INVASIVE SPECIES II



Fragment type and water nutrient interact and affect the survival and establishment of *Myriophyllum aquaticum*

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Abstract *Myriophyllum aquaticum* is a semi-submerged exotic macrophyte that was introduced to China for many years. This species may be found in an emergent form in aquatic environments or in an amphibious form under drained conditions. Nuisance growth of this species has often been attributed to excessive amounts of nutrients. Therefore, we tested the following hypotheses: (1) high nutrient availability facilitates the establishment of *M. aquaticum* and (2) fragment type interacts with nutrient availability to determine the colonization and regeneration capacities of *M. aquaticum*. Two types of fragments were grown in water solutions with two levels of phosphorous.

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After 3 weeks, the survival rates showed no significant difference between the phosphorous treatments. However, emergent fragments showed higher RGR in the low and high phosphorous treatments than amphibious fragments. In addition, emergent fragments also showed higher regeneration capacities, indicating higher invasiveness in emergent fragments compared to amphibious fragments. Moreover, the high phosphorous concentration caused emergent fragments to produce more branches, indicating that nutrient availability may increase *M. aquaticum* propagule pressure. Our study highlights that nutrient supply increased emergent fragment establishment and shaped the invasion dynamics of macrophytes, which could help predict the spread and potential impact of exotic macrophytes in natural aquatic ecosystems.

Keywords Biological invasion · Colonization · Invasiveness · Regeneration · Resource availability

Introduction

Propagule pressure is one of the most important factors driving biological invasion (Lockwood et al., 2005; Simberloff, 2009; Thomaz et al., 2015). Propagule pressure may heavily increase the probability of non-native species population establishment and expansion (Simberloff, 2009). In fact, previous studies have shown a positive relationship between propagule

pressure and invasion success among introduced plants (Xie et al., 2013; You et al., 2016). Despite an increasing number of studies related to propagule pressure (mostly evaluating the frequency of arriving propagules), there are relatively fewer studies focusing on the characteristics of the propagules themselves, such as the phenotypic qualities of propagules, which could also drive invasion success (Lange & Marshall, 2016).

Fragments of aquatic macrophytes can be considered to be propagule sources, and they are able to disperse within/between water bodies through water flow. Macrophyte stem fragments can be produced by extreme water flow, animal feeding activities, or human activities (Riis & Sand-Jensen, 2006). These fragments may exacerbate the process of invasion in aquatic macrophytes (Zedler & Kercher, 2004). For many submerged macrophytes, stem fragments are considered to be the most common dispersal units (Santamaría, 2002). For instance, fragment dispersal could be considered to be the main driving force of Elodea canadensis Michx. and Hydrilla verticillata (L.f.) Royle invasion in Europe, New Zealand, and Brazil (Silveira et al., 2009; Redekop et al., 2016). In southern China, fragments of Cabomba caroliniana A. Gray and Elodea nuttallii (Planch.) H. St. John are also widespread in water bodies and irrigation ditches, and fragment dispersal promotes successful invasion in these species (Huang et al., 2016; Wang et al., 2016).

In fact, macrophyte stem fragments are highly suited for dispersal in aquatic habitats (Xie et al., 2010) because compared to other types of asexually produced propagules, stem fragments have the advantages of easy production and dispersal, long periods of survival, and high colonization and regeneration capacities (Riis & Sand-Jensen, 2006). The high colonization and regeneration capacities of submerged macrophyte fragments have been revealed in many previous studies (Barrat-Segretain & Bornette, 2000; Umetsu et al., 2012) and usually increase with fragment size (Barrat-Segretain et al., 1999; Xie et al., 2013). Barrat-Segretain et al. (1998) reported that under laboratory conditions, even stem fragments with four internodes established within several weeks. However, even though the colonization and regeneration capacities of submerged macrophyte fragments have been well studied, only a few papers consider that environmental variables may induce differences in the colonization and regeneration capacities of submerged macrophyte fragments (e.g., Xie et al., 2007; Puijalon et al., 2008; Xie et al., 2010).

Resource availability is another important factor driving biological invasion, as invasion dynamics can be altered by increases in resource availability due to resource enrichment or frequent disturbances within ecosystems (Davis et al., 2000). For instance, there are several types of globally harmful aquatic weeds (e.g., water hyacinth and alligator weed) that are typically better adapted to eutrophic conditions and the decreased biomass of native species in such environments (Zhang et al., 2010; You et al., 2013c, 2014). Although increases in resource availability will sometime facilitate the native community and reduce the establishment of invaders (Xie et al., 2013; Teixeira et al., 2017), resource availability may interact with propagule pressure (e.g., the sustained arrival of new propagules) and cause successful invasions (Xie et al., 2013; You et al., 2016). Therefore, a better understanding of the interactions between propagule pressure and resource availability is of scientific interest.

Myriophyllum aquaticum (Vell.) Verdc. is a semisubmerged macrophyte that is native to the Amazon River in South America and has become a harmful weed species in North America since it was introduced, likely in the late 1800s (Sutton, 1985). During the twentieth century, this species colonized areas in South Africa, East Asia, Europe, New Zealand, and Australia (Sheppard et al., 2005; Thiébaut, 2007; Wang et al., 2016). After being introduced into East Asia as an ornamental species, it escaped into the wild and became widely distributed in aqueducts in Japan, Taiwan, and mainland China (Xie et al., 2001; Wang et al., 2016). M. aquaticum is dioecious, but staminate plants are rare, even in its native range, and seed production is therefore limited in natural habitats (Sutton, 1985). The plants lack structures for storage, dispersal, and perennation (e.g., tubers or turions), and the stem fragments and rhizomes (older submersed stems without leaves) are believed to serve all of these functions in both its native and invasive ranges (Sytsma & Anderson, 1993a). These fragments are able to float for a long time (several months) and disperse over great distances (several kilometers) between water bodies (Wersal & Madsen, 2010).

Zedler & Kercher (2004) noted that wetlands are especially vulnerable to invasion due to flood pulses and the materials in flows. Although a growing number of studies concluded that high water levels/ sediment moisture and nutrients will promote the invasion outcomes of *M. aquaticum*, relatively few studies have focused on the establishment process (e.g., Hussner et al., 2009; You et al., 2013a). M. aquaticum usually invades shallow aquatic habitats, such as wetlands, littoral zones of lakes, ponds, sloughs, and backwaters (Wersal & Madsen, 2011b). An ecotype of this species produces amphibious fragments that can survive on sediments without an overlying water column for several months and increase in biomass (Hussner et al., 2009). Elongation of the creeping stem forces the emergent stem to extend along the water surface and form adventitious roots and new branches from the internodes, creating dense mats (Sytsma & Anderson, 1993b; Hussner et al., 2009) and generating an ecotype with emergent fragments. Thus, two ecotypes with different types of fragments occur: those with emergent fragments (on the water surface) and those with amphibious fragments (on the sediment without overlying water). Therefore, considering that the invader growth form may largely cause differences in invasion impacts (Hussner et al., 2017), it is essential to understand whether fragment type may interact with nutrient availability and increase propagule pressure.

To predict the population growth and distribution of M. aquaticum, we compared the colonization and regeneration capacities of fragments originating from different habitats characterized by contrasting water contents. We tested the hypotheses that (1) high nutrient availability facilitates the establishment of M. aquaticum and that (2) fragment type interacts with nutrient availability to determine the colonization and regeneration capacities of M. aquaticum. We expected fragments with greater biomass under high-nutrient conditions, which would indicate successful establishment. Additionally, our previous studies observed that water/nutrients supplement both stimulated the branch production of *M. aquaticum* (Xie et al., 2010; You et al., 2013b), therefore, we expected that more branches would be found among emergent fragments under high-nutrient conditions, which would suggest an increased capacity of colonization and regeneration in M. aquaticum.

Materials and methods

Field survey and plant preparation

The *M. aquaticum* materials were collected from five field survey plots $(50 \text{ m} \times 50 \text{ m})$ at the National Wetland Ecosystem Field Station of Taihu Lake, which is located in Sanshan Island National Wetland Park, Taihu Lake, Suzhou, Jiangsu Province, China $(31^{\circ}01'40''N, 120^{\circ}17'37''E)$. The field survey plots are located at least 800 m from one another, and the vegetative cover in the field survey plots was dominated by Potamogeton crispus L., M. aquaticum, and Nelumbo nucifera Gaerth., accounting for approximately 30-80% of the standing biomass in the field survey plots. The water depth at the site was 1–2 m, nitrogen (TN) was 0.67 ± 0.11 mg/l total (mean \pm SE) and total phosphorus (TP) was 0.046 ± 0.007 mg/l. The water TP concentrations at the site correspond with trophic state index (TSI) value 50-60 (TP: 0.024-0.048 ml/l) (Carlson, 1977), which meets mesotrophic to eutrophic conditions.

We collected 20 fragments of *M. aquaticum* (10 emergent fragments and 10 amphibious fragments) from each field survey plot, considering each plot as a different plant population. The plant fragments were apical unbranched portions from multiple parent individuals. After collection, the plants were washed using tap water and were transported back to the lab (Nanjing Forestry University) within 24 h. In the lab, the plant material was transplanted into container with clean sand (approximately 10 cm) and filled with 30 cm of tap water. The plant fragments were cultivated in a greenhouse (water temperature: $17.53 \pm 2.49^{\circ}$ C; irradiance: $1241 \pm 25.94 \mu$ mol photons/m² s, mean \pm SE) for 2 weeks until the experiments began.

Experimental design

The experiment was conducted in a greenhouse located at the campus of Nanjing Forestry University, Nanjing, Jiangsu Province, China $(32^{\circ}04'45''N, 118^{\circ}48'46''E)$. The experiment was conducted during a 3-week period (from April to May 2016) using a factorial design, crossing two levels of nutrient availability (low and high) and two fragment types (amphibious and emergent) of *M. aquaticum*. Nutrient levels were created by using only tap water to simulate

a low phosphorus concentration (generally below 0.05 mg/l of TN and 0.01 ± 0.002 mg/l of TP, mean \pm SE—the detection limit for our analyses) and by the addition of a nutrient solution (monosodium phosphate) to the tap water to simulate a high phosphorus concentration (below 0.05 mg/l of TN and 0.04 ± 0.005 mg/l of TP, mean \pm SE). The high phosphorus treatment corresponds with the same phosphorus concentration level as that at Sanshan Island (TSI values 50-60). To prepare the plant fragments for addition to the nutrient treatments, the amphibious and emergent fragments of M. aquaticum were cut to a length of 7 cm. Then, five fragments (same ecotype but one from each survey plotdifferent populations) were mixed and planted in a plastic box $(40 \times 30 \times 20 \text{ cm})$ with 2 1 water. The same process was performed with both fragment types. Each treatment was replicated five times, totaling 20 experimental units, and therefore 100 plant individuals of similar size were used (fresh weight: amphibious fragments 0.46 ± 0.03 g and emergent fragments 0.49 ± 0.02 g, mean \pm SE). The experimental units were kept in a room with temperature and light intensity controlled and recorded during the experimental period (water temperature 20.21 \pm 4.11°C; light intensity 1232 \pm 38.23 µmol m⁻² s⁻¹, mean \pm SE). To reduce the influence of algae on plant growth, the water in the boxes was changed every 3 days.

At the end of the experiment, plants from each treatment were carefully harvested and then rinsed. We used the percentage of living fragments to quantify fragment survival. The survival of the fragments was calculated using the following equation:

Survival (%) = $FN_S/FN_o \times 100\%$,

where FN_s is the number of surviving fragments, and FN_o is the initial number of fragments ($FN_o = 5$). Moreover, we measured the fragment length relative growth rate (RGR), the fragment weight relative RGR, the branch number and length, and the root number and length as proxies to evaluate the colonization and regeneration capacities of fragments (Barrat-Segretain & Bornette, 2000). Additionally, branch number was also used as a proxy for potential propagule supplementation, since branch number strongly correlates with the abundance of plant asexual propagules (stem fragments) in aquatic macrophytes (Xie et al., 2010). The fragments were evaluated twice a week (every 3–

4 days) for survival, and dead plant material was removed from the boxes. The relative growth rate was calculated using the following equation:

 $RGR = [ln(final dry weight or length) \\ - ln(initial dry weight or length)]/$ experiment time span

Before the experiment began, an additional 10 fragments of each fragment type were used to calculate the water content to obtain the initial dry weight. Fresh weight and stem length were measured for each plant individual. Then, plant tissue samples were dried at 80°C for 72 h and then weighed.

Statistical analysis

All measured plant attributes (fragment survival, fragment length and weight RGR, branch number and length, root number and length) were analyzed using two-way ANOVA, with nutrient availability (low and high nutrient concentration) and fragment type (amphibious and emergent) as fixed factors. Tukey's post hoc tests were used to evaluate the significance of the levels within factors (P < 0.05). All data were analyzed using R version 3.2.0 (http:// www.r-project.org).

Results

Survival

High survival rates ($\geq 60\%$) were observed for all fragments of *M. aquaticum* during 3 weeks of the experiment. There were significant differences in survival between amphibious fragments and emergent fragments. The emergent fragments from the low phosphorus treatment had highest survival rates among treatments. However, nutrient availability had no significant effect on the survival of fragments, and the interactions between fragment types and nutrient availability were not significant (Fig. 1).

Weight and length relative growth rate

The interaction between fragment type and nutrient availability significantly determined the weight RGR and length RGR (Fig. 2). However, the main effects of



Fig. 1 Fragment survival rates between amphibious fragments (AF, black bars) and emergent fragments (EF, white bars) in high and low phosphorous treatments. The data are presented as the mean \pm SE (n = 5). The F and P values of two-way ANOVA are shown. Bars sharing the same letters indicate no significant differences between the treatments (Tukey's post hoc test, P > 0.05)



Fig. 2 Dry weight RGR (a) and fragment length RGR (b) between amphibious fragments (AF, black bars) and emergent fragments (EF, white bars) in high and low phosphorous treatments. The data are presented as the mean \pm SE (n = 5). The F and P values of two-way ANOVA are shown. Bars sharing the same letters indicate no significant differences between treatments (Tukey's post hoc test, P > 0.05)

fragment type and nutrient availability significantly affected macrophyte weight RGR, while only the effect of fragment type significantly affected macrophyte length RGR. In the high phosphorus treatment, the amphibious fragments had negative weight and length RGRs, but emergent fragments had positive and higher weight and length RGRs than amphibious fragments. Similarly, in the low phosphorous treatment, emergent fragments had a higher weight RGR than amphibious fragments, although the length RGR was similar between the two fragment types (Fig. 2).

Colonization and regeneration capacities

The regeneration rates were high in both the amphibious and emergent fragments, with approximately 86% of all fragments regenerating (i.e., produced branches) within the 3-week experimental period. In the high phosphorous treatment, the number and length of branches that sprouted were lower in amphibious fragments than in emergent fragments (Fig. 3a, b; Table 1). In the low phosphorous treatment, the number and length of branches were similar between the two types of fragments (Fig. 3a, b; Table 1). Regarding the roots, in the high phosphorous treatment, the number of roots was similar between the two types of fragments. However, the root length of amphibious fragments was lower than that of emergent fragments (Fig. 3c, d; Table 1). In the low phosphorous treatment, the amphibious fragments produced a similar number of roots but longer roots compared to those from emergent fragments (Fig. 3c, d; Table 1).

Discussion

M. aquaticum is one of the major invaders growing in a variety of aquatic habitats in China, including static and flowing waters with high nutrient regimes (Xie et al., 2013; You et al., 2013a; Wang et al., 2016). The spread of *M. aquaticum* is similar to that of most submerged aquatic plants, largely based on the dispersal of vegetative fragments (Sytsma & Anderson, 1993a; Xie et al., 2010; You et al., 2013a). In the present study, we observed that both fragment types had high survival rates independent of the nutrient availability, which is consistent with our previous results (Xie et al., 2010, 2013). The higher the survival rates of the vegetative fragments produced, the more likely the dispersal of a given individual within/between connected water bodies (e.g., rivers and irrigation systems) when these fragments are dispersed by water flow (Riis & Sand-Jensen, 2006; Boedeltje et al.,

Fig. 3 Branch number per fragment (a), branch length per fragment (b), root number per fragment (c), and root length (d) between amphibious fragments (AF, black bars) and emergent fragments (EF, white bars) in high and low phosphorous treatments. The data are presented as the mean \pm SE (n = 5). Bars sharing the same letters indicate no significant differences between treatments (Tukey's post hoc test, P > 0.05)



 Table 1
 Two-way ANOVA F and P values for phosphorous treatment and fragment type for branch number per fragment, branch length per fragment, root number per fragment, and root length

	d.f.	Branch number		Branch length		Root number		Root length	
		F	Р	F	Р	F	Р	F	Р
Phosphorous	1.16	3.099	> 0.05	6.193	0.024	26.255	< 0.001	21.955	< 0.001
Ecotype	1.16	21.61	0.002	13.393	0.002	4.949	0.041	39.996	< 0.001
Interaction	1.16	6.995	0.017	0.664	> 0.05	0.332	> 0.05	4.324	> 0.05

2008). Because of the high survival rates of many invasive macrophytes, even when a small number of individuals is released into a native community, negative influences on growth of native species are also expected after its initial arrival (Fleming & Dibble, 2015).

Previous studies have revealed that *M. aquaticum* prefers nutrient-rich sites (Sytsma & Anderson, 1993a, b; Wersal & Madsen, 2011b). However, our results show that the growth (fragment weight RGR and length RGR) of amphibious fragments decreased in the high phosphorous treatment. The bottom parts of the amphibious fragments died and decayed after the experiment began, which is consistent with phenotypic plasticity (e.g., transformation between amphibious and emergent) in *M. aquaticum* fragments (Hussner et al., 2009; Malheiro et al., 2013). Amphibious fragments of *M. aquaticum* stems are relatively old

stems, which are brittle and easily fragmented (Wersal & Madsen, 2011b). In addition, the mechanical resistance (breaking force and strength) of aquatic plants is significantly reduced under nutrient-rich conditions due to their biomechanical properties (Lamberti-Raverot & Puijalon, 2012). Although a previous study found that the growth of *M. aquaticum* could be limited by nitrogen, M. aquaticum did not increase in biomass unless the water nitrogen concentration was above 1.8 mg/l (Wersal & Madsen, 2011b), and high growth was observed in emergent fragments. This is consistent with the stem fragments of *Myriophyllum* species containing large amounts of storage resources (e.g., carbohydrates, nitrogen, and phosphorous), helping these fragments adapt to and survive in heterogeneous environments (Wersal et al., 2011; Xie & Yu, 2011; Xie et al., 2013). Based on our results, the nutrients stored in fragments can obviously sustain high biomass production in *M. aquaticum* at least over the short term (e.g., days to weeks). In addition, the emergent fragments produced more biomass than the amphibious fragments in the low phosphorous treatment, which can be explained by *M. aquaticum* storing most resources in emergent tissues (Sytsma & Anderson, 1993a). However, our experimental period was relatively short, and the results might differ if long-term experiment and different nutrient treatments are performed.

Most of the colonization and regeneration traits of fragments were significantly affected by resource availability, consistent with our previous study on stem fragments of M. aquaticum and other Myriophyllum species (Xie et al., 2010, 2013; Xie & Yu, 2011). Although growth in the high phosphorous treatment did not increase the survival rates of either of these two fragment types, it significantly increased the branch number and length in emergent fragments. These results are consistent with those of previous studies showing that M. aquaticum prefers nutrientrich sites (Hussner et al., 2009), and plants tend to allocate more resources to branch biomass under nutrient-rich conditions (Wersal & Madsen, 2011b), especially species with high invasiveness. A greater number and longer roots were produced by fragments under the low phosphorous conditions, which was also consistent with previous studies showing that M. aquaticum has high tolerance to low nutrient conditions (Hussner et al., 2009; Wersal & Madsen, 2011b).

Our first hypothesis that high nutrient availability will facilitate the establishment of *M. aquaticum* was rejected, while our second hypothesis that fragment type will interact with nutrient availability to determine the colonization and regeneration capacities of M. aquaticum was not. Emergent fragments had a higher RGR and colonization and regeneration capacities than the amphibious fragments at both phosphorous levels. This result suggests that despite the minor importance of nutrient availability in fragment establishment, nutrient availability may increase propagule pressure (i.e., the number of branches), especially in emergent fragments of *M. aquaticum*. Bickel (2016) reported that under laboratory conditions, even stem fragments with single internodes established (Cabomba caroliniana, 50% establishment success) within several weeks. Similarly, M. aquaticum reestablish quickly after harvesting (You et al., 2013a) and dropping harvested plant material close to the managed water body would cause additional invasive pressure. Previous studies have indicated that M. aquaticum takes up and transports nutrients (e.g., nitrogen and phosphorous) and water mainly from the water column through adventitious roots (Sytsma & Anderson, 1993b). An increase in root porosity was observed when M. aquaticum grew in deep water (17-56% increase in root porosity when grown at a water level of 10-20 cm above the soil surface) (Hussner et al., 2009), indicating that M. aquaticum prefers phosphorous from the water column. Indeed, these adventitious roots grow from each node of the fragment, where growth will begin once the fragments touch the water (after 3 days in our experiment). The emergent fragments in our experiment had higher branch and root growth, also indicating that these fragments are more suitable for water/nutrient transportation because amphibious tissues are usually vascularized, thick and stiff, which may limit water/ nutrient transportation (Wersal & Madsen, 2011a). Therefore, to control and prevent the further invasion of M. aquaticum, in addition to the management of nutrient availability, water level control could be important in reducing habitat suitability for emergent fragments. Moderate water level fluctuations could be effective, especially in high-nutrient habitats. However, more research is still needed to determine how nutrients interact with water uptake/transport, which influence the growth of M. aquaticum.

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References

- Barrat-Segretain, M. H. & G. Bornette, 2000. Regeneration and colonization abilities of aquatic plant fragments: effect of disturbance seasonality. Hydrobiologia 421(1): 31–39.
- Barrat-Segretain, M. H., G. Bornette & A. Hering-Vilas-Bôas, 1998. Comparative abilities of vegetative regeneration among aquatic plants growing in disturbed habitats. Aquatic Botany 60: 201–211.
- Barrat-Segretain, M. H., C. P. Henry & G. Bornette, 1999. Regeneration and colonization of aquatic plants fragments in relation to the disturbance frequency of their habitat. Archiv für Hydrobiologie 145: 111–127.
- Bickel, T. O., 2016. Processes and factors that affect regeneration and establishment of the invasive aquatic plant *Cabomba caroliniana*. Hydrobiologia. doi:10.1007/ s10750-016-2995-0.
- Boedeltje, G., W. A. Ozinga & A. Prinzing, 2008. The trade-off between vegetative and generative reproduction among angiosperms influences regional hydrochorous propagule pressure. Global Ecology and Biogeography 17(1): 50–58.
- Carlson, R. E., 1977. A trophic state index for lakes. Limnology and Oceanography 22(2): 361–369.
- Davis, M. A., J. P. Grime & K. Thompson, 2000. Fluctuating resources in plant communities: a general theory of invasibility. Journal of Ecology 88(3): 528–534.
- Fleming, J. P. & E. D. Dibble, 2015. Ecological mechanisms of invasion success in aquatic macrophytes. Hydrobiologia 746(1): 23–37.
- Huang, W., H. Shao, W. Li, H. Jiang & Y. Chen, 2016. Effects of urea on growth and photosynthetic metabolism of two aquatic plants (*Cabomba caroliniana* A. Gray and *Elodea nuttallii* (Planch.) H. St. John). Aquatic Botany. doi:10. 1016/j.aquabot.2016.04.003.
- Hussner, A., C. Meyer & J. Busch, 2009. The influence of water level and nutrient availability on growth and root system development of *Myriophyllum aquaticum*. Weed Research 49: 73–80.
- Hussner, A., I. Stiers, M. J. J. M. Verhofstad, E. S. Bakker, B. M. C. Grutters, J. Haury, J. L. C. H. van Valkenburg, G. Brundu, J. Newman, J. S. Clayton, L. W. J. Anderson & D. Hofstra, 2017. Management and control methods of invasive alien freshwater aquatic plants: a review. Aquatic Biology 136: 112–137.
- Lamberti-Raverot, B. & S. Puijalon, 2012. Nutrient enrichment affects the mechanical resistance of aquatic plants. Journal of Experimental Botany 63(17): 6115–6123.
- Lange, R. & D. J. Marshall, 2016. Propagule size and dispersal costs mediate establishment success of an invasive species. Ecology 97(3): 569–575.
- Lockwood, J. L., P. Cassey & T. Blackburn, 2005. The role of propagule pressure in explaining species invasions. Trends in Ecology & Evolution 20(5): 223–228.
- Malheiro, A. C. E., P. Jahns & A. Hussner, 2013. CO₂ availability rather than light and temperature determines growth and phenotypical responses in submerged *Myriophyllum aquaticum*. Aquatic Botany 110: 31–37.
- Puijalon, S., T. J. Bouma, J. V. Groenendael & G. Bornette, 2008. Clonal plasticity of aquatic plant species submitted

to mechanical stress: escape versus resistance strategy. Annals of Botany 102:989–996.

- Redekop, P., D. Hofstra & A. Hussner, 2016. *Elodea canadensis* shows a higher dispersal capacity via fragmentation than *Egeria densa* and *Lagarosiphon major*. Aquatic Botany 130: 45–49.
- Riis, T. & K. Sand-Jensen, 2006. Dispersal of plant fragments in small streams. Freshwater Biology 51(2): 274–286.
- Santamaría, L., 2002. Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. Acta Oecologica 23: 137–154.
- Sheppard, A. W., R. H. Shaw & R. Sforza, 2005. Top 20 environmental weeds for classical biological control in Europe: a review of opportunities regulations and other barriers to adoption. Weed Research 46: 93–117.
- Silveira, M. J., S. M. Thomaz, R. P. Mormul & F. P. Camacho, 2009. Effects of desiccation and sediment type on early regeneration of plant fragments of three species of aquatic macrophytes. International Review of Hydrobiology 94(2): 169–178.
- Simberloff, D., 2009. The role of propagule pressure in biological invasions. Annual Review of Ecology, Evolution, and Systematics 40: 81–102.
- Sutton, D. L., 1985. Biology and ecology of Myriophyllum aquaticum. In Proceeding, 1st International Symposium on watermilfoil (Myriophyllum spicatum) and Related Haloragaceae Species, Vancouver, B. C., 1985: 59–71.
- Sytsma, M. D. & L. W. J. Anderson, 1993a. Biomass, nitrogen, and phosphorus allocation in parrotfeather (*Myriophyllum aquaticum*). Journal of Aquatic Plant Management 31: 244–248.
- Sytsma, M. D. & L. W. J. Anderson, 1993b. Transpiration by an emergent macrophyte: source of water and implications for nutrient supply. Hydrobiologia 271(2): 97–108.
- Teixeira, M. C., L. M. Bini & S. M. Thomaz, 2017. Biotic resistance buffers the effects of nutrient enrichment on the success of a highly invasive aquatic plant. Freshwater Biology 62(1): 65–71.
- Thiébaut, G., 2007. Invasion success of non-indigenous aquatic and semi-aquatic plants in their native and introduced ranges. A comparison between their invasiveness in North America and in France. Biological Invasions 9(1): 1–12.
- Thomaz, S. M., R. P. Mormul & T. S. Michelan, 2015. Propagule pressure, invasibility of aquatic ecosystems by non-native macrophytes and their impacts on populations, communities and ecosystems: a review of tropical freshwater ecosystems. Hydrobiologia 746: 39–59.
- Umetsu, C. A., H. B. A. Evangelista & S. M. Thomaz, 2012. The colonization, regeneration, and growth rates of macrophytes from fragments: a comparison between exotic and native submerged aquatic species. Aquatic Ecology 46: 443–449.
- Wang, H., Q. Wang, P. A. Bowler & W. Xiong, 2016. Invasive aquatic plants in China. Aquatic Invasions 11(1): 1–9.
- Wersal, R. M. & J. D. Madsen, 2010. Comparison of subsurface and foliar herbicide applications for control of Parrotfeather (*Myriophyllum aquaticum*). Invasive Plant Science and Management 3: 262–267.

- Wersal, R. M. & J. D. Madsen, 2011a. Comparative effects of water level variations on growth characteristics of *Myriophyllum aquaticum*. Weed Research 51: 386–393.
- Wersal, R. M. & J. D. Madsen, 2011b. Influences of water column nutrient loading on growth characteristics of the invasive aquatic macrophyte *Myriophyllum aquaticum* (Vell.) Verdc. Hydrobiologia 665: 93–105.
- Wersal, R. M., J. C. Cheshier, J. D. Madsen & P. D. Gerard, 2011. Phenology, starch allocation, and environmental effects on *Myriophyllum aquaticum*. Aquatic Botany 95: 194–199.
- Xie, D. & D. Yu, 2011. Size-related auto-fragment production and carbohydrate storage in auto-fragment of *Myriophyllum spicatum* L. in response to sediment nutrient and plant density. Hydrobiologia 658: 221–231.
- Xie, Y., Z. Li, W. P. Gregg & D. Li, 2001. Invasive species in China-an overview. Biodiversity and Conservation 10(8): 1317–1341.
- Xie, Y., W. Luo, B. Ren & F. Li, 2007. Morphological and physiological responses to sediment type and light availability in roots of the submerged plant *Myriophyllum spicatum*. Annals of Botany 100: 1517–1523.
- Xie, D., D. Yu, L. F. Yu & C. H. Liu, 2010. Asexual propagations of introduced exotic macrophytes *Elodea nuttallii*, *Myriophyllum aquaticum*, and *M. propinquum* are improved by nutrient-rich sediments in China. Hydrobiologia 655: 37–47.
- Xie, D., D. Yu, W. H. You & C. X. Xia, 2013. The propagule supply, litter layers and canopy shade in the littoral community influence the establishment and growth of *Myriophyllum aquaticum*. Biological Invasions 15: 113–123.

- You, W., D. Yu, C. Liu, D. Xie & W. Xiong, 2013a. Clonal integration facilitates invasiveness of the alien aquatic plant Myriophyllum aquaticum L. under heterogeneous water availability. Hydrobiologia 718(1): 27–39.
- You, W., D. Yu, C. Liu, D. Xie & W. Xiong, 2013b. Clonal integration facilitates invasiveness of the alien aquatic plant *Myriophyllum aquaticum* L. under heterogeneous water availability. Hydrobiologia 718: 27–39.
- You, W., D. Yu, D. Xie & L. Yu, 2013c. Overwintering survival and regrowth of the invasive plant *Eichhornia crassipes* are enhanced by experimental warming in winter. Aquatic Biology 19: 45–53.
- You, W. H., D. Yu, D. Xie, C. Han & C. Liu, 2014. The invasive plant *Alternanthera philoxeroides* benefits from clonal integration in response to defoliation. Flora 209: 666–673.
- You, W. H., C. Han, L. Fang & D. Du, 2016. Propagule pressure, habitat conditions and clonal integration influence the establishment and growth of an invasive clonal plant, *Alternanthera philoxeroides*. Frontiers in Plant Science 7: 568.
- Zedler, J. & S. Kercher, 2004. Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. Critical Reviews in Plant Sciences 23(5): 431–452.
- Zhang, Y. Y., D. Y. Zhang & S. C. H. Barrett, 2010. Genetic uniformity characterizes the invasive spread of water hyacinth (*Eichhornia crassipes*), a clonal aquatic plant. Molecular Ecology 19(9): 1774–1786.