



## ARRIVAL ORDER AND AQUATIC MACROPHYTE COMMUNITY ORGANIZATION IN A TROPICAL ESTUARY

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**Abstract:** The arrival order in species colonization may be important to the communities' organization of perennial plants along environmental gradients. In estuarine gradients, the competitive exclusion may have great importance to species zonation regardless of their arrival order. However, priority effect has also been considered, since founder species may remain in the environment even with the later arrival of species of greater competitive ability. We evaluated whether the arrival order and priority effect influence the interspecific interaction between adult individuals (early-colonizing individuals) and young individuals (later-arriving individuals) of the perennial emergent estuarine aquatic macrophytes *Spartina alterniflora* (S) and *Crinum americanum* (C). We conducted a laboratory experiment with four cultures (monocultures of early-colonizing individuals of S; monocultures of early-colonizing individuals of C; cultures of early-colonizing individuals of S with introduction of later-arriving individuals of C; and cultures of early-colonizing individuals of C with introduction of later-arriving individuals of S) and two sediment types (lower and middle estuary sediment: salinity 32 and 20, respectively). We hypothesized that, due to the priority effect, in both sediment types (i) the later-arriving species would not establish itself in adult early-colonizing vegetation and (ii) the later-arriving species would not limit the growth of the early-colonizing species. Our results corroborate our hypotheses as the later-arriving individuals of *S. alterniflora* and *C. americanum* did not grow up and establish themselves in the heterospecific cultures of early-colonizing individuals in both sediment types. Furthermore, the growth of the early-colonizing individuals was not limited by the presence of the later-arriving individuals. We conclude that the arrival order and priority effect can influence the interspecific competition, communities' organization and spatial distribution of aquatic macrophytes in tropical estuaries.

**Keywords:** colonization; competitive exclusion; *Crinum americanum*; environmental gradient; *Spartina alterniflora*.

### INTRODUCTION

The interspecific competition and abiotic stress are important factors for the community organization and spatial distribution of aquatic macrophytes in estuaries (Crain *et al.* 2004, Engels

& Jensen 2010). The importance of these factors may vary along the environmental gradient (Greenwood & Macfarlane 2009) and both of them may act on its extremes (Nunes & Camargo 2018). However, the different historical sequences of species arrival in colonization may also have

an influence on the communities' organization along environmental gradients (Chase 2003, Kardol *et al.* 2013).

The arrival order may have great importance in perennial plant communities, that is, when founder species (early-colonizing species) remain in the environment and are not excluded in a succession process by species of later colonization (later-arriving species) (Körner *et al.* 2008, Viana *et al.* 2016). In this context, when the early-colonizing species has an advantage over the later-arriving one there is the ecological priority effect, as called by Fukami (2015). This priority effect can allow species that arrive first to be able to persist in the environment even when later-arriving species are more competitive (Sarneel *et al.* 2016) with long-term dominance or monopolization (De Meester *et al.* 2016). If the species have similar resource requirements the priority effect tends to be inhibitory, that is, the early-colonizing species negatively affects the colonization and growth of the later-arriving species (Fukami 2015). However, if these species are equally good founders and competitors under certain environmental conditions they may even coexist in the same area, resulting in niches overlapping (Bockelmann & Neuhaus 1999) or competition for dominance (Angelini *et al.* 2011).

To date there is a limitation of ecological studies focusing on arrival order and priority effect (García-Girón *et al.* 2021). Most of them have addressed communities of microbes (Zee & Fukami 2018), vertebrates (Stroud *et al.* 2019) and terrestrial plants (Sikes *et al.* 2016) in a context of exotic species and bioinvasion (García-Girón *et al.* 2021). In freshwater ecosystem, some studies have already studied the assembly history and composition (Fried-Petersen *et al.* 2020, Ge *et al.* 2021), but there are still few researches on species priority effects and their interaction with environmental variation (García-Girón *et al.* 2021), especially on aquatic plant communities. These communities can greatly contribute to these ecological perspectives, as they are composed by organisms with vegetative regeneration, capacity of emerging from propagule banks and colonization by immigrants arriving from the regional species pool (Viana *et al.* 2016).

Aquatic macrophyte communities of native species in estuaries are considered perennial

and founder-controlled, that is, the species are good colonizers and have similar competitive abilities (Barrat-Segretain 1996, Emery *et al.* 2001). However, the ability of plant species to suppress the growth of other ones is not always associated with their ability to resist suppression (Goldberg & Fleetwood 1987). These competitive abilities and establishment success in new areas may vary among various aspects with, the plant life stage (Shipley *et al.* 1989, Callaway 1995, Rojas-Sandoval & Meléndez-Ackerman 2012) and size of the colonizer propagules (Bickel 2017). All these factors are also related to the evolutionary history of taxonomic entities. The vegetative propagules of aquatic macrophytes, including their clonal shoots, play a major role in dispersion, colonization and establishment in new areas (Barrat-Segretain 1996, Capers 2003). However, these young individuals may not be able to establish themselves in heterospecific adult vegetation (Paradis *et al.* 2014), since conspecific adult individuals tend to form dense clonal stands and vegetation units, limiting the occurrence of heterospecific propagules (Bertness & Ellison 1987, Schwarz *et al.* 2015).

In the Itanhaém River Estuary (Southeastern Brazil) the most abundant aquatic macrophyte species present are *Spartina alterniflora* Loisel. (Poaceae) and *Crinum americanum* L. (Amaryllidaceae). In this estuary, there are monospecific stands of *S. alterniflora* in the lower estuary and mixed stands of both species in the middle estuary. However, *C. americanum* is absent from the lower estuary area. Prior to this study, we carried out a laboratory experiment to evaluate these species growth and competition at lower, middle and upper estuary sediment. We found that *C. americanum* grew up in the lower estuary sediment (high salinity level) both in monoculture and in the presence of *S. alterniflora* (Nunes & Camargo 2018). Therefore, salinity does not impede the growth of *C. americanum* (Nunes & Camargo 2018, Nunes & Camargo 2020). For explaining the absence of *C. americanum* from the lower estuary of the Itanhaém River we presume now that *S. alterniflora* firstly colonized the lower estuary area. Some mechanisms can also avoid the interspecific competition and allow their coexistence in the middle estuary. Thus, the historical sequences of these species colonization

may also have been important for the community organization and zonation of *S. alterniflora* and *C. americanum* in this estuary.

Here we evaluated whether the arrival order and priority effect influence on the interspecific interaction between adult individuals (early-colonizing individuals) and young individuals (later-arriving individuals) of *S. alterniflora* and *C. americanum* in the lower and middle estuary conditions. We hypothesized that, due to the priority effect, (i) the later-arriving species would not establish itself in the adult early-colonizing vegetation and (ii) the later-arriving species would not limit the growth of the early-colonizing species regardless of the environmental conditions (sediment characteristics). To test these hypotheses we conducted a laboratory experiment evaluating the species growth in different arrival order cultures and in two sediment types (lower and middle estuary conditions).

## MATERIAL AND METHODS

### Study area

The Itanhaém River Estuary (Southeastern Brazil) has a relatively constant water flow over the year, seawater influence for about 10 km (downstream-upstream) and sediment salinity gradient from 0 to 32 ppt. The maximum tidal amplitude is 1.5 m (micro-tidal regime) with two complete cycles of low tide and high tide per lunar day (24 h and 50 min) (Tessler *et al.* 2006). The region is marked by small seasonal variations in climatic characteristics, mainly because the rains are well distributed over the year and there is no dry season (Monteiro 1973). The average temperature in the summer months is 27.9 °C and in the winter months it is 21.1 °C. The average annual rainfall is 2,260 mm with the average month rainfall in summer of 256.4 mm and in winter of 92.7 mm (Embrapa 2015).

In the lower Itanhaém River Estuary the emergent aquatic macrophyte *S. alterniflora* is the only herbaceous species present. This species forms monospecific stands associated to the mangrove forest where water and sediment have greater salinity levels (Nunes & Camargo 2018). In the middle estuary *S. alterniflora* and *C. americanum* coexist along the depth gradient in marginal stands of aquatic macrophytes, although

*S. alterniflora* populations are denser at greater depths (Nunes & Camargo 2020).

### Species description

The species *S. alterniflora* and *C. americanum* are emergent, clonal, rhizomatous and perennial macrophytes and are considered native to the American Atlantic coast. These species grow up from rhizomes through clonal emergencies above the sediment. These clonal emergencies can be identified as “individuals”. The individuals of *S. alterniflora* form stems and those of *C. americanum* have leaves that are distributed in rosettes (Wang *et al.* 2010, Nunes & Camargo 2017). *S. alterniflora* is a halophyte species adapted to a wide range of salinity (Céccoli *et al.* 2015). This halophyte occurs in patches in tropical estuaries in the short frontal fringes of mangroves (Schaeffer-Novelli *et al.* 1990) and in subtropical and temperate estuaries it forms salt marshes in extensive intertidal plains (Costa *et al.* 2003, Wieski & Pennings 2014). *C. americanum* is a non-halophyte tolerant to low salinity levels (Meerow *et al.* 2003, Ribeiro *et al.* 2011). In Brazil, it is commonly found in brackish and oligohaline habitats in coastal ecosystems (Nunes & Camargo 2018) and also in freshwater environments in Pantanal, Cerrado (tropical savannah) and Amazon (Dutilh & Oliveira 2020).

### Experiment

We conducted a manipulative experiment with two factors (4 cultures x 2 sediment types) with five experimental units each treatment in a greenhouse lasting 230 days to evaluate the growth and interspecific interaction of adult individuals (called here as “early-colonizing individuals”) and young individuals (called here as “later-arriving individuals”) of *S. alterniflora* and *C. americanum* in the lower and middle estuary sediment of the Itanhaém River (sediment types) (Figure 1).

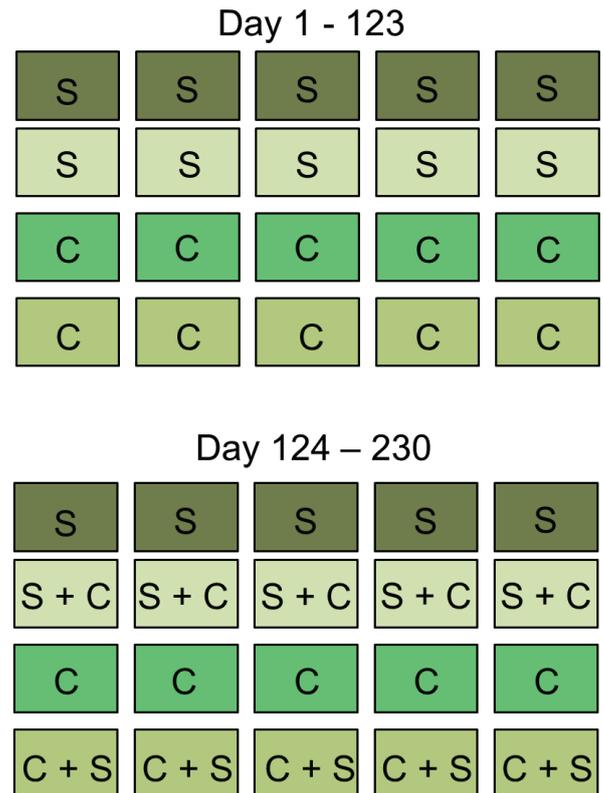
The experiment was carried out from September 2017 to May 2018 in an unheated “umbrella-type” greenhouse at the São Paulo State University (city of Rio Claro). During the experiment the average maximum temperature was 30.7 °C and the average minimum temperature was 18.0 °C. The average maximum relative humidity was 90.1% and the average minimum relative humidity was

54.6%. These data was obtained from the Rio Claro Meteorological Station located close to the greenhouse (about 700 m). The greenhouse conditions meet the environmental conditions in the Itanhaém River Estuary, as the climatic conditions in Rio Claro during the period (Spring/Summer) in which the experiment was carried out are not very different from those in Itanhaém.

Although *S. alterniflora* and *C. americanum* are rhizomatous species, the growth form of their shoots and roots enables the identification of ramets (*S. alterniflora* stems and *C. americanum* rosettes) (Nunes & Camargo 2017). Thus, we considered each ramet as an “individual”. We collected ten early-colonizing individuals of *S. alterniflora* and *C. americanum* similar size (adult individuals from 0.50 to 0.70 m) per experimental unit in their mixed stands in the Itanhaém River Estuary. The early-colonizing individuals were planted in plastic boxes (experimental units) with a volume of 26 liters and an area of 0.13 m<sup>2</sup>.

From day 1 to day 123 of the experiment we maintained 20 experimental units of monoculture of *S. alterniflora* (10 in each sediment type) and 20 experimental units of monoculture of *C. americanum* (10 in each sediment type). On day 124 of the experiment in 10 experimental units of each species monocultures and in each sediment type we introduced five young individuals of the other species. That is, in the cultures of early-colonizing individuals of *S. alterniflora* we introduced five later-arriving individuals of *C. americanum*, and in the cultures of early-colonizing individuals of *C. americanum* we introduced five later-arriving individuals of *S. alterniflora*. Thus, from day 124 of the experiment, 20 mixed cultures (10 in each sediment type) were started and 20 monospecific cultures (10 in each sediment type) were maintained (Figure 1). These later-arriving individuals of *S. alterniflora* and *C. americanum* were also collected in the mixed stands in the Itanhaém River Estuary and had similar sizes (approximately 0.30 m) and appearances (green and healthy leaves).

We collected the sediment in the lower and middle estuary in the Itanhaém River close to the macrophyte stands. The sediment from each



**Figure 1.** Experimental design scheme used with each sediment type (lower and middle estuary sediment) totaling 40 experimental units. Cultures: Monocultures: monocultures of the early-colonizing individuals of *Spartina alterniflora* (S), monocultures of the early-colonizing individuals of *Crinum americanum* (C); Mixed cultures: cultures of the early-colonizing individuals of *S. alterniflora* with introduction of the later-arriving individuals of *C. americanum* (S + C), cultures of the early-colonizing individuals of *C. americanum* early-colonizing individuals with introduction of later-arriving individuals of *S. alterniflora* (C + S) on day 124.

stand was collected with a shovel to a maximum depth of about 20 cm, homogenized and disposed in the experimental units. Before planting, 50 ml samples of sediment were taken from each experimental unit to determine the salinity and the contents of total nitrogen (TN) (Allen *et al.* 1974) and total phosphorus (TP) (Golterman *et al.* 1978). The average values (standard deviation) of salinity (ppt), TN (% dry mass) and TP (% dry mass) of the lower estuary sediment were, respectively, 32.1 (1.9), 0.167 (0.057) and 0.014 (0.001); and the middle estuary sediment were, respectively, 23.9 (1.6), 0.247 (0.165) and 0.013 (0.002). We tested for significant differences

( $p < 0.05$ ) in these variables between the lower and middle estuary sediment ( $N = 20$ ). For that, we applied the non-parametric Mann-Whitney using the GraphPad Prism® 5.0 software (GPW5-066646-RCG7389) (GraphPad Software 2007). The sediments of the two estuarine portions were significantly different only in relation to the salinity level (Appendix 1). After planting, the experimental units were constantly watered with tap water to maintain the approximate level of 2.0 cm above the sediment surface. The greenhouse is supplied by water from an artesian well; therefore this tap water does not contain chlorine or fluorine.

To estimate the aquatic macrophyte aboveground biomass we used the non-destructive method and its regression equations developed by Nunes & Camargo (2017). These authors generated these equations through significant simple linear regressions ( $p < 0.05$ ) between individual height and aboveground dry mass of *S. alterniflora* and *C. americanum* (Equations 1 and 2). We performed measurements of the height of the early-colonizing individuals on days 1, 63, 81, 104, 124, 153, 183, 203 and 230 of the experiment. On days 124, 153, 183, 203 and 230 of the experiment we also measured the height of the later-arriving individuals introduced into the cultures. Using data on the individual heights we calculated through equations (Equation 1 and Equation 2) the aboveground biomass of each species in each experimental unit over time.

$$\begin{aligned} S. \textit{alterniflora} \text{ aboveground dry mass} = \\ 10.64 * (\text{individual height} - 0.5451 \text{ m}) \quad (1) \\ (R^2 = 0.8279) \end{aligned}$$

$$\begin{aligned} C. \textit{americanum} \text{ aboveground dry mass} = \\ 16.03 * (\text{individual height (m)} - 6.0077) \quad (2) \\ (R^2 = 0.7532) \end{aligned}$$

To estimate the aquatic macrophyte belowground biomass (roots and rhizomes) we used the non-destructive method and its regression equations also used by Nunes & Camargo (2018) and Nunes & Camargo (2020). These authors applied simple linear regressions ( $p < 0.05$ ) between belowground volume and belowground dry mass of the individuals of *S. alterniflora* and *C. americanum* (Equation 3 and Equation 4). The root and rhizome volumes were obtained by measuring water displacement in a

graduated recipient. We performed measurements of belowground volume before planting the aquatic macrophytes (Day 1) and after the plant removal at the ending of the experiment (Day 230).

$$\begin{aligned} S. \textit{alterniflora} \text{ belowground dry mass} = \\ 0.0932 * (\text{belowground volume (mL)} + 0.3244) \quad (3) \\ (R^2 = 0.7965) \end{aligned}$$

$$\begin{aligned} C. \textit{americanum} \text{ belowground dry mass} = \\ 0.0504 * (\text{belowground volume (mL)} + 1.241) \quad (4) \\ (R^2 = 0.9315) \end{aligned}$$

We evaluated the difference between the final and initial biomass (total biomass, aboveground biomass and belowground biomass) (respectively,  $\Delta TB$ ,  $\Delta AB$  and  $\Delta BB$ ) of the early-colonizing and later-arriving individuals in the experiment (Equation 5).

$$\Delta B_{ax} = Bf_{ax} - Bi_{ax} \quad (5)$$

, where  $\Delta B_{ax}$  = difference of biomass (grams of dry mass per square meter =  $\text{g m}^{-2}$ ) of the species *a* in the treatment *x*;  $Bf_{ax}$  = final biomass ( $\text{g m}^{-2}$ ) of the species *a* in the treatment *x*; and  $Bi_{ax}$  = initial biomass ( $\text{g m}^{-2}$ ) of the species *a* in the treatment *x*.

To the belowground biomass we calculated its difference between the first day (day 1 for the early-colonizing individuals and day 124 for the later-arriving individuals) and last day (day 230) of the experiment. However, at some treatments the plants had an initial reduction in aboveground biomass probably due to transport and replanting stress. Thus, to the aboveground biomass we considered the biomass difference between the lowest value of initial biomass (day 1 or 63) and the greatest value of final biomass (day 203 or 230) because they varied among the replicates. We also calculated the relative growth rate of total biomass (RGR-TB) of the later-arriving individuals introduced into the cultures (Equation 6).

$$RGR_{ax} = \frac{\ln B_{2ax} - \ln B_{1ax}}{(t_2 - t_1)} \quad (6)$$

, where: RGR = relative growth rate ( $\text{g m}^{-2} \text{d}^{-1}$ ) of the species *a* in the treatment *x*;  $\ln B_{2ax}$  = ln of biomass of the species *a* in the treatment *x* at time 2;  $\ln B_{1ax}$  = ln of biomass of the species *a* in the treatment *x* at time 1;  $t_2 - t_1$  = time variation (days).

### Data analysis

We tested for significant differences ( $p < 0.05$ ) of

the  $\Delta TB$ ,  $\Delta AB$  and  $\Delta BB$  of the early-colonizing individuals of *S. alterniflora* and *C. americanum*; and  $\Delta TB$ ,  $\Delta AB$ ,  $\Delta BB$  and RGR of the later-arriving individuals of both species in the different cultures (arrival orders) and sediment types (difference of biomass and RGR = response variable; culture and sediment type = categorical predictors). Previously, we evaluated and confirmed that the conditions of data normality and homoscedasticity had been met. Then, we applied the two-way analysis of variance (Factorial ANOVA) and the Tukey's test a posteriori. We applied the t-test for the  $\Delta TB$ ,  $\Delta AB$  and  $\Delta BB$  and RGR-TB of the later-arriving individuals of *S. alterniflora*, and the  $\Delta AB$  and RGR-TB of the later-arriving individuals of *C. americanum*. For the  $\Delta TB$  and  $\Delta BB$  of *C. americanum* we applied the non-parametric Mann-Whitney test.

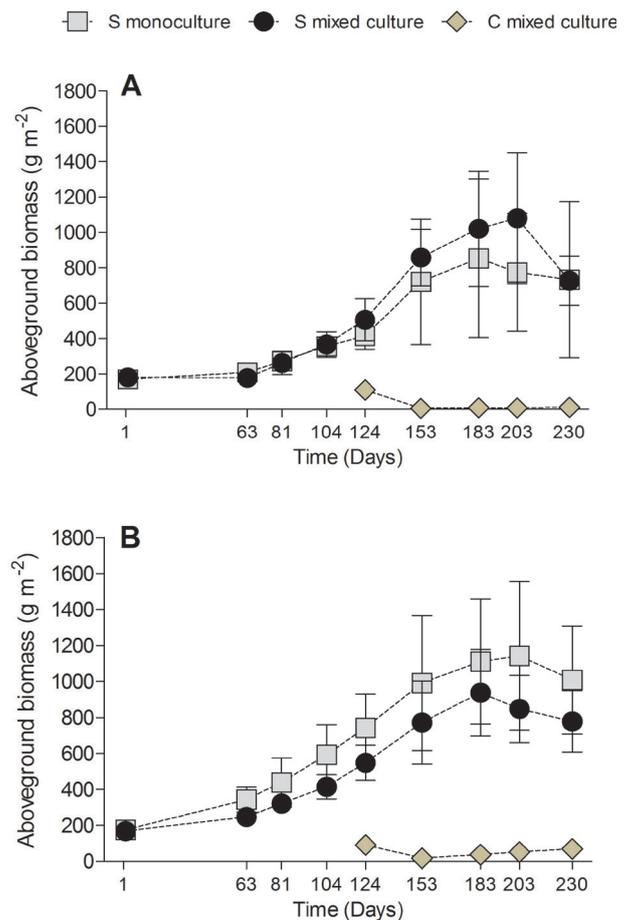
We applied these statistical analyses (ANOVA, t-test and Mann-Whitney test) using the Statistica 7.1 software (AX505B150718FA) (StatSoft 2005). We elaborated the growth curves of aboveground biomass and the graphs of difference of biomass using the GraphPad Prism 5.0 software (GPW5-066646-RCG7389) (GraphPad Software 2007).

## RESULTS

The growth curves of aboveground biomass of the early-colonizing individuals of *S. alterniflora* and *C. americanum* showed that these species grew up in the two sediment types both in monocultures and in mixed cultures in which heterospecific later-arriving individuals were introduced (Figure 2; Figure 3). However, the early-colonizing individuals of *C. americanum* showed a reduction in aboveground biomass during the acclimatization period in both sediment types, mainly in the lower estuary sediment (Figure 3).

The growth of the early-colonizing individuals of *S. alterniflora* and *C. americanum* were not limited by the presence of the later-arriving individuals of *C. americanum* and *S. alterniflora*, respectively, regardless of sediment type (Figure 4; Figure 5; Appendix 2).

The later-arriving individuals of *S. alterniflora* and *C. americanum* introduced into the heterospecific cultures of early-colonizing individuals suffered reduction in biomass in both sediment types throughout the experiment

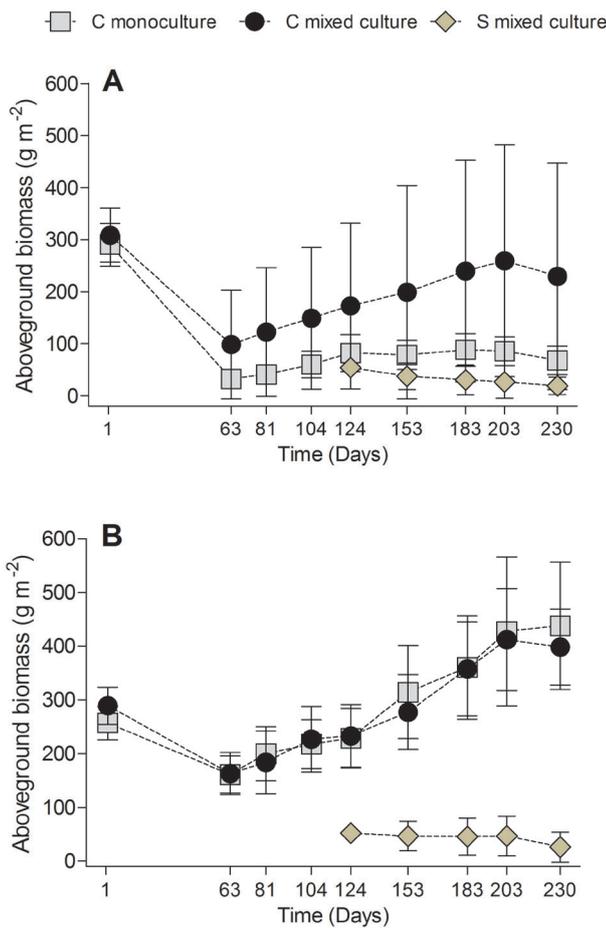


**Figure 2.** Average values and standard deviation of the aboveground biomass of the early-colonizing individuals of *Spartina alterniflora* (S) in monoculture (gray squares) and in mixed cultures (black circles), and the aboveground biomass of the later-arriving individuals of *Crinum americanum* (C) after introduction (day 124) in the early-colonizing individual cultures of *S. alterniflora* (brown diamonds) in the experiment in the lower (A) and middle estuary sediment (B).

(Figure 2; Figure 3; Appendix 3). Both species had negative values of  $\Delta TB$ ,  $\Delta AB$  and  $\Delta BB$  of their later-arriving individuals (except *S. alterniflora*  $\Delta BB$  in the middle estuary sediment) (Table 1).

## DISCUSSION

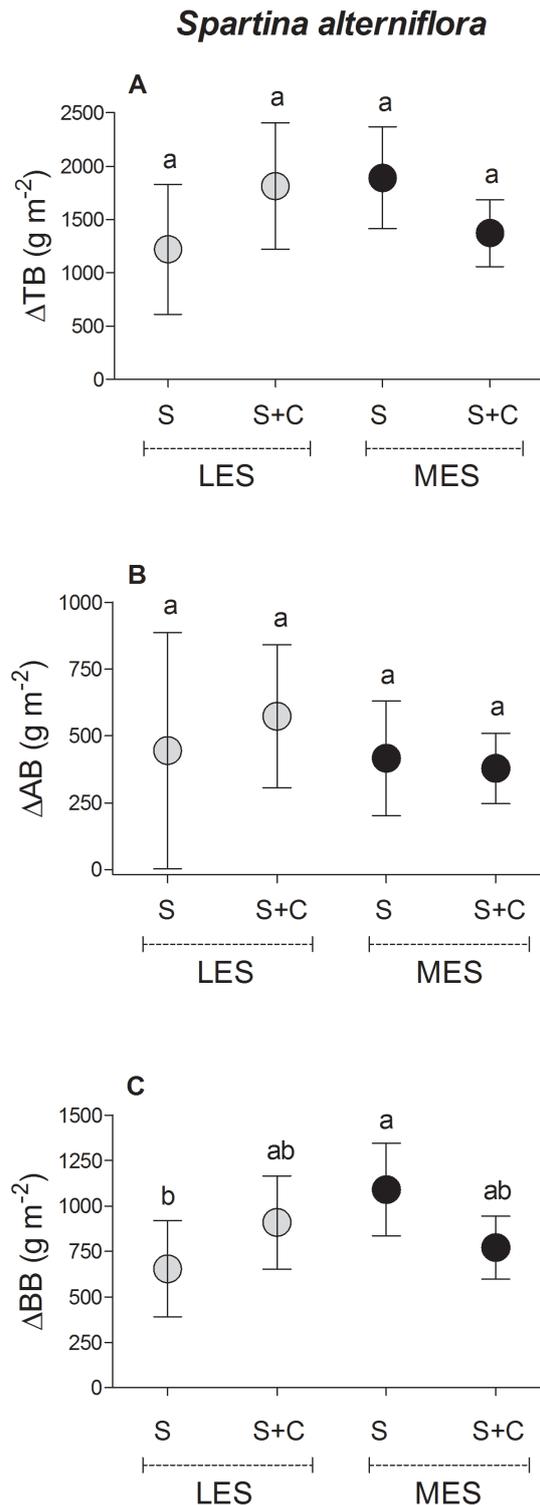
The results of our experiment corroborated our hypotheses. In both sediment types the later-arriving individuals of *S. alterniflora* and *C. americanum* did not grow up and establish themselves in the heterospecific cultures of early-colonizing individuals. In addition, the growth of the early-colonizing individuals was not limited by the presence of the later-arriving individuals.



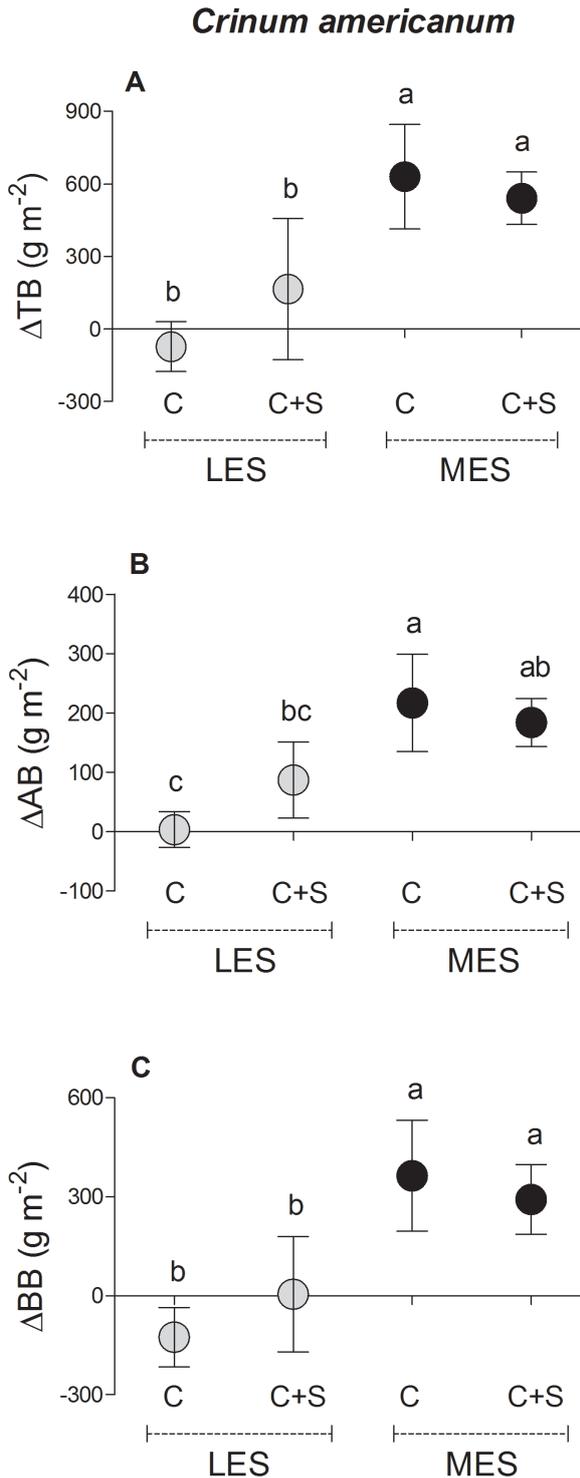
**Figure 3.** Average values and standard deviation of the aboveground biomass of the early-colonizing individuals of *Crinum americanum* (C) in monoculture (gray squares) and in mixed cultures (black circles), and the aboveground biomass of the later-arriving individuals of *Spartina alterniflora* (S) after introduction (day 124) in the early-colonizing individual cultures of *C. americanum* (brown diamonds) in the experiment in the lower (A) and middle estuary sediment (B).

These results indicate that the arrival order of these species and their priority effect influence the interaction between young and adult individuals of *S. alterniflora* and *C. americanum* in the lower and middle estuary sediment.

In the Itanhaém River Estuary, *S. alterniflora* forms monospecific stands in the lower estuary, and *S. alterniflora* and *C. americanum* form mixed stands in the middle estuary (Nunes & Camargo 2018). The halophyte *S. alterniflora* is considered a stress-tolerant founder in lower estuarine areas due to its ability to colonize both newly deposited sediments in the frontal fringes of tropical mangroves (Schaeffer-Novelli *et al.* 1990) as well as margins in moderate erosion processes



**Figure 4.** Average values and standard deviation of the difference of total biomass ( $\Delta TB$ ), aboveground biomass ( $\Delta AB$ ) and belowground biomass ( $\Delta BB$ ) (g m<sup>-2</sup>) of the early-colonizing individuals of *Spartina alterniflora* in monoculture (S) and mixed cultures (S + C) in the lower (LES) and middle estuary sediment (MES). The graphs have different scales. Distinct letters indicate significant differences (p < 0.05).



**Figure 5.** Average values and standard deviation of the difference of total biomass ( $\Delta TB$ ), aboveground biomass ( $\Delta AB$ ) and belowground biomass ( $\Delta BB$ ) ( $\text{g m}^{-2}$ ) of the early-colonizing individuals of *Crinum americanum* in monoculture (C) and mixed cultures (C + S) in the lower (LES) and middle estuary sediment (MES). The graphs have different scales. Distinct letter indicate significant differences ( $p < 0.05$ ).

(Cao *et al.* 2018), and also to tolerate hypersaline environments (Angelini *et al.* 2011). Although *C. americanum* does not occur in the lower estuary of the Itanhaém River, under experimental conditions of the lower estuary and in monoculture this species grows up moderately as shown by the results of Nunes & Camargo (2018) and those of this present work. Thus, we suggest that *S. alterniflora* was the first species to colonize and establish itself in the lower estuary of the Itanhaém River and it did not allow the establishment of later-arriving individuals of *C. americanum*.

In restored saltmarshes in the UK, Sullivan *et al.* (2018) found that some macrophyte species can establish outside their normal realized niche where there was an initial availability of bare sediment and then inhibit subsequent colonization of other species. Moore & Franklin (2012) also observed in an experiment that early-colonizing individuals of emergent macrophytes maintained their competitive advantage over later-arriving individuals regardless of the abiotic stress. In our experiment, the greater salinity level of the lower estuary sediment limited the gain in belowground biomass of the early-colonizing individuals of *S. alterniflora* when compared to its growth in the middle estuary sediment. However, even so, this species possibly dominated the use of available resources, such as space and nutrients, and maintained the inhibitory priority effect on *C. americanum* individuals.

In the middle estuary conditions *C. americanum* had great growth both in monoculture and in culture with *S. alterniflora* introduction on day 124. Therefore, the middle estuary conditions that are favorable for *C. americanum* and *S. alterniflora* do not affect their growth. The same result was observed for *S. alterniflora* that had great growth in the middle estuary condition both in monoculture and in the culture with *C. americanum* introduction. These results show that the middle estuary conditions are favorable for both species growth, and also that introduction of the other species does not affect their growth. These experimental results explain the occurrence of mixed stands in this estuarine area. However, Nunes & Camargo (2018) observed in a pair-wise experiment that in conditions of the middle

**Table 1.** Average values and standard deviation of the differences of total biomass ( $\Delta$ TB), aboveground biomass ( $\Delta$ AB) and belowground biomass ( $\Delta$ BB) ( $\text{g m}^{-2}$ ), and relative growth rate of total biomass (RGR-TB) ( $\text{g m}^{-2} \text{d}^{-1}$ ) of the later-arriving individuals of *Crinum americanum* and *Spartina alterniflora* introduced into the early-colonizing heterospecific cultures in the lower (LES) and middle estuary sediment (MES). The symbol (\*) indicates significant differences in  $\Delta$ TB,  $\Delta$ AB,  $\Delta$ BB and RGR-TB of each species between the two sediment types ( $p < 0.05$ ).

	Sediment type	$\Delta$ TB	$\Delta$ AB	$\Delta$ BB	RGR-TB
<i>C. americanum</i>	LES	-136.95 (68.65)	-99.67 (3.17)*	-37.29 (61.22)	-0.01259 (0.00647)*
	MES	-16.63 (160.22)	-21.05 (17.76)*	-34.60 (14.17)	-0.00353 (0.00179)*
<i>S. alterniflora</i>	LES	-35.18 (17.41)	-45.88 (24.99)	-10.71 (9.79)	-0.00996 (0.00679)
	MES	-25.98 (29.00)	-22.47 (40.29)	3.51 (12.03)	-0.00668 (0.00821)

Itanhaém estuary, *S. alterniflora* had greater competitive ability than *C. americanum*. Our experiment results may indicate that the initial colonization by *C. americanum* prevented its limitation by *S. alterniflora* resulted, probably in a fugitive strategy in space by the propagules of *S. alterniflora*, and led to the coexistence of both species in adjacent habitats in the same estuarine area. We emphasize that although both species occur in the middle estuary, *S. alterniflora* occurs closer to the channel and *C. americanum* close to terrestrial vegetation, showing lateral segregation. Although interspecific competition may occur in the contact zone between heterospecific individuals (Woo & Zedler 2002), the spatial segregation tends to decrease its intensity (Barot 2004) and the chances of a species invades the portion occupied by another by lateral expansion (Stoll & Prati 2001). Mixed stands with a lateral segregation of emergent aquatic macrophytes was also observed by Levine *et al.* (1998) in New England salt marshes (Northern USA) where *S. alterniflora*, *Spartina patens* and *Juncus gerardi* form parallel stands. According to Angelini *et al.* (2011), when an early-colonizing species inhibits the colonization of its interstitial spaces by others, the later-arriving species can colonize adjacent spaces and form stands laterally segregated in stable population densities.

In a controlled greenhouse experiment, Sarneel *et al.* (2016) found that a great biomass reduction and mortality of later-arriving riparian plant species may occur if the environmental conditions were not favorable to them. However, in our experiment we observed a reduction in biomass of the later-arriving individuals even under favorable abiotic conditions for their early-

colonizing individuals. This result, in addition to showing the priority effect, indicates that the interspecific interaction outcomes vary between young and adult plants. In fact, in a study of 25 species of emergent macrophytes, Shipley *et al.* (1989) observed that there may be no association between juvenile and adult attributes and suggested that discussions about the plant ecological strategies should be based on their life stages.

Since the importance of interspecific competition for the macrophytes spatial distribution in the Itanhaém River Estuary has already been reported (Nunes & Camargo 2018, Nunes & Camargo 2020) we also point out that, possibly, both biotic interactions and arrival order may be important for the macrophyte zonation in this tropical estuary. In estuaries in Georgia (southern USA), Guo *et al.* (2014) found that stochastic processes, such as arrival order, are less important in relation to deterministic processes, such as abiotic stress and biotic interactions, for the structuring of aquatic plant communities. However, Kim *et al.* (2016) suggested that different deterministic and historically contingent factors act simultaneously on the vegetation dynamics in salt marshes in Denmark.

Finally, we conclude that there is an inhibitory priority effect of both *S. alterniflora* and *C. americanum* in both estuarine conditions analyzed, that is, the first species to colonize and establish itself has a competitive advantage over the species that arrives later. So, this early-colonizing species is not limited by interspecific interaction and it prevents the establishment of the later-arriving species. Thus, the arrival order can also be determinant for the communities' organization and spatial distribution of aquatic

macrophytes in tropical estuaries. This study provides unprecedented information on the importance of arrival order for aquatic macrophyte zonation in tropical estuaries.

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## APPENDIX

**Appendix 1.** Results of the Mann-Whitney test (U) applied to salinity and content of total nitrogen (TN) and phosphorus (TP) between the two sediment types (lower and middle estuary sediment).

Variables	U	p
Salinity	0.0	< 0.01
TN	172.0	0.46
TP	157.0	0.25

**Appendix 2.** Results of the Factorial ANOVA applied to the gain of total biomass (GTB), gain of aboveground biomass (GAB) and gain of belowground biomass (GBB) of the early-colonizing individuals of *Spartina alterniflora* and *Crinum americanum* in the different cultures and sediment types.

	df	F	p
<i>Spartina alterniflora</i>			
<b>GTB</b>			
Culture	1	8.218	0.008
Sediment type	2	0.419	0.662
culture*sediment type	2	0.130	0.879
<b>GAB</b>			
Culture	1	0.128	0.726
Sediment type	1	0.758	0.397
culture*sediment type	1w	0.423	0.525
<b>GBB</b>			
Culture	1	0.088	0.77
Sediment type	1	1.92	0.185
culture*sediment type	1	7.141	< 0.05
<i>Crinum americanum</i>			
<b>GTB</b>			
Culture	1	0.302	0.083
Sediment type	2	1.037	0.370
culture*sediment type	2	1.625	0.218
<b>GAB</b>			
Culture	1	0.94	0.347
Sediment type	1	36.02	< 0.01
culture*sediment type	1	5.06	< 0.05
<b>GBB</b>			
Culture	1	0.22	0.646
Sediment type	1	38.57	< 0.01
culture*sediment type	1	2.62	0.125

**Appendix 3.** Results of the t-test (t) and Mann-Whitney test (U) applied to the aplicados the gain of total biomass (GBT), gain of aboveground biomass (GAB), gain of belowground biomass (GBB) and relative growth rate (RGR-TB) of the later-arriving individuals of *Spartina alterniflora* and *Crinum americanum* between the two sediment types.

		<b>df</b>	<b>p</b>
<i>Spartina alterniflora</i>			
GBT	t = 1.104	8	0.301
GAB	t = 0.6077	8	0.562
GBB	t = 1.834	8	0.104
RGR - TB	t = 0.6138	8	0.556
<i>Crinum americanum</i>			
GBT	U = 6		0.222
GAB	t = 9.749	8	< 0.01
GBB	U = 5		0.151
RGR - TB	t = 2.702	8	0.027