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The colonization, regeneration, and growth rates of macrophytes from fragments: a comparison between exotic and native submerged aquatic species

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Abstract The propagation of most aquatic macrophytes occurs vegetatively, via fragments. After dispersal, the fragments may exhibit two survival strategies: colonization (the ability to develop roots) and regeneration (the ability to develop new propagules that can disperse). Invasive species, for example, *Hydrilla verticillata*, are known to have a high potential for dispersal and growth. We experimentally evaluated the colonization and regeneration abilities and growth rates of this species in the early stage of development and compared these properties to the corresponding characteristics of two native species of Hydrocharitaceae (*Egeria densa* and *Egeria najas*). The following hypotheses were tested: (1) fragments with apical tips have greater colonization and regeneration abilities and higher growth rates than fragments without apical tips and (2) *H. verticillata* has greater colonization and regeneration abilities and higher growth rates than other native species of Hydrocharitaceae. Our results showed that both types of fragments had the same ability to colonize, whereas fragments without tips had a higher ability to regenerate. However, fragments with apical tips showed higher growth rates. It is probable that these higher growth rates resulted from apical dominance. *H. verticillata* had the greatest propagation potential. Its colonization and regeneration abilities and growth rates were greater than those of the native species. These differences can produce competitive advantages for the exotic species in the early stages of development in new habitats and may contribute to understanding the success of the exotic compared to the native species.

Keywords Colonization · Regeneration · Growth rates · Fragment · Exotic · *Hydrilla verticillata*

Introduction

Submerged aquatic species are especially vulnerable to physical and biological disturbances associated, for example, with the water regime (Barrat-Segretain and Cellot 2007) and herbivory (Li et al. 2010). These disturbances help to propagate the plants by causing

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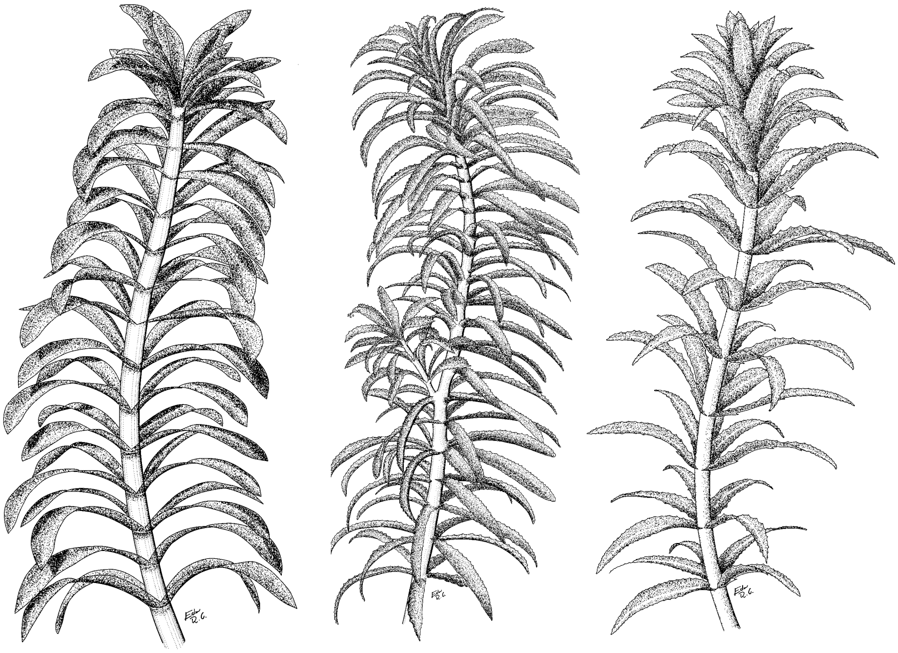

Fig. 1 Drawings of *E. densa* (*left*), *E. najas* (*center*), and *H. verticillata* (*right*) showing architectural similarity among them (by Eduardo Ribeiro da Cunha)

the release of a variety of vegetative plant parts, including entire plants, rhizomes, stolons, tubers, turions, and, more frequently, fragments (Riis and Sand-Jensen 2006). Fragments, which are mainly produced by the mechanical breakage of the plants, disperse from the original colonies and settle in the sediment (Madsen and Smith 1999). These fragments can exhibit two survival strategies: the development of roots and rapid establishment in the sediment (colonization) and the development of new propagules that can be dispersed (regeneration) (Southwood 1988; Barrat-Segretain and Bornette 2000).

Particular species may show a greater ability to colonize and regenerate in new environments. In certain cases, it is probable that these species will expand beyond their natural range and population density, thus becoming invasive (Richardson et al. 2000) and often causing ecological and economic damages (Vitousek et al. 1997; Pimentel et al. 2001). *Hydrilla verticillata* (L.f.) Royle, for example, has been one of the most problematic invasive species worldwide. This species is a submerged aquatic macrophyte, native to Asia and Australia (Cook and Lüönd 1982) and considered “the perfect aquatic weed” (Langeland 1996) because it has many traits commonly attributed to invasive species, including rapid growth and reproductive and physiological characteristics that allow it to become a dominant species in a broad range of freshwater ecosystems (Cook and Lüönd 1982; Langeland 1996; Chadwell and Engelhardt 2008; Bianchini et al. 2010; Sousa 2011). In the Upper Paraná River Floodplain System—UPRFS, *H. verticillata* was first recorded in 2005 (Sousa et al. 2009). This record is most likely the first for South America. Previous studies have shown that this species is highly competitive (Van et al. 1999; Mony et al. 2007; Wang et al. 2008) and can compete with the native species *Egeria densa* Planchon and *Egeria najas* Planchon in the UPRFS (Sousa et al. 2010). These three species have very similar morphology (Fig. 1) and ecological features, and these similarities can cause intense competitive interactions among the species (Sousa et al. 2009). Observations in the habitats of the UPRFS indicate that these species begin to establish from small fragments.

A study similar to ours showed that fragments of *H. verticillata* have higher colonization and regeneration abilities than fragments of the native species after desiccation stress (Silveira et al. 2009). However, it is unknown whether fragments without apical tips, which are also frequently dispersed by flowing water and by birds, have the same colonization and regeneration abilities as those with tips. To better understand how the attributes of one exotic species can contribute to their invasiveness compared with native species, we conducted experiments with plant fragments to compare the colonization and regeneration abilities and growth rates of *H. verticillata* with the corresponding characteristics of two phylogenetically related native species, *E. densa* and *E. najas*. Based on the studies performed on other continents and in South America (Mony et al. 2007; Riis et al. 2009; Sousa et al. 2010), we tested the following hypotheses: (1) fragments with apical tips have greater colonization and regeneration abilities and higher growth rates than fragments without apical tips and (2) *H. verticillata* has greater colonization and regeneration abilities and higher growth rates than other native species of Hydrocharitaceae.

Materials and methods

Samples of *E. densa*, *E. najas*, and *H. verticillata* were collected in the Itaipu Reservoir in April 2010. Healthy fragments were stored in open plastic containers with water collected at the same site and immediately transported to the laboratory. The fragments were washed in running tap water to remove material attached to the surface. In contrast to the material used by Silveira et al. (2009), we used two types of 10-cm vegetative fragments from each species: fragments with an apex (apical stem) and fragments without an apex (mid-stem). For each fragment type, we selected 15 replicates. In total, we analyzed 90 samples. Each fragment was individually placed in 0.8-L plastic containers (15.5 cm in height and 10 cm in diameter) containing tap water, which remained without changes throughout the experiment, and 3 cm of substrate (horticultural peat). The substrate used in this experiment was chosen because it did not limit plant growth, as shown by a pilot experiment. The fragments were individually incubated to avoid interspecific competition. They were not planted in the substrate but were simply dropped into the containers. As in the nature, the fragments sink to bottom and began to root after a few days (Langeland and Sutton 1980). The position of the plastic containers was randomized in the greenhouse. The physical and chemical parameters of the water were measured weekly. The plants were grown at a temperature of 17.86 ± 0.50 °C, a dissolved oxygen concentration of 10.15 ± 0.23 mg L^{-1}, a pH of 8.78 ± 0.03 and a conductivity of 510.75 ± 18.74 µS cm^{-1} (mean ± standard deviation).

By 38 days after the beginning of the experiment, most fragments had rooted in the sediment and grown to the surface of the microcosm. We then terminated the experiment. We measured the number of roots, the dry weight (DW) of the roots, and the root ratio (calculated as the DW of the roots divided by the DW of the entire individual) to evaluate the colonization ability of the plant fragments. To assess the regeneration ability of the plant fragments, we measured the number of shoots (including any secondary shoots that emerged during the experiment), the DW of the shoots, and the shoot ratio (calculated as the DW of the secondary shoots divided by the DW of the entire individual). Root ratio and shoot ratio were used as the traits of growth in biomass accumulation and allocation. The plants were oven-dried at 60 °C until they reached a constant weight.

Relative growth rates (RGR) were calculated for the fragments alone (considering only the growth of the original fragments and excluding any shoots and roots that appeared during the experiment) and for the total biomass (considered as the total investment in the growth and production of new biomass, that is, the growth of the fragment plus new shoots and roots): $\mathrm{RGR} = (\ln W_f – \ln W_i)/t_{\mathrm{days}}$, where $W_f =$ final DW and $W_i =$ initial DW. The initial DW of the fragments was estimated from 15 fragments of each species with a size similar to those used in the experiment.

We used a two-way ANOVA to test the effect of the species and fragment type as well as the effect of the interaction between these factors on the colonization ability of the fragments (the number and the DW of the roots and the root ratio), the regeneration ability of the fragments (the number and the DW of the shoots and the shoot ratio), and fragments and total RGR. When ANOVA showed no significant interaction between species and fragment type, we just tested for the main effect and multiple comparisons of the attributes were performed using Fisher's test. The level of significance of the tests was set at 0.05.

Results

The number of shoots was significantly affected by the species and fragment type (Table 1). In general, the mid-stem fragments had a greater shoot number than the apical stems. *H. verticillata* had the highest number of shoots for both fragment types (Fig. 2a). The number of roots was affected only by the species (Table 1), and again, *H. verticillata* exhibited the

Table 1 Two-way analysis of variance for the effects of *E. najas*, *E. densa*, and *H. verticillata* and the type of fragment (with or without apices) with their interactions on the shoot and root number; shoot and root dry weight (DW); shoot and root ratios; and fragment and total relative growth rate (RGR)

Variable response	Treatments					
	Species $F_{2,83}$	*P*	Type $F_{1,83}$	*P*	*S*T* $F_{2,83}$	*P*
Shoot number	31.23	<0.001	7.505	0.007	0.789	0.457
Root number	19.54	<0.001	1.596	0.210	0.520	0.596
Shoot DW (mg)	16.59	<0.001	25.90	<0.001	2.050	0.135
Root DW (mg)	7.758	<0.001	2.367	0.127	1.341	0.267
Shoot ratio (%)	9.925	<0.001	2.582	<0.001	31.89	0.218
Root ratio (%)	8.487	<0.001	3.495	0.065	1.548	0.218
Fragment RGR	14.68	<0.001	13.79	<0.001	13.43	<0.001
Total RGR	22.16	<0.001	2.920	0.091	11.81	<0.001

Shoot and root ratios were calculated as shoot/root dry weight divided by dry weight of the whole individual

highest number of roots for both fragment types, followed by *E. najas* (Fig. 2b).

The shoot DW was significantly affected by the species and fragment type (Table 1). The mid-stem fragments exhibited higher values of shoot DW, and *E. densa* had the highest values of shoot DW for both fragment types (Fig. 2c). The root DW was significantly affected only by species (Table 1). *E. densa* and *E. najas* had the highest values of root DW for both types of fragments, whereas *H. verticillata* had the lowest values (Fig. 2d).

The shoot ratio was significantly affected by the species and the fragment type (Table 1). The highest shoot ratios were observed in the mid-stem fragments. *H. verticillata* generally had the highest shoot ratios (Fig. 2e). The root ratio was affected only by the species (Table 1), and *H. verticillata* again showed higher values than the other two species (Fig. 2f).

Both the fragment RGR and the total RGR were significantly affected by the interaction between species and fragment type (Table 1). In general, higher values were obtained in the apical stems than in the mid-stem fragments. *E. densa* exhibited the lowest RGR for both fragments and its mid-stem fragment RGR differed significantly from the other species (Fig. 2g, h). The significant interaction indicates that the values for both the fragment RGR and the total RGR were lower in the mid-stem fragments of *E. densa* and *E. najas* but higher in *H. verticillata* (Figs. 2g, h).

Discussion

The results of this experiment only partially support the hypothesis that apical fragments have greater regeneration and colonization abilities and higher growth rates than mid-stem fragments. In fact, the apical stems exhibited higher growth rates than the mid-stems. However, there was no significant difference in colonization ability between fragment types because both types had, on average, the same root number, the same root DW, and the same root ratio. Moreover, mid-stem fragments exhibited a greater ability to regenerate than apical stems because they allocated more biomass to produce secondary shoots. These secondary shoots contributed to produce the highest values of shoot DW and shoot ratio. Similar results were found in previous studies with several species of aquatic macrophytes (Wu et al. 2007; Jiang et al. 2009, Riis et al. 2009). Unlike mid-stem fragments, apical fragments invested more in growth and presented higher values of fragment RGR, supporting the concept of apical dominance (Cline 1997), defined as the control exerted by the shoot apex over the outgrowth of the lateral buds in association with the investment made only in growth by the apex.

The results of the study indicated that although the species of Hydrocharitaceae have similar architecture, they differ in efficiency based on their colonization and regeneration ability. The exotic *H. verticillata* was more efficient compared with native species because it

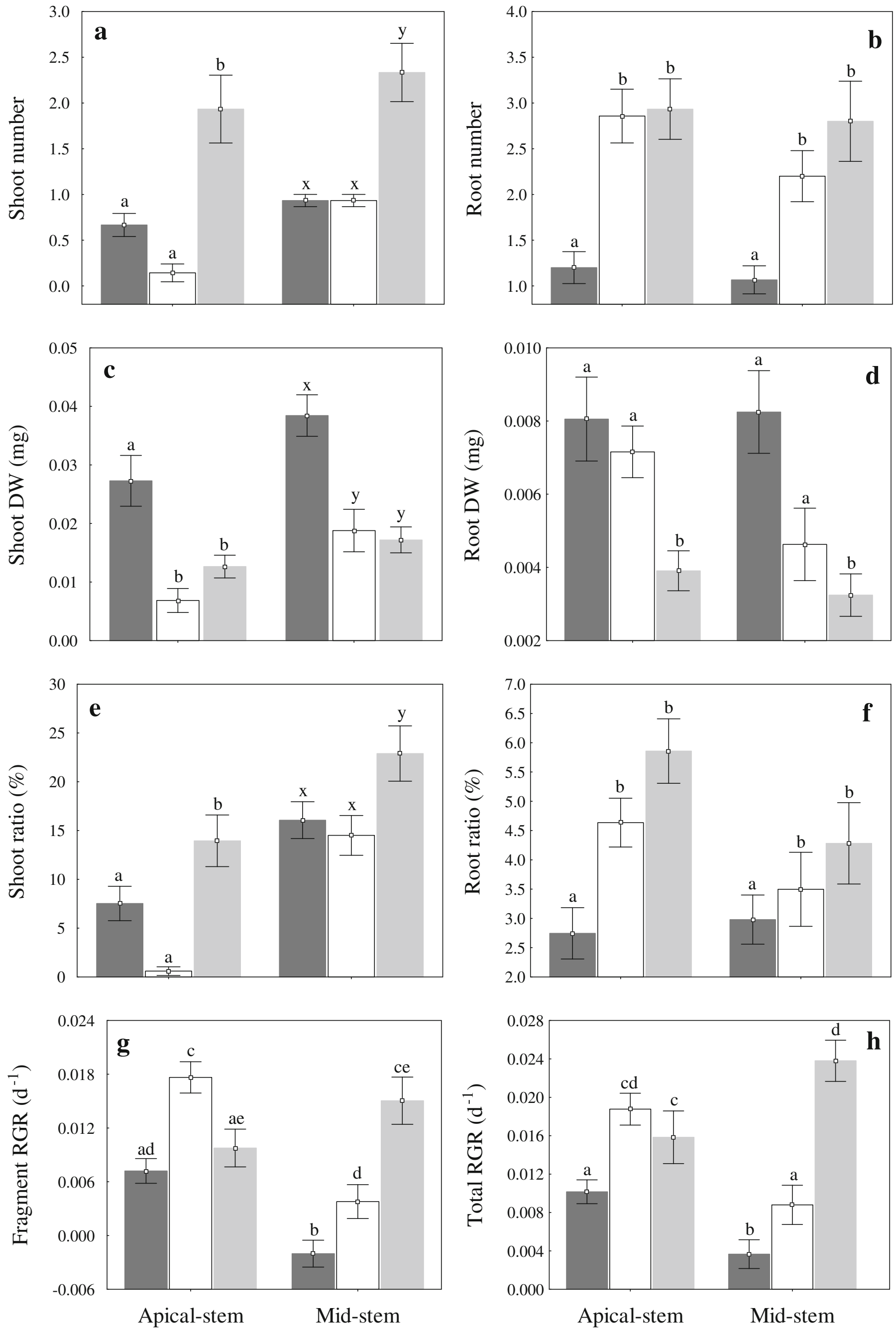

Fig. 2 Mean (± SE) shoot number (**a**), root number (**b**) shoot DW (**c**), root DW (**d**), shoot ratio (**e**), root ratio (**f**) fragment RGR (**g**), and total RGR (**h**) for species according to the type of fragment: apical stem and mid-stem. Different letters above columns indicate the significant differences between treatments at $P < 0.05$, following Fisher's test. *Black bar E. densa*, *White bar E. najas*, and *Gray bar, H. verticillata*

had higher numbers of shoots and roots for both fragment types, an additional factor that indicates its advantages over the native species and contributes to its ability to disperse and establish in novel sites. Despite the lower values of shoot and root DW shown by the exotic species, the proportions (shoot and root ratio) of these structures in relation to total biomass (including fragments) were more representative. Its adventitious roots, which are fine and filiform (Blackburn et al. 1969), gave it the lowest DW, but this morphology would not represent a disadvantage because fine elongated roots may be more efficient at nutrient uptake than thicker roots (Xie and Yu 2003; Xie et al. 2006). According to P'yankov and Ivanov (2000), the individual contribution of each plant part in relation to its total biomass can identify the primary strategy of the species. In this case, such individual contributions shown by the exotic species would indicate its investment in both survival strategies: colonization and regeneration. The native *E. densa* and *E. najas* had a lower number of sprouts and roots and lower proportions of these structures in relation to total weight but had higher values of DW. These differences are simply the consequences of the structure of these species, which have short internodes, large leaves, and thick roots (Blackburn et al. 1969).

Hydrilla verticillata deserves attention because it showed, in general, higher values of fragment RGR and total RGR. These findings clearly indicate that this species invests in the growth of its own vegetative fragments and in new structures (shoots and roots). The significant interaction between species and fragment type highlights the lower growth rates of the mid-stem of the native plants compared with *H. verticillata* (see Figs. 2g, h). Thus, our results supported our hypothesis that this exotic species has greater colonization and regeneration abilities and higher growth rates than the native species.

The simultaneous allocation of resources for colonization and regeneration and the greater regeneration ability of *H. verticillata* from mid-stem fragments compared with native plants may offer a competitive advantage to the exotic species because the fragmentation of stems certainly creates a greater number of mid-stem fragments than apices. Previous studies demonstrated a competitive advantage for *H. verticillata* over *E. densa* (Mony et al. 2007) and *E. najas* (Sousa et al. 2010) as well as other aquatic macrophytes with lower relative growth rates (Wang et al. 2008). Recent studies comparing only apical tips also demonstrated that fragments of *H. verticillata* regenerate more rapidly from desiccation stress than the native *E. densa* and *E. najas* (Silveira et al. 2009). These strategies allow this species to establish more rapidly than native species after disturbances. In fact, Sousa et al. (2010) found that after a very large flood in the Paraná River, *E. najas* and *H. verticillata* were able to regenerate at nearly the same time. However, the former species grew more rapidly than the latter.

Our experiment highlights species differences in colonization and regeneration ability. These results may be useful for better understanding the dynamics of establishment and the effects of exotic plants on native species and may also assist in management and ecological restoration. In this regard, the substantial regeneration ability of *H. verticillata*, regardless of fragment type, indicates that mechanical control techniques that result in the formation of fragments may in fact facilitate the invasion of novel sites by this species. The native plants showed poorer colonization and regeneration abilities. This result indicates that the control techniques that produce fragments would facilitate more the success of this exotic compared with the native species. However, studies in situ are needed to better determine the colonization and regeneration abilities of native and exotic species in natural ecosystems.

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