UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
(BIOLOGIA VEGETAL)

ECOLOGIA DE MACRÓFITAS AQUÁTICAS EM RIOS COSTEIROS DE SÃO PAULO: DIVERSIDADE, COMPETIÇÃO INTERESPECÍFICA E DISTRIBUIÇÃO ESPACIAL EM GRADIENTES AMBIENTAIS

LAÍS SAMIRA CORREIA NUNES

Rio Claro – SP
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LAÍS SAMIRA CORREIA NUNES

Orientador: Prof. Dr. Antonio Fernando Monteiro Camargo

Tese apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutora em Ciências Biológicas (Biologia Vegetal).

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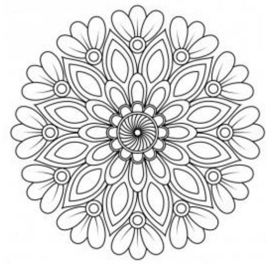

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☼

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Apresentação

Sobre o formato da tese

Esta tese está dividida em seis capítulos organizados em formato de artigos. Os artigos estão formatados (principalmente citações e lista de referências) de acordo com as normas específicas de cada periódico científico em que foram publicados ou para os quais foram submetidos. Todos os capítulos estão redigidos em língua inglesa, mas nós adicionados resumos em português em cada um deles. Para fins de melhor fluidez de leitura e compreensão do conteúdo, as figuras e tabelas estão apresentadas ao longo do texto em cada capítulo. Nesta tese, há também uma introdução geral e conclusões envolvendo a inter-relação entre os temas abordados e os resultados encontrados em todos os capítulos.

Os três primeiros capítulos correspondem a estudos em campo em oito rios costeiros do estado de São Paulo (municípios de Ubatuba, São Sebastião, Bertioga, Itanhaém e Peruíbe), localizados em planícies costeiras com diferentes características. O primeiro capítulo foi publicado na seção especial “Ecology of Wetlands” de *Oecologia Australis* (Nunes *et al*., 23(4):829–845, 2019, DOI: 10.4257/oeco.2019.2304.09). Neste capítulo, nós apresentamos um inventário de espécies de macrófitas aquáticas elaborado em parceria com Dra. Cristiane Akemi Umetsu (Programa de Pós-Graduação em Aquicultura – UNESP – Jaboticabal), Ma. Maria Estefânia Fernandes Rodrigues (Programa de Pós-Graduação em Botânica – USP – São Paulo) e Profa. Dra. Vali Joana Pott (vice-curadora do herbário CGMS – UFMS – Campo Grande). No segundo capítulo, nós avaliamos se a heterogeneidade ambiental dos rios costeiros e a distância geográfica entre eles influenciam a riqueza de espécies e de formas de vida e a composição de espécies de macrófitas aquáticas. Este estudo foi elaborado também em parceria com Dra. Cristiane Akemi Umetsu. Este artigo foi publicado na revista *Freshwater Biology* (Nunes *et al*. 00:1–12, 2020, DOI: 10.1111/fwb.13586). No terceiro capítulo, nós estudamos a diversidade beta de espécies e de formas de vida de macrófitas aquáticas dos rios costeiros em duas escalas espaciais, e avaliamos se as características das planícies costeiras influenciam a variação das comunidades. O planejamento deste estudo e sua elaboração ocorreram em parceria com o Prof. Dr. Victor Satoru Saito (Departamento de Ciências Ambientais – UFSCar – São Carlos). Este artigo foi aceito para publicação na revista *Freshwater Science* (Setembro/2020).

Os três últimos capítulos correspondem a estudos experimentais em casa de vegetação, tendo como base o estuário do rio Itanhaém (litoral sul de São Paulo), sobre a competição entre as macrófitas emergentes *Spartina alterniflora* (Poaceae) e *Crinum americanum* (Amaryllidaceae). No quarto capítulo, nós avaliamos se a competição entre estas espécies é dependente da densidade inicial de indivíduos jovens em sedimento de baixo e médio estuário do rio Itanhaém. Este artigo foi publicado em *Aquatic Botany* (Nunes & Camargo, 164:1–8, 2020, DOI: 10.1016/j.aquabot.2020.103233). No quinto capítulo, nós avaliamos se a ordem de chegada e o efeito de prioridade influenciam os resultados da competição entre *S. alterniflora* e *C. americanum* nos dois tipos de sedimento. No sexto capítulo, nós avaliamos os efeitos da salinidade sobre o crescimento, a interação competitiva e o conteúdo de nitrogênio total destas espécies, através do cultivo das plantas em substrato artificial em três níveis de salinidade. Este experimento foi desenvolvido em parceria com a Ma. Rachel Santini Paulino (Programa de Pós-Graduação em Aquicultura – UNESP – Jaboticabal) que avaliou o estresse oxidativo nas plantas provocado pela salinidade. Este artigo foi publicado na revista *Aquatic Ecology* (Nunes & Camargo, 2020, DOI: 10.1007/s10452-020-09787-5).

Frequentemente na literatura, a salinidade da água e do sedimento tem sido expressa em ppt (parts per thousand) ou psu (practical salinity units). No entanto, de acordo com o Sistema Internacional de Unidades em Oceanografia da UNESCO (The international system of units (SI) in oceanography, UNESCO Technical Papers in Marine Science n. 45, IAPSO Publication Scientifique n. 32, Paris, 1985), a salinidade prática não possui unidades, porém deve ser baseada na escala ppt (= ‰ ou g L^{-1}); por isso em alguns dos capítulos desta tese a salinidade foi expressa sem unidade de medida.

Trajetória de elaboração do projeto de pesquisa e de desenvolvimento da tese

Comecei como estagiária no Laboratório de Ecologia Aquática no segundo ano da minha graduação em Ciências Biológicas. Na época fui procurar o Prof. Antonio porque eu tinha interesse em ecologia aquática e, especialmente, porque soube que o professor trabalhava há muito tempo no litoral. Cresci em Bertioga e, graças ao meu pai ambientalista, sempre amando água: do mar ou do rio Itapanhaú, nadando, andando de barco ou pescando siri. Então, o estágio com Ecologia em uma bacia costeira era a grande oportunidade. Logo que comecei a ajudar em experimentos e no campo, me encantei pelas macrófitas. Rapidamente surgiu a possibilidade de uma bolsa de iniciação científica, e por sugestão do Prof. Antonio, estudei a variação temporal de biomassa e composição química de *C. americanum* na bacia do rio Itanhaém, que resultou no meu TCC. Depois de tantas idas a campo, fiquei curiosa para entender a distribuição desta espécie no gradiente de salinidade do estuário. Foi a partir disso que surgiu a ideia do projeto de mestrado antes mesmo que eu ingressasse no PPG em Ciências Biológicas (Biologia Vegetal). No mestrado, nós desenvolvemos um experimento para avaliar o crescimento e a competição entre *S. alterniflora* e *C. americanum* com sedimento do alto, médio e baixo estuário, e para entender se a competição e a tolerância à salinidade seriam responsáveis pelo padrão de distribuição destas espécies. Tivemos sucesso no experimento, publicamos um artigo em *Hydrobiologia*, mas ainda continuamos curiosos para compreender melhor os resultados.

Com a possibilidade da seleção contínua no doutorado na Biologia Vegetal, nós elaboramos o projeto de doutorado. As ideias iniciais eram descobrir quais espécies ocorreriam em outros estuários de São Paulo, quais as características destes estuários e se havia um padrão de distribuição espacial de espécies; e ainda investigar se os fatores 'densidade' e 'quem chegou primeiro' também teriam importância para o resultado da interação interespecífica e para a zonação e organização dos bancos de *S. alterniflora* e *C. americanum* no gradiente estuarino. Assim, no projeto de pesquisa estavam previstos a realização de um inventário de espécies, a caracterização ambiental de estuários do litoral paulista e dois experimentos em casa de vegetação. Fizemos coleta nos rios costeiros e encontramos resultados muito interessantes, e até mesmo inesperados, os quais nos motivaram a investir bastante nos dados de campo que resultaram em três manuscritos. Os experimentos previstos foram realizados e ainda conseguimos desenvolver um terceiro no qual parte dos resultados foram utilizados no capítulo 6 desta tese e parte em outra tese de doutorado do nosso grupo de pesquisa.

Já no final do terceiro ano de curso, e depois de outras duas pré-tentativas não bem sucedidas de estágio no exterior, eu entrei em contato com o Prof. Dr. Steven Pennings da University of Houston nos EUA, especialista em ecologia da vegetação herbácea em wetlands costeiras. O Prof. Steve (como ele gosta de ser chamado) prontamente e gentilmente me aceitou para uma visita técnica de uma semana em seu laboratório em Houston em março de 2019, em que definimos a realização de um estudo (ainda em preparação) em conjunto e comparativo sobre a variação espacial da biomassa, riqueza e pool de espécies de plantas aquáticas entre ecossistemas costeiros da Georgia (EUA) e de São Paulo (Brasil). Em setembro do mesmo ano retornei aos EUA para um estágio de 20 dias em que acompanhei as coletas e auxiliei no trabalho de campo na Georgia.

Durante meu doutoramento confirmei minha paixão por ecologia de ecossistemas aquáticos costeiros, e a beleza das macrófitas floresceu-a e frutificou-a.

Resumo

Esta tese de doutorado teve como objetivo estudar a diversidade, a competição interespecífica e a distribuição espacial de macrófitas aquáticas em rios costeiros do estado de São Paulo, através de estudos em campo (oito rios costeiros) e de experimentos em casa de vegetação. Nós elaboramos um inventário de espécies e de formas de vida de macrófitas aquáticas nos rios costeiros e encontramos um total de 45 táxons, 24 famílias e 6 formas de vida. As macrófitas emergentes foram mais frequentes e encontradas em todos os rios, porém as flutuantes e submersas foram registradas em somente dois rios. Nós observamos um gradiente de distribuição de espécies no gradiente norte-sul do litoral, com apenas 4 espécies ocorrendo em grande parte do trecho estudado e 43,5% dos táxons ocorrendo em somente um dos rios amostrados. Nós observamos que as diferenças de características das planícies costeiras (largura e inclinação) ao longo do litoral influenciam as características dos rios costeiros, especialmente sua heterogeneidade ambiental, seus comprimentos e seus gradientes longitudinais de salinidade. Nós encontramos que a heterogeneidade ambiental influencia positivamente a riqueza de formas de vida de macrófitas e a variação na composição de espécies entre os rios, no entanto, não influencia a riqueza de espécies. Os rios costeiros com heterogeneidade ambiental semelhante foram aqueles com maior similaridade na composição de espécies de macrófitas aquáticas independentemente de suas distâncias geográficas. Nós observamos elevada diversidade beta de espécies e de formas de vida de macrófitas aquáticas em escala local e regional. Na escala local, o *turnover* de espécies e o aninhamento de formas de vida foram explicados pelos gradientes ambientais longitudinais dos rios. Na escala regional, a diversidade beta de formas de vida foi explicada pelo comprimento dos rios; no entanto, a variação de espécies pareceu estar mais relacionada a fatores bióticos e históricos na dispersão e colonização. A partir dos estudos em campo, nós concluímos que as diferenças nas características das planícies costeiras promovem diferentes características ambientais nos rios costeiros que levam à formação de diferentes comunidades e padrões de diversidade e distribuição espacial de macrófitas aquáticas ao longo do litoral de São Paulo. Em relação aos estudos experimentais, nós encontramos que a competição entre as macrófitas emergentes *Spartina alterniflora* e *Crinum americanum* em sedimento natural de baixo e médio estuário do rio Itanhaém não é dependente das densidades iniciais de indivíduos jovens, pois *S. alterniflora* apresentou maior habilidade do que *C. americanum* em qualquer densidade nos dois tipos de sedimento. Nós encontramos também que a distribuição e a organização dos bancos destas espécies no estuário do rio Itanhaém podem ter siso influenciadas por suas ordens de chegada na colonização e efeitos de prioridade, pois indivíduos jovens não se estabeleceram em culturas heteroespecíficas de indivíduos adultos. Em experimento com substrato artificial, nós encontramos que a salinidade não foi limitante para o crescimento em monocultura de *S. alterniflora* e *C. americanum* e não influenciou o conteúdo de nitrogênio total em *S. alterniflora*. No entanto, em maior salinidade (30), *C. americanum* apresentou maior conteúdo de nitrogênio total em relação à condição não salina (0), possivelmente, como uma estratégia de tolerância à salinidade. No tratamento de maior salinidade ocorreu competição interespecífica assimétrica com maior habilidade competitiva de *S. alterniflora.* Os resultados de nossos experimentos sugerem que, possivelmente, em baixo estuário do rio Itanhaém *C. americanum* foi excluída competitivamente por *S. alterniflora* independentemente de suas densidades de colonização, e que em médio estuário a ordem de chegada destas espécies tem importância para a organização de seus bancos mistos.

Palavras-chave: plantas aquáticas, ecossistemas aquáticos costeiros, estuários, gradiente de salinidade, diversidade beta, interação biótica, pressão de propágulos, ordem de chegada, efeito de prioridade.

Abstract

This Ph.D. thesis aimed to study the diversity, interspecific competition and spatial distribution of aquatic macrophytes in coastal rivers in the São Paulo State, through field studies (eight coastal rivers) and greenhouse experiments. We elaborated an inventory of macrophyte species and life forms in the coastal rivers and found a total of 45 taxa, 24 families and 6 life forms. The emergent macrophytes were the most frequent ones and they were found in all rivers, but the floating and submerged macrophytes were recorded in only two rivers. We observed a gradient of species distribution in the north-south gradient of the coast, with only 4 species occurring in a large part of the studied coastal length and 43.5% of the taxa occurring in only one of the sampled rivers. We observed that the differences in the characteristics of the coastal plains (width and slope) influence the characteristics of the coastal rivers, especially their environmental heterogeneity (EH), lengths and longitudinal salinity gradients. We found that EH positively influences life form richness and species composition variation among the rivers; however, it does not drive species richness. The coastal rivers with similar EH were those with the greatest similarity in species composition regardless of their geographical distances. We observed great beta diversity of macrophyte species and life forms at local and regional scales. At the local scale, the species turnover and life form nestedness were explained by the rivers' longitudinal environmental gradients. At the regional scale, the life form beta diversity was explained by rivers' length; however, species composition variation was probably related to biotic and historical factors in dispersion and colonization. From the field studies, we conclude that the differences in the characteristics of the coastal plains promote different environmental characteristics of the coastal rivers and lead to different communities and patterns of diversity and spatial distribution of aquatic macrophytes along the São Paulo coastal region. In relation to the experimental studies, we found that the competition between the emergent macrophytes *Spartina alterniflora* and *Crinum americanum* in natural sediment of the lower and middle Itanhaém River Estuary is not density-dependent of young individuals, as *S. alterniflora* had greater ability than *C. americanum* in any density in both sediment types. We also found that the distribution and stand organization of these species in the Itanhaém River Estuary may be influenced by their arrival order in colonization and priority effects, as young individuals did not establish themselves in heterospecific cultures of adult individuals. In an experiment using artificial substrate, we found that salinity was not limiting for the *S. alterniflora* and *C. americanum* growth in monoculture and it did not influence the *S. alterniflora* total nitrogen content. However, in higher salinity (30), *C. americanum* had higher total nitrogen content in relation to the treatment with no salt addition (0), possibly as a strategy of salt tolerance. In the higher salinity treatment, asymmetric interspecific competition occurred with greater competitive ability of *S. alterniflora*. The results of our experiments suggest that, possibly, in the lower Itanhaém River Estuary *C. americanum* was competitively excluded by *S. alterniflora* regardless of their colonization densities, and that in the middle estuary the arrival order was important for the organization of their mixed stands.

Keywords: aquatic plants, coastal wetlands, estuaries, salinity gradient, beta diversity, biotic interaction, propagules pressure, arrival order, priority effect.

Sumário

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Introdução geral

As bacias hidrográficas costeiras percorrem as planícies costeiras em terrenos baixos e planos paralelos à linha de costa e deságuam no oceano (BRANCO, 1992). À sua jusante, nas áreas de transição entre os ambientes fluvio-continental e marinho, formam-se os estuários. Os estuários são ambientes de misturas entre águas doces vindas dos rios e águas salgadas do oceano (WOLANSKI, 2007). Quando há uma ligação permanente entre os rios e o oceano estes estuários são chamados de regulares. Os estuários regulares são influenciados diretamente e diariamente pelos ciclos de marés, possuem características de ambientes lóticos e podem ter uma inversão periódica de fluxo com o aporte de águas de preamar (TESSLER *et al.*, 2006). No entanto, alguns estuários têm sua conexão com o oceano periodicamente interrompida (por meses ou anos), devido à formação de barras de areia, e são chamados de estuários irregulares ou cegos. Nestes estuários irregulares, a influência das marés só ocorre quando a barra de areia é rompida e, por isso, ocorrem mais frequentemente características de ambientes lênticos (MIRANDA *et al.*, 2012).

Ao longo dos rios costeiros, especialmente aqueles com zonas estuarinas regulares, ocorrem variações espaciais graduais em suas características ambientais tanto ao longo de seus canais (gradiente longitudinal) quanto nas áreas alagáveis às suas margens (gradiente lateral). Estes gradientes, principalmente de salinidade, variação do nível de água e concentração de nutrientes, promovem heterogeneidade espacial e diversidade de habitats (BERTNESS, 1991). Os rios costeiros e suas zonas estuarinas são fundamentais para a manutenção dos ciclos biogeoquímicos e da biodiversidade aquática (TUNDISI; TUNDISI, 2008), com elevada importância ecológica como áreas de reprodução, deslocamento, alimentação e crescimento de várias espécies marinhas e de água doce. As suas comunidades biológicas são adaptadas às variações abióticas e seus padrões de distribuição espacial estão relacionados a estes gradientes ambientais (NETO *et al.*, 2019).

Ao longo de parte do litoral sudeste e sul do Brasil a planície costeira é delimitada pela presença da “Serra do Mar”, uma cadeia de montanhas, paredões e escarpas que se estende por 1500 km entre os estados do Rio de Janeiro e de Santa Catarina. A Serra do Mar foi formada no final do Cretáceo, de 130 a 40 milhões de anos atrás, durante a separação entre América do Sul e África. A separação destes continentes gerou uma intensa movimentação de rochas graníticas e reativações tectônicas que levaram ao soerguimento desta serrania a uma altitude média de 800 m (ALMEIDA, 1976). Durante o Quaternário, entre a Serra do Mar e o oceano, ocorreram eventos transgressivo-regressivos do nível do mar (SUGUIO; MARTIN, 1978) com deposição de argilas transportadas pelos rios e de areias vindas do mar, formando as planícies costeiras (Figura 1) (BRANCO, 1992).

Figura 1. Imagem em perspectiva com destaque para a Serra do Mar, planície costeira e a foz dos rios Itapanhaú (em amarelo) e Guaratuba (em azul) em Bertioga/SP. Fonte: Google LLC (2020).

As planícies costeiras de São Paulo apresentam larguras bastante variadas e diferentes características geomorfológicas. Na região norte do litoral, entre Ubatuba e São Sebastião, a Serra do Mar se aproxima da linha de costa formando planícies costeiras estreitas (aproximadamente entre 1,5 e 4 km de largura) e encostas recortadas com pequenas praias, enseadas e baías. Na região sul, entre Cananeia e Praia Grande, a Serra do Mar se distancia da linha de costa formando extensas planícies costeiras (entre 5 e 15 km de largura) e linhas de praias contínuas e retilíneas. Na região central, entre Santos e São Sebastião, a planície costeira tem características das regiões norte e sul, como uma zona de transição (IPT, 1981; TESSLER *et al*., 2006). A largura da planície costeira, ou seja, a distância entre a linha de costa e a Serra do Mar, influencia sua topografia e elevação, promovendo diferenças nas características limnológicas, morfométricas e hidrológicas dos ecossistemas aquáticos localizados nesta região (TESSLER *et al.*, 2006). Desta forma, as bacias costeiras de São Paulo possuem características únicas (SOUZA; CUNHA, 2011), além de serem geograficamente isoladas entre si, uma vez que apresentam suas cabeceiras em elevadas altitudes na Serra do Mar e são drenadas diretamente para o mar (ALMEIDA; CARNEIRO, 1998).

Em regiões tropicais, as planícies costeiras são ocupadas pelas florestas de mangue e de restinga. Nos corpos de água nas bordas destas florestas formam-se estreitas faixas com deposição de sedimentos onde ocorrem franjas de vegetação herbácea caracterizada pelas macrófitas aquáticas (Figura 2) (SCHAEFFER-NOVELLI *et al*., 1990). Em regiões costeiras subtropicais e temperadas, onde o manguezal não ocorre, as macrófitas aquáticas ocupam diferentes elevações em extensas planícies intertidais formando marismas e *salt marshes* (Figura 3) (COSTA *et al*., 2003; GUO; PENNINGS, 2012). As macrófitas aquáticas desempenham importantes serviços ecossistêmicos, como formação de habitats, regulação de nutrientes, retenção de sedimentos, prevenção de erosão, acúmulo de matéria orgânica e produtividade (WIESKI *et al*., 2010).

Figura 2. Franja de *Spartina alterniflora* na borda do manguezal no baixo estuário do rio Itanhaém, Itanhaém/SP. Foto em baixamar. Autor: Laís S. C. Nunes.

Figura 3. *Salt marsh* subtropical em Sapelo Island, Georgia, sul dos EUA. Ao fundo o canal do estuário. Foto em baixamar. Autor: Laís S. C. Nunes.

No sudeste do Brasil, os rios costeiros são colonizados por várias espécies de macrófitas aquáticas, incluindo as espécies emergentes *Spartina alterniflora* Loisel. (Poaceae), *Crinum americanum* L. (Amaryllidaceae), *Schoenoplectus californicus* (C.A.May) Soják (Cyperaceae), *Rhynchospora corymbosa* (L.) Britton (Cyperaceae) e *Typha domingensis* Pers. (Typhaceae) (RIBEIRO *et al*., 2011 a, NUNES; CAMARGO, 2018). No litoral sul de São Paulo, como no estuário do rio Itanhaém, *S. alterniflora* e *C. americanum* são as espécies mais abundantes e frequentes e se distribuem em mosaicos. *Spartina alterniflora* ocorre em bancos monoespecíficos na porção inferior do estuário (baixo estuário), *C. americanum* ocorre em bancos monoespecíficos na porção superior do estuário (alto estuário) e bancos contendo as duas espécies ocorrem na porção intermediária do estuário (médio estuário) (NUNES; CAMARGO, 2018). No entanto, em estuários do litoral norte, *C. americanum* forma um gradiente de distribuição longitudinal com *T. domingensis* e *Eleocharis interstincta* (Vahl) Roem & Schul (Cyperaceae), e a ocorrência de *S. alterniflora* não foi registrada (RIBEIRO *et al*., 2011 b).

A distribuição espacial de macrófitas em estuários tem sido explicada pela hipótese da competição-estresse (competition-to-stress hypothesis). Esta hipótese, elaborada a partir de estudos em estuários de regiões temperadas, considera que algumas espécies são excluídas dos ambientes mais próximos da linha de costa (baixo estuário) devido ao estresse abiótico, e que outras espécies são excluídas dos ambientes mais distantes da linha de costa (alto estuário) devido à competição interespecífica (CRAIN *et al*., 2004; ENGELS; JENSEN, 2010). No entanto, outros estudos encontraram que o estresse abiótico e a competição interespecífica podem atuar também nos extremos contrários do gradiente, ou seja, respectivamente, em alto e baixo estuário (BOCKELMANN; NEUHAUS, 1999; NUNES; CAMARGO, 2018). Por exemplo, Nunes e Camargo (2018) sugeriram que a ausência de *S. alterniflora* em alto estuário do rio Itanhaém se deve ao estresse abiótico (principalmente menor disponibilidade de fósforo e sulfatos e menor salinidade), e que a ausência de *C. americanum* em baixo estuário é resultado da competição com *S. alterniflora*. De fato, segundo Guo e Pennings (2012), para estuários tropicais e subtropicais, a hipótese da competição-estresse é uma simplificação de processos complexos e de múltiplos fatores, os quais podem atuar em conjunto para determinar a distribuição da vegetação de acordo com as variações das características destes ambientes.

A maioria dos estudos envolvendo a competição entre macrófitas aquáticas tem analisado a resposta de plantas adultas, entretanto, a competição interespecífica pode atuar já sobre indivíduos jovens (ou propágulos) nos processos de colonização e estabelecimento. Desta forma, os resultados de interações interespecíficas de macrófitas podem ser dependentes de suas densidades iniciais (THOMAZ, 2002). Além disso, a ordem de chegada e seu efeito de prioridade também têm ganhado destaque para explicar a estruturação de comunidades e a distribuição espacial de macrófitas aquáticas (PARADIS *et al.*, 2014), principalmente, considerando espécies estuarinas as quais tendem a ser perenes e a monopolizar o ambiente, pois podem ser tanto boas colonizadoras quanto boas competidoras (BOCKELMANN; NEUHAUS, 1999). Em muitos casos, os processos determinísticos (competição e estresse abiótico) e estocásticos (ordem de chegada e dispersão) podem atuar simultaneamente ou variar ao longo de gradientes ambientais (GUO *et al.*, 2014).

A riqueza e a diversidade beta de macrófitas aquáticas também podem apresentar diferentes padrões em diversas escalas espaciais, principalmente relacionados às diferenças de heterogeneidade ambiental (GUO *et al*., 2015). Em grandes escalas espaciais, pode haver um grande pool regional de espécies de macrófitas em ecossistemas costeiros com a coexistência de diferentes comunidades, no entanto, em menores escalas espaciais uma pequena proporção do pool regional de espécies tende a ocorrer devido à combinação de amplitude de nicho e filtragem ambiental (KUNZA; PENNINGS, 2008). Entretanto, a variação destas comunidades pode ser maior em escala reduzida do que em escala regional devido aos fortes gradientes ambientais locais dos estuários (GUO *et al*., 2015).

Grande parte dos estudos sobre distribuição de macrófitas em ambientes costeiros tem sido realizada em regiões temperadas e subtropicais (COSTA *et al*., 2003; GUO; PENNINGS, 2012; MARANGONI; COSTA, 2012; GUO *et al*., 2015). Ainda há poucos inventários de espécies e estudos ecológicos com macrófitas aquáticas em regiões costeiras tropicais, especialmente aqueles que abordem a diversidade e distribuição espacial de comunidades ao longo de gradientes ambientais (RIBEIRO *et al*., 2011 a; RIBEIRO *et al*., 2011 b; RIBEIRO *et al*., 2012; RIBEIRO *et al*., 2013; RIBEIRO *et al*., 2015; NUNES; CAMARGO, 2018). Além disso, a influência de fatores bióticos e abióticos sobre a distribuição espacial de macrófitas aquáticas em geral, e sobre *S. alterniflora* em particular, ainda é controversa. Portanto, estudos experimentais e em campo podem contribuir para elucidar a importância destes fatores na ocorrência e distribuição espacial de macrófitas aquáticas em ecossistemas aquáticos costeiros tropicais, gerando conhecimento para práticas de conservação da vegetação herbácea e de conservação e restauração de rios costeiros.

Esta tese de doutorado teve como objetivo estudar a diversidade, a competição interespecífica e a distribuição espacial de macrófitas aquáticas em rios costeiros do estado de São Paulo (com zonas estuarinas regulares e ainda bem conservadas) e responder os seguintes questionamentos:

i) Quais espécies e formas de vida de macrófitas aquáticas ocorrem nos rios costeiros? Há um padrão de distribuição de espécies no gradiente norte-sul do litoral?

ii) A heterogeneidade ambiental dos rios costeiros influencia sua riqueza de espécies e de formas de vida e sua composição de espécies de macrófitas aquáticas? A distância geográfica entre os rios costeiros também é importante para a variação na composição de espécies?

iii) Como ocorre a variação na composição das comunidades de macrófitas aquáticas (diversidade beta) em escala local (dentro dos rios) e em escala regional (entre os rios costeiros)? Fatores ambientais relacionados às diferenças nas características das planícies costeiras (largura e inclinação) explicam a diversidade beta de macrófitas aquáticas nas duas escalas espaciais?

iv) Diferentes densidades iniciais de indivíduos jovens influenciam os resultados da competição entre *S. alterniflora* e *C. americanum*, sua distribuição espacial e organização dos bancos em baixo e médio estuário?

v) A ordem de chegada e o efeito de prioridade influenciam a interação interespecífica entre indivíduos adultos "*early-colonizing*" (fundadores – que colonizaram primeiro) e indivíduos jovens "*later-arriving*" (que chegaram depois) de *S. alterniflora* e *C. americanum,* sua distribuição espacial e organização dos bancos em baixo e médio estuário?

vi) Quais são os efeitos isolados da salinidade sobre o crescimento, a interação interespecífica e o conteúdo de nitrogênio total de *S. alterniflora* e *C. americanum* cultivadas em substrato artificial em casa de vegetação?

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Capítulo 1

INVENTÁRIO DE ESPÉCIES DE MACRÓFITAS AQUÁTICAS EM RIOS COSTEIROS DO ESTADO DE SÃO PAULO, BRASIL

Crinum americanum, Rio Una, São Sebastião/SP

Inventory of aquatic macrophyte species in coastal rivers of the São Paulo State, Brazil*

Resumo: A região costeira do estado de São Paulo (sudeste do Brasil) é marcada pela presença da Serra do Mar, uma cadeia de montanhas com altitude de até 1000 m. Devido às diferenças de proximidade entre a Serra do Mar e a linha de costa, as planícies costeiras têm diferentes larguras. Como consequência, os rios que atravessam estas planícies também têm diferentes comprimentos, maior ou menor influência de águas marinhas e variação de inclinação. Nós realizamos um inventário de espécies de macrófitas aquáticas para avaliar a riqueza de espécies e de formas de vida e sua distribuição latitudinal nessa região. As macrófitas foram inventariadas em 100 locais de amostragem em oito rios (entre 9 e 19 locais por rio) em março de 2017. Foram exploradas descrições gerais sobre aspectos taxonômicos, formas de vida e frequência de ocorrência das macrófitas. Nós registramos 45 táxons de macrófitas aquáticas pertencentes a 24 famílias. Três espécies são exóticas, mas apresentaram baixas frequências de ocorrência. A grande maioria dos táxons tem forma de vida emergente. Macrófitas flutuantes e submersas foram encontradas em apenas dois rios. As espécies mais frequentes foram *Crinum americanum* L. (Asparagales, Amaryllidaceae), *Spartina alterniflora* Loisel. (Poales, Poaceae) e *Schoenoplectus californicus* (C. A. Mey.) Soják (Poales, Cyperaceae). A maioria dos táxons é rara em termos de ocorrência. Apenas quatro espécies ocorreram ao longo de grande parte do trecho norte-sul amostrado e estas, possivelmente, têm uma ampla variação no requerimento de recursos e tolerância à salinidade. O gradiente norte-sul de ocorrência de táxons parece estar relacionado à diversidade de características ambientais devido a diferenças no comprimento dos rios e na largura das planícies costeiras.

Palavras-chave: plantas aquáticas; bacia costeira; estuário; formas de vida.

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Abstract: The coastal region of the São Paulo state (Southeastern Brazil) is marked by the presence of the Serra do Mar, a system of mountain ranges with altitude up to 1,000 m. Due to the difference of proximity of the mountain range to the coastline, the coastal plains have different width. As a consequence, the rivers that cross the plains also have different length, greater or less influence of marine waters and slope variation. We carried on an inventory of aquatic macrophyte species in order to assess the species and life form richness and latitudinal distribution in this region. Macrophytes were inventoried at 100 sampling sites in eight rivers (between 9 and 19 sites per river) in March 2017. General descriptions on taxonomic aspects, life forms and frequency of occurrence of the macrophytes were explored. We recorded 45 taxa of aquatic macrophytes belonging to 24 families. Three species are exotic, but they presented low frequencies of occurrence. The vast majority of the taxa have emergent life form. Floating and submerged macrophytes were found in only two rivers. The most frequent species were *Crinum americanum* L. (Asparagales, Amaryllidaceae), *Spartina alterniflora* Loisel. (Poales, Poaceae) and *Schoenoplectus californicus* (C. A. Mey.) Soják (Poales, Cyperaceae). Most taxa are rare in terms of occurrence. Only four species occurred along a large part of the north-south stretch sampled and these, possibly, have a wide tolerance to the variation in resource requirements and salinity. The north-south gradient of the taxa occurrence may be related to the diversity of environmental characteristics due to differences in the rivers length and coastal plains width.

Keywords: aquatic plants; coastal river basin; estuary; life forms.

INTRODUCTION

Coastal ecosystems, especially the estuarine zones, have great ecological importance for many marine and freshwater species due to the presence of mangroves in most of the Brazilian coast (Pinheiro *et al.* 2008, Pinto-Coelho & Havens 2015). Currently, these ecosystems are among the most impacted environments due to the urban occupation and varied human activities (Pinheiro *et al.* 2008, Pinto-Coelho & Havens 2015). The estuaries are also vulnerable ecosystems to the influence of climate change, mainly by the increasing in sea-level rise and saltwater intrusion and alteration in the amount of freshwater (Scavia *et al.* 2002).

Coastal rivers are marked by the influence of salinity and water level variation due to the tidal regime (Wolanski 2007). In these ecosystems, there are environmental gradients that promote distribution patterns and provide different habitats for the occupation of aquatic vegetation (Bertness 1991). Aquatic macrophyte distribution in estuaries forms a longitudinal gradient in the coastal rivers (from river mouth to headwater), with species adapted to salinity and water level variation in the low river zone and species adapted to oligohaline and freshwater conditions in the upper river zone (Ribeiro *et al.* 2011, Nunes & Camargo 2018). However, salinity may have an influence on plants through the salt spray (Boyce 1954), and thus, even plants occurring in the upper and farther areas from the coastline may be exposed to salt stress (Ribeiro *et al.* 2011).

From the geographical standpoint, there is also a latitudinal distribution of macrophyte species in coastal ecosystems. At global and very wide scales, the processes that drive the macrophyte distribution in low- and high-latitude estuaries are mainly related to the differences of precipitation, temperature and solar radiation (Pennings & Bertness 1999). At these scales, the studies are based on the comparison of single areas (Fariña *et al.* 2017). At more regional and local scales, it is possible to focus on continuous areas and, although the importance of climate gradient is relevant (Fariña *et al.* 2017), the edaphic and geomorphological differences of the aquatic ecosystems may be the principal drivers to the north-south distribution of macrophyte species (Isacch *et al.* 2006, Fariña *et al.* 2017).

In the coastal region of the São Paulo state, southeastern Brazil, there is the Serra do Mar, a system of mountain ranges and escarpments with a length of about 1,000 km and altitude up to 1,000 m. The presence of the Serra do Mar forms a north-south gradient of coastal plain width (IPT 1981, Almeida & Carneiro 1998, Tessler *et al.* 2006). The watersheds located in these plains are influenced by the variation in topography and altitude

(Souza & Cunha 2011). They cross areas with different sedimentary formations (Suguio *et al.* 1978) and disembogue on beaches of different typologies (Tessler *et al.* 2006). Due to the diversity of environmental characteristics of the São Paulo coast it is expected that different aquatic macrophyte species and life forms occur in the coastal north-south gradient.

The knowledge on aquatic macrophyte distribution still presents large gaps (Chambers *et al.* 2008) and such studies in coastal regions are even scarcer. Most studies on coastal aquatic ecosystems assess physiological tolerances and ecological interactions among only a few species (Castillo *et al.* 2000, Costa *et al.* 2003, Touchette 2006, Guo & Pennings 2012, Nunes & Camargo 2018), and species lists and inventories are poorly published (Ribeiro *et al.* 2011, Ferreira *et al.* 2017). Species inventories represent a consistent and efficient method to generate information on aquatic macrophytes distribution, to monitor biodiversity, and for conservation actions of species and aquatic ecosystems (Brooks *et al.* 2004, Thomaz *et al.* 2004). Therefore, the aim of this study was to perform an inventory of aquatic macrophyte species in coastal rivers of São Paulo state in order to assess the species and life form richness and latitudinal distribution in this region.

MATERIAL AND METHODS

Study area

The São Paulo state coast can be compartmentalized into three main regions according to the coastal plains width. The southern region (from the municipality of Ilha Comprida to the municipality of Praia Grande) is marked by large coastal plains, about 15 km wide, interspersed by hills between the Serra do Mar and the continuous and rectilinear beaches. The northern region (from São Sebastião Island to the municipality of Ubatuba) has narrow coastal plains and crenellated hillside forming small beaches, creeks and bays. And the central region of the coast (from the municipality of Santos to São Sebastião Island) presents characteristics of both northern and southern regions (Tessler *et al.* 2006).

The study area presents a small seasonal variation of climatic characteristics, an absence of flood pulses in the aquatic ecosystems, humid tropical climate, mild winters, rainfall in all months of the year and absence of a defined dry season (Monteiro 1973, Camargo & Florentino 2000). The average annual temperature corresponds to 23.6ºC, with slightly higher averages in the central region (24.6ºC) than in the northern region (21.9ºC)

(Embrapa 2015). The average annual rainfall is 2,140 mm, with an average of about 2,500 mm in the northern, 2,000 mm in the central and 1,900 mm in the southern region (Embrapa 2015).

The selection of the rivers was based on the north-south gradient of distancing between the Serra do Mar and the coastline and the coastal plain width, and the occurrence of yet well-conserved estuaries (Table 1). Eight coastal rivers were selected in five municipalities along a coastal stretch of about 280 km. They are: Ubatumirim River, Puruba River and Itamambuca River (municipality of Ubatuba) in the northern region; Una River (municipality of São Sebastião), Guaratuba River and Itapanhaú River (municipality of Bertioga) in the central region; and Itanhaém River (municipality of Itanhaém) and Guaraú River (municipality of Peruíbe) in the southern coast of the São Paulo state (Figure 1).

Table 1. Number of sampling sites per river, sampled river length, the rivers length on the coastal plain, and the coastal plain width in each basin.

Coastal rivers	**Number of sampling sites**	**Sampled river length (km)**	**River length on coastal plain (km)**	**Coastal plain width (km)**
Ubatumirim	09	2.13	2.43	2.00
Puruba	10	2.45	2.62	2.80
Itamambuca	10	1.84	1.84	1.50
Una	11	3.60	3.28	3.90
Guaratuba	12	7.65	8.18	6.10
Itapanhaú	17	25.70	35.84	4.90
Itanhaém	19	18.90	19.00	14.50
Guaraú	12	9.20	10.13	5.50

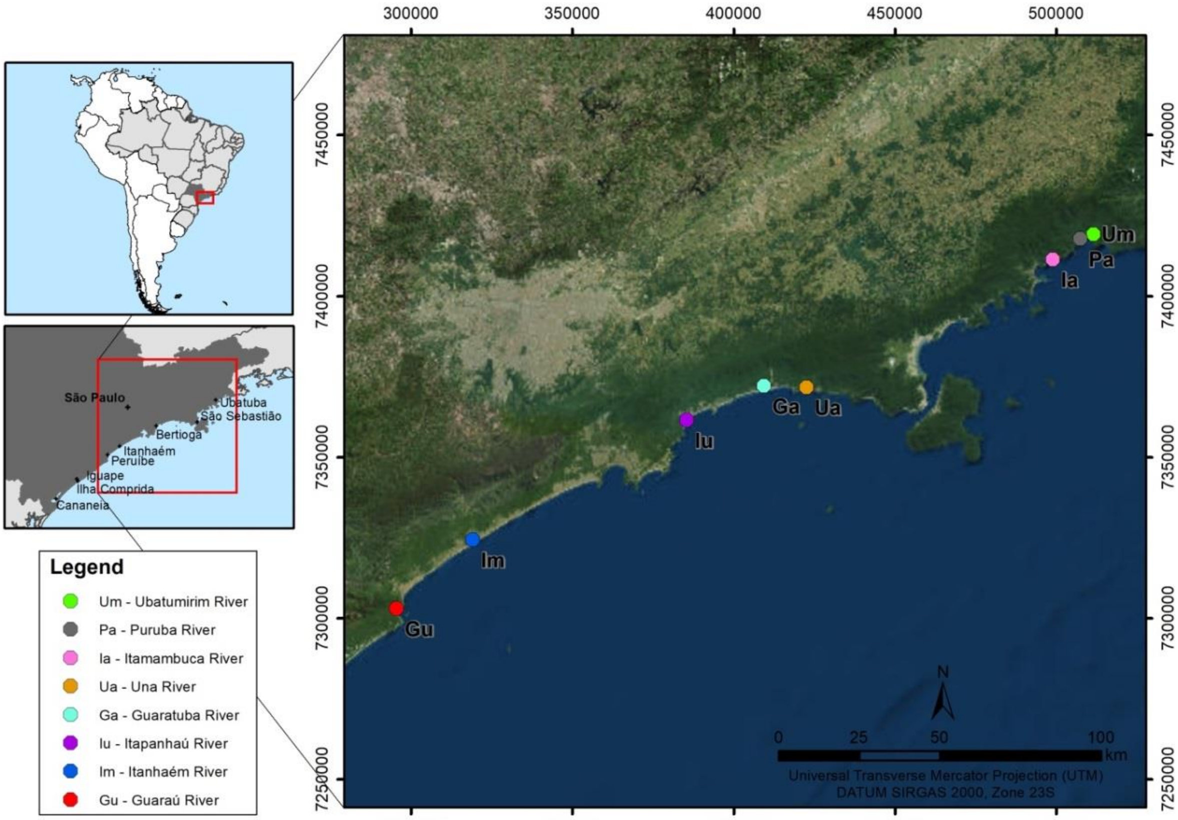

Figure 1. Maps of South America, Brazil and São Paulo State, highlighting the location of the sampled rivers in northern (Ubatumirim, Puruba and Itamambuca), central (Una, Guaratuba and Itapanhaú) and southern (Itanhaém and Guaraú) coastal regions of the São Paulo state.

Data collection

We collected the data in March 2017 and chose to perform a single collecting since most aquatic macrophyte species from estuarine regions are perennial (Engels 2010). We went through the main river of the coastal basins on a small boat from the mouth to the farthest point of possible navigation. Then we returned to the river mouth stopping at all observed macrophyte banks and recording the species occurrence. We sampled between 9 and 19 sites per river (Table 1) based on the macrophyte occurrence. In total, we sampled 100 sites along 59.30 km of rivers (Table 1).

Submerged species were sampled using a hook. We included the amphibious plants found on the sandbanks in the river channels and on the margin up to 2 m away from the water bodies. The aquatic macrophyte species found were recorded and when unidentified in the field they were collected and herborized for later identification. The aquatic macrophytes were identified at the lowest possible taxonomic level using the following literature: Pott & Pott (2000), Amaral *et al.* (2008) and Rodrigues *et al.* (2017). The fertile specimens were included in the herbarium HRCB (Herbário Rioclarense, Instituto de Biociências, UNESP).

The taxa were classified into their life forms, according to Chambers *et al.* (2008): emergent (rooted plants with the vegetative parts emerging above the water surface), free-floating (plants floating on water surface), rooted floating (rooted plants with floating leaves and flowers on the water surface), rooted submerged (plants with predominantly submerged vegetative parts) and free submerged (plants with submerged vegetative parts, but not rooted in the substrate). In addition, the amphibious (or semiaquatic) species have also been considered, as they colonize wetlands but are able to survive for varying periods on a dry substrate.

Data analysis

The general descriptions for taxonomic aspects, life forms and distribution of the aquatic macrophytes were explored considering the number of taxa per taxonomic family, number of taxa per life form, the frequency of occurrence and latitudinal distribution.

The frequency of occurrence (FO) was calculated from the number of occurrence of each species in relation to the total number of sampling sites. The species were classified as: constant = FO > 50%, common = 10% < FO ≤ 50%, or rare = FO ≤ 10% (Lobo & Leighton 1986).

The graphs were drawn up using the GraphPad Prism 5.0 software (GPW5-066646-RCG7389) (GraphPad Software 2007). The interpolated and extrapolated taxa accumulation curve was developed in the R environment 3.5.1 (R Development Core Team 2018) using the iNEXT package (Hsieh *et al.* 2018) and its sample-size-based protocol (incidence raw data).

RESULTS

We recorded 45 taxa of aquatic macrophytes belonging to 24 families (Table 2; Figure 2). One Cyperaceae taxum could not be identified since it was not fertile when collected. Of the total, 41 taxa are native and the species *Panicum repens* L. (Poales, Poaceae), *Urochloa arrecta* (Hack. ex. T. Durand & Schinz) Morrone & Zuloaga (Poales, Poaceae) and *Hedychium coronarium* J. Koenig (Zingiberales, Zingiberaceae) are exotic (Flora do Brasil 2020 2018).

The families with the highest number of taxa were Cyperaceae (N = 10) and Poaceae (N = 8) (Figure 2). Most species (60%) have the emergent life form (N = 27) (Figure 3). The species *Cyperus blepharoleptos* Steud. (Poales, Cyperaceae) (syn. *Oxycaryum cubense* (Poepp. & Kunth) Lye), often considered epiphytic, was classified in our study as emergent because it was found rooted directly in the river sediment.

The greatest species (N = 25) and life form (N = 6) richness were found in the Itanhaém River. Submerged and floating species were found only in the Itapanhaú and Itanhaém Rivers. In the other rivers, we only recorded the occurrence of emergent and/or amphibious taxa (Table 2).

Crinum americanum L. (Asparagales, Amaryllidaceae), *Spartina alterniflora* Loisel. (Poales, Poaceae) and *Schoenoplectus californicus* (C. A. Mey.) Soják (Poales, Cyperaceae) were the most frequent species (frequency of occurrence respectively 39%, 25% and 24%). These emergent macrophytes along with *Salvinia molesta* D.S. Mitch. (Salviniales, Salviniaceae), *Egeria densa* Planch. (Alismatales, Hydrocharitaceae) and *Eichhornia crassipes* (Mart.) Solms (Commelinales, Pontederiaceae) were classified as common species (13.3% of the total species). The other taxa were classified as rare. No taxum was considered constant in terms of frequency of occurrence (Table 2).

The exotic species *P. repens* and *U. arrecta*, plus eleven native species occurred in only one location. The exotic *H. coronarium* was recorded in two rivers. *Crinum americanum* and *S. californicus* were recorded in seven of the eight sampled rivers (Table 2).

Table 2. List of the aquatic macrophyte species recorded in the coastal rivers of the São Paulo state, Brazil (UM = Ubatumirim; PA = Puruba; IA = Itamambuca; UA = Una; GA = Guaratuba; IU = Itapanhaú; IM = Itanhaém; GU = Guaraú), life forms (LF; EM = emergent; AM = amphibious; FF = free-floating; RF = rooted floating; FS = free submerged; RS = rooted submerged), taxa codes used in Figure 4, Herbarium (HRCB) vouchers, and frequency of occurrence (FO).

Species	LF	Code	HRCB Voucher	UM	PA	IA	UA	GA	IU	IM	GU	FO (%)
ACANTHACEAE												
Hygrophila costata Nees & T. Nees	EM	Hcos	-						X	X		2
AMARYLLIDACEAE												
Crinum americanum L.	EM	Came	-	X	X		X	X	X	X	X	39
ARACEAE												
Pistia stratiotes L.	FF	Pstr	-						X	X		5
ARALIACEAE												
Hydrocotyle ranunculoides L. f.	EM	Hran	-							X		1
ASTERACEAE												
Pluchea sagittalis (Lam.) Cabrera	AM	Psag	-			X						1
Sphagneticola trilobata (L.) Pruski	AM	Stri	73014				X					1
CABOMBACEAE												
Cabomba furcata Schult. & Schult. f.	RS	Cfur	73002						X	X		5
CYPERACEAE												
Cyperus blepharoleptos Steud.	EM	Cble	-			X				X		2
Cyperus odoratus L.	EM	Codo	73004		X							1
Eleocharis acutangula (Roxb.) Schult.	EM	Eacu	73005			X		X				1
Eleocharis interstincta (Vahl) Roem. & Schul.	EM	Eint	73006		X			X				2
Eleocharis minima Kunth	EM	Emin	-							X		1
Fimbristylis dichotoma (L.) Vahl	AM	Fdic	73007		X	X	X					5
Fuirena umbellata Rottb.	EM	Fumb	73008					X				1
Rhynchospora corymbosa (L.) Britton	EM	Rcor	73013			X		X	X	X	X	8
Schoenoplectus californicus (C.A.Mey.) Soják	EM	Scal	-	X	X	X	X	X	X	X		24
Unidentified	EM	Cype	-	X	X							9
HALORAGACEAE												
Myriophyllum aquaticum (Vell.) Verdc.	EM	Maqu	-							X		1

HYDROCHARITACEAE												
Egeria densa Planch.	RS	Eden	-						X	X		10
Limnobium laevigatum (Humb. & Bonpl. ex Willd.) Heine	FF	Llae	-							X		1
JUNCACEAE												
Juncus cf. *marginatus* Rostk.	EM	Jmar	-				X					1
LENTIBULARIACEAE												
Utricularia foliosa L.	FS	Ufol	-							X		1
NYMPHAEACEAE												
Nymphaea rudgeana G. Mey.	RF	Nrud	-						X	X		3
ONAGRACEAE												
Ludwigia decurrens Walter	EM	Ldec	73010			X						3
ORCHIDACEAE												
Habenaria repens Nutt.	EM	Hrep	-							X		1
PLANTAGINACEAE												
Bacopa monnieri (L.) Pennell	EM	Bmon	73002				X					1
POACEAE												
Hymenachne amplexicaulis (Rudge) Nees	EM	Hamp	73009		X							1
Leersia hexandra Sw.	EM	Lhex	-						X	X		3
Panicum repens L.	EM	Prep	73011				X					3
Paspalidium geminatum (Forssk.) Stapf	EM	Pgem	-	X	X							2
Paspalum virgatum L.	EM	Pvir	-		X							1
Spartina alterniflora Loisel.	EM	Salt	-					X	X	X	X	25
Steinchisma laxum (Sw.) Zuloaga	AM	Slax	-		X	X						6
Urochloa arrecta (Hack. ex. T. Durand & Schinz) Morrone & Zuloaga	EM	Uarr	-							X		4
POLYGONACEAE												
Polygonum acuminatum Kunth	EM	Pacu	-		X	X						4
Polygonum punctatum Elliott	EM	Ppun	73012		X	X						2
Eichhornia azurea (Sw.) Kunth	RF	Eazu	-							X		6
Eichhornia crassipes (Mart.) Solms	FF	Ecra	-						X	X		10
PTERIDACEAE												
Acrostichum danaeifolium Langsd. & Fisch.	AM	Adan	-	X		X	X	X	X	X		9

RICCIACEAE									
Ricciocarpos natans (L.) Corda	FF	Rnat	-					X	1
RUBIACEAE									
Richardia grandiflora (Cham. & Schltdl.) Steud.	AM	Rgra	-			X			1
SALVINIACEAE									
Azolla filiculoides Lam.	FF	Afil	-					X	1
Salvinia molesta D.S. Mitch.	FF	Smol	-				X	X	11
TYPHACEAE									
Typha domingensis Pers.	EM	Tdom	-		X			X	4
ZINGIBERACEAE									
Hedychium coronarium J. Koenig	AM	Hcor	-	X	X				3

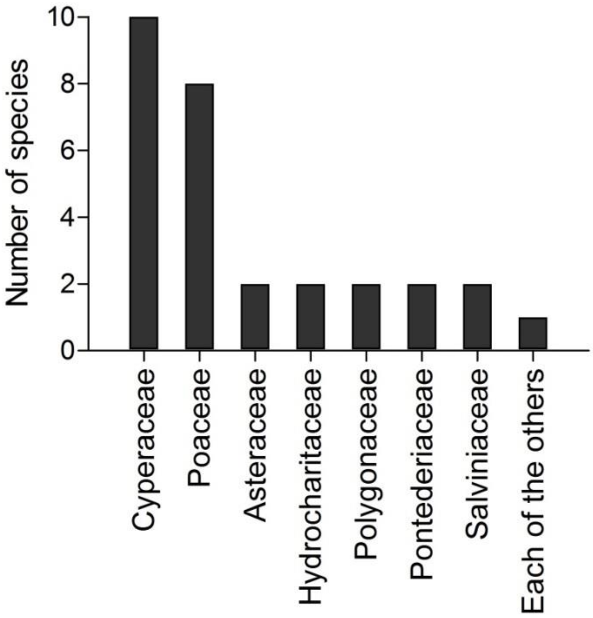

Figure 2. Number of species of aquatic macrophyte per family.

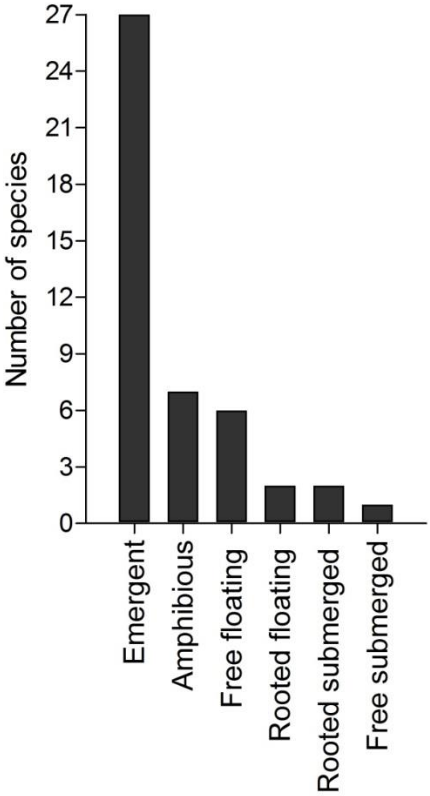

Figure 3. Number of species of aquatic macrophyte per life form.

The aquatic macrophyte distribution occurs in a latitudinal gradient, with only four species (*C. americanum*, *S. californicus*, *Acrostichum danaeifolium* Langsd. & Fisch. (Polypodiales, Pteridaceae) and *Rhynchospora corymbosa* (L.) Britton (Poales, Cyperaceae) (8.9% of the total taxa) occurring along much of the north-south stretch sampled. Eleven taxa occurred only in the northern region, six in the central region and ten taxa occurred only in the southern region of the sampled coastal stretch (Figure 4).

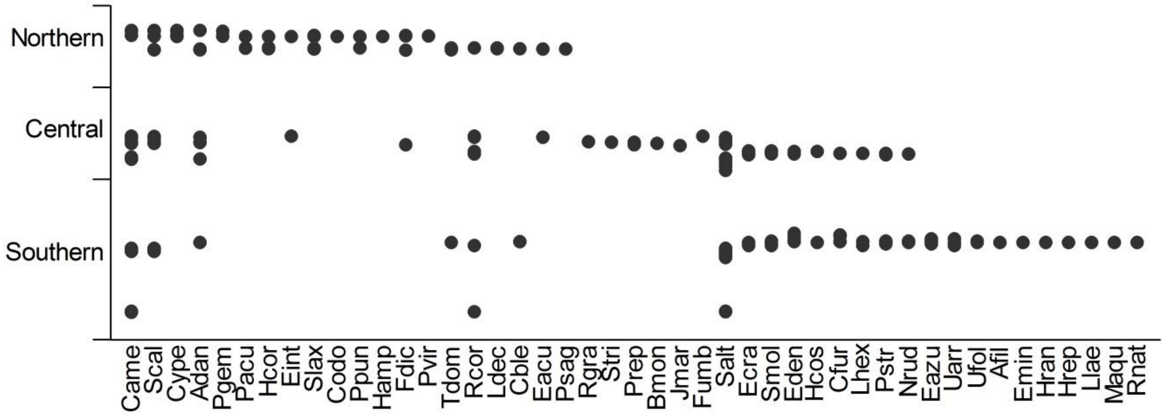

Figure 4. Occurrence of aquatic macrophyte species in the coastal rivers in northern, central and southern coastal regions of the São Paulo state. Taxa codes are shown in Table 2.

The recorded number of aquatic macrophyte taxa increased with the sampling effort, but the interpolated taxa accumulation curve did not reach an asymptote (Figure 5). The extrapolation-sampling curve indicated that other eleven species could still be recorded doubling the number of sampling sites, totaling 56 species (Figure 5). Nevertheless, in 100 sampling sites we recorded 80.36% of the macrophyte species richness in the coastal rivers. Our survey was limited to the main rivers of the coastal basins and whether the sampling was expanded to the tributaries the richness would increase in 19.64%.

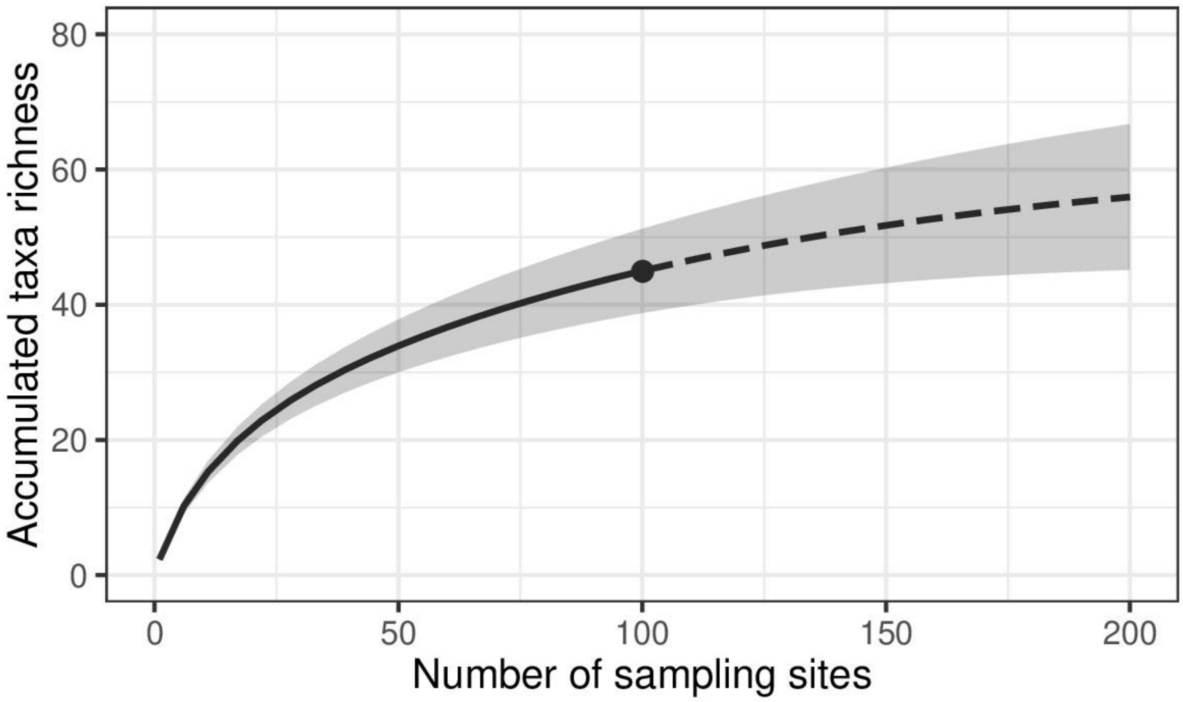

Figure 5. Taxa accumulation curve of the aquatic macrophytes sampled. The solid line is the interpolated rarefaction curve, the dashed line represents the extrapolation-sampling curve and the grey highlight represents the confidence interval (95%) around the curve.

DISCUSSION

From the total taxa recorded in the coastal rivers of São Paulo, 51.1% of these taxa were also found in the Guarapiranga Reservoir, in the São Paulo state Metropolitan Region (Rodrigues *et al.* 2017), 35.5% in the Upper Paraná River (Floodplain ecosystems in Southern Brazil) (Souza *et al.* 2017a) and 24.4% in coastal lakes in the Santa Catarina state (Southern Brazil) (Ferreira *et al.* 2017). Although the aquatic environments are quite distinct, that is, coastal rivers, reservoir, floodplain and coastal lakes; the greatest similarity occurs due to the geographically close locations. Many aquatic macrophyte species found in the São Paulo coast have extensive geographical distributions and are native to tropical America (Lorenzi 2000), such as: *S. californicus*, *E. crassipes* and *P. stratiotes* (Flora do Brasil 2020 2018).

The most frequent species in our study were also found in coastal ecosystems in southern Brazil and in the Atlantic coast of North America. In the Patos Lake estuary, *S. californicus* and *S. alterniflora* are two of the three main aquatic macrophyte species

(Hickenbick *et al.* 2004). In the San Francisco Estuary (West Coast, USA), Watson & Byrne (2009) also recorded the occurrence of *S. californicus*. *Spartina alterniflora* is dominant in estuarine regions on the Atlantic coast of North America (Valilela *et al.* 1978, Adair *et al.* 1994), however its occurrence in Brazil is more restricted to the southeast coast (Flora do Brasil 2020 2018). Although these species are directly influenced by salinity because they occur in stands close to the river mouth, the other species can withstand the salt spray action at different distances of the river mouth and the coastline (Boyce 1954).

We observed that Cyperaceae and Poaceae species corresponded to 22.2% and 17.7% of the total species recorded in the coastal rivers of São Paulo. Similar percentages of Cyperaceae were recorded by Ribeiro *et al.* (2011) (23.7%) and by Ferreira *et al.* (2017) (21.5%) in coastal ecosystems. Cyperaceae and Poaceae are two of the three richer families in aquatic macrophyte species currently known in the main biogeographical areas (Chambers *et al.* 2008). The great occurrence of these families in the coastal rivers is due to the fact that Cyperaceae includes a large number of facultative halophytic species (Sabovljevic & Sabovljevic 2007, Aslam *et al.* 2011) and Poaceae is one of the principal families in which species with great variation in terms of salt tolerance are found (Marcum 2008, Aslam *et al.* 2011, Flowers & Colmer 2015).

We found similar species richness, but greater life form richness than the inventory of aquatic and amphibious plants (50 taxa classified as herbaceous plants in macrophyte banks) made by Ribeiro *et al.* (2011) only in the Massaguaçu River (Northern coast of São Paulo). This river is characterized for being an irregular estuary, that is, it remains some periods without connection with the ocean and, therefore, it frequently presents characteristics of lentic environments (Ribeiro *et al.* 2013). Besides that, in irregular estuaries opportunistic amphibious species may occur in addition to their aquatic ones (Ribeiro *et al.* 2011). The coastal rivers we sampled have regular estuarine zones, that is, they are always connected to the ocean and, therefore, they are lotic environments (L. S. C. Nunes, personal communication). The longitudinal gradient of salinity and flooding in rivers with regular estuaries may be limiting for species richness (Smith *et al.* 2002, Crain *et al.* 2004). However, the marine influence and the different rivers length can promote life form richness and diversity of species composition among the studied coastal region.

The species richness recorded in inventories is varied and this variation may be related to the sampling effort, approach and area (Moura-Júnior *et al.* 2013), as well as the aquatic ecosystems heterogeneity (Souza *et al.* 2017b) and habitat diversity (Moura-Júnior *et al.* 2013). For example, Souza *et al.* (2017a) sampled the main river channel, tributaries and floodplain lakes in the Upper Paraná River (Southern Brazil) totaling 230 km of river stretch and 71 taxa; and Henry-Silva *et al.* (2010) sampled 40 macrophyte taxa in about 210 km long of the Apodi/Mossoró River Basin (Northeast Brazil). We sampled only the main rivers of the coastal basins totaling less than 60 km of rivers and, nevertheless, we found species richness (N = 45) in the same order of magnitude of these other inventories. Thus, we suggest that the diversity of environmental characteristics of the São Paulo coastal rivers favors the aquatic macrophyte richness and diversity in the entire region.

In relation to the sampling effort, we observed from the extrapolation of species richness provided by the taxa accumulation curve that more than 80% of the total expected richness of the coastal region was sampled in our study. Similar percentage was also sampled by Souza *et al.* (2017a). Thus, probably increasing the sampling effort we would record other rare species in some tributaries. Perhaps it would be more interesting that future investigations in this coastal region include other rivers rather than expanding the sampling in these basins that have already been inventoried.

In large geographical scales, environmental factors such as climate and salinity are responsible for the spatial variation of species occurrence and ecological processes among aquatic macrophytes in estuarine areas (Pennings & Bertness 1999, Fariña *et al.* 2017). For example, Fariña *et al.* (2017) observed that over a latitudinal gradient of 2,000 km in the Pacific coast of Chile the variation in the macrophyte species distribution in the aquatic ecosystems is explained by the variation of climatic and edaphic factors and by the tidal regime variation. Those authors also observed that only one species occurs throughout the studied stretch. In our study conducted on a reduced geographical scale, we also observed a north-south gradient of species occurrence that is probably related to the coastal plains width and estuaries characteristics. The species richness of the northern (N = 20) and central-southern regions (N = 25) is not very different, however, the species composition differs greatly between the northern and southern. The species found in the central region are common to the other regions. We observed that few species occur along the entire north-south sampled stretch. These species possibly have a wide tolerance to variation in resource requirements and to salinity.

Although the species richness did not show much variation among the coastal regions, the life form richness did. We found greater life form richness in the central and southern than in the northern region. Emergent and amphibious species were recorded in all regions, but floating and submerged species were not found in the northern. Floating and submerged macrophytes were recorded only in the two largest rivers, possibly because they present backwater and semi-abandoned meandering areas with lower current velocity and sediment deposition due to the greatest rivers length and coastal plains width. Although well distributed in Neotropical aquatic ecosystems (Pott *et al.* 2011, Souza *et al.* 2017a), floating species can be limited by salt (Paudel *et al.* 2018) and water flow (Camargo *et al.* 2003) in coastal lotic ecosystems. Some submerged macrophyte species may be salt tolerant, but they occur in lower richness in coastal areas (Adair *et al.* 1994, Abu-Hena *et al.* 2010, Henry-Silva *et al.* 2010, Ferreira *et al.* 2017).

As also observed by other authors in coastal lotic ecosystems of different geographical regions (Hickenbick *et al.* 2004, Rumrill & Sowers 2008, Watson & Byrne 2009, Ribeiro *et al.* 2011, Janousek & Folger 2014), emergent life form was the dominant one among aquatic macrophytes in the sampled coastal rivers. Emergent species, especially estuarine ones, have propagation, growth and persistence strategies through clonal shoots and long-term rhizomes or stolons (Bertness & Ellison 1987). Many species are tolerant of water level variation (Santos & Esteves 2004, Zhou *et al.* 2018) and the species occurring near the coastline can be positively related to salinity (Janousek & Folger 2014). The amphibious macrophytes, second most frequent life form group in our study, are also tolerant of water stress and have adaptations to occupy dry and wet substrates (Matias *et al.* 2003). However the amphibious are in disadvantage comparing to the emergent ones in terms of growth strategies (Lycarião & Dantas 2017). In relation to these two life forms, there are still difficulties and controversies about the most appropriate classification and characterization for each environment (Bove *et al.* 2003).

Of the total species recorded in the coastal rivers of São Paulo state, only three species are exotic. These species had low frequency of occurrence and were restricted to certain coastal regions. *Urochloa arrecta* was recorded in the Itanhaém River (southern region), *P. repens* in the Una River (central region), and *H. coronarirum* in the Ubatumirim and Puruba Rivers (northern region). These exotic species have been reported as aggressive invaders in aquatic ecosystems of other tropical and subtropical regions, negatively affecting the richness, diversity and abundance of native aquatic macrophytes (Fernandes *et al.* 2013, Michelan *et al.* 2013, Amorim *et al.* 2015, Castro *et al.* 2016, Overholt & Franck 2017).

The African Poaceae *U. arrecta* (syn. *Brachiaria arrecta (Hack. ex T.Durand & Schinz) Morrone & Zuloaga* and *B. subquadripara* (Trin.) Hitchc.) has already been recorded in all Brazilian regions (Flora do Brasil 2020 2018) and it has been observed in different aquatic ecosystems such as Pantanal wetlands (Pott *et al.* 2011), reservoirs (Michelan *et al.* 2010, Rodrigues *et al.* 2017) and coastal rivers and lakes (Amorim 2015, Ferreira *et al.* 2017).

Panicum repens is native to Australia and it is considered an invasive of difficult control in the southern United States (Sutton 1996). In Brazil, this species was recorded in disturbed flooding areas of the Pantanal (Pott *et al.* 2011) and reservoirs in the Paraná River Basin (Agostinho *et al.* 2005) however, its invasive status is still less documented in the country. The amphibious *H. coronarium* is native to Tropical Asia and it has been very common in the Brazilian coastal zone where it is invasive in wetlands, along water courses and in the sub-forest of the Atlantic Rainforest (Soares & Barreto 2008). Although these three exotic species did not present expressive frequencies of occurrence in the São Paulo state coast, their monitoring and management can be actions to avoid their dispersion and establishment in new areas, and consequently avoid changes in ecosystem functioning (Bove *et al.* 2003, Souza *et al.* 2017a).

In conclusion, we observed a north-south gradient of macrophyte species distribution in the coastal rivers of São Paulo state. Only three species and only emergent and amphibious life forms occur along the entire sampled coastal stretch. We found the greatest life form richness in the central-southern region, although the species richness of the northern and central-southern is not very different. Thus, we suggest that the macrophytes diversity and the north-south gradient of species distribution may be related to the diversity of environmental characteristics of the coastal rivers due to differences in the coastal plains width and rivers length by the presence of the Serra do Mar.

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Capítulo 2

A HETEROGENEIDADE AMBIENTAL INFLUENCIA A RIQUEZA DE FORMAS DE VIDA E A COMPOSIÇÃO DE ESPÉCIES, MAS NÃO A RIQUEZA DE ESPÉCIES DE MACRÓFITAS AQUÁTICAS EM RIOS COSTEIROS TROPICAIS

Cyperus odoratus, Rio Puruba, Ubatuba/SP

Environmental heterogeneity drives life form richness and species composition but not species richness of aquatic macrophytes in tropical coastal rivers*

Resumo:

1. Os rios costeiros do estado de São Paulo no sudeste do Brasil têm diferentes comprimentos e influências de águas marinhas. Nós avaliamos se (i) a heterogeneidade ambiental influencia a riqueza de espécies e formas de vida de macrófitas aquáticas; e (ii) a heterogeneidade ambiental e a distância geográfica moldam a composição de espécies nos rios costeiros.

2. Nós registramos a ocorrência de espécies e formas de vida de macrófitas aquáticas e coletamos variáveis explanatórias da água, do sedimento e do canal do rio em 100 pontos de coleta em 8 rios. Nós aplicamos a Análise de Componentes Principais (PCA) às variáveis explanatórias e calculamos a heterogeneidade ambiental dos rios usando a variação dos escores da PC1. Nós determinamos a posição de cada foz dos rios ao longo da linha de costa para medir a distância geográfica entre os rios. Nós usamos Modelos Lineares Generalizados quasi-Poisson (GLM) para avaliar os efeitos a heterogeneidade ambiental sobre a riqueza de espécies e formas de vida. Para determinar o efeito a heterogeneidade ambiental e da distância geográfica (matrizes de distância Euclidiana) na variação na composição de espécies (matriz de dissimilaridade de Jaccard) entre os rios nós aplicamos Regressões Múltiplas em Matrizes de Distância (MRM).

3. Nós encontramos que os rios variavam em até cinco vezes em termos de heterogeneidade ambiental. A salinidade do sedimento, a largura do rio, o fósforo total da água e a distância da foz foram as variáveis que mais contribuíram para a heterogeneidade ambiental dos rios. A heterogeneidade ambiental não explicou a variação na riqueza de espécies; no entanto, teve uma relação positiva significativa com a riqueza de formas de vida. O efeito da heterogeneidade ambiental foi maior do que o efeito da distância geográfica na variação da composição de espécies entre os rios. Os pares de rios com heterogeneidade ambiental mais semelhante foram os mais semelhantes em composição de espécies, porém nem todos os rios foram geograficamente próximos.

4. Nós concluímos que a heterogeneidade ambiental influencia a riqueza de formas de vida, mas não influencia a riqueza de espécies de macrófitas aquáticas nos rios costeiros que estudamos; entretanto, a heterogeneidade ambiental molda a composição de espécies independentemente da distância geográfica entre os rios.

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Palavras-chave: plantas aquáticas, composição de macrófitas, estuários, heterogeneidade de rios, planície costeira.

Abstract:

1. The coastal rivers of the São Paulo State in SE Brazil have different lengths and seawater influence. We evaluated whether (i) environmental heterogeneity (EH) drives the species and life form richness of aquatic macrophytes; and (ii) EH and geographical distance shape species composition in these coastal rivers.

2. We recorded the macrophyte species and life form occurrence and collected explanatory variables characterizing the water, sediment and river channel at 100 sampling sites over 8 rivers. We applied a Principal Component Analysis (PCA) to the explanatory variables and calculated the rivers' EH using the range of PC1 scores. We also determined the position of each river mouth along the coastline to measure the distance between the rivers. We used quasi-Poisson Generalized Linear Models (GLM) to evaluate the effects of EH on richness of species and life forms. To determine the effect of EH and geographical distance (Euclidean distance matrices) on the variation in species composition (Jaccard dissimilarity matrix) among the rivers we applied Multiple Regressions on Distance Matrices (MRM).

3. We found that the rivers varied up to five times in terms of EH. Sediment salinity, river width, total phosphorus of water and distance from river mouth were the most important variables contributing to the rivers' EH. EH did not explain variation in species richness; however, it had a significant positive relationship with life form richness. The effect of EH was greater than that of the geographical distance on the variation in species composition among the rivers. The pairs of rivers with the most similar EH were the most similar in species composition, but not all of them were geographically close.

4. We conclude that EH drives the life form richness but does not influence the species richness of aquatic macrophytes in the coastal rivers we studied; however, EH does shape the species composition regardless of the rivers' geographical distance.

Keywords: aquatic plants, macrophyte composition, estuaries, river heterogeneity, coastal plain.

Introduction

Environmental heterogeneity (EH) has been reported as one of the main factors that explain species diversity (Reynolds, Hungate, Chapin, & D'Antonio, 1997). EH can promote high species richness (MacArthur & MacArthur, 1961) due to the increase in habitat complexity and availability of resources and niches (Tews, Brose, Grimm, & Tielbörger, 2004; Stein, Gerstner, & Kreft, 2014). Different environmental conditions and EH may also shape community composition (Xiu, Keming, & De, 2012). However, the relationship between EH and community structure in ecosystems may be dependent on both the spatial scale analysed (Pringle, Naiman, Bretschko, Karr, Oswood, Webster, … Winterbourn, 1988; Tamme, Hiiesalu, Laanisto, Szava-Kovats, & Pärtel, 2010; Bar-Massada & Wood, 2014) and characteristics of the taxonomic groups in question (Allouche, Kalyuzhny, Moreno-Rueda, Pizarro, & Kadmin, 2012; Xiu et al., 2012). Furthermore, the term "heterogeneity" has been used for various aspects of environmental variability (Shi, Ma, Wang, Zhao, & He, 2010; Constanza, Moody, & Peet, 2011; Alahuhta, Kosten, Akasaka, Auderset, Azzella,…& Heino, 2017) and its different uses may lead to different results related to communities (Stuefer, 1996).

The communities' composition may also be influenced by geographical distance, because the distance between ecosystems may lead to differences in their environmental characteristics and EH, and limit species dispersal (Barrat-Segretain, 1996; Bini, Thomaz, & Souza, 2001; Capers, Selski, & Bugbee, 2010). Thus, ecosystems that are geographically closer to each other tend to be more similar in their species composition than more distant ones (Kuglerová, Jansson, Sponseller, Laudon, & Malm-Renöfālt, 2015).

Coastal lotic ecosystems are heterogeneous at different spatial scales (Cooper, Barmuta, Sarnelle, Kratz, & Diehl, 1997). At the local scale, coastal rivers have longitudinal gradients of salinity, flooding and nutrient concentration due to the influence of seawater (Bertness, 1991). At the regional scale, these ecosystems vary in terms of dimension and tidal influence due to the topographic and geomorphological characteristics of the coastal plain (Costa, Marangoni, & Azevedo, 2003; Morzaria-Luna, Callaway, Sullivan, & Zedler, 2004; Kunza & Pennings, 2008). The differences in regional characteristics in aquatic ecosystems may affect local conditions promoting EH (Schneider, Cunha, Spíndola, Marchese, & Thomaz, 2019), and thus, variation in community structure at different spatial extents (Barton, Cunningham, Manning, Gibb, Lindemayer, & Didham, 2013; Heino, Melo, & Bini, 2015).

Aquatic macrophyte communities play an important role in productivity, habitat formation and retention of sediments and nutrients in coastal wetlands (Wieski, Guo, Craft, & Pennings, 2010). The structure and composition of these communities may be mainly shaped by variation in environmental conditions, especially those related to the ecosystems' differences in salinity and morphometry at regional scales (Odum, 1988), as many macrophyte species are not strongly dispersal-limited even at large spatial extents (Alahuhta, Kanninen, Hellsten, Vuori, Kuoppala, & Hämäläinen, 2013). There are still few studies testing hypotheses related to EH and macrophyte communities' structure. These studies have been carried out mainly at high latitudes (Alahuhta, 2015; Fernández-Aláez, García-Criado, García-Giron, Santiago, & Fernández-Aláez, 2020) and in some cases at small spatial scales (Shi, Ma, Wang, Zhao, & He, 2010; Xiu et al., 2012). Thus, there is a lack of studies with this approach in tropical and subtropical regions, at large spatial scales, and especially in coastal wetlands. One of the few papers focusing on the effect of EH on macrophyte assemblages in coastal wetlands in South America was developed by Rolon, Lacerda, Maltchik, and Guadagnin (2008). These authors found that macrophyte richness and composition in coastal lacustrine and palustrine environments of southern Brazil were significantly explained by habitat diversity in addition to other environmental factors (Rolon et al., 2008).

The rivers located in the coastal region of the São Paulo State (SE Brazil) are isolated from each other and have different lengths and seawater influence due to the variation in the characteristics of the coastal plain. These features make these rivers appropriate to evaluate the role of EH on macrophyte richness and composition. We evaluated whether (i) EH drives the species and life form richness of aquatic macrophytes, and (ii) EH and geographical distance shape the species composition in the coastal rivers. We expected that (i) EH would be positively related to both species and life form richness, thus, similar EH would drive similar richness; and that (ii) the rivers with similar EH would have similar species composition regardless of their geographical distance. Therefore, EH would be a better predictor to the variation in species composition among the coastal rivers than their geographical distance.

Methods

Study area

The Atlantic mountain range (Serra do Mar) is present along the entire 400 km coastline of the São Paulo State (SE Brazil, South America) (Almeida & Carneiro, 1998). Along this coastal region, there is considerable variation in distance between the Serra do Mar and the coastline (Ponçano, Tessler, Freitas, & De Mahiques, 1999; Tessler, Goya, Yoshikawa, & Hurtado, 2006). Thus, the coastal plain is varied in terms of width, slope, elevation (Souza & Cunha, 2011) and geological formation (Suguio & Martin, 1978). The coastal basins are isolated from each other because the headwaters are located at high altitudes in the Serra do Mar and the rivers drain directly into the sea (Almeida & Carneiro, 1998). In general, in the southern and central coastal region of São Paulo the Serra do Mar is further from the coastline, thus, the coastal plain is larger and flatter. The southern and central rivers are longer and have a stronger salinity gradient due to the great seawater influence on them. Instead, in the northern coast the Serra do Mar is nearest to the coastline, thus, the coastal plain is shorter and steeper. The northern rivers are shorter and have freshwater predominance due to the weak seawater influence (Table 1).

Table 1. River length on the coastal plain, coastal plain width, coastal plain slope and upstream limit of the salinity influence in sediment.

Coastal rivers	**River length on the coastal plain (km)**	**Coastal plain width (km)**	**Coastal plain slope (e^{-3} %)**	**Upstream limit of the salinity influence (km)**
Ubatumirim	2.43	2.00	3.42	0.82
Puruba	2.62	2.80	4.60	1.54
Itamambuca	1.84	1.50	4.92	0.39
Una	3.28	3.90	3.58	1.89
Guaratuba	8.18	6.10	2.92	6.18
Itapanhaú	35.84	4.90	0.93	13.50
Itanhaém	19.00	14.50	0.70	9.25
Guaraú	10.13	5.50	1.74	9.20

The São Paulo coastal region has small seasonal variations in climatic characteristics (Monteiro, 1973) and a relatively constant and stable water flow in aquatic ecosystems over the year. The *Köppen-Geiger climate classification* is Af, that is, tropical humid climate, mild winters, rainfall in all months of the year and absence of a defined dry season (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2013). The average annual temperature in the region is 23.6ºC (average summer temperature = 26.46°C and average winter temperature = 20.15°C), and the average annual rainfall is 2,260 mm (average summer rainfall = 892 mm and average winter rainfall = 315 mm) (Embrapa, 2015). Tides are micro-tidal, regular, lunar and semidiurnal. That is, the tidal range is less than two meters and there are two complete cycles of tides (high tide and low tide) per lunar day (24 h and 50 min) (Tessler et al., 2006).

We selected 8 rivers to study along a coastline length of 280 km (Figure 1) based on the following criteria: (i) variation in the north-south distance gradient between the Serra do Mar and the coastline, (ii) the occurrence of regular estuarine zones (permanent connection of the rivers to the ocean) and (iii) lack of heavy human influence. These criteria were determined since we were interested in sampling the possible variation of the rivers in terms of EH due to the differences in the characteristics of the coastal plain.

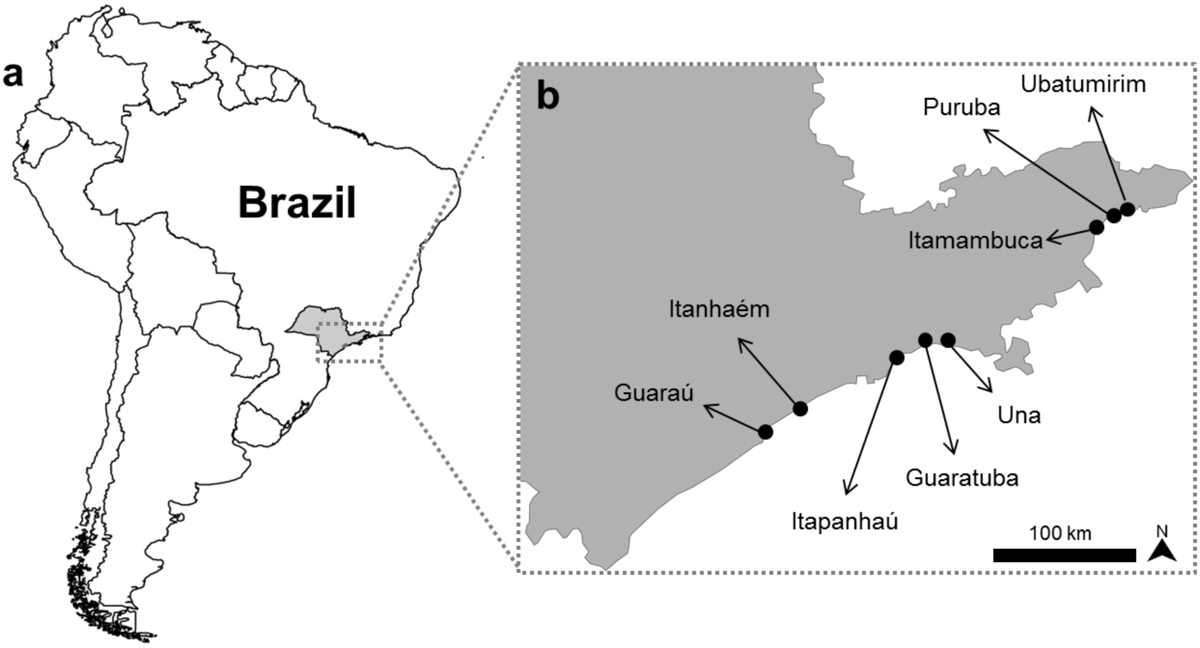

Figure 1. Maps of South America, Brazil (a) and the coastal region of São Paulo State (b), highlighting the rivers' locations on the coastline.

Data sampling in the field

Tropical coastal rivers of Brazil are vegetated by mangrove and Restinga forests (tidal arboreal vegetation) in the intertidal plains, and by macrophyte stands that fringe the forests (Schaeffer-Novelli et al., 1990). Macrophyte stands occur in short (up to 20 m width) and shallow areas (up to 1.5 m deep) of sediment deposition external to the forest and are distributed in a mosaic, forming herbaceous patches along the rivers' longitudinal gradient (Nunes & Camargo, 2020). At low tide, the macrophytes (including the submerged ones) are partially or fully exposed, which allows viewing of all stands along the rivers. Thus, the distribution of macrophytes in these tropical coastal rivers is different from that in temperate and subtropical ones, where macrophytes occur in different elevational zones in intertidal marshes (Engels & Jensen, 2010).

We collected the data in March 2017, sampling only once because most estuarine macrophytes are perennial (Engels, 2010), the rains are constant throughout the year, and the temperature does not fluctuate much in the study region. We used a small boat to travel along the main rivers of the coastal basins from the furthest upstream point of possible navigation to the estuarine mouth. We sampled all macrophyte stands on both sides of the river and recorded the presence of species regardless of stand size. Each macrophyte stand corresponded to a sampling site. We considered a macrophyte stand each macrophyte patch isolated from each other regardless of their size and composition (mixed or monospecific). The macrophyte stands varied in length from 2 to 300 m parallel to the littoral. We sampled both hydrophyte and amphibious (plants that colonize wetlands but are able to survive for varying periods in dry substrate) macrophytes on sand banks in the river channel and on the margins up to 1.5 m away from the water towards the dry substrate. We collected and archived macrophyte taxa that could not be identified in the field for later identification using Pott and Pott (2000), Amaral, Bittrich, Faria, Anderson, and Aona (2008), and Rodrigues, Souza, and Pompêo (2017). The macrophytes were classified into six life forms according to Chambers, Lacoul, Murphy, and Thomaz (2008): emergent, free floating, rooted floating, rooted submerged, free submerged and amphibious. The number of sampling sites varied from 9 to 19 per river, totaling 100 sampling sites across all rivers. The variation of the number of sampling sites per river was due to the different number of macrophyte stands in each river.

At each sampling site, that is, in each macrophyte stand, we collected samples from water (up to 30 cm depth) and sediment (up to 15 cm belowground) using plastic bottles and cups, respectively. We measured pH and dissolved oxygen of water, the littoral slope and river width in the sampling sites (Table 2). In the laboratory, we determined total phosphorus and nitrogen of water and sediment, and sediment salinity (Table 2). We performed granulometric analysis and classified the sediment grain-size distribution (silt, clay and sand) according to the Udden-Wentworth scale (Wentworth, 1922). After that, we defined three sediment types and classified the sediment of each sampling site into them. The sediment types we used were: (1) 50% or more very fine grains (silt, clay and very fine sand), (2) 50% or more fine or medium grains (fine and medium sand) and (3) 50% or more coarse grains (coarse and very coarse sand) (Table 2).

Table 2. Local and basin variables measured in the coastal rivers of the São Paulo State and their respective codes and methods.

Variables	**Code**	**Methods**
From field sampling		
Water:		
Total phosphorus (µg L^{-1})	TPw	Golterman, Climo, and Ohnstad (1978)
Total nitrogen (mg L^{-1})	TNw	Mackereth, Heron, and Talling (1978)
Dissolved oxygen (mg L^{-1})	DO	Horiba U-50 Multi-parameter water quality checker
pH		
Sediment:		
Total phosphorus (% dry mass)	TPsed	Drying oven (60°C), Golterman et al. (1978)
Total nitrogen (% dry mass)	TNsed	Drying oven (60°C), Mackereth et al. (1978)
Salinity	salinity	Digital salt-meter Instrutemp Nutra-Wand Combo
Granulometry (%) and sediment type	Sediment type	Drying oven (60°C), Wentworth (1922), Sediment type 1, 2 and 3
River channel:		
Littoral slope (%)	Littoral slope	Measuring tape
River width (m)	width	Laser telemeter Bushnell Yardage Pro 500
From remotely sensed images		
For each sampling site:		
Distance from river mouth (km)	mouth distance	GPS Garmin and Google Earth Pro
Distance from coastline (km)	coastline distance	
For each river:		
Geographical distance (km)		Google Earth Pro

Obtaining data from remotely sensed images

For each sampling site we obtained the distance from river mouth (following the river channel) and the distance from coastline (in a straight line) (Table 2). We also determined the position of each river's mouth on the coastline (Table 2), setting most northernly study river (the Ubatumirim River) at zero and the river located furthest south (the Guaraú River) as the maximum value (280 km). Thus, the geographical distance between any pairs of rivers was their difference in km. To obtain these data we used remotely sensed images from Google Earth Pro 7.1 (Google LLC 2020, Mountain View, USA).

Statistical analysis

To determine the EH of each coastal river, we applied a Principal Component Analysis (PCA) to the explanatory (all the variables in Table 1 except for geographical distance). We then calculated the range of PC1 scores (maximum minus minimum values) for the sampling sites of each river. We used the range of PC1 scores as a measure of EH. A similar method for measuring EH was done by Pinha, Tramonte, Bilia, and Takeda (2017) using PC1 scores and their coefficient of variation. Although we found that the explanation of the PC2 is not trivial (20.69%), we opted to retain only PC1 because it represented most of the data variation (37.66%).

The most commonly used EH measurements are the coefficient of variation, variance or range of some abiotic variables or groups of them (Shi et al., 2010; Constanza, Moody, & Peet, 2011; Alahuhta, Kosten, Akasaka, Auderset, Azzella,…& Heino, 2017). We chose to use the range of PC1 scores so we could summarize and consider the variation of the entire dataset with only one EH measure, considering that PC1 did not increase with the number of sampling sites per river.

To evaluate whether EH influenced macrophyte richness, we used Generalized Linear Models (GLM), which are extensions of standard linear models that accommodate various non-normal error distributions (Nelder & Wedderburn, 1972). The species richness (number of species) and life form richness (number of life forms) were modeled using quasi-Poisson models because of overdispersion in the count data. Goodness of fit was measured by deviance statistics (D). The half-normal plot (Atkinson, 1985) was used for model diagnostics (if the model is well adjusted most of the points will be within the confidence bands and randomly distributed).

To assess the similarity in macrophyte species composition among the coastal rivers, we applied cluster analysis using the Jaccard dissimilarity index and the UPGMA method (Unweighted pair-group method using arithmetic averages) to the data of species presence and absence in each river. We validated the efficiency of the applied *clustering* method through the cophenetic correlation, which measures how faithfully a dendrogram preserves the pairwise distances between the original unmodeled data points (Saraçli, Doğan, & Doğan, 2013). We assumed that a cophenetic correlation coefficient (CPCC) greater than 0.70 indicated good clustering.

We calculated the Euclidean distance between pairs of rivers using EH and geographical distance. To determine whether EH and geographical distance explained variation in macrophyte species composition among the coastal rivers, we applied Multiple Regressions on Distance Matrices (MRM) among its Euclidean distance matrices and the dissimilarity matrix of species composition (Jaccard dissimilarity matrix). Prior to the MRM application, we tested the Pearson's correlation between EH and geographical distance and verified that they were not strongly correlated ($r = 0.54$).

We calculated the sole and shared effect of EH and geographical distance to variation in macrophyte species composition among the coastal rivers with variation partitioning equations (Borcard, Legendre, & Drapeau, 1992). Variation partitioning measures the amount of variation in the response matrix that can be attributed to a single explanatory matrix and the variation explained by two or more matrices.

The analyses were performed in the R environment 3.6.1 (R Core Team 2019, Vienna, Austria) using the vegan (Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., ... & Solymos, P., 2019) and ecodist (Goslee & Urban, 2007) packages. Graphic illustrations were created using GraphPad Prism 5.0 software (GraphPad Software 2007, San Diego, USA) and the ggplot2 package (Wickham, 2016) in the R environment.

Results

We recorded a total of 45 taxa and 6 life forms of aquatic macrophytes (Figure 2, Table S1). The number of taxa per river varied from 3 to 25, with the greatest and lowest richness found in the Itanhaém and Guaraú River, respectively (Figure 2 a). The Itanhaém and Itapanhaú Rivers presented the greatest life form richness (6 and 5, respectively). In the

Guaraú River only emergent aquatic macrophytes occurred. In the other rivers, we recorded the occurrence of emergent and amphibious macrophytes (Figure 2 b).

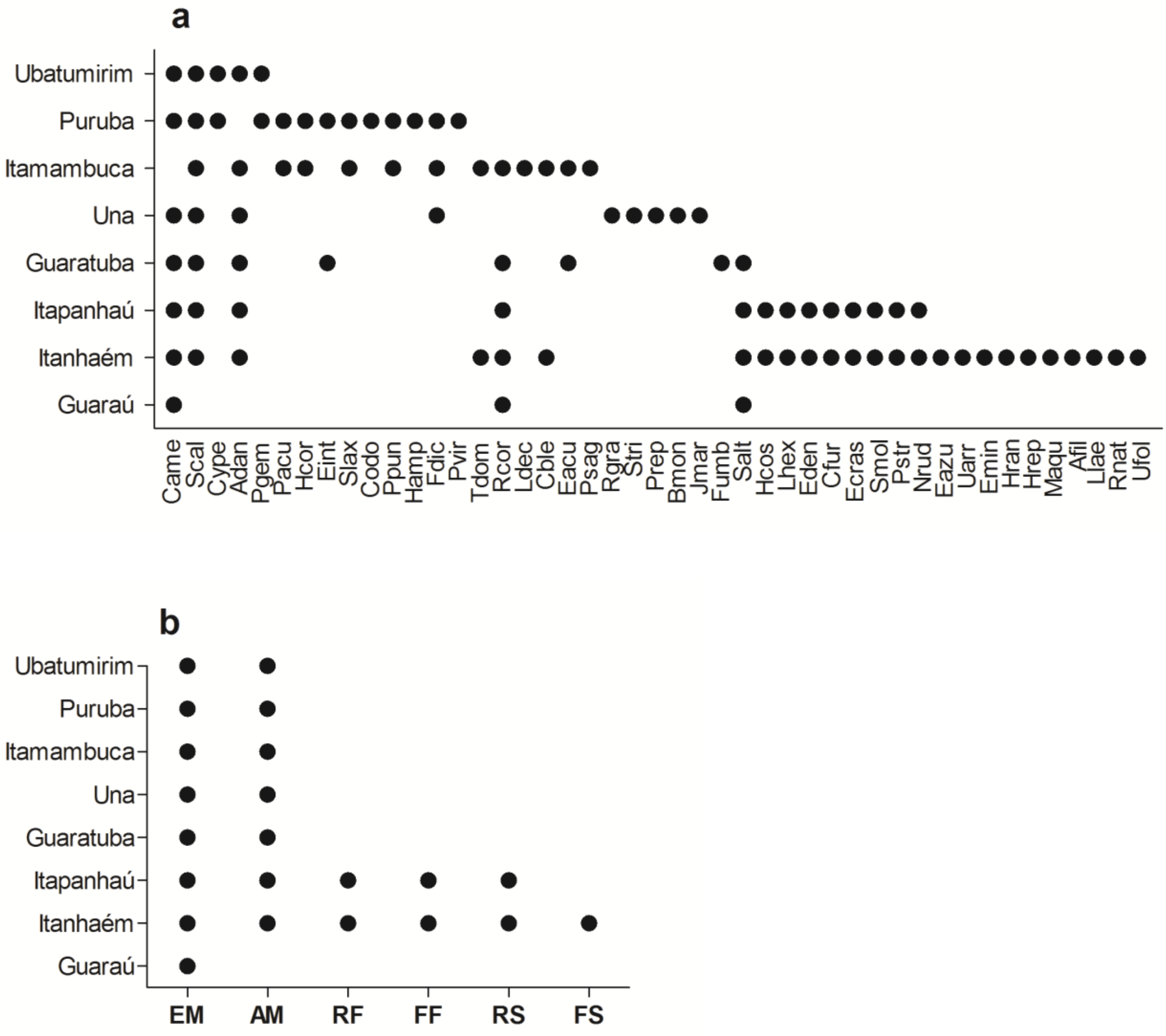

Figure 2. Occurrence of the macrophytes taxa (a) and life forms (b) in the São Paulo coastal rivers. **Macrophyte taxa:** *Crinum americanum* (Came), *Schoenoplectus californicus* (Scal), Cyperaceae unidentified (Cype), *Acrostichum danaeifolium* (Adan), *Paspalidium geminatum* (Pgem), *Polygonum acuminatum* (Pacu), *Hedychium coronarium* (Hcor), *Eleocharis intestincta* (Eint), *Steinchisma laxum* (Slax), *Cyperus odoratus* (Codo), *Polygonum punctatum* (Ppun), *Hymenachne amplexicaulis* (Hamp), *Fimbristylis dichotoma* (Fdic), *Paspalum virgatum* (Pvir), *Thypha domingensis* (Tdom), *Rhynchospora corymbosa* (Rcor), *Ludwigia decurrens* (Ldec), *Cyperus blepharoleptos* (Cble), *Eleocharis acutangula* (Eacu), *Pluchea sagittalis* (Psag), *Richardia grandiflora* (Rgra), *Sphagneticola trilobata* (Stri), *Panicum repens* (Prep), *Bacopa monnieri* (Bmon), *Juncus* cf. *marginatus* (Jmar), *Fuirena umbellata* (Fumb), *Spartina alterniflora* (Salt), *Hygrophila costata* (Hcos), *Leersia hexandra* (Lhex), *Egeria densa* (Eden), *Cabomba furcata* (Cfur), *Eichhornia crassipes* (Ecra), *Salvinia molesta* (Smol), *Pistia stratiotes* (Pstr), *Nymphaea rudgeana* (Nrud), *Eichhornia azurea* (Eazu), *Urochloa arrecta* (Uarr), *Eleocharis minima*, (Emin), *Hydrocotyle ranunculoides* (Hran), *Habenaria repens* (Hrep), *Myriophyllum aquaticum* (Maqu), *Azolla filiculoides* (Afil), *Limnobium laevigatum* (Llae), *Ricciocarpus natans* (Rnat), *Utricularia foliosa* (Ufol). **Life forms:** emergent (EM), amphibious (AM), rooted floating (RF), free floating (FF), rooted submerged (RS), free submerged (FS).

The eight rivers differed in their range of PC1 scores (Figure 3). Sediment salinity, river width, total phosphorus concentration in the water and distance from the river mouth were the strongest variables ($r > 0.60$) related to axis 1 of the PCA, and thus they were the most determinant variables to the ordination of the sampling sites (Table 3). The minimum and maximum values of each explanatory variable in each river are shown in Table S2.

Some rivers (e.g., rivers 3, 4, 6 and 8) also had a considerable range of scores along the second axis of the PCA (Figure 3). Although we did not include PC2 in our measure of rivers' EH, variation across PC2 was mainly related to sediment nutrient content (TNsed and TPsed) (Fig. 3, Table 2).

Based on PC1, the rivers varied considerably in terms of EH. The Itanhaém, Itapanhaú, Guaraú and Guaratuba Rivers had a greater EH than the Ubatumirim, Puruba, Una and Itamambuca Rivers (Table 4). The most heterogeneous river (Itanhaém) had heterogeneity score about five times greater than the least heterogeneous river (Itamambuca) (Table 4).

The Ubatumirim, Puruba and Itamambuca Rivers were the geographically closest rivers to each other and the most distant from the other rivers. The Itamambuca and Una Rivers, and the Itapanhaú and Itanhaém Rivers were the pairs of rivers with the greatest geographical distance between them (respectively, 106 and 81 km) (Table 4).

The most heterogeneous river (the Itanhaém River) had the greatest species richness (Figure 4 a) and the two most heterogeneous rivers (the Itanhaém and Itapanhaú Rivers) had the greatest life form richness (Figure 4 b).

Species richness was not significantly related to EH ($D = 23.64$; $P = 0.33$) (Table 5). However, life form richness increased with EH ($D = 3.20$; $P = 0.02$) (Table 5).

The cluster dendrogram based on the dissimilarity of macrophyte species (CPCC = 0.79) showed that the Itanhaém and Itapanhaú Rivers were the most similar rivers in terms of species composition, followed by the Guaratuba and Guaraú Rivers. The species composition of the Puruba and Itamambuca Rivers was less similar to that of the six other rivers (Figure 5).

The results of the MRM showed that EH (R^2=0.37) and geographical distance (R^2=0.22) were both important in explaining the variation in species composition among rivers (Table 6); however, the sole effect of EH (17.95%) was greater than that of the geographical distance (2.70%). The shared effect of these two variables to the variation in species composition was 19.16% and the unexplained variation was 60.19% (Table 6).

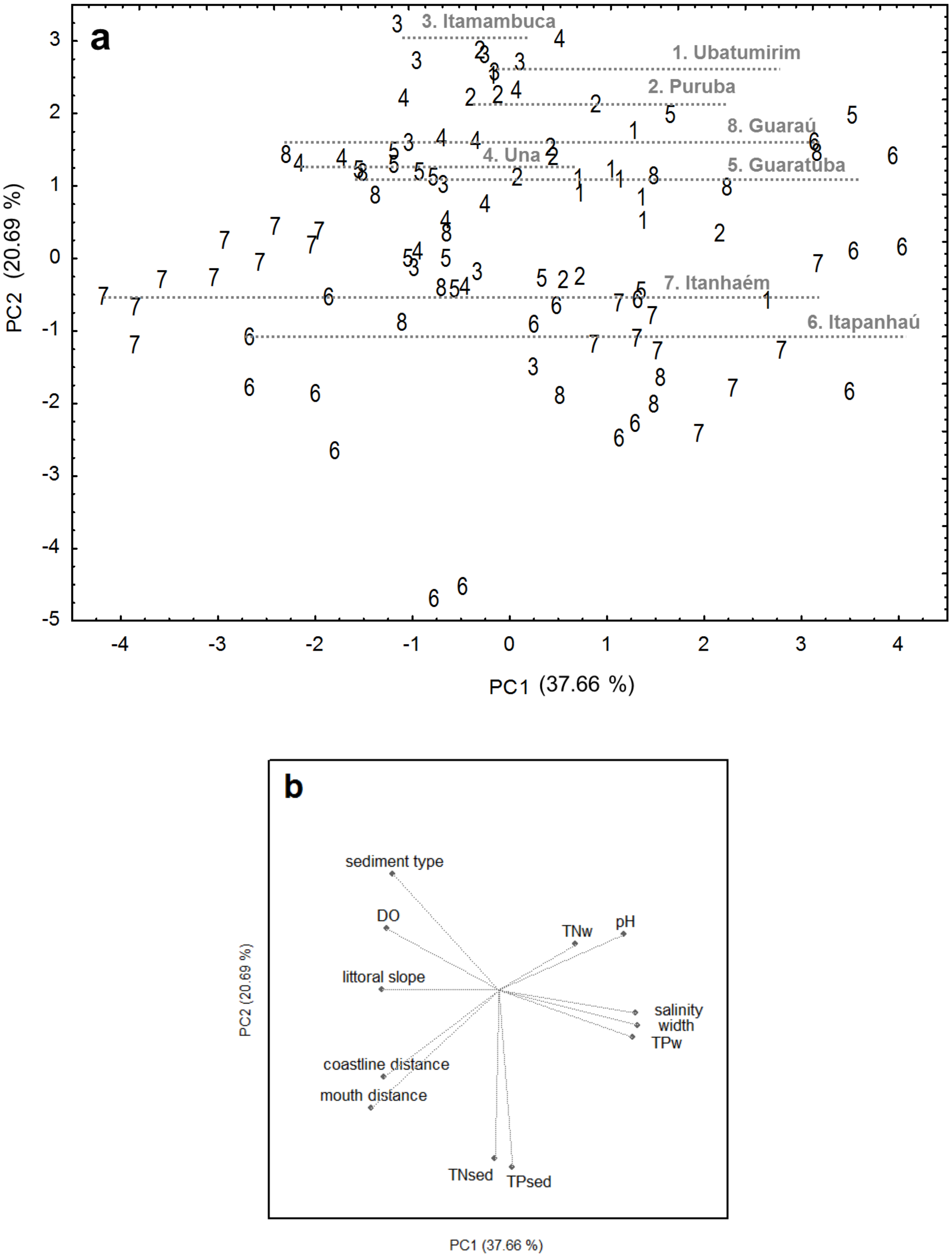

Figure 3. Biplot of the Principal Components Analysis showing the ordination of the sampling sites (a) and the explanatory variables related to the axis (b). The dotted lines of panel (a) represent the range of PC1 scores of each river. The eigenvector of the variables in relation to the axis 1 and 2 of the PCA are shown in Table 3. Coastal Rivers: Ubatumirim (1), Puruba (2), Itamambuca (3), Una (4), Guaratuba (5), Itapanhaú (6), Itanhaém (7) and Guaraú (8). The variables' codes are shown in Table 2.

Table 3. The eigenvector of the variables in relation to axes 1 and 2 of the Principal Components Analysis; and the proportion explained by both axes. The most important variables loading onto PC1 and PC2 (r > 0.60) are highlighted in bold. The variables' codes are shown in Table 2.

Variables	PC1	PC2
salinity	**0.67**	-0.17
sediment type	-0.50	0.56
mouth distance	**-0.61**	-0.57
coastline distance	-0.55	-0.42
width	**0.66**	-0.11
Littoral slope	-0.56	0.001
TNw	0.37	0.22
TPw	**0.64**	-0.23
TNsed	-0.02	**-0.81**
TPsed	0.06	**-0.85**
DO	-0.54	0.29
pH	0.60	0.26
% explained	37.66	20.69

Table 4. Maximum and minimum PC1 scores used to calculate the environmental heterogeneity (EH) of each coastal river, and the geographical distance of the rivers from North to South along the coast.

Coastal Rivers	**Maximum PC1 scores**	**Minimum PC1 scores**	**EH**	**Geographical distance (km)**
Ubatumirim	2.65	-0.17	2.82	0
Puruba	2.16	-0.40	2.56	5
Itamambuca	0.24	-1.15	1.39	16
Una	0.52	-2.16	2.68	122
Guaratuba	3.52	-1.55	5.07	138
Itapanhaú	4.04	-2.67	6.71	167
Itanhaém	3.17	-4.17	7.34	248
Guaraú	3.16	-2.30	5.46	280

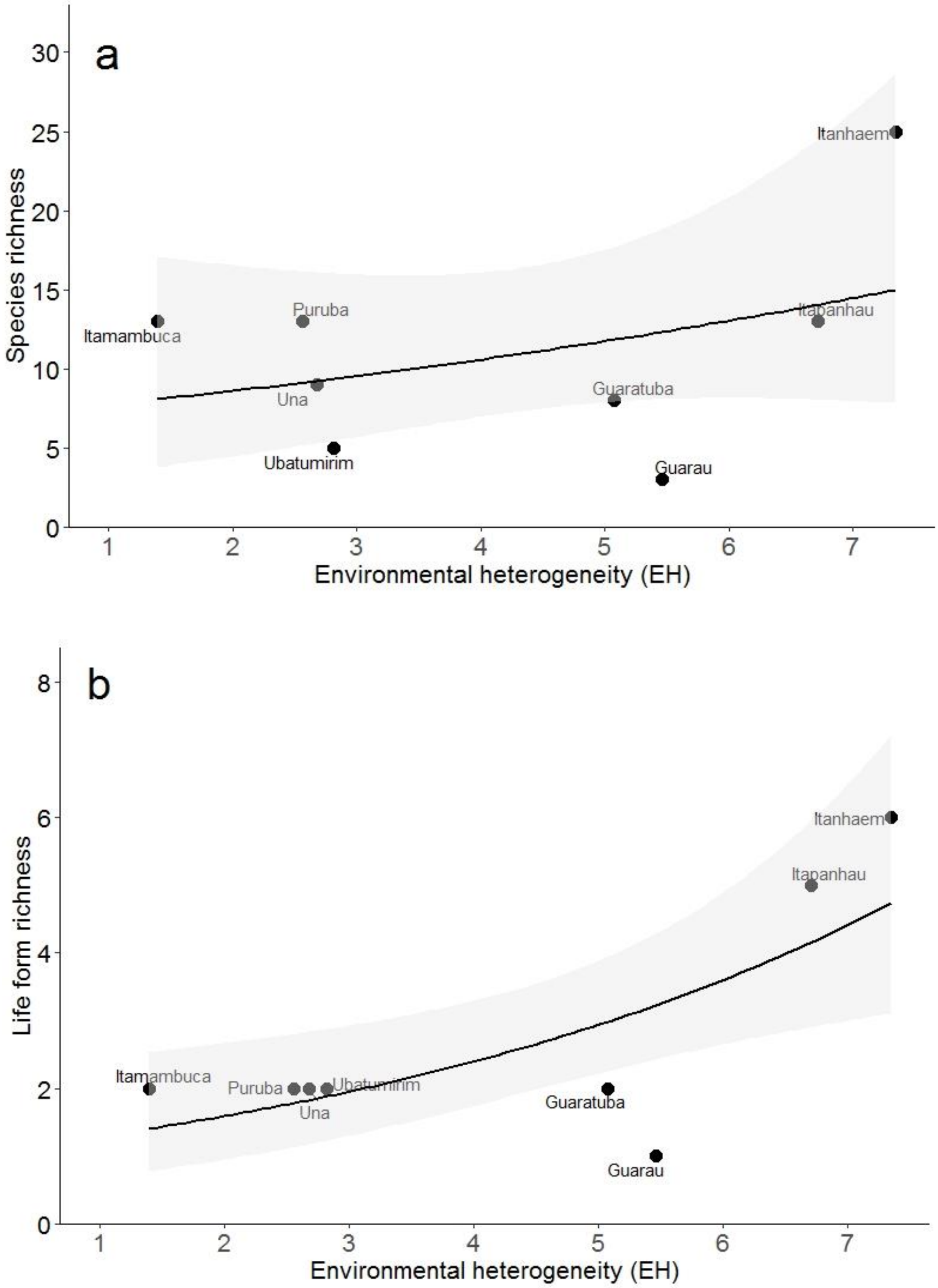

Figure 4. Species richness (a) and life form richness (b) as a function of environmental heterogeneity for the quasi-Poisson GLM with 95% confidence intervals.

Table 5. Summary statistics of the Generalized Linear Models relating environmental heterogeneity (EH) to species richness and life form richness.

	Species richness		**Life form richness**	
Parameter	Intercept	EH	Intercept	EH
Estimation	1.94	0.10	0.06	0.23
Standard error	0.50	0.10	0.39	0.07
t - statistic	3.84	1.05	0.15	2.85
P	<0.01	0.33	0.89	0.02

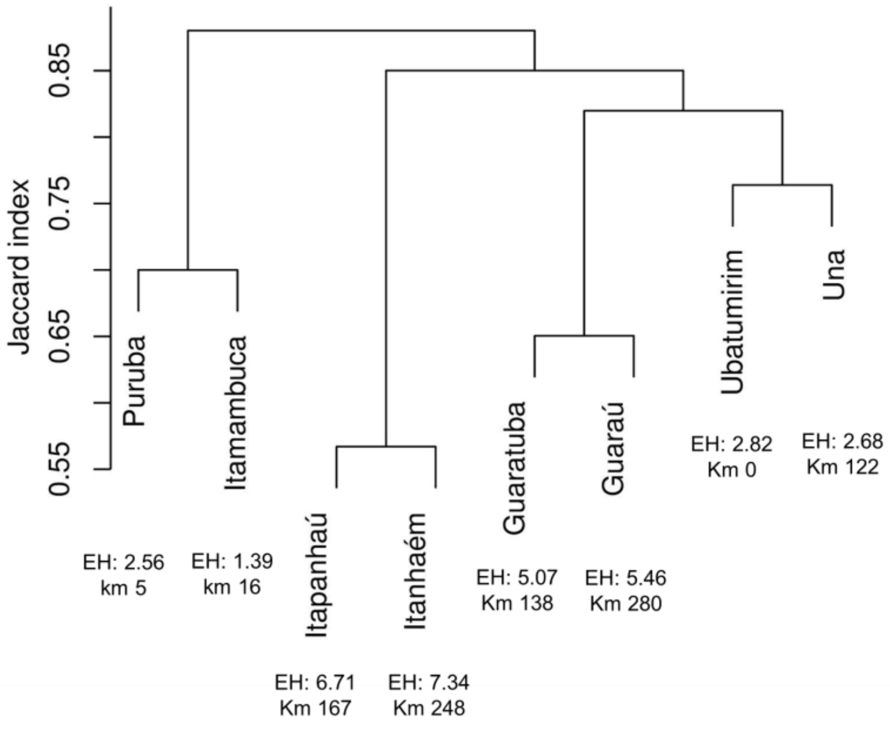

Figure 5. Cluster dendrogram of the dissimilarity of macrophyte species composition among the coastal rivers using the Jaccard index and the UPGMA method. The EH and geographical distance along the coastline are listed below the name of each river.

Table 6. Summary of multiple regressions on distance matrices (MRM), variation partitioning and residuals. The response matrix was species composition and explanatory matrices were environmental heterogeneity (EH) and geographical distance.

Matrices	**MRM**	**Variation partitioning**
Species composition ~ EH	$R^2 = 0.37$ $P = 0.0008$	**17.95%**
Species composition ~ geographical distance	$R^2 = 0.22$ $P = 0.0083$	**2.70%**
Species composition ~ EH + geographical distance	$R^2 = 0.40$ $P = 0.0013$	**19.16%**
Residuals		**60.19%**

Discussion

Our results partially confirmed our hypothesis that EH would be positively related to macrophyte species and life form richness. Our findings indicate that EH did not drive species richness. Although the most heterogeneous river (the Itanhaém River) had the greatest species richness, other rivers with very different EH values (the Itapanhaú, Puruba and Itamambuca Rivers) did not differ in species richness. In contrast, life form richness was positively related to EH. In fact, the two most heterogeneous rivers (the Itanhaém and Itapanhaú Rivers) had the greatest life form richness and the greatest similarity in species composition.

Different spatial scales and extents of the environmental gradients analyzed may affect the strength and shape of the richness-heterogeneity relationship (Constanza et al., 2011; Bar-Massada & Wood, 2014), with richness peaks related to the maximum heterogeneity (Lundholm & Larson, 2003) or even flat responses (Allouche, Kalyuzhny, Moreno-Rueda, Pizarro, & Kadmon, 2012). In our study we observed a peak of macrophyte species richness at maximum heterogeneity, but species richness remaind relatively constant over lower values of in EH. We suggest that there is a threshold of EH in the sampled coastal rivers at which macrophyte species richness is favored. This threshold may lead to positive or unimodal curves at broader spatial scales, as observed by Bar-Massada and Wood (2014).

In contrast, we found that EH significantly predicted the number of life forms. This finding indicates that, in the coastal rivers we studied, as the environments become more heterogeneous, a greater richness of life forms of aquatic macrophyte is supported. Thus, heterogeneous environments can sustain the occurrence of emergent, submerged and floating macrophytes, because their different growth forms allow them to colonize habitats that vary in environmental characteristics and potential niches. A similar conclusion was reached by Gantes and Caro (2001) for the macrophyte assemblages of plain streams of the Buenos Aires Province (Argentina). These authors observed that less heterogeneous environments tended to have only emergent macrophytes, which are more independent of some limnological and hydrological variables. We suggest that this is also true for the emergent and amphibious macrophytes in the rivers we studied.

Thus, we observed that life form richness seems to better reflect the differences in the rivers EH than does species richness. This is because most of the rivers (6 of the 8 sampled rivers) had no pattern of variation in number of species versus EH. For example, rivers with great EH (e.g., Itapanhaú and Guaraú) had equal or lower species richness than rivers with the lowest EH (e.g., Puruba and Itamambuca). In addition, these rivers had only emergent and/or amphibious macrophytes, which tend to colonize habitats with more similar conditions along environmental gradients. However, the two most heterogeneous rivers (EH = 6.71 and 7.35) had the greatest number of life forms (5 and 6) and these different life forms occurred in diverse habitat types.

The most important variables promoting high EH of the rivers we studied were sediment salinity, river width, total phosphorus of water and distance from river mouth. This result highlights that the characteristics of the coastal plain (mainly slope and width) where each river is located are directly important to the rivers' EH. Rivers located in flatter and larger coastal plains are more influenced by salinity and phosphorus inputs from seawater (Adam, 1990), and also have wider and longer channels along the plain (Tessler et al., 2006). These characteristics promote environmental gradients and a diversity of habitat types along the coastal rivers that favor increased life form richness of aquatic macrophytes.

In addition, the coastal plain characteristics also influence other topographic and morphometric-related variables that we did not measure here, such as depth and channel structure, that contribute to habitat diversity and hydromorphological variation favoring high macrophyte life form diversity (Schneider, Cunha, Marchese, & Thomaz, 2018; Gyosheva, Kalchev, Beshkova, & Valchev, 2019). The most heterogeneous rivers (Itanhaém and Itapanhaú) have meandering channels because they are located on large and flat coastal plains and this morphometry possibly also favors the greatest life form richness. Although we did not include variation in the PC2 as an input to the river EH score, some rivers had considerable variation in PC2, which was driven by sediment nutrient content, which may be associated to organic matter content, and may be important to the distribution of the rooted macrophytes.

As we expected, we found that EH was important for shaping the species composition of the coastal rivers, and that the rivers with similar heterogeneity had the greatest similarity in their species composition. However, we found that geographical distance did not explain much variation in species composition. Only the Puruba and Itamambuca Rivers are geographically close. Besides that, the Puruba and Itamambuca Rivers were the less heterogeneous rivers and their species composition was less similar to that of the other ones. Although the Itanhaém and Itapanhaú Rivers have similar EH scores, they are located at a relatively large distance from each other. Thus, we suggest that their characteristics and geomorphological resemblance may influence the similarity in their heterogeneity and species composition. These rivers are located in lowlands where lagoonal formations occurred before the quaternary formations. They are also marked by the extensive presence of mangroves and the deposition of very fine and organic matter-rich sediment (Suguio & Martin, 1978). Thus, the other pairs of rivers with similar species composition and EH may also have similar geomorphological characteristics.

We observed that most plant species had restricted occurrences, and that the rivers had relatively different species pools, but the fact that variation in species composition was not strongly explained by geographical distance suggests little dispersal limitation (Capers et al., 2010), even with the marked isolation of the coastal rivers that we studied. For some salt-tolerant macrophytes the ocean currents can play an important role in dispersing their fruits and seeds between estuaries (Huiskes, Koutstaal, Herman, Beeftink, Markusse, & Munck, 1995). However, for most species of coastal macrophytes, the ectozoochorous transport of propagules by migratory waterfowl may be the main vector of successful dispersal over long distances (Vivian-Smith & Stiles, 1994). Thus, it is not surprising that isolation and dispersal limitation are not as important as local factors when they are evaluated separately (Viana, Figueola, Schwenk, Manca, Hob,...& Santamaría, 2015). Alahuhta (2015) found that the composition of macrophyte assemblages in lakes in Minnesota (USA) was better explained by local patterns (e.g., EH) than by spatial factors and processes, such as geographical distance and dispersal limitation, as we also observed in our study.

The shared effect of EH and geographical distance on variation in species composition among the coastal rivers was greater than the sole effect of geographical distance, but close to the effect of EH alone. This shared effect may be a result of the combination of the rivers' geographical location on the coastal plain and spatial structure in the environmental factors (EH) due to spatial variation in the characteristics of the coastal plain. However, we found that about 60% of the variation in species composition among the coastal rivers was not explained by EH or geographical distance. This unexplained variation may be due to stochastic and biotic factors, as also pointed out by Xiu et al. (2012) for the macrophytes composition of the Yellow River Estuary (China), or due to environmental variables that we did not evaluate in our study (Ricklefs, 1987; Capers et al., 2010; Mikulyuk, Sharma, Egeren, Erdmann, Nault, & Hauxwell, 2011). In coastal ecosystems, the topographic heterogeneity at various scales and consequently the gradients of elevation, current velocity, flooding and water dynamics may also have a considerable influence on community diversity (Sánchez, Izco, & Medrano, 1996; Blanchard & Bourget, 1999; Morzaria-Luna et al., 2004). These variables were not measured in our study, however, they may be structured along the north-south coastal gradient, due to the differences in the coastal plain characteristics.

Especially in coastal wetlands, studies on benthic macroinvertebrate and fish assemblages have found that their richness and diversity may be supported by the variation in ecosystem characteristics and environmental gradients and heterogeneity (Medeiros, Costa, Lima, Oliveira, Cavalcanti-Júnior, …& Molozzi, 2016; Teichert, Lepage, Chevillot, and Lobry, 2018). Although global patterns of variation in macrophyte communities have been related to EH (Alahuhta et al., 2017), its importance relative to other spatial factors at regional and local scales is not well known. At smaller spatial scales, variation in the macrophyte communities may not be related to the environmental conditions of ecosystems. For example, in boreal rivers and lakes, Alahuhta, Rääpysjärvi, Hellsten, Kuoppala, and Aroviita (2015) found that macrophyte communities were structured by environmental factors, but their structure was not related to habitat specificity despite variation of the characteristics of the aquatic environments, as we similarly observed for species richness in this study.

We conclude that EH drives the life form richness but does not influence the species richness of aquatic macrophytes in the coastal rivers we studied; however, EH does shape the species composition regardless of the rivers' geographical distance. The variation in environmental conditions of the rivers promotes different macrophyte communities among them. The rivers differ in their species pools in ways that may not have been shaped by dispersal limitation and isolation, given the little effect of geographical distance on species composition. The variation in environmental conditions among the rivers drives the community composition and the occurrence of diverse life forms. Therefore, preservation of each river's local characteristics is important for maintaining the regional diversity of the macrophyte community.

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Supporting information

Table S1. List of the aquatic macrophyte taxa recorded in the coastal rivers of the São Paulo State in SE Brazil; and their life forms (LF): emergent (EM), free floating (FF), rooted floating (RF), rooted submerged (RS), free submerged (FS) and amphibious (AM). The codes were used in Figure 2.

Family	**Species**	**Code**	**LF**
ACANTHACEAE	*Hygrophila costata* Nees & T. Nees	Hcos	EM
AMARYLLIDACEAE	*Crinum americanum* L.	Came	EM
ARACEAE	*Pistia stratiotes* L.	Pstr	FF
ARALIACEAE	*Hydrocotyle ranunculoides* L. f.	Hran	EM
ASTERACEAE	*Pluchea sagittalis* (Lam.) Cabrera	Psag	AM
	Sphagneticola trilobata (L.) Pruski	Stri	AM
CABOMBACEAE	*Cabomba furcata* Schult. & Schult. f.	Cfur	RS
CYPERACEAE	*Cyperus blepharoleptos* Steud.	Cble	EM
	Cyperus odoratus L.	Codo	EM
	Eleocharis acutangula (Roxb.) Schult.	Eacu	EM
	Eleocharis interstincta (Vahl) Roem. & Schul.	Eint	EM
	Eleocharis minima Kunth	Emin	EM
	Fimbristylis dichotoma (L.) Vahl	Fdic	AM
	Fuirena umbellata Rottb.	Fumb	EM
	Rhynchospora corymbosa (L.) Britton	Rcor	EM
	Schoenoplectus californicus (C. A. Mey.) Soják	Scal	EM
	Unidentified	Cype	EM
HALORAGACEAE	*Myriophyllum aquaticum* (Vell.) Verdc.	Maqu	EM
HYDROCHARITACEAE	*Egeria densa* Planch.	Eden	RS
	Limnobium laevigatum (Humb. & Bonpl. ex Willd.) Heine	Llae	FF
JUNCACEAE	*Juncus* cf. *marginatus* Rostk.	Jmar	EM
LENTIBULARIACEAE	*Utricularia foliosa* L.	Ufol	FS
NYMPHAEACEAE	*Nymphaea rudgeana* G. Mey.	Nrud	RF
ONAGRACEAE	*Ludwigia decurrens* Walter	Ldec	EM
ORCHIDACEAE	*Habenaria repens* Nutt.	Hrep	EM
PLANTAGINACEAE	*Bacopa monnieri* (L.) Pennell	Bmon	EM
POACEAE	*Hymenachne amplexicaulis* (Rudge) Nees	Hamp	EM
	Leersia hexandra Sw.	Lhex	EM
	Panicum repens L.	Prep	EM
	Paspalidium geminatum (Forssk.) Stapf	Pgem	EM
	Paspalum virgatum L.	Pvir	EM
	Spartina alterniflora Loisel.	Salt	EM
	Steinchisma laxum (Sw.) Zuloaga	Slax	AM

	Urochloa arrecta (Hack. ex. T. Durand & Schinz) Morrone & Zuloaga	Uarr	EM
POLYGONACEAE	*Polygonum acuminatum* Kunth	Pacu	EM
	Polygonum punctatum Elliott	Ppun	EM
PONTEDERIACEAE	*Eichhornia azurea* (Sw.) Kunth	Eazu	RF
	Eichhornia crassipes (Mart.) Solms	Ecra	FF
PTERIDACEAE	*Acrostichum danaeifolium* Langsd. & Fisch.	Adan	AM
RICCIACEAE	*Ricciocarpos natans* (L.) Corda	Rnat	FF
RUBIACEAE	*Richardia grandiflora* (Cham. & Schltdl.) Steud.	Rgra	AM
SALVINIACEAE	*Azolla filiculoides* Lam.	Afil	FF
	Salvinia molesta D.S. Mitch.	Smol	FF
TYPHACEAE	*Typha domingensis* Pers.	Tdom	EM
ZINGIBERACEAE	*Hedychium coronarium* J. Koenig	Hcor	AM

Table S2. Minimum and maximum values of the explanatory variables sampled in the coastal rivers of the São Paulo State in SE Brazil. Their respective codes and methods are shown in Table 2.

Coastal Rivers	**salinity**	**sediment type**	**mouth distance (km)**	**coastline distance (km)**	**width (m)**	**litoral slope (%)**	**TNw (mg L^{-1})**	**TPw (µg L^{-1})**	**TNsed (% dry mass)**	**TPsed (% dry mass)**	**DO (mg L^{-1})**	**pH**
Ubatumirim	0-1	1-2	0.43-2.13	0.22-0.50	15-100	4.5-15.8	0.182-0.392	22.77-163.80	0.020-0.200	0.005-0.018	3.6-7.8	6.46-6.88
Puruba	0-1	1-3	0.30-2.45	0.07-0.44	20-165	0.0-28.8	0.168-0.462	16.25-198.20	0.012-0.268	0.001-0.021	5.1-6.6	5.62-6.22
Itamambuca	0-0	1-3	0.39-1.84	0.16-1.15	21-115	3.3-22.5	0.084-0.560	12.47-30.70	0.039-0.530	0.004-0.028	8.5-10.5	6.16-6.83
Una	0-2	1-2	0.18-3.60	0.16-1.31	12-80	5.0-62.5	0.238-0.644	10.44-35.54	0.050-0.290	0.003-0.016	5.9-8.5	5.29-6.44
Guaratuba	0-24	1-2	0.30-7.65	0.38-3.40	25-280	5.0-22.5	0.070-0.182	15.19-54.89	0.038-0.299	0.003-0.011	6.1-7.8	5.86-7.74
Itapanhaú	0-19	1-2	0.52-25.70	1.80-4.00	20-333	4.2-13.3	0.098-0.252	32.50-91.48	0.055-0.564	0.003-0.050	4.5-7.3	4.58-7.73
Itanhaém	0-15	1-2	1.70-18.9	1.00-10.70	35-190	5.3-60.6	0.090-0.530	16.48-100.00	0.130-0.350	0.005-0.027	2.6-8.5	5.56-6.81
Guaraú	0-21	1-2	0.57-9.20	0.55-4.68	7-110	8.3-40.0	0.168-0.252	14.51-95.39	0.019-0.451	0.006-0.022	5.5-7.2	6.15-7.73

Capítulo 3

FATORES LOCAIS E REGIONAIS DETERMINANTES DA DIVERSIDADE BETA DE MACRÓFITAS AQUÁTICAS EM RIOS COSTEIROS TROPICAIS

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Local and regional drivers of macrophyte beta diversity in tropical coastal rivers*

Resumo: Estudos sobre diversidade beta em diferentes escalas espaciais podem fornecer informações sobre os fatores ecológicos que moldam as comunidades e os mecanismos de manutenção e perda de diversidade. Os rios costeiros no sudeste do Brasil têm diferentes comprimentos e gradientes de salinidade, devido à variação altitudinal em relação à largura e inclinação da planície costeira. Essas diferenças de características ambientais promovem gradientes naturais dentro e entre os rios, e padrões de variação das comunidades de macrófitas aquáticas. Para investigar os fatores determinantes da diversidade beta de macrófitas, nós registramos a ocorrência de espécies e de formas de vida e coletamos variáveis limnológicas em 100 pontos de coleta em 8 rios não conectados entre si e que são drenados diretamente para o oceano. Em cada ponto de coleta, nós coletamos variáveis da água (nutrientes, oxigênio dissolvido e pH), do sedimento (nutrientes, salinidade e tipo de grão) e do canal (largura, declividade da margem e distância da foz e da linha de costa). Nós também medimos o comprimento dos rios e seus gradientes de salinidade. Nós estimamos a diversidade beta de macrófitas e seus componentes aninhamento e *turnover* dentro e entre os rios costeiros (escala local e regional) usando métricas tradicionais e uma métrica que considera o efeito da amostragem. Nós avaliamos o potencial das variáveis ambientais para explicar a diversidade beta local e regional usando a Análise de Redundância baseada na distância (db-RDA) e regressões lineares. Como esperado, na escala local o elevado *turnover* de espécies e o elevado aninhamento de formas de vida foram explicados predominantemente pelo gradiente longitudinal dos rios (principalmente salinidade, fósforo e largura do canal). Na escala regional, nós encontramos que o comprimento do rio explica o aninhamento de formas de vida entre os rios (rios pequenos são subconjuntos dos rios maiores); entretanto, nem o comprimento do rio nem o gradiente de salinidade explicaram o elevado *turnover* de espécies entre os rios. Nós sugerimos que a limitação da dispersão entre os rios e a contingência histórica da colonização moldaram o pool de espécies em cada rio, resultando em comunidades com composição de espécies quase que completamente distintas. Esse elevado *turnover* na escala regional sugere que as estratégias de conservação para macrófitas aquáticas devem considerar múltiplos locais (rios) e não apenas os rios maiores. No geral, nossos resultados sugerem um forte papel da variação ambiental longitudinal relacionada à influência costeira na determinação da diversidade beta de macrófitas dentro e entre rios tropicais.

Palavras-chave: *turnover*, aninhamento, plantas aquáticas, formas de vida, salinidade, comprimento do rio, influência marinha, heterogeneidade ambiental, estuário.

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Abstract: Studies on beta diversity across different spatial scales can yield insight into the ecological factors that shape communities and the mechanisms of maintenance and loss of diversity. The coastal rivers in SE Brazil have different lengths and salinity gradients, because of the altitudinal variation of the coastal plain width and slope. These differences in environmental characteristics promote natural gradients within and among rivers, and patterns of macrophyte community variation. To investigate the drivers of macrophyte beta diversity we recorded the occurrence of species and life-forms and collected limnological variables at 100 sampling sites over 8 unconnected rivers that run directly to the sea. At each sampling site, we collected variables from water (nutrients, dissolved oxygen and pH), sediment (nutrients, salinity and grain type) and channel (width, littoral declivity, distance from mouth and coastline). We also measured river length and salinity gradient. We estimated macrophyte beta diversity and its nestedness and turnover components within and among coastal rivers (local and regional scale) using traditional metrics and a metric that accounts for sampling effect. We evaluated the potential of environmental variables to explain local beta diversity and regional beta diversity using distance-based Redundancy Analysis (db-RDA) and linear regressions. As expected, at the local scale, high species turnover and life-form nestedness were predominantly explained by the rivers' longitudinal gradient (mainly salinity, phosphorus and channel width). At the regional scale, we found that river length explained life-form nestedness among rivers (small rivers are subsets of the largest rivers); however neither river length nor salinity gradient explained the high species turnover among rivers. We suggest that dispersal limitation among rivers and historical contingency of colonization shaped the species pool in each river resulting in almost completely distinct community compositions. This high turnover at the regional scale suggests that conservation strategies for macrophytes must consider multiple rivers and not only the largest ones. Overall, our results suggest a strong role of the longitudinal environmental variation related to the coastal influence in determining macrophyte beta diversity within and among tropical rivers.

Key words: turnover, nestedness, aquatic plants, life forms, salinity, river length, seawater influence, environmental heterogeneity, estuary.

INTRODUCTION

Understanding differences in community composition along spatial gradients and through time is a major goal in ecology and biogeography and is important for the management of ecosystems (Alahuhta et al. 2017). Community differences can be quantified as beta diversity, which in turn can be partitioned into turnover and nestedness components (Baselga 2007). Turnover occurs when species present at one site are absent at another site, but are replaced by other species absent from the first (Socolar et al. 2016). Nestedness occurs when the biota of sites with low richness is a nested subset of the biota of the richest sites as a non-random species loss due to local factors that constrain assemblage development (Ulrich and Gotelli 2007, Baselga 2010). High turnover along spatial and environmental gradients across natural sites indicates that conservation actions must account for this variation by targeting multiple sites, while nestedness suggests that actions can prioritize the richest sites (Socolar et al. 2016). Studies on beta diversity across different spatial scales can also yield insight into the ecological factors that shape communities (Guo et al. 2015) and the mechanisms of maintenance and loss of regional diversity (Socolar et al. 2016).

Beta diversity of aquatic macrophytes has been studied in several types of aquatic environments at different geographical regions (Guo et al. 2015, Boschilia et al. 2016, Alahuhta et al. 2017, Bertuzzi et al. 2019, Schneider et al. 2019). However, there is still a lack of beta diversity studies in tropical coastal wetlands, which are usually regions under strong anthropogenic pressures due to urbanization and tourism (Lee et al. 2006). Macrophyte communities play an important role in structuring and maintenance of aquatic ecosystems and are a good biological group to test hypotheses about spatial diversity patterns, because of their high rates of asexual growth and dispersal that enable them to have a heterogeneous distribution in space (Boschilia et al. 2008). The spatial variation in composition of these communities has been related to variation in regional factors such as altitude (Lukács et al. 2015), geomorphology (Schneider et al. 2019) and connectivity degree (Viana et al. 2015), and local characteristics related to limnology (Boschilia et al. 2016), morphometry (Thomaz et al. 2003) and hydrology (Kunza and Pennings 2008). Differences in the regional characteristics of aquatic ecosystems may affect their local environmental gradients promoting habitat diversity at different spatial extents (Schneider et al. 2019) and, thus, community variation at different spatial scales (Barton et al. 2013, Heino et al. 2015). However, besides these studies at multiple scales, joint assessments of macrophyte beta diversity at both local and regional scales are still scarce.

At regional scales, coastal rivers and estuaries vary in terms of dimension and seawater influence due to the topographic and geomorphological characteristics of the coastal plains (Costa et al. 2003, Morzaria-Luna et al. 2004, Kunza and Pennings 2008). At local scales, these ecosystems have longitudinal gradients of salinity, flooding and nutrient concentration due to the tidal cycle (Bertness 1991). The aquatic macrophyte communities of these coastal wetlands are perennial and influenced by variation in environmental factors that can favor different ecological processes and promote different diversity patterns (Kunza and Pennings 2008). Macrophyte life-form diversity can be differently shaped by environmental conditions in comparison to species diversity (Alahuhta 2014, Fu et al. 2019). Life-form diversity may be favored by habitat heterogeneity; however species diversity may not follow this pattern (Nunes et al. 2020), mainly because species are also limited by dispersal at large scales and the history of colonization that shapes the species pool (McGill et al. 2007). Thus, studies with functional approaches could reveal if niche-based assembly processes (e.g., niche differentiation) promote beta diversity of functional groups which may not be evident in species-level patterns, because they usually do not consider functional similarities among communities (Swenson 2011).

The coastal plains of the São Paulo State in southeast Brazil have different widths, slopes (Tessler et al. 2006), topography (Souza and Cunha 2011) and bedrock and superficial geology (Suguio et al. 1978) in the north-south coastal length due to the presence of the Atlantic mountain range. The coastal basins in this region are isolated from each other and their rivers have different lengths and salinity gradients, and consequently, diversity of habitat types and niches (Nunes et al. 2020). The rivers located in the flattest and largest coastal plains are longer, more influenced by seawater and heterogeneous than the rivers in the steepest and shortest coastal plains (Adam 1990; Nunes et al. 2020). Thus, the study of species and life-forms of aquatic macrophyte in environmental gradients at different spatial scales is ideal in these coastal plains, for example, within a river and among different rivers.

Here, we estimated species and life-form beta diversity of aquatic macrophytes within and among coastal rivers, i.e., at the local scale in the rivers' longitudinal gradient and at the regional scale in the coastal north-south gradient. We then evaluated whether (i) the environmental variables related to rivers' longitudinal gradient drive macrophyte beta diversity at the local scale (within each river) and (ii) river length and salinity gradient explain regional beta diversity (among rivers). At the local scale, we expected high species turnover within the rivers with the greatest length and salinity gradient, due to species replacement along the strongest environmental gradient, and high nestedness of species within the rivers with the lowest length and salinity gradient due to the weak environmental gradient. We expected life-form nestedness associated with the longitudinal environmental gradient, because emergent macrophytes (most of the herbaceous plants in tropical tidal lotic ecosystems) occur downstream and along the entire gradient, as opposed to the floating and submerged ones that tend to occur farther from the river mouth. At the regional scale, we expected higher nestedness of macrophyte species and life-form than turnover, because macrophyte communities of short rivers would be subsets of rivers with the greatest length and salinity gradient. That would promote a non-random species and life-form loss in the north-south gradient. Thus, the differences in river length and salinity gradient in the north-south gradient would explain this pattern of macrophyte beta diversity among rivers.

MATERIALS AND METHODS

Study area

In part of the southeastern and southern coast of Brazil there is the Atlantic mountain range called Serra do Mar. This mountain range is approximately 1,000 km long and has up to 1,000 m elevation (Almeida and Carneiro 1998), and it follows a coastline length of 400 km in the São Paulo State (Fig. 1).

The differences in the distances between the Serra do Mar and the coastline cause variations in the width and slope of the coastal plain (Nunes et al. 2020). The watersheds located in these coastal plain are thus influenced by the variation in topography and altitude (Souza and Cunha 2011), crossing areas with different geological formations (Suguio et al. 1978) and debouching on beaches of different typologies (Tessler et al. 2006). In general, the southern region of the São Paulo coast is marked by large coastal plain and continuous and rectilinear beaches due to the greatest distance between the Serra do Mar and the coastline. The northern region instead has narrow coastal plain and crenelated hills forming small beaches, creeks and bays due to the shortest path between the Serra do Mar and the coastline (Ponçano et al. 1999, Tessler et al. 2006). The coastal basins of São Paulo are isolated from each other because the headwaters are located at higher elevations in the Serra do Mar and the rivers drain directly into the sea (Almeida and Carneiro 1998).

The study region has small seasonal variations in climatic characteristics and a relatively constant and stable water flow in the aquatic ecosystems over the year (Monteiro 1973). The Köppen-Geiger climate classification is Af, that is, tropical rainfall climate with no dry season. The average annual temperature in the region is 23.6ºC (average temperature in summer = 26.46°C and average temperature in winter = 20.15°C), and the average annual rainfall is 2,260 mm (average rainfall in the summer months = 297.5 mm and average rainfall in the winter months = 182.3 mm) (Embrapa 2015). The region has a micro-tidal regime and regular lunar and semidiurnal tidal patterns, that is, the tidal range is less than two meters and there are two complete cycles of tides (high tide and low tide) per lunar day (24 h and 50 min) (Tessler et al. 2006).

We selected eight rivers in a north-south coastal length of 280 km of the São Paulo State. They were: the Ubatumirim, Puruba and Itamambuca Rivers in the northern region, and the Una, Guaratuba, Itapanhaú, Itanhaém and Guaraú Rivers in the central-southern region (Fig. 1). The choice of the studied rivers was based on the following criteria: (i) north-south distance gradient between the Serra do Mar and coastline, (ii) coastal plain width, and (iii) the occurrence of still well-preserved coastal rivers (lack of heavy human influence). The coastal plain width varies from 1.50 to 14.50 km in this coastal length. All the coastal rivers studied have regular estuarine zones, that is, they are always connected to the ocean. Thus, there are no disturbances such as drought and few periods of saline intrusion.

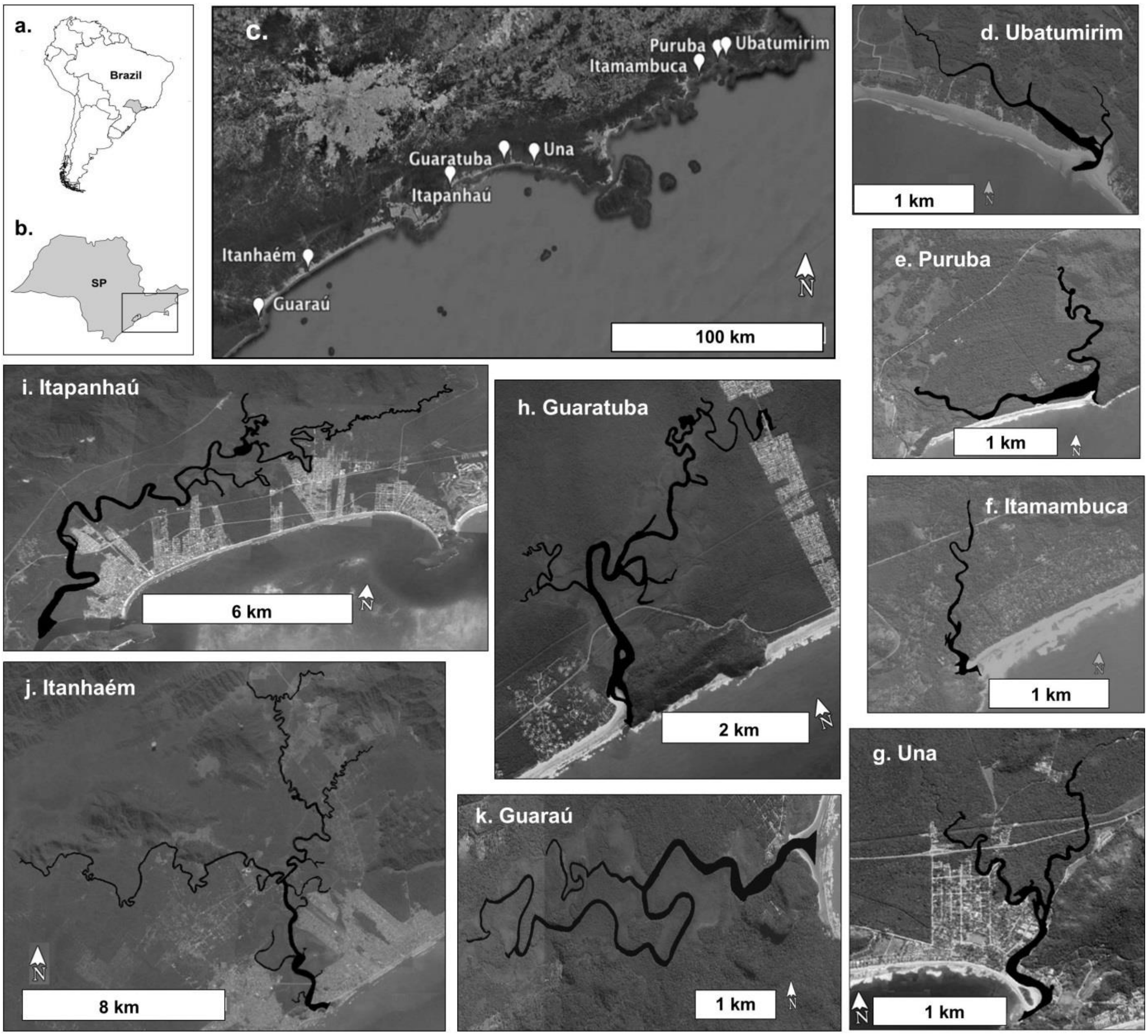


Figure 1. Maps of South America, Brazil (a) and the São Paulo State (b), highlighting the rivers' location on the coast (c); and images (different scales) of each sampled river: the Ubatumirim River (d), the Puruba River (e), the Itamambuca River (f), the Una River (g), the Guaratuba River (h), the Itapanhaú River (i), the Itanhaém River (j) and the Guaraú River (k).

Field sampling

Macrophytes occurrence

In temperate and subtropical estuaries, aquatic macrophytes occur in different elevational zones in extensive intertidal marshes where there is no arboreal vegetation (Engels and Jensen 2010). However, the intertidal plains in tropical estuaries are vegetated by mangroves and Atlantic forests, thus, macrophytes occur only in these forests' fringes (Schaeffer-Novelli et al. 1990). Macrophytes form short herbaceous patches (up to 20 m width), considered as stands, in shallow areas (up to 1.5 m deep) along the rivers' longitudinal gradient (Nunes and Camargo 2020) (Fig. S1). At high tide these stands are flooded, but at low tide the macrophytes (including the submerged ones) are partially or fully exposed which permits field observations and sampling (Nunes et al. 2020).

We performed a single sampling campaign in March 2017, because most estuarine macrophyte species are perennial (Engels 2010) and there are small seasonal climatic variations in the region and absence of disturbances occurrence in the estuaries. We went through the main river of the coastal basins on a boat from the mouth to the furthest point of possible navigation. We returned to the river mouth stopping at all macrophyte stands on both sides of the margins and recording the presence of species. Each macrophyte stand corresponded to a sampling site and a community. We considered a macrophyte stand each macrophyte patch isolated from each other regardless of their size and composition (mixed or monospecific) (Fig. S1). We found and sampled from 9 to 19 sites (macrophyte stands) per river, totaling 100 sampling sites. The number of sampling sites per river varied due to the different number of macrophyte stands present in each river. The aquatic macrophyte species found were recorded and when unidentified in the field they were collected and archived for later identification. Submerged species were sampled using a hook. We included the amphibious plants found on sand banks in the river channel and in the margin up to 1.5 m away from the water body towards the dry substrate. The aquatic macrophytes were identified using the following literature: Pott and Pott (2000), Amaral et al. (2008), and Rodrigues et al. (2017). The species were classified into their life-forms according to Chambers et al. (2008): emergent (rooted plants with the vegetative parts emerging above the water surface), free-floating (plants floating on water surface), rooted floating (rooted plants with floating leaves and flowers on the water surface), rooted submerged (plants with predominantly submerged vegetative parts), free submerged (plants with submerged vegetative parts, but not rooted in the substrate) and amphibious (semiaquatic plants that colonize wetlands, but are also able to survive for varying periods on dry substrates).

Local environmental variables

At each sampling site (macrophyte stand), we measured pH and dissolved oxygen (DO) of water (Horiba U-50 multi-parameter water quality checker), littoral declivity (measuring tape) and channel width (Laser telemeter Bushnell Yardage Pro 500). We also collected samples from water (up to 30 cm depth) and sediment (up to 15 cm belowground). In the laboratory, we determined total phosphorus (TP) (Golterman et al. 1978) and total nitrogen (TN) (Mackereth et al. 1978) of water and sediment, and sediment salinity (Digital salt-meter Instrutemp Nutra-Wand Combo). We performed a granulometric analysis, defined three sediment types according to the Udden-Wentworth grain-size scale (Wentworth, 1922) and classified each sediment sample into: (1) silt, clay and very fine sand; (2) fine and medium sand; and (3) coarse and very coarse sand. From remotely sensed images (Google LLC 2019), we obtained the distance of each sampling site from river mouth.

Regional environmental variables

We obtained river length on the coastal plain (following the river channel), disregarding the river length on the highlands of the Serra do Mar, also from Google LLC (2019). We calculated the rivers' longitudinal salinity gradient as the length (km) from river mouth up to the farthest sampling site at which sediment salinity was measurably greater than zero.

Statistical analysis

Estimating beta diversity

We calculated total beta diversity and its components (turnover and nestedness) (Baselga et al. 2013) among sites within each river (local scale – longitudinal gradient) and among rivers (comparing complete composition from all sites within each river - regional scale –north-south gradient) through a Jaccard dissimilarity matrix with presence and absence data for both macrophyte species and life-forms. We used the beta diversity metric for multiple sites (Baselga et al. 2013). We calculated the ratio of turnover and nestedness based on the total beta diversity value, and considered them as the response variables instead of the value of turnover or nestedness itself (Dobrovolski et al. 2012). This reduces the effect of total beta diversity, which could be influenced by the different number of sampled sites, to focus only on the mechanism promoting turnover or nestedness.

Since sampling effort could influence the quantification of total beta diversity in each river, we also calculated total beta diversity using two approaches that are not influenced by sampling effort. First we used Permutational Analysis of Multivariate Dispersions (PERMDISP), which is based on the distance to centroid in a multi-dimensional space obtained in a Principal Coordinates Analysis (PCoA) (Anderson et al. 2006). Second, we calculated the rarefied beta diversity for each river through normalized non-directional beta diversity as a function of sampling effort (Ricotta et al. 2019). The non-directional curve assumes that sampling site position in an environmental space is not relevant. This rarefied beta diversity analysis measures how multiple-site dissimilarity changes in relation to sampling effort and allows the comparison of the amount of community variation among rivers with different number of sampling sites (Ricotta et al. 2019). We compared the rivers' beta diversity based on an equal sampling effort, that is, this comparison was controlled by the river with the lowest number of sampling sites (The Ubatumirim River).

Explaining beta diversity

For the local scale analyses, we applied a Principal Component Analysis (PCA) to ordinate the sampling sites of all river in relation to the variables from water, sediment and channel. We used the PC axes (and their related environmental variables) to explain macrophyte community variation (Jaccard beta diversity) on the local scale (within rivers). For that, we applied distance-based Redundancy Analysis (db-RDA), which is a variation of RDA to cope with dissimilarity matrices as response matrices and find linear relationships between all the Principal Coordinate Analysis (PCoA) axes (summarizing a dissimilarity matrix between sites) and their related environmental variables (Legendre and Anderson 1999). We ran one db-RDA for each river and so, local beta diversity was analyzed for each river individually.

At the regional scale, we applied db-RDA using the species and life-forms composition of the rivers (a dissimilarity matrix of summed occurrence of species for each river) as response variables and river length and salinity gradient as explanatory variables. Similarly, we estimated the relationship among the regional rarefied beta diversity (a vector of eight rarefied values of beta diversity), the river length and salinity gradient using one multiple linear regression.

To calculate the total beta diversity (Jaccard) and its components we used the betapart and vegan packages and the beta.multi function (Baselga et al. 2013, Oksanen et al. 2019); to calculate the beta diversity from PERMDISP we used the vegan package and the betadisper function (Anderson et al. 2006); to calculate the rarefied beta diversity we used the Beta_N function provided by Ricotta et al. (2019); to apply the PCA and db-RDA we used the vegan package and the rda and dbrda function (Oksanen et al. 2019) in the R environment 3.6.1 (R Development Core Team 2019). To draw up the graphs we used R and GraphPad Prism 5.0 software (GPW5-066646-RCG7389) (GraphPad Software 2007).

RESULTS

General biotic results

We recorded a total of 45 taxa and 6 life-forms of aquatic macrophytes. The Itanhaém River (25 species and 6 life-forms) and the Guaraú River (3 species and 1 life-form) had the greatest and lowest local richness of species and life-forms, respectively. Only three macrophyte species (*Crinum americanum*, *Schoenoplectus californicus* and *Acrostichum danaeifolium*) occurred along a large part of the north-south gradient. The emergent species *C. americanum* and *S. californicus* occurred in seven rivers and the amphibious species *A. danaeifolium* in six rivers. Twenty-one recorded taxa occurred in only one of the sampled rivers. Floating and submerged life-forms were recorded only in the Itapanhaú and Itanhaém Rivers. In other rivers we recorded only emergent and/or amphibious macrophytes (Fig. 2).

Estimating beta diversity

At the local scale, we found that turnover was the most important component of beta diversity. Within all rivers, except in the Ubatumirim River, the species turnover ratio contribution (turnover ratio from 0.91 to 0.97) to beta diversity was greater than the nestedness one (nestedness ratio from 0.03 to 0.07) (Fig. 3 b). For life-forms, we found that the components of beta diversity varied among rivers, with nestedness corresponding to larger ratios in most comparisons (Fig. 3 g).

For the regional scale, we found that Jaccard average beta diversity (not controlling by sampling effort) was very high (Fig. 3 a). Turnover corresponded to 92% of this beta diversity, showing a strong compositional replacement among rivers. In contrast, life-form beta diversity was characterized by nestedness, indicating that rivers constitute subsets of macrophyte life-forms. When controlling for sampling effort, the rarefied beta diversity showed large variation among rivers (Fig. 3 d and e). We found that rarefied beta diversity was the lowest for the Ubatumirim River (Um) and highest for the Puruba River (Pa) (Fig. 3 d). Similar effects of sampling effort on beta diversity could be found when looking into life-forms at regional scale with lowest rarefied beta diversity at the Puruba River (Pa) and highest at the Itanhaém River (Im) (Fig. 3 i and j). The PERMDISP approach resulted in similar patterns to those using rarefied beta diversity.

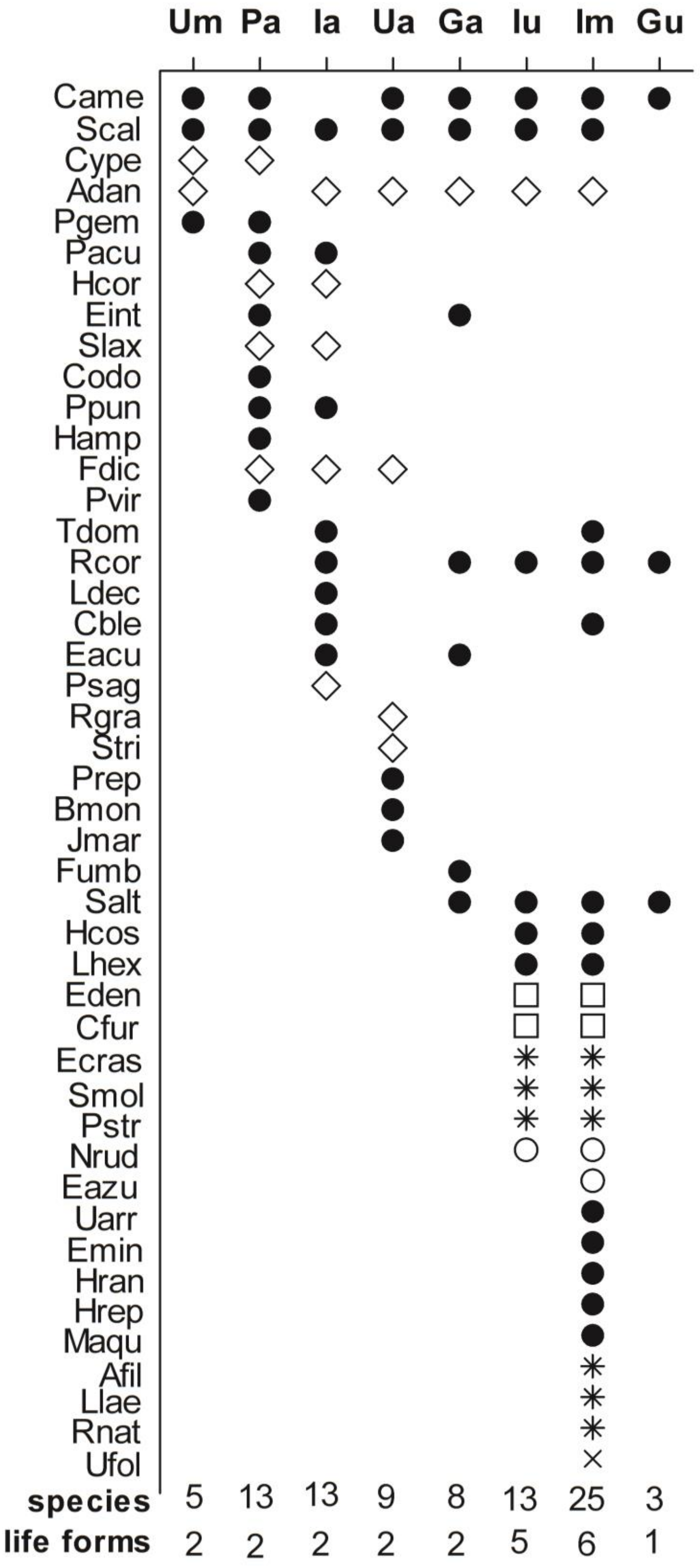


Figure 2. Aquatic macrophyte occurrence in the coastal rivers sampled and the number of taxa and life-forms. **Life-forms:** emergent (black circle), amphibious (open diamond), rooted submerged (open square), free floating (asterisk), rooted floating (open circle), free

submerged (ex). **Macrophyte taxa:** *Crinum americanum* (Came), *Schoenoplectus californicus* (Scal), Cyperaceae unidentified (Cype), *Acrostichum danaeifolium* (Adan), *Paspalidium geminatum* (Pgem), *Polygonum acuminatum* (Pacu), *Hedychium coronarium* (Hcor), *Eleocharis intestincta* (Eint), *Steinchisma laxum* (Slax), *Cyperus odoratus* (Codo), *Polygonum punctatum* (Ppun), *Hymenachne amplexicaulis* (Hamp), *Fimbristylis dichotoma* (Fdic), *Paspalum virgatum* (Pvir), *Thypha domingensis* (Tdom), *Rhynchospora corymbosa* (Rcor), *Ludwigia decurrens* (Ldec), *Cyperus blepharoleptos* (Cble), *Eleocharis acutangula* (Eacu), *Pluchea sagittalis* (Psag), *Richardia grandiflora* (Rgra), *Sphagneticola trilobata* (Stri), *Panicum repens* (Prep), *Bacopa monnieri* (Bmon), *Juncus* cf. *marginatus* (Jmar), *Fuirena umbellata* (Fumb), *Spartina alterniflora* (Salt), *Hygrophila costata* (Hcos), *Leersia hexandra* (Lhex), *Egeria densa* (Eden), *Cabomba furcata* (Cfur), *Eichhornia crassipes* (Ecra), *Salvinia molesta* (Smol), *Pistia stratiotes* (Pstr), *Nymphaea rudgeana* (Nrud), *Eichhornia azurea* (Eazu), *Urochloa arrecta* (Uarr), *Eleocharis minima*, (Emin), *Hydrocotyle ranunculoides* (Hran), *Habenaria repens* (Hrep), *Myriophyllum aquaticum* (Maqu), *Azolla filiculoides* (Afil), *Limnobium laevigatum* (Llae), *Ricciocarpus natans* (Rnat), *Utricularia foliosa* (Ufol). **Rivers:** Ubatumirim (Um), Puruba (Pa), Itamambuca (Ia), Una (Ua), Guaratuba (Ga), Itapanhaú (Iu), Itanhaém (Im), Guaraú (Gu).

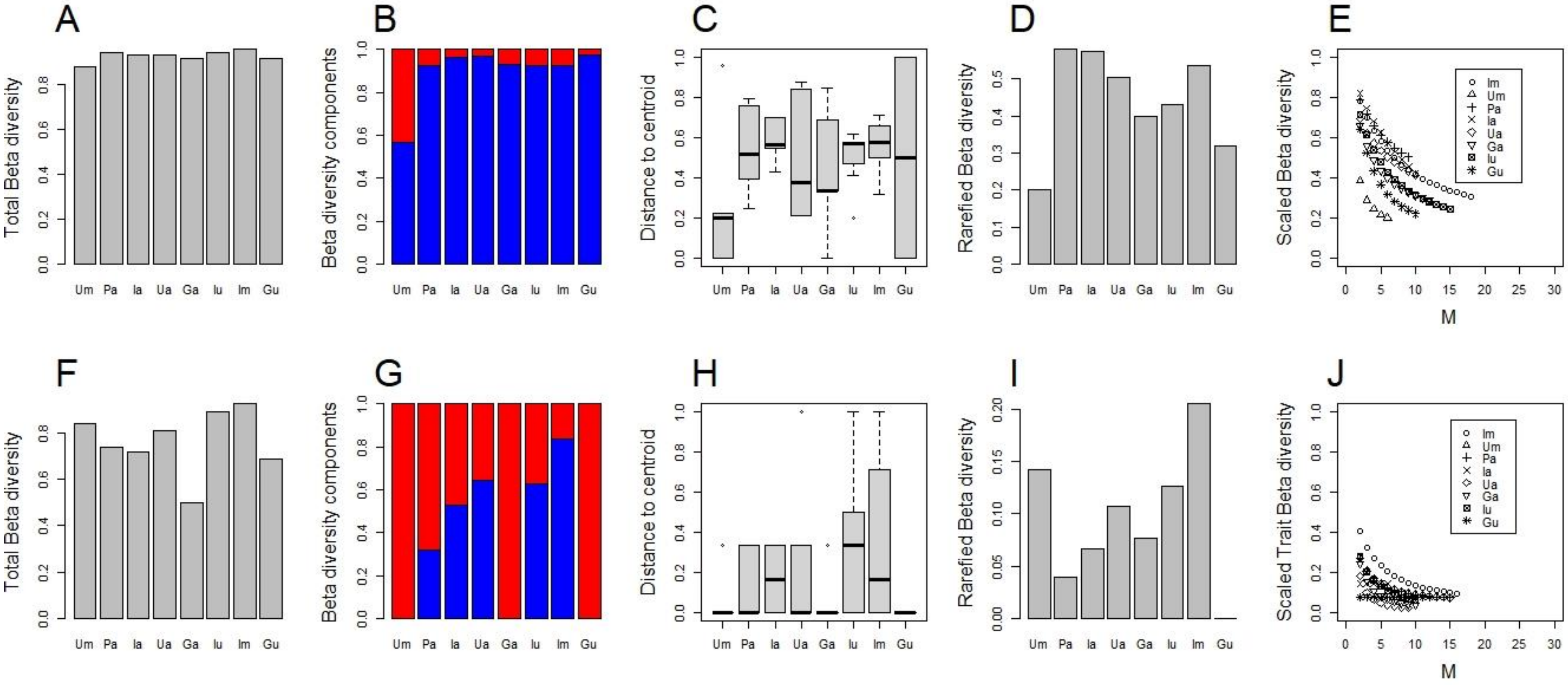


Figure 3. Results of species (A-E) and life-form (F-J) beta diversity. Total Jaccard beta diversity of species (A) and life-forms (F); Ratio of turnover (blue) and nestedness (red) of species (B) and life-forms (G); Total beta diversity of species (C) and life-forms (H) using PERMDISP; Rarefied beta diversity of species (D) and life-forms (I) controlling for the lowest sampled river; Relationship between expected beta diversity of species (E) and life-forms (J) and sampling effort.

The results of Jaccard beta diversity and rarefied beta diversity were different, which indicates that river beta diversity was affected by sampling effort. However, these differences in beta diversity were consistent, as the curves of rarefied beta diversity had similar decay and they did not overlap; which means that rivers had indeed different amounts of variation and thus, the relative importance of turnover and nestedness should be consistent (Ricotta et al. 2019). The life-form curves stabilized and decayed with a lower sampling effort than the species curves, because there was a maximum number of life-forms (6) to be found.

Local environmental variables

PCA axes mainly summarized the strong longitudinal gradient within rivers. The first four PCA axes explained 27, 20, 13 and 9% of local environmental variation totalizing 72% of the whole environmental variation in the dataset (these were the axes used in subsequent analyses). The first axis was positively related to local salinity, channel width and TP of water. Thus, PC1 partly represents a longitudinal gradient of seawater influence. The second axis was positively related to sediment type and negatively related to distance from mouth and TN of sediment and water. The third axis was positively related to salinity, distance from coastline, channel width and pH. Thus, PC3 also represents a clear longitudinal gradient within rivers. Finally, the forth axis was negatively related to littoral declivity and TN of water and positively related to DO. These relevant variables had eigenvalues higher than 0.35 (Fig. 4).

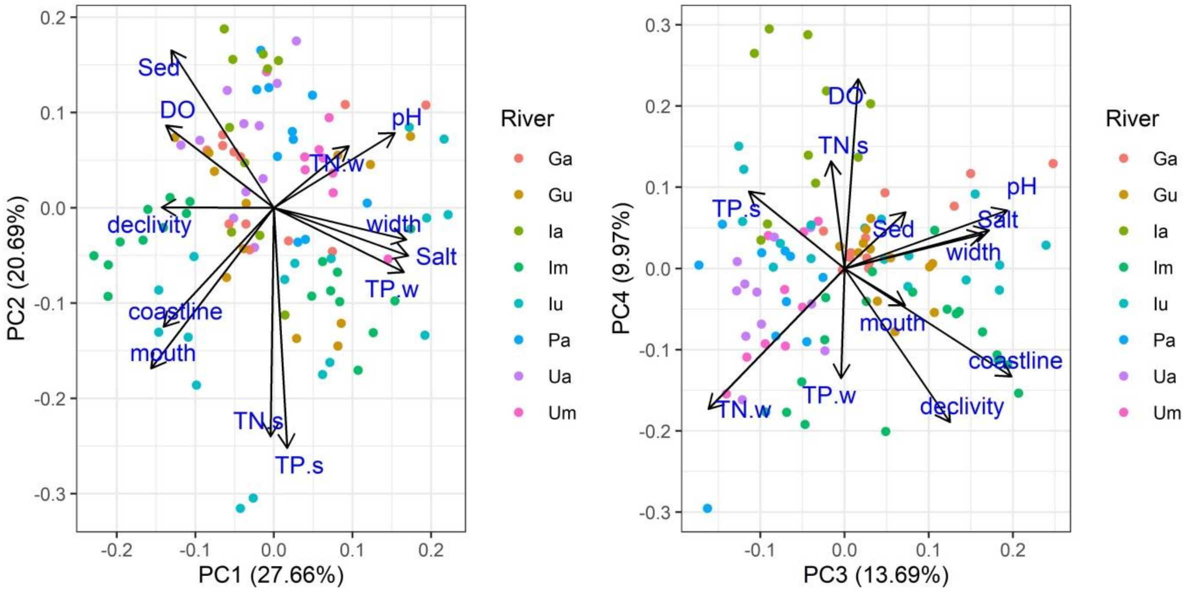


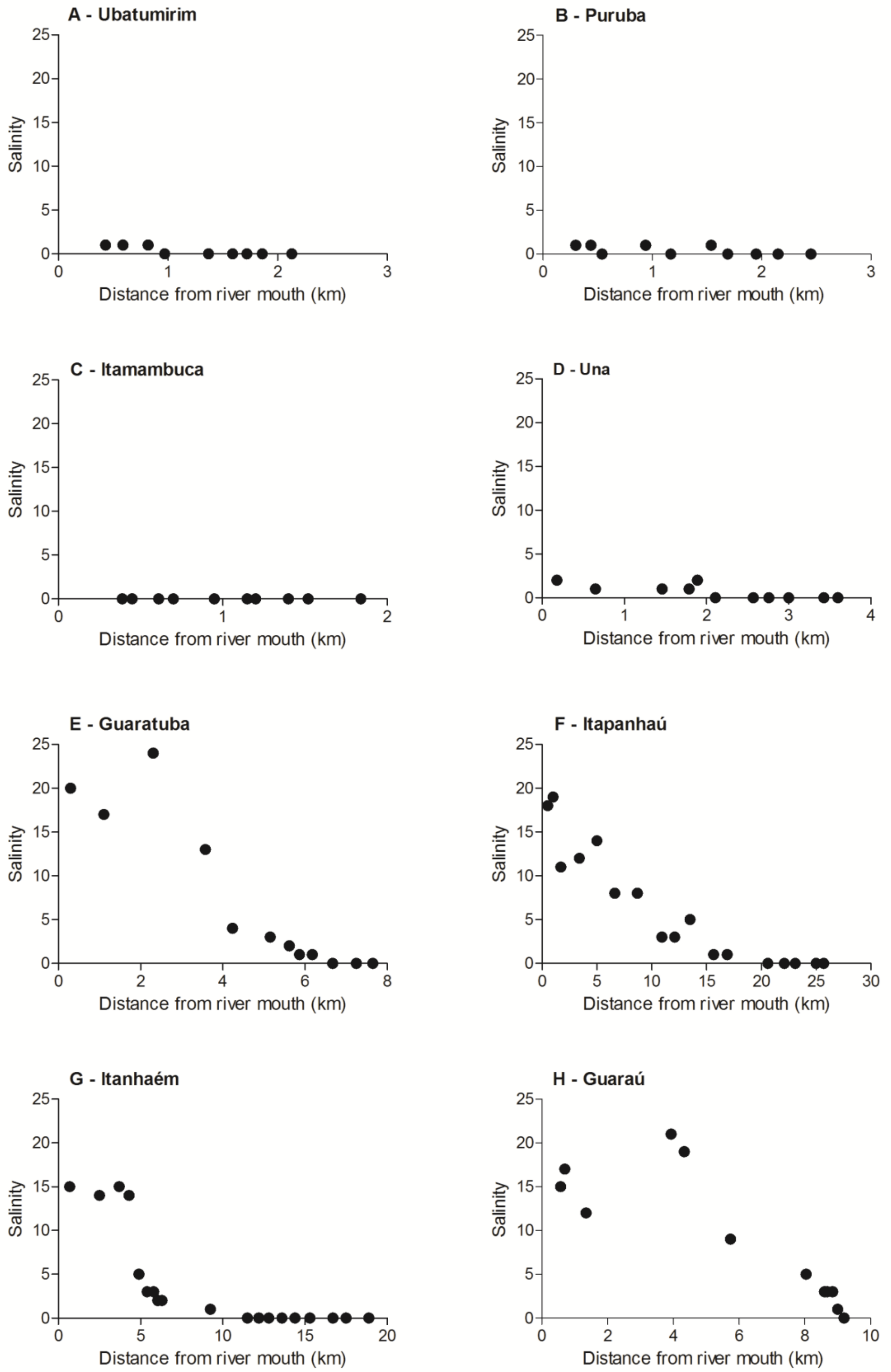
Figure 4. Plots of the Principal Components Analysis (PC1, PC2, PC3 and PC4) showing the ordination of the sampling sites at each river and the environmental variables. **Variables:** total phosphorus of water (TP.w) and sediment (TP.s), total nitrogen of water (TN.w) and sediment (TN.s), dissolved oxygen (DO) and pH of water, sediment salinity (Salt), sediment type (Sed), channel width (width), littoral declivity (declivity), distance from river mouth (mouth) and distance from coastline (coastline). **Rivers:** Ubatumirim (Um), Puruba (Pa), Itamambuca (Ia), Una (Ua), Guaratuba (Ga), Itapanhaú (Iu), Itanhaém (Im) e Guaraú (Gu).

Rivers' characteristics

The Itapanhaú, Itanhaém, Guaraú and Guaratuba Rivers were the longest rivers and had the greatest longitudinal salinity gradient (Table 1; Fig. 5). The Una, Puruba, Ubatumirim and Itamambuca Rivers were the shortest rivers and had essentially no longitudinal salinity gradient (Table 1; Fig. 5). Salinity gradient varied about fifty times between the rivers with the greatest and lowest gradients (Itapanhaú and Itamambuca, respectively) (Table 1).

Table 1. Number of sampling sites per river, river length on coastal plain (km) and salinity gradient (km).

Rivers	Number of sampling sites	River length on coastal plain (km)	Salinity gradient (km)
Ubatumirim	09	2.43	0.97
Puruba	10	2.80	1.69
Itamambuca	10	1.84	0.39
Una	11	3.90	2.11
Guaratuba	12	8.18	6.68
Itapanhaú	17	35.84	20.60
Itanhaém	19	19.00	11.50
Guaraú	12	10.13	9.20



A - Ubatumirim

B - Puruba

C - Itamambuca

D - Una

E - Guaratuba

F - Itapanhaú

G - Itanhaém

H - Guaraú

Figure 5. Sediment salinity at each sampling site along the length of the coastal rivers we studied. The graphs have different scales at axis x.

Explaining beta diversity at the local scale

The db-RDA at the local scale for species composition identified that five of the eight rivers had their beta diversity significantly explained by environmental variables (Table 2). The forward selection procedure indicated that axis 1 was the most common explanatory variable, but axis 3 and 4 were also significant for some rivers. These axes were mainly related to the longitudinal gradient variables like salinity, channel width, TP of water and littoral declivity. Surprisingly, the three rivers with non-significant db-RDA had very different distances from coastline.

Similar results from db-RDA for life-form composition were found at the local scale (Table 2). However, only three out of eight rivers had significant db-RDA with axis 1 and 3 being the selected variables by forward selection. Overall, when significant, db-RDA explained a large proportion of community variation.

Table 2. Significant results of db-RDA for species and life-form composition.

Response matrix	River	Selected variables	Adj. R²	Df	F	P value
Species	Ubatumirim	Axis 4	0.52	1	6.47	0.01
	Una	Axis 1	0.10	1	2.06	0.03
		Axis 3	0.28	1	2.94	<0.01
	Itapanhaú	Axis 1	0.39	1	10.13	<0.01
	Itanhaém	Axis 1	0.31	1	8.75	<0.01
	Guaraú	Axis 1	0.49	1	0.78	<0.01
Life-forms	Una	Axis 3	0.68	1	20.25	<0.01
	Itapanhaú	Axis 1	0.49	1	14.62	<0.01
	Itanhaém	Axis 1	0.50	1	18.43	<0.01

Explaining beta diversity at the regional scale

At the regional scale we found that river length and salinity gradient could not explain species composition among rivers (summed occurrences within rivers) (db-RDA, $P<0.05$). In contrast, life-form variation at the regional scale (among rivers comparison) was strongly related with river length (Ajd. $R^2=0.46$, $F=7.01$, $P=0.03$).

Finally, the multiple linear regression between rarefied beta diversity at the regional scale and salinity gradient and river length did not result in significant models. Life-form variation likewise was not explained by any of the tested predictor variables.

DISCUSSION

Beta diversity at the local scale

We expected higher species turnover within rivers with the greatest length and salinity gradient, and nestedness of species within rivers with the lowest length and salinity gradient. However, we found that turnover was the most important component for beta diversity of macrophyte species within all coastal rivers. High turnover of salt marsh plant species was also observed by Guo et al. (2015) within estuaries in the southern USA. These authors found that strong local environmental gradients (salinity and flooding) play an important role on this diversity pattern (Guo et al. 2015), as competition and abiotic stress drive plant composition and zonation in these coastal environments (Crain et al. 2004, Engels and Jensen 2010). In fact, we found that community variation within rivers was explained by environmental variables that consistently vary along their longitudinal gradients, such as salinity, TP of water, channel width and littoral declivity. Gradients of salinity and phosphorus along coastal rivers are positively related to the seawater influence on them (Vitousek and Howarth 1991, Tessler et al. 2006). These abiotic gradients explained the species turnover especially in the central-southern rivers we sampled, which are the seawater influenced ones. However, in the northern rivers we sampled, their weakest seawater influence leads to weak gradients of salinity and phosphorus; thus, channel width and littoral declivity may be more important variables to the species turnover within them. These rivers cross narrow coastal plains with relief variations (Ross and Fierz 2018), which can greatly influence their channel structure and morphometry (Bledsoe and Shear 2000).

In the longitudinal gradient, we expected life-form nestedness in all rivers because emergent and amphibious macrophytes usually occur downstream and along the entire gradient in tidal rivers, as opposed to the floating and submerged macrophytes that tend to occur farther from river mouth. Indeed, in most rivers we found higher relative importance of nestedness than turnover. However, only emergent and amphibious macrophytes occurred along them, which did not totally agree with our prediction about the ordered loss of life-forms in the rivers' longitudinal gradient. Thus, probably because of this absence of other life-forms (floating and submerged macrophytes) in these rivers, the environmental variation in the rivers' longitudinal gradient were less important to drive the life-form beta diversity than species turnover. Sharpe and Baldwin (2009) and Weilhoefer et al. (2013) found that the changes in habitat characteristics lead to the replacement of macrophyte groups along spatial gradient in coastal wetlands in the northern USA. We found that two of the three rivers with high life-form turnover (Itanhaém and Itapanhaú) had emergent, amphibious, submerged and floating macrophytes. Thus, for the Itanhaém and Itapanhaú, variables related to the longitudinal gradient explained their life-form beta diversity likely by promoting habitat diversity that supports different groups of macrophytes (Nunes et al. 2020).

Beta diversity at the regional scale

At the regional scale, we found a large variation in macrophyte species among the coastal rivers with higher importance of turnover than nestedness to beta diversity, contrary to our expectation, which indicates that each river has a distinct species composition. At the same time, the life-form variation among the coastal rivers was represented by nestedness, as we expected. Fu et al. (2019) also highlighted the importance of functional nestedness to macrophyte beta diversity in lakes in China at a coarse spatial scale, because of the loss of functional richness along the spatial gradient (Fu et al. 2019). We found that only river length explained rarefied beta diversity of macrophyte life-forms. In our study, the emergent and amphibious life-forms were the only ones present in the vast majority of the sampled rivers (six rivers) and the greatest life-form richness occurred only in the two largest rivers (the Itanhaém and Itapanhaú Rivers). The Itanhaém and Itapanhaú Rivers form very sharp meanders, which contribute to their great lengths on the coastal plain (19 and 35.84 km, respectively). In the meanders backwater areas with low current velocity occur and they contribute to the variety of habitat conditions (Garcia et al. 2012) and hydromorphology variation that favors the occurrence of various macrophyte life-forms (Gyosheva et al. 2019).

Thus, we highlight that variation in river length and consequently habitat and niche loss among the coastal rivers contribute to the life-forms nestedness in the north-south gradient.

At this regional scale, species turnover was explained neither by river length nor salinity gradient, which indicates that this pattern of species variation among rivers may be shaped by other factors that we did not consider here, such as dispersal limitation enhancing the turnover patterns we found. Given the almost complete compositional variation at the local scale, it is possible that each river sustains a distinct species pool to colonize and recolonize, with little connection among rivers. This dispersal limitation would be likely to occur due to the lack of hydrological connectivity among these rivers that run directly to the sea (Almeida and Carneiro 1998) and also due to the isolation barriers (mainly the Serra do Mar and ocean). In regions with high topographic variations the macrophyte community may be strongly influenced by spatial dispersal processes and history at regional scales (Alahuhta et al. 2018). Although aquatic plants tend to have broad distributions and are weakly limited (Santamaría 2002), Oikonomou and Stefanidis (2020) found that the compositional variation of these communities among lakes of the mountainous region of Southern Balkan (Greece) was primarily shaped by dispersal. Although this role of isolation and dispersion is not as expected as that of local factors (Viana et al. 2015), we suggest that they may have been important especially for species turnover and local species pool, given the spatial configuration of the studied coastal region. In general, at the estuarine local scales the dominant species of the regional pool are expected to occur due to the strong local environmental filtering (Kunza and Pennings 2008, Tamme et al. 2010, Guo et al. 2015). However, in our study, we observed a lack of species dominance and a restrictive occurrence of species in the north-south gradient, which possibly highlights the importance of the dispersal and isolation for shaping the particular species pool of each river.

Final remarks

Understanding the environmental drivers of macrophyte beta diversity and the patterns of turnover and nestedness can help in choosing the most appropriate strategies and actions for biodiversity conservation at various spatial scales (Fu et al. 2019). Based on the statements of Socolar et al. (2016), we highlight that at the regional scale, the high species turnover and life-forms nestedness we found in our study indicate that it is important to consider all these rivers in the north-south gradient and their environmental variations to species diversity conservation with attention to the longest rivers to maintain life-forms diversity of aquatic macrophytes. At the local scales, ensuring that the local environmental gradients are maintained is important for conservation of macrophyte diversity within these coastal rivers.

In summary, we found great variation in the macrophyte communities in the São Paulo coastal rivers at both spatial scales and different beta diversity patterns of species and life-forms. We conclude that the rivers' longitudinal gradients explained species turnover and life-form nestedness at the local scale and river length explained life-form beta diversity among the coastal rivers at the regional scale. We suggest that dispersion and historical contingency of colonization shaped species pool in each river, as rivers' species composition at the regional scale was not explained by the environmental variables we tested.

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Supplementary material

Figure S1. Monospecific stand of *Crinum americanum* that fringes the riverine forest in a narrow and shallow stretch in the littoral zone of the Itanhaém River (SE Brazil). Author: Laís S. C. Nunes.

Capítulo 4

A COMPETIÇÃO INTERESPECÍFICA DE MACRÓFITAS AQUÁTICAS ESTUARINAS TROPICAIS NÃO É DEPENDENTE DA DENSIDADE

Crinum americanum e *Spartina alterniflora*, Rio Itanhaém, Itanhaém/SP

The interspecific competition of tropical estuarine macrophytes is not density-dependent*

Resumo: A competição interespecífica de macrófitas aquáticas pode ser dependente da densidade e seu resultado tem importância para a distribuição de bancos monoespecíficos e mistos em estuários tropicais. Para avaliar o crescimento de *Spartina alterniflora* e *Crinum americanum* e o efeito das densidades iniciais em sua competição, nós desenvolvemos um experimento de laboratório usando cinco densidades de plantio de indivíduos jovens (8:0, 5:3, 4:4, 3:5 e 0:8) e dois tipos de sedimento (sedimento de baixo e médio estuário: salinidade 20,90 e 6,60, respectivamente). Nós testamos as hipóteses de que (i) em sedimento de baixo estuário, *S. alterniflora* teria maior capacidade competitiva do que *C. americanum,* mesmo em menores densidades iniciais, devido à sua grande tolerância à salinidade; (ii) No entanto, em sedimento de médio estuário, o resultado da competição dependeria das densidades iniciais das plantas, devido às condições salobras favoráveis para ambas as espécies. Nossos resultados corroboram nossa primeira hipótese, já que em sedimento de baixo estuário, *S. alterniflora* limitou a biomassa emergente de *C. americanum* por competição assimétrica, independentemente de suas densidades de plantio. Entretanto, nossa segunda hipótese não foi confirmada, pois *S. alterniflora* teve maior capacidade competitiva e limitou *C. americanum* a qualquer densidade de plantio em sedimento de médio estuário. Nós concluímos que a competição entre *S. alterniflora* e *C. americanum* nos dois tipos de sedimento não é dependente da densidade. Nós sugerimos que em baixo estuário, *S. alterniflora* possa ter excluído competitivamente *C. americanum*, independentemente de suas densidades de colonização; e que em médio estuário a ordem de chegada pode ser importante para a organização dos bancos mistos.

Palavras-chave: *Spartina alterniflora, Crinum americanum,* colonização, pressão de propágulos, densidade de plantio, exclusão competitiva, gradiente de salinidade.

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Abstract: The interspecific competition of aquatic macrophytes may be density-dependent and its outcome has importance for the monospecific and mixed stands zonation in tropical estuaries. To evaluate the growth of *Spartina alterniflora* and *Crinum americanum* and the effect of the initial densities on their competition we developed a laboratory experiment using five planting densities of young individuals (8:0, 5:3, 4:4, 3:5 and 0:8) and two sediment types (lower and middle estuary sediment: salinity 20.90 and 6.60, respectively). We tested the hypotheses that (i) in the lower estuary sediment *S. alterniflora* would have greater competitive ability than *C. americanum* even at lower initial densities, because of its great salt tolerance; (ii) However, at the middle estuary sediment the competitive outcome would depend on initial plant densities, because of the favorable brackish conditions for both species. Our results corroborated our first hypothesis, since in the lower estuary sediment *S. alterniflora* limited the *C. americanum* aboveground biomass by asymmetrical competition regardless of their planting densities. However, our second hypothesis was not confirmed as *S. alterniflora* had greater competitive ability and limited *C. americanum* at any planting density in the middle estuary sediment. We conclude that the competition between *S. alterniflora* and *C. americanum* in the two sediment types is not density-dependent. We suggest that in the lower estuary *S. alterniflora* might have competitively excluded *C. americanum* regardless of their colonization densities; and that in the middle estuary the arrival order might be important to the mixed stands organization.

Keywords: *Spartina alterniflora, Crinum americanum,* colonization, propagules pressure, planting density, competitive exclusion, salinity gradient.

Highlights:

The competition between *S. alterniflora* and *C. americanum* is not density-dependent.

S. alterniflora had greater competitive ability than *C. americanum* regardless of their density.

Colonization density was not determinant for species zonation in the estuary.

INTRODUCTION

Macrophyte zonation in estuaries has been explained by the competition-to-stress hypothesis that states that tolerance to abiotic stress (salinity and flooding) and competition act on the opposite extremes of the estuarine gradient (Crain et al., 2004; Engels and Jensen, 2010). Stress tolerance would be responsible for the tolerant species occurrence near the coastline (lower estuary). Competition would be responsible for exclusion of the tolerant species in freshwater (upper estuary) by the less tolerant ones with great competitive ability (Crain et al., 2004; Engels and Jensen, 2010). However, the importance of these factors may vary along salinity gradient (Barbour, 1978; Kenkel et al., 1991; Greenwood and Macfarlane, 2009; Weilhoefer et al., 2013) and even competition may have great importance in saline areas (Bockelmann and Neuhaus, 1999; Nunes and Camargo, 2018), once tolerant species can also be efficient competitors (Ungar, 1998; Emery et al., 2001; Costa et al., 2003).

Most of the estuarine macrophytes are perennial and the plant communities in the middle and lower estuary may be composed of good colonizer as well as good competitor species (Barrat-Segretain, 1996; Emery et al., 2001). Thereby, plants can develop strategies to avoid competition and then species coexistence and mixtures may occur (Watson and Byrne, 2009). However some halophytes may monopolize these brackish and saline habitats (Odum, 1988; Costa et al., 2003, Di Bella et al., 2014) because their adaptations to salt stress (like salt gland) (Hester et al., 2001; Flowers and Colmer, 2008) permit them to act as pioneer shoots and persistent adults (Costa et al., 2003).

Spartina alterniflora Loisel. (Poaceae) and *Crinum americanum* L. (Amaryllidaceae) are the main macrophyte species that occur in the southern coast of the São Paulo state (southeast Brazil, South America). *S. alterniflora* is a halophyte species adapted to a wide range of salinity levels (Wang et al., 2006) and commonly associated to mangrove forest and saline estuarine habitats in Brazil (Nunes and Camargo, 2018). This species is considered native from the Atlantic seacoast but it is invasive in the western USA, Europe and China (Bortolus et al., 2015). *C. americanum* occurs in flooded environments of the American seacoast, usually in brackish and freshwater habitats (Ribeiro et al. 2009; Nunes and Camargo, 2018). In temperate and subtropical estuaries the herbaceous species occur in extensive tidal marshes along a vertical elevation (low, mid and high marshes) (Engels and Jensen, 2010). However, in tropical estuaries there are the mangrove and Restinga forests (tidal arboreal vegetation), thus *S. alterniflora* and *C. americanum* occur at these forest's fringes (Schaeffer-Novelli et al., 1990), that is, in short areas (up to 20 m) in the edge of the arboreal vegetation, really close to the river and strongly influenced by the water level variation. In the Itanhaém River Estuary there are *S. alterniflora* monospecific stands in the lower estuary and mixed stands with these two species in the middle estuary. In a previous study, Nunes and Camargo (2018) suggested that *S. alterniflora* might be excluded from the upper estuary due to its intolerance of freshwater condition and that this species might have competitively excluded *C. americanum* from the lower estuary.

The competitive strategies and abilities of plants may differ not only with environmental condition (Wiegleb and Brux, 1991) but also with the life stage (Shipley et al., 1989; Callaway, 1995; Rojas-Sandoval and Meléndez-Ackerman, 2012). Most of the studies on macrophytes distribution in estuarine regions analyzed the response of adult plants (Crain et al, 2004; Pennings et al., 2005; Engels and Jensen, 2010); however, many mechanisms responsible for plant zonation will possibly have already an effect on the colonization and establishment processes of young plants (Scholten et al., 1987; Engels et al., 2011). Thus, the interspecific interaction outcomes during colonization may depend on the propagule and young plant densities (Gleason et al., 1979; Thomaz, 2002; Xie et al., 2006) and even the arrival order and priority effect (Fukami, 2015). For macrophytes, the vegetative propagules and young individuals play a major role on colonization, establishment, development and perenniality in new areas (Barrat-Segretain, 1996; Capers, 2003), since they are able to disperse, regenerate and propagate by clonal expansion (Keddy, 1976; Barrat-Segretain, 1996; Silinski et al., 2016). However, there are not so many studies testing the effects of plant density and salinity on macrophyte species growth and competition at their establishment phase in tropical estuarine environments.

Here we evaluated whether different initial densities of young individuals influence on interspecific competition between *S. alterniflora* and *C. americanum* in the lower and middle estuary conditions in order to understand the spatial distribution of these species in the Itanhaém River Estuary. We hypothesized that (i) at the lower estuary conditions (lower estuary sediment) *S. alterniflora* would have greater competitive ability than *C. americanum* even at lower initial densities, because of its great salt tolerance; (ii) However, at the middle estuary conditions (middle estuary sediment) the competitive outcome would depend on initial plant densities, because of the favorable brackish conditions for both species. To test these hypotheses, we conducted a laboratory experiment evaluating the species growth under two sediment types and five planting densities.

METHODS

Study area

The Itanhaém River Basin (southern coast of the São Paulo state, southeast Brazil) is strongly influenced by the tidal regime. Its estuarine zone, mixture of freshwater and seawater, has a salinity gradient (sediment salinity from 26 to 0) of about 10 km. The region has a micro-tidal regime and regular, lunar and semidiurnal tidal patterns, that is, the tidal range is less than two meters and there are two complete cycles of tides (high tide and low tide) per lunar day (24 h and 50 min) (Tessler et al. 2006). This region has a relatively constant and stable water flow in aquatic ecosystems and small seasonal variations in climatic characteristics over the year (Monteiro, 1973). The climate is classified as tropical rainfall climate with no dry season (Af climate according to the Köppen-Geiger classification). The average temperature in summer is 27.9°C and in winter 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).

The stands of *S. alterniflora* and *C. americanum* occur along the salinity gradient in the Itanhaém River Estuary (Fig. S1). The *S. alterniflora* monospecific stands are located in the lower estuary and really close to the mangrove forest in habitats of saline water and sediment. The *C. americanum* monoespecific stands are located in the upper estuary associated to Restinga forest and oligohaline water and sediment. In the middle estuary, there are mixed stands of these two species (Fig. S2) associated to brackish conditions and higher sediment nutrient and organic matter availability (Nunes and Camargo, 2018).

Experiment

We developed a randomized experiment lasting 150 days in a greenhouse comprising two factors (initial density x sediment type) to evaluate *S. alterniflora* and *C. americanum* growth and their interspecific competition in different initial densities of young individuals under two different sediment types (salinity levels). We applied the substitutive series method for our experimental design. This method consists of keeping a constant initial total density and varying the species proportion. It has been used to test the effect of factors (e.g. sediment type) on species interaction (Gibson et al., 1999) and it is appropriate for studies based on the competitors' similarity in terms of resource requirement (Sackville-Hamilton, 1994).

We used natural sediment of the lower and middle estuary of the Itanhaém River as substrate to evaluate the plant growth in the experiment. The sediments were collected near the macrophyte stands in the two estuarine portions, homogenized and put in the experimental units. After that, we used sediment samples of each experimental unit to determine the sediment salinity level, total nitrogen (TN) and phosphorus (TP) concentration and organic matter content (OM). The average values (and standard deviation) of salinity, TN (% dry mass), TP (% dry mass) and OM (% dry mass) of the lower estuary sediment were: 20.90 (1.30), 0.17 (0.03), 0.010 (0.001) and 15.30 (4.85); and those ones of the middle estuary sediment were: 6.60 (1.20), 0.22 (0.02), 0.011 (0.001) and 10.83 (2.89), respectively. We tested for significant differences ($p<0.05$) in these variables between the middle and lower estuary sediment ($n = 25$). For that, we applied the t-test (TN and OM) and the non-parametric Mann-Whitney test (salinity and TP) using GraphPad Prism 5.0 software (GPW5-066646-RCG7389) (GraphPad Software, 2007). The sediments of the two estuarine portions are significantly different in relation to salinity, TN, TP and OM (Table S1), so we called them "sediment types".

Most of the emergent macrophytes present clonal development, so it is difficult to identify individuals (Thomaz and Esteves, 2011). Although *S. alterniflora* and *C. americanum* are rhizomatous species, the growth form of both species allows the identification of "individuals", because *S. alterniflora* forms stems and *C. americanum* has rosette philotaxis (Nunes and Camargo, 2017). Thus, we considered as individual each stem and rosette, i.e., each plant emergence above the sediment (Wang et al., 2010).

We collected young individuals (up to 0.50 m) of *C. americanum* and *S. alterniflora* in their mixed stands from the middle estuary of the Itanhaém River. We chose to collect the plants in this estuarine portion so that they were in the same field environmental conditions at the experiment planting. The individuals were planted in the experimental units (plastic boxes: volume = 26 liters and area = 0.13 m^2) in quintuplicate. We used a total planting density (initial total density) similar to that observed in the field. It was of 8 individuals per experimental unit, 8:0 and 0:8 in the monoculture and 5:3, 4:4 e 3:5 in the mixed culture treatments (5 planting densities x 2 sediment types x 5 replicas = 50 experimental units). The experimental units were constantly watered with tap water to maintain the approximate level of 2.0 cm above the sediment surface.

To estimate macrophytes biomass we used the non-destructive method and its equations (Eq. 1, 2. 3 and 4) proposed by Nunes and Camargo (2017). Seventy individuals each of *S. alterniflora* and *C. americanum* of different sizes were collected in the Itanhaém River Estuary. In the laboratory, the height of each individual was measured considering the height of the *S. alterniflora* stem and the *C. americanum* highest leaf. The root and rhizome volumes of both species were estimated by measuring water displacement in a graduated recipient. The plant aboveground and belowground fractions were dried in an oven at 60°C to constant mass and then weighed on a scale. The equations were obtained by significant ($p<0.05$) simple linear regressions between individual height and aboveground dry mass (Eq. 1 and 2), and between volume and belowground dry mass (Eq. 3 and 4).

Equation 1: *S. alterniflora* aboveground dry mass = 10.64 * (individual height (m) – 0.5451)

($R^2 = 0.8279$)

Equation 2: *C. americanum* aboveground dry mass = 16.03 * (individual height (m) – 6.0077)

($R^2 = 0.7532$)

Equation 3: *S. alterniflora* belowground dry mass = 0.0932 * (belowground volume (mL) + 0.3244)

($R^2 = 0.7965$)

Equation 4: *C. americanum* belowground dry mass = 0.0504 * (belowground volume (mL) + 1.241)

($R^2 = 0.9315$)

We performed measurements of the plant height on days 23, 38, 64, 83, 104, 119 and 150. On these same days we counted the number of individuals per species in each experimental unit. The belowground volume was assessed at the beginning (day 1) and end of the experiment. To obtain the values of the plant aboveground and belowground biomass, we used the regression equations cited above.

We adjusted the logistic and simple linear regression models to the growth curves between aboveground biomass and time (from day 23 to day 150) for each replica *S. alterniflora* and *C. americanum* and aboveground biomass to compare their growth in the different treatments. The logistic model allows evaluating the response of the aboveground biomass through the carrying capacity and the intrinsic growth rate; and the linear regression model through the slope of the regression line. For all *S. alterniflora* replicas the fit of both growth models of aboveground biomass was significant ($p<0.05$). However, for 35% of the total *C. americanum* replicas we found no significant adjustment of any growth model. Thus, we chose to evaluate the species aboveground growth through gain of biomass.

We evaluated the variation in gain of total biomass (GTB), gain of aboveground biomass (GAB) and gain of belowground biomass (GBB) in the experiment using the following equation (Eq. 5).

Equation 5: $GB_{ax} = Bf_{ax} – Bi_{ax}$,
where GB_{ax} = gain in biomass (grams of dry mass per square meter = g m^{-2}) of the species *a* in the treatment *x*; Bf_{ax} = final biomass (g m^{-2}) of the species *a* in the treatment *x*; and Bi_{ax} = initial biomass (g m^{-2}) of the species *a* in the treatment *x*.

We calculated the GBB through the biomass increasing between the first and last experiment days, that is, day 1 and 150. However, to calculate the GAB we considered the biomass increasing between the lowest initial biomass value (day 1 or 23) and the greatest final biomass value (day 119 or 150) because they varied among the replicas.

Data analysis

We tested for significant differences ($p<0.05$) among the GTB, GAB and GBB of *S. alterniflora* and *C. americanum* in the different initial densities and sediment types (gain of biomass = response variable; initial density and sediment type = categorical predictors). For this, we evaluated and confirmed that the conditions of data normality and homoscedasticity had been met. For the values of GTB of *C. americanum* we performed the logarithmic transformation so that the normality and homoscedasticity conditions were met. Then, we applied the two-way analysis of variance (Factorial ANOVA) and the Tukey's test a posteriori using the Statistica 7.1 software (SN AX505B150718FA) (StatSoft, 2005).

We elaborated the gain of biomass graphs (GTB, GAB and GBB), variation in number of individuals and growth of aboveground biomass using the GraphPad Prism 5.0 software (GPW5-066646-RCG7389) (GraphPad Software, 2007).

RESULTS

The gain of biomass (GTB, GAB and GBB) of *S. alterniflora* was not limited by the presence of *C. americanum* in any density and sediment type (Fig. 1, Table S1). The values of GTB and GAB of *S. alterniflora* in monoculture showed no significant differences between the sediment types (Fig. 1 A and B, Table S1). However, the values of GBB of *S. alterniflora* in monoculture and mixed culture in the lower estuary sediment were significantly lower than the values in monoculture in the middle estuary sediment, which indicates that salinity was limiting for the belowground biomass growth of this species (Fig. 1 C, Table S1).

The gain of biomass of *C. americanum* (GTB, GAB and GBB) was significantly different among the densities and sediment types (Fig. 2, Table S1). The presence of *S. alterniflora* at any density limited the GAB of *C. americanum* in both sediment types (Fig. 2 B). However, the GTB and GBB of *C. americanum* were limited by the presence of *S. alterniflora* at any density only in the middle estuary sediment (Fig. 2 A and C). In the lower estuary sediment, the values of GTB and GBB of *C. americanum* in monoculture and mixed culture were significantly lower than the values in the middle estuary sediment, which indicates that salinity was more limiting for the total and belowground biomass growth of this species than interspecific competition (Fig. 2 A and C).

The number of *S. alterniflora* individuals increased throughout the experiment in all densities and sediment types (Fig. 3); however the number of *C. americanum* individuals remained nearly constant over time in all treatments (Fig. 4).

The growth curves of *S. alterniflora* presented a sigmoid pattern (logistic model) for all densities and sediment types (Fig. 5). The growth curves of *C. americanum* varied depending the densities and sediment types, and the *C. americanum* growth was more moderate than the *S. alterniflora* growth (Fig. 5 and 6). In the lower estuary sediment, *C. americanum* had a reduction of aboveground biomass in the early experimental period (Fig. 6 A).

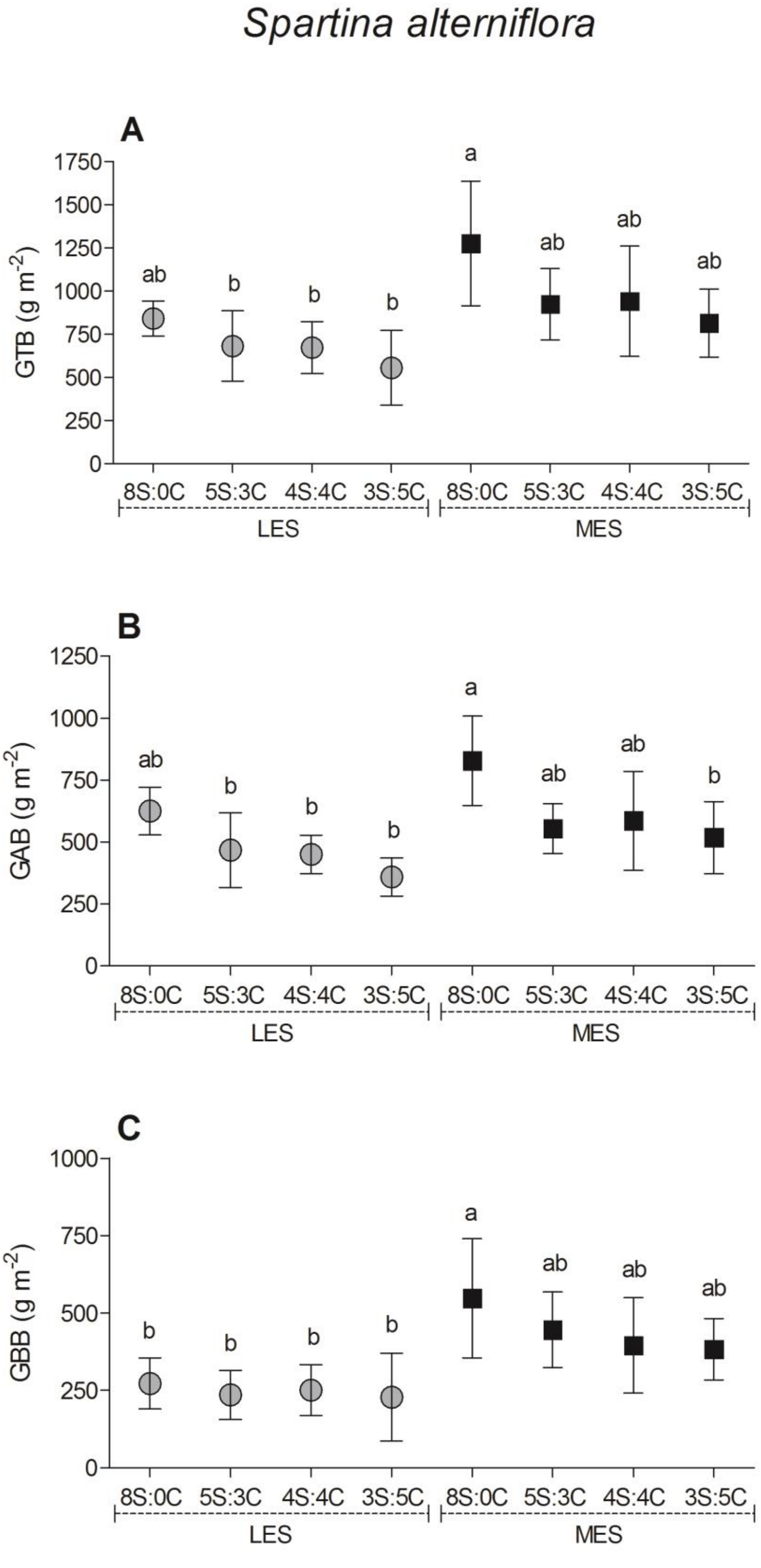

Figure 1. Average values and standard deviation of (A) Gain of total biomass - GBT, (B) Gain of aboveground biomass - GAB and (C) Gain of belowground biomass - GBB (grams of dry mass per m^2) of *Spartina alterniflora* at the different initial densities and sediment types. The graphs have different scales. Distinct letters indicate significant difference ($p < 0.05$). LES: Lower estuary sediment; MES: Middle estuary sediment; C: *Crinum americanum*; S: *S. alterniflora.*

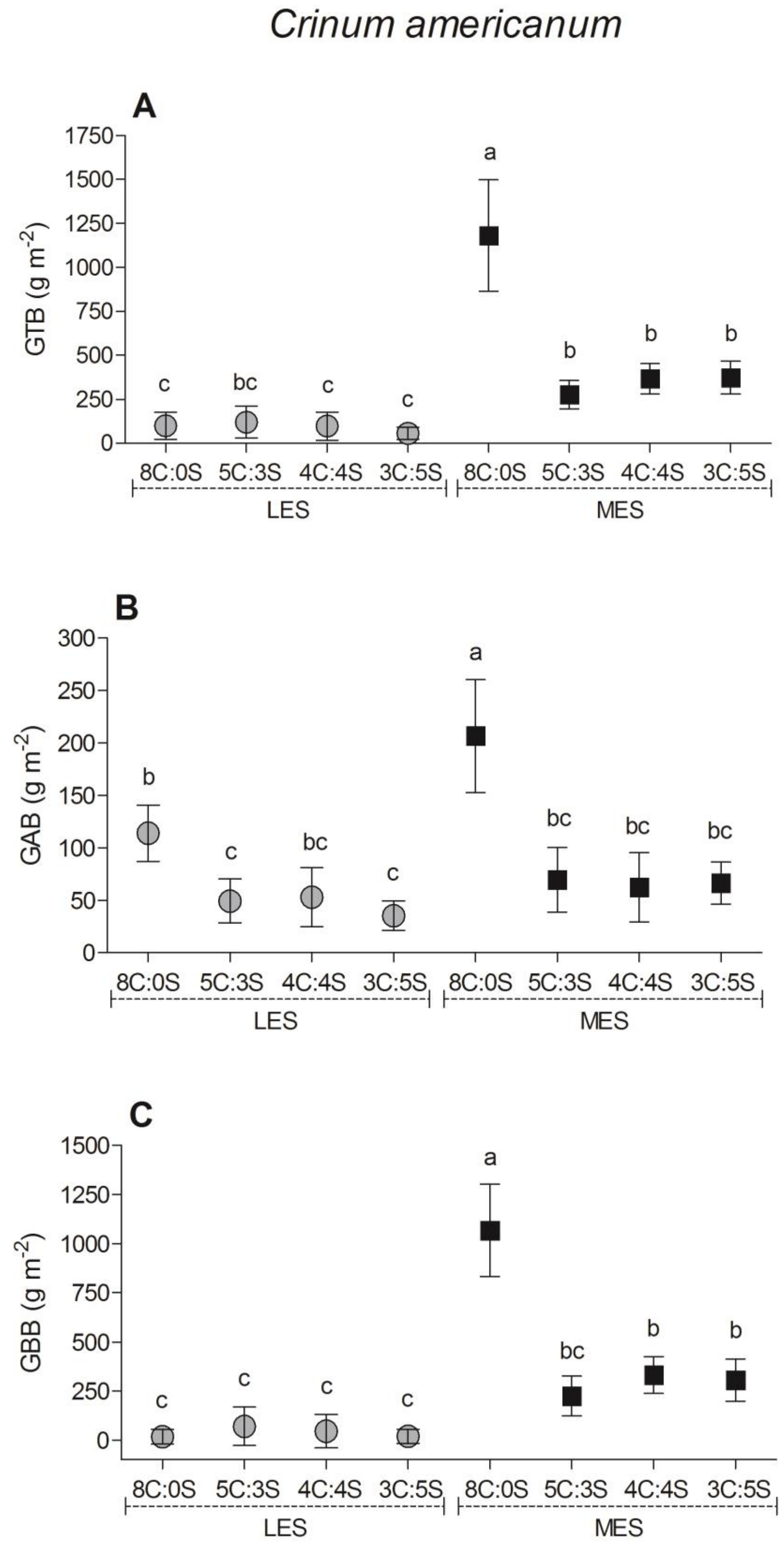

Figure 2. Average values and standard deviation of (A) Gain of total biomass - GBT, (B) Gain of aboveground biomass - GAB and (C) Gain of belowground biomass - GBB (grams of dry mass per m^2) of *Crinum americanum* at the different initial densities and sediment types. The graphs have different scales. Distinct letters indicate significant difference ($p < 0.05$). LES: Lower estuary sediment; MES: Middle estuary sediment; C: *C. americanum*; S: *Spartina alterniflora.*

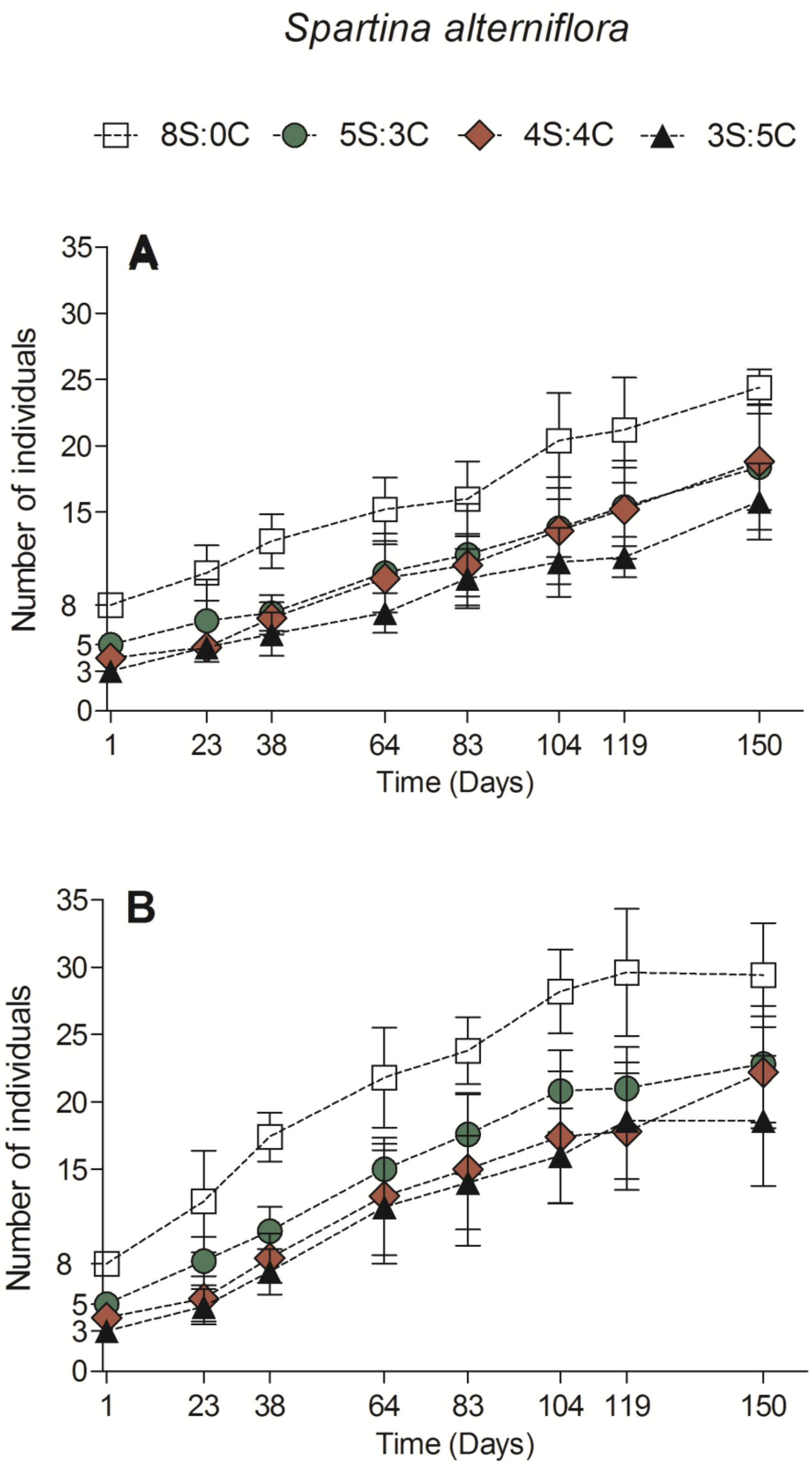

Figure 3. Average values and standard deviation of the number of *Spartina alterniflora* individuals per experimental unit (0.13 m^2) throughout the experiment at the different initial densities in (A) Lower estuary sediment and (B) Middle estuary sediment. C: *Crinum americanum*; S: *S. alterniflora.*

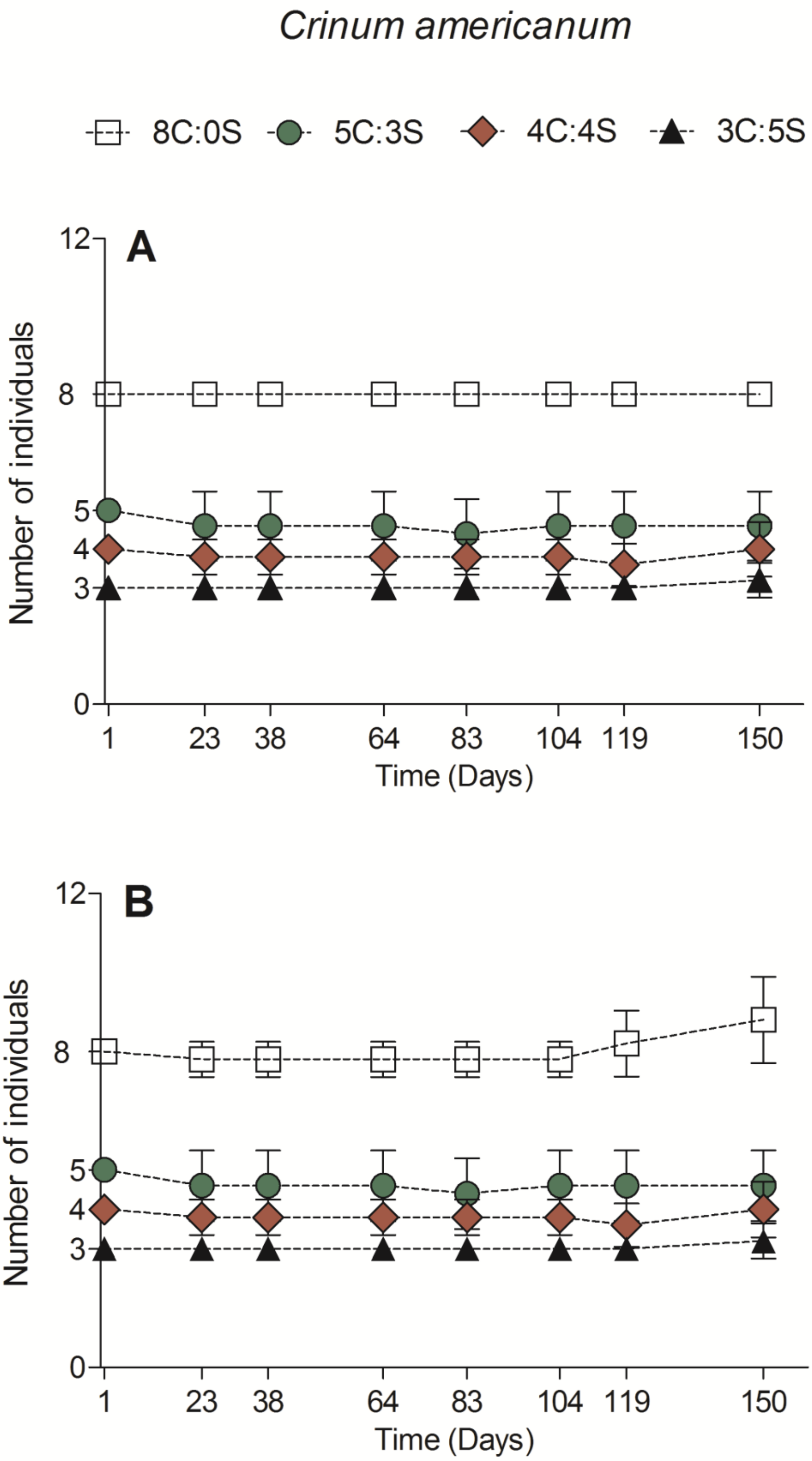

Figure 4. Average values and standard deviation of the number of *Crinum americanum* individuals per experimental unit (0.13 m^2) throughout the experiment at the different initial densities in (A) Lower estuary sediment and (B) Middle estuary sediment. C: *C. americanum*; S: *Spartina alterniflora*.

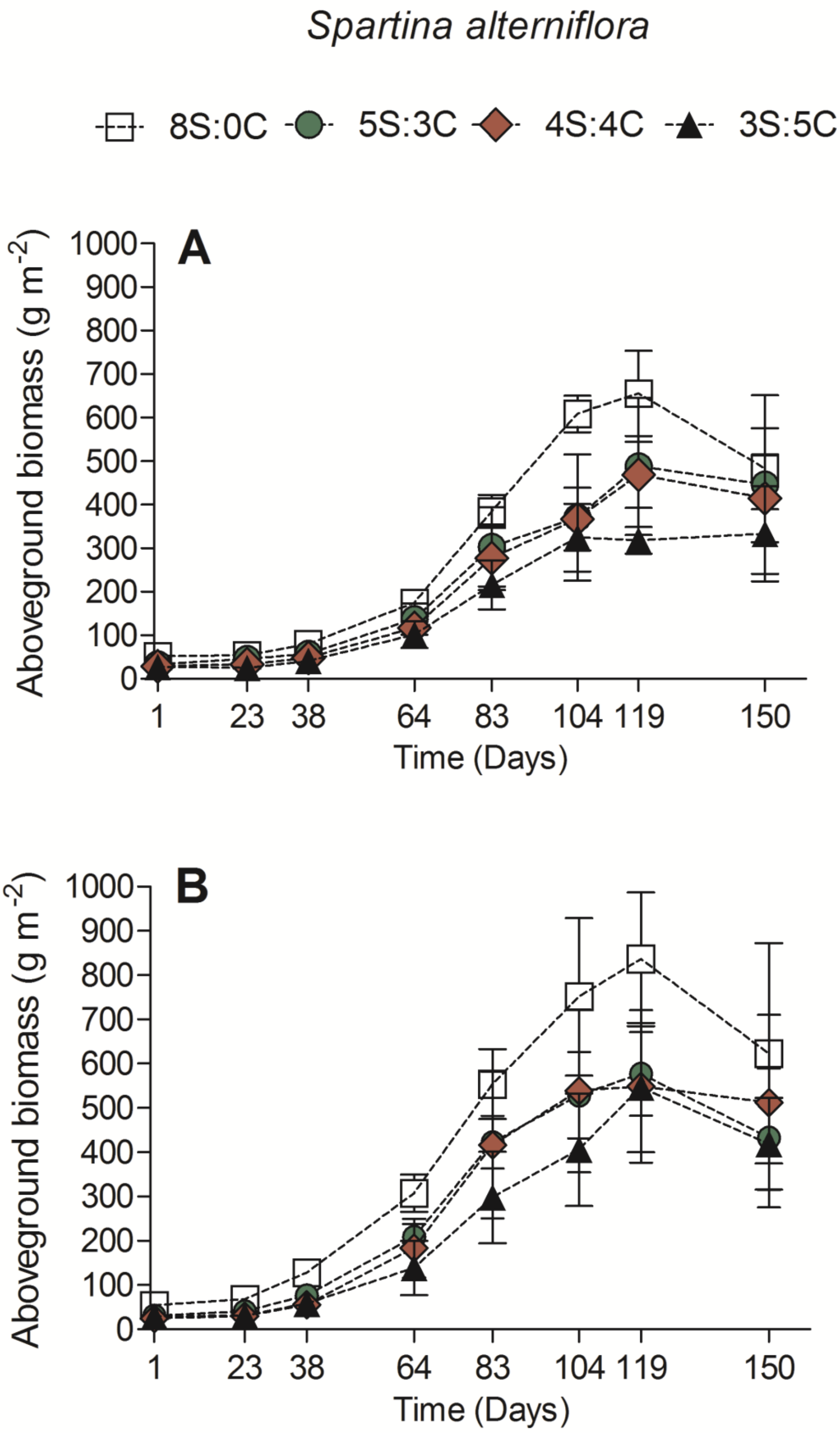

Figure 5. Average values and standard deviation of *Spartina alterniflora* aboveground biomass (grams of dry mass per m^2) throughout the experiment at the different initial densities in (A) Lower estuary sediment and (B) Middle estuary sediment. C: *Crinum americanum*; S: *S. alterniflora*.

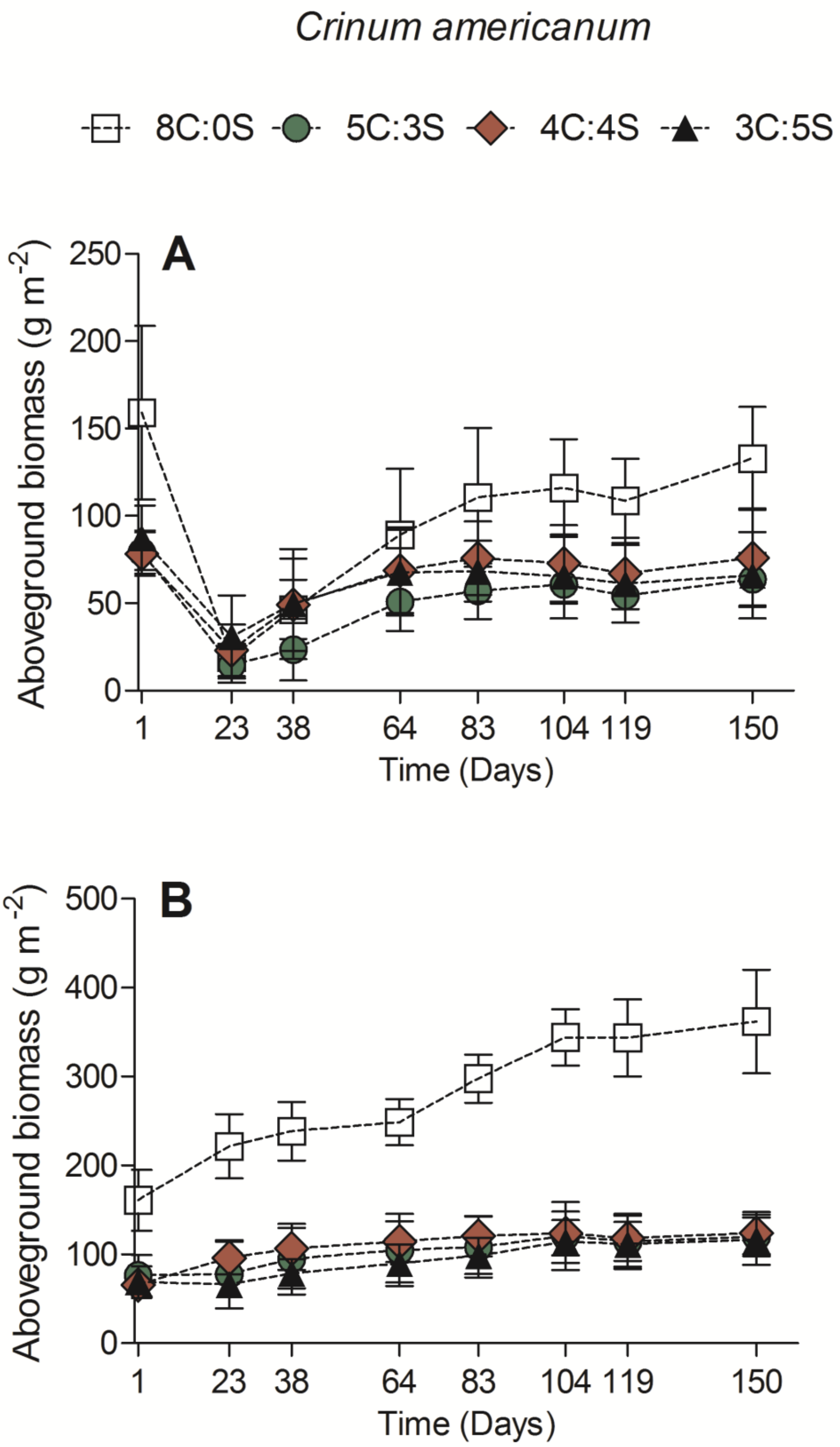

Figure 6. Average values and standard deviation of *Crinum americanum* aboveground biomass (grams of dry mass per m^2) throughout the experiment at the different initial densities in (A) Lower estuary sediment and (B) Middle estuary sediment. The graphs have different scales. C: *C. americanum*; S: *Spartina alterniflora.*

DISCUSSION

The results of our experiment showed that *S. alterniflora* growth was not limited by *C. americanum* in any sediment type. However, *S. alterniflora* may limit *C. americanum* growth in both sediment types and regardless of density, with asymmetric competition occurring. Our first hypothesis that the halophyte *S. alterniflora*, regardless of the initial density, would have greater competitive ability than *C. americanum* in the lower estuary sediment was corroborated for aboveground biomass competition. In this sediment type, *S. alterniflora* at any density limited the growth of *C. americanum* aboveground biomass. However, the conditions of the lower estuary sediment were more limiting than interspecific competition for the growth of total and belowground biomass of *C. americanum*. Our second hypothesis that in the middle estuary sediment the competitive outcome would depend on initial plant densities was not corroborated. In this sediment type, *S. alterniflora* had greater competitive ability since it limited the *C. americanum* growth at any density.

We found that competition between *S. alterniflora* and *C. americanum* in both sediment types was not dependent on the initial density of young individuals. Some studies have shown that interspecific competition between submerged macrophytes can be density-dependent (Michelan et al., 2018; Silveira et al., 2018); however, especially for emergent macrophytes in estuarine regions the density effect on interspecific competition is still uncertain (Zhou et al., 2017). In general, what has been observed in estuary studies is that initial density may not have interfered with plant growth (Bockelman and Neuhaus, 1999; Zhou et al., 2017) and that species with great investment in clonal expansion (Bertness and Elisson, 1987; Paradis et al., 2014) and aboveground biomass tend to have greater competitive ability (Guo and Pennings, 2012). In addition, according to Barrat-Segretain (1996) if different macrophyte species colonized the same open area at the same time, those that develop more aboveground biomass will have the greatest competitive advantage. In our experiment, this was the case for *S. alterniflora* that showed the greatest increasing in number of individuals, gain of aboveground biomass and competitive ability compared to *C. americanum*.

We observed that *S. alterniflora* and *C. americanum* presented different growth strategies at our experimental conditions. *Spartina alterniflora* showed great investment in clonal production, increasing the number of individuals and consequently biomass throughout the experiment; and *C. americanum* showed no clonal investment, so its moderate increase in biomass occurred due to the investment in growth of each individual itself. In an experiment, Paradis et al. (2014) observed that the halophyte *Phragmites australis* limited the *Thypha angustifolia* growth at all tested salinity levels (0, 6 and 12), but that *T. angustifolia* did not limit the *P. australis* growth. The authors related the greatest competitive advantage of *P. australis* to its greater clonal expansion and thus to the increasing in number of individuals and biomass, which resulted in asymmetric competition possibly due to differences in density increase between these species. Thus, we suggest that the faster increment in density of *S. alterniflora* compared to *C. americanum*, leading the former to reach higher density than the latter one in our experiment, also played an important role in the competitive advantage of *S. alterniflora* over *C. americanum*.

The lack of influence of initial density on the interspecific interaction of emergent macrophytes in salinity conditions was also observed by Zhou et al. (2017) in an experimental study. These authors observed that different initial densities did not affect the interaction between *Scirpus planiculumis* and *Suaeda salsa* at no salt (0) and low salinity (8) conditions. However, density was important for facilitation under high salinity conditions (15). In general, interspecific interactions tend to be less negative in stressful habitats, for example, saline habitats (Keammerer and Hacker, 2013). However, in our experiment there was no tendency for facilitation in the lower estuary sediment, and *C. americanum* had its aboveground growth limited by both *S. alterniflora* and sediment type. In estuarine conditions, the facilitation may occur mainly due to the mechanism of amelioration of sediment salinity conditions and salt stress alleviation from a species over another one (Bertness and Hacker, 1994). Considering this mechanism, it is possible that the conditions of the lower estuary sediment in our experiment were not harsh enough for facilitation, but they were limiting enough for the *C. americanum* growth. A similar fact was suggested by Engels and Jensen (2010) that did not find a facilitation effect on *Phragmites australis* in the lower estuary of the Elbe River (Germany) either.

Although *C. americanum* was limited by both competition and sediment conditions in our experiment, it was able to survive and grow moderately in the lower estuary sediment. In a study on salt marshes in the Netherlands, Bakker et al. (1985) observed that non-halophyte species may develop as young plants in the lower estuary, but as adults they end up being suppressed by the halophyte species. Although the competition is less common in more stressful areas, the competitive exclusion of macrophyte species from lower estuary zones has already been reported by other studies on salt marshes in Europe (Bockelmann and Neuhaus, 1999) and the northern USA (Medeiros et al., 2013). The possibility of competitive exclusion of *C. americanum* by *S. alterniflora* in the lower estuary of the Itanhaém River had also been evidenced by Nunes and Camargo (2018) from the result of previous pairwise experiment; however, the authors could not confirm a significant negative effect of *S. alterniflora* on *C. americanum*. Thus, we indicate here that possibly the less favorable conditions of the lower estuary sediment and the combination of salt stress of aboveground and belowground fractions and aboveground biomass limitation by interspecific competition may indeed have led to the exclusion of *C. americanum* in the lower estuary of the Itanhaém River.

We observed that the conditions of the middle estuary sediment were favorable for the *S. alterniflora* and *C. americanum* development, once these species presented great average values of gain of biomass in monoculture in this sediment type. In brackish and middle estuarine areas high productivity may occur (Guo and Pennings, 2012), as macrophyte species found in both freshwater (upper estuary) and salt habitats (lower estuary) may show higher growth under these conditions (Nunes and Camargo, 2018). In middle estuaries, high species diversity and coexistence have also been reported (Watson and Byrne, 2009; Wieski et al., 2010), because in these brackish areas there may be a balance among increased nutrient and organic matter availability and intermediate levels of salinity and dissolved sulfur (Craft, 2007; Wieski et al., 2010) favoring the estuarine macrophytes development. However, in contrast, intense interspecific competition can also occur due to these abiotic factors and higher productivity in these areas (Guo and Pennings, 2012), as we observed in our experiment.

In the middle estuary sediment, we observed asymmetric competition with *S. alterniflora* limiting the total, aboveground and belowground biomass of *C. americanum* regardless of their initial densities, but not the opposite. In the middle estuary of the Itanhaém River, the species *S. alterniflora* and *C. americanum* co-occur, which is in conflict with the result of our experiment that showed the greatest competitive ability of *S. alterniflora*. However, these mixed stands have a lateral organization of the species, where *C. americanum* occurs near the arboreal vegetation and farther from the river channel, whereas *S. alterniflora* occurs near the river channel in locations with greater variation in water level (Nunes and Camargo, 2018). One possibility we suggest is that *C. americanum* colonized the middle estuary areas prior to *S. alterniflora* and the way the species organized in the mixed stands prevented *C. americanum* from being excluded by competition for *S. alterniflora*. Thus, the arrival order may have been important for macrophyte zonation in the middle estuary, since our results showed that the different species densities did not influence on their interspecific interaction.

The arrival order influences on the assembly structure and organization (Fukami, 2015). Newly arrived plant propagules may not be able to interfere with already established species due to the priority effect and thus the early colonizer species tends to persist even when more competitive ones arrive later (Sarneel et al., 2016). In addition, other factors that influence macrophytes may reduce the occurrence of competitive exclusion and promote competitive displacement and species coexistence. Some studies have indicated that *S. alterniflora* is quite tolerant of different flooding levels (Brown et al., 2006; Visser and Sandy, 2009; Voss et al., 2013) and may even be required for intermittent tidal flooding and saturated sediment (Adams and Bate, 1995); and *C. americanum* may be less flood tolerant (Ribeiro et al., 2011). Thus, the specific flood tolerances should also be taken into account to the mixed macrophyte stands organization in estuaries (Levine et al., 1998).

Finally, we conclude that the initial density of young individuals did not influence the interaction between *S. alterniflora* and *C. americanum*; and that possibly the initial density was not determinant for the species zonation in the Itanhaém River Estuary. We indicate that in the lower estuary *S. alterniflora* may have competitively excluded *C. americanum* regardless of the colonization density of these species. We suggest that in the middle estuary the colonization density of these species was not important either and the arrival order may have been more important for the mixed stand organization. Possibly the early colonization of *C. americanum* allowed species coexistence and avoided its competitive exclusion by *S. alterniflora*.

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Supporting information

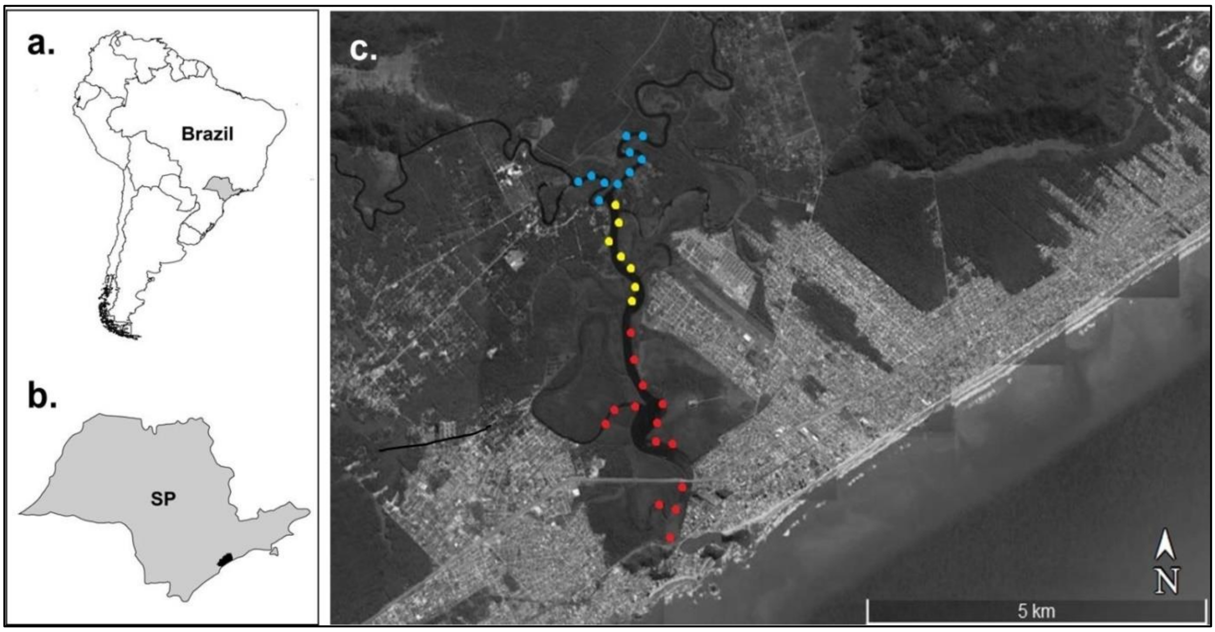

Figure S1. Maps of South America, Brazil (a) and the São Paulo State (b), highlighting the Itanhaém River Estuary (c); and the location of the monospecific stands of *Spartina alterniflora* in the lower estuary (red circles), mixed stands of *S. alterniflora* e *Crinum americanum* in the middle estuary (yellow circles), and *C. americanum* monospecific stands in the upper estuary (blue circles).

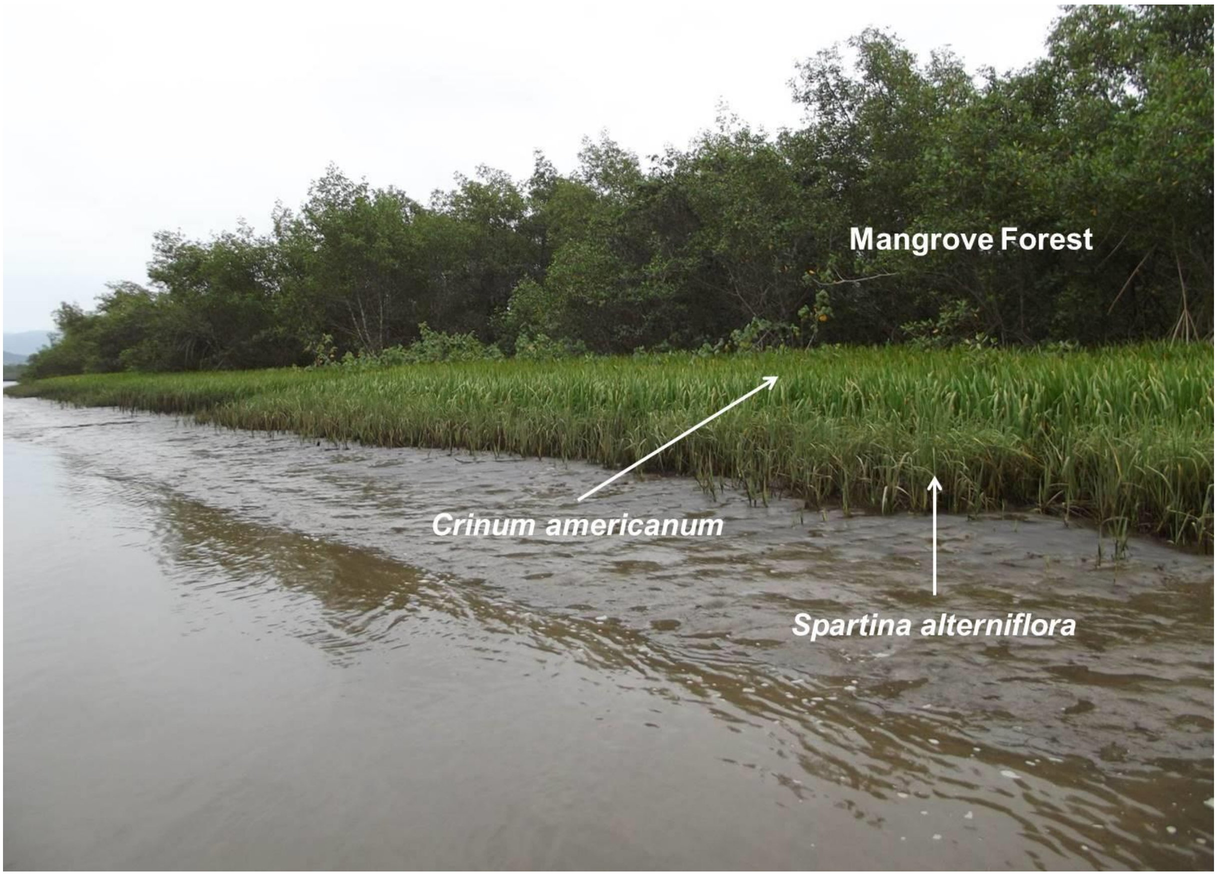

Figure S2. Mixed stand of *Spartina alterniflora* and *Crinum americanum* in the middle estuary of the Itanhaém River at low tide. The mixed stands have a species lateral organization, where *C. americanum* occurs near the arboreal vegetation (mangrove forest) and farther from the river channel, whereas *S. alterniflora* occurs near the river channel.

Table S1. Results of the t-test and Mann-Whitney test applied to salinity, total nitrogen (TN) and phosphorus (TP) concentration, and organic matter content (OM) between the two sediment types (lower and middle estuary sediment).

Variables	Applied test	Results	
Salinity	Mann-Whitney test	U=0.0	p<0.01
TN	t-test	t=5.96 df=48	p<0.01
TP	Mann-Whitney test	U=131.0	p<0.01
OM	t-test	t=3.96 df=48	p<0.01

Table S2. 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 *Spartina alterniflora* and *Crinum americanum* in the different initial densities and sediment types. The significant values are highlighted in bold ($p<0.05$).

	df	F	p
S. alterniflora			
GTB			
Density	3	4.54	< **0.01**
Sediment type	1	16.72	< **0.01**
Density*Sediment type	3	0.37	0.776
GAB			
Density	3	8.35	< **0.01**
Sediment type	1	11.56	< **0.01**
Density*Sediment type	3	0.32	0.814
GBB			
Density	3	1.33	0.281
Sediment type	1	24.57	< **0.01**
Density*Sediment type	3	0.58	0.635
C. americanum			
GTB			
Density	3	27.20	< **0.01**
Sediment type	1	150.60	< **0.01**
Density*Sediment type	3	31.90	< **0.05**
GAB			
Density	3	29.21	< **0.01**
Sediment type	1	15.73	< **0.01**
Density*Sediment type	3	3.71	< **0.05**
GBB			
Density	3	27.20	< **0.01**
Sediment type	1	150.60	< **0.01**
Density*Sediment type	3	31.90	< **0.01**

Capítulo 5

A IMPORTÂNCIA DA ORDEM DE CHEGADA E DO EFEITO DE PRIORIDADE PARA A ORGANIZAÇÃO DA COMUNIDADE DE MACRÓFITAS AQUÁTICAS EM UM ESTUÁRIO TROPICAL

Spartina alterniflora, Rio Itanhaém, Itanhaém/SP

The importance of arrival order and priority effect for the macrophyte community organization in a tropical estuary

Resumo: A ordem de chegada de espécies na colonização pode ser importante para a organização de comunidades vegetais perenes ao longo de gradientes ambientais. Devido ao efeito de prioridade, espécies fundadoras (“*early-colonizing*”) se mantêm no ambiente mesmo com a chegada posterior de espécies (“*later-arriving*”) de maior habilidade competitiva. Para avaliar se a ordem de chegada e o efeito de prioridade influenciam a interação interespecífica entre indivíduos adultos “*early-colonizing*” e indivíduos jovens “*later-arriving*” das macrófitas emergentes estuarinas perenes *Spartina alterniflora* (S) e *Crinum americanum* (C), nós desenvolvemos um experimento em laboratório com quatro culturas (monoculturas de indivíduos “*early-colonizing*” de S; monoculturas de indivíduos “*early-colonizing*” de C; culturas de indivíduos “*early-colonizing*” de S com introdução de indivíduos “*later-arriving*” de C; e culturas de indivíduos “*early-colonizing*” de C com introdução de indivíduos “*later-arriving*” de S) e dois tipos de sedimento (sedimento de baixo e médio estuário: salinidade 32 e 20, respectivamente). As hipóteses que testamos foram que em ambos os tipos de sedimento, devido ao efeito de prioridade, (i) a espécie “*later-arriving*” não se estabeleceria em vegetação adulta “*early-colonizing*” e (ii) a espécie “*later-arriving*” não interferiria no crescimento da espécie “*early-colonizing*”. Nossos resultados corroboram nossas hipóteses, pois nos dois tipos de sedimento os indivíduos “*later-arriving*” de *S. alterniflora* e *C. americanum* não cresceram e não se estabeleceram em culturas heteroespecíficas de indivíduos “*early-colonizing*”. E ainda, o crescimento de indivíduos “*early-colonizing*” não foi limitado pela presença dos indivíduos “*later-arriving*”. Nós concluímos que a ordem de chegada e o efeito de prioridade podem influenciar a competição interespecífica, a organização de comunidades e a distribuição espacial de macrófitas aquáticas em estuários tropicais.

Palavras-chave: *Spartina alterniflora, Crinum americanum,* colonização, exclusão competitiva, efeito de prioridade inibitório, gradiente ambiental, gradiente de salinidade.

Abstract: The arrival order in species colonization may be important to the communities' organization of perennial plants along environmental gradients. Due to the priority effect, founder species 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 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: *Spartina alterniflora, Crinum americanum,* colonization, competitive exclusion, inhibitory priority effect, environmental gradient, salinity.

INTRODUCTION

The interspecific competition and abiotic stress are important factors for the organization of communities and spatial distribution of aquatic macrophytes in estuaries (Crain et al, 2004; Engels and Jensen, 2010). The importance of these factors may vary along the environmental gradient (Greenwood and Macfarlane, 2009) and both of them may act on its extremes (Nunes and 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 and Neuhaus, 1999) or competition for dominance (Angelini et al., 2011).

Aquatic macrophyte communities 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 and Fleetwood, 1987). These competitive abilities and establishment success in new areas may vary according to the plant life stage (Shipley et al., 1989; Callaway, 1995; Rojas-Sandoval and Meléndez-Ackerman, 2012) and size of the colonizer propagules (Bickel, 2017). 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 and Ellison, 1987; Schwarz et al., 2015).

In the Itanhaém River Estuary (Southeastern Brazil) the most abundant macrophyte species present are *Spartina alterniflora* Loisel. (Poaceae) and *Crinum americanum* L. (Amaryllidaceae). In this region, there are monospecific stands of *S. alterniflora* in the lower estuary and mixed stands of both species in the middle estuary. Nunes and Camargo (2018) suggested that *S. alterniflora* may have, in a long-term, competitively excluded *C. americanum* from the lower estuary and that, possibly, some mechanisms 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 organization of communities and zonation of *S. alterniflora* and *C. americanum* in this estuary.

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).

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. 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 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 and Camargo, 2018). In the middle estuary, *S. alterniflora* and *C. americanum* form mixed stands with a lateral segregation between them, as *S. alterniflora* occupies the stand portion closest to the river channel and *C. americanum* occupies the portion closest to the arboreal vegetation (Nunes and 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 and 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 and 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 and Camargo, 2018) and also in freshwater environments in Pantanal, Cerrado (tropical savannah) and Amazon (Dutilh and Oliveira, 2020).

Experiment

We conducted a manipulative experiment with two factors (4 cultures x 2 sediment types x 5 replicates = 40 experimental units) 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) (Fig. 1).

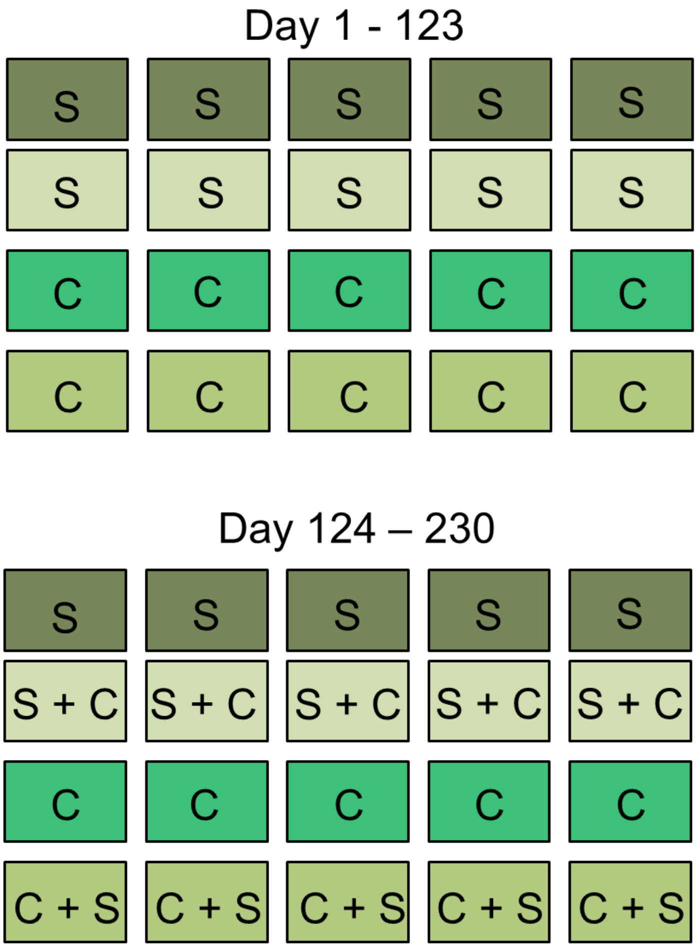

Figure 1. Experimental design scheme used with each sediment type (lower and middle estuary sediment) totaling 40 experimental units. Cultures: **Monocultures:** monocultures of the early-colonizing individuals of *Spartina alterniflora* (S), monocultures of the early-colonizing individuals of *Crinum 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.

We collected the early-colonizing individuals of *S. alterniflora* and *C. americanum* similar size (adult individuals from 0.50 to 0.70 m) 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 m². The initial total density, similar to that observed in the field, was ten individuals per experimental unit.

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.

The 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.

The experimental units were constantly watered with tap water to maintain the approximate level of 2.0 cm above the sediment surface.

We collected the sediment in the lower and middle estuary in the Itanhaém River close to the macrophyte stands. The sediment from each stand was collected with a shovel to a maximum depth of about 20 cm, homogenized and disposed in the experimental units. Then, the sediment samples from each experimental unit were used to determine the salinity and the contents of total nitrogen (TN) and total phosphorus (TP) at the beginning of the experiment. The average values (standard deviation) of salinity, 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 (Table S1).

To estimate the macrophyte aboveground and belowground biomass we used the non-destructive method and its regression equations (Eq. 1 to 4) developed by Nunes and Camargo (2017). These authors generated the equations through significant simple linear regressions ($p < 0.05$) between individual height and aboveground dry mass (Eq. 1 and 2), and between volume and belowground dry mass (Eq. 3 and 4) (Nunes and Camargo, 2017).

Equation 1: *S. alterniflora* aboveground dry mass = 10.64 * (individual height (m) – 0.5451)

($R^2 = 0.8279$)

Equation 2: *C. americanum* aboveground dry mass = 16.03 * (individual height (m) – 6.0077)

($R^2 = 0.7532$)

Equation 3: *S. alterniflora* belowground dry mass = 0.0932 * (belowground volume (mL) + 0.3244)

($R^2 = 0.7965$)

Equation 4: *C. americanum* belowground dry mass = 0.0504 * (belowground volume (mL) + 1.241)

($R^2 = 0.9315$)

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. The belowground fraction volume was obtained on the first and last days of the experiment. To estimate the macrophytes aboveground and belowground biomass we used the equations cited above (Eq. 1 to 4).

We evaluated the difference between the final and initial biomass (total biomass, aboveground biomass and belowground biomass) (ΔTB, ΔAB and ΔBB) of the early-colonizing and later-arriving individuals in the experiment (Eq. 5).

Equation 5: $\Delta \mathrm{Ba}x = \mathrm{Bf}ax - \mathrm{Bi}ax$, where ΔB_{ax} = difference of biomass (grams of dry mass per square meter = g m^{-2}) of the species *a* in the treatment *x*; Bf_{ax} = final biomass (g m^{-2}) of the species *a* in the treatment *x*; and Bi_{ax} = initial biomass (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, 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 (Eq. 6).

Equation 6: $RGRax = (\ln B_2ax - \ln B_1ax)/(t_2 - t_1)$,

where: RGR = relative growth rate (g m^{-2} 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 gain or loss of total biomass, aboveground biomass and belowground biomass of *S. alterniflora* and *C. americanum* in the different cultures (arrival orders) and sediment types (difference of biomass = 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 using the Statistica 7.1 software (SN AX505B150718FA) (StatSoft, 2005).

We tested for significant differences ($p<0.05$) of the ΔTB, ΔAB and ΔBB of the later-arriving individuals of *S. alterniflora* and *C. americanum* in the two 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 t-test for the the ΔTB, ΔAB and ΔBB and RGR-TB of *S. alterniflora*, and the difference of aboveground biomass and RGR-TB of

C. americanum. For the ΔTB and ΔBB 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 sing 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 (Fig. 2 and 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 (Fig. 3). The later-arriving individuals of *S. alterniflora* and *C. americanum* introduced into the heterospecific cultures of early-colonizing individuals suffered reduction in aboveground biomass throughout the experiment (Fig. 2 and 3).

The ΔBB of the early-colonizing individuals of *S. alterniflora* in monoculture was significantly lower in the lower estuary sediment than the ΔBB in monoculture in the middle estuary sediment, indicating that the greater salinity level was limiting for the aboveground biomass growth of this species (Fig. 4 C, Tab. S2). The ΔTB, ΔAB and ΔBB of the early-colonizing individuals of *S. alterniflora* were not limited by the presence of the later-arriving individuals of *C. americanum* in any sediment type (Fig. 4, Tab. S3).

The ΔTB and ΔBB of the early-colonizing individuals of *C. americanum* in monoculture and mixed culture and the ΔAB in monoculture were significantly lower in the lower estuary sediment than in the middle estuary sediment, indicating that the greater salinity level was limiting for the species growth (Fig. 5, Tab. S3). The ΔTB, ΔAB and ΔBB of the early-colonizing individuals of *C. americanum* were not limited by the presence of the later-arriving individuals of *S. alterniflora* in any sediment type (Fig. 5, Tab. S3).

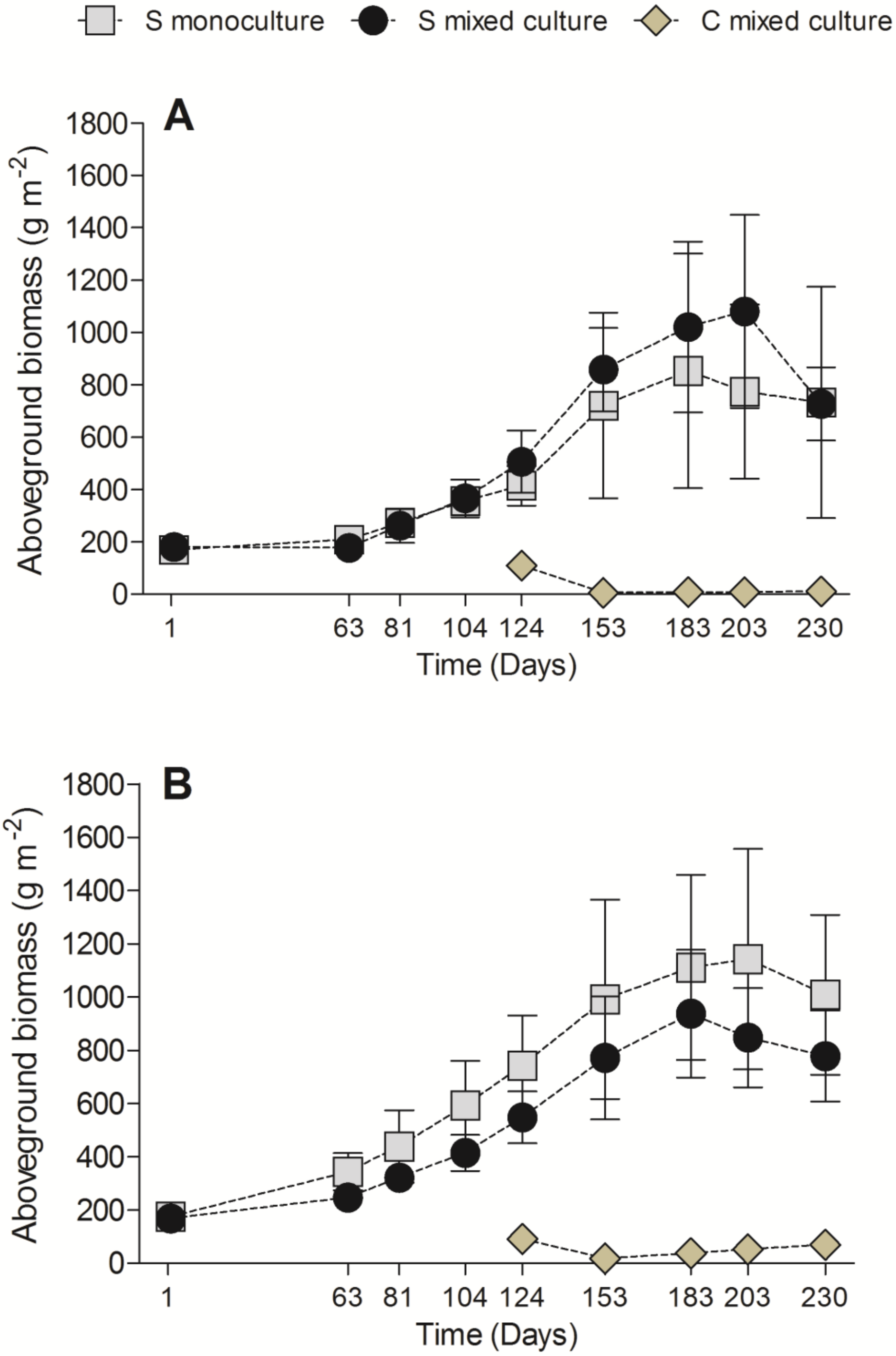

Figure 2. Average values and standard deviation of the aboveground biomass of the early-colonizing individuals of *Spartina alterniflora* (S) in monoculture (gray squares) and in mixed cultures (black circles), and the aboveground biomass of the later-arriving individuals of *Crinum americanum* (C) after 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).

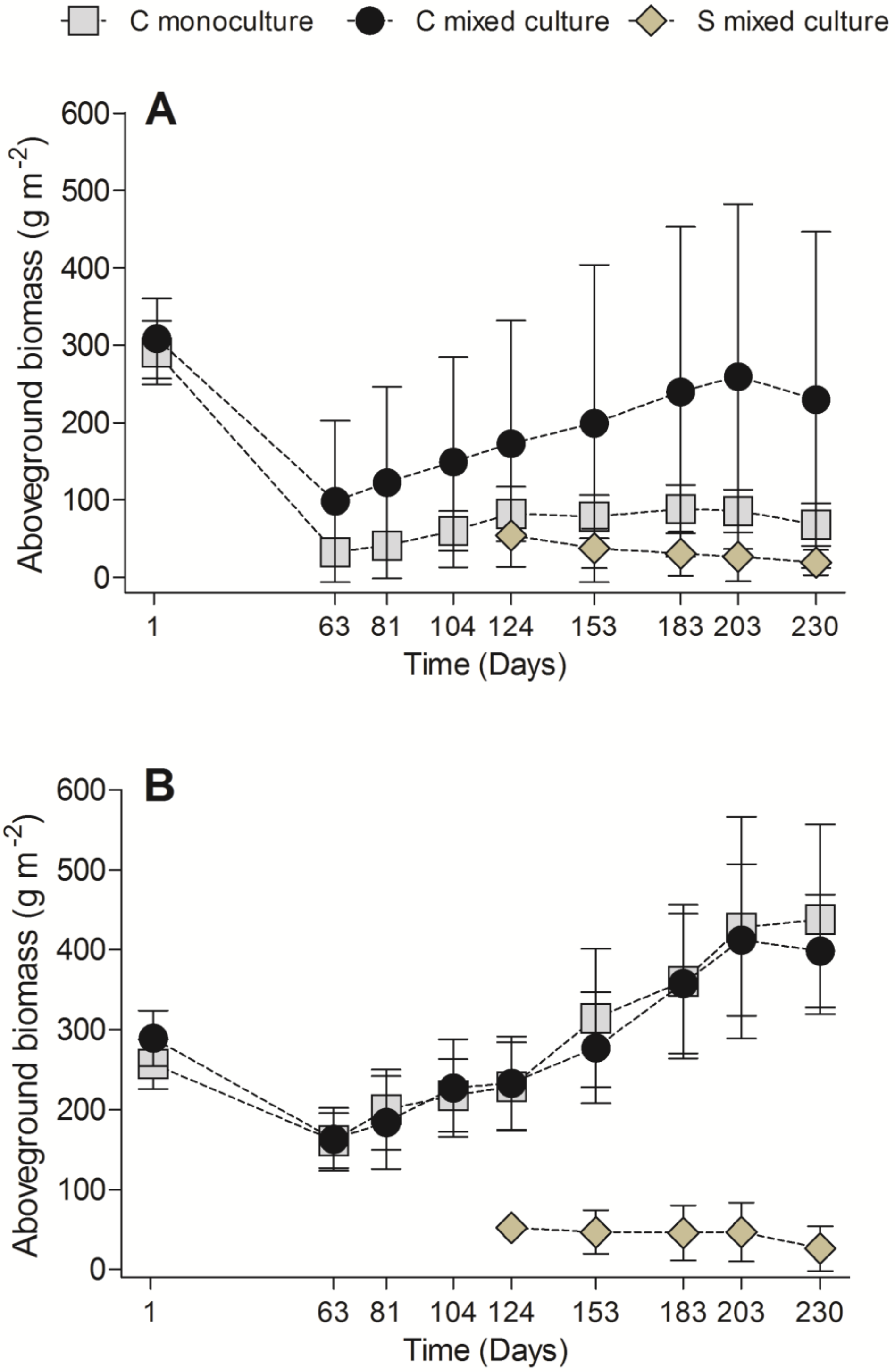

Figure 3. Average values and standard deviation of the aboveground biomass of the early-colonizing individuals of *Crinum americanum* (C) in monoculture (gray squares) and in mixed cultures (black circles), and the aboveground biomass of the later-arriving individuals of *Spartina alterniflora* (S) after 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).

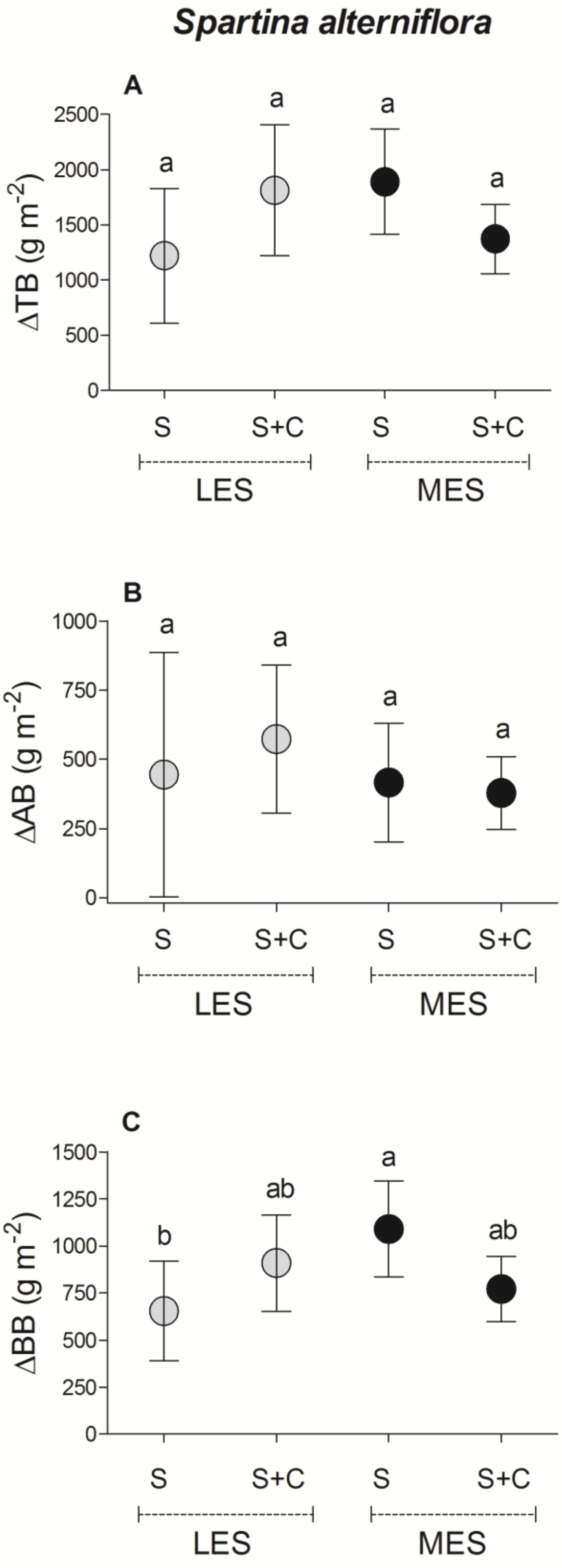

Figure 4. Average values and standard deviation of the difference of total biomass (ΔTB), aboveground biomass (ΔAB) and belowground biomass (ΔBB) (g m^{-2}) of the early-colonizing individuals of *Spartina alterniflora* in monoculture (S) and mixed cultures (S + C) in the lower (LES) and middle estuary sediment (MES). The graphs have different scales. Distinct letter indicate significant differences ($p<0.05$).

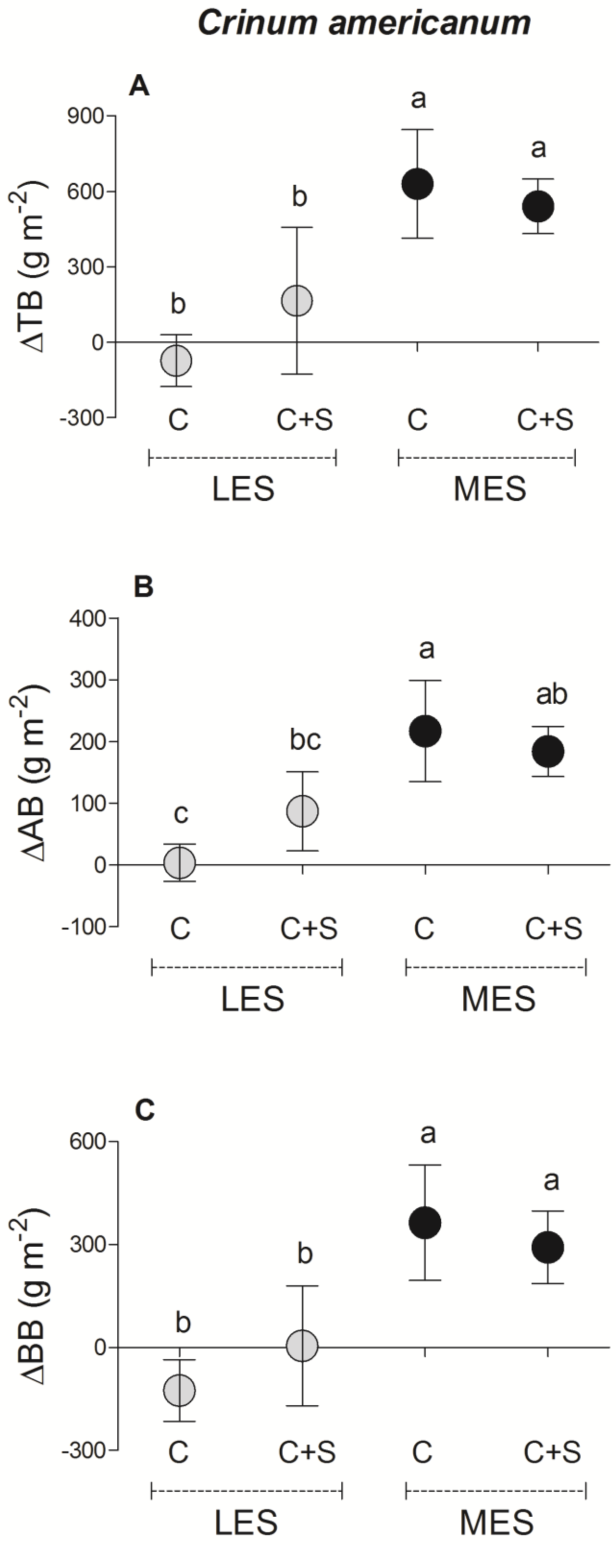

Figure 5. Average values and standard deviation of the difference of total biomass (ΔTB), aboveground biomass (ΔAB) and belowground biomass (ΔBB) (g m^{-2}) of the early-colonizing individuals of *Crinum americanum* in monoculture (C) and mixed cultures (C + S) in the lower (LES) and middle estuary sediment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

The later-arriving individuals of *C. americanum* and *S. alterniflora* suffered reduction in aboveground biomass in both sediment types (Fig. 2 and 3) with negative values of ΔTB, ΔAB and ΔBB (except *S. alterniflora* in the middle estuary sediment) and RGR-TB (Tab. 1 and 2).

The ΔTB and ΔBB of the later-arriving individuals of *C. americanum* were not significantly different between the sediment types. However, the average value of the ΔAB was significantly lower in the lower estuary sediment than in the middle estuary sediment (Tab. 1, Tab. S3). The average value of the RGR-TB of the later-arriving individuals of *C. americanum* in the lower estuary sediment was 3.5 times significantly greater than that in the middle estuary sediment, indicating that the reduction in biomass of the later-arriving individuals of *C. americanum* was faster in greater salinity (Tab. 1).

The ΔTB, ΔAB and ΔBB and RGR-TB of the *later-arriving* individuals of *S. alterniflora* were not significantly different between the sediment types (Tab. 2, Tab. S2).

Table 1. Average values and standard deviation of the differences of total biomass (ΔTB), aboveground biomass (ΔAB) and belowground biomass (ΔBB) (g m^{-2}), and relative growth rate of total biomass (RGR-TB) (g m^{-2} d^{-1}) of the later-arriving individuals of *Crinum americanum* introduced into the early-colonizing individual cultures of *Spartina alterniflora* in the lower (LES) and middle estuary sediment (MES). The symbol (*) indicates significant differences ($p<0.05$).

Sediment type	**ΔTB**	**ΔAB**	**ΔBB**	**RGR-TB**
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)*

Table 2. Average values and standard deviation of the differences of total biomass (ΔTB), aboveground biomass (ΔAB) and belowground biomass (ΔBB) (g m^{-2}), and relative growth rate of total biomass (RGR-TB) (g m^{-2} d^{-1}) of the later-arriving individuals of *Spartina alterniflora* introduced into the early-colonizing individual cultures of *Crinum americanum* in the lower (LES) and middle estuary sediment (MES).

Sediment type	**ΔTB**	**ΔAB**	**ΔBB**	**RGR-TB**
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)

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. 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.

We observed that *S. alterniflora* and *C. americanum* when first colonizers had an inhibitory priority effect on both sediment types, although some studies indicate that the priority effect may vary with environmental conditions (Kardol et al., 2013) and even be reduced under more severe ones (Chase, 2007), as for example in greater salinity level. Moore and 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 greatest salinity level of the lower estuary sediment limited the gain of belowground biomass of the early-colonizing individuals of *S. alterniflora* and the gain of total, aboveground and belowground biomass of *C. americanum*. However, even so, possibly the species dominated the available resources uses, such as space and nutrients, and maintained the inhibitory priority effect on the heterospecific later-arriving individuals.

In both sediment types, the later-arriving individuals of *S. alterniflora* and *C. americanum* showed expressive reduction in biomass. According to Sarneel et al. (2016), 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 conditions for their early-colonizing individuals, that is, in the middle estuary sediment. 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 plants ecological strategies should be based on their life stages.

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 and Camargo, 2018). 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.* 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 (Cao et al., 2018), and also to tolerate hypersaline environments (Angelini et al., 2011). Some studies indicate that, once established, *S. alterniflora* can facilitate the colonization of other plant species (Bruno and Kennedy, 2000) or limit potential new colonizers (Tognella De Rosa et al., 2009). Thus, according to our experiment results, we suggest that the monopolization of *S. alterniflora* in the lower estuary of the Itanhaém River may be the result of the inhibitory priority effect, possibly enhanced by the environmental stress conditions tolerated by it, but more limiting factors for non-halophyte species such as *C. americanum*.

In the middle estuary of the Itanhaém River, *S. alterniflora* and *C. americanum* form mixed stands with a lateral segregation between them in parallel to the margin. This same zonation pattern of emergent macrophytes was 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.

Nunes and Camargo (2018) observed in a pair-wise experiment that in conditions of the middle of the Itanhaém River, *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 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. Although interspecific competition may occur in the contact zone between heterospecific individuals (Woo and 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 and Prati, 2001).

In estuaries in Georgia (southern USA), Guo et al. (2014) found that stochastic processes, such as the 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. Since the importance of interspecific competition for the macrophytes spatial distribution in the Itanhaém River Estuary has already been reported (Nunes and Camargo, 2018; Nunes and 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.

Our results may have been influenced by the density and biomass of the later-arriving individuals that we introduced into the early-colonizing individual cultures, as the growth of propagules and young individuals from emergent macrophytes in already established heterospecific populations may be dependent on their densities and initial biomasses (Michelan et al., 2018). The density is not a restriction for primary colonization of macrophytes in a new area (Riis, 2008), but it can be limiting for the colonization success in areas with the presence of other plant species (Chadwell and Engelhardt, 2008). In this way, studies with the introduction of different sizes and densities of later-arriving individuals can contribute to the understanding of arrival order, colonization, priority effect and structuring of aquatic macrophyte communities.

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 organization of communities and spatial distribution of aquatic macrophytes in tropical estuaries.

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Supporting information

Table S1. Results of the Mann-Whitney test applied to salinity and content of total nitrogen (TN) and phosphorus (TP) between the two sediment types (lower and middle estuary sediment).

Variables	Results	p value
Salinity	U = 0.0	$p < 0.01$
TN	U = 172.0	$p = 0.46$
TP	U = 157.0	$p = 0.25$

Table S2. Resuls of the Factorial ANOVA applied to the gain of total biomass (GBT), 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.

Spartina alterniflora	dF	F	p
GTB			
Culture	1	8.218	0.008
Sediment type	2	0.419	0.662
culture*sediment type	2	0.130	0.879
GAB			
Culture	1	0.128	0.726
Sediment type	1	0.758	0.397
culture*sediment type	1	0.423	0.525
GBB			
Culture	1	0.088	0.77
Sediment type	1	1.92	0.185
culture*sediment type	1	7.141	< 0.05

Crinum americanum			
GTB			
Culture	1	0.302	0.083
Sediment type	2	1.037	0.370
culture*sediment type	2	1.625	0.218
GAB			
Culture	1	0.94	0.347
Sediment type	1	36.02	< 0.01
culture*sediment type	1	5.06	<0.05
GBB			
Culture	1	0.22	0.646
Sediment type	1	38.57	< 0.01
culture*sediment type	1	2.62	0.125

Table S3. Results of the t-test and Mann-Whitney test 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.

Spartina alterniflora			
GTB	t = 1.104	dF = 8	p = 0.301
GAB	t = 0.6077	dF = 8	p = 0.562
GBB	t = 1.834	dF = 8	p = 0.104
RGR - TB	t = 0.6138	dF = 8	p = 0.556

Crinum americanum			
GTB	U = 6		p = 0.222
GAB	t = 9.749	dF = 8	p < 0.01
GBB	U = 5		p = 0.151
RGR - TB	t = 2.702	dF = 8	p = 0.027

Capítulo 6

EFEITOS DA SALINIDADE SOBRE O CRESCIMENTO, A INTERAÇÃO COMPETITIVA E O CONTEÚDO DE NITROGÊNIO TOTAL DE DUAS ESPÉCIES DE MACRÓFITAS AQUÁTICAS ESTUARINAS CULTIVADAS EM SUBSTRATO ARTIFICIAL

Crinum americanum, Bacia do Rio Itanhaém, Itanhaém/SP

Effects of salinity on growth, competitive interaction and total nitrogen content of two estuarine macrophyte species cultivated on artificial substrate*

Resumo: A salinidade pode comprometer o crescimento de macrófitas aquáticas influenciando seu conteúdo de nutrientes e as interações interespecíficas. Em estuários, as características do sedimento variam tanto em salinidade como também em disponibilidade de nutrientes, granulometria e microbiota. O uso de substratos artificiais em experimentos manipulativos permite avaliar o efeito isolado da salinidade sobre as plantas. Nós avaliamos os efeitos da salinidade sobre o crescimento, a interação interespecífica e o conteúdo de nitrogênio total (NT) das macrófitas aquáticas emergentes estuarinas *Spartina alterniflora* e *Crinum americanum* cultivadas em vermiculita expandida. Nós desenvolvemos um experimento de dois fatores (3 culturas x 3 salinidades x 5 réplicas = 45 unidades experimentais) com duração de 185 dias em casa de vegetação. Nós cultivamos monoculturas (8:0 e 0:8) e culturas mistas (4:4) das espécies em salinidades 0, 20 e 30 ppt. Nós adicionamos solução nutritiva de mesma concentração em todas as unidades experimentais. Nós encontramos que a salinidade não influenciou o crescimento em monocultura de *S. alterniflora* e *C. americanum.* Nós observamos que *S. alterniflora* limitou o crescimento da fração emergente de *C. americanum* em maior salinidade, porém *C. americanum* não limitou o crescimento de *S. alterniflora* em nenhum dos tratamentos. Neste caso, houve competição assimétrica com limitação de *C. americanum* por *S. alterniflora* em maior salinidade. A salinidade não influenciou o conteúdo de NT em *S. alterniflora*. No entanto, *C. americanum* apresentou maior conteúdo de NT na fração emergente em maior salinidade, possivelmente, como uma estratégia de tolerância à salinidade. O conteúdo de NT na fração emergente de *C. americanum* foi menor quando em presença de *S. alterniflora*, indicando um possível efeito da interação competitiva entre as espécies.

Palavras-chave: *Spartina alterniflora*, *Crinum americanum*, competição interespecífica, compostos nitrogenados, vermiculita expandida, estresse salino, gradiente de salinidade.

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Abstract: Salinity can compromise the growth of aquatic macrophytes, influencing their nutrient content and interspecific interactions. In estuaries, the sediment characteristics vary in salinity as well as nutrient availability, granulometry and microbiota. The use of artificial substrates in manipulative experiments allows the evaluation of the isolated effect of salinity on plants. We evaluated the effects of salinity on growth, interspecific interaction and total nitrogen (TN) content of the estuarine macrophytes *Spartina alterniflora* and *Crinum americanum* cultivated on expanded vermiculite. We conducted a two-factor experiment (3 cultures x 3 salinities x 5 replicates = 45 experimental units) lasting 185 days in a greenhouse. We cultivated monocultures (8:0 and 0:8) and mixed cultures (4:4) at 0, 20 and 30 ppt salinity levels with the same concentration of nutrient solution. We found that salinity did not influence the growth of *S. alterniflora* and *C. americanum* in monoculture. In mixed culture, there was an asymmetric competition, as *S. alterniflora* limited the growth of the aboveground fraction of *C. americanum* at higher salinity, however *C. americanum* did not limit the growth of *S. alterniflora* at any treatment. Salinity did not influence the TN content in *S. alterniflora*. However, *C. americanum* had greater TN content in the emergent fraction at higher salinity, possibly as a strategy of salt tolerance. The TN content in the aboveground fraction of *C. americanum* was lower in the mixed cultures than in the monocultures, indicating an effect of the interspecific interaction.

Keywords: *Spartina alterniflora*, *Crinum americanum*, interspecific competition, nitrogenous compounds, expanded vermiculite, salt stress.

Introduction

Salinity is the main abiotic factor that limits plant development and primary production in coastal ecosystems (Houle et al. 2001), since it may cause ionic imbalance, stress and toxicity in plants (Esteves and Suzuki 2008). In general, there is a negative relationship between salinity and plant growth, mainly due to the reduction in biomass, density, height, leaf expansion and number of leaves (Esteves and Suzuki 2008). However, even among salt tolerant plant species, the effect of salinity on their growth can vary (Zhang et al. 2015). Some macrophyte species from coastal environments can grow in both saline and freshwater habitats. These species may either not suffer a significant negative effect of salinity (Tang et al. 2014) or have better growth in non-saline conditions (Crain et al. 2004) or even require a certain salinity condition for optimal growth (Partridge and Wilson 1987).

The nutrient absorption by plants, especially nitrogen (N), tends to decrease with increasing salinity (Chambers et al. 1998; Brown et al. 2006; Gomes et. al. 2011), and its deficiency can quickly reduce the plant growth (Hu and Schmidhalter, 2005). In natural estuarine habitats, sediments with higher salinity tend to have lower nitrogen availability and plants stock and use part of this already limited nitrogen for their osmoregulation, resulting in reduced growth (Cavalieri and Huang 1981). In this case, N is accumulated in the leaves and roots in the form of nitrogenous compounds that act as biomarkers on osmoregulation (Levine et al. 1998; Gomes et al. 2017) to deal with salt stress (Mansour 2000). However, the accumulation of these nitrogenous compounds may be more important for non-halophyte species than for the halophyte ones, since some halophyte macrophytes have glands in the leaves for salt excretion and osmotic control (Céccoli et al. 2015) and, therefore, their nutrient assimilation, N content and growth may not be greatly influenced by salinity (Brown 1998).

Salinity can indirectly mediate competition and, consequently, community organization patterns of aquatic macrophytes in coastal aquatic ecosystems by affecting their growth and nutrient absorption (Pennings and Callaway 1992). Although the role of interspecific competition of macrophytes in coastal wetlands has been widely reported in the freshwater habitats (Crain et al. 2004; Engels and Jensen 2010; Guo and Pennings 2012), some studies have observed that this biotic factor can also be important in the brackish and saline environments (Bockelmann and Neuhaus 1999; Medeiros et al. 2013; Nunes and Camargo 2018). In the southeastern and southern estuaries of Brazil, *Spartina alterniflora* Loisel. (Poaceae) and *Crinum americanum* L. (Amaryllidaceae) are the common macrophyte species found along the salinity gradient. In general, *S. alterniflora* occurs in the lower estuary (salinity from 20 to 30 ppt), *C. americanum* occurs in the upper estuary (salinity from 0 to 5 ppt) and both species form mixed stands in the middle estuary (salinity from 5 to 20 ppt) (Nunes and Camargo 2018). From experimental studies on aquatic macrophytes conducted in the Itanhaém River Estuary (southeastern Brazil), Nunes and Camargo (2018) suggested that the absences of *S. alterniflora* in the upper estuary and *C. americanum* in the lower estuary are due, respectively, to the lower nutrient availability in the sediment and interspecific competition, factors indirectly related to the salinity gradient. These authors also found that the middle estuary conditions are favorable for both species, possibly due to the balance between brackish water and sediment with greater nutrient availability.

Along the environmental gradient in estuaries, the sediment characteristics vary in terms of salinity as well as nutrient availability, sulfides and organic matter content, water retention capacity, aeration and granulometry. All of these sediment characteristics can have a joint effect on growth and interspecific competition of macrophytes (Huckle et al. 2000; Guo and Pennings 2012). In addition, the specific benthic macroinvertebrate and microorganism (mycorrhizal fungi and N-fixing bacteria) communities to each sediment type can also influence plant growth through processes related to nutrition (Barko et al. 1991; Gribben et al. 2017) and salt tolerance (Rodriguez et al. 2008; Yuan et al. 2016). Thus, the use of artificial substrates in manipulative experiments allows the evaluation of the isolated effect of a given variable on plants. Artificial substrates have been used in studies involving the effect of salinity on seed germination (Middleton 2016) and growth of emergent macrophytes (Ma et al. 2011).

We evaluated the effects of salinity on growth, interspecific interaction and total nitrogen (TN) content of the emergent macrophyte species *S. alterniflora* and *C. americanum* cultivated on artificial substrate in a greenhouse. Our hypotheses were:

- Plant growth: (i) salinity would not limit the growth of *S. alterniflora* in monoculture, as this species is able to tolerate high salinity due to its mechanism of salt tolerance (salt glands) and also can grow in freshwater habitats without competition; (ii) the growth of *C. americanum* in monoculture would be limited by salinity due to salt stress;

- Interspecific competition: (iii) *S. alterniflora* would have greater competitive ability at higher salinity and (iv) *C. americanum* would have greater competitive ability in the absence of salinity;

- TN content: (v) for *S. alterniflora* there would be no relationship between its TN content and salinity; (vi) for *C. americanum* there would be a positive relationship between its TN content and salinity as a strategy of salt tolerance. However, (vii) the TN content of both species would be reduced when in interspecific competition due to their limitation of N assimilation.

Methods

Species description

The species *S. alterniflora* and *C. americanum* are emergent aquatic macrophytes and very common in estuaries on the coast of the São Paulo State (Southeastern Brazil) (Nunes and Camargo 2018). They form monospecific and mixed stands that fringe mangrove and riverine forests (Schaeffer-Novelli et al. 1990). Although both species are clonal and rhizomatous, they can have different growth strategies. That is because *S. alterniflora* can invest more in clonal production than *C. americanum*, which prioritizes the increase in the biomass of the individuals themselves (Nunes and Camargo 2020). *Spartina alterniflora* is a halophyte species, has salt glands in the leaves and is adapted to a wide range of salinity (Céccoli et al. 2015). Some studies have indicated that low salinity levels (e.g., 4 ppt) may be the most favorable ones for *S. alterniflora*; however this species is able to tolerate salinity higher than seawater (35 ppt) (Courtney et al. 2016) and even hypersaline habitats (e.g., 50 ppt or 850 mol m^{-3}) (Pezeshki and DeLaune 1995). *Crinum americanum* occurs in coastal rivers and lagoons in Brazil, as well as in diverse continental freshwater environments (Pantanal, Cerrado and Amazon) (Dutilh and Oliveira 2015). This species is not able to excrete salts from its tissues, but it can occur in brackish environments (salinity from 5 to 20 ppt) (Meerow et al. 2003; Ribeiro et al. 2011).

Experiment

We conducted a two-factor manipulative experiment (3 cultures x 3 salinities x 5 replicates = 45 experimental units) to evaluate the growth, competitive interaction and TN content in *S. alterniflora* and *C. americanum* at different salinity levels on artificial substrate. The experiment lasted 185 days and was developed in a greenhouse. Our treatments consisted of monospecific (8:0 and 0:8) and mixed cultures (4:4) at three salinity levels (0, 20 and 30 ppt).

We used the substitutive design in our experiment, which consists of maintaining a constant initial total density of species and varying their proportion in mixed culture. The substitutive design has been used to test the effect of some factors on species interaction (Gibson et al. 1999) and is appropriate for studies based on the similarity of competitors in terms of resource requirements (Sackville-Hamilton 1994). The initial total density of plants we used (8) was similar to that observed in the field. We considered each stem of *S. alterniflora* and each rosette of *C. americanum* as an individual, that is, each clonal emergence above the substrate (Nunes and Camargo 2017).

We collected adult individuals of *S. alterniflora* and *C. americanum* of similar size (≥ 0.50 m) and appearance in the mixed stands of these species in the middle estuary of the Itanhaém River. The macrophytes were washed to remove sediment and other material adhered to the leaves, roots and rhizomes, and planted in the substrate in the experimental units.

As substrate for plant development, we used 13 liters of fine-grained expanded vermiculite (grains from 0.30 to 0.50 mm) in each experimental unit (plastic boxes with a volume of 26 liters and an area of 0.13 m^2). The vermiculite grains consist of flakes expanded into accordion-like particles of micaceous minerals that act as a biologically inert artificial substrate (Ugarte et al. 2008) with uniform chemical composition and great capacity of water retention and aeration (Martins et al. 2009).

After planting, the plants were kept in acclimatization for 38 days. During this period, we added 700 mL of modified Hoagland nutritive solution once a week to the vermiculite in each experimental unit. After this period, we added 2 liters of saline solution of 20 and 30 ppt to the experimental units every three weeks, respectively, in the salinity treatments of 20 and 30 ppt, and 700 mL of the nutrient solution. In the treatment of 0 ppt (no salt addition), replacing the saline solution, we added 2 liters of tap water. In addition, the experimental units were watered with tap water every three days to maintain the approximate level of 2 cm above the substrate surface. We monitored salinity level in vermiculite every three weeks. For that, we mixed 3g of a fresh substrate sample in 100 mL of distilled water and measured salinity (ppt) of the solution using a digital salt content meter (Instrutemp Nutra-Wand Combo). We observed that salinity level in vermiculite of the experimental units at each treatment showed little variation thought the experiment: 0 ppt (no variation), 20 ppt (standard deviation = 4.97) and 30 ppt (standard deviation = 4.68). Salinity was significantly different ($p<0.05$) among the treatments.

The salt concentrations we manipulated were based on that of water and sediment salinity in the middle and lower estuary of the Itanhaém River. For the saline solutions preparation, we used formulated sea salt (Aquaforest Sea Salt®) which, in addition to sodium chloride, contains sulfates, calcium, potassium, magnesium and other minerals found in seawater. The concentration and volume of the modified Hoagland nutrient solution (Hoagland and Arnon 1950) we used were based on the experiment of Mendelssohn et al. (2001). This modified Hoagland nutritive solution was composed of 2 mM $MgSO_4$, 4mM KH_2PO_4, 5 mM $CaCl_2$, 10 mM NH_4Cl, 1.8 mM Fe-EDTA, 46 mM H_3BO_3, 0.1µM H_2MoO_4, 0.4 µM $MnCl_2$, 0.76 µM $ZnSO_4$ and 0.32 µM $CuSO_4$ (Hoagland and Arnon 1950; Mendelssohn et al. 2001).

To estimate the macrophyte aboveground and belowground biomass we used the non-destructive method and its regression equations (Eq. 1 to 4) developed by Nunes and Camargo (2017). These authors generated the equations through significant simple linear regressions ($p <0.05$) between individual height and aboveground dry mass (Eq. 1 and 2), and between volume and belowground dry mass (Eq. 3 and 4) (Nunes and Camargo 2017).

Equation 1: *S. alterniflora* aboveground dry mass = 10.64 * (individual height (m) – 0.5451)

($R^2 = 0.8279$)

Equation 2: *C. americanum* aboveground dry mass = 16.03 * (individual height (m) – 6.0077)

($R^2 = 0.7532$)

Equation 3: *S. alterniflora* belowground dry mass = 0.0932 * (belowground volume (mL) + 0.3244)

($R^2 = 0.7965$)

Equation 4: *C. americanum* belowground dry mass = 0.0504 * (belowground volume (mL) + 1.241)

($R^2 = 0.9315$)

Before planting, we calculated the aboveground and belowground biomass of *S. alterniflora* and *C. americanum* of each experimental unit (Eq. 1 to 4). We performed plant height measurements on days 1, 39, 68, 95,109, 116, 123, 130, 136, 143, 150, 157, 164, 171, 178 and 185 of the experiment to assess the variation of aboveground biomass over time.

We intended to apply the simple linear regression between aboveground biomass and time, and compare the growth of *S. alterniflora* and *C. americanum* at the different treatments through the slope of the regression line, as done by Nunes and Camargo (2018). For all replicates of S. *alterniflora*, the adjustment of this linear model was significant ($p < 0.05$); however, we did not find significant linear adjustments for any replicate of *C. americanum*. In this way, we chose to evaluate the growth of both species through the gain of biomass (Eq. 5). We considered the gain of aboveground biomass (GAB) from day 109 to 185, and the gain of belowground biomass (GBB) from day 1 to 185.

Equation 5: $GBax = Bfax – Biax$,

where GB_{ax} = gain of biomass (grams of dry mass per square meter = g m^{-2}) of the species *a* in the treatment *x*; Bf_{ax} = final biomass (g m^{-2}) of the species *a* in the treatment *x*; and Bi_{ax} = initial biomass (g m^{-2}) of the species *a* in the treatment *x*.

At the end of the experiment, we separated the aboveground (leaves and stems) and belowground (roots and rhizomes) fractions of the individuals of *S. alterniflora* and *C.*

americanum from each experimental unit. Although the roots and rhizomes of the two species were tangled at the end of the experiment, they could be visually distinguished because they have different appearances. For the separation of the belowground fraction of each species, we washed all vermiculite out and put the roots and rhizomes in a tank with water to cut and separate them. The aboveground and belowground fractions were dried in an oven at 60°C and crushed in a mill. From the crushed plant material, we determined the total nitrogen (TN) content (organic and inorganic nitrogen) in the aboveground and belowground fractions of each species by the Kjeldahl method (Allen et al. 1974) in laboratory.

Data analysis

We tested for significant differences ($p<0.05$) in GAB, GBB and TN content of *S. alterniflora* and *C. americanum* at the different salinity levels and cultures (gain of biomass and TN content = response variables; salinity levels and cultures = predictor variables). For that, we evaluated and confirmed that the conditions of normality and homoscedasticity of the data were met. For the TN content of the belowground fraction of *S. alterniflora* and TN content of the aboveground fraction of *C. americanum*, we performed square root transformation so that the conditions of normality and homoscedasticity were met. Then, we applied the two-way analysis of variance (Factorial ANOVA) and the Tukey’s test when necessary.

We elaborated the graphs of gain of biomass (GAB and GBB), variation of aboveground biomass and TN content using the GraphPad Prism software (GPW5-066646-RCG7389) (GraphPad Software 2007). We applied the statistical analysis using the Statistica 7.1 software (SN AX505B150718FA) (StatSoft 2005).

Results

The gain of biomass (GAB and GBB) of *S. alterniflora* was not significantly different among the salinity levels and cultures (Figure 1, Table S1), indicating that salinity and the presence of *C. americanum* did not influence its growth.

The GBB of *C. americanum* were not significantly different among the salinity levels and cultures either (Figure 1 B, Table S1), indicating that salinity and the presence of *S. alterniflora* did not influence its growth of belowground and total fraction. However, the GAB of *C. americanum* was significantly lower at the higher salinity (30 ppt) and in mixed culture in relation to the GAB at other salinities (0 and 20 ppt) and in monoculture (Figure 1 A, Table S1), indicating that at higher salinity the growth of the aboveground fraction of *C. americanum* was limited both by salinity and competition.

The species *S. alterniflora* and *C. americanum* had different behaviors of aboveground fraction growth in the experiment (Figure 2), because *S. alterniflora* had greater growth of aboveground fraction at all salinities and cultures (Figure 2 A, B and C) than *C. americanum* (Figure 2 D, E and F).

The TN content of the aboveground fraction of *S. alterniflora* was not significantly different among the salinity levels and among the monocultures and mixed cultures (Figure 3 A, Table S2), however, its content in the belowground fraction in the mixed culture at 30 ppt was significantly higher than in monoculture at 0 ppt (Figure 3 B, Table S2).

The TN content in the aboveground fraction of *C. americanum* in monoculture was significantly higher at 30 ppt than at 0 ppt (Figure 3 A, Table S2). At all salinity levels, the TN content in the aboveground fraction of *C. americanum* was significantly higher in monoculture than in mixed culture (Figure 3 A, Table S2). The TN content of the belowground fraction of *C. americanum* was not significantly different between the monocultures at the different salinity levels, but it was significantly higher in monoculture at 20 ppt than in mixed culture at 0 ppt (Figure 3 B, Table S2).

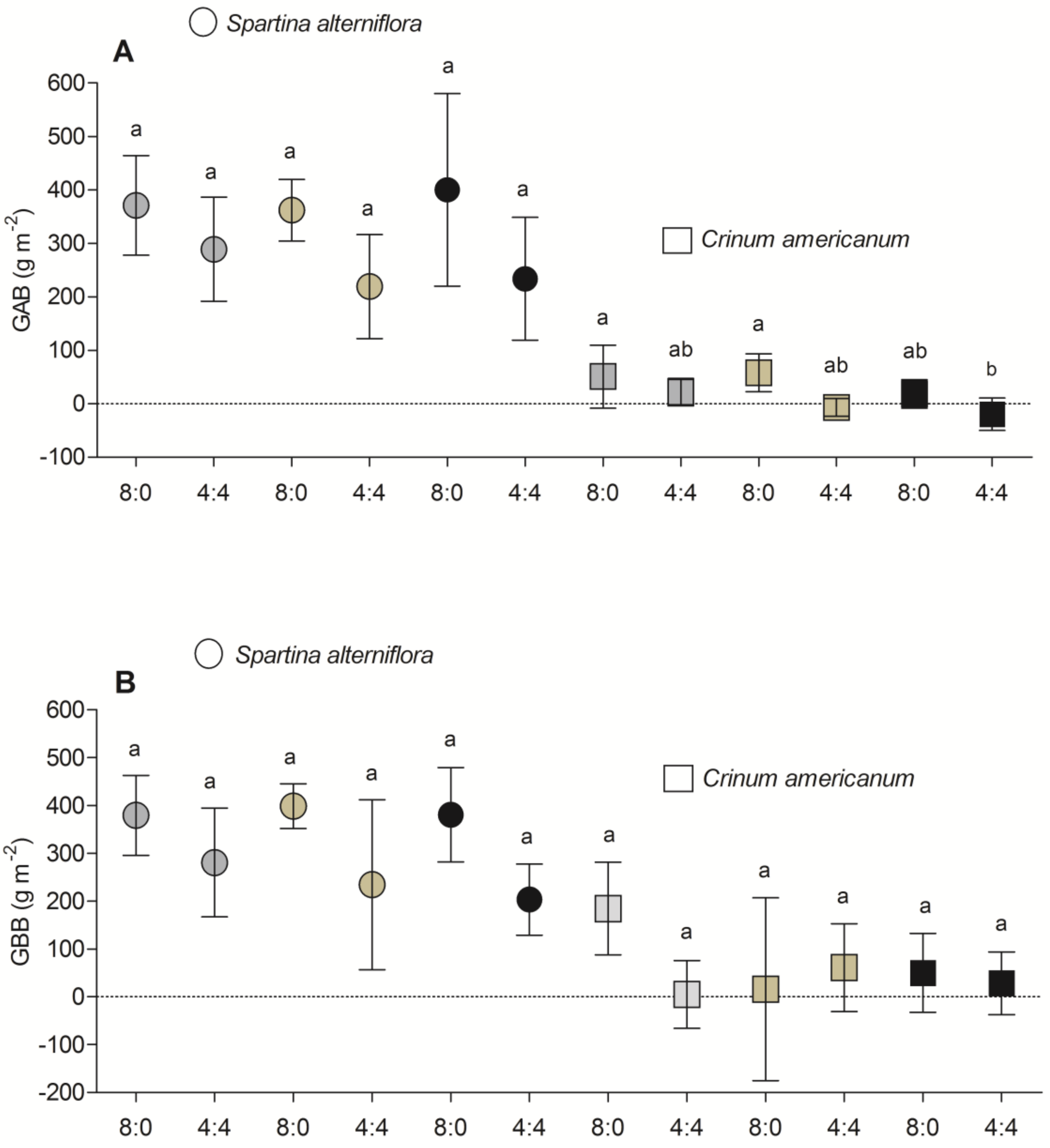

Figure 1. Mean values and standard deviation of (A) gain of aboveground biomass (GAB), and (B) gain of belowground biomass (GBB) of *Spartina alterniflora* (circles) and *Crinum americanum* (squares) in monoculture (8:0) and mixed culture (4:4) at 0 ppt (grey), 20 ppt (brown) and 30 ppt (black) salinities. Distinct letters indicate significant differences in gain of biomass of each species among the treatments ($p<0.05$).

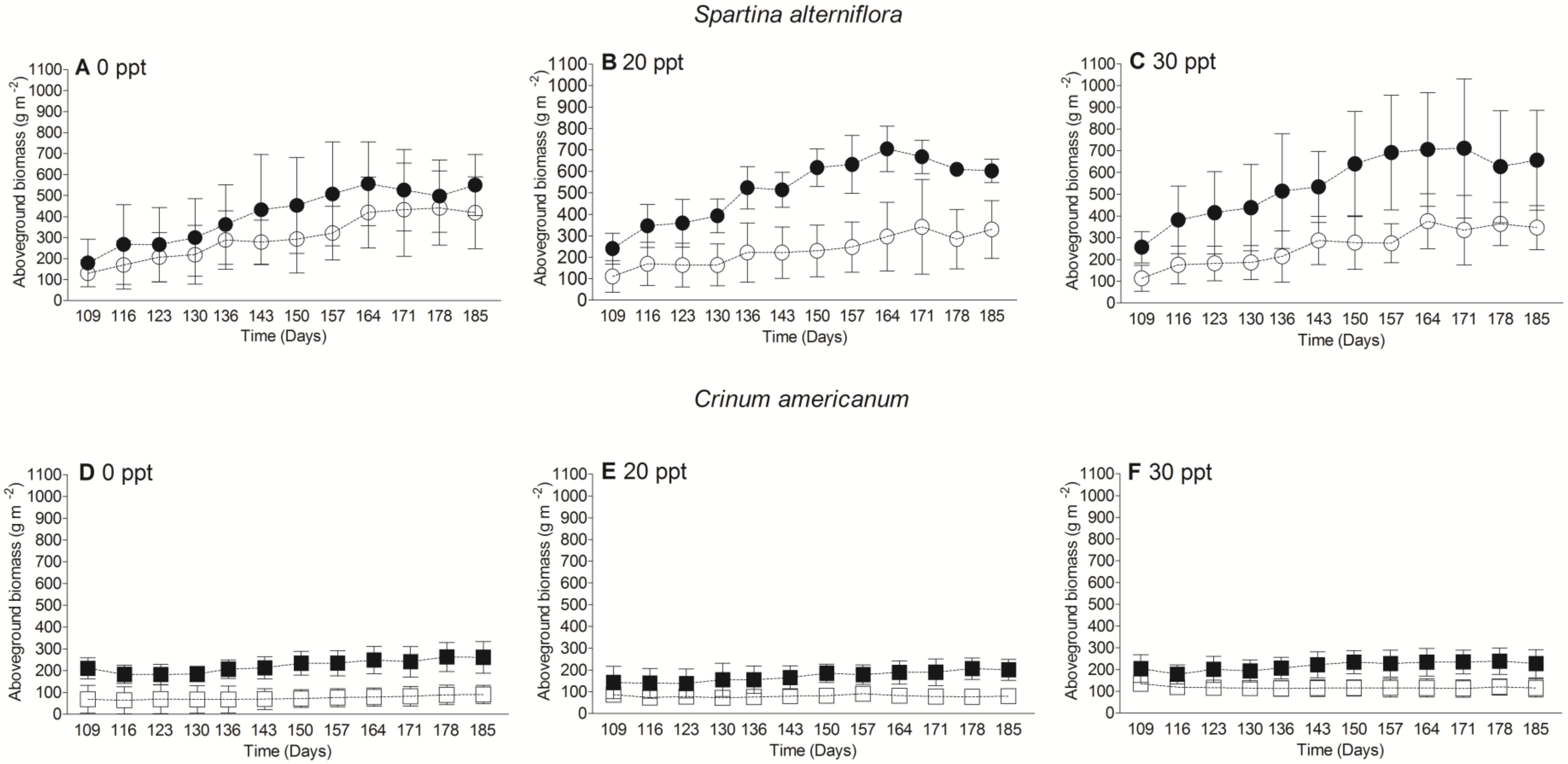

Figure 2. Mean values and standard deviation of aboveground biomass of *Spartina alterniflora* throughout the experiment in monoculture (black symbols) and mixed culture (open symbols) at (A) 0 ppt, (B) 20 ppt and (C) 30 ppt salinities; and of *Crinum americanum* in monoculture and mixed culture at (D) 0 ppt, (E) 20 ppt and (F) 30 ppt salinities.

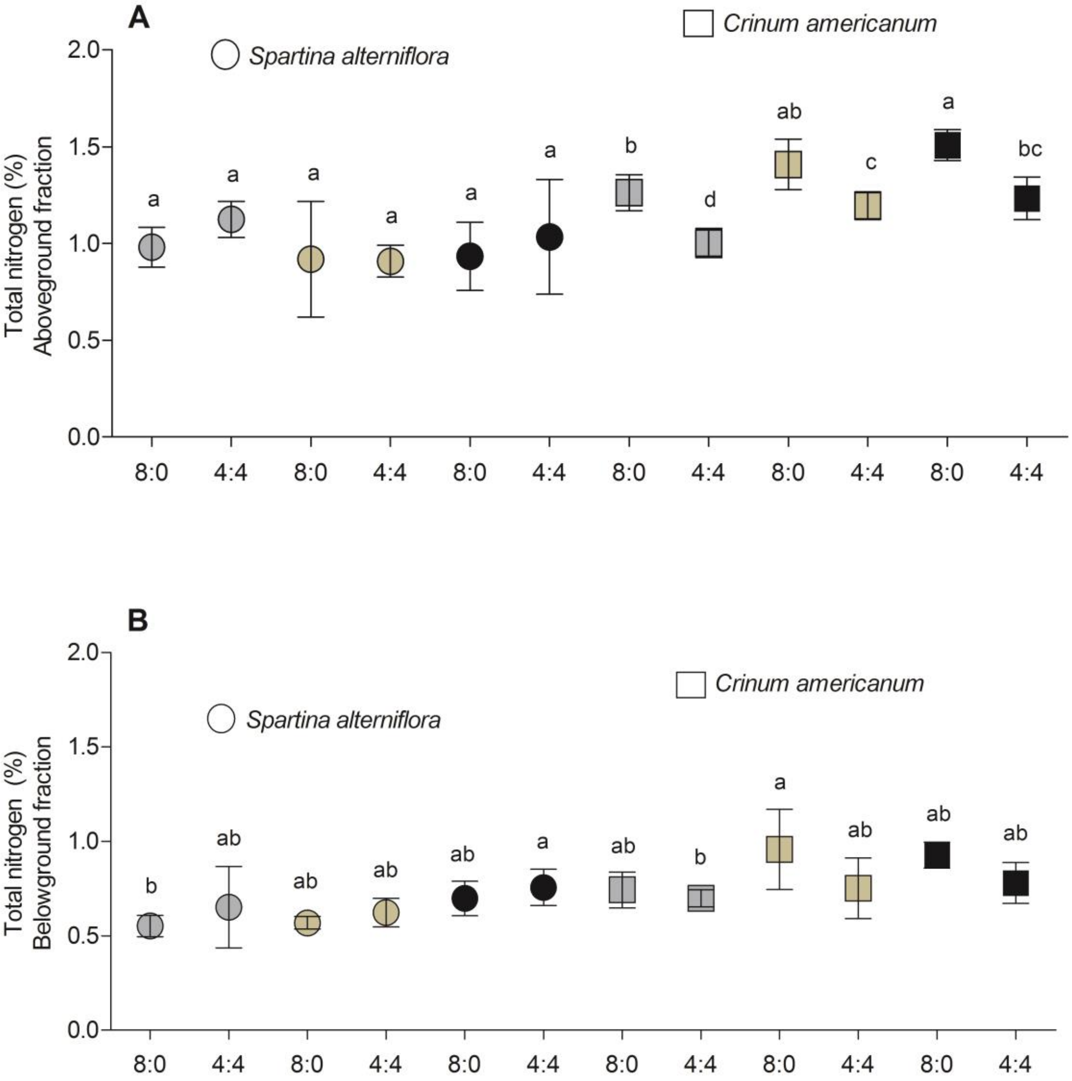

Figure 3. Mean values and standard deviation of total nitrogen content (TN) in (A) aboveground fraction and (B) belowground fraction *Spartina alterniflora* (circles) and *Crinum americanum* (squares) in monoculture (8:0) and mixed culture (4:4) at 0 ppt (grey), 20 ppt (brown) and 30 ppt (black) salinities. Distinct letters indicate significant differences in TN content of each species among the treatments ($p<0.05$).

Discussion

The results of our experiment showed that there was no difference in the growth of *S. alterniflora* and *C. americanum* in monoculture among the salinity levels we tested, which corroborate our hypothesis for the growth of *S. alterniflora*, but contradict our hypothesis for the growth of *C. americanum*. We observed that *S. alterniflora* limited the growth of the aboveground fraction of *C. americanum* at higher salinity; however *C. americanum* did not limit the growth of *S. alterniflora* at any treatment. These results partially confirmed our hypothesis that *S. alterniflora* would have greater competitive ability at higher salinity, however, our hypothesis that *C. americanum* would have greater competitive ability at the treatment with no salt addition was not corroborated.

Our results are different from the results obtained in other studies on these same species. For *S. alterniflora*, Nunes and Camargo (2018) observed a reduction in its biomass and mortality in oligohaline conditions (4 ppt) of the Itanhaém River Estuary (Southeastern Brazil). These authors conducted this greenhouse experiment using sediment collected from the estuary with no addition of nutrient solution. On the other hand, in field experiments, Crain et al. (2004) found greater growth of this species in freshwater condition in estuaries in Rhode Island (Northern USA), and Guo and Pennings (2012) observed its greater biomass in brackish environments (14 ppt) in estuaries in Georgia (Southern USA). For *C. americanum,* Ribeiro et al. (2011) observed a positive relationship between growth and salinity (from 0 to 5 ppt) in the blind estuary of the Massaguaçu River (Southeastern Brazil); however, Nunes and Camargo (2018) observed greater gain in biomass of this species in sediment with intermediate salinity (12 ppt).

The studies cited above were carried out on natural sediments. According to Tootoonchi and Gettys (2019) the response of aquatic plants to salt stress may also be influenced by differences in other substrate characteristics. In estuaries, in addition to salinity, nutrient availability and other sediment characteristics also vary along the environmental gradient (Pennings and Callaway 1992) and between ecosystems in different geographical regions (Eyre and Balls 1999). Possibly, the different growth outcomes of *S. alterniflora* and *C. americanum* to the salinity gradient found in these studies are due to these environmental differences. In our experiment, *S. alterniflora* and *C. americanum* were cultivated on the same type of artificial substrate and nutrient concentration, eliminating differences in the characteristics of natural sediments. Thus, we observed that salinity itself did not influence the species growth; and we highlight that, probably, joint effects of the sediment characteristics are responsible for different responses of the macrophytes growth in environmental gradients of estuaries.

Our results showed that *S. alterniflora* and *C. americanum* grew at the different salinity levels we tested. However, *S. alterniflora* had greater growth than *C. americanum* at all treatments. Although the growth strategies, such as biomass allocation, height growth and clonal investment, may vary at different salinities (Howard et al. 2006; Xiao et al. 2011), our results indicated that these species may have different growth strategies regardless of salinity, which was also observed by Nunes and Camargo (2020). These authors found that *S. alterniflora* invests in increasing biomass and height of its individuals as well as clonal production, which generate great growth; and *C. americanum* has a more moderate growth due to its greater investment in increasing biomass of the individuals in relation to the clonal production.

We found that salinity has an influence on the competitive interaction between *S. alterniflora* and *C. americanum*. We expected greater competitive ability of *S. alterniflora* at higher salinity and greater competitive ability of *C. americanum* at lower salinity. In fact, we observed a tendency of asymmetric competition with limitation of aboveground biomass of *C. americanum* by *S. alterniflora* at higher salinity. Our results agree with those obtained by Nunes and Camargo (2018). In an experiment with these same species in natural sediment, these authors also found that *S. alterniflora* limited the growth of *C. americanum* at higher salinity (lower estuary sediment of the Itanhaém River, Brazil). Thus, we suggest that salinity has a direct effect on the interaction between these species both in natural sediment and artificial substrate, reducing the effects of other sediment characteristics on competition. However, at our treatment with no salt addition *C. americanum* did not limit the growth of *S. alterniflora* as we expected. Many studies have shown that in freshwater environments *S. alterniflora* is limited by non-halophyte species that have greater competitive ability in the absence of salinity (Crain et al. 2004; Guo and Pennings 2012). However, we did not observe the same fact in our study. In this case, we suggest that other characteristics of natural sediments may have a greater influence on the mediation of interspecific interaction between halophyte and non-halophyte species in freshwater conditions.

Regarding the relationship between plant TN content and salinity, our hypothesis was corroborated for the belowground fraction of *S. alterniflora* and for the aboveground fraction of *C. americanum*. In general, the greatest N content in plants in saline conditions may be related to mechanisms of salt tolerance (Twilley and Barko 1990; Mansour 2000; Crain 2007), which possibly occurred in our experiment. The results for *S. alterniflora* indicated that this species was not limited by salinity, as there was no difference in its TN content among the treatments. Although *S. alterniflora* had greater TN content in its belowground fraction at the higher salinity treatment in mixed culture than at the treatment with no salt addition in monoculture, this result does not seem to be a response to salt stress; it seems to be a strategy to maintain its competitive ability. However, the aboveground fraction of *C. americanum* was limited by both salinity and competition. The TN content in *C. americanum* in monoculture and in mixed culture was significantly greater at 20 and 30 ppt than at the treatment with no salt addition; however, in mixed culture its TN content was more reduced in relation to monocultures.

In an experimental study in estuaries in Maine (Northern USA), Crain (2007) observed that the N content in *Spartina patens* was significantly greater with increasing salinity. The author attributed this result to the species physiological demand for N to help its salt tolerance and not due to the local N availability, since environments with higher salinity tend to have less availability of this nutrient. The increase in TN content in plants exposed to salinity occurs due to the production of nitrogenous compounds for osmoregulation (Flowers et al. 2015). Thus, we indicate that this same process may have occurred with *C. americanum* in our experiment and that its greater TN content in higher salinity is a strategy of salt tolerance. Although there is a lack of studies on salt stress in *C. americanum*, Tram et al. (2002) related that at diverse stress conditions some species of *Crinum* can produce alkaloids (N-containing organic compounds) to prevent oxidation and hydrolysis, which can contribute to explaining our results. At salt conditions the production of proline by *S. alterniflora* can increase (Li et al. 2010). However, this species has salt glands and, therefore, the absence of differences in its TN content among the salinity levels we found in our experiment may indicate its great ability to tolerate salinity, as also suggested by Courtney et al. (2016).

Angelstein et al. (2009) found lower N content in the tissues of the aquatic macrophyte *Myriophyllum spicatum* when in mixed culture with *Elodea nuttalli* in relation to their N content in monoculture, and they related this result to the interspecific competition. The authors suggested that possibly *E. nuttalli* had greater ability to assimilate N from the sediment and, thus, presented greater growth and, consequently, overcrowding. This *E. nuttalli* overcrowding caused the shading of *M. spicatum* and impaired its N assimilation. Although we did not observe significant differences in the growth of the aboveground fraction of *C. americanum* between the monocultures and mixed cultures in our experiment, we also suggest that the interspecific interaction may have limited the TN content in the aboveground fraction of *C. americanum.*

We conclude that salinity did not influence the growth of *S. alterniflora* and *C. americanum*. We observed a tendency of asymmetric competition of aboveground fraction at higher salinity and we suggest that, perhaps in a long term, *C. americanum* may be limited by *S. alterniflora*. In general, salinity did not limit the TN content in *S. alterniflora*. However, *C. americanum* had higher TN content in the aboveground fraction at higher salinity than in the treatment with no salt addition, possibly as a strategy of salt tolerance. Regardless of salinity, *C. americanum* had lower TN content in the aboveground fraction when in the presence of *S. alterniflora*, which may indicates an effect of the competitive interaction between these species.

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Supporting information

Table S1. Resuls of the Factorial ANOVA applied to the gain of aboveground biomass (GAB) and gain of belowground biomass (GBB) of *Spartina alterniflora* and *Crinum americanum* at the different salinities and cultures.

Spartina alterniflora	dF	F	p
GAB			
Culture	1	11.46	< 0.01
Salinity	2	0.51	0.608
Culture*salinity	2	0.54	0.509
GBB			
Culture	1	14.02	< 0.01
Salinity	2	0.33	0.072
Culture*salinity	2	0.39	0.681
Crinum americanum			
GAB			
Culture	1	11.74	< 0.01
Salinity	2	2.94	0.072
Culture*salinity	2	0.71	0.502
GBB			
Culture	1	1.72	0.201
Salinity	2	0.89	0.424
Culture*salinity	2	22.84	0.078

Table S2. Resuls of the Factorial ANOVA applied to the total nitrogen content (TN) in the aboveground fraction (AF) and belowground fraction (BF) of *Spartina alterniflora* and *Crinum americanum* at the different salinities and cultures.

Spartina alterniflora	dF	F	p
TN - AF			
Culture	1	1.165	0.291
Salinity	2	1.232	0.309
Culture*salinity	2	0.406	0.671
TN - BF			
Culture	1	3.068	0.093
Salinity	2	5.492	< 0.05
Culture*salinity	2	0.083	0.921

Crinum americanum			
TN - AF			
Culture	1	0.050	0.824
Salinity	2	1.090	0.352
Culture*salinity	2	0.895	0.422
TN - BF			
Culture	1	0.010	0.902
Salinity	2	0.101	0.904
Culture*salinity	2	0.410	0.668

Conclusões

As planícies costeiras do estado de São Paulo possuem diferentes características ao longo do gradiente norte-sul do litoral, especialmente aquelas relacionadas à suas larguras e inclinações. Estas caracaterísticas das planícies costeiras fazem com que os seus rios apresentem diferenças de heterogeneidade ambiental, de influência de águas marinhas e de comprimento, que por sua vez, levam à formação de diferentes comunidades, e diferentes padrões de diversidade e distribuição espacial de macrófitas aquáticas nesta escala regional (gradiente norte-sul). Os rios da região norte do litoral paulista estão localizados em planícies costeiras mais estreitas e mais inclinadas, e por isso, são mais curtos, têm menor largura do canal e sofrem pouca ou nenhuma influência marinha. Os rios da região central e sul estão localizados em planícies costeiras mais largas e mais planas, e por isso, são mais compridos e meândricos, possuem canais mais largos e sofrem forte influência de águas marinhas com marcados gradientes longitudinais de salinidade. As diferenças de influência de águas marinhas nos rios também estão indiretamente relacionadas às variações em seus aspectos hidrológicos, como por exemplo, direção do fluxo de água, inversão periódica do fluxo de água (fluxos invertidos em baixamar e preamar), amplitude de variação do nível de água, velocidade de corrente (TESSLER *et al*., 2006); e limnológicos, como a presença de sulfetos e fosfatos vindos do mar (HOWARTH, 1984; BENITES-NELSON, 2000). Todas estas características ambientais dos rios costeiros evidenciam a complexidade das interrelações entre os conjuntos de variáveis limnológicas, hidrológicas e morfométricas e a estrutura das comunidades de macrófitas aquáticas costeiras.

Diferentemente das hipóteses gerais em ecologia para os padrões de diversidade de espécies (RICKLEFS, 1977; STEIN *et al*., 2014) e do observado em outros estudos sobre macrófitas aquáticas (ROLON *et al*., 2008; SHI *et al*., 2010; ALAHUHTA *et al*., 2017), nós não encontramos relação entre a hetorogeneidade ambiental e a riqueza de espécies de macrófitas nos rios costeiros que estudamos. No entanto, nós encontramos que a heterogeneidade ambiental influencia positivamente a riqueza de formas de vida de macrófitas aquáticas, devido à maior diversidade de tipos de hábitats em rios mais heterogêneos. Apesar dos rios costeiros serem isolados e geograficamente distantes entre si em uma escala de até centenas de quilômetros, nós encontramos pouca explicação da distância geográfica entre os rios para a variação da composição de espécies de macrófitas entre os rios; resultados também contrários àqueles encontrados em outros estudos com esta abordagem (HEEGARD, 2004; CAPERS *et al*., 2009).

Os rios costeiros de São Paulo possuem diferentes pools de espécies de macrófitas aquáticas com comunidades praticamente distintas entre si, gerando grande diversidade beta e elevado *turnover* de espécies e aninhamento de formas de vida de macrófitas na escala regional. Este padrão de diversidade beta de formas de vida de macrófitas foi explicado pela diferença de comprimento dos rios. No entanto, a variação de espécies de macrófitas nesta escala regional parece estar mais relacionada a fatores bióticos e históricos na dispersão e colonização. Em uma escala local, de uma forma geral, também predominaram *turnover* de espécies e aninhamento de formas de vida, que foram explicados pelos gradientes ambientais longitudinais dos rios. De fato, a partir de nossos estudos experimentais nós sugerimos que ao longo do gradiente estuarino, a competição interespecífica independentemente da densidade de espécies, a ordem de chegada e o efeito de prioridade podem ter importância para a ocorrência e distribuição espacial de macrófitas aquáticas.

Nós não encontramos efeitos isolados da salinidade sobre o crescimento de *S. alterniflora* e *C. americanum* em substrato artificial, no entanto, encontramos que a salinidade pode ser importante para a interação interespecífica. Em maior salinidade em substrato artificial, houve uma tendência de competição assimétrica de fração emergente de *C. americanum* por *S. alterniflora.* Os resultados deste experimento indicam que a salinidade e as diferenças em outras características dos sedimentos naturais são responsáveis por diferentes respostas do crescimento e competição de macrófitas aquáticas em gradientes ambientais de estuários.

Nós encontramos que a densidade não é um fator determinante para a resposta da competição entre *S. alterniflora* e *C. americanum* em sedimento natural de baixo e médio estuário do rio Itanhaém. Nossos resultados mostraram que *S. alterniflora* limitou o crescimento de *C. americanum* e que *C. americanum* não limitou o crescimento de *S. alterniflora* independentemente de suas densidades iniciais em ambos os tipos de sedimento. Desta forma, *S. alterniflora* apresentou maior habilidade competitiva (em qualquer densidade) do que *C. americanum*. No entanto, quando nós avaliamos os fatores estocásticos de ordem de chegada e efeito de prioridade, nós encontramos que a primeira a colonizar o ambiente de baixo e médio estuário tem efeito de prioridade sobre a espécie que chegar posteriormente. Isto é, mesmo que *S. alterniflora* tenha maior habilidade competitiva do que *C. americanum* quando em colonização simultânea, essa vantagem competitiva não se mantém se *C. americanum* for a espécie fundadora em um determinado local. Assim, a ausência de *C. americanum* em baixo estuário pode estar relacionada ao fato de que *S. alterniflora* colonizou o ambiente primeiramente. A ocorrência de bancos mistos das duas espécies em médio estuário também deve estar relacionada à ordem de chegada. Nestes bancos, as espécies ocorrem com uma separação espacial lateralmente à margem do rio, ou seja, formam agrupamentos heteroespecíficos segregados no espaço. Possivelmente, *C. americanum* foi a primeira espécie a colonizar essas áreas e a colonização posterior de *S. alterniflora* levou-a a ocupar o espaço adjacente aos bancos já existentes de *C. americanum.*

Além de ser um resultado da ordem de chegada na colonização, a segregação espacial interespecífica de macrófitas aquáticas tem sido relatada como uma forma de organização estratégica que permite a coexistência entre diferentes espécies e diminui a competição (LEVINE *et al*., 1998; BAROT, 2004; ANGELINI *et al*., 2011). Esta forma de organização permite que espécies com iguais capacidades competitivas particionem o espaço e se utilizem dos mesmos recursos, porém também impede que a espécie de maior habilidade competitiva (em nosso caso, *S. alterniflora*) invada a porção ocupada pela espécie menos competitiva (*C. americanum*) (BAROT, 2004). Além disso, alguns estudos indicaram um potencial alelopático de *C. americanum* (RIBEIRO *et al*., 2009; RIBEIRO; LIMA, 2011), porém nós não encontramos indicativos e evidências em nossos experimentos de que suas possíveis substâncias alelopáticas possam ter efeito negativo sobre *S. alterniflora*. No entanto, não podemos descartar a possibilidade de que a alelopatia também tenha papel em manter a segregação das espécies nos bancos mistos em médio estuário.

Os resultados de nossos experimentos vão de encontro à recente hipótese proposta por Bortolus *et al.* (2015) sobre a ocorrência de *S. alterniflora* na América do Sul. A partir de levantamentos de registros históricos em herbários e de estudos genéticos e morfológicos, estes autores sugerem que embora *S. alterniflora* tenha sido, até então, considerada nativa da costa Atlântica de todo o continente americano, esta espécie pode ter sido introduzida na América do Sul no século 19 a partir de populações nativas do Golfo do México. Essa introdução pode ter ocorrido acidentalmente, por meio de sementes e fragmentos vegetativos vindos em águas de lastro de navios, ou intencionalmente, como uma tentativa de viabilizar uma alternativa alimentar para cavalos e bois. Estes autores acreditam que *S. alterniflora* tenha sido introduzida no Brasil em 1817 pelo porto do Rio de Janeiro e se expandido primeiramente em direção ao norte, chegando na Guiana em 1840, e depois em direção ao sul, chegando na Argentina em 1900. As sementes e propágulos de *S. alterniflora* podem flutuar no oceano e alcançar curtas distâncias em meses (DAEHLER; STRONG, 1996). No Brasil, especialmente no sudeste e parte da região sul, *S. alterniflora* teria colonizado áreas de sedimentos expostos ("*bare mudflats*") sem vegetação na borda do manguezal (BORTOLUS *et al.*, 2015). Esta espécie é considerada uma "*ecological engineer*" em estuários, isto é, é capaz de modificar o funcionamento e alguns processos do ambiente que ocupa por meio da agregação de sedimentos, redução da turbulência da água, expansão de superfícies de margens (LAMBRINOS; BANDO, 2007) e aumento da capacidade de armazenamento de C, N e S no sedimento (WAN *et al.*, 2020), facilitando a colonização de propágulos de mangue e a expansão do manguezal (SCHAEFFER-NOVELLI *et al.*, 1990). Desta forma, segundo Bortolus *et al.* (2015), a maioria das áreas estuarinas na América do Sul podem atualmente ser "miragens ecológicas", ou seja, paisagens geradas no tempo que impedem com que percebamos como era a natureza dos ecossistemas nativos pré-existentes à colonização de *S. alterniflora*. Nestas considerações, a introdução de *S. alterniflora* não levou a aspectos negativos relevantes, visto que, provavelmente, não ocupou o nicho de outras espécies de macrófitas nem de espécies de mangue (WAN *et al.*, 2009); oposto ao ocorrido com sua invasão em estuários no sul da China, por exemplo (PENG *et al.*, 2018).

Nossos resultados experimentais e proposições sobre os efeitos da densidade, da ordem de chegada e da salinidade na competição interespecífica de *S. alterniflora* e *C. americanum* valem para explicar o padrão de distribuição espacial destas espécies em estuários tropicais com grande influência marinha e gradientes de salinidade, como os estuários da região central e sul do litoral de São Paulo. Em estuários com pouca influência de águas marinhas e ausência de gradientes de salinidade, como naqueles da região norte do litoral paulista, estes resultados não devem explicar os padrões de distribuição de espécies, no entanto, podem contribuir para explicar a ausência de *S. alterniflora* nestes rios. Possivelmente, *S. alterniflora* não foi capaz de colonizar os rios da região norte, não por seus ambientes de água doce, mas sim devido a outros fatores como pequena área de margem disponível para sua colonização. Nestes rios, as áreas de manguezal são bastante reduzidas em comparação àquelas dos rios costeiros da região central e sul e as margens potencialmente colonizáveis já estariam ocupadas por outras espécies. Além disso, alguns estudos indicam que *S. alterniflora* pode requerer uma variação constante do nível de água para um ótimo crescimento e sobrevivência (VOSS *et al.*, 2013; SMITH; LEE, 2015), assim, caso a colonização desta espécie tenha ocorrido, seu desenvolvimento pode ter sido negativamente influenciado pela menor variação do nível de água, devido à pequena influência marinha, nos estuários da região norte do litoral de São Paulo.

Como perspectivas futuras a partir dos estudos desenvolvidos nesta tese, nós consideramos que experimentos em campo poderão ainda contribuir para o entendimento dos fatores responsáveis pelos padrões de distribuição e organização de assembleias de macrófitas aquáticas ao longo dos estuários. Quanto à escala regional, nós indicamos que a ampliação espacial da amostragem (inclusão de rios tributários das bacias já amostradas ou de outras bacias ainda não amostradas) e a coleta e análise de variáveis ambientais não consideradas aqui somariam na explicação dos padrões de diversidade e variação de comunidades de macrófitas aquáticas entre os rios costeiros.

Atualmente, as zonas costeiras abrigam a maior parte das áreas urbanas e regiões metropolitanas no mundo e, por isso, os ecossistemas aquáticos costeiros estão entre os ambientes mais impactados dos últimos dois séculos. Apesar de o Brasil possuir uma extensa e diversa zona costeira e da necessária urgência de ações para prevenir sua degradação ambiental, ainda pouco se conhece sobre a ecologia de seus ecossistemas aquáticos (PRATES *et al.*, 2012). No país, 13 das 27 capitais e cerca de 60% dos habitantes estão localizadas na costa litorânea de quase 11 mil km, indicativos do alto nível de pressão a que seus ecossistemas estão submetidos (PRATES *et al.*, 2012). Um exemplo disso é a zona costeira do estado de São Paulo que vem sofrendo os impactos desta expansão e adensamento urbano e industrial, pois abriga a região metropolitana da Baixada Santista e parte da região metropolitana do Vale do Paraíba - Litoral Norte, a maior zona portuária da América Latina (Porto de Santos) e um grande pólo de indústrias petroquímicas e siderúrgicas (Cubatão) (ZÜNDT, 2006). Esta zona costeira possui características próprias (SOUZA; CUNHA, 2011), e é historicamente uma das regiões mais exploradas e ocupadas do estado, porém estes impactos antrópicos têm ocorrido com maior intensidade desde 1950 devido, principalmente, à ocupação urbana desordenada, aterramento de áreas de manguezais, rebaixamento do lençol freático, despejo de efluentes e poluentes, retificação de rios, dragagem de canais, tráfego náutico, pesca e turismo pedratórios, extração de areia em praias e corpos de água (TESSLER *et al.*, 2006; PINHEIRO *et al.*, 2008; PINTO-COELHO; HAVENS, 2015).

Os ecossistemas aquáticos costeiros e suas comunidades biológicas também são sensíveis aos impactos provocados pelas mudanças climáticas, principalmente pela elevação do nível do mar (SIMAS *et a*l., 2001). Estima-se que o nível do mar suba 3,2 mm por ano em uma média global e entre 1,8 e 4,2 mm por ano no Brasil. Como consequências também são esperadas alterações nas frequências de inundação, erosão de margens e maior intrusão salina nas bacias costeiras, e assim, os ambientes aquáticos costeiros com micro-marés (amplitude de até 2 m), como os do Sudeste do Brasil tendem a ser os mais afetados (WARD *et al*., 2017). Além disso, a construção de reservatórios e a captação de água doce à montante destas bacias também podem levar à redução da vazão de água doce e, consequentemente, à maior salinização de suas águas e sedimentos (PINTO-COELHO; HAVENS, 2015).

Desta forma, nós finalizamos destacando a importância de estudos ecológicos em rios costeiros e zonas estuarinas visando a geração de conhecimento sobre sua ecologia e informações sobre a ocorrência e distribuição de espécies e composição de comunidades, que permitirão embasar os planos de ação e gestão de gerenciamento costeiro, o monitoramento da biodiversidade e da qualidade ambiental, o manejo de espécies e a conservação e restauração destes ecossistemas.

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