Biological interactions in the co-occurrence of *Eichhornia azurea* and free-floating macrophytes

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Introduction

Facilitation and competition are important biotic mechanisms which influence the structure of plant communities (CALLAWAY, 1995; PUGNAIRE; LUQUE, 2001; ESPIGARES et al., 2004). These interactions co-occur in a dynamic way in nature and responses of plant communities to them vary according to several factors, such as fluctuations in abiotic conditions, organisms life stage and initial population size (HOLMGREN et al., 1997; CALLAWAY; PUGNAIRE, 1999; CALLAWAY et al., 2002).

These interactions have received less attention in freshwater, compared to terrestrial ecosystems (GOPAL; GOEL, 1993; BOSCHILIA et al., 2008). Macrophytes colonize the majority of lentic and lotic ecosystems and they have an important role in the structure and functioning of these ecosystems (ESTEVES, 1998, WETZEL, 2001). The ecological importance of macrophytes is emphasized by several researchers and is related mainly to the increase of spatial heterogeneity, which provides a variety of habitats for macro and micro-organisms, fishes and aquatic birds (ESTEVES, 1998). In addition, macrophytes may...
contribute with organic matter for aquatic food webs (LOPES et al., 2007).

The majority of Brazilian inland water ecosystems are shallow, which facilitates the existence of large littoral zones, which are considered the main sites concerning primary production (ESTEVES, 1998). There are several investigations that detected positive interactions between macrophyte species in temperate regions (ESPINAR et al., 2002; FRITZ et al., 2004; ERVIN, 2005; PROFFITT et al., 2005). However, the few studies suggesting facilitation between macrophytes in tropical ecosystems employed a purely observational approach (THOMAZ et al., 2005; BOSCHILIA et al., 2008). These last authors suggested, based on high values of co-occurrences (54-80%), that facilitation between Eichhornia azurea Kunth (1843) and several free-floating species may be a common phenomenon.

Because of the importance of macrophytes to the structure and functioning of aquatic ecosystems, the main mechanisms regulating the structure of this community becomes of interest for aquatic ecology. In this work we investigated the hypothesis that E. azurea facilitates free-floating species. Facilitation would occur basically through changing the micro-habitat characteristics, such as reducing water velocity and wind disturbance, and by creating a favorable environment in terms of temperature, light and nutrients. This hypothesis was based on the high rates of co-occurrence between E. azurea and free-floating species, demonstrated by others (MURPHY et al., 2003; THOMAZ et al., 2005; BOSCHILIA et al., 2008).

Material and methods

This study was conducted in three backwaters (lakes permanently connected to the Paraná river channel), in the upper Paraná river floodplain: Leopoldo (22°45’24”S; 53°16’7.98”W), Pau Veio (22°44’50.76”S; 53°15’11.16”W) and Osni (22°47’6.36”S; 53°20’13.67”O) (Figure 1).

Figure 1. Map showing the position of the three back-waters investigated.
Based on previous data about co-occurrences between *E. azurea* and free-floating species obtained in this floodplain (BOSCHILIA et al., 2008), we chose four species: *Eichhornia crassipes* (Mart.) Solms, 1883, *Salvinia minima* Baker, 1886, *Salvinia auriculata* Aubl., 1775, and *Limnobium laevisatum* (Humb. & Bonpl. ex Willd.) (Heine, 1968). These species were chosen because they were highly abundant and had the highest rates of association with *E. azurea*.

To test the hypothesis that *E. azurea* improves the abiotic conditions in its micro-habitat, getting it favorable to the development of free-floating species, we carried experiments in situ, with four replicates per backwater (N = 12), between October and December 2007. Free floating species were left to grow, individually, in the presence of *E. azurea* (inside the littoral zone) and in its absence (in the limnetic region, close to the littoral zone, but outside the stands). Plants remained inside 0.5 x 0.5 m floating quadrats. A net was added in the bottom of the quadrats to ensure that plants did not leave the quadrats if wind or current disturbance occurred (plants did not remain inside the quadrats in preliminary tests which did not use nets). Each replicate contained a quadrat where a specific free floating species grew in the presence or absence of *E. azurea*.

Free floating plants were collected in floodplain lakes and taken to the laboratory where they were weighed (fresh weight). Following the biomass determination, they were immediately taken to the backwaters, where they remained inside the quadrats for 11 days (*E. crassipes* and *S. minima*) or 17 days (*S. auriculata* and *L. laevisatum*). The fresh and dry weights were obtained after six days in an oven at 105°C. The obtained dry weight: fresh weigh ratios were used to estimate the initial dry weigh.

The relative growth rates (RGR) were estimated as \[ \text{RGR} = (\ln DW_{t} - \ln DW_{0}) t^{-1} \], where \( DW_{t} \) and \( DW_{0} \) are final and initial biomass, respectively, and \( t \) is the time in days.

The relative interaction index (RII; ARMAS et al., 2004) was used to quantify the effects of the presence of *E. azurea* on the growth (in biomass) of the free-floating macrophytes. This index was estimated as \[ \text{RII} = (B_{w} - B_{0})/(B_{w} + B_{0}) \], where \( B_{w} \) represents the biomass of the free-floating species growing together with *E. azurea* and \( B_{0} \) the biomass of the free-floating species growing in the absence of *E. azurea*. Results from this index varies from -1 (competition is the most important mechanism) to +1 (facilitation is the most important mechanism).

The following abiotic factors were measured inside and outside *E. azurea* stands: temperature (digital thermometer), pH and conductivity (digital field equipments; Digimed), photosynthetic active radiation (quanta-meter LiCor), total phosphorus and nitrogen. These variables were measured in the beginning and at the end of the experiments, in all quadrats. Light was obtained in several points inside the stands, because *E. azurea* leaves create patches with different light intensities. For total N and total P, samples were preserved in a freezer (at -20°C) until determination (spectrophotometer; Mackereth et al. 1978) for nitrogen and Giné et al. (1980) for phosphorus.

To test for differences of RGR and abiotic variables obtained inside and outside stands of *E. azurea*, we used a one-way Anova. The significance of the RII values was tested with a Test \( t \) for one sample. Both analyses were carried with the software Statistica 7.

**Results**

In general, the lowest RGR values were recorded for *S. minima* and *E. crassipes* (ca. 0 - 0.04 \( d^{-1} \) and 0.001 - 0.05 \( d^{-1} \), respectively), and the highest values for *L. laevisatum* and *S. auriculata* (0.01 - 0.16 \( d^{-1} \) and 0.04 - 0.13 \( d^{-1} \), respectively). However, RGR values did not differ significantly when free-floating macrophytes grew at *E. azurea* presence or absence (Anova: *E. crassipes*: \( df = 1, F = 0.091, p = 0.764; \) *S. minima*: \( df = 1, F = 3.231, p = 0.094; \) *S. auriculata*: \( df = 1, F = 0.049, p = 0.945; \) *L. laevisatum*: \( df = 1, F = 2.06, p = 0.170 \) (Figure 2).

Mean values of RII for *E. crassipes*, *S. minima* and *S. auriculata* did not differ significantly from zero (*E. crassipes*: \( df = 12, t = -0.46, p = 0.65; \) *S. minima*: \( df = 8, t = -1.70, p = 0.13 \) and *S. auriculata*: \( df = 11, t = 1.33, p = 0.21 \)). It indicates that the interaction between these species and *E. azurea* was neutral (i.e., neither competition nor facilitation occurred). However, RII was negative and significantly different from zero for *L. laevisatum* (\( df = 9, t = -3.49, p = 0.007 \)) (Figure 3).

The abiotic factors measured inside and outside the littoral zone colonized by *E. azurea* did not differ significantly (Table 1). These results indicate that the habitat in which the floating species grow did not contribute to explain possible differences of their RGR growing together to or separated from *E. azurea*.
Discussion

Observations made on an investigation that assessed patterns of macrophyte co-occurrences carried out in the upper Paraná river floodplain showed significant greater rates of occurrences of *E. azurea* with several free-floating species (BOSCHILIA et al., 2008). These results indicate that *E. azurea*, which is dominant in a variety of habitats in this floodplain and in several Neotropical freshwater ecosystems, could facilitate the occurrence of other, smaller free-floating plants (THOMAZ et al., 2005; BOSCHILIA et al., 2008). *E. azurea* could have a physical role for anchoring of the free-floating species and furnishing a favorable habitat. A possible mechanism related to this potential facilitation could be a decrease of water velocity and protection from wind, inside the stands of *E. azurea*, what could favor the colonization and development of the free-floating species. In fact, these species are affected by these disturbances in more exposed (limnetic) areas (CAMARGO et al., 2003). In addition, the existence of a micro-habitat below *E. azurea* leaves and stems could create a favorable environment in terms of light, temperature and nutrients.
During our experiments, we noted that when free-floating plants were left in the pelagic zone, they dispersed and reached quickly the littoral zones, even when wind was not so evident (personal observation). Such observations reinforce the physical role of *E. azurea* in anchoring free-floating plants. However, results of physical and chemical parameters measured inside *E. azurea* stands did not differ from those measured outside its stands and thus, there is no evidence that this species facilitates the free-floating ones through changes in the physical-chemic habitat. Accordingly, there were no differences of RGR of the four species growing inside and outside *E. azurea* stands (Figure 2).

Similar values of the limnological variables in the presence and absence of *E. azurea* may be associated with the hydrological dynamic inside these shallow lakes, which is directly influenced by constant exchanges with the Paraná river main channel. Thus, we suppose that the exchanges of water between littoral and limnetic zones prevent great differences between these habitats. However, these results are typical of low water periods and from the specific lakes analyzed. In another study which investigated 36 lagoons and canals in the upper Paraná river floodplain it was shown that littoral differed significantly from limnetic regions concerning several abiotic factors (ROCHA, 2003). In addition, the method employed in our investigation may have been contributed to these results because the net used under our quadrats to avoid plants to be dislodged by wind and current may have created a different micro-habitat inside quadrats. Thus, although we did not find any evidence of facilitation mediated by changes in the physico-chemistry medium, this mechanism can not be discarded in other, more isolated floodplain lakes, neither in other experiments employing different protocols (e.g., without protection nets under the quadrats).

Concerning RII, which is an indicative of biotic interactions between pairs of species, there was no indication of any effect of *E. azurea* on *E. crassipes*, *S. minima* and *S. auriculata* and thus, there is no indication of facilitation. Thus, these results corroborate what was found with the RGR of these three species. On the other hand, the negative (and significantly different from zero) value of RII found for *L. laevigatum* suggests that this species competes with *E. azurea*.

According to Bertness and Callaway (1994), the importance of facilitation increases in habitats subject to elevated abiotic stresses, because neighborhood would protect organisms against harsh abiotic conditions. Unfavorable habitats may restrict the ability of plants to acquire resources and thus, any improvement of the physical habitat by neighbors favors the growth of the facilitated species (CALLAWAY; WALKER, 1997). On the other hand, competition would be a more important mechanism under less stressed conditions, where resources can be quickly obtained (BERTNESS; CALLAWAY, 1994).

Investigations carried out in several biomes, such as deserts (CALLAWAY et al., 1991; HOLZAPFEL; MAHALL, 1999) “savannas”, temperate and tropical forests (CALLAWAY et al., 1996; CALLAWAY; WALKER, 1997; BAUMEISTER; CALLAWAY, 2006), shrub and Mediterranean vegetation (GÓMEZ-APARICIO et al., 2004), salt-marshes (BERTNESS; LEONARD, 1997) and grasslands (GREENLEE; CALLAWAY, 1996), showed that both negative and positive interactions may occur in plant communities, but the importance of each depends largely on the environmental stress (KELLMAN; KADING, 1992; CALLAWAY; WALKER, 1997). Greenlee and Callaway (1996), for example, showed that competition was the main interaction between grasses and *Lesquerella carinata* in Montana grasslands (USA) during rainy years while facilitation predominated during dry years. Kikvidze et al. (2001) suggested that in sub-alpine fields the competitive effects on biomass production were equilibrated and even altered by facilitation. The equilibrium between such interactions may increase species co-existence. Taking these considerations into account, we state that our results can be viewed as limited to the type of habitat and period under which our study was carried out and its consistence has to be checked in other habitats and hydrological periods.

In summary, despite several suggestions that *E. azurea* facilitates free-floating species increasing protection against wind and flow disturbances (e.g., THOMAZ et al., 2005; BOSCHILIA et al., 2008), our experiments did not indicate facilitation to occur, at least in the sense of an increasing in plant growth (measured as RGR). In addition, an improvement of micro-habitats by presence of *E. azurea* was not found. As a result, RGR of the four free-floating species was not increased in the presence of *E. azurea*. The absence of facilitation was confirmed by RII results. Lack of facilitation may be expected in habitats where abiotic stress is not severe (BERTNESS; CALLAWAY, 1994), as is probably the case of the investigated lakes. However, although the tested free-floating species did not grow faster together with *E. azurea*, they did not colonize more exposed sites in the floodplain lakes and the role of this last species in retaining this plants ("anchoring")
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