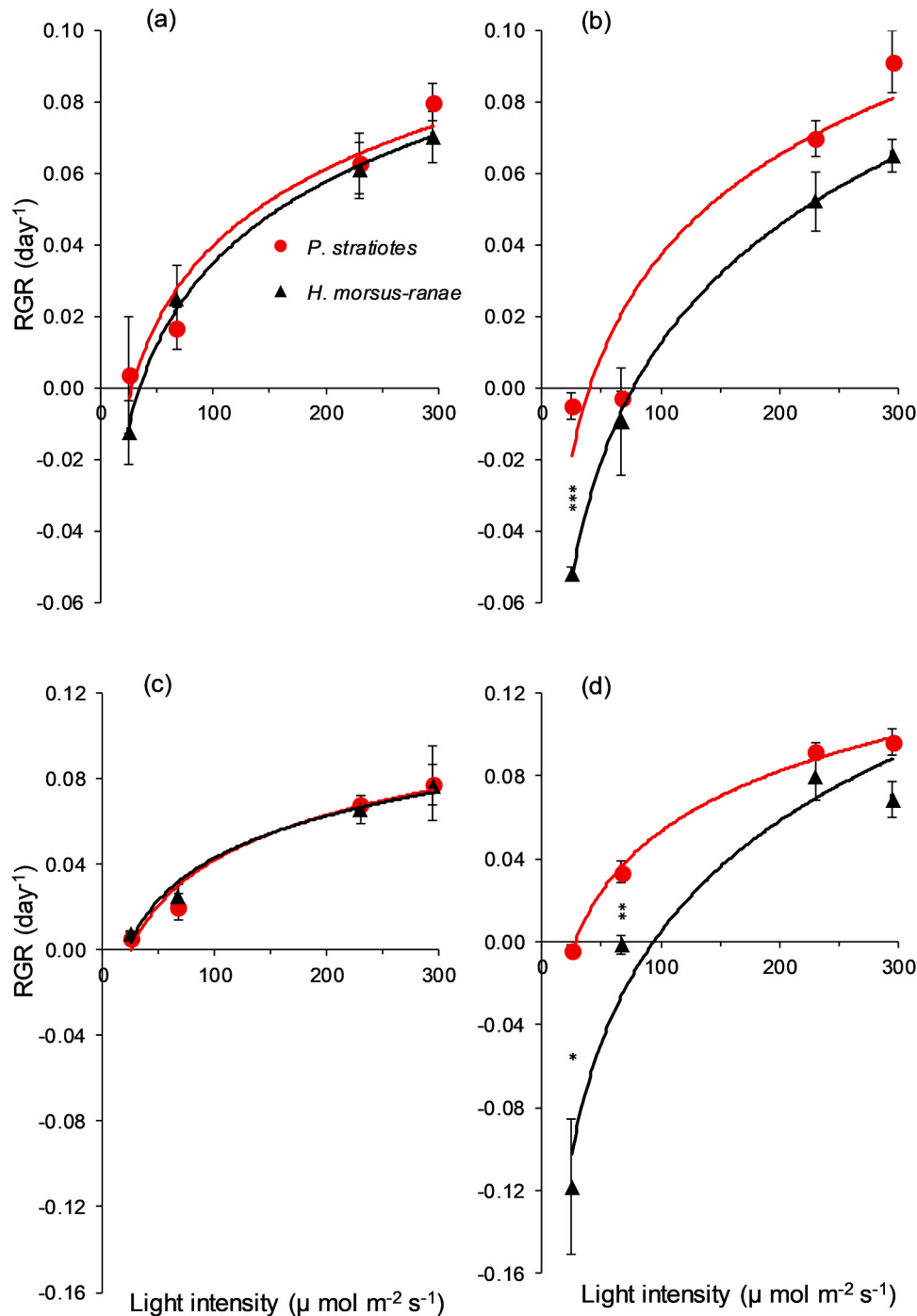
### 2.3. Density dependent interactions in mixed cultures

We co-cultured *P. stratiotes* and *H. morsus-ranae* at six different biomass ratios: 5:25; 10:20; 15:15; 20:10; 25:5; 30:0 g biomass respectively in 12-L plastic containers (height: 23 cm, diameter: 31 cm). The co-cultures were cultivated in a general-purpose culture solution medium (Barko and Smart, 1985) at high ( $4 \text{ mg L}^{-1} \text{ N}$ ;  $1 \text{ mg L}^{-1} \text{ P}$ ) nutrient concentration. Half of the culture medium was renewed twice a week. The cultures were incubated under  $25 \text{ }^\circ\text{C}$  ( $\pm 0.5$ ) at two different light intensities,  $65 \pm 5$  (shaded) and  $300 \pm 10$  (well illuminated)  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  PAR photon flux density. The shading effect was provided by a

shading mesh (80% light filtration). Each treatment ( $7 \times 2 = 14$ ) was replicated three times meaning that we used 42 buckets. We harvested the plants after 28 days of incubation. We measured fresh mass and dry mass of the plants detailed in monoculture experiment. The RGR of the plants was calculated as detailed in monoculture experiment. Yield of the plant species was calculated as:  $\text{FW}_t - \text{FW}_0$ , where  $W_0$  represents the initial and  $W_t$  the final fresh weight of the plants in each aquarium.

### 2.4. Statistical analyses

Normality of the variables was checked by the Kolmogorov–Smirnov



**Fig. 1.** Relative growth rate (RGR) of *Pistia stratiotes* and *Hydrocharis morsus-ranae* cultures grown at different light levels and treatments (a) low nutrient and low temperature; (b) low nutrient, high temperature; (c) high nutrient, low temperature; (d) high nutrient and high temperature) (mean  $\pm$  SE,  $N = 3$ ). Asterisks indicate a significant difference (PC) between the species (\*  $p < 0.05$ , \*\*  $p < 0.01$ ). Data are based on dry weight of the plants.

test. As the measured variables (RGR, SLA, LDMC, yield) were normally distributed ( $P > 0.05$ ), general linear model (GLM) was used to test the significance of the effects of different factors (light intensity, nutrient concentration, temperature, species identity, biomass ratio) and their interactions on these variables. We evaluated homogeneity of variances by Levene's test. In co-culture experiment, we applied Tukey's post hoc tests to or pairwise comparisons (PC) evaluate which-biomass-ratio treatments differed from each other. We used PC to test the variables for significant differences between the two species, the two nutrient level and the two temperature level, furthermore among light intensity levels (L1-L4) as well; where the mean difference (MD)  $\pm$  standard error (SE) was indicated. All analyses were made using SPSS 26.0 software.

### 3. Results

#### 3.1. Effects of abiotic factors on plant traits in monocultures

##### 3.1.1. Relative growth rate

All independent variables (light intensity, nutrient concentration, and temperature) have a significant effect on the RGR of either or both species (Table A1). Elevated nutrient concentration significantly increased the RGR of *P. stratiotes* (MD =  $0.011 \pm 0.004$  SE day<sup>-1</sup>;

$p < 0.006$ , PC) and had no effect on *H. morsus-ranae* (Table A1). Light intensity significantly increased the RGR of both species from L1 to L2 (MD =  $0.04 \pm 0.01$  SE day<sup>-1</sup>;  $p < 0.001$ , PC), and from L2 to L3 (MD =  $0.06 \pm 0.01$  SE day<sup>-1</sup>;  $p < 0.001$ , PC) light intensities (Fig. 1).

Water temperature had no effect on the growth of *P. stratiotes*, however, at lower light intensity (L1-L2) at the higher temperature, the RGR of *H. morsus-ranae* was significantly ( $p < 0.005$ , PC) less (MD =  $-0.045 \pm 0.012$  SE day<sup>-1</sup>) than at lower temperature (MD =  $0.011 \pm 0.012$  SE day<sup>-1</sup>) (Fig. 1b, d). At the low temperature, no difference could be detected between RGR of the two species (Fig. 1a, c), however, at the high temperature with the lowest (L1) light intensity, RGR of *P. stratiotes* (MD =  $-0.004 \pm 0.015$  SE day<sup>-1</sup>) was significantly higher ( $p = 0.003$ , PC) than that of *H. morsus-ranae* (MD =  $-0.085 \pm 0.015$  SE day<sup>-1</sup>) (Fig. 1b, d). At the high temperature with the highest (L4) light intensity, RGR of *P. stratiotes* (MD =  $0.094 \pm 0.005$  SE day<sup>-1</sup>) was also significantly higher ( $p = 0.003$ , PC) than that of *H. morsus-ranae* (MD =  $0.067 \pm 0.005$  SE day<sup>-1</sup>) (Fig. 1b, d).

##### 3.1.2. Specific leaf area and leaf dry matter content

All independent variables have a significant effect on the SLA (Table A2). Increasing the light intensity from L1 to L4 significantly decreased the SLA of both species: *P. stratiotes* from  $35.9 \pm 1.1$ – $18.6 \pm$

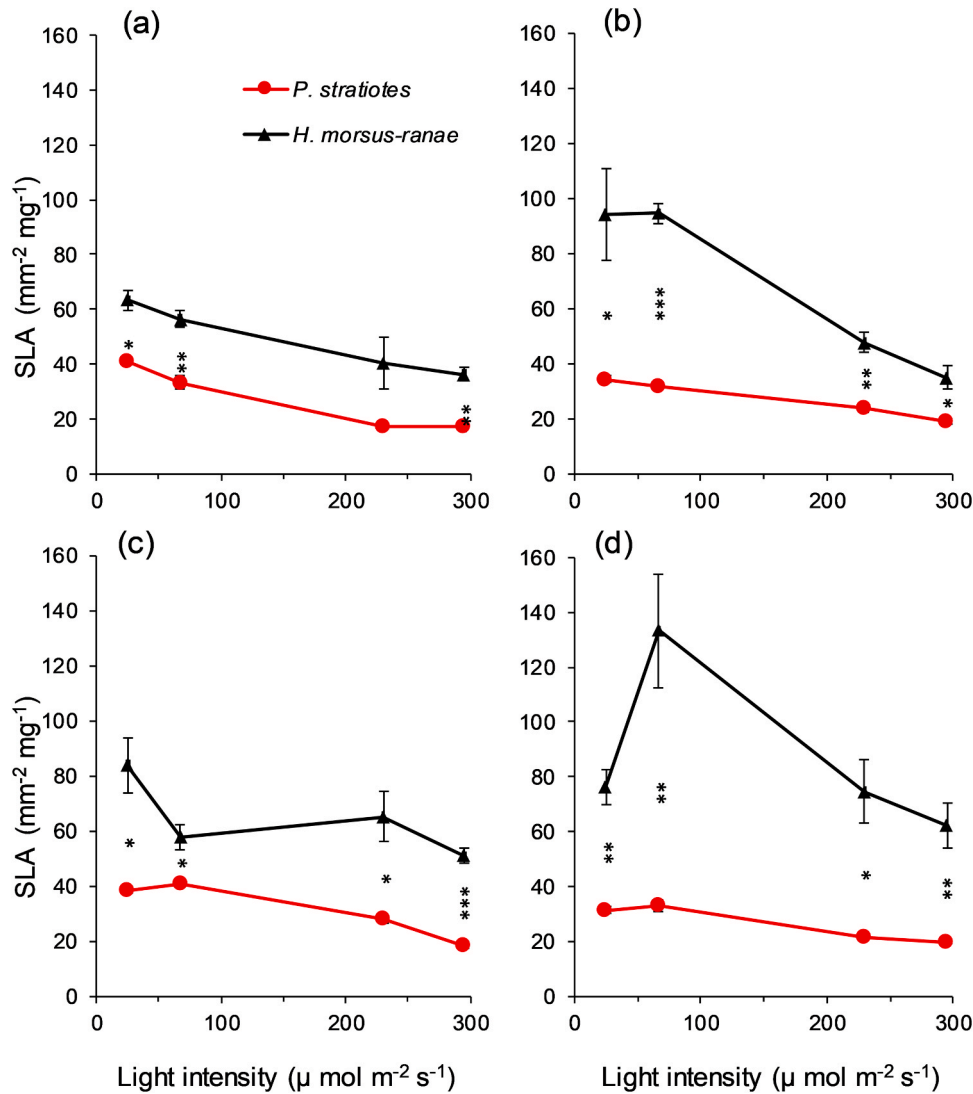


Fig. 2. Specific leaf area (SLA) of *Pistia stratiotes* and *Hydrocharis morsus-ranae* cultures grown at different light levels and treatments (a) low nutrient and low temperature; (b) low nutrient, high temperature; (c) high nutrient, low temperature; (d) high nutrient and high temperature) (mean  $\pm$  SE, N = 3). Asterisks indicate a significant difference (PC) between the species (\*  $p < 0.05$ , \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ).

1.1 mm<sup>2</sup> mg<sup>-1</sup>; *H. morsus-ranae* from 79.3 ± 6.9–46.0 ± 6.9 mm<sup>2</sup> mg<sup>-1</sup>) (Fig. 2). Furthermore, the SLA of *H. morsus-ranae* (67.5 ± 4.1 mm<sup>2</sup> mg<sup>-1</sup>) was significantly (p=0.001, PC) higher compared to *P. stratiotes* (28.0 ± 1.2 mm<sup>2</sup> mg<sup>-1</sup>) (Fig. 2a). While increased nutrient concentration (MD 16.98 ± 4.62 SE mm<sup>2</sup> mg<sup>-1</sup>; p=0.001, PC) and temperature (MD 20.40 ± 4.62 SE mm<sup>2</sup> mg<sup>-1</sup>; p<0.001, PC) significantly increased the SLA of *H. morsus-ranae*, it did not affect the SLA of *P. stratiotes* (Fig. 2).

All independent variables have a significant effect on the LDMC (Table A3). The LDMC of both species increased with increasing light intensity (Fig. 3). The LDMC of *H. morsus-ranae* was significantly higher compared to *P. stratiotes* at L1 (*H. morsus-ranae* 0.077 ± 0.006 mg g<sup>-1</sup>, *P. stratiotes* 0.055 ± 0.006 mg g<sup>-1</sup>) and L2 (*H. morsus-ranae* 0.073 ± 0.005 mg g<sup>-1</sup>, *P. stratiotes* 0.055 ± 0.005 mg g<sup>-1</sup>) light intensity (Fig. 3) however, not at L3 and L4. Both the elevated nutrient concentration (MD 0.26 ± 0.005 mg g<sup>-1</sup>; p<0.001, PC) and temperature (MD 0.15 ± 0.005 SE mg g<sup>-1</sup>; p=0.005, PC) significantly decreased the LDMC of *H. morsus-ranae* (Fig. 3).

### 3.2. Interactions in co-cultures

#### 3.2.1. Relative growth rate

The independent variables (light intensity, biomass ratio), have a significant effect on the RGR of both species (Table A4). In mixed cultures, the RGR of *P. stratiotes* was significantly (p<0.001, PC) higher at both low (*P. stratiotes* 0.037 ± 0.005 day<sup>-1</sup>; *H. morsus-ranae* -0.056 ± 0.021 day<sup>-1</sup>) and high (*P. stratiotes* 0.081 ± 0.003 day<sup>-1</sup>, *H. morsus-ranae* 0.040 ± 0.009 day<sup>-1</sup>) light intensities compared to *H. morsus-ranae* (Fig. 4). The relative growth rate of *P. stratiotes* was twice as much as *H. morsus-ranae* (mean RGR = 0.081 vs. 0.040 day<sup>-1</sup>) at high light intensity. At both light intensities, the RGR of *P. stratiotes* continuously decreased with its increasing biomass ratios (Fig. 4a, b). Compared to *P. stratiotes*, the RGR of *H. morsus-ranae* showed opposite trend at the low light intensity, as its relative growth rate was significantly higher (MD 0.076 ± 0.027 SE day<sup>-1</sup>; p<0.014) with increasing biomass ratio (from 33% to 100%) (Fig. 4a). At the high light intensity, RGR of *P. stratiotes* decreased with increasing biomass ratio however, the RGR of *H. morsus-ranae* did not differ across the biomass ratios (17–100%) and was always around 0.04 day<sup>-1</sup> (Fig. 4b).

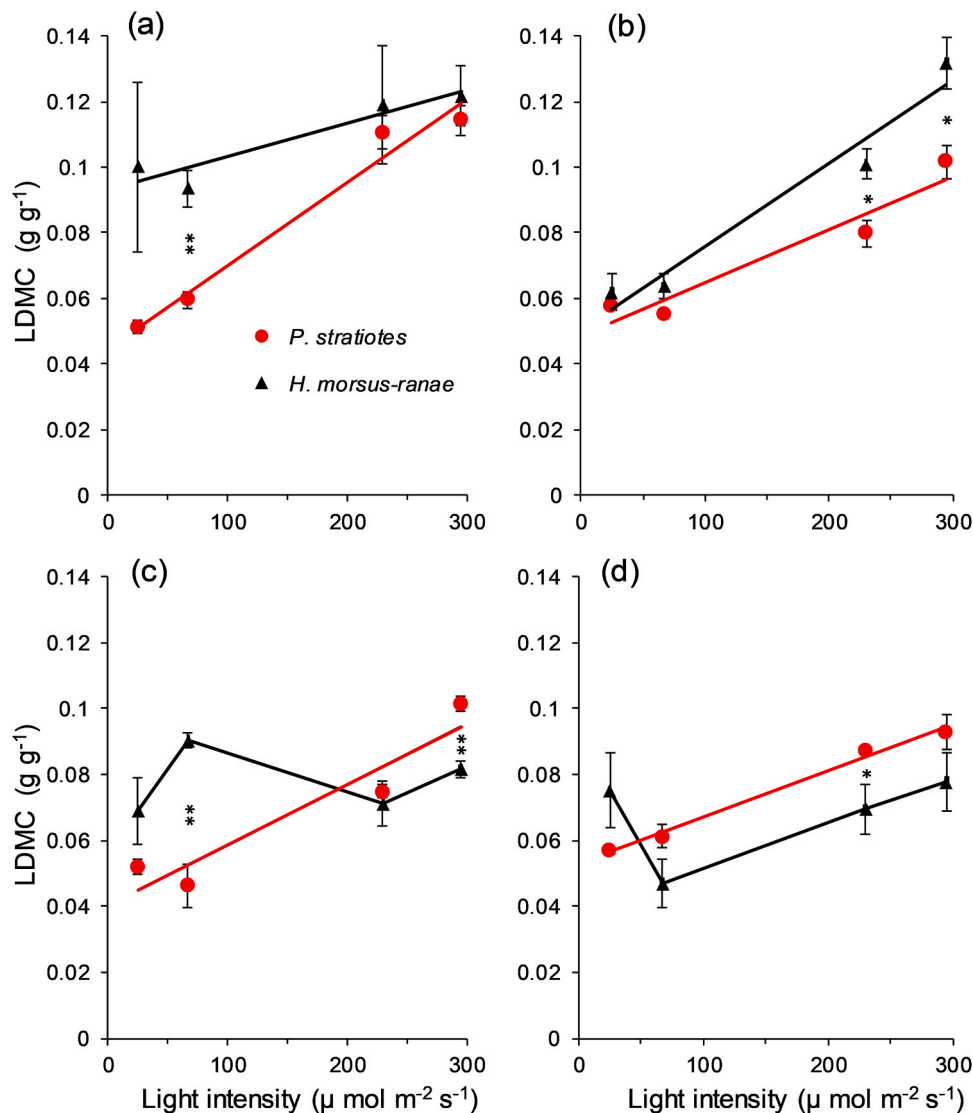
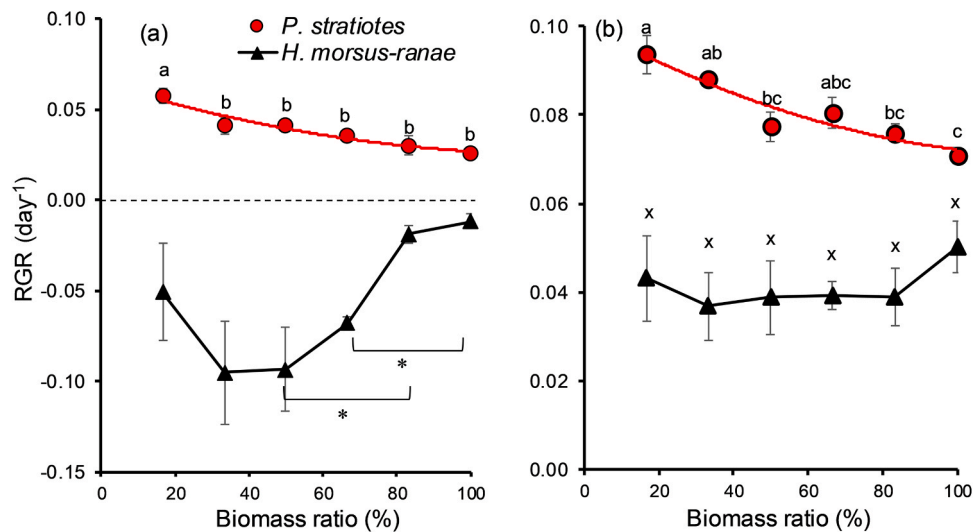


Fig. 3. Leaf dry matter content (LDMC) of *Pistia stratiotes* and *Hydrocharis morsus-ranae* cultures grown at different light levels and treatments (a) low nutrient and low temperature; (b) low nutrient, high temperature; (c) high nutrient, low temperature; (d) high nutrient and high temperature) (mean ± SE, N = 3). Asterisks indicate a significant difference (PC) between the species (\* p<0.05, \*\* p<0.01; \*\*\*p < 0.001).



**Fig. 4.** Relative growth rate (RGR) of *Pistia stratiotes* and *Hydrocharis morsus-ranae* co-cultures grown at low (a) and high (b) light intensity under different initial biomass ratios (mean  $\pm$  SE,  $N = 3$ ). Significant differences among biomass ratio treatments are indicated with different lowercase letters (Tukey's test,  $P < 0.05$ ). Asterisks indicate a significant difference (PC) between the biomass ratios (\*  $p < 0.05$ , PC). Note that the scales of Y axes are different.

### 3.2.2. Biomass yield

The biomass yield of species was significantly different (MD = 69.1  $\pm$  12.6 g FW;  $p < 0.001$ ) in co-cultures (Fig. 5), and *P. stratiotes* had almost 6 times higher biomass yield than *H. morsus-ranae* (mean biomass yield = 72.9 vs. 13.6 g FW). Regardless of the light intensity, the higher the initial *P. stratiotes* biomass, the higher the biomass yield. However, at low light intensity, the yield of *H. morsus-ranae* decreased continuously until 67% of its biomass ratio (Fig. 5a), while at high light intensity, the biomass yield of *H. morsus-ranae* continuously increased with increasing initial plant biomass (Fig. 5b).

### 3.2.3. Changes in biomass

In co-cultures at low light intensity, *H. morsus-ranae* showed a strong decrease in biomass, while *P. stratiotes* still doubled it. Here all five cases *P. stratiotes* overgrew *H. morsus-ranae*, resulting in near complete decay (Fig. 6a). At high light intensity, both *H. morsus-ranae* (by 240%) and *P. stratiotes* (by 805%) increased their biomass. Here, four of the five cases (from 33% to 83% biomass ratio of *P. stratiotes*) *P. stratiotes*

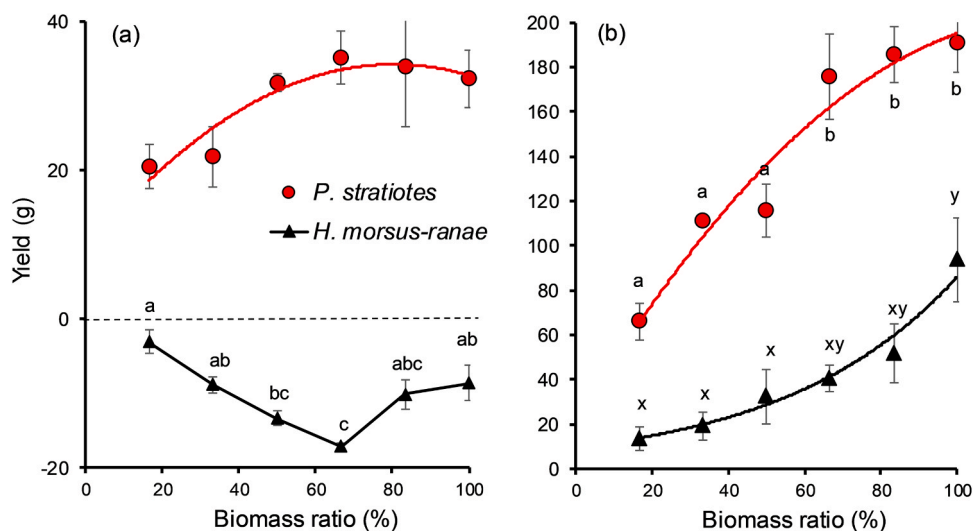
overgrew *H. morsus-ranae* (Fig. 6b).

## 4. Discussion

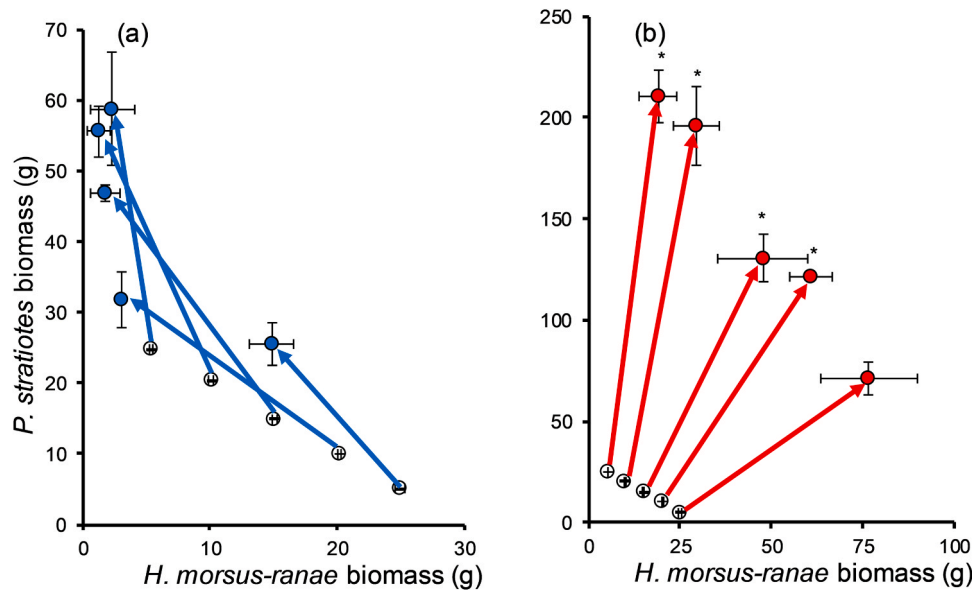
The present study clearly demonstrated how the abiotic factors (light intensity in combination with temperature) may modify the competitive outcome between alien and native free-floating plants.

### 4.1. Changes in RGR in monocultures

Increased light intensity strongly increased the growth rate of the plants, that is in line with the finding of Smith (2022). The lowest applied light intensity was below the light compensation point of *H. morsus-ranae*, while it did not cause significant biomass loss for *P. stratiotes*. This higher shade tolerance suggests that at elevated temperature under shaded conditions, the growth of *P. stratiotes* is less limited compared to *H. morsus-ranae*. Knowing the trend of climate change, according to which a 2–4 °C increase in water temperature is



**Fig. 5.** Biomass yield of *Pistia stratiotes* and *Hydrocharis morsus-ranae* in co-cultures grown at low(a) and high (b) light intensity under different initial biomass ratios (mean  $\pm$  SE,  $N = 3$ ). Significant differences among biomass ratio treatments are indicated with different lowercase letters (Tukey's test,  $P < 0.05$ ). Note that the scales of Y axes are different.



**Fig. 6.** Change in biomass in *Pistia stratiotes* and *Hydrocharis morsus-ranae* co-cultures between the initial (open circles) and final (28th) day of incubation under low (a) and high (b) light intensity (mean  $\pm$  SE, N = 3). Note that the scales of Y axes are different. Asterisks indicate where *P. stratiotes* exceeded the biomass of *H. morsus-ranae* at high light intensity (b).

forecasted by 2100 (Pachauri et al., 2014), this trait may be an advantage for *P. stratiotes* over *H. morsus-ranae* where they occur together. This is especially important if the plants are growing in a slightly shaded area by trees or reeds. Under lower light level (L1–2) the growth rate of *H. morsus-ranae* significantly decreased with increased temperature, resulting in much lower growth rate compared to *P. stratiotes*. On the other hand, under high light levels, *P. stratiotes* increased the growth with elevated temperature. This clearly implies that under elevated temperature and higher light intensity, the two species likely to co-exist with predominance of *P. stratiotes*. On the other hand, under shaded conditions *P. stratiotes* will gradually replace *H. morsus-ranae*.

#### 4.2. Leaf traits differences in monocultures

We found that SLA of the plants decreased with increasing light intensity, which is in line with several field studies as well (Spence et al., 1973; Corré, 1983). Our results are in line with studies (Lukács et al., 2017) that reported similar values as our results. Based on this, the SLA of *P. stratiotes* is between 28 and 36 mm<sup>2</sup> mg<sup>-1</sup> and the SLA of *H. morsus-ranae* is approximately 150 mm<sup>2</sup> mg<sup>-1</sup> which was significantly higher than that of *P. stratiotes*, regardless of the light intensity (Fig. 2a). This anticipates an uncommon scenario of competition arising between native and alien species, specifically with a potential bias favouring the native species. We could assume that *H. morsus-ranae* can be more successful in a competitive situation, since higher SLA indicate faster and more efficient carbon sequestration, and higher growth-rate (Pyšek and Richardson, 2008; Kleunen et al., 2010). However, our results appear to contradict the commonly observed correlation that invasive alien species are those that have higher SLA (Hamilton et al., 2005) but that this is valid in large scale multispecies comparisons. In turn, our results underscore the context-dependent nature of the relationship between SLA and invasiveness.

However, examining the LDMC values, there was also significant difference between the two species (Fig. 2b), and showed similar results to SLA, since the LDMC values of *P. stratiotes* were lower than *H. morsus-ranae*, mainly at low light intensity. Overall, plants with lower LDMC may have a disadvantage in physical resistance (Lukács et al., 2017), which is an additional drawback against herbivores or physical impacts (e.g. waves). Based on our results, we can state, that our first hypothesis -as *P. stratiotes* will be more adaptable to various environmental factors-

proved to be only partially justified, since the applied treatments had less influence on the RGR, SLA and LDMC of *P. stratiotes* compared to *H. morsus-ranae*. Therefore, we can assume that *P. stratiotes* is a species with much wider tolerance, since the applied treatments probably did not reach those extreme values that would cause greater changes in the examined traits.

#### 4.3. Biomass change in co-cultures

In the co-cultures, there was a marked difference between the RGR and biomass yield of the two species. RGR of *P. stratiotes* decreased with the increase of its own initial biomass ratio while *H. morsus-ranae* showed the opposite trend. It implies that the growth rate of *P. stratiotes* is mainly influenced by intraspecific competition, on the other hand *H. morsus-ranae* was predominantly by the competition of *P. stratiotes*. In the case of *P. stratiotes*, it is obvious that the decreasing RGR with increasing initial biomass comes from the self-shading effect under overcrowding of plants. The results of co-cultures experiment are in line with the results of monocultures, because at low light intensity, *H. morsus-ranae* biomass decreased drastically in all cases regardless of its initial biomass. This is especially important if the plants are growing in a shaded area. These results strengthened our second hypothesis that *P. stratiotes* will outgrow *H. morsus-ranae* in the co-cultures.

#### 4.4. Complexity in field conditions

Our experimental results with monocultures pointed out that the three abiotic factors strongly modified the investigated plant traits of either or both species. However, it is also well known that these conditions continuously change over the growing season. For instance, in stands of dense vegetation due to plant nutrient uptake, inorganic nitrogen and phosphorus concentration of the water shows continuous decrease over the growing season (Szabó et al., 2022a, b). In contrast, bioturbation of benthic fauna may increase nutrient release from the sediment to the water body (Chen et al., 2016). Beyond the increasing temperature over the growing season, free-floating vegetation itself can strongly modify the water temperature due to reduced turbulence and shading. Natural pests and consumers may strongly modify the competition between the two species (Center et al., 2005). Overall, in temperate regions over the growing season, both biotic and abiotic

conditions are frequently altering. Therefore, it is obvious that our laboratory results do not directly reflect the complexity of field conditions which consequently, may not directly reveal the competitive outcome between the two free-floating species. Several questions may arise regarding the duration of the experiment. Although an 8-day period experiment may seem relatively short, the reason we finished the experiment after 8 days was to avoid overcrowding of the plants in 2-L aquaria. In case of overcrowding, the changes in traits are no longer just due to the treatments (light, nutrient, and temperature), but also due to self-shading (intraspecific competition). However, in 28 days co-culture experiment, intraspecific competition played a significant role in the interplays.

## 5. Conclusions

Experimental results showed that under higher temperature, the light compensation point of alien *P. stratiotes* was much lower compared to native *H. morsus-ranae*. Although *P. stratiotes* had lower SLA and LDMC (former is a disadvantage, the latter is an advantage in terms of invasiveness), our results in co-culture experiment has obviously proved that *P. stratiotes* can outgrow *H. morsus-ranae*, and the invasion success of *P. stratiotes* is regardless of its biomass. The emergent life form of *P. stratiotes* makes it suitable for shading and outcompete *H. morsus-ranae* and other free-floating plants. In order to maintain emergent life form, more biomass is needed to form a unit of leaf surface, but despite this, the plants gain an advantage in the competition for light. For this reason, „the higher the SLA value the higher the invasiveness” theory can not be used between species of different life forms. Warmer temperature under shaded conditions ultimately led to the stable dominance of *P. stratiotes*. Due to its extremely rapid growth rate it leads to a decrease in the composition of animal communities and the biodiversity of native aquatic macrophytes (Ercolini, 2008; Brunel et al., 2010), furthermore, largely contributing to the development of several ecological and economic problems.

## Appendix

**Table A1**

Analysis of variance (One-way ANOVA) of the relative growth rate (RGR) of plant species (S) (*Pistia stratiotes* and *Hydrocharis morsus-ranae*) grown in aquaria under different light (L), nutrient (N) and temperature (T) levels.

Source/Trait	df	F	Sig.
<b>RGR</b>			
N	1	2.11	0.151
L	3	190.23	<b>0.000</b>
S	1	30.28	<b>0.000</b>
T	1	13.09	<b>0.001</b>
N * L	3	2.82	<b>0.046</b>
L * S	3	6.21	<b>0.001</b>
L * T	3	13.31	<b>0.000</b>
S * T	1	24.43	<b>0.000</b>
N * L * T	3	4.80	<b>0.004</b>
N * S * T	1	4.359	<b>0.041</b>
L * S * T	3	3.89	<b>0.013</b>
Error	64		

## CRedit authorship contribution statement

**Julie A Coetzee:** Writing – review & editing, Writing – original draft, Conceptualization. **Balázs András Lukács:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Sándor Szabó:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **János Korpónai:** Writing – review & editing, Visualization, Software. **Gergő Koleszár:** Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Data will be made available on request.

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**Table A2**

Analysis of variance (One-way ANOVA) of specific leaf area (SLA) of plant species (S) (*Pistia stratiotes* and *Hydrocharis morsus-ranae*) grown in aquaria under different light (L), nutrient (N) and temperature (T) levels.

Source/Trait	df	F	Sig.
<b>Specific leaf area</b>			
N	1	15.54	0.000
L	3	33.73	0.000
S	1	268.76	0.000
T	1	14.58	0.000
N * S	1	10.37	0.002
L * S	3	4.72	0.005
L * T	3	5.89	0.001
S * T	1	22.90	0.000
L * S * T	3	8.27	0.000
N * L * S * T	3	3.31	0.026
Error	61		

**Table A3**

Analysis of variance (One-way ANOVA) of the leaf dry matter content (LDMC) of plant species (S) (*Pistia stratiotes* and *Hydrocharis morsus-ranae*) grown in aquaria under different light (L), nutrient (N) and temperature (T) levels.

Source/Trait	df	F	Sig.
<b>Leaf dry matter content</b>			
N	1	37.80	0.000
L	3	50.55	0.000
S	1	18.12	0.000
T	1	9.94	0.002
N * L	3	6.67	0.001
N * S	1	13.15	0.001
N * T	1	6.36	0.014
L * S	3	3.42	0.023
S * T	1	6.36	0.014
N * L * T	3	2.89	0.042
L * S * T	3	5.28	0.003
N * L * S * T	3	4.24	0.009
Error	63		

**Table A4**

Analysis of variance (One-way ANOVA) of the relative growth rate (RGR) of plant species (*Pistia stratiotes*; *Hydrocharis morsus-ranae*) grown in mixed cultures under different light and biomass ratios.

Source	df	F	Sig.
Species	1	257.0	<0.001
Light	1	278.6	<0.001
Biomass ratio	5	2.9	0.025
Species * Light	1	42.8	<0.001
Species * Biomass ratio	5	6.2	<0.001
Species * Light * Biomass ratio	5	2.7	0.031

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