

Reciprocal interactions between the native *Mentha aquatica* and the invasive *Ludwigia hexapetala* in an outdoor experiment.

Gabrielle Thiébaud \*, Hector Rodriguez –Perez, Jambon O,

<sup>1</sup>ECOBIO, UMR 6553 CNRS, Université de Rennes 1, Rennes, France

\*Corresponding Author: [gabrielle.thiebaud@univ-rennes1.fr](mailto:gabrielle.thiebaud@univ-rennes1.fr)

### Highlights

- A high ratio of *M. aquatica* stimulated *L. hexapetala*'s flowering
- The total biomass and branching of *L. hexapetala* were lowest in the monoculture
- *M. aquatica* root/above-ground biomass was reduced by a high ratio of *L. hexapetala*

**Abstract**

The invasion of aquatic ecosystems by non-native species often leads to competitive interactions with native species. However, non-native and native species can co-occur. This study presents a comparison between two wetlands species differing in origin: the invasive plant *Ludwigia hexapetala* (Lh) and the native plant *Mentha aquatica* (Ma). Both species were grown for three months in monocultures and in mixtures at different planting ratios (6Ma/0Lh, 5Ma/1Lh, 3Ma/3Lh, 1Ma/5Lh; 0Ma/6Lh). We assessed species performance in an experimental, outdoor garden. The shoot length, and flower production were measured weekly. At the end of the experiment, root and shoot biomasses were determined, the number of lateral branches was counted, and shoot lengths were measured. Based on biomass, two competition indices were calculated: the Relative Yield Total (RYT<sub>ab</sub>) index, and the Relative Competition Intensity (RCI) index. The RCI index suggested a facilitation effect for *Mentha* and for *Ludwigia* at the ratio of 1Ma/5Lh, whereas competition was established for both species at the ratio of 3Ma/3Lh. Both the total biomass of *L. hexapetala* and its production of lateral branches were significantly reduced when grown as a monoculture, suggesting intra-specific competition within *L. hexapetala* populations. The number of flowers produced by *L. hexapetala* was highest in the presence of a high ratio of *M. aquatica* individuals. However, when *L. hexapetala* outnumbered *M. aquatica*, its ratio of below/above-ground biomass decreased, indicating an inter-specific competition effect. The intra-specific competition for *L. hexapetala* was much stronger than interspecific competition.

**Keywords:** plant performance; functional traits; plant ratio; positive interactions; competition.

## 1. Introduction

Non-native species must overcome numerous geographical and biological barriers to become invasive in their introduced range (Richardson et al., 2000; Blackburn et al., 2011). The establishment success of invasive species relies on the assumption of niche differences to native species, involving either the exploitation of unused resources (empty niche) (Elton, 1958) or competition mechanisms to access a shared resource (niche replacement). Species sharing the same resources commonly coexist in nature. However, competition can also result in the extinction of native species (competitive exclusion). There is a limit to niche overlap or similarity in resource use between native and non-native species (i.e., the theory of limiting similarity) (MacArthur and Levins, 1967). When niches overlap, the species with superior competitive ability prevails (MacDougall et al., 2009). Differences among species in how they use resources determine the outcome of this interaction.

Numerous experiments in aquatic ecosystems have repeatedly shown competitive advantages of invasive aquatic plants over native ones (Spencer and Rejmanek 2010; Martin and Coetzee, 2014), and even over other non-native plants (Barrat-Segretain and Elger, 2004; Mony et al., 2007; Gerard et al., 2014). The competitive advantage of invading species is often attributed to traits related to physiology and morphology, such as leaf-area allocation, shoot allocation, growth rate, size, and fitness (van Kleunen et al., 2010; Cuda et al., 2015). However, invasive species are not always superior to native species (Daehler, 2003). The biotic resistance hypothesis (Elton, 1958) partly explains the failures of many invasion attempts. According to this hypothesis, native species can repel invasive plants through competitive exclusion (MacDougall et al., 2009). Therefore, the outcome of competition among non-native and native species depends both on environmental conditions (Daehler, 2003), such as sediment fertility and nutrient

availability (Mony et al., 2007; Stiers et al., 2011; Gérard et al., 2014; Martin and Coetzee, 2014) and on the functional group identity of the native species (Petruzzella et al., 2018). These complex interactions may support native species' persistence and coexistence with invaders (Gurevitch and Padilla, 2004; Sax and Gaines, 2008), yet studies focusing on the reciprocal impact of native species on invasive species are rare (Leger and Espeland, 2010).

This study examined a non-native versus a native species, *Ludwigia hexapetala* (Hook. and Arn.) Zardini, H. Y. Gu and P. H. Raven (syn. *L. grandiflora* subsp. *hexapetala*), and *Mentha aquatica* L. respectively, as biological models. Water primrose *L. hexapetala* is an invasive species in Europe, native to South America (Thouvenot et al., 2013a). Water primrose was introduced voluntarily into south-eastern France in approximately 1820 (Thouvenot et al., 2013a). It is a perennial aquatic plant forming dense mats. It grows horizontally on water (or mud) and can emerge above the water surface. Early growth consists of rosette-like clusters of rounded leaves on the water's surface. It is mainly aquatic but is also able to colonise terrestrial habitats, such as riverbanks or wet meadows (Thouvenot et al., 2013a). This plant is able to outcompete both submerged and emergent native species (Dandelot et al., 2005; Stiers et al., 2011). *Ludwigia hexapetala* produces allelochemicals (Dandelot et al., 2008; Santonja et al., 2018), which could be implicated in the outcome of the interactions between water primrose and native species. In France, *L. hexapetala* has not been imported since 2007 because its sale and introduction in natural areas has been forbidden by law. It was included in the first European Union list of 37 invasive species, adopted by the European Commission in July 2016. According to EU criteria, species included in the list can cause damage on a scale that justifies dedicated measures applicable across the European Union.

These measures effectively prevent, minimise, or mitigate the adverse impact of these species in a cost-effective manner.

*Mentha aquatica* (Lamiaceae) is a native macrophyte from the northern temperate regions of Europe. It has a creeping rhizome with submerged leaves, and the erect stems possess aerial leaves. *Mentha aquatica* is most common along wet or waterlogged riverbanks with the shoots exposed to the air, but it can also grow fully submerged during wet seasons. According to the biotic resistance hypothesis, *M. aquatica* could repel *L. hexapetala* through competitive exclusion (MacDougall et al., 2009). The invasive *L. hexapetala* and the native *M. aquatica* may co-occur in the wild in European aquatic ecosystems.

The aim of this study was to investigate, experimentally, in a common garden, the effect of neighbour (*M. aquatica* versus *L. hexapetala*) on the performance (biomass, production of flowers and life-cycle completion) of two species at a constant density. It was hypothesised that the performance of *L. hexapetala* and of *M. aquatica* would depend on the ratio and/or on the identity of the neighbouring species.

## 2. Materials and methods

Two macrophyte species were selected for the study: the non-native *Ludwigia hexapetala* and the native *Mentha aquatica*.

### 2.1. Experimental design

In mid-March 2017, 150 shoots each of *L. hexapetala* (Lh) and *M. aquatica* (Ma) were collected from a channelized stream (Marais de Mainguy, 47°07'21.7"N 2°00'14.9"W) and from a pond (Pornic, 47°07'22.5"N 2°05'22.0"W), respectively, in western France. At these two sampling sites, the two species did not co-occur. Shoots (hereafter called individuals) derived from a single stand either of *L. hexapetala* (Lh) or

*M. aquatica* (Ma) were acclimatised for two weeks in dechlorinated tap water at room temperature.

For each individual, an apical shoot, without buds or lateral stems, was cut to 7 cm in length. The mean initial fresh weights of *L. hexapetala* and of *M. aquatica* were, respectively,  $0.6 \pm 0.24$  g FW and  $1.59 \pm 0.74$  g FW. In the laboratory, the apical shoots (150 per species) were gently rinsed with tap water to remove invertebrates, algae, and debris in preparation for planting. Each shoot was planted in a pot (22 cm in diameter and 30 cm in height), containing a fertile agricultural soil (NPK= 14:10:18 kg/m<sup>3</sup>, pH=6). The effects of the experimental conditions were tested by combining different ratios of *M. aquatica* and *L. hexapetala* individuals for a total density of six individuals per pot, following a replacement series design (Cousens, 1991). In the standard replacement design, the same density is used for both species in their respective monocultures; mixtures are formed by substituting an equal number of plants of one species for that of the other, so that the total number of plants per unit area is constant. The overall density per pot (six individuals per pot, i.e. six individuals/0.038m<sup>2</sup>) corresponds to the density typically observed in the field (from 100 to 200 individuals/m<sup>2</sup>) (G. Thiebaut, unpubl. data). Experimental conditions consisted of monospecific pots (6Ma/0Lh or 0Ma/6Lh; containing six individuals of one plant species, Ma or Lh, respectively), mid-ratio pots (3Ma/3Lh; including three individuals of each species), high ratio (5Ma/1Lh; including five individuals of the focal species Ma, and one single individual of the alternative species Lh), and a low ratio treatment (1Ma/5Lh; including one single individual of the focal species Ma, and five individuals of the alternative species Lh). Each treatment had ten replicates. The experiment was conducted outdoors in the experimental garden ECOBIO Research Facility at the University of Rennes 1 (48° 7'4.50"N; 1°38'22.96"W) from April (14<sup>th</sup> week of the year) to July (26<sup>th</sup> week of the year). According to the

Köppen climate classification, the climate type is categorised as a Cfb temperate oceanic climate zone, in which the mean annual temperature is 13°C and the mean annual precipitation is 694 mm.

The bottom of the pots were kept in tap water (*ca.* 10 cm depth). The water chemistry was basic with moderate nutrient concentrations (mean annual value according to French Water Agency data: conductivity = 400  $\mu\text{S cm}^{-1}$ ; pH= 8.10;  $[\text{NO}_3^- \text{ N}] = 1.44 \text{ mg L}^{-1}$ ;  $[\text{NH}_4^+ \text{ N}] = 0.03 \text{ mg L}^{-1}$ ;  $[\text{PO}_4^{3-} \text{ P}] = 0.05 \text{ mg L}^{-1}$ ).

## 2.2. Measurement of morphological traits

The number of flowers and the length of the main shoot (of all individuals) were measured in all of the pots each week for 11 weeks. The Relative Growth Rate (RGR;  $\text{d}^{-1}$ ) was also calculated on a weekly basis, as suggested by Hunt (1990):

$$\text{RGR stem} = (\ln L_2 - \ln L_1) / (T_2 - T_1)$$

where  $L_1$  and  $L_2$  represent total length at time  $T_1$  (beginning of the experiment) and  $T_2$  (end of the experiment).

At the end of the experiment, the main shoot length was measured, the number of lateral branches was counted, and the roots and shoots were harvested. The above-ground and below-ground parts of the plants were dried separately at 65°C for 72 hours and weighed. The below-ground/above-ground ratio was calculated. RGR and stem length are indicators of apical growth, whereas the number of lateral branches is an indicator of lateral growth and the plant's ability to regenerate (Barrat-Segretain et al., 1998). The number of roots is an indicator of the plant's ability to colonise (Barrat-Segretain et al., 1998).

## 2.3. Indices of plant competition

Indices were used to quantify and express several attributes of plant competition, including competition intensity and importance, competitive effects and responses, and

the outcome of competition (Weigelt and Joliffe, 2003). Two indices were selected: the Relative Yield Total index ( $RYT_{ab}$ ) (de Witt and Van den Bergh, 1965) reflects how plant performance is influenced by population density or neighbour size; the Relative Competition Intensity (RCI) index (Grace, 1995) quantifies the intensity of the competition. The  $RYT_{ab}$  (de Witt and Van den Bergh, 1965) was calculated for each ratio treatment, using the following equation:

$$RYT_{ab} = RY_a + RY_b$$

Where  $RY_a$  and  $RY_b$  corresponded to the individual relative yields of each species for the given magnitude.

The relative yield of a species is:

$$RY = Y_{mix}/Y_{contr}$$

in which  $Y_{mix}$  and  $Y_{contr}$  are the biomasses in mixture and in monoculture.

Individual relative yield divides the individual biomass for each plant of the given species in the given treatment by the average yield of the biomass of the same species cultivated in monoculture conditions. According to Snaydon (1991), an  $RYT_{ab}$  value of 2 would indicate that the species did not share any common limiting resource, i.e. it did not compete and showed full resource complementarity, while  $RYT_{ab}$  values greater than 1 indicate that the species did not fully share common limiting resources, i.e. it competed partially and showed partial resource complementarity, and  $RYT_{ab}$  values of 1 indicate that the species fully shared the same limiting resources, i.e. competed fully and showed no resource complementarity.

the RCI (Grace 1995), with the following formulation:

$$RCI = (P_{mono} - P_{mix})/P_{mono}$$

Where  $P_{mono}$  represents the performance of a plant (hereafter total biomass) in a monoculture and  $P_{mix}$  represents the performance (i.e. total biomass) of a plant in a



mixture. RCI compares the performance of plants growing in a mixture with control plants growing alone.

The RCI score was calculated for each species at each planting ratio in mixtures (i.e. 1Ma/5Lh, 3Ma/3Lh, 5 Ma/1Lh). According to Goldberg et al. (1999), a negative score indicates a better performance of the plants growing in mixture than individuals growing in monoculture, suggesting facilitation interactions between the two species. A positive value indicates a weaker performance of the plants in mixture in comparison with plants coming from a monoculture, suggesting competitive interactions between the two species. There is no minimum RCI value for facilitation, but there is a maximum value of 1 indicating maximal competition. An RCI of 0 indicates null interaction effects.

#### 2.4. Data analyses

RGRs were analysed on a repeated-measures basis by means of non-parametric testing (Naguchi et al., 2012), since data did not meet criteria for parametric analyses (i.e., homoscedasticity and normality of residuals). Whenever the interaction between treatment (planting ratio) and time was significant, pairwise comparison among treatments was performed within a given sampling time using the Mann–Whitney–Wilcox test. Data pairwise comparisons were only run for the periods when growth rates peaked and a subsequent Benjamini–Yekutieli correction for dependent multiple tests was applied to the multiple test series (Benjamini and Yekutieli, 2001).

Morphological traits matrices were analysed independently for each species by means of Metric Multidimensional Scaling (MDS) based on Euclidean distance with standardised values. Since the graphical results suggested treatment effects on plant morphology, univariate analyses were used to explore treatment effects on the below/above-ground biomass ratio, total biomass, main stem length, number of flowers (total number of flowers produced throughout the whole experiment), and the number of

branches. Whenever possible, a linear model approach was used for univariate analysis. A type II sum of squares was used in each case for testing the significance of the planting ratio treatment effect, owing to the unbalanced design (Langsrud, 2003). A linear model approach was used to analyse treatment effect on the harvested biomass. In the case of the *M. aquatica* data set, biomass was log-transformed prior to the analysis in order to meet normality and homoscedasticity requirements. Residuals were explored graphically to assess departure from the assumptions of the analysis (Bolker et al., 2009). Number of branches and number of flowers were analysed with a general linear model (GLM) using a Poisson distribution. Patterns in residuals were explored graphically to assess departure from the assumptions of the analysis (Bolker et al., 2009). As the number of flowers for *M. aquatica* did not meet normality and homoscedasticity requirements, a Kruskal–Wallis test was run. Whenever the treatment effect of the planting ratio was significant, HSD Tukey pairwise comparisons were run to determine significant differences among the levels of the experimental factor. Pairwise comparisons based on a non-parametric Wilcox test and subsequent Bonferroni corrections were used for the different ratios when a significant p-value was obtained with the Kruskal–Wallis univariate test. All analyses were performed with R software (R Core Team, 2016). The nparLD package (Naguchi et al., 2012) was used for nonparametric analysis of longitudinal data; the multcomp package for HSD Tukey pairwise comparisons (Hothorn and Bretz, 2008); and the vegan package (Oksanen et al., 2017) for MDS analysis.

### 3. Results

The calculation of the  $\text{RYT}_{ab}$  index indicated that the two species did not share any common limiting resources, suggesting there was no competition between *M. aquatica* and *L. hexapetala* (Fig 1A). The highest biomass total yield (RYT) was observed in pots at the planting ratio 1Ma/5Lh and, to a lesser extent, in pots at the planting ratio

5Ma/1Lh (Fig 1A). We observed an increase in biomass production RY of *L. hexapetala* RY of *M. aquatica* (over-yielding) for both species in mixtures at the planting ratio 1Ma/5Lh, and an increase in biomass production (RY) of *L. hexapetala* only at the planting ratio 5Ma/1Lh (Fig. 1A).

The RCI standardises competition intensity, allowing the comparison of species with different RGR in the absence of interaction. Negative RCI values were found for both species at the ratio of 1Ma/5Lh, whereas positive values were calculated at the ratio 3Ma/3Lh (Fig. 1B). The RCI was close to 0 for *M. aquatica* and negative for *L. hexapetala* at the ratio of 5Ma/1Lh.

The highest RGRs for both *L. hexapetala* and *M. aquatica* were documented during the third growth period (week 3). For *L. hexapetala*, it was significantly lower at the ratio of 5Ma/1Lh than when the individual grew in a monoculture or at the 1Ma/5Lh ratio (Table 1; Fig. 2A). On the other hand, *M. aquatica* exhibited highest growth rates in the 1Ma/5Lh and 3Ma/3Lh ratio treatments for the same periods (Fig. 2B).

The overlap of the treatments was greater for *M. aquatica* than for *L. hexapetala* (Fig. 3). Taking into account centroids in MDS analysis (Fig. 3A), the traits of *L. hexapetala* shoots issued from 5Ma/1Lh and 1Ma/5Lh treatments differed from those coming from the monoculture treatment (0Ma/6Lh). The shoots of *M. aquatica* coming from the 1Ma/5Lh treatment were visually distinct from those coming from other treatments (Fig. 3B). It is worth noting that the greatest variability was consistently observed in the 5Ma/1Lh treatment for *L. hexapetala* and the 1Ma/5Lh treatment for *M. aquatica*.

The total biomass of *Ludwigia hexapetala* individuals was significantly smaller in monoculture than in mixture (1Ma/5Lh and 5Ma/1Lh treatments). The individuals of *L. hexapetala* had significantly fewer branches in monoculture than in the 1Ma/5Lh

treatment (Table 2; Fig. 4). On the other hand, *L. hexapetala* individuals produced significantly more flowers in 5Ma/1Lh than in 1Ma/5Lh and in monoculture (Table 2; Fig. 4). At the ratio of 1Ma/5Lh, *M. aquatica* individuals had the lowest below/above-ground biomass ratio, by a significant margin (Table 2; Fig. 4).

#### 4. Discussion

##### 4.1 Impacts of *Mentha aquatica* on the performance of *Ludwigia hexapetala*

Although most studies on the interactions among invasive and native aquatic plants have focused on competition (Spencer and Rejmanek, 2010; Martin and Coetzee, 2014; Gérard et al., 2014), there is increasing recognition that facilitation can be important in such interactions (Rodriguez, 2006). ‘Facilitation’ is defined as an interaction by which one species positively impacts the performance of another. The RCI indices suggested a facilitation effect for *L. hexapetala* in the presence of a low or high ratio of *M. aquatica*. The number of lateral branches and the total biomass of *L. hexapetala* were stimulated by the presence of one individual of *M. aquatica*. Moreover, the study findings also showed a positive effect of the presence of *M. aquatica* on the total biomass and on the production of flowers by *L. hexapetala* when water primrose was out numbered by *M. aquatica*. It is plausible that these positive effects were mediated by some change in the soil environment such as inhibition of denitrification to enhance release of nutrients that also benefit the water primrose (indirect facilitation). The allocation of energy to lateral growth, biomass, and flowering suggests an efficient strategy for avoiding competition with *M. aquatica*.

Surprisingly, no negative impact of *M. aquatica* on the invasive *L. hexapetala* was found. A possible explanation is the low level of competition for resources in the mesocosms, as indicated by the value of the RYT<sub>ab</sub>. During the experiment, *L. hexapetala* adopted a creeping mode of growth, whereas *M. aquatica* produced erect stems. These two types of

architecture, creeping versus erect, meant competition for space and light was avoided, and suggests niche partitioning. Competition is expected to occur mostly between species of similar growth form and occupying similar niches. The individuals of *L. hexapetala* had less biomass in monoculture than at 1Ma/5Lh and 5Ma/1Lh treatments, and produced fewer lateral branches in monoculture than at 1Ma/5Lh treatment and less flowers in monoculture than at 5Ma/1Lh treatment, suggesting strong intraspecific competition for resources between the individuals of *L. hexapetala*. Competition for light is likely to have led the plants in monoculture to reduce their investment in branching (lateral growth). The number of meristems that can potentially produce reproductive organs can be inhibited by competition for light (Bonser and Aarssen, 2003), negatively influencing the number of flowers. The stem lengths of *L. hexapetala* individuals in monocultures were generally small. Smaller individuals with low reproductive allocation may simply have been growing and developing more slowly, and might have reached the same biomass if they had had more time to develop. Plants were also measured after three weeks, when a lower relative growth rate of *M. aquatica* in monoculture and at the high planting ratio (5Ma/1Lh) suggested a competition for resources within the species. This hypothesis is also supported by competition indices (RCI) results.

#### 4.2. Impacts of *Ludwigia hexapetala* on the performance of *Mentha aquatica*

The results did not show a negative impact of *L. hexapetala* on the growth of *M. aquatica*, whereas in the field the presence of *L. hexapetala* affects the abundance of native species such as *Alisma plantago-aquatica*, *Ceratophyllum demersum*, and *Lycopus europaeus* (Stiers et al., 2011). However, this study established that the below/above-ground biomass ratio of *M. aquatica* was reduced in the presence of a high planting ratio of *L. hexapetala* (1Ma/5Lh). In a previous study, the authors showed that *M. aquatica* root length decreased at a higher *L. hexapetala* ratio, and no effect was found of the

presence of *L. hexapetala* on the relative growth rate of *M. aquatica* (Thouvenot et al., 2013b). These results are congruent with Vila and Weiner's (2004) review of pairwise experiments on non-native and native plant species, which supports the general perception that the effect of invasive species on native species is usually stronger, and not vice versa. The lower root biomass of *M. aquatica* observed in the presence of five individuals of *L. hexapetala* could be due to the synthesis of allelochemicals by the water primrose roots (pedunculagin and an ellagic acid) (Marcellin-Gros, 2015) and a subsequent inhibition of *M. aquatica*'s root growth. Indeed, ellagic acid is a rooting inhibitor (Qin et al., 2006). However, in our study, we did not specifically test this hypothesis. Surprisingly, the presence of five individuals of *L. hexapetala* could benefit the growth rate of *M. aquatica*. However, this result was only measured once, during the most active growing period (after three weeks). At this phenological stage, there was no competition for light between water primrose and *M. aquatica*.

The absence of a significant effect of different ratios on the traits of the native species related to growth (stem length, number of lateral branches, total biomass) could be explained by (a) similar competitive abilities of the two species; (b) high variability of competitive abilities within species; or (c) because the intensity of competition was not sufficiently high to cause significant differences in species performance within the time scale of the study. This study has shown that a native plant species can coexist with an invasive plant species at a certain ratio, suggesting the possibility of adaptive evolutionary responses in this plant to the invasive plant. Indeed, it has been hypothesised that strong competition from invasive plants eliminates native plant genotypes that cannot resist or tolerate strong competition, resulting in an accumulation of native plant genotypes that can resist or tolerate such competition within the native populations (Leger and Espeland, 2010). The greater fitness of these native populations could be a result of their evolved

tolerance or competitive ability against non-native species (Leger and Espeland, 2010; Oduor, 2013). In the field, the two species can co-exist, they can develop a common interaction history, allowing the native species to partially resist the invasive *L. hexapetala*.

The results of this study suggest that the outcome of the interactions (Competition, Facilitation) between *L. hexapetala* and *M. aquatica* was partially determined by the planting ratio of the species. The study has shown strong intraspecific competition within *L. hexapetala* individuals and facilitation interactions between *L. hexapetala* and *M. aquatica*, indicating that species coexistence is favoured over competitive exclusion. The findings can be extrapolated to the broader context of how native and non-native species can exist together (or not), although this study investigated only two species, one example of a native and non-native species interacting.

Thus, the strength of competitive interactions between invasive and native plants that might result in evolutionary responses from the native species may be context-dependent, varying with both habitat productivity and the inherent traits of the invasive and native plants under consideration (Funk and Vitousek, 2007; Leger and Espeland, 2010).

### **Acknowledgements**

The authors would like to express their gratitude to Quentin Gaudet, Milin Morgane, and Leo Barthelemie for their assistance in setting up the experiment and measuring the traits. We thank also the staff of the EcolEx platform. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## References

- Barrat-Segretain, M. H., Bornette, G., Hering-Vilas-Bôas, A, 1998. Comparative abilities of vegetative regeneration among aquatic plants growing in disturbed habitats. *Aquat. Bot.* 60, 201–211.
- Barrat-Segretain, M.H., Elger, A., 2004. Experiments on growth interactions between two invasive macrophytes species. *J. Veg. Sci.* 15, 109-114.
- Benjamini, Y., Yekutieli, D., 2001. The control of the false discovery rate in multiple testing under dependency. *Ann. Stat.* 29, 1165-1188.
- Blackburn, T.M., Pyšek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarosik, V., Wilson, J.R.U., Richardson, D.M., 2011. A proposed unified framework for biological invasions. *TREE* 26, 333–339.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R. Stevens, M.H.H., White, J-S.S., 2009. Generalized linear models : a practical guide for ecology and evolution. *TREE* 24(3), 127-135.
- Bonser, S.P., Aarssen, L.W., 2003. Allometry and development in herbaceous plants: functional responses of meristem allocation to light and nutrient availability. *Am. J. Bot.* 90, 404–412.
- Cousens, R., 1991. Aspects of the design and interpretation of competition (interference) experiments. *Weed Technol.* 5, 664–673
- Čuda, J., Skálová, H., Janovský, Z., Pyšek, P., 2015. Competition among native and invasive *Impatiens* species: the roles of environmental factors, population density and life stage. *AoB Plants* 7, plv033. <http://doi.org/10.1093/aobpla/plv033>



- Daehler, C. C., 2003. Performance comparisons of co-occurring native and alien invasive plants: Implications for conservation and restoration. *Annu. Rev. Ecol. Evol. Syst.* 34, 183-211.
- Dandelot S., Matheron, R., Le Petit, J., Verlaque, W., Cazaubon, A., 2005. Temporal variations of physicochemical and microbiological parameters in three freshwater ecosystems (south-eastern France) invaded by *Ludwigia* spp. *C.R. Biol.* 328, 991-999.
- Dandelot, S., Robles, C., Pech, N., Cazaubon, A., Verlaque, R., 2008. Allelopathic potential of two invasive alien *Ludwigia* spp. *Aquat. Bot.* 88, 311-316
- de Wit, C.T., Van den Bergh, J.P., 1965. Competition between herbage plants. *NJAS* 13 (2), 212–221.
- Elton, C.S., 1958. *The ecology of invasion by animals and plants*, Chapman and Hall, London
- Funk, J.L., Vitousek, P.M., 2007. Resource-use efficiency and plant invasion in low-resource systems. *Nature* 446, 1079–1081
- Gérard J, Brion, N., Triest, L., 2014. Effect of water column phosphorus reduction on competitive outcome and traits of *Ludwigia grandiflora* and *L. peploides*, invasive species in Europe. *Aquat Invasions* 9 (2), 157–166
- Goldberg, D.E., Rajaniemi, T., Gurevitch, J., Stewart-Oaten, A., 1999. Empirical approaches to quantifying interaction intensity: competition and facilitation along productivity gradients. *Ecology* 80, 1118–1131.
- Grace, J.B., 1995. On the measurement of plant competition intensity. *Ecology* 76, 305–308.
- Gurevitch, J., Padilla, D.K., 2004. Are invasive species a major cause of extinctions? *TREE* 19, 470–474

- Hothorn, T., Bretz, F., 2008. Simultaneous inference in general parametric models. *Biom. J.* 50(3), 346-363.
- Hunt, R., 1990. *Basic growth analysis*. Unwin Hyman, London.
- Langsrud, Ø., 2003. ANOVA for unbalanced data: use Type II instead of Type III sums of squares. *Stat. Comput.* 13, 163-167.
- Leger, E.A., Espeland, E.K., 2010. Coevolution between native and invasive plant competitors: implications for invasive species management. *Evol Appl.* 3(2), 169–178
- Mac Dougall, A.S., Gilbert, B., Levine, J.M., 2009. Plant invasions and the niche. *J. Ecol.* 97, 609–615.
- MacArthur RH, Levins R., 1967. The limiting similarity, convergence and divergence of coexisting species. *Am. Nat.* 101, 377–385. (doi:10.1086/282505)
- Marcellin-Gros, R., 2015. Caractérisation des métabolites secondaires des différentes formes de croissances chez *Ludwigia grandiflora*. Master Report. pp25
- Martin, G.D., Coetzee, J.A., 2014. Competition between two aquatic macrophytes, *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae) and *Myriophyllum spicatum* Linnaeus (Haloragaceae) as influenced by substrate sediment and nutrients. *Aquat. Bot* 114, 1-11.
- Mony, C., Koschnick, T.J., Haller W.T., Muller, S., 2007. Competition between two invasive Hydrocharitaceae (*Hydrilla verticillata* (L.f.) (Royle) and *Egeria densa* (Planch)) as influenced by sediment fertility and season. *Aquat. Bot.* 86, 236-242.
- Naguchi, K., Gel, Y.R., Brunner, E., Konietzschke, F., 2012. nparLD: An R software for the nonparametric analysis of longitudinal data in factorial experiments. *J. Stat. Softw.* 50(12), 1-23
- Oduor, A.M., 2013. Evolutionary responses of native plant species to invasive plants: a review. *New phytol* 200 (4), 986-92.

- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner H., 2017. *vegan*: Community Ecology Package. R package version 2.4-3.
- Petruzzella A., Manschot J., van Leeuwen C.H.A., Grutters B.M.C., Bakker E.S., 2018. Mechanisms of Invasion Resistance of Aquatic Plant Communities. *Front. Plant Sci.* 9, 1–11.
- Qin, B., Perry, L.G., Broeckling, C.D., Jiang, D.J., Stermitz, F.R., Paschke, M.W., Vivanco, J.M., 2006. Phytotoxic allelochemicals from roots and root exudates of leafy spurge (*Euphorbia esula*). *Plant Signal Behav.* 1(6), 323–327.
- R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Richardson, D.M., Pysek, P., Rejmanek, M., Barbour, M.G., Panetta, F.D., West, C.J., 2000. Naturalization and invasion of alien plants: concepts and definitions. *Divers. Distrib* 6, 93–107.
- Rodriguez, L.F., 2006. Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. *Biol Invasions* 8, 927–939
- Santonja, M., Le Rouzic, B., Thiébaud, G., 2018. Seasonal dependence and functional implications of macrophyte-phytoplankton allelopathic interactions. *Freshw Biol.* 63 (9), 1161-1172
- Sax, D.F., Gaines, S.D., 2008. Species invasions and extinction: the future of native biodiversity on islands. *PNAS* 105, 11490–11497
- Snaydon, R.W. 1991. Replacement or additive designs for competition studies?. *J Appl Ecol* 28(3), 930-946.

- Spencer, D.F., Rejmanek, M., 2010. Competition between two submersed aquatic macrophytes, *Potamogeton pectinatus* and *Potamogeton gramineus*, across a light gradient. *Aquat. Bot* 92, 239–244.
- Stiers, I., Njambuya, J., Triest, L., 2011. Competitive abilities of invasive *Lagarosiphon major* and native *Ceratophyllum demersum* in monocultures and mixed cultures in relation to experimental sediment dredging. *Aquat. Bot.* 95, 161-166
- Thouvenot, L., Haury, J., Thiébaud, G., 2013a. A success story: water primroses, aquatic plant pests. *Aquat Conserv.* 23, 790-803.
- Thouvenot, L., Puech, C., Martinez, L., Haury, J., Thiébaud, G. 2013b. Strategies of the invasive macrophyte *Ludwigia grandiflora* in its introduced range: competition, facilitation or coexistence with native and exotic species? *Aquat. Bot* 107, 8-13
- van Kleunen, M., Weber, E., Fischer, M., 2010. A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecol. Lett.* 13, 235–245.
- Vila, M., Weiner, J., 2004. Are invasive plant species better competitors than native plant species? Evidence from pair-wise experiments. *Oikos* 105, 229–238.
- Weigelt, A., Jolliffe, P., 2003. Indices in plant competition. *J. Ecol.* 91, 707-720

## Figure caption

Figure 1. Competition indices based on the total biomass and calculated according to the treatment ratio. Fig.1A: The score of Relative Yield Total index (RYT<sub>ab</sub>) was calculated for each mixture treatments. It shows the individual Relative Yield of each species contributing to the RYT. Relative Yield scores of *L. hexapetala* : black circles. Relative Yield scores of *M. aquatica* : black triangles. Relative Yield Total index (RYT<sub>ab</sub>): black squares. Fig. B: Relative Competition Intensity (RCI). *L. hexapetala*: black circles, and *M. aquatica*: black triangles

Figure 2. Mean Relative Growth Rate (RGR) plus standard error by period (two weeks measurement) for the two species: *Ludwigia hexapetala* (A) and *Mentha aquatica* (B). Symbols marked with the same letter were not significantly different for treatment factor within the same period ( $p > 0.05$ ) according Mann-Whitney-Wilcox pairwise comparisons corrected by Benjamini-Yekutieli correction.

Figure 3. MDS plot based on Euclidean distance and morphological traits after standardisation. Fig 2A showed ellipsoids at 95% SE around the centroid for each treatment for *L. hexapetala*. Fig 2B showed ellipsoids at 95% SE around the centroid for each treatment for *M. aquatica*.

Figure 4. Mean values + standard errors of morphological traits (Total biomass, root/above-ground biomass, total main stem length, number of branches and total number of counted flowers) for *L. hexapetala* (A, B, C, D, E) or *M. aquatica* (F,G, H, I, J). Bars marked with the same letter were not significantly different for treatment factor within the same period ( $p > 0.05$ ) according to HSD Tukey pairwise comparisons.

Fig 1

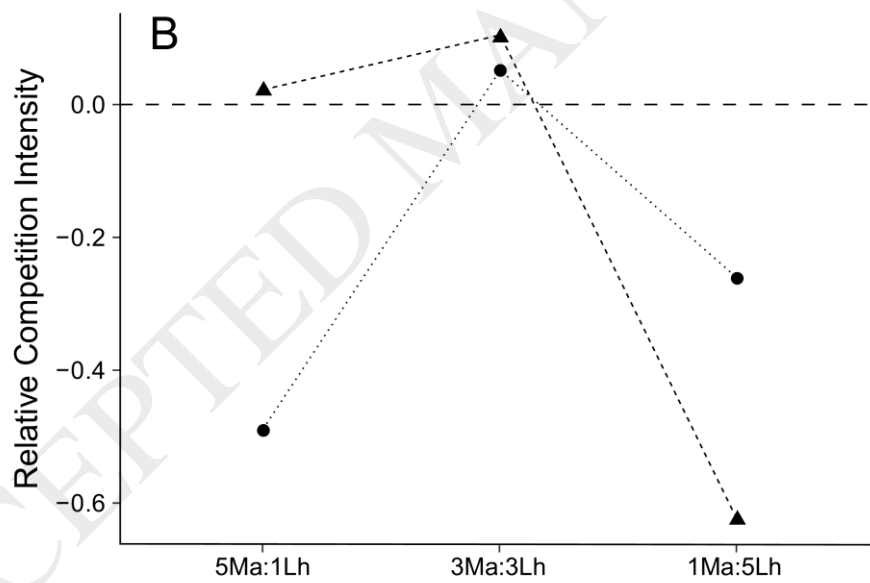
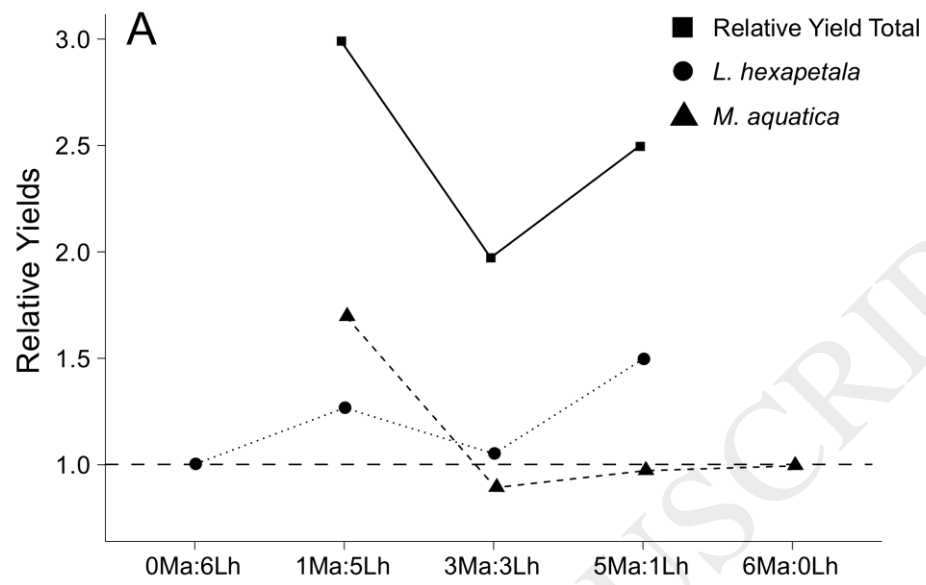


Fig 2

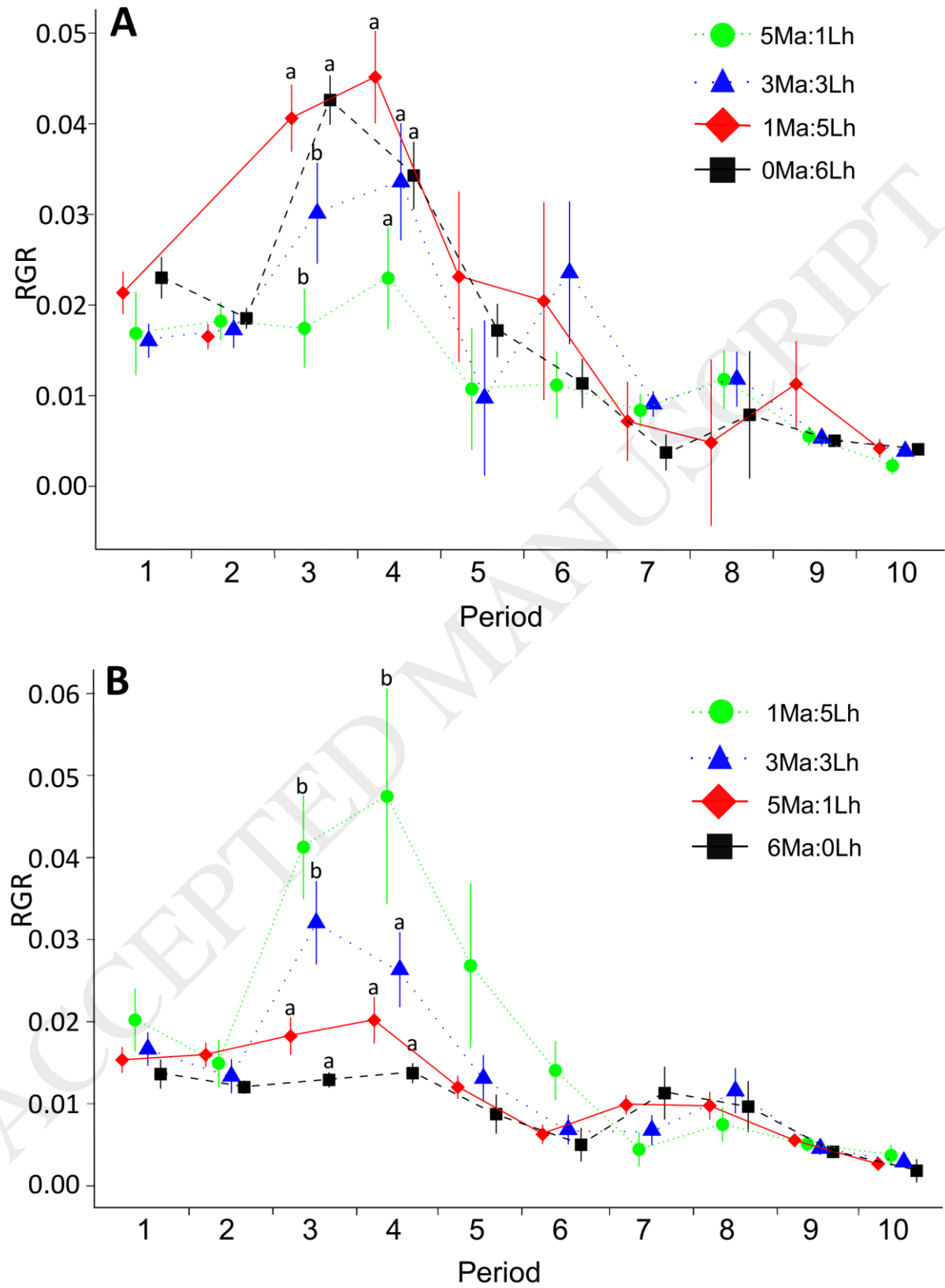


Fig 3

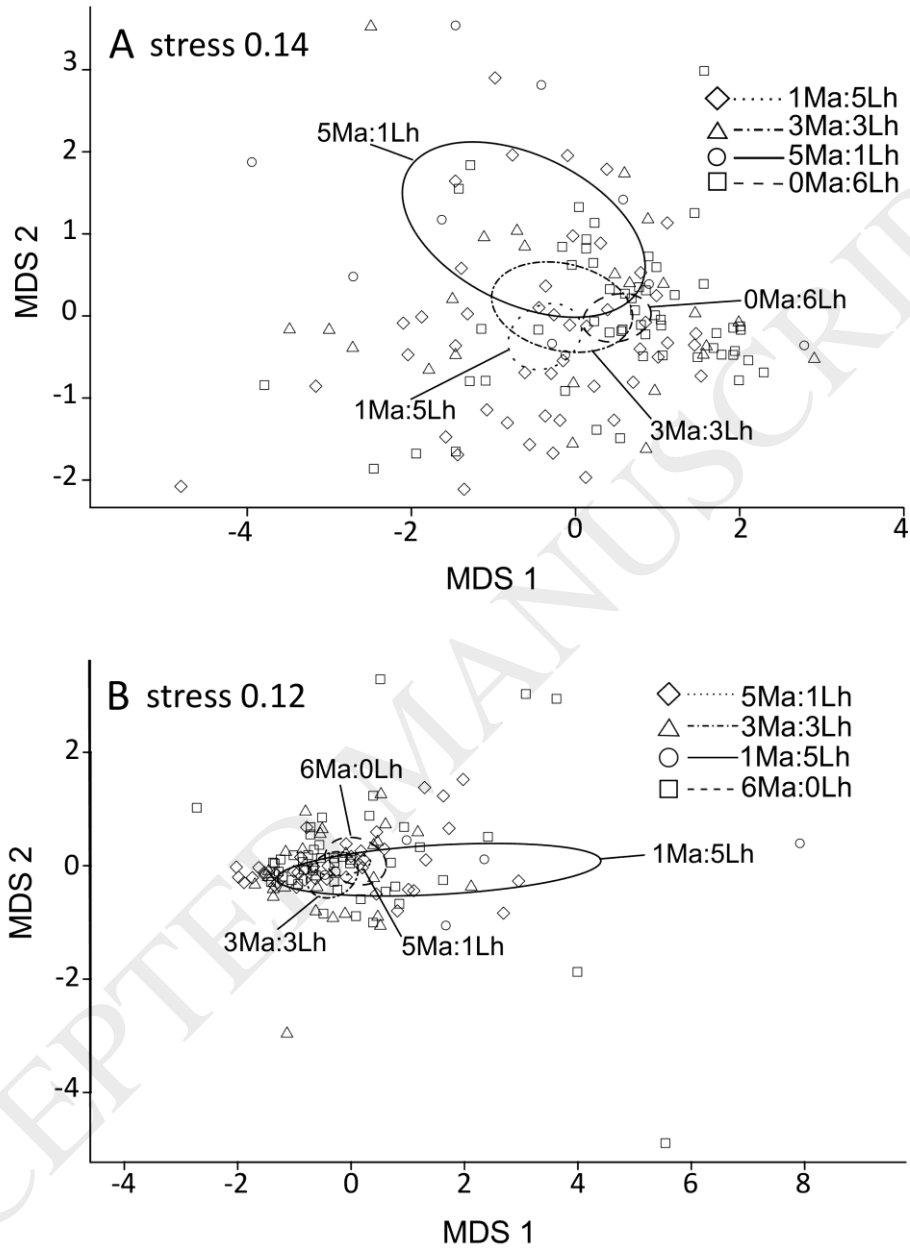




Fig 4

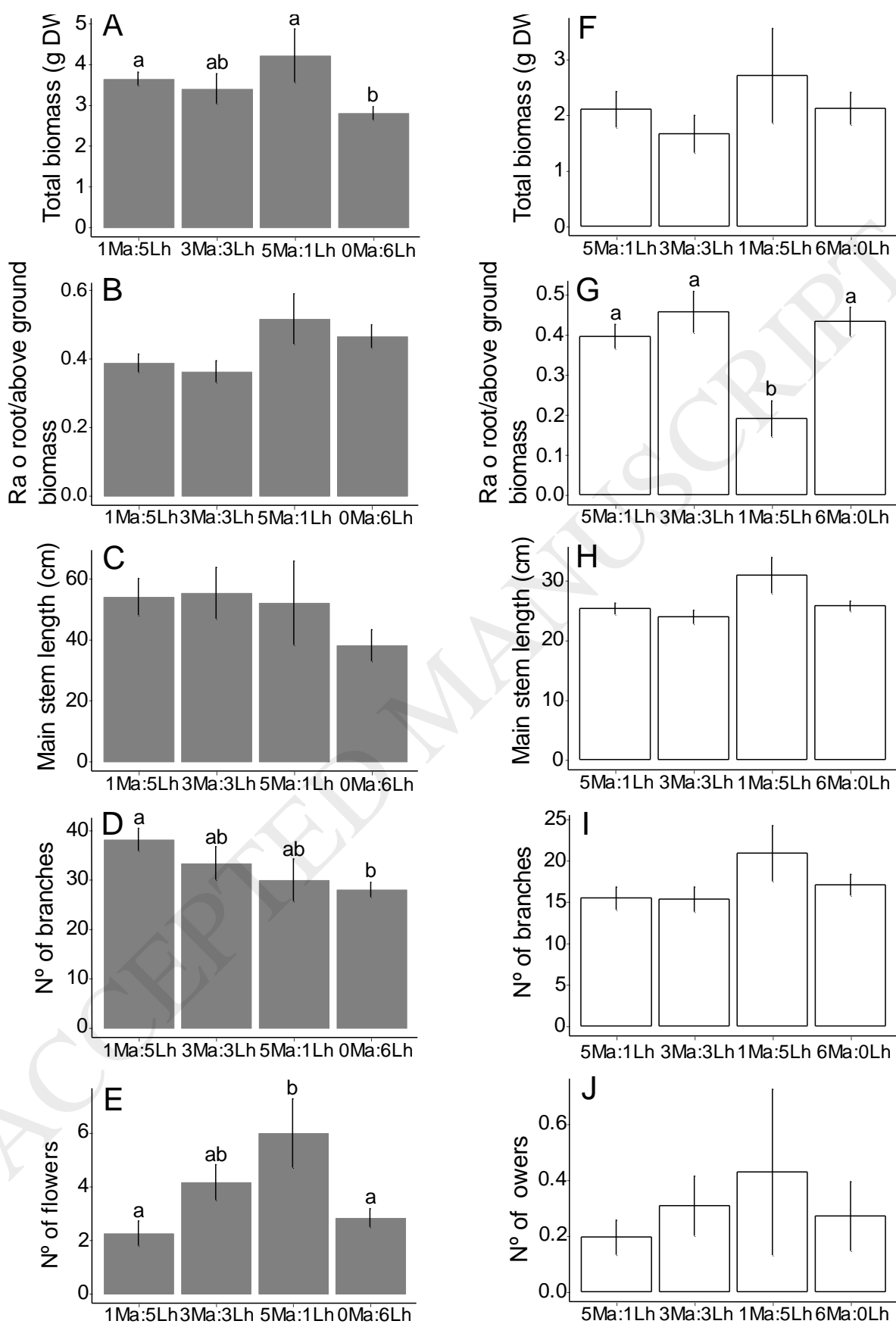


Table 1. Longitudinal data analysis results based on non-parametric repeated measures. RGR is Relative Growth Rate. ATS denotes Anova-type Statistic. Significant p-values at 5% significance level are in bold.

		<i>L. hexapetala</i>			<i>M. aquatica</i>		
		ATS	df	p	ATS	df	p
RGR	Density	2.51	2.54	0.07	1.62	1.64	0.09
	Time	31.28	4.80	<b>&lt;0.001</b>	68.31	6.33	<b>&lt;0.001</b>
	Density x Time	2.49	8.41	<b>0.01</b>	3.05	10.93	<b>&lt;0.001</b>

Table 2. Univariate analyses of morphological traits results. The first three variables were analyzed with a linear model and type II sum of squares. Lateral Branches and Flowers were analyzed by means of glm following a Poisson error distribution, except for Number of flowers variable in the case of *M. aquatica* analyzed with a Kruskal-Wallis test. Significant p-values at 5% significance level are in bold.

	<i>L. hexapetala</i>				<i>M. aquatica</i>			
	SS	df	F	p	SS	df	F	p
Total Biomass	28.88	3	4.55	<b>0.004</b>	6.92	3	0.56	0.6
Stem Length	4.98	3	2.18	0.09	271.4	3	2.27	0.08
Below/ above ground biomass	0.27	3	2.10	0.1	4.71	3	5.27	<b>0.002</b>
	$\chi^2$	df	p		$\chi^2$	df	p	
Lateral Branches	13.45	3	<b>0.004</b>		2.77	3	0.4	
Number Flowers	13.06	3	<b>0.005</b>		1.96	3	0.6	