



Universidade Federal do Rio Grande
Instituto de Ciências Biológicas
Pós-graduação em Biologia de
Ambientes Aquáticos Continentais



**Influência da fisiografia, paisagem, fatores
espaciais e locais, e sobreposição espacial de
macrófitas aquáticas em áreas úmidas do sul do
Brasil**

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Tese apresentada ao Programa de Pós-graduação em Biologia de Ambientes Aquáticos Continentais como requisito parcial para a obtenção do título de Doutora em Biologia de Ambientes Aquáticos Continentais.

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**ATA DE DEFESA DE TESE DE DOUTORADO EM BIOLOGIA DE AMBIENTES
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Às 09:00 h (nove horas) do dia 07 (sete) do mês de maio de 2021 (dois mil e vinte e um), via Webconferência (endereço eletrônico: ([https://conferenciaweb.rnp.br/webconf/ana-silvia-ro lon-ana](https://conferenciaweb.rnp.br/webconf/ana-silvia-rolon-ana))) reuniram-se docentes, discentes e comunidade em geral, para a Defesa Pública da Tese de Doutorado da acadêmica **Karine Massia Pereira**. A Tese intitulada **“Influência da fisiografia, paisagem, fatores espaciais e locais, e sobreposição espacial de macrófitas aquáticas em áreas úmidas do sul do Brasil”** foi avaliada pela Banca Examinadora composta pela Prof^a. Dra. Ana Silva Rolon (Orientadora), Prof^a Dra Sônia Marisa Hefler (Co-orientadora), Prof^o Dr. Eduardo Dias Forneck (FURG), Prof^a Dra Raquel Lüdtke (UFPEL) e Prof^o Dr. Antonio Fernando Monteiro Camargo (UNESP). Após a defesa e arguição pública, a Banca Examinadora reuniu-se, para deliberação final, e considerou a acadêmica **APROVADA**. Desta forma, a acadêmica concluiu mais uma das etapas necessárias para a obtenção do grau de **DOUTORA EM BIOLOGIA DE AMBIENTES AQUÁTICOS CONTINENTAIS**. Nada mais havendo a tratar, às 13:20 h (treze horas e vinte minutos) foi lavrada a presente ata, que lida e aprovada, foi assinada pelos membros da Banca Examinadora, pela Acadêmica e pelo Coordenador do Curso.

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Dedico essa tese *in memorium* ao meu pai, Edi Terra Pereira, o grande incentivador dos meus sonhos e quem sempre esteve ao meu lado na minha caminhada.

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RESUMO

Entender as relações entre comunidades biológicas e o seu meio ambiente é o tema central da ecologia de comunidades. As assembleias de macrófitas aquáticas desempenham fundamental importância ecológica em ambientes aquáticos, cujos quais apresentam papéis importantes à sociedade e à biodiversidade. O objetivo geral da tese é avaliar os fatores determinantes para a riqueza e composição das assembleias de macrófitas aquáticas em áreas úmidas do sul do Brasil (regiões fisiográficas Encosta do Sudeste e Litoral). Foram avaliados desde fatores da paisagem (quantidade de habitats, isolamento e uso da terra), fisiográficos e climáticos; espaciais e ambientais; até interações interespecíficas. Além disso, avaliou-se se esses fatores interferem de maneira distinta sobre os diferentes grupos funcionais de macrófitas aquáticas. Características da paisagem foram mensuradas através de técnicas de processamento de imagens de satélite e Sistemas de Informação Geográfica. Fatores espaciais foram obtidos através de Análises de Coordenadas Principais de Matrizes Vizinhas. Interações interespecíficas foram mensuradas através do Índice de Sobreposição Espacial desenvolvido especialmente para macrófitas aquáticas. Ao todo foram amostradas 249 espécies de macrófitas aquáticas; 221 na Encosta do Sudeste e 144 no Litoral. Verificamos que a disponibilidade de habitats na paisagem relacionou-se negativamente com a riqueza, enquanto o isolamento e áreas agrícolas relacionaram-se positivamente. A fisiografia e outras características da paisagem também foram importantes para a composição. Fatores espaciais também estruturaram as assembleias de macrófitas aquáticas, mas sua importância relativa diferiu entre escalas espaciais de observação e grupos funcionais. Em relação às interações interespecíficas, verificou-se uma possível vantagem competitiva de *Luziola peruviana* sobre *Eleocharis minima*. Pode-se concluir que para entender a estruturação de assembleias de macrófitas aquáticas é necessário avaliar um amplo espectro de fatores. Essa tese abordou aspectos paisagísticos e de interações interespecíficas ainda não estudados em áreas úmidas do sul do Brasil, assim como contribuiu para o conhecimento florístico dessa região.

Palavras-chave: Encosta do Sudeste, fatores climáticos, isolamento, Litoral, metacomunidades.

ABSTRACT

Understanding the relationship between biological communities and their environment is the central theme of community ecology. The assemblages of aquatic macrophytes play a fundamental ecological importance in aquatic environments, which are important for society and biodiversity. The general aim of the thesis is to evaluate the determining factors for the richness and composition of the assemblages of aquatic macrophytes in ponds of southern Brazil (physiographic regions *Encosta do Sudeste* and *Litoral*). It was evaluated from landscape factors (number of habitats, isolation and land use), physiographic and climatic; space and environmental; to interspecific interactions. In addition, it was evaluated whether these factors influence differently on the different functional groups of aquatic macrophytes. Landscape factors were measured using satellite image processing techniques and Geographic Information Systems. Spatial factors were obtained through Principal Coordinate Analysis of Neighboring Matrices. Interspecific interactions were measured using the Spatial Overlap Index developed especially for aquatic macrophytes. A total of 249 species of aquatic macrophytes were sampled: 221 on the *Encosta do Sudeste* and 144 on the *Litoral*. We found that the availability of habitats in the landscape was negatively related to richness, while isolation and agricultural areas were positively related to richness. The physiography and other characteristics of the landscape were also important for the composition. Spatial factors also structured the assemblages of aquatic macrophytes, but their relative importance differed between spatial scales of observation and functional groups. In relation to interspecific interactions, there was a possible competitive advantage of *Luziola peruviana* over *Eleocharis minima*. It can be concluded that to understand the structuring of aquatic macrophyte assemblages, it is necessary to evaluate a wide spectrum of factors. This thesis addressed landscape aspects and interspecific interactions not yet studied in ponds of southern Brazil, as well as contributed to the floristic knowledge of this region.

Key-words: Encosta do Sudeste, climatic factors, isolation, Litoral, metacommunities.

APRESENTAÇÃO

A tese foi estruturada em uma introdução geral, quatro capítulos, e considerações finais e perspectivas. A introdução geral contém uma breve revisão sobre características da paisagem, metacomunidades e interações biológicas de macrófitas aquáticas. Cada capítulo corresponde a um artigo científico produzido pela autora e colaboradores. O primeiro capítulo/artigo é intitulado “*Influences of Landscape and Climatic Factors on Aquatic Macrophyte Richness and Composition in ponds*”, o qual foi aceito para publicação na revista Flora. O segundo capítulo/artigo é intitulado “*The role of spatial factors and environmental filters on aquatic macrophyte metacommunities in subtropical ponds*” e foi submetido à Inland Waters. O terceiro capítulo/artigo é intitulado “*Spatial niche overlap of aquatic macrophytes in intermittent and permanent ponds*” e foi submetido ao periódico Hydrobiologia. Já o quarto artigo, “*Aquatic macrophytes of ponds in two physiographic regions of southern Brazil: floristic, assemblage structure, and beta diversity*”, está sob revisão na Acta Botanica Brasílica. Ao final da tese encontram-se em anexo outros trabalhos produzidos pela autora durante o período do doutorado.

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INTRODUÇÃO GERAL

Entender as relações entre comunidades biológicas e o seu meio ambiente é o tema central da ecologia de comunidades (VELLEND, 2010). O modo como as comunidades biológicas estão organizadas depende de vários fatores e da interação entre eles (LEIBOLD et al., 2004). Esses fatores envolvem desde características mais amplas, como características geomorfológicas regionais da paisagem e capacidade de dispersão dos organismos e sua consequente estruturação espacial, até interações biológicas e condições ambientais locais.

As áreas úmidas são de extrema importância para a biodiversidade e sociedade (MITSCH; BERNAL; HERNANDEZ, 2015), e dentre os organismos que dependem e contribuem para diversos serviços ecossistêmicos das áreas úmidas temos as macrófitas aquáticas (REJMANKOVA, 2011). Essa introdução geral visa resumir o conhecimento científico sobre fatores paisagísticos, de metacomunidades e de interações biológicas que atuam sobre assembleias de macrófitas aquáticas.

1. Macrófitas Aquáticas

As macrófitas aquáticas são “vegetais visíveis a olho nu, cujas partes fotossintetizantes estão total ou parcialmente submersas ou flutuantes em água doce ou salobra” (IRGANG; GASTAL JR, 1996). Elas possuem papel ecológico importante nos ecossistemas, pois são produtores primários, fazem parte da ciclagem de nutrientes e gases, além de atuarem como abrigo e berçário para diversos organismos (REJMANKOVA, 2011). Elas podem interferir nas condições do meio ambiente (e.g. velocidade das correntes aquáticas e luminosidade da coluna d’água) (PINERO-RODRÍGUEZ et al., 2019; YAN et al., 2019), assim como condições ambientais podem interferir na sua estrutura (BORNETTE; PUIJALON, 2011; LACOUL; FREEDMAN, 2006), alterando sua riqueza e composição, por exemplo.

Muitos estudos avaliaram a interferência de condições ambientais sobre a estrutura das assembleias de macrófitas aquáticas. Bini et al. (1999) e Ferreira et al. (2015) verificaram a influência de características físico-químicas da água na distribuição e cobertura da assembleia, verificando que, por exemplo, espécies flutuantes geralmente estão presentes em altas concentrações de nitrogênio e fósforo, enquanto a penetração de luz na coluna d’água é o

principal preditor da ocorrência e composição de espécies submersas. Mauhs et al. (2006) e Spindola (2007) verificaram que a profundidade da lâmina d'água interfere na riqueza específica estando inversamente relacionada à mesma. Jeffries (1988) e Rolon et al. (2008) verificaram que o tamanho da área úmida geralmente apresenta relação positiva com a riqueza, assim como a diversidade de habitat, que também está relacionada com o tamanho da área úmida. Maltchik et al. (2007) afirmam que o hidroperíodo também pode ser considerado responsável por diferenças na riqueza e composição de macrófitas aquáticas, visto que, pequenas variações hidrológicas podem modificar a composição de macrófitas aquáticas.

As macrófitas aquáticas possuem características biológicas que as possibilitam habitar o ambiente aquático de diferentes maneiras. Vários autores propõem classificações de acordo com a sua forma de vida nesses ambientes (CRONK; FENNESSY, 2001; IRGANG; GASTAL-JR, 1996). Irgang & Gastal Jr (1996) classificam as macrófitas aquáticas em anfíbias, emergentes, epífitas, flutuantes fixas, flutuantes livres, trepadeiras e submersas (Fig. 1). As anfíbias habitam o ambiente terrestre, mas suportam períodos de alagamento. As emergentes são fixas no substrato e emergem suas estruturas vegetativas e reprodutivas da superfície da água. As epífitas desenvolvem-se sobre outras macrófitas. As flutuantes podem ser fixas no substrato e suas folhas flutuarem sobre a água, ou podem flutuar livremente na superfície da água (flutuantes livres). As submersas desenvolvem-se totalmente imersas na água, podendo apenas suas estruturas reprodutivas emergirem em época de reprodução. Elas também podem ser fixas no substrato ou livres de baixo da água, prendendo-se a caules e estruturas de outras macrófitas aquáticas.



Figura 1. Formas de vida de macrófitas aquáticas: 11, 12 e 13) flutuantes livres; 21) submersas; 22) flutuantes fixas; 23 e 24) emergentes; 25) trepadeiras; e 26) anfíbias. Fonte: Irgang & Gastal Jr (1996).

As diferentes formas de vida também podem estar relacionadas aos mecanismos de dispersão das macrófitas aquáticas. As anfíbias e emergentes dispersam seus diásporos principalmente pelo vento ou por animais, que alcançam maiores distâncias (SOONS, 2006). Já as hidrófitas (plantas que vivem submersas ou flutuantes na coluna d'água, estando enraizadas ou não) dispersam seus diásporos principalmente por hidrocoria e são mais sensíveis a mudanças ambientais, como na qualidade da coluna d'água (CRONK; FENNESSY, 2001).

Em estudos ecológicos, os autores geralmente agrupam as espécies de acordo com suas funções no ambiente, de modo a refletir respostas ecológicas a determinados fatores. Esses agrupamentos podem ser chamados de grupos funcionais. As formas de vida das macrófitas muitas vezes são agrupadas para refletir suas funções no ambiente. Por exemplo, Alahuhta & Heino (2013) classificaram as macrófitas aquáticas em três grupos funcionais: emergentes (incluindo anfíbias), submersas (fixas) e macrófitas não enraizadas (submersas livres e flutuantes livres) para verificar a influência de fatores espaciais e ambientais sobre as comunidades de macrófitas aquáticas de lagos temperados, e se esses fatores interferiam de modo diferente nas macrófitas. Desse modo, classificar as macrófitas aquáticas em grupos funcionais pode ser eficaz no entendimento de processos ecológicos que atuam nessas comunidades (NUNES; UMETSU; CAMARGO, 2020; TRINDADE; LANDEIRO; SCHNECK, 2018).

2. Ecologia de paisagem de macrófitas aquáticas

A ecologia de paisagens aborda questões geográficas e ecológicas, cujo foco é o estudo dos efeitos de padrões espaciais sobre processos ecológicos (METZGER, 2001; TURNER, 1989). A paisagem pode ser definida como uma “área espacialmente heterogênea”, formada por mosaicos ambientais (*habitat patches*/manchas) e suas interconexões, e sua delimitação depende do ponto de vista em que é observada (escala espacial) (TURNER, 1989). Fatores estruturantes da paisagem, como a fisiografia, quantidade de habitats (*habitat amount*), isolamento e uso da terra são investigados como fatores influentes na estrutura de comunidades biológicas, tais como, as assembleias de macrófitas aquáticas (AKASAKA et al., 2010).

A fisiografia, ou geografia física, reflete a história geomorfológica das paisagens naturais e pode influenciar a estrutura de assembleias de macrófitas aquáticas (ALAHUHTA et al., 2018), visto que, influencia nas taxas de extinção e colonização de espécies nas manchas.

Porém, a fisiografia é um fator ainda pouco considerado em estudos que avaliam os fatores estruturantes de assembleias de macrófitas aquáticas em áreas úmidas (e.g. ALAHUHTA; VUORI; LUOTO, 2011; SASS et al., 2010). Sass et al. (2010) avaliaram a influência de fatores paisagísticos sobre macrófitas aquáticas de lagos em duas “ecorregiões”, mas não relacionaram as “ecorregiões” com a estrutura da assembleia. Alahuhta et al. (2011) adicionaram variáveis geomorfológicas, como topografia, altitude e tipos de solo, para investigar os fatores influentes sobre macrófitas emergentes de lagos boreais.

A quantidade de habitats na paisagem (*habitat amount*) pode ser entendida como o quanto de habitat está disponível para a colonização de determinado grupo de organismos. A quantidade de habitats pode ser mensurada através do número de manchas, tamanho de cada mancha (*patch size* ou área), tamanho total de manchas e da forma que essas manchas apresentam na paisagem (TURNER, 1989). O tamanho das manchas é comumente investigado como preditor da estrutura das assembleias de macrófitas aquáticas e geralmente apresenta relação positiva com a riqueza (BOSIACKA; PIENKOWSKI, 2012; HOULAHAN et al., 2006; MORAES et al., 2014; SCHÖPKE et al., 2019), mas isso nem sempre é verificado (AKASAKA et al., 2010; ROLON; ROCHA; MALTCHIK, 2012). O'Hare et al. (2012) utilizaram o perímetro dos lagos como uma métrica de disponibilidade de habitats, mas encontraram pouco efeito detectável dessa métrica sobre a assembleia de macrófitas aquáticas. Porém, uma teoria relativamente recente - *Habitat Amount Hypothesis* - afirma que o efeito do tamanho da mancha e do isolamento da paisagem na riqueza de espécies é devido principalmente ao efeito da área amostral, mas que outros fatores de uso da terra também são influentes na riqueza de espécies (FAHRIG, 2013).

O isolamento tende a tornar as comunidades mais dissimilares entre si em termos de composição e riqueza de espécies (BORNETTE; AMOROS; LAMOUROUX, 1998; ROLON; ROCHA; MALTCHIK, 2012). Rolon et al. (2012) verificaram que o isolamento da área úmida fonte diminui a riqueza da assembleia de macrófitas aquáticas de áreas úmidas costeiras do Rio Grande do Sul, mas constataram que isso varia entre grupos funcionais. Porém, nem sempre há relação entre isolamento e riqueza de macrófitas aquáticas (e.g. BOSIACKA; PIENKOWSKI, 2012; SCHÖPKE et al., 2019).

Mudanças no uso da terra que conduzam à perda de habitats naturais e/ou diminuição da heterogeneidade ambiental, principalmente atividades antrópicas como a expansão da agricultura e urbanização, tendem a reduzir a diversidade de plantas em ambientes naturais (COUSINS et al., 2015). Estudos que avaliam a influência dos usos da terra sobre macrófitas

aquáticas são mais abundantes. Houlahan et al. (2006) verificaram que a riqueza de macrófitas aquáticas de áreas úmidas do Canadá esteve positivamente relacionada com a cobertura vegetada e negativamente relacionada com a densidade de construções urbanas. Akasaka et al. (2010) também encontraram relação negativa entre a proporção de área urbana e a diversidade de macrófitas aquáticas emergentes, flutuantes livres e submersas de áreas úmidas do Japão. O desenvolvimento urbano e agrícola ao redor de rios da Austrália e lagos dos EUA aumentou a abundância de macrófitas invasoras (QUINN; SCHOOLER; VAN KLINKEN, 2011; SASS et al., 2010). Porém, a intensificação na formação de canais de drenagem em sistemas agrícolas aumentou a abundância de macrófitas emergentes em lagos boreais da Finlândia (ALAHUHTA; VUORI; LUOTO, 2011), podendo acarretar outros problemas devido ao crescimento exagerado desses organismos nos ambientes aquáticos.

O avanço de geotecnologias como o uso de imagens de satélite, ferramentas de SIG (Sistemas de Informação Geográfica) e softwares específicos possibilitou mensurar métricas ambientais à nível de paisagem (e.g. usos da terra, isolamento e quantidade de habitats naturais na paisagem). A análise dessas métricas por meio de modelos estatísticos permite relacionar tais métricas com características das comunidades a fim de verificar como esses fatores influenciam a estrutura de comunidades biológicas (e.g. riqueza e composição de espécies).

3. Características ambientais versus espaciais: uma perspectiva de metacomunidades de macrófitas aquáticas

De acordo com a definição de Wilson (1992), metacomunidades são “um conjunto de comunidades locais interligadas por dispersão de espécies que potencialmente interagem entre si”. Os estudos de metacomunidades procuram avaliar a influência e a importância de fatores espaciais, fatores ambientais e os efeitos conjuntos desses fatores sobre as comunidades biológicas como representantes (*proxies*) de processos de dispersão e de nicho ambiental (LEIBOLD et al., 2004; LOGUE et al., 2011). A dispersão das espécies pode ser considerada como o principal mecanismo regional estruturador das comunidades, podendo os seus efeitos sobrepor-se aos efeitos de nicho (LEIBOLD et al., 2004).

Dependendo da contribuição de cada porção (espacial, ambiental ou ambas), as metacomunidades podem ser reconhecidas em quatro paradigmas não inter-excluentes: dinâmica de manchas (*patch dynamics*), filtragem de espécies (*species sorting*), efeito de massa (*mass effect*), e dinâmica neutra (*neutral model*) (LEIBOLD et al., 2004). A perspectiva “*patch*

dynamics” assume que a dispersão é limitante para a diversidade de espécies, sendo a porção espacial mais importante que a ambiental na estruturação das comunidades (LEIBOLD et al., 2004). A perspectiva “*species sorting*” enfatiza que a dispersão é alta o suficiente para permitir que as espécies preencham nichos dentro dos habitats por causa da diversificação do nicho (LOGUE et al., 2011), sendo a porção ambiental mais importante na estruturação das comunidades. No terceiro modelo, “*mass effects*” tanto a dispersão (fatores espaciais) quanto os fatores ambientais são importantes. Em sistemas em que as espécies podem ser excluídas por serem competidores inferiores, a migração para outro ambiente pode ser uma alternativa. E o “*neutral model*” assume que as espécies são equivalentes ecologicamente em uma comunidade troficamente definida (HUBBELL, 2005).

As metacomunidades de macrófitas aquáticas são tradicionalmente entendidas como pouco limitadas pela dispersão. Essa ideia resulta da suposição de que a maioria das espécies é amplamente distribuída e pelo potencial de produzirem propágulos vegetativos que colonizam em longas distâncias (CHAMBERS et al., 2008; SANTAMARÍA, 2002). Porém, Murphy et al. (2019) avaliaram a distribuição global de mais de três mil espécies de macrófitas aquáticas e constataram que a maioria delas (aproximadamente 80%) tem distribuição restrita, ocupando menos de 10% da área global potencial para o estabelecimento desse grupo.

Mais recentemente, estudos que abordam fatores espaciais que organizam as metacomunidades de macrófitas aquáticas mostram-se divergentes quanto aos padrões encontrados: Padial et al. (2014) e García-Girón et al. (2019) verificaram que fatores espaciais são mais importantes na estruturação dessas comunidades do que os ambientais. Porém, Alahuhta et al. (2013, 2014), Mikulyuk et al. (2011) e O’Hare et al. (2012) encontraram o contrário. Já Capers et al. (2010), Grimaldo et al. (2016), Heino et al. (2010) e Kuglerova et al. (2015) verificaram a importância conjunta de fatores regionais (dispersão) e locais (ambientais) para a diversidade e composição de macrófitas aquáticas, assim como de processos estocásticos, provavelmente associados à colonização e persistência das plantas.

Os padrões encontrados podem ser divergentes devido aos diferentes tipos de ambientes estudados (rios, lagos, banhados), regiões geográficas, extensão espacial, escalas de observação e grupos funcionais (ALAHUHTA et al., 2013; ALAHUHTA; HEINO, 2013; HEINO et al., 2015). A maioria dos estudos avaliou metacomunidades de macrófitas aquáticas em lagos e rios em regiões temperadas e tropicais (e.g. Alahuhta et al., 2013; Grimaldo et al., 2016). Porém, pouco se conhece sobre processos que estruturam essas metacomunidades em áreas úmidas rasas subtropicais (TRINDADE; LANDEIRO; SCHNECK, 2018).

4. Interações biológicas interespecíficas de macrófitas aquáticas

Em escala local, a dinâmica das interações entre espécies é de importância central para a compreensão da estrutura das comunidades (OKLAND; SKARPAAS; KAUSRUD, 2009; WIENS, 2011). Os principais tipos de interações investigadas entre espécies de plantas aquáticas são a competição e a facilitação. Porém, para avaliar essas interações é necessário o emprego de experimentos que demandam maior tempo de execução e dão respostas em nível de espécie-espécie. Investigar a sobreposição espacial permite avaliar as interações biológicas em nível de comunidades. A sobreposição espacial pode refletir tanto mecanismos de competição quanto de facilitação (*latu sensu*). Se a competição interespecífica é um forte estruturador das comunidades, espécies ecologicamente similares tendem a apresentar intervalos espaciais em escala local que não se sobrepõem (HOFER; BERSIER; BORCARD, 2004). Se há sobreposição espacial entre determinadas espécies, isso pode indicar mecanismos de facilitação (van ZUIDAN et al., 2018).

Estudos que avaliam a competição parecem ser mais abundantes do que estudos que avaliam a facilitação entre macrófitas aquáticas, principalmente aqueles que avaliam espécies submersas e/ou invasoras. Dentre esses podemos citar: McCreary (1991) que fez uma breve revisão de trabalhos que abordam competição entre macrófitas submersas; Spencer & Rejmánek (1989) que verificaram que o tipo de propágulo de *Hydrilla verticillata* (L. f.) Royle (submersa invasora) interfere na sua capacidade de competição, sendo que as plantas originadas de turiões são menos competitivas do que aquelas originadas de tubérculos; Doyle et al. (2003) investigaram a competição entre duas espécies submersas, indicando *Hygrophila polysperma* (Roxb.) T. Anderson (invasora) compete sobre *Ludwigia repens* J.R. Forst. (nativa); Wang et al. (2008) que verificaram que *H. verticillata* (invasora) apresenta vantagens sobre *Myriophyllum spicatum* L. (nativa); Martin & Coetzee (2014) avaliaram a competição entre duas macrófitas submersas invasoras *Lagarosiphon major* Moss ex Wager e *Myriophyllum spicatum* em diferentes concentrações de nutrientes e tipos de substrato e verificaram que *L. major* compete sobre *M. spicatum* em sedimentos com alta concentração de nutrientes, e que *L. major* leva vantagem em sedimentos de barro e *M. spicatum* na areia.

Porém, também podemos mencionar um estudo que avaliou o potencial de competição, sob diferentes condições ambientais, de uma espécie submersa nativa endêmica de Taiwan e ameaçada de extinção, *Isoetes taiwanensis* De Vol (YU; LUR; CHANG, 2013). Os autores

investigaram em que condições de profundidade da água essa espécie consegue competir com outras a fim de auxiliar no desenvolvimento de protocolos de conservação. Eles verificaram que *I. taiwanensis* tem vantagem sobre as outras espécies em profundidades de 46,8 a 185,6 cm, e que essa espécie não cresce em locais com menos de 30 cm de profundidade.

Estudos que avaliaram a competição de macrófitas aquáticas de outras formas de vida também foram desenvolvidos. Kankanamge & Kodithuwakku (2017) verificaram que a obtenção de nutrientes de *Pistia stratiotes* L., *Limnocharis flava* (L.) Buchenau e *Ipomoea aquatica* Forssk. em ambientes aquáticos ricos em nutrientes depende da competição entre as espécies. Dickinson & Miller (1998) verificaram que a competição tem papel importante, mas limitado, entre três espécies flutuantes livres, indicando que *Salvinia minima* Baker é uma boa competidora sobre *Azolla caroliniana* Willd. e *Spirodella punctata* (G.Mey.) C.H.Thomps., mas que os efeitos da competição foram sazonais em áreas úmidas da Flórida, EUA. Murillo et al. (2019) avaliaram a competição e respostas às flutuações no nível de água de duas macrófitas emergentes e verificaram que *Polygonum acuminatum* Kunth é melhor competidora do que *Polygonum ferrugineum* Wedd, onde a primeira coloniza locais mais rasos que a segunda, mas também apresenta adaptações à inundação, sobrevivendo melhor em ambientes intermitentes. A competição entre outras duas macrófitas emergentes, *Crinum americanum* L. e *Spartina alterniflora* Loisel., também tem sido testada. Nunes & Camargo (2018) verificaram, em condições laboratoriais, que o crescimento de *C. americanum* é prejudicado na presença de *S. alterniflora* em ambientes de alta salinidade, indicando que uma competição assimétrica entre essas duas espécies pode ser responsável pela ausência de *C. americanum* na parte inferior de um estuário tropical. Nunes & Camargo (2020) apontaram, através de experimentos em laboratório, que *S. alterniflora* limita o crescimento de *C. americanum* independentemente da densidade inicial de colonização dessas espécies tanto em condições de alta salinidade quanto em condições salobras.

Aparentemente poucos estudos avaliam puramente a facilitação de macrófitas aquáticas. Hidding et al. (2014) avaliaram se a presença de uma espécie flutuante (*Stratiotes aloides* L.) facilitaria o estabelecimento de outras espécies em períodos de inundação, mas não obtiveram evidências de que isso ocorra com as variáveis mensuradas. Alguns estudos avaliaram tanto a facilitação quanto a competição (MARQUES-SILVA; THOMAZ, 2009; THOUVENOT et al., 2013). Marques-Silva & Thomaz (2009) avaliaram os efeitos de *Eichornia azurea* (Sw.) Kunth, uma macrófita fixa de folha flutuante, sobre o crescimento de macrófitas flutuantes livres (*Eichornia crassipes* (Mart.) Solms, *Salvinia minima* Baker, *Salvinia auriculata* Aubl. e

Limnobium laevigatum (Humb. & Bonpl. ex Willd.) Heine) na bacia de inundação do rio Paraná. Eles verificaram que *E. azurea* compete sobre *L. laevigatum*, mas não encontraram evidências de que haja facilitação de *E. azurea* sobre as outras espécies, embora não descartem a possibilidade de uma facilitação “mecânica” contra o vento e as ondas. Já Amorim et al. (2015) verificaram que *E. azurea* facilita o estabelecimento de macrófitas submersas livres e fixas, ao contrário da exótica invasora *Urochloa arrecta* (Hack. ex T.Durand & Schinz) Morrone & Zuloaga. Thouvenot et al. (2013) avaliaram as interações biológicas de uma macrófita anfíbia invasora (*Ludwigia grandiflora* (Michx.) Greuter & Burdet) nas áreas úmidas da Europa sobre outras macrófitas invasoras de mesma origem (América do Sul – *Myriophyllum aquaticum* (Vell.) Verdc. e *Egeria densa* Planch.) e sobre macrófitas nativas da região (*Ceratophyllum demersum* L. e *Mentha aquática* L.). Os autores verificaram que o estabelecimento e o crescimento de *L. grandiflora* não parece ser facilitado por outras espécies exóticas, porém *L. grandiflora* inibiu tanto a produção de raízes quanto o crescimento da nativa *M. aquatica* e facilitou o estabelecimento da invasora *E. densa*.

A sobreposição espacial já foi abordada para algumas plantas terrestres (veja Silander & Antonovics 1982; Higuchi et al. 2011), mas apenas o trabalho de Lombardo & Mjelde (2014) fez essa abordagem para macrófitas aquáticas. Quantificar a sobreposição espacial é importante para entender a riqueza e composição de espécies em comunidades. Lombardo & Mjelde (2014) propuseram um índice para quantificar a sobreposição de nicho espacial especialmente para macrófitas aquáticas, a partir do Índice α de Levins (LEVINS, 1968), o qual é aplicado para organismos não sésseis e possui em sua base matemática as equações de competição de Lotka-Volterra. O índice de Lombardo e Mjelde (2014) tenta considerar que a acessibilidade das plantas ao ambiente não é imediata, ao contrário dos organismos móveis, e que em condições em que há a aglomeração de espécies (alta cobertura/dominância) a distribuição da comunidade é afetada. Até o presente momento, estudos testando a aplicabilidade desse índice não foram desenvolvidos.

5. Áreas Úmidas

Áreas Úmidas (AUs), ou *wetlands* em inglês, são “ecossistemas na interface entre ambientes terrestres e aquáticos, continentais ou costeiros, naturais ou artificiais, permanentemente ou periodicamente inundados por águas rasas ou com solos encharcados, doces, salobras ou salgadas, com comunidades de plantas e animais adaptadas à sua dinâmica

hídrica” (JUNK; PIEDADE, 2015). As áreas úmidas promovem diversos serviços ecossistêmicos como: regulação e abastecimento dos níveis de água de corpos aquáticos; redução do risco de enchentes; recarga dos aquíferos e lençol freático; fornecimento de água para a população humana e para animais; retenção de sedimentos; estocagem de carbono orgânico; ecoturismo e recreação; dentre outros (CUNHA; PIEDADE; JUNK, 2014)

Existem diversas classificações dessas áreas devido às suas peculiaridades e aos diferentes termos regionais utilizados (e.g. banhados, pântanos, charcos, lagoas, várzeas etc.). No Brasil houve um esforço para classificá-las a fim de facilitar o desenvolvimento de políticas públicas para a sua conservação e para padronizar os termos utilizados na literatura internacional. Junk et al. (2014) classificaram-nas em três níveis hierárquicos: sistemas, unidades definidas por fatores hidrológicos, e unidades definidas por plantas superiores. O primeiro nível pode ser categorizado em: a) áreas úmidas costeiras (*coastal wetlands*), b) áreas úmidas interiores (*inland wetlands*), c) áreas úmidas antropogênicas (*artificial wetlands*). O segundo nível subdivide os sistemas em subsistemas considerando aspectos hidrológicos das AUs. Por exemplo, as AUs interiores são subdivididas em AUs com nível de água relativamente estável e AUs com flutuações no nível de água. Já o terceiro nível hierárquico considera a estrutura da vegetação superior, como por exemplo, a dominância de vegetação arbórea ou de vegetação herbácea (JUNK et al., 2014).

As áreas úmidas são bons modelos para o estudo de fatores paisagísticos e de metacomunidades, principalmente para organismos aquáticos obrigatórios, por serem bem delineadas na paisagem (e.g. limites da própria lâmina d’água e por estarem cercados de ambiente terrestre) e pelo alto número desses ambientes (DE MEESTER et al., 2005). Além dessas características, as áreas úmidas apresentam ampla variação espaço-temporal, variam em seu grau de conectividade e abrigam grupos organizacionais altamente distintos (HEINO et al., 2015). Em regiões tropicais e subtropicais os ambientes aquáticos lênticos são numerosos e possuem pequena profundidade e grandes regiões litorâneas, o que favorece o estabelecimento de macrófitas aquáticas de diversos grupos funcionais (CHAMBERS et al., 2008; ESTEVES, 1998).

6. Área de estudo

No total, foram amostradas 60 áreas úmidas de água doce no Rio Grande do Sul durante o desenvolvimento da tese: 40 nas regiões fisiográficas Encosta do Sudeste (20) e Litoral (20);

e 20 no município de Rio Grande (Fig. 2). Os dados coletados nas 40 áreas úmidas das regiões fisiográficas compõem os capítulos um, dois e quatro da tese (Fig. 3, 4, 5 e 6), enquanto os dados coletados nas 20 áreas úmidas de Rio Grande compõem o capítulo três (Fig. 7 e 8).

As áreas úmidas foram amostradas em propriedades privadas com autorização prévia dos proprietários. Elas são pequenas (< 2 ha; média, 0,58 ha) e rasas (< 1,5 m de profundidade). As 40 primeiras foram distribuídas a pelo menos 10 km uma da outra, e as 20 de Rio Grande foram amostradas dentro de uma área de 275 km² para evitar potenciais influências do gradiente climático ou espacial sobre a riqueza e composição das assembleias de macrófitas aquáticas. De acordo com Junk et al. (2014), elas podem ser classificadas como áreas úmidas costeiras com flutuações no nível de água separadas do mar, lagos temporários e lagoas. No capítulo três elas foram classificadas em intermitentes (10) e permanentes (10). As áreas úmidas permanentes são permanentemente inundadas, e as intermitentes são inundadas em pelo menos alguns períodos do ano, secando principalmente na estação seca/verão austral (MALTCHIK et al., 2004). O clima na região de estudo é subtropical úmido.

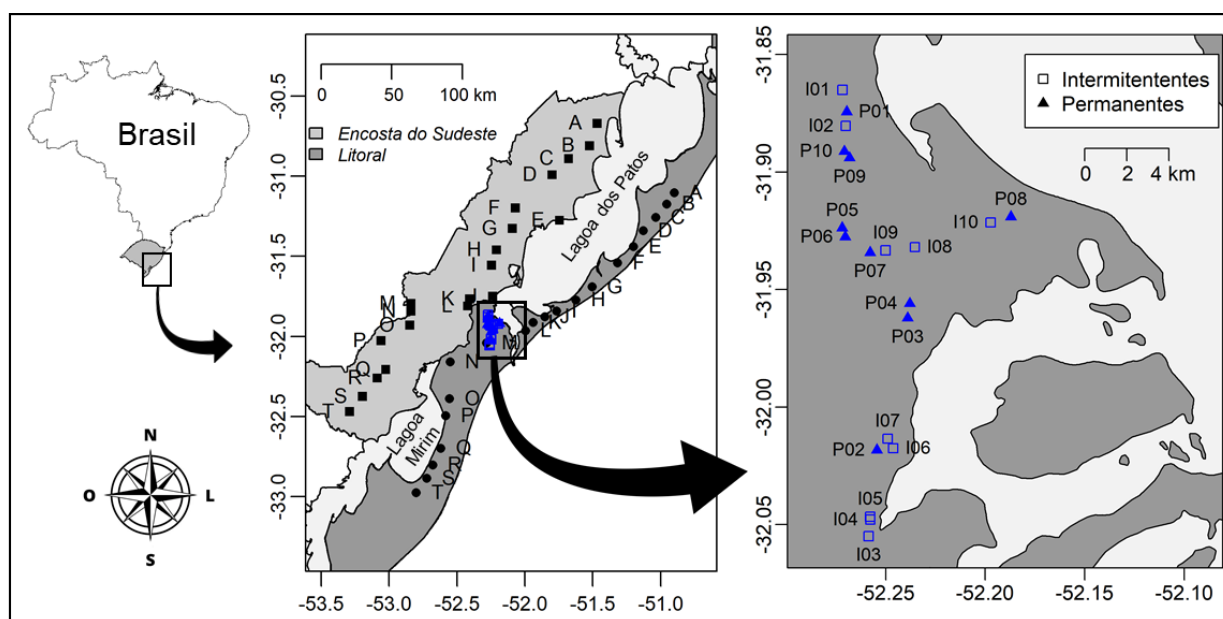


Figura 2. Áreas úmidas amostradas no desenvolvimento da tese.

As regiões fisiográficas Encosta do Sudeste e Litoral encontram-se ao redor de duas grandes lagoas litorâneas (Lagoa dos Patos e Mirim), onde a Encosta do Sudeste localiza-se a oeste e o Litoral a leste dessas lagoas. A Encosta do Sudeste apresenta uma faixa de sedimentos arenosos e aluviões holocênicos que provavelmente foram formados entre os períodos

quaternário superior e terciário (~2,5 mya), enquanto o Litoral foi formado por um sistema deposicional laguna-barreira nos últimos 0,4 mya (VILLWOCK; TOMAZELLI, 2007). Embora tenham formações geológicas distintas, essas regiões possuem tamanhos semelhantes: a Encosta do Sudeste ocupa uma área de 14.981 km² e o Litoral 14.905 km², segundo Fortes (1959). O solo de Encosta do Sudeste é em sua maioria plano e a altitude geralmente permanece abaixo de 30 m, mas também apresenta esporões rochosos que chegam a 200 m (BRENA, 2002). O solo do Litoral é plano a ligeiramente ondulado e a altitude é baixa (de 5 a 10 m) (BRENA, 2002).

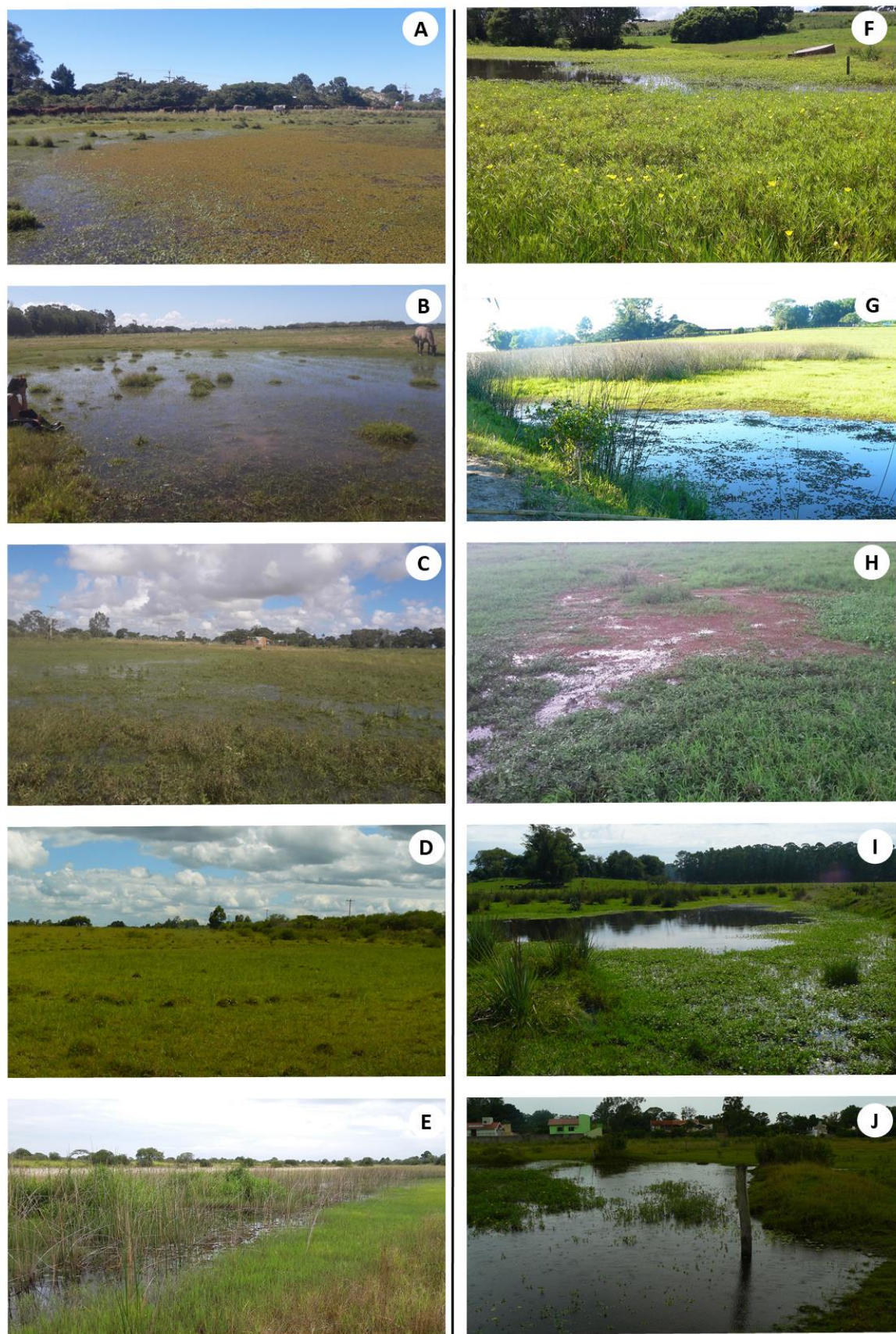


Figura 3. Fotografias das áreas úmidas amostradas na Encosta do Sudeste (A-J).

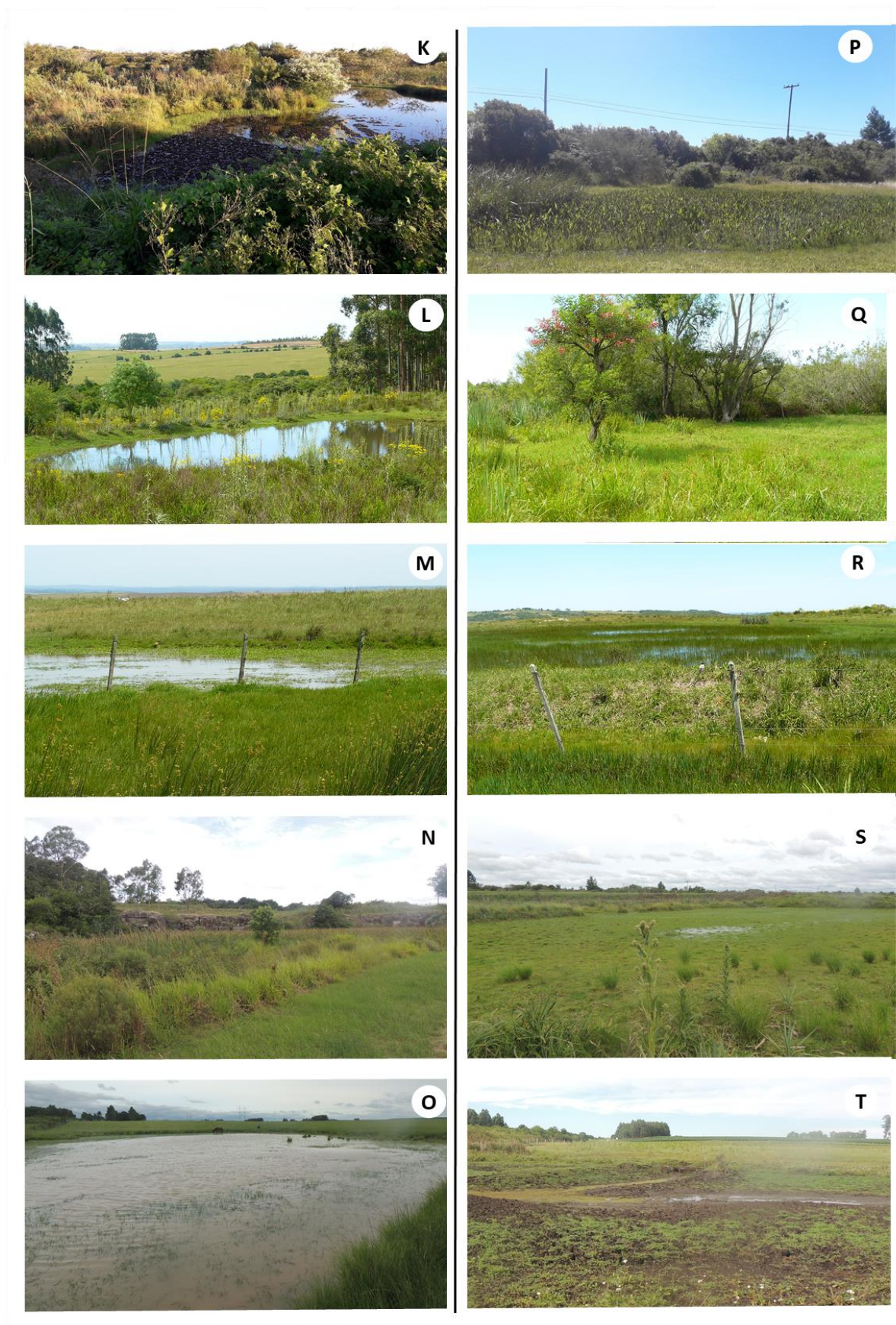


Figura 4. Fotografias das áreas úmidas amostradas na Encosta do Sudeste (K-T).

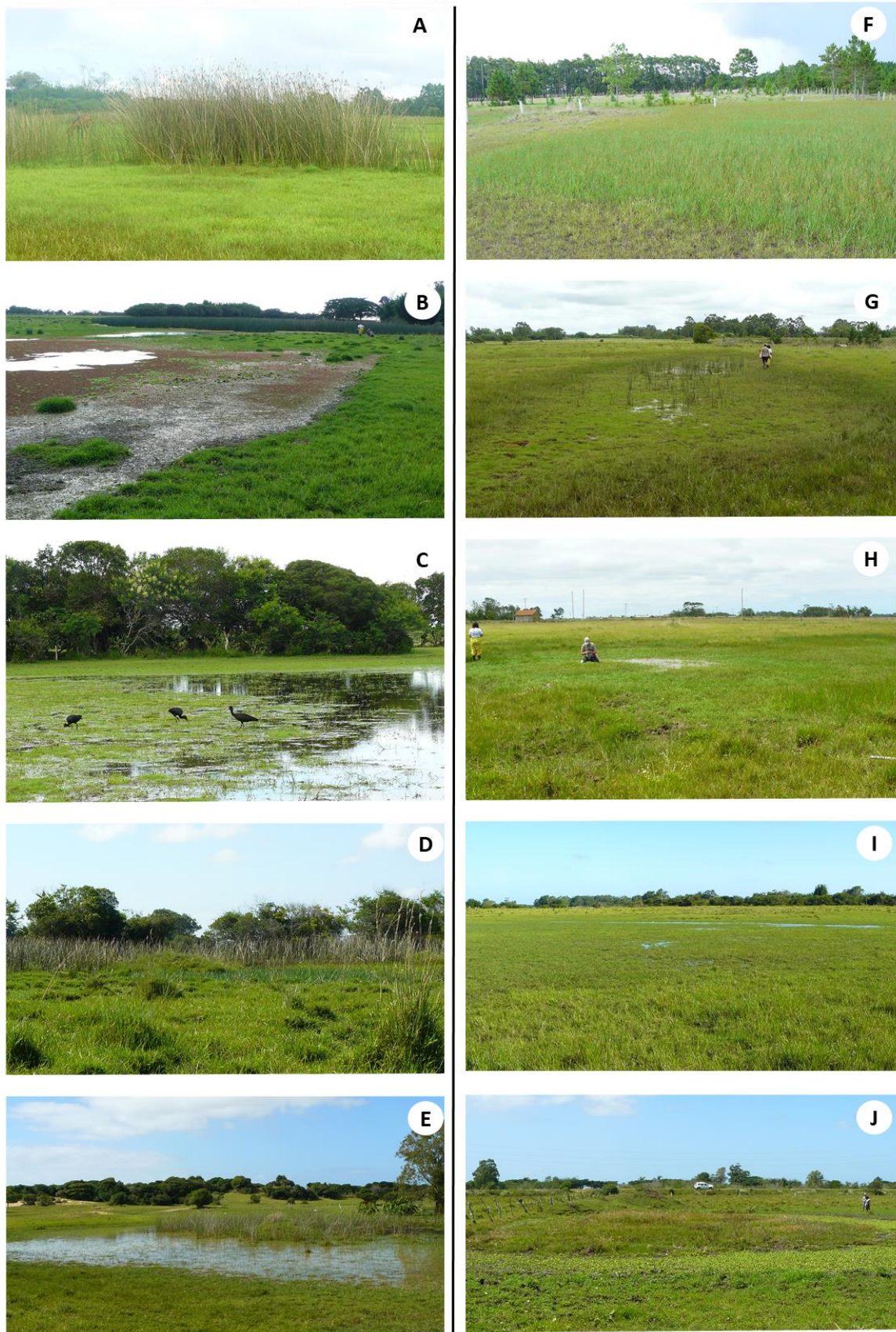


Figura 5. Fotografias das áreas úmidas amostradas no Litoral (A-J).

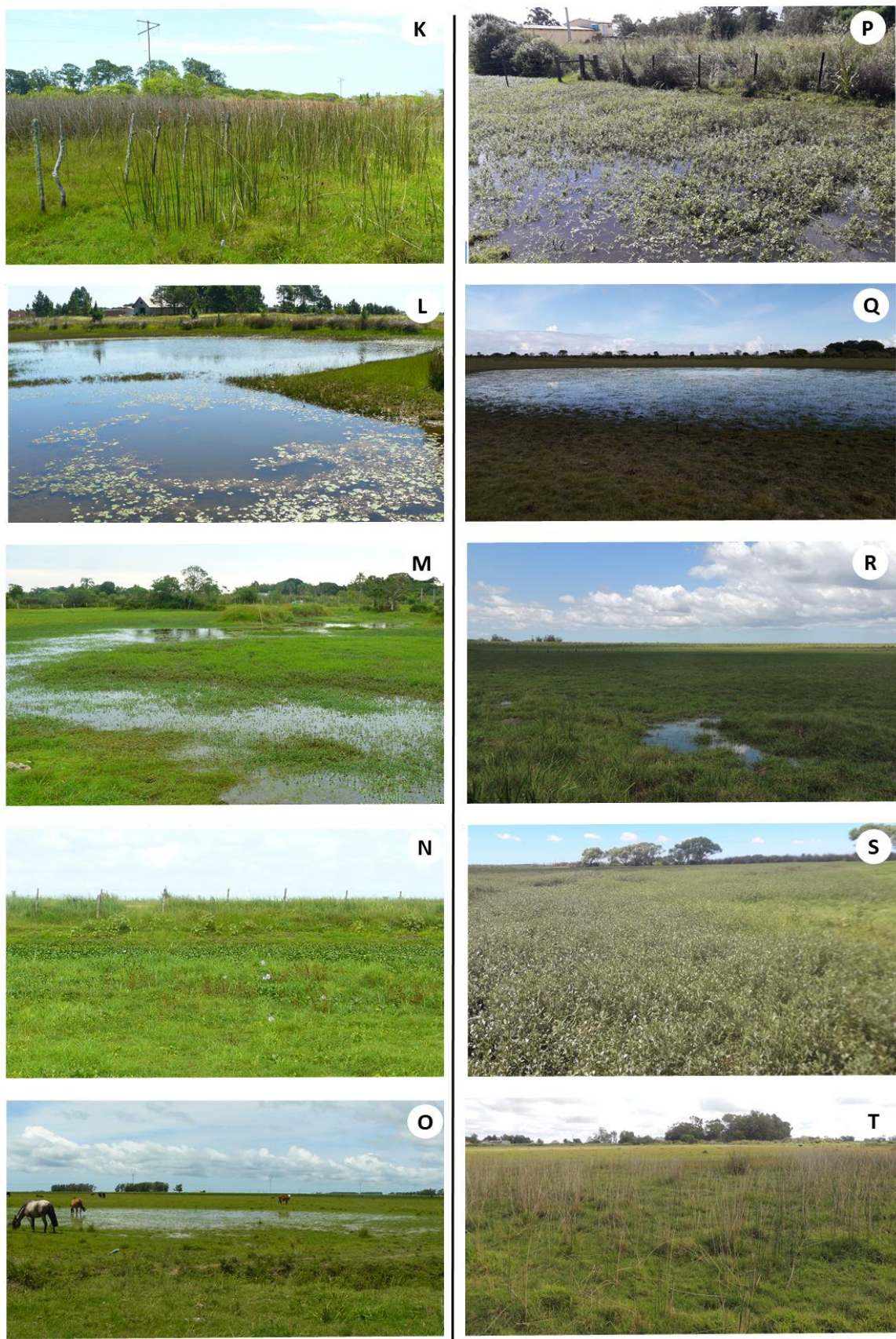


Figura 6. Fotografias das áreas úmidas amostradas no Litoral (K-T).



Figura 7. Fotografias das áreas úmidas intermitentes amostradas no município de Rio Grande.



Figura 8. Fotografias das áreas úmidas permanentes amostradas no município de Rio Grande.

JUSTIFICATIVA

Considerando a complexidade no entendimento dos fatores influentes na riqueza e composição das assembleias de macrófitas aquáticas, avaliar a contribuição dos fatores paisagísticos, espaciais, ambientais locais e interações biológicas locais torna-se importante. Dessa forma:

- Avaliar a influência das características da paisagem (quantidade de habitats, isolamento e uso da terra) juntamente com a fisiografia e fatores climáticos sobre riqueza e composição de macrófitas e formas de vida pode auxiliar no entendimento de características que atuam a nível regional em áreas úmidas subtropicais;

- Avaliar a importância de fatores espaciais e ambientais em metacomunidades de macrófitas aquáticas e seus diferentes grupos funcionais em diferentes escalas espaciais pode contribuir para o entendimento do papel da dispersão e de nicho ambiental nessas comunidades de áreas úmidas subtropicais;

- Avaliar a sobreposição espacial de macrófitas aquáticas em nível de assembleias pode auxiliar no entendimento de mecanismos de competição ou facilitação na estrutura das assembleias de macrófitas aquáticas em áreas úmidas.

OBJETIVOS

O objetivo geral da tese é avaliar os fatores determinantes para a riqueza e composição das assembleias de macrófitas aquáticas em áreas úmidas rasas do sul do Brasil.

Os objetivos específicos são:

- 1) Verificar como fatores da paisagem (quantidade de habitats, isolamento e uso da terra), fisiografia (região fisiográfica), e fatores climáticos (temperatura e precipitação) influenciam a riqueza e composição de macrófitas aquáticas em áreas úmidas subtropicais, bem como, verificar se esses fatores influenciam diferentemente dos grupos funcionais (anfíbias, emergentes, flutuantes e submersas).

- 2) Determinar a contribuição relativa dos fatores espaciais e ambientais (locais, paisagem, climáticas e regionais) na estruturação das metacomunidades de macrófitas aquáticas de áreas úmidas do sul do Brasil; verificar se a contribuição relativa dos fatores espaciais e ambientais varia entre os grupos funcionais de macrófitas aquáticas (anfíbias, emergentes e hidrófitas); verificar se a contribuição desses fatores varia em diferentes escalas espaciais.

3) Avaliar o quanto da estrutura da assembleia de macrófitas aquáticas pode ser explicada localmente por interação biológica através de um índice de sobreposição de nicho espacial adaptado para macrófitas aquáticas.

4) Realizar um levantamento florístico de macrófitas aquáticas em áreas úmidas da Encosta do Sudeste e do Litoral do Rio Grande do Sul para contribuir com o conhecimento da diversidade deste grupo.

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CAPÍTULO 1 - Influences of Landscape and Climatic Factors on Aquatic Macrophyte Richness and Composition in Ponds

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1 **Influences of Landscape and Climatic Factors on Aquatic Macrophyte Richness and**
2 **Composition in ponds**

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Abstract

There has been no consensus reached regarding the influences of landscape and climatic factors on aquatic macrophyte assemblages and their functional groups in wetlands. This study aimed to verify: 1) if landscape factors (habitat amount, isolation and land use), physiography and climatic factors (temperature and precipitation) relate to richness and composition of aquatic macrophytes in subtropical ponds; 2) whether these factors relate to functional groups differently (amphibious, emergent, floating and submerged). To accomplish these aims, plants were collected from 40 ponds in two physiographic regions of differing geomorphological age. Landscape variables were measured using GIS tools and FRAGSTATS software. The climatic variables were obtained from the WorldClim database. Habitat amount was negatively related to total species richness, while isolation, agricultural area and precipitation were positively related to it, possibly due to second-order effects, e.g., interspecific interactions or trophic control. Physiography, pond size and diversity of landscape use were related to species composition. Number of patches and pond size were positively related to emergent and floating species, and isolation was negatively associated with the richness of amphibious species, likely due to their dispersal strategies. We conclude that physiography was related to the structure of some aquatic macrophyte functional groups of subtropical ponds.

Keywords: habitat amount; isolation; land use; functional groups; spatial scale.

1.Introduction

Understanding which factors determine the characteristics of biological communities is a central theme in community ecology (Vellend, 2010). Understanding how these factors are related to natural assemblages of freshwater environments is important in terms of conservation (e.g., biodiversity and ecological process). In addition, these environments promote many ecosystem services including drinking water, recreation and ecotourism, recharge of groundwater and flood control (Junk et al., 2014).

Studies conducted on the landscape-scale allow to assess the influence of the spatial configuration of landforms on ecological processes and features (Metzger, 2001; Turner, 1989), such as the composition and richness of species in local communities. At the landscape scale, some land use practices (e.g., urbanization and advancement of agriculture) decrease the amount of natural habitat and increase the isolation of patches and, consequently, decrease richness (Cousins et al., 2015), change species composition and increase the dissimilarity between the communities (Bornette et al., 1998; Rolon et al., 2012). With the advance of geotechnologies such as the use of satellite imagery, GIS tools and specific software it is possible to measure metrics at the landscape level (e.g., land uses, isolation and quantity of natural habitats in the landscape). With the aid of statistical models, it is possible to relate these characteristics with features of biological communities (e.g., species richness and composition). Several studies have used this approach to determine the influence of patch size, isolation (distances between neighboring patches), and land use on the structure of aquatic plants assemblage (e.g., Bosiacka and Pieńkowski 2012; Houlahan et al. 2006; Sun et al. 2018). However, these studies differed in how the determining factors affected these assemblages. Some of them attribute greater importance to patch size (Bosiacka and Pieńkowski, 2012; Houlahan et al., 2006) while for others land use is more influential (Sun et al., 2018).

Patch size and isolation are discussed as the main landscape factors influencing species richness, according to the landscape models and Theory of Island Biogeography (Fahrig, 2013; MacArthur and Wilson, 1967). Patch size usually present a positive relationship with species richness (Jeffries, 1988; Rolon et al., 2008), but this is not always the case (Rolon et al., 2012; Spiesman et

al., 2018). Increased isolation tends to make communities more dissimilar in terms of species composition and richness (Bornette et al., 1998; Bosiacka and Pieńkowski, 2012; Rolon et al., 2012), but it does not always affect the richness of aquatic macrophytes (Schöpke et al., 2019). However, the Habitat Amount hypothesis postulates that the effects of patch size and isolation in the landscape on species richness are predominantly the result of the sampling area effect, but other land use factors can also influence species richness (Fahrig, 2013). Urban and agricultural land use generally decreases the richness of aquatic macrophytes (Akasaka et al., 2010, 2018), but this relationship was not observed in some studies. For example, Quinn et al. (2011) observed that urban and agricultural land use were related to a greater richness of alien aquatic macrophytes while it did not affect the richness of native species. In addition, different types of land use can interfere on aquatic bodies by increasing the availability of nutrients in oligotrophic water bodies which, consequently, results in an alteration of water quality (Gorman et al., 2014). This alteration can homogenize species composition or increase species richness (Salgado et al., 2018).

Physiography, that is, the physical and geological formation of the landscapes, can also influence the richness and composition of biological communities (Alahuhta et al., 2011) since it influences extinction rates and colonization of species in patches. Physiography has been approached as a possible influential factor affecting the structure of aquatic macrophyte assemblages in rivers (Dong et al., 2016; Manolaki and Papastergiadou, 2016). However, physiography is often overlooked in studies addressing structuring factors and aquatic macrophyte assemblages of ponds. Climate can interfere with the structure of communities of aquatic environments on broader spatial scales (Toivanen et al., 2019) due to latitudinal gradients (Alahuhta et al., 2011). These factors can interfere with the distribution of species that are adaptable to a certain climatic condition. Therefore, physiography and climatic factors should also be included when evaluating the influences on aquatic macrophyte characteristics in ponds at landscape scale.

Aquatic macrophyte assemblages in wetlands are made up of species that present different physical relationship to the water and soil (e.g., hydrophytes, emergent and amphibious) (Cronk and Fennessy, 2001). These species have different strategies for the dispersal of vegetative propagules (e.g., water, wind, animals) (Boedeltje et al., 2003; Capers et al., 2010). The emergent and amphibious

species obtain nutrients from substrates, assimilate gases from the atmosphere, and disperse their diaspores, mainly, by wind (Soons, 2006). Hydrophytes have a higher level of dependence on water, since they are either submerged or float across the water column (submerged and floating, respectively). Hydrophytes disperse their diaspores mainly by hydrochory and are more sensitive to changes in water quality than emergent and amphibious species (Cronk and Fennessy, 2001). Although hydrochory can disperse plant propagules over long distances in rivers (Jansson et al., 2005), dispersal via wind can reach greater distances than water dispersion (Soons, 2006), mainly for wetland plants. Due to the different dispersal strategies, the ecological effects of landscape factors on aquatic macrophytes can differ among functional groups (Akasaka et al., 2010). Plants with a greater dispersive potential tend to be less sensitive to isolation than plants that are dispersed over shorter distances, because a greater dispersal potential can neutralize isolation effects (Schöpke et al., 2019). However, studies addressing the influence of landscape factors on aquatic macrophytes and their functional groups in ponds are still scarce (Akasaka et al., 2018; Bosiacka and Pieńkowski, 2012; Rolon et al., 2012) compared to studies investigating abiotic (Bornette and Puijalon, 2011; Fernández-Aláez et al., 2020; Rolon et al., 2008) and biotic factors (García-Girón et al., 2020; Silknetter et al., 2020). Therefore, the inclusion of functional groups of aquatic macrophytes in landscape approaches may be useful in the clarification of finer ecological patterns affecting groups in ponds (Trindade et al., 2018; Weiher et al., 2011).

Understanding the factors determining aquatic macrophyte assemblages at a landscape scale in subtropical regions is important, especially as there still is no consensus regarding the influence of these factors on assemblages of aquatic macrophytes. Furthermore, it is unclear how these factors influence the different functional groups of aquatic macrophytes of subtropical ponds. Therefore, the aims of this study were to verify: 1) how landscape factors (habitat amount, isolation and land use), physiography (physiographic region), and climatic factors (temperature and precipitation) are related to species richness and composition of aquatic macrophytes in subtropical ponds; 2) whether these factors differentially relate to the species richness and composition of functional groups of aquatic macrophytes (amphibious, emergent, floating and submerged).

We predict that: (I) aquatic macrophyte richness will be greater in ponds with larger habitat amount (pond size and landscape wetland area), lower in isolated ponds, and lower in ponds with greater agricultural and urban areas (Cousins et al., 2015); (II) the richness and composition of floating and submerged species will be related to higher habitat amount, lesser isolation, and to landscapes with lesser agricultural and urban areas, due to their lower dispersal potential and greater sensitivity to changes in water quality (Alahuhta et al., 2020; Cronk and Fennessy, 2001; Schöpke et al., 2019; Soons, 2006); and (III) the richness and composition of amphibious and emergent species will be mainly related to physiographic and climatic factors because these factors are proxies of colonization history and processes acting on broader scales (Heino et al., 2016).

2. Methods

2.1. Study Area

The study was carried out in spatially isolated ponds of two physiographic regions that represent distinct geological formations of southern Brazil (Fig. 1): *Encosta do Sudeste* (ES) and *Litoral* (L) (Fortes, 1959). The *Encosta do Sudeste* is mainly composed of a depositional system of alluvial fans that were probably formed between the late tertiary and early quaternary periods, ~2.5 mya (oldest). The *Encosta do Sudeste* covers an area of 14,981 km². This region, according to Brena (2002), presents a range of sandy sediments and holocenic alluvions along rivers. The ground is usually flat, and the altitude generally remains below 30 meters, but presents rocky spurs reaching up to 200 meters (Brena, 2002). Between rocky spurs are ponds formed by the accumulation of water.

The *Litoral* region was formed by lagoon-barrier depositional systems within the last 0.4 mya (most recent) (Villwock and Tomazelli, 2007). This region covers the Atlantic Coastal Plain and comprises an area of approximately 14,905 km² (Fortes, 1959). This physiographic region was geologically formed by sands moved and deposited by water and winds, which form poor soils that are partially saline, poorly consolidated, and permeable (Brena, 2002). The *Litoral* region contains a

high level of diversity and abundant wetlands. The ground is flat to slightly wavy and the altitude is low (from 5 to 10 meters) (Brena, 2002).

The study area in the two physiographic regions correspond to the same latitudinal segment of the west and east boundaries of *Lagoa dos Patos* (largest lagoon in South America) and *Lagoa Mirim* (the largest lake in Brazil). These regions have a high number of wetlands compared to other regions of southern Brazil (Maltchik, 2003). The climate is moist subtropical and the mean annual temperature varies between 16 and 20°C (Nimer, 1977). Mean annual rainfall ranges from 1,000 to 1,500 mm (Villwock and Tomazelli, 2007).

2.2. Survey of aquatic macrophytes

To select ponds for aquatic macrophytes sampling, we first surveyed the geographic locations of ponds in the study regions using GPS. Then we plotted the approximate coordinates of the ponds in Google Earth to visualize their distribution and approximate sizes. Finally, we randomly selected 40 ponds that were small (<2 ha; average, 0.58 ha), shallow (<1.5 m depth) and were distributed at least 10 km from each other.

Aquatic macrophytes were sampled from the 40 ponds between January 2017 and April 2017; 20 in *Encosta do Sudeste* and 20 in *Litoral* regions (Fig. 1). Non-linear transects based on time (3 min) were established traversing the ponds by foot. The transects followed the variation of the pond structure (along the border of the pond and from the border towards the center). To achieve sample sufficiency, sampling was exhaustive, and we finished sampling after two transects (6 min) without a new species occurrence (Rolon et al., 2012). Therefore, the number of transects varied according to the size and heterogeneity of the ponds and ranged from 5 to 34 transects (mean 11.7).

Species identification of aquatic macrophytes was carried out in the field and in the laboratory using a Leica EZ4D stereomicroscope. The taxonomic framework followed the procedures outlined in APG IV (2016) for angiosperms and Smith et al. (2006) for pteridophytes. The scientific names of the species are in accordance with Flora of Brazil (Forzza, 2020). We used the broad concept of aquatic macrophytes, which includes hydrophytic (submerged and floating),

and emergent and amphibious species (herbaceous and woody) (Cronk and Fennessy, 2001). To define functional groups, aquatic plants were classified as amphibious, emergent, floating or submerged. We included in the emergent category the epiphytes, which live on other aquatic plants without rooting to the substrate, and lianas, which are rooted in the substrate, but require support from other plants. The expression "all macrophytes" refers to the set of all aquatic macrophytes sampled without distinguishing them into functional groups.

2.3. Survey of landscape variables – GIS analysis

Landscape variables were obtained using a supervised digital classification technique developed for this study from satellite images. The satellite images were captured by the Operational Land Imager (OLI) sensor, with 30 meters of spatial resolution, aboard the Landsat-8 satellite. These images were made freely available by the National Institute for Space Research (INPE). We selected four scenes with a smaller proportion of clouds and for the same period of survey of aquatic macrophytes. Their orbits/points were 221/081, 221/082, 221/083 and 222/082; the first three images were taken on 12/12/2016 and the final one was taken on 01/20/2017. They were redesigned for the projected coordinate systems UTM Zone 22 South and Datum WGS84. Windows of 10 km² centered in each pond were cut for each band, totaling 40 windows (landscapes). From these cut-outs, falsecolor compositions (432, 654, 564, and 543) were generated for sampling of regions of interest (ROIs) of the classes of land use and cover. The classes used in the interpretation key of the supervised classification included 1) wetlands, 2) agricultural land (not further distinguished), 3) bare soil (natural exposed soil), 4) native grassland, and 5) native forest. The wetland class included continental aquatic bodies such as streams, marshes, and ponds. Three classes were included after classification to act as masked pixels to be excluded in the supervised classification: Sea, Urban, and Lacustrine Systems. The class Sea included areas of sea in some images. The class Urban included urban perimeters and highways. The Lacustrine Systems class included two large coastal lagoon/lakes (*Lagoa dos Patos* and *Lagoa Mirim*) that have different characteristics from those of small aquatic bodies.

Supervised Digital classification was accomplished using the MAXVER routine (Maximum likelihood algorithm) within Idrisi Taiga software (Eastman, 2009). Tests were performed and, due to the size of the ponds, the threshold for rejection was set to zero, so that all pixels could be classified. Masking layers were digitized for the classes "Sea", "Urban", and "Lacustrine Systems". These masking layers were converted into raster format with the same size and spatial resolution of the windows. Then, we overlapped the images by multiplying the classified images (the products of the MAXVER routine) by the rasterized mask images. The final images (Anexo 4) were reclassified attributing the class values to their respective numbers. Other techniques of post classification were not applied because of the small size of ponds. We contrasted field observations of the ponds and the images available at Google Earth software (which have higher spatial resolution) with the classifications to validate them. In addition, an Overall Accuracy Index was applied to verify the percentage classification accuracy (Congalton, 1991). The file with the data locations of the 40 sampled ponds (the same ponds of the aquatic macrophytes survey) was used to verify if the classification could detect the wetland class in these sites effectively. The calculation consisted of dividing the number of ponds correctly classified as wetlands by the total number of ponds sampled (40) (Congalton, 1991). The calculated value of the index was 62.5%, which was considered good when the spatial resolution of the images used (30 m), the study scale (extension of approximately 300 km), and the size of the ponds (small; average, 0.58 ha) were considered (Baschuk et al., 2012). The classified images generated were exported in tiff format to FRAGSTATS software (McGarigal et al., 2012) and the "Rfrag" package from R software (McGarigal, 2014) was used to calculate metrics.

We considered as *Habitat amount* metrics: the area of the wetland class within each landscape, the Landscape Wetland Area (LWA); the Number of Patches in the wetland class (NP); the proportion (%) of the largest patch of the wetland class relative to the total size of the class, Largest Patch Index (LPI); the Shape Index (SHAPE), which represents the complexity of patch shapes within the landscape; and the Pond Size (PS). Although the Pond Size is a local factor and not a landscape one, it represents the local portion of the habitat colonizable by aquatic macrophytes. *Isolation* metrics consisted of the Mean of the Euclidean Distances from the Nearest Neighbor

(ENN_MN) among wetland patches, and the Proximity Index (PROX), which consisted of a radius of 500 meters of search. *Land use* metrics corresponded to the Size of the Agricultural Class (Agr), the Size of the Urban Class (Urb), and the Landscape Shannon's Diversity Index (SHDI), which represented the diversity of the use and type of landscape cover.

2.4. Survey of physiographic and climatic variables

The physiographic regions *Encosta do Sudeste* (code ES) and *Litoral* (code L) were treated as categories of the qualitative binary variable *Physiography* in the analyses. *Climatic variables* consisted of the Mean Annual Temperature (T) and Annual Precipitation (P) of the central coordinate of each sample pond. These variables were obtained through the "getData" function of the "raster" package in R (Hijmans, 2016) from the WorldClim database (Fick and Hijmans, 2017). The climatic variables database downloaded has 2.5 arc minutes of spatial resolution.

2.5. Data analysis

The relationships between environmental variables were tested using Spearman's correlations. Relationships for which the correlation coefficients were below -0.7 or above 0.7, and for which *p*-values were below 0.05, were considered as correlated variables. The influence of the physiography, landscape (habitat amount, isolation, land use) and climate-related (mean annual temperature and annual precipitation) variables on the richness of aquatic macrophytes was evaluated by Linear Regression Models with stepwise selection through the "stepAIC" function of the "MASS" package in R (Venables and Ripley, 2002). These models were performed separately for all macrophytes and for each one of the functional groups examined (amphibious, emergent, floating and submerged).

We analyzed the data of the presence and absence of species using Principal Coordinate Analysis (PCoA). A PCoA was performed for all macrophytes and for each of the functional groups, using the Jaccard coefficient to generate distance matrices among all sampled

sites (Venables and Ripley, 2002). To relate physiography, landscape and climatic variables with the species composition of ponds, these variables were adjusted to ordinations using the “envfit” function of the “vegan” package in the R environment (Oksanen et al., 2016). These variables were also adjusted to the ordinations of each functional group to verify whether the relationship between species composition and environmental variables differed. These models were performed with 4999 permutations to get a more precise estimation of p -value.

In addition, we performed a Permutational Analysis of Multivariate Variance (PERMANOVA) test to assess whether physiography influences the species composition of all macrophytes and each one of the functional groups. We also used the Jaccard coefficient to generate distance matrices among all sampled sites (Venables and Ripley, 2002). The PERMANOVA tests were performed using the “adonis” function of the “vegan” package in the R environment (Oksanen et al., 2016). We considered p -values < 0.05 as statistically significant.

3.Results

A total of 243 species of aquatic macrophytes were identified in the ponds studied (Table S1), of which 185 were amphibious, 41 emergent, 14 floating and 3 were submerged (Table 1). A total of 221 species were recorded in the ponds of *Encosta do Sudeste* (mean = 40.7; standard deviation = 13.3) and 138 were found in *Litoral* (mean = 28.8; standard deviation = 8.7) (Table 1). Units, means, and standard deviations of landscape and climatic variables are summarized in Table 2.

LWA was positively correlated to the LPI ($r = 0.81$; $p < 0.001$) and to PROX ($r = 0.72$; $p < 0.001$) (Table 3). LPI was positively correlated to PROX ($r = 0.81$; $p < 0.001$). The Size of Agricultural Class was negatively related to SHDI ($r = -0.95$; $p < 0.001$). Mean Annual Temperature was positively related to Annual Precipitation ($r = 0.72$; $p < 0.001$) (Table 3).

The richness of all macrophytes was influenced negatively by LWA and positively by PROX, Size of Agricultural Class, and the Annual Precipitation ($R^2_{adj} = 0.426$; $F = 5.13$; $p < 0.001$) (Table 4). Regarding the composition of all macrophytes, the first two axes of the PCoA explained 19% of the variation and the *ordination axes* showed a subtle distinction, represented by Jaccard

dissimilarity, between the physiographic regions. The composition of all macrophytes was related to Physiography, PS and SHDI (Fig. 2a). The results of PERMANOVA showed differences between species composition of the physiographic regions for all macrophytes ($F = 2.68$; $p = 0.001$).

The richness of amphibious species was influenced negatively by ENN_MN and the Mean Annual Temperature, and it was influenced positively by Annual Precipitation ($R^2_{adj} = 0.4871$; $F = 6.293$; $p < 0.001$). The emergent species richness was influenced negatively by LWA, and it was influenced positively by NP, PROX, Size of Agricultural Class and Size of Urban Class ($R^2_{adj} = 0.331$; $F = 3.093$; $p = 0.009$) (Table 4). The floating species richness was influenced positively by NP, PS, ENN_MN and Mean Annual Temperature, and it was influenced negatively by Annual Precipitation ($R^2_{adj} = 0.423$; $F = 3.379$; $p = 0.015$) (Table 4). The richness of submerged species was influenced positively by Physiography (Litoral), LWA and Size of Urban Class, and it was influenced negatively by LPI and Shape Index ($R^2_{adj} = 0.849$; $F = 14.09$; $p < 0.001$) (Table 4).

The first two axes of the PCoA explained 20% of the variation in the composition of amphibious species, 24% of the variation in the composition of emergent, 42% of the variation in the composition of floating species and 1% of the variation in the composition of submerged species. The two axes of ordinations showed a subtle distinction, represented by Jaccard dissimilarity, between the physiographic regions regarding the amphibious and emergent species. The composition of amphibious species was related to SHDI (Fig. 2b). The composition of emergent species was related to Physiography (Fig. 2c). No variable considered in this study was related to the composition of floating species (Fig. 2d), and only the Size of Urban Class was related to the composition of the three submerged species.

The results of PERMANOVA showed differences between species composition in the physiographic regions for the amphibious ($F = 3.05$; $p = 0.001$) and emergent species ($F = 2.16$; $p = 0.004$). There were no differences of floating ($F = 0.53$; $p = 0.835$) or submerged species composition ($F = 2.28$; $p = 0.181$) between the two physiographic regions.

4. Discussion

The richness of aquatic macrophytes found in this study is representative of southern Brazil, considering that Irgang and Gastal Jr (1996) observed a richness of 331 species in this studied area. Other studies conducted in these regions observed lower levels of species richness. However, we should consider the decreased spatial extension and the lesser degree of wetland diversity sampled in other studies (Rolon et al., 2008, 2012; Trindade et al., 2018). In our study, aquatic macrophytes are predominantly amphibious (76% of the species) and only 7% of the species are hydrophytes. This is likely due to characteristic of the ponds analyzed, which are small, shallow, and mostly intermittent. These features favor the establishment of amphibious species and limit the occurrence of floating and submerged species. Our results agree with previous studies conducted in tropical and subtropical wetlands of Brazil (Boschilia et al., 2008; Bove et al., 2003; Cervi et al., 2009; Pott et al., 2011; Rolon et al., 2010), which also found greater diversity of amphibious and emergent species. It should be noted that our conclusions about the submerged group are limited due to the low representativeness of this group in our study (only three taxa: *Cabomba caroliniana*, *Utricularia gibba*, and *Utricularia* sp.).

Physiography played an important role in structuring the composition of all macrophytes, and the amphibious and emergent species in the ponds studied. This partially corroborated our third prediction, since Physiography did not influence the richness of these groups. We considered Physiography as a proxy variable for the different geological formations and ages of the studied regions. Since biological communities are structured by geological formations (O'Hare et al., 2012; Swanson et al., 1988), different geomorphological features and ages of the studied regions (Encosta do Sudeste ~2.5 mya; Litoral ~0.4 mya) could be one of the explanatory elements for the differences in species composition between the physiographic regions for these groups. Some authors state that higher geomorphological age can indirectly provide greater species richness (Borges and Brown, 1999; Zaitsev et al., 2013). We did not observe it for the richness of assemblages of aquatic macrophytes, although Physiography did influence the species composition. Studies addressing the influence of physiography on aquatic macrophytes of small lentic ecosystems are still scarce (Liu et al., 2016). Therefore, it is difficult to assess and compare the importance of physiography in these ecosystems. However, our results can contribute to future investigations on this issue.

Different variables used to quantify habitat amount in the landscape had different effects on species richness. There were positive relationships between Landscape Wetland Area and the richness of submerged species; between Number of Patches and emergent and floating species richness; and between Pond Size and floating species richness. Some studies have revealed that increases in habitat amount increase species richness (Bolpagni et al., 2018; Fahrig, 2013; Ferenc et al., 2016; Matias et al., 2010). Nevertheless, there are distinct relationships between habitat amount and the functional group of aquatic macrophytes in question. At the landscape level, distinct relationships may occur because of different dispersal strategies of different functional groups. However, the Landscape Wetland Area negatively influenced the richness of all macrophytes and emergent species, and the Largest Patch Index negatively influenced submerged richness. Decreases in habitat amount and higher isolation can increase species richness, which results from second-order influences, such as interspecific interactions (for example, competition and facilitation) and trophic control (for example, absence of fish species) (García-Girón et al., 2020; McCullough et al., 2019; Scheffer et al., 2006). Some studies have shown that in small, shallow, and hydrologically isolated waterbodies fish are commonly absent (McCullough et al., 2019), which results in greater diversity of macroinvertebrates (McDevitt-Galles and Johnson, 2018), zooplankton (Kuczyńska-Kippen and Pronin, 2018), amphibians (Holbrook and Dorn, 2016), and plants (Van Geest et al., 2003). A low abundance of fish may reflect a high diversity of aquatic macrophytes due to low herbivory (Vejříková et al., 2018).

Isolation was positively related to all macrophytes richness, emergent and floating macrophytes (partially refuting our first and second hypotheses), and it was negatively associated with amphibious species richness. This also reveals that there are distinct effects of isolation that influence the richness of the aquatic macrophyte functional groups. According to the Theory of Island Biogeography, the greater the isolation the lesser the species richness (MacArthur and Wilson, 1967), and this is a common expectation when the configuration of the landscape influences biodiversity (Spiesman et al., 2018). However, the positive relationship between isolation and species richness has been reported when habitat amount was considered (Fahrig, 2017).

The statistical positive effect of isolation on species richness may result from a biased relationship since geographical isolation does not necessarily correspond to functional connectivity of

patches. Connectivity is defined as “the degree to which the landscape facilitates or impedes movement among resource patches” (Taylor et al., 1993). Two types of connectivity can be distinguished in landscape ecology: structural and functional connectivity. Structural connectivity concerns the structural/physical configuration of the patches in the landscape, and functional connectivity includes, besides the structuring of the patches, the behavioral responses of the organisms that allow the dispersion among the patches (Goodwin, 2003). In our study, the positive relationship between the isolation metric (which reflects the landscape structure) and the richness of all macrophytes and the richness of emergent and floating species, may indicate the existence of functional connectivity even if there is no structural connectivity in the landscape.

Though dispersal is important or limiting for some groups of aquatic macrophytes (Alahuhta et al., 2020; Padial et al., 2014), our results suggest that floating and emergent species could disperse among spatially isolated patches, at least at the spatial scale evaluated. Floating macrophytes usually reproduce asexually (Boedeltje et al., 2003). Asexual fragments detached from the body of aquatic macrophytes can reach long distances (Philbrick and Les, 1996), especially in hydrologically connected environments (Li, 2014). The dispersal of asexual fragments in spatially isolated environments, such as ponds, can occur through anemochory, anthropolochory (Philbrick and Les, 1996), endozoochory (Silva et al., 2020) or epizoochory, mainly through birds (Coughlan et al., 2017). In addition, the effects of interspecific interactions, such as facilitation (e.g., birds and floating propagules) and/or competition, on species richness may outweigh the effects of isolation (Spiesman et al., 2018). However, the effects of biotic interactions on structuring functional groups of aquatic macrophytes is commonly neglected (García-Girón et al., 2020).

The expansion of agricultural and urban areas impacts natural communities in several ways (Adhikari and Hansen, 2018), which include the discharge of contaminants, water drainage and eutrophication (Akasaka et al., 2018). This expansion can also interfere with other landscape variables, increasing isolation and fragmentation between communities (Vukov et al., 2018). Agricultural Class was related positively (and tenuously) to the richness of all macrophytes and emergent species (contradicting our first and second hypotheses partially). A large part of the areas classified as Agricultural areas is used for irrigated rice production in both regions. However, it was not possible to

further detail the types of agricultural systems in the classification because is difficult to distinguish, by means of spectral images, irrigated rice areas in the post-harvest period from other types of agriculture (without surface water). This type of cultivation may serve as a complementary habitat for aquatic macrophytes (Linke et al., 2014; Rolon et al., 2017). Therefore, the Agricultural Class may not have had a negative effect on the richness of all macrophytes and emergent species due to the proportion of the active irrigated rice fields present in this class.

Urban areas influenced the richness of emergent species, as well as being related to the three submerged species. The landscapes with greater urbanization were associated with the presence of *Cabomba caroliniana*, while less urbanized regions were associated with *Utricularia* species (*Utricularia gibba* and *Utricularia* sp.). *Cabomba caroliniana* tolerates eutrophic environments (Huang et al., 2017), while *Utricularia* species prefer oligotrophic environments (Bini et al., 1999). Perhaps, submerged aquatic macrophytes suffer more from the presence of urban and agricultural areas than other groups. However, we must take into consideration that the diversity of this group, in the studied region, was low in general. Hence generalizations must be made with caution.

With regard to climate variables, Mean Annual Temperature was negatively correlated with the richness of amphibious species and was correlated positively to floating species richness. Conversely, Annual Precipitation was positively related to all macrophytes and amphibious richness, and it was negatively related to the floating species richness. Therefore, climatic factors also influenced floating species richness and not just amphibious species as predicted. Trindade et al. (2018) used temperature and precipitation variables, such as evapotranspiration, as proxies of the hydroperiod to evaluate the drivers of the structuration of macrophyte functional groups. The hydrological regime is extremely important for the structuring of the aquatic macrophyte assemblages in wetlands (Casanova and Brock, 2000). Although temperature is expected to act in structuring aquatic macrophyte assemblages at broader scales (Alahuhta et al., 2018; Bolpagni et al., 2018), it was also important at the regional scale of our study for more than one functional group.

5. Conclusions

We conclude that habitat amount and isolation influenced the richness of aquatic macrophytes assemblages in the opposite way than we had expected. Landscape Wetland Area was negatively related to the richness of all macrophytes and emergent species. Isolation was positively related to all macrophytes, and emergent and floating species richness. Negative relationships between habitat amount and macrophyte richness, as well as positive relationships between isolation and species richness, may have been due to second-order effects on richness. For example, they may have occurred because of interspecific interactions. However, other habitat amount variables (Number of Patches and Pond Size) were positively related to emergent and floating species, and isolation was negatively associated with the richness of amphibious species. Therefore, we observed that different functional groups were affected by landscape factors differently, and this may be related to their dispersal strategies.

In addition, physiography was related to the structure of some functional groups of aquatic macrophyte assemblages of subtropical ponds. One of the elements that may have contributed to this relationship is the geomorphological age of the studied physiographic regions. Besides that, landscape factors in terms of agriculture had no negative effect on species richness of all macrophytes and emergent species, contrary to our predictions. Although we have not distinguished the types of agriculture in the classification of satellite images, this lack of a negative effect of agriculture on species richness may have been due to the proportion of irrigated rice present within the agricultural class, as irrigated rice fields may act as a complementary habitat for aquatic macrophytes.

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695 0

696 **Table 1.** Species richness and respective percentages of all macrophytes and functional groups of
697 aquatic macrophytes sampled from subtropical ponds. ES = *Encosta do Sudeste* and L = *Litoral*.

Attribute	All macrophytes	Amphibious	Emergent	Floating	Submerged
Total richness	243	185 (76.1%)	41 (16.9%)	14 (5.8%)	3 (1.2%)
ES richness	221	169 (76.5%)	35 (15.8%)	14 (6.3%)	3 (1.4%)
L richness	138	95 (68.8%)	28 (20.3%)	13 (9.4%)	2 (1.4%)

Table 2. Mean and standard deviation of operational variables^e (categorized in theoretical variables) of 40 subtropical pond landscapes of two physiographic regions

Theoretical variables	Operational variables	<i>Encosta do Sudeste</i>		<i>Litoral</i>	
		Mean	Standard deviation	Mean	Standard deviation
Habitat amount	LWA (ha)	991.3	1072.37	1458.45	1009.98
	NP	517.2	423.64	740.75	360.24
	LPI (%)	3.59	7.32	7.41	7.37
	SHAPE	1.21	0.1	1.21	0.06
	PS (ha)	0.61	0.51	0.56	0.5
Isolation	ENN_MN (m)	172.76	114.75	109.23	24.91
	PROX	96.33	211.48	95.94	100.83
Land use	Agr (ha)	6888.89	2380	5502.78	1835.54
	Urb (ha)	286.87	458.51	86.83	85.96
	SHDI (≥ 0)	0.87	0.42	1.21	0.32
Climatic	T (°C)	18.2	0.48	18.1	0.4
	P (mm)	1338.25	52.59	1280.6	60.62

^e LWA = Landscape Wetland Area; NP = Number of Patches; LPI = Largest Patch Index; SHAPE = Shape Index; PS = Pond Size; ENN_MN = Mean of the Euclidean Nearest Neighbor; PROX = Proximity Index (radius 500 m); Agr = Size of Agricultural Class; Urb = Size of Urban Class; SHDI = Landscape Shannon's Diversity Index; T = Mean Annual Temperature; P = Annual Precipitation

Table 3. Matrix of Spearman's correlations between environmental variables^f of subtropical ponds. The correlation coefficients are displayed in bold and the *p*-values in regular font. Correlation coefficients below -0.7 and above 0.7 are underlined. Values of *p* < 0.05 are marked with asterisk (*).

	LWA	NP	LPI	SHAPE	PS	ENN_MN	PROX	Agr	Urb	SHDI	T	P
LWA	1.00	0.00*	0.00*	0.03*	0.18	0.00*	0.00*	0.00*	0.68	0.00*	0.74	0.68
NP	0.47	1.00	0.16	0.31	0.55	0.00*	0.28	0.05	0.73	0.01*	0.04*	0.03*
LPI	<u>0.81</u>	0.23	1.00	0.94	0.55	0.04*	0.00*	0.00*	0.91	0.03*	0.26	0.44
SHAPE	0.35	0.16	0.01	1.00	0.42	0.05	0.34	0.12	0.87	0.23	0.11	0.48
PS	0.22	-0.10	0.10	0.13	1.00	0.40	0.11	0.09	0.82	0.17	0.14	0.28
ENN_MN	-0.51	-0.60	-0.33	-0.32	-0.14	1.00	0.06	0.00*	0.89	0.00*	0.03*	0.61
PROX	<u>0.72</u>	0.17	<u>0.81</u>	0.15	0.26	-0.30	1.00	0.00*	0.63	0.01*	0.84	0.74
Agr	-0.55	-0.31	-0.48	-0.25	-0.27	0.45	-0.57	1.00	0.24	0.00*	0.36	0.76
Urb	0.07	0.06	0.02	-0.03	0.04	-0.02	0.08	-0.19	1.00	0.19	0.65	0.18
SHDI	0.45	0.39	0.35	0.20	0.22	-0.46	0.40	<u>-0.95</u>	0.21	1.00	0.18	0.59
T	0.05	0.33	-0.18	0.26	0.24	-0.35	-0.03	-0.15	-0.07	0.22	1.00	0.00*
P	0.07	0.34	-0.13	0.11	0.17	-0.08	0.05	-0.05	0.22	0.09	<u>0.72</u>	1.00

^f LWA = Landscape Wetland Area; NP = Number of Patches; LPI = Largest Patch Index; SHAPE = Shape Index; PS = Pond Size; ENN_MN = Mean of the Euclidean Nearest Neighbor; PROX = Proximity Index (radius 500 m); Agr = Size of Agricultural Class; Urb = Size of Urban Class; SHDI = Landscape Shannon's Diversity Index; T = Mean Annual Temperature; P = Annual Precipitation

705 **Table 4.** Linear regression models with full stepwise selection among richness of all macrophytes and
 706 functional groups of aquatic macrophytes and statistically significant variables[§] ($p < 0.05$). * for $p <$
 707 0.05 , ** for $p < 0.01$, *** for $p < 0.001$.

Stepwise regression model	R ² adj	F	p-value
All macrophytes richness ~ - LWA* + PS - ENN_MN + PROX** + Agr* - T + p***	0.426	5.13	< 0.001
Amphibious richness ~ - Physiography (Litoral) - LWA - ENN_MN* + PROX + Agr - T* + P**	0.487	6.293	< 0.001
Emergent richness ~ + Physiography (Litoral) - LWA* + NP* + PS + PROX*** + Agr* + Urb* + T - P	0.331	3.093	0.009
Floating richness ~ - LWA + NP*** + PS** + ENN_MN** + PROX + Urb + T* - P**	0.423	3.379	0.015
Submerged richness ~ + Physiography (Litoral)* + LWA* + NP - LPI* - SHAPE* + PS + PROX + Urb*** - SHDI	0.849	14.09	< 0.001

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[§]LWA = Landscape Wetland Area; NP = Number of Patches; LPI = Largest Patch Index; SHAPE = Shape Index; PS = Pond Size; ENN_MN = Mean of the Euclidean Nearest Neighbor; PROX = Proximity Index (radius 500 m); Agr = Size of Agricultural Class; Urb = Size of Urban Class; SHDI = Landscape Shannon's Diversity Index; T = Mean Annual Temperature; P = Annual Precipitation

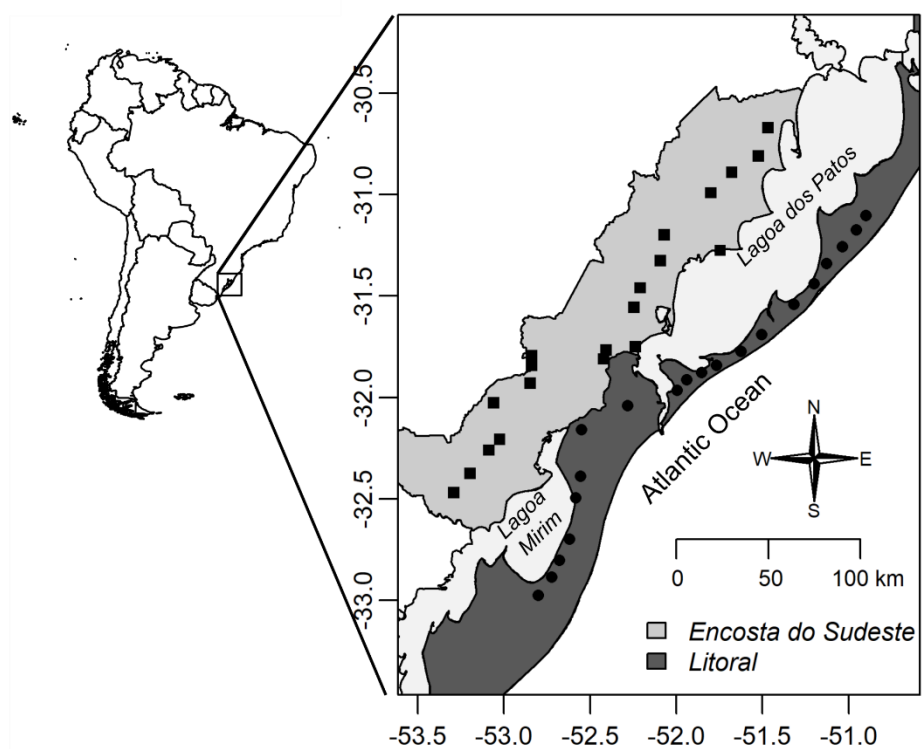


Figure 1. Ponds and the physiographic regions from *Encosta do Sudeste* (■) and *Litoral* (●), Southern Brazil, sampled in the study

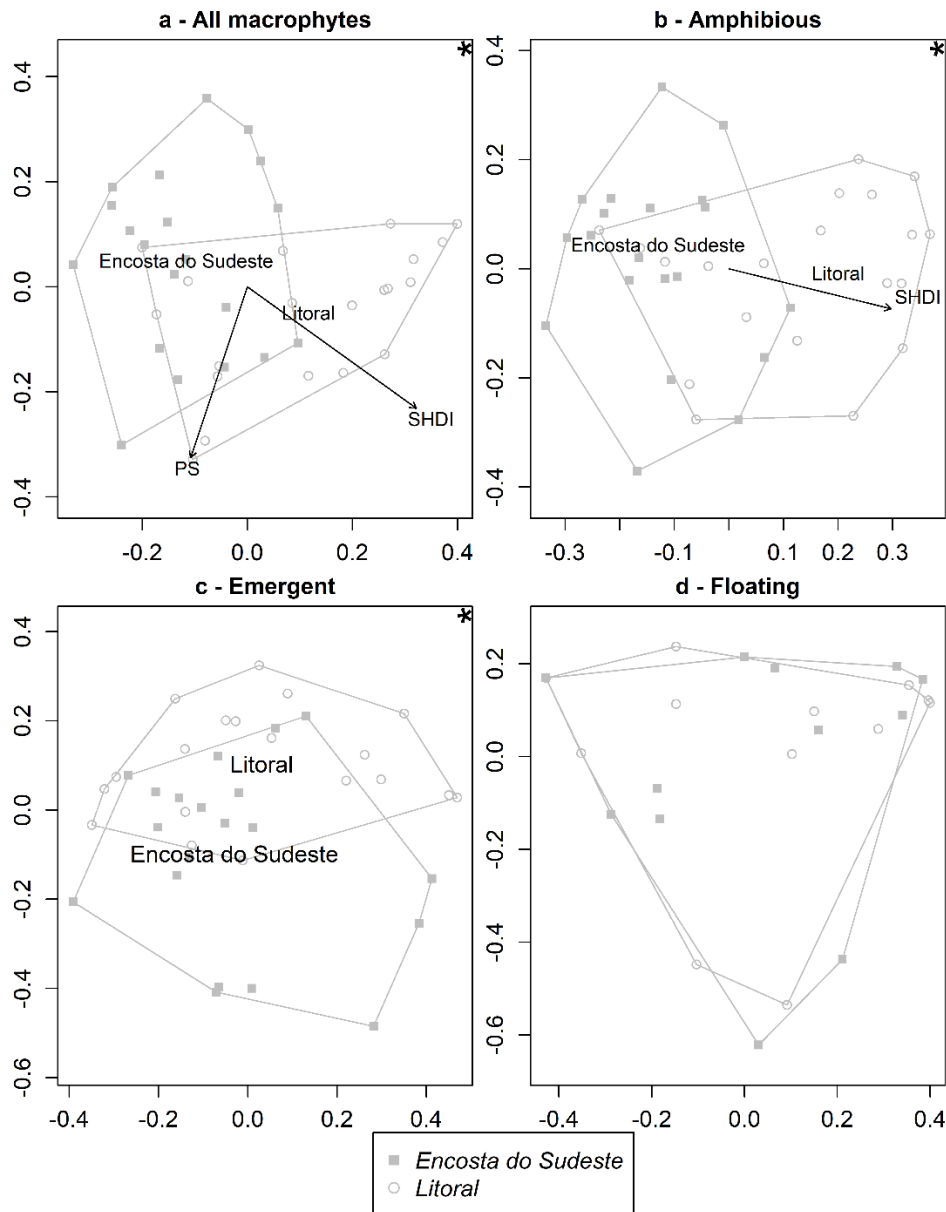


Figure 2. Variables^h adjusted for principal coordinates analysis of all macrophytes and functional groups of aquatic macrophytes of subtropical ponds. *Encosta do Sudeste* (■) and *Litoral* (○). The asterisk (*) in the top-right corner of the plots represent significant results of Permutational Analysis of Multivariate Variance (differences between composition of aquatic macrophytes of the physiographic regions).

^h PS = Pond Size; SHDI = Landscape Shannon's Diversity Index

CAPÍTULO 2 - The role of spatial factors and environmental filters on aquatic macrophyte metacommunities in subtropical ponds

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The role of spatial factors and environmental filters on aquatic macrophyte metacommunities in subtropical ponds

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The role of spatial factors and environmental filters on aquatic macrophyte metacommunities in subtropical ponds

Aquatic macrophytes are traditionally understood as not extremely limited by dispersion. Nevertheless, assessing this group in a metacommunity approach can clarify the role of dispersion (spatial factors) and environmental niche (environmental factors) on these communities. We evaluate the relative contribution of spatial and environmental factors (local, landscape, climate and regional) on structure of aquatic macrophyte metacommunities (richness and species composition) in subtropical ponds at two spatial scales in southern Brazil. Furthermore, we verified whether the relative contribution of these factors varied among functional groups (amphibious, emergent, and hydrophytes) on a broader scale. To accomplish these aims, we employed regression and partial redundancy analyses with variation partitioning. We verified that spatial factors were influential on all macrophytes composition at the broader spatial scale, whilst it was not important at a medium scale. We also verified that the richness of amphibious species was explained only by the spatial portion, whilst the richness of hydrophytes was explained only by environmental portion. The composition of amphibious and emergent species were mostly explained by spatial factors, while the composition of hydrophytes was explained mostly by environmental ones. Our results reveal that, at a broader scale, patch dynamics probably structure metacommunities of all macrophytes and the functional groups, amphibious and emergent, whilst species sorting seems to organize the hydrophytes. On a medium spatial scale, species sorting seems to structure all macrophytes.

Keywords: climatic factors, environmental factors, land use, partitioned variation, Principal coordinates of neighbour matrices.

Introduction

Metacommunities are “a set of local communities that are linked by dispersal of multiple potentially interacting species” (Wilson 1992). Studies of metacommunities seek to evaluate the influence and the importance of spatial and environmental factors and joint effects of these factors on biological communities as proxies of dispersal and the environmental processes

(Leibold et al. 2004, Logue et al. 2011). Aquatic macrophyte metacommunities are traditionally understood as not extremely limited by dispersion. This idea results from the assumption that most species are widely distributed and produce vegetative propagules that colonize over long distances (Santamaría 2002, Chambers et al. 2008). However, Murphy et al. (2019) evaluated the global distribution of more than 3000 aquatic macrophyte species and found that most of them (approximately 80%) have restricted distribution, occupying less than 10% of the potential global area for the establishment of this group. Therefore, these communities are potentially structured by both spatial factors (related to dispersal mechanisms) and by environmental factors (related to the selection of environmental niches).

Studies on aquatic macrophyte metacommunities have been divergent regarding the patterns found; some of them point to a greater importance of the spatial component compared to the environmental component in structuring these metacommunities (Padial et al. 2014); whereas others point to the opposite (e.g. Alahuhta et al. 2013, and Alahuhta and Heino 2013) and still other suggest the joint importance of spatial and environmental factors (e.g., Capers et al. 2010; Kuglerova et al. 2015; Grimaldo et al. 2016; García-Girón et al. 2019b). These different findings may be due to the different types of environments studied (rivers, lakes, and wetlands), geographical regions, spatial extent, observation scales, and functional groups (Alahuhta and Heino 2013, Alahuhta et al. 2013, Heino et al. 2015). Most studies have evaluated aquatic macrophyte metacommunities in lakes and rivers in temperate and tropical regions (e.g., Alahuhta et al. 2013, Grimaldo et al. 2016). However, little is known about processes that structure these metacommunities in subtropical ponds (Trindade et al. 2018).

Metacommunity analysis can and should be conducted in different geographic regions as proxies of different spatial scales as well as different ecological contexts (e.g., Alahuhta and Heino 2013; Alahuhta et al. 2014; Grimaldo et al. 2016). The greater the spatial observation scale, it is expected that dispersion processes (spatial factors) will have a greater contribution

in explaining the structuring of communities (Leibold et al. 2004, Capers et al. 2010). Consequently, the importance of environmental factors is expected to increase as the spatial scale under investigation decreases (Cottenie 2005).

Regarding environmental factors, few studies assess the contribution of climate (Grimaldo et al. 2016, Alahuhta et al. 2018) and land use variables (Mikulyuk et al. 2011, Alahuhta et al. 2015), while most studies are restricted to local variables, such as wetland size, pH, conductivity, turbidity, and mean depth, among others (Capers et al. 2010, Padial et al. 2014). Climatic variables have latitudinal gradients (Alahuhta et al. 2011) that can interfere in community organization on a broader scale (Toivanen et al. 2019). Land use variables may increase the percentage of explanation of the environmental portion in the organization of these metacommunities (Grimaldo et al. 2016). Land use may also be important because it is indirectly related to the eutrophication processes that negatively affects the richness and composition of aquatic macrophytes (Sass et al. 2010).

Aquatic macrophytes can be classified into functional groups (e.g., amphibious, emergent, and hydrophytes) that have different biological features to survive in aquatic ecosystems, as well as different dispersion strategies (Boedeltje et al. 2003, Capers et al. 2010). Spatial factors tend to be more important for species with lower dispersal abilities than for species that disperse at greater distances (Heino et al. 2015). Amphibious are plants that survive both in the absence and presence of water (Cronk and Fennessy 2001), while emergent species emerge their vegetative and reproductive structures above the water surface (Lacoul and Freedman 2006). Hydrophytes live submerged or floating in water, whether rooted or not, and are more sensitive to environmental changes, such as water quality (Cronk and Fennessy 2001). Amphibious and emergent species disperse their diaspores mainly by wind, so they reach greater distances, whilst hydrophytes disperse their diaspores mainly by water (Soons 2006). Therefore, hydrophytes species are likely to be more influenced by spatial factors than

amphibious and emergent ones because wind disperses across greater distances than water (Soomers et al. 2013). However, little is known about how spatial and environmental factors act on functional groups of aquatic macrophytes (Alahuhta et al. 2013, Heino et al. 2015, García-Girón, Wilkes, et al. 2019).

Evaluating the importance of spatial and environmental factors in aquatic macrophyte metacommunities and their different functional groups of subtropical ponds at different spatial scales can contribute to understanding the role of dispersion and environmental niches in these communities. The objectives of this study were to verify: 1) the relative contribution of spatial and environmental factors (local, landscape, climatic, and regional) in the structure of aquatic macrophyte metacommunities of ponds in southern Brazil; 2) whether the relative contribution of spatial and environmental factors varies between functional groups of aquatic macrophytes (amphibious, emergent, and hydrophytes), and 3) whether the contribution of these factors varies at different spatial scales (30,000 km² and 15,000 km²), since the physiographic regions have distinct geological formations. We hypothesized that a) the contribution of environmental factors will be greater than the contribution of spatial factors in explaining the organization of all sampled aquatic macrophyte species and of emergent and amphibious functional groups, since restrictions imposed by environmental factors will be predominant in relation to their dispersion ability (related to spatial factors); b) for the functional group hydrophytes, the contribution of spatial factors will be relatively greater than the environmental portion due to their dispersion limitation, and c) the patterns are scale-dependent, being that environmental portion will be predominant in the organization of aquatic macrophytes in medium spatial scales.

Methods

Study area

The study was conducted in 40 spatially isolated (distributed at least 10 km from each other) subtropical ponds located in southern Brazil (Fig. 1). We selected ponds that were small (< 2 ha) and shallow (< 1.5 m depth). We used two spatial scales of study: one corresponds to the Coastal Plain of Rio Grande do Sul (Villwock and Tomazelli 2007), and another corresponds to two distinct physiographic regions: *Encosta do Sudeste* and *Litoral* (Fortes 1959). The extension of the first scale is of approximately 30,000 km² and the second one is of approximately 15,000 km². We denominated these scales as “broader” and “medium”, respectively, to indicate that the first spatial extent is greater than the second. If we draw a straight line in the southwest–northeast direction between our sample points, we have a length of approximately 300 km.

According to Fortes (1959), these physiographic regions have distinct geological formations. The *Encosta do Sudeste* is mainly composed of a depositional system of alluvial fans that were formed between the late tertiary and early quaternary periods, ~2.5 mya (oldest), while the *Litoral* region was formed by lagoon-barrier depositional systems within the last 0.4 mya (most recent) (Villwock and Tomazelli 2007). The *Encosta do Sudeste* covers an area of 14981 km² and is situated west of a large lake (*Lagoa Mirim*) and lagoon (*Lagoa dos Patos*) systems. This region, according to Brena (2002), presents a range of sandy sediments and holocenic alluvions along rivers. The ground is usually flat, and the altitude generally remains below 30 meters, but presents rocky spurs reaching up to 200 meters (Brena 2002). Between rocky spurs are ponds formed by the accumulation of water. The *Litoral* area covers the Atlantic Coastal Plain and comprises an area of approximately 14905 km² (Fortes 1959). This physiographic region was geologically formed by sands moved and deposited by water and winds that form poor soils and are partially saline, poorly consolidated, and permeable (Brena 2002). The *Litoral* region contains a high level of diversity and abundant wetlands. The ground

is flat to slightly wavy, and the altitude is low (from 5 to 10 meters) (Brena 2002). The study area in the two physiographic regions corresponds to the same latitudinal segment of the west and east boundaries of *Lagoa dos Patos* (the largest lagoon in South America) and *Lagoa Mirim* (the largest lake in Brazil). These regions have a high number of wetlands compared to other regions of Southern Brazil (Maltchik 2003). The climate in these regions is moist subtropical. The mean annual temperature varies between 16 °C and 20 °C, the mean temperature of the warmest month varies between 22 °C and 26 °C, and the mean temperature of the coldest month varies between 10 °C and 15 °C (Nimer 1977). The annual rainfall ranges from 1000 to 1500 mm (Villwock and Tomazelli 2007).

Macrophytes sampling

Aquatic macrophytes were collected between January and April 2017. Time-based nonlinear transects (3 minutes) were performed across the ponds on foot. Each transect consists to record the macrophyte species visualized by the collector in an imaginary transect walking through the pond for three minutes. The transects followed the variation of the pond structure (at the border of the pond and from the border towards the center). To achieve sufficient samples, the number of transects varied according to the size and heterogeneity of the pond (from 5 to 34, with a mean of 11.7). The sampling was exhaustive, ending after two transection (6 minutes) with no new record of species occurrence (Rolon et al. 2012).

Species identification was carried out in the field and in the laboratory using a Leica EZ4D stereomicroscope. The taxonomic framework followed the procedures outlined in APG IV (2016) for angiosperms and Smith et al. (2006) for pteridophytes. The scientific names of the species are in accordance with the Flora of Brazil (2020). Fertile specimens were herborized and included in the HURG herbarium (*Herbário Universidade do Rio Grande*). We used functional group as a proxy for dispersal mode because species-specific dispersion has not been

adequately documented. Species were classified into three functional groups: amphibious, emergent and hydrophytes (floating + submerged) (Cronk and Fennessy 2001). We included epiphytes and lianas in the emergent category, which live on other aquatic plants without rooting to the substrate and are rooted in the substrate but require support from other plants, respectively. We joined floating and submerged in the same functional group (hydrophytes) due to the similarity that these groups have in relation to the dependence on water for dispersion and due to the low diversity of submerged ones generally found in the study region (Rolon et al. 2010). The term “All macrophytes” will be used to refer to the total set of sampled aquatic macrophyte species. We used matrices of presence and absence of the species in the sampled ponds as composition in the statistical analyses.

Survey of environmental and spatial variables

The predictive variables in structuring aquatic macrophytes were classified in environmental and spatial variables. The environmental variables were subdivided into local, landscape, climate, and regional variables. The local environmental variables were water temperature (WT—°C), pH, *Oxidation Reduction Potential* (ORP—mV), electric conductivity (EC— $\mu\text{S}\cdot\text{cm}^{-1}$), dissolved oxygen concentration (DO— $\text{mg}\cdot\text{cm}^{-1}$), percentage of dissolved oxygen (pDO—%), total dissolved solids (TDS— $\text{g}\cdot\text{L}^{-1}$), diversity of habitats, maximum depth (cm), pond size (ha), and altitude (m). Water temperature, pH, ORP, EC, DO, pDO, and TDS were measured at points randomly distributed in each pond (minimum of three points) using a multiparameter probe (Horiba U-500). The mean of each variable was used in the subsequent analyses. Diversity of habitats index was quantified based on the cumulative number of habitats considering water depth, substrate characteristics, and wind protection, as described by Rolon et al. (2008). We surveyed depth values with a ruler in at least in five points randomly distributed in each pond. The maximum depth corresponds to the highest depth value measured.

Size and altitude of each pond were measured in the field with the use of a GPS while walking around the ponds. When it was not possible to walk around the perimeter of some ponds to measure the size, it was calculated using Google Earth software.

The landscape environmental variables were used to predict the influence of natural and artificial landscapes around each pond (squared buffer/windows of 10 km²). These variables included the Landscape Wetland Area (LWA) as a natural landscape, and Size of Agricultural Area (Agr) and Size of Urban Area (Urb) as artificial landscapes. These variables were obtained by supervised digital classification of satellite images using GIS tools and FRAGSTATS software (McGarigal et al. 2012) as described in (Pereira et al. 2021). In other macrophyte studies, landscape variables were determined within a 1 km² buffer around aquatic bodies (Alahuhta and Heino 2013, Weilhoefer et al. 2013, Alahuhta et al. 2014). However, we obtained landscape variables at windows of 10 km² due to the likely dispersion of aquatic macrophytes by birds (Thomaz 2002), which can reach greater dispersion distances among aquatic bodies (Bugoni et al. 2005), and due to the large spatial resolution of the satellite images.

The climatic environmental variables were obtained from WorldClim database (Fick and Hijmans 2017). The climatic variables considered were Annual Mean Temperature (Temp), Temperature Seasonality (TS), Mean Temperature of Wettest Quarter, (MTWeQ), Mean Temperature of Driest Quarter (MTDQ), Mean Temperature of Warmest Quarter (MTWaQ), Mean Temperature of Coldest Quarter (MTCQ), Annual Precipitation (P), Precipitation Seasonality (PS), Precipitation of Wettest Quarter (PWeQ), Precipitation of Driest Quarter (PDQ), Precipitation of Warmest Quarter (PWaQ), and Precipitation of Coldest Quarter (PCQ). We used the central geographic coordinates of each pond to extract the data from WorldClim matrix of 2.5 minutes of spatial resolution. This matrix was obtained by “getData” function of

“raster” package in R environment (Hijmans 2016). The regional environmental variable is categorical with two levels: *Encosta do Sudeste* and *Litoral*.

The spatial variables correspond to their spatial correlation gradient. They were obtained by Principal Coordinate Analysis of Neighboring Matrices (PCNM) (Borcard and Legendre 2002) using the “pcnm” function of the Vegan package (Oksanen et al. 2016). This analysis allows obtaining spatial structure of metacommunities at different spatial scales (Alahuhta and Heino 2013). The geographic coordinates of the center of each pond were used to calculate Euclidean distances among the ponds. The eigenvectors (PCNMs) with larger eigenvalues (the first eigenvectors) reveals broader spatial structuring, and PCNMs with smaller eigenvalues (the latest eigenvectors) reveals finer spatial structuring (Griffith and Peres-Neto 2006).

Data analysis

To reduce multicollinear environmental variables, we removed the variables that obtained values of Variance Inflation Factor (VIF) below 10 using the “vif” function of the “faraway” R package (Faraway 2016). Thus, the matrix of environmental variables contained: pH, ORP, diversity of habits, maximum depth, altitude, pond size, LWA, Agr, Urb, and MTDQ. The spatial matrix is composed of the PCNMs axes. Maps for significant PCNM values were constructed using Jenks Natural Breaks optimization to determine the best arrangement of values into two different classes.

Global models and selection of variables

All environmental (after removing multicollinear variables) and all spatial variables were tested as a function of the richness and of the composition of aquatic macrophytes (global

environmental and global spatial models) by multiple linear regression and redundancy analysis, respectively. If the global model was significant ($p < 0.05$) for a given group of variables (environmental and/or spatial), then the environmental variables were selected by the step-forward method, and the spatial variables (PCNMs) by the Forward Selection With Double Stopping Criterion (FWD approach) (Blanchet et al. 2008). The FWD approach was used to select the eigenvectors that better described the spatial variation of aquatic macrophyte assemblages. According to Bauman et al. (2018), this approach performed well at broad and medium scales. We performed the step-forward selection using “forward.sel” of the “adespatial” package of the software R (Jombart et al. 2019), and the FWD approach according to Bauman et al. (2018).

Multiple Regressions and Partial Redundancy Analysis

To evaluate the relative contributions of environmental (local, landscape, climatic, and regional) and spatial variables (PCNM) in explaining richness and composition of aquatic macrophyte assemblages, we performed variation partition by multiple regressions and Partial Redundancy Analysis (RDAP) (Borcard et al. 1992) using the “lm” function and “varpart” function of the “vegan” package of the software R (Oksanen et al. 2016). The variation in the community matrix was divided into [a] purely environmental, [b] both spatial and environmental (spatial + environmental), [c] purely spatial, and [d] residual or unexplained explanatory components (Borcard et al. 1992). The global models, hence the selection of variables, and the multiple regressions and RDAP were performed for both richness and composition of all macrophytes and of each one of the functional groups (amphibious, emergent, and hydrophytes) to verify differences in the portion of environmental and spatial factors of the functional groups.

Spatial Scale Analysis

These analyses were also performed separately for two different spatial scales for all macrophytes: one of a broader scale of 40 ponds (Coastal Plain of Rio Grande do Sul) and two of medium scales each with 20 ponds (Encosta do Sudeste and Litoral). We compared the results of these analyses to verify if the spatial scales could be interfering with richness and composition of aquatic macrophytes.

Results

A total of 243 species of aquatic macrophytes were sampled in 40 ponds of Southern Brazil. Of those, 185 were amphibious, 41 were emergent, and 17 were hydrophytes. The mean, minimum and maximum values of environmental variables observed are summarized in [Table 1](#).

Broader spatial scale (40 sampled sites)

Species Richness

None of the global regression models explained the richness of all macrophytes and of the emergent species. The richness of amphibious species was explained by the pure spatial portion ([Tab. 2](#)), which explained 21% of variance. The selected spatial variables in this model were both broader spatial patterns (PCNMs 2, 3, 5, 7, and 10) and finer spatial patterns (PCNMs 14 and 26) ([Fig. 2](#)). The richness of hydrophyte species was explained by pure environmental portion (42%), these included diversity of habitats and ORP ([Tab. 2](#))

Species Composition

Both environmental and spatial global models explained the composition of all macrophytes and each one of the functional groups. All portions analyzed (pure environmental,

environmental + spatial, and pure spatial) explained part of variation of the composition of all macrophytes, amphibious, and emergent species. For all macrophytes, both pure environmental and pure spatial portions each explained 3% of the variance, and the joint environmental and spatial portion explained 1% (Tab. 2). For amphibious and emergent species, the pure spatial portion was more important than the other portions, but the pure environmental portion was not significant (Tab. 2). Only the purely environmental and purely spatial portions explained the hydrophytes composition, with the environmental being greater than the spatial portion.

Regarding the environmental variables that explained the data variation, we could verify that local and landscape variables (diversity of habitats, altitude, size of agricultural area) influenced the composition of all macrophytes (Tab. 2). Both local, landscape (land use), and climate variables (diversity of habitats, altitude, and MTDQ) influenced composition of amphibious species (Tab. 2). For emergent species, only one landscape variable (size of urban area) influenced composition. For hydrophytes, only one local variable (diversity of habitats) influenced composition.

Regarding the spatial variables in species composition, both eigenvectors (PCNM) that reveal broader and finer spatial structure were observed (Fig. 2). The PCNMs selected for all macrophytes were PCNMs 2, 3, and 10. In this case, the first two demonstrate broad spatial structure (Fig. 2a, b), and only the last one shows fine spatial structure (Fig. 2g). The PCNMs selected for amphibious were PCNMs 2, 3, 5, 7, 10, 14, and 26 (both broad and fine spatial structure). The PCNMs selected for emergent species were PCNMs 1, 2, 3, 10, and 21. Only one PCNM spatially structured the composition of hydrophytes: PCNM 6 (Tab. 2). PCNMs 1, 2, and 3 appeared to show broader spatial patterns. PCNMs 1 (Fig. 2a) showed a differentiated center/south portion, while PCNM 2 (Fig. 2b) separates the ponds of the *Litoral* above the channel that connects *Lagoa dos Patos* to the Atlantic Ocean, and PCNM3 (Fig. 2c) showed a

north/south portion. The other PCNMs are more locally structured, showing no evident spatial pattern (Fig. 2d-j).

Medium Spatial Scales (Encosta do Sudeste and Litoral)

The species composition of *Encosta do Sudeste* was explained only by the pure environmental portion (seasonal precipitation and MTDQ) (Table 3). None of the global regression models among the richness and all macrophytes was significant for both physiographic regions (*Encosta do Sudeste* and *Litoral*). None of the environmental or spatial portions explained variation in the species composition of the *Litoral*.

Discussion

In this study, the environmental, environmental + spatial, and spatial portions together explained from 7 to 14% of the variation in species composition of aquatic macrophytes. The explanation percentage of aquatic macrophyte composition is generally low in studies that include the spatial portion in the partitioned variation analysis of similar spatial extensions, reaching a maximum of 50% of the variation (Cottenie 2005, Grimaldo et al. 2016). For example, based on a similar spatial extent (10^4 - 10^5 km²), Grimaldo et al. (2016) observed that spatial, environmental and the spatially-structured environmental portions explained together between 3 and 11% of the variation of species composition. In a similar location, Trindade et al. (2018) observed percentages of explanation of these portions between 13 and 18% of the species composition of aquatic macrophytes and their functional groups. Grimaldo et al. (2016) suggests that including environmental factors such as regional land use could increase the percentage of explanation on the richness and composition of aquatic macrophyte communities in calcareous rivers. Although we have included in our study land use variables, such as the

size of agriculture and urban area, the explanations percentages are still low in explaining species composition. For richness explanation, the values were higher: 21% of the variation in amphibious species was explained by spatial variables and 42% of the variation in hydrophyte richness was explained by environmental variables. Similar patterns were reported by Grimaldo et al. (2016) and Trindade et al. (2018), who observed a maximum of 26 and 39% of the variation in richness of aquatic macrophytes.

Although we found low percentages of explanation of the variables analyzed about species composition, it is possible to draw some cautious conclusions from our results. They indicate that spatial factors explained the richness of amphibious species of aquatic macrophytes and was the only portion that explained the composition of amphibious and emergent species in ponds of Southern Brazil. In addition, the environmental portion explained macrophyte richness and had greater contribution than the spatial portion in explaining the composition of hydrophytes. These results contradicted our assumptions that the environmental portion would be the most important in structuring all macrophytes, amphibious, and emergent assemblages, and that the spatial portion would be the most important portion for hydrophytes. This reveals that metacommunity-level processes can act differently among functional groups of aquatic macrophytes in subtropical ponds. In general, all sampled aquatic macrophytes, amphibious, and emergent functional groups are likely to be structured accordingly with the patch dynamics perspective in subtropical ponds. The perspective of species sorting seems to structure the functional group of hydrophytes in those ponds. In addition, this study found that metacommunity processes act differently depending on the scale of observation and the geographic context (physiographic region).

None of the factors addressed in this study (spatial, environmental, and spatial + environmental factors) explained the richness of all macrophytes, but did explain the composition, which was equally explained by the purely spatial and environmental portions

(3%). The composition of aquatic macrophytes from temperate calcareous rivers (Grimaldo et al. 2016) and subtropical wetlands (Trindade et al. 2018), as well as the richness of aquatic macrophytes from tropical calcareous rivers (Grimaldo et al. 2016), were explained mostly or solely by spatial factors. García-Girón et al. (2019a) found greater importance of the joint portion of dispersal processes (spatial factors) and species sorting (environmental factors) in aquatic macrophytes of Mediterranean ponds. The spatial extensions of these studies were similar to our study ($\sim 10^4$ - 10^5 km²). This indicates that the spatial portion is probably important in structuring aquatic macrophytes, reinforcing the idea that macrophytes assemblages are affected by dispersal processes (Murphy et al. 2019). This may also depend on the environment in which these assemblages are found. In ponds, there is a tendency for dispersion limitation to be predominant in relation to environmental factors due to the greater isolation and ephemerality of these aquatic bodies (Heino et al. 2015).

We observed divergences in the explanations of environmental and spatial portions among functional groups of aquatic macrophytes, although these divergences were not as we hypothesized. The fact that the environmental portion was the only influential portion of the hydrophyte richness (submerged + floating) is, to some extent, corroborated by Trindade et al. (2018), who found the same for the floating functional group in a similar study area. Regarding species composition, although all portions (spatial, environmental, and spatial + environmental) were influential for all macrophytes, amphibious, and emergent, the explanation of spatial portion was relatively greater for amphibious and emergent species than for all macrophytes and hydrophytes. In temperate ponds and lakes, submerged species were more influenced by environmental factors than by water dispersion processes (Akasaka and Takamura 2011, Mikulyuk et al. 2011), and the opposite was found for floating (Akasaka and Takamura 2011).

The most important variables that explained macrophyte richness and composition of the studied subtropical ponds were diversity of habitats, altitude, ORP, MTDQ, Size of

agricultural Area, and Size of Urban area. Some studies indicate that phosphorus concentration, conductivity, and turbidity are the most influential variables on aquatic macrophyte communities (O'Hare et al. 2012, Alahuhta et al. 2013, 2016, Viana et al. 2014). Agriculture and urbanization can increase nutrient concentrations (including phosphorus) in aquatic environments (Sass et al. 2010, Alahuhta et al. 2011) and, therefore, may indirectly explain the structure of these communities. Diversity of habitats can be understood as a proxy for pond size /area ($r = 0.65$, see Tab. S1—Supporting Information) besides habitat availability. The higher these variables, the more environment is available for colonization of aquatic macrophytes. Altitude, although a local factor, is probably a reflection of spatially structured processes (O'Hare et al. 2012). Few studies of aquatic macrophyte metacommunities include climate factors in their analyses. Grimaldo et al. (2016) verified the importance of a minimum temperature of the coldest month for macrophyte composition of temperate rivers and importance of evapotranspiration for macrophyte composition of tropical rivers. The temperature of the driest quarter may have been important for ponds in Southern Brazil by probably influencing the hydroperiod of these ponds, which often dry out during dry periods of the year. The hydroperiod directly influences the richness and composition of aquatic macrophytes (Rolon et al. 2010).

Different metacommunity patterns probably act in aquatic macrophyte communities due to the spatial scale of observation. The PCNMs demonstrated that, in addition to spatial factors influencing communities in broader spatial extent (revealing a possible patch dynamics paradigm), these factors act at different scales; the communities showed clear broad spatial structure (such as PCNMs 1, 2, and 3) and presented more local spatial structuring (such as PCNMs 5, 6, 7, 10, 14, 21, and 26). Mikulyuk et al. (2011) point out that PCNMs which reveal broader spatial structure may reflect processes related to climate and geology, while PCNMs

that reveal finer spatial structure probably reflect hydrology and more local geological processes.

However, data of one medium spatial scale (*Encosta do Sudeste*) demonstrated that the spatial portion was not important in structuring aquatic macrophytes, but the environmental portion does, revealing a species sorting pattern for this region. Similar results were found by Alahuhta and Heino (2013) in a spatial extension similar to *Encosta do Sudeste* (~15000 km²), where the importance of the environmental portion was greater than the spatial and joint portions in structuring aquatic macrophytes of temperate lakes of the United States. However, in that same region, Alahuhta et al. (2014) found no differences in metacommunity patterns at different spatial scales that they analyzed. But in much higher spatial extensions (over 300000 km²), Grimaldo et al. (2016) found greater importance of the spatial portion on calcareous river macrophyte assemblages of temperate and tropical zones. Our study revealed that considering higher spatial scales (above 15000 km²), even if not as high as those analyzed by Grimaldo et al. (2016), may reveal greater importance of the spatial portion (patch dynamics), especially in subtropical ponds. Viana et al. (2014) pointed out that dispersion limitation for aquatic macrophytes may be important in spatial extensions above 300 km, a similar extension of this study. Our results corroborate the expectation that on larger spatial scales, the spatial portion will be more influential, and, on smaller spatial scales, the environmental portion may be more important. However, this was not verified in the other physiographic region of similar extension (*Litoral*), revealing that these patterns can also depend on the geographic region of study (dependency context), besides other stochastic processes.

Conclusions

Based on findings of our study, we can draw the following conclusions: 1) the processes that structure the metacommunities were different among the functional groups of subtropical

aquatic macrophytes and 2) these processes were also differentiated according to the spatial scale of study. On a broader spatial scale (*Encosta do Sudeste + Litoral*), dispersal limitation appears to be important for all macrophytes and amphibious and emergent functional groups, while niche dependent processes appear to be more important for hydrophytes. However, on a medium spatial scale (*Encosta do Sudeste*), environmental factors appear to be more important in the composition of aquatic macrophytes. However, other factors not evaluated, besides stochastic processes, probably act in these communities due to the high unexplained percentage of variance, although these high values are common in metacommunity studies. These conclusions are relevant in the conservation aspect of southern Brazilian ponds. Anthropogenic impacts that lead to declines in water quality of these environments, such as agriculture and urbanization expansion (as nutrient enhancement proxies in aquatic environments), are also associated with fragmentation and changes in habitat availability in the landscape. Poor water quality and fragmentation of habitats can have negative impacts on hydrophyte species. On the other hand, impacts that interfere in dispersal mechanisms of aquatic macrophytes, such as agricultural expansion and urbanization itself or climate change, may affect the entire macrophyte assemblage. These effects are likely to exceed the local scale of impact, so it is important to evaluate communities on more than one spatial scale.

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Table 1. Mean, standard deviation, minimum and maximum values of environmental variables of 40 ponds sampled in Southern Brazil.

Environmental variables	Mean	Standard deviation	Minimum	Maximum
Water Temperature - WT (°C)	28.0	3.3	20.6	33.4
pH	6.5	1.0	3.7	8.5
Oxidation Reduction Potential – ORP (mV)	192.7	94.1	5.3	345.3
Electric Conductivity – EC ($\mu\text{S.cm}^{-1}$)	0.1	0.1	0.0	0.2
Dissolved Oxygen Concentration – DO (mg.L^{-1})	7.3	2.5	0.9	12.8
Dissolved Oxygen Percentage – pDO (%)	93.9	32.4	11.6	166.2
Total Dissolved Solids – TDS (g.L^{-1})	0.1	0.0	0.0	0.1
Diversity of Habits - DH	3.4	1.3	2.0	6.0
Maximum Depth - MD (cm)	35.9	22.5	4.0	100.0
Altitude (m)	29.2	39.5	2.0	188.0
Pond size (ha)	0.6	0.5	0.1	1.8
Landscape Wetland Area – LWA (ha)	1224.9	1055.1	32.1	4184.4
Size of Agricultural Area - Agr (ha)	6195.8	2212.2	1166.7	9249.6
Size of Urban Area – Urb (ha)	186.8	341.0	20.7	2043.5
Annual Mean Temperature – Temp (°C)	18.2	0.4	17.3	19.0
Temperature Seasonality - TS	360.7	10.8	341.6	377.2
Mean Temperature of Wettest Quarter – MTWeQ (°C)	14.5	0.6	13.7	15.9
Mean Temperature of Driest Quarter – MTDQ (°C)	21.2	1.0	19.0	22.5
Mean Temperature of Warmest Quarter – MTWaQ (°C)	22.8	0.4	21.7	23.3
Mean Temperature of Coldest Quarter – MTCQ (°C)	13.6	0.5	12.8	14.7
Annual Precipitation – P (mm)	1309.4	63.2	1200.0	1393.0
Precipitation Seasonality – PS	14.7	2.2	10.0	19.0
Precipitation of Wettest Quarter – PWeQ (mm)	374.1	19.9	333.0	401.0
Precipitation of Driest Quarter - PDQ (mm)	266.5	16.3	233.0	301.0
Precipitation of Warmest Quarter - PWaQ (mm)	333.5	19.4	303.0	373.0
Precipitation of Coldest Quarter - PCQ (mm)	368.0	20.4	331.0	397.0

Table 2. Spatial and environmental models that explained variation in richness and composition of aquatic macrophytes in ponds on a broader spatial extension (*Encosta do Sudeste + Litoral*) in Southern Brazil. Adjusted R² portions: [a] purely environmental, [b] both spatial and environmental, [c] purely spatial, and [d] residual or unexplained.

Aquatic Macrophytes	Selected environmental variables [‡]	Selected spatial variables (PCNMs)	p value of Global Environmental model	p value of Global Spatial model	p value of Environmental portion	p value of Spatial portion	[a]	[b]	[c]	[d]
RICHNESS										
All macrophytes	-	-	0.617	0.072	-	-	-	-	-	-
Amphibious	-	2, 3, 5,7,10,14,26	0.477	0.030*	-	0.04*	-	-	0.21	0.79
Emergent	-	-	0.362	0.243	-	-	-	-	-	-
Hydrophytes	DH, ORP	-	0.048	0.414	-	0.000*	0.42	-	-	0.58
COMPOSITION										
All macrophytes	DH, Altitude, Agr	2, 3, 10	0.001*	0.001*	0.007*	0.003*	0.03	0.01	0.03	0.93
Amphibious	Altitude, DH, Agr, MTDQ	2, 3, 5,7,10,14,26	0.004*	0.003*	0.051	0.001*	0.02	0.03	0.09	0.86
Emergent	Urb	1,10, 21	0.035*	0.006*	0.097	0.001*	0.01	0.00	0.06	0.93
Hydrophytes	DH	6	0.015*	0.045*	0.002*	0.016*	0.09	-	0.03	0.88

[‡]Agr: size of agricultural area; DH: Diversity of Habitats; MTDQ: Mean Temperature of Driest Quarter; ORP: Oxidation Reduction Potential; Urb: size of urban area.

Table 3. Spatial and environmental models that explained variation in richness and composition of aquatic macrophytes in ponds in two regions (medium spatial extensions—*Encosta do Sudeste* and *Litoral*) in Southern Brazil. Adjusted R² portions: [a] purely environmental, [b] both spatial and environmental, [c] purely spatial, and [d] residual or unexplained.

Regions	Selected environmental variables [§]	Selected spatial variables (PCNMs)	p value of Global Environmental model	p value of Global Spatial model	p value of Environmental portion	p value of Spatial portion	[a]	[b]	[c]	[d]
RICHNESS										
<i>Encosta do Sudeste</i>	-	-	0.719	0.279	-	-	-	-	-	-
<i>Litoral</i>	-	-	0.488	0.996	-	-	-	-	-	-
COMPOSITION										
<i>Encosta do Sudeste</i>	MTDQ, PS	-	0.003*	0.858	0.001	-	0.153	-	-	0.847
<i>Litoral</i>	-	-	0.608	0.352	-	-	-	-	-	-

[§]MTDQ: Mean Temperature of Driest Quarter; PS: Precipitation Seasonality.

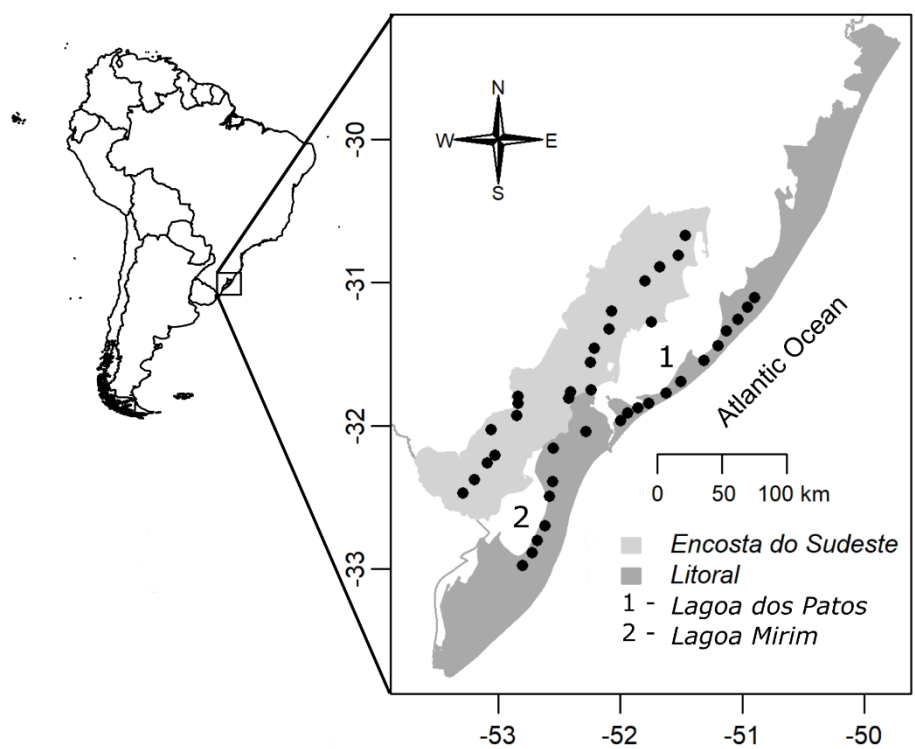


Figure 1. (•) Subtidal ponds sampled in Southern Brazil.

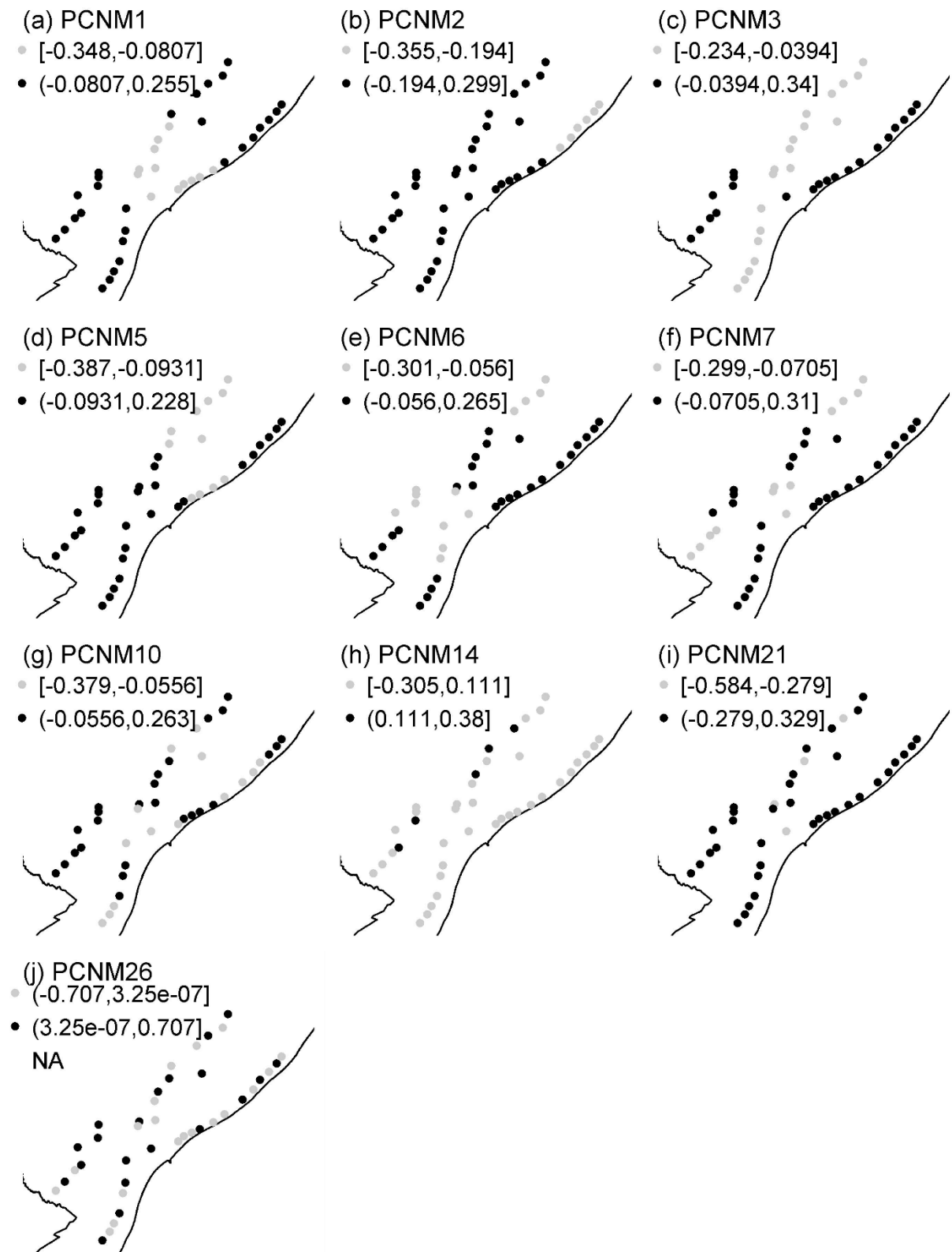


Figure 2. Spatial variables selected in models that explained the variation in richness and

composition of aquatic macrophytes in ponds of Southern Brazil. The values of the two classes are result of Jenks Natural Breaks optimization.

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1 **CAPÍTULO 3 - Spatial niche overlap of aquatic macrophytes in intermittent and**
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1 **Spatial niche overlap of aquatic macrophytes in intermittent and permanent ponds**

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3

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ABSTRACT

The dynamics of the interactions among species are of great importance for understanding the communities' structure. This study aimed to verify whether: a) the interspecific spatial overlap ("species packing") of aquatic macrophytes is different between intermittent and permanent ponds; b) the spatial overlap influences the species richness and the composition; c) an abiotic variable (depth) influences the "species packing;" d) frequent species have a different biological interaction pattern between permanent and intermittent ponds. To accomplish these aims, we verified the horizontal coverage of aquatic macrophytes in 10 permanent wetlands and 10 intermittent wetlands. We calculated the indexes of pairwise spatial overlap of all sampled species and the spatial overlap index of the 20 assemblages ("species packing"). The spatial overlap was similar between permanent and intermittent ponds; it did not influence the richness and explained 30% of the species composition. The depth did not influence the "species packing." The pairs that showed great importance on assemblages were between *Luziola peruviana* and *Nymphoides humboldtiana*, *L. peruviana* and *Eleocharis minima*, and *E. minima* and *N. humboldtiana*. *L. peruviana* probably dominates over *E. minima*. Future studies are necessary to clarify relations between spatial overlap and the structure of assemblages of macrophytes in aquatic environments.

Keywords: Interspecific relationships, Levin's overlap index, Lombardo and Mjelde index, plant-plant relationships, "species packing."

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32

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37 **Code availability** Not applicable

38 **Authors' contributions:** **Karine Massia Pereira:** Conceptualization, Methodology, Formal
39 Analysis, Investigation, Data curation, Writing- Original draft preparation, Writing-
40 Reviewing and Editing; **Ana Silvia Rolon:** Conceptualization, Methodology, Investigation,
41 Writing- Original draft preparation, Writing- Reviewing and Editing, Supervision.

42 **Ethics approval** Not applicable

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INTRODUCTION

The ecological niche theory involves the knowledge of multiple dimensions/factors that interfere in the distribution and structure of communities, such as the capacity of dispersion of species, spatial landscape configuration, biotic interactions, and abiotic factors (Soberón & Peterson, 2005). The importance of these dimensions in community structure can vary depending on the analyzed spatial scale (Li et al., 2018). The spatial niche overlap at a local scale gives a greater focus to interspecific interactions of species that co-occur in small sample units (Cardillo & Warren, 2016). Similar ecologically species (that use similar resources, which are usually scarce) tend to show spatial intervals that do not overlap (Hofer et al., 2004). In comparison, species that use different resources or that share abundant resources in the environment can coexist spatially. Although quantifying the spatial niche overlap is a challenging task, its evaluation can elucidate patterns in which the processes of competition, facilitation, or other types of interspecific interactions can be further tested (Lombardo & Mjelde, 2014).

In aquatic environments, the co-occurrence of plants can suggest the existence of both facilitation and competition depending on the life form (functional group) of species that potentially interact among themselves. For example, the co-occurrence of aquatic macrophytes of the same functional group can show mechanisms of competition due to sharing similar resources (e.g., Henry-Silva & Camargo, 2005; Martin & Coetzee, 2014). However, the co-occurrence of different functional groups, like emergent and floating aquatic macrophytes, can show the absence of competition or a possible facilitation (van Zuidam et al., 2019). We can cite the stands of *Salvinia* spp. (floating) as examples, which are often used as substrate to the growth of *Oxycarium cubense* (Poepp. & Kunth) Lye (emergent; Lycarião & Dantas, 2017), and the usual co-occurring of *Schoenoplectus californicus* (C.A.Mey.) Soják (emergent) with small floating species as *Ricciocarpus natus* (L.) Corda, *Lemna* spp., and *Wolffia* spp.. Nevertheless, there may be competition by light and nutrients when floating and submerged species co-occur (Scheffer et al., 2003; Armitage & Jones, 2019).

Aquatic macrophytes are important organisms in aquatic environments because they are primary producers on the trophic chains, they serve as shelter and nursery grounds for other species, and they are essential for nutrient cycling (Rejmankova, 2011). Studies about the interactions between aquatic macrophytes and other biological groups are common, such as with associated macroinvertebrates (e.g., Ferreira et al., 2011; Walker et al., 2013; Wolters

et al., 2018) or fish (Ferrer-Montañó & Dibble, 2002; Dias et al., 2011; Mello et al., 2016). Studies regarding the interactions of aquatic macrophytes considered as invasive/pests with other assemblages are also frequent (Schultz & Dibble, 2012; Ribas et al., 2017). There are some studies regarding the interactions between macrophyte-macrophyte species in natural aquatic environments (e.g., Nunes & Camargo, 2018; Nunes & Camargo, 2020). However, studies that clarify the non-invasive, natives or subspontaneous macrophyte interactions at assemblage level. The spatial overlap has already been discussed for some terrestrial plants (see Silander & Antonovics, 1982; Higuchi et al., 2011), but the study by Lombardo & Mjelde (2014) was innovative, proposing an index to measure the spatial niche overlap specifically for aquatic macrophytes.

Lombardo & Mjelde (2014) proposed an index to quantify the spatial niche overlap, especially for aquatic plants, based on Levin's α index (Levins, 1968). Levin's index applies to nonmobile organisms, has Lotka-Volterra competition equations as a mathematical basis (Volterra, 1928), and does not consider population densities. The Lombardo & Mjelde (2014) $\hat{\alpha}$ index considers that the accessibility (reachability) of plants to the environment is not immediate, unlike the mobile organisms, which adjust their distribution in the environment in response to intense interspecific interactions. For plants, the space is relatively fixed, and changes in their distribution occur slowly. In contrast, the density/coverage of plants in the environment can be a reflex of interspecific interactions between vegetal species because, for example, in high density (coverage) conditions of a certain species, other species may not get established due to spatial competition. Therefore, the density of vegetal species, expressed by percentage of coverage, is essential to evaluate the spatial niche overlap. The index of Lombardo & Mjelde (2014) allows quantifying both the spatial overlap between pairwise species as the average community interaction strength ($\hat{\alpha}_c$ or "species packing" 0; (Lombardo & Mjelde, 2014). These authors also affirm that more information can be obtained by integrating this index to local environmental or species ecological information. Nevertheless, new ecological interferences can emerge from the integration of the index with habitat analysis. Until the present moment, studies applying this index were not carried out.

Wetlands presents high aquatic macrophyte biodiversity (Mitsch & Gosselink, 2007), especially in the neotropical region (Chambers et al., 2008; Murphy et al., 2019). In southern Brazil, there is a high abundance of ponds (Maltchik et al., 2004). Ponds are shallow and small wetlands that can be flooded permanently (permanent ponds) or only in some periods of the year (intermittent ponds; Junk et al., 2014; Maltchik et al., 2004). The hydroperiod

fluctuations in permanent ponds are less intensive than in intermittent ponds, which allows better establishment and possible dominance of some species of aquatic macrophytes (Rolon et al., 2012). Since the hydroperiod is an important aquatic macrophyte structure (Maltchik et al., 2007), permanent and intermittent ponds can present differences in richness and composition of aquatic macrophytes, as well as in abiotic features (e.g., water nutrients concentration; Rolon et al., 2008). However, it is not known how biological interactions between aquatic macrophytes occur in these environments.

We aimed to evaluate the spatial overlap of macrophyte species in permanent and intermittent ponds using the spatial niche overlap index of Lombardo & Mjelde (2014), developed especially for aquatic macrophytes. Thereby, it was evaluated whether: a) the spatial niche overlap is different between permanent and intermittent ponds; b) the spatial overlap influences the richness and the composition of macrophyte species in permanent and intermittent wetlands; c) the abiotic variable (depth) influences the “species packing;” d) the most frequent species have a different biological interaction pattern between permanent and intermittent ponds.

We hypothesized that: 1) the “species packing” ($\hat{\alpha}_c$) is different between intermittent and permanent ponds due to differences in the hydroperiod; 2) the spatial overlap of aquatic macrophytes influence the richness and composition of aquatic macrophytes assemblages, considering that in conditions of high density, the richness may be reduced, and the dominant species could be determinants in the composition of the assemblages; 3) the increase of depth is related to the reduction of the spatial overlap of species, since it potentially limits the occurrence of emergent and amphibious species; and 4) the patterns of biological interaction of species ($\hat{\alpha}$'s) is different in intermittent and permanent ponds also due to the differences in hydroperiod.

MATERIAL AND METHODS

Study area and aquatic macrophytes sampling

Aquatic macrophytes were sampled in 10 permanent ponds (P01-P10) and 10 intermittent ponds (I01-I10) in the extreme of southern Brazil (Rio Grande – Rio Grande do Sul, Brazil; [Fig. 1](#)). Species were identified in the field and in the laboratory, and then fertile specimens were herborized and included in the HURG herbarium (*Herbário Universidade do*

Rio Grande). The ponds were sampled within an area of 275 km² to avoid potential influences of climatic or spatial gradient on the richness and composition of the assemblages of aquatic macrophytes.

The climate of the region is classified as moist subtropical (Cfa), according to the classification of Köppen-Geiger. The annual temperature average is 19 °C, and the annual minimum and maximum average temperatures are 13.2 and 24.2 °C, respectively (Reboita & Krusche, 2018). The annual precipitation average is approximately 1350 mm (Reboita & Krusche, 2018).

The permanent ponds are permanently flooded, and the intermittent ponds are flooded at least in some periods of the year, drying out mainly in the dry season/austral summer, with the presence of saturated soils (Maltchik et al., 2004). To classify ponds as permanent and intermittent, field surveys before the macrophyte sampling were carried out in the dry season (January-February 2018) to demarcate and verify which ponds were with and without water depth. The sampling was carried out in October 2019 (southern spring) to collect flowering plants to facilitate the species identification and assure that intermittent areas still had some water depth.

The percentage of taxa coverage was sampled in nine 0.5m x 0.5m squares (here called patches) randomly distributed in each of the 20 wetlands, totaling 180 squares analyzed. The percentage of coverage (%cover) of each species in each square was calculated through the weighted average of the class intervals according to the visual scale adapted from Domin-Krajina (Ellenberg & Mueller-Dombois, 1974), with the following adaptations: 1 = 1–5%; 2 = 6–10%; 3 = 11–20%; 4 = 21–40%; 5 = 41–60%; 6 = 61–80%; and 7 = 81–100, and the mean values of the classes were used in the analyses. The samples were obtained at depths between 10 and 80 cm, aiming to standardize the collections between permanent and intermittent ponds. We recorded the depth of the water in the center of each square using a centimeter stick.

Calculation of spatial niche overlap indexes

The spatial niche overlap for each species' pair of each pond was calculated according to Lombardo & Mjelle (2014) through the following equations: $\hat{\alpha}_{ij} =$

$$\alpha_{ij} \times \frac{\%cover_w}{100} \times \left(\frac{n_w^* + N_w}{N_w} \right) \text{ and } \hat{\alpha}_{ji} = \alpha_{ji} \times \frac{\%cover_w}{100} \times \left(\frac{n_w^* + N_w}{N_w} \right), \text{ where } \alpha_{ij} \text{ and } \alpha_{ji} \text{ are the spatial}$$

niche overlap indexes proposed by Levins (1968) of species i over species j , and of species j over species i , respectively; %cover_w is the mean of the total vegetation density (%cover), which is calculated through the mean percentage of coverage classes (described above) of the patches N_w in which both taxa occur; and n^*_w is the number of patches of high plant density (> 90%). The %cover represents, as described above, the weighted mean of coverage classes of the taxa in each patch.

The community's spatial niche overlap index ($\hat{\alpha}_c$), which quantifies the mean strength interaction within a community, or "species packing," was calculated through the following equation: $\hat{\alpha}_c = \bar{\alpha}_c \times \frac{\%cover_{total}}{100} \times \left(\frac{n^*_{total} + N_{total}}{N_{total}} \right)$; where $\bar{\alpha}_c$ is the Levin's community index (or average Levin's index); %cover_{total} is the coverage weighted mean of the total number of patches that described the community (N_{total}); and n^*_{total} is the number of patches of high density (> 90%). The higher the value of $\bar{\alpha}_c$, the greater the spatial overlap of species in the aquatic macrophyte assemblage.

We have calculated the standard error of $\hat{\alpha}_c$ (SE $\hat{\alpha}_c$) according to Lombardo & Mjelde (2014), through the equation: $SE\hat{\alpha}_c = \frac{SD\bar{\alpha}_c}{\sqrt{S \times (S-1)}} \times \frac{\%cover_{total}}{100} \times \left(\frac{n^*_{total} + N_{total}}{N_{total}} \right)$. SD $\bar{\alpha}_c$ is the standard deviation of Levin's index, and S is the species richness of the pond. The SE $\hat{\alpha}_c$ establishes the thresholds which the $\hat{\alpha}'$ s can be distinguished of $\hat{\alpha}_c$.

Data Analysis

Previously, through parametric and non-parametric tests, we analyzed possible abiotic interferences of the hydroperiod (permanent and intermittent) in density (percentage of coverage), richness and composition of the macrophyte assemblage. We verified that there was no significant difference in total coverage (W = 26; $p = 0.075$), richness (t = -1.692; $p = 0.108$), and species composition between permanent and intermittent ponds (F = 1.3294; $p = 0.1934$). We did not verify a significant richness-area relationship (F = 1.504; $p = 0.236$).

A t-test was applied between the community overlap indexes ($\hat{\alpha}_c$) of permanent and intermittent ponds to verify whether the biological interaction of aquatic macrophytes was different between these areas. To achieve data normality, community overlap indexes ($\hat{\alpha}_c$) were log-transformed. Linear regression and redundancy analysis (RDA) were applied to verify whether the spatial overlap of the community ($\hat{\alpha}_c$) influences the species richness and composition of aquatic macrophytes, respectively. For the RDA, the composition matrix was

generated with the mean coverage values of the species in the patches of each pond, and the Bray-Curtis distance was used to generate the distance matrix. Linear regression was also applied to verify whether the maximum depth influences the “species packaging” ($\hat{\alpha}_c$) of the ponds.

The index of “importance” of the taxa within the communities was estimated by comparing the paired index of taxa ($\hat{\alpha}_{ij}$) with the community index ($\hat{\alpha}_c$). When $\hat{\alpha}_{ij} > \hat{\alpha}_c$, this indicates that these taxa contribute to the aquatic macrophyte density at values above the mean in the pond. When $\hat{\alpha}_{ij} >> \hat{\alpha}_c$ and $\hat{\alpha}_{ji} << \hat{\alpha}_c$, this may indicate a dominance of i over j . Lombardo & Mjelde (2014) did not describe how much higher these indexes should indicate dominance, so we interpret as dominant species those whose index pairs were at least twice as much $\hat{\alpha}_c$. When both pairs are higher than $\hat{\alpha}_c$, this may indicate a strong two-way interaction between these taxa. Although we have calculated the indexes for all species pairs (see Online Resource), we have detailed the comparisons only for the three most frequent species of the sampling.

The assumptions of normal distribution and homogeneity of variances were verified by Shapiro–Wilk and Durbin–Watson tests, respectively. The analyses were performed in the software R version 3.6.0 (R Core Team 2019), and we considered $p < 0.05$ as significant.

RESULTS

Sixty species of aquatic macrophytes were recorded in ponds in the extreme south of Brazil (Table 1): 47 in intermittent ponds (mean = 12.4; standard deviation = 5.5) and 52 in permanent ponds (mean = 16.4; standard deviation = 5). The most frequent species in the studied sites were *Luziola peruviana* Juss. ex J.F.Gmel. (100% of the sampled sites), *Nymphoides humboldtiana* (Kunth) Kuntze (95%), and *Eleocharis minima* Kunth (80%) (Anexo 5). The $\hat{\alpha}_c$ values ranged from 0.130 to 1.556, and $SE\hat{\alpha}_c$ ranged from 0.011 to 0.077 (Fig. 2).

There was no difference in species packaging ($\hat{\alpha}_c$) between permanent and intermittent ponds ($t = -1.875$; $p = 0.078$). The regression between $\hat{\alpha}_c$ and species richness was not significant ($F = 1.303$; $r^2_{adj} = 0.016$, $p = 0.269$). The first axis of the RDA explained 30% of the data variation in species composition ($F = 7.643$; $p = 0.001$). In the RDA graph (Fig. 3), species packaging was more evident in the permanent ponds P05 and P08, which

were related to the presence of *Azolla filiculoides* Lam. ($r = 0.92$). The regression between maximum depth and $\hat{\alpha}_c$ was not significant ($F = 0.017$; $p = 0.897$).

The indexes of the pairs $\hat{\alpha}$ *Luziola peruviana* (LP) – *Nymphoides humboldtina* (NH) were higher than $\hat{\alpha}NH - LP$ in 18 ponds (95%), being that just in I06, $\hat{\alpha}LP - NH < \hat{\alpha}NH - LP$ (Fig. 4a). The pairs $\hat{\alpha}LP - NH$ were higher or equal to twice their respective $\hat{\alpha}_c$ in 14 of the 19 ponds (73%; Fig. 4a). Still, $\hat{\alpha}NH - LP$ was higher than or equal to twice that of $\hat{\alpha}_c$ in six of the 19 ponds (31%; Fig. 4a).

The pairs $\hat{\alpha}$ *Luziola peruviana* (LP) – *Eleocharis minima* (Emi) were higher than $\hat{\alpha}Emi - LP$ in 13 of the 16 ponds where these taxa co-occur (81%), while in 19% of the ponds (I03, I07, and P05) $\hat{\alpha}Emi - LP$ was higher than $\hat{\alpha}LP - Emi$ (Fig. 4b). $\hat{\alpha}LP - Emi$ was higher than $\hat{\alpha}_c$ in 100% of the ponds, and it was higher than or equal to twice $\hat{\alpha}_c$ in eight ponds (50%). Otherwise, $\hat{\alpha}Emi - LP$ was higher than $\hat{\alpha}_c$ in 11 ponds (69%), and lower in five (31%; Fig. 4b). $\hat{\alpha}Emi - LP$ was at least twice as much $\hat{\alpha}_c$ in six ponds (37%).

The pairs $\hat{\alpha}$ *Eleocharis minima* (Emi) – *Nymphoides humboldtiana* (NH) were higher than $\hat{\alpha}NH - Emi$ in 11 of the 16 ponds (69%) where both taxa co-occur, and $\hat{\alpha}NH - Emi$ was higher than $\hat{\alpha}Emi - NH$ in five ponds (I05 I10, P01, P02, and P09; Fig. 4c). $\hat{\alpha}Emi - NH$ was higher than $\hat{\alpha}_c$ in 15 ponds (94%), and at I07, it was less than $\hat{\alpha}_c$. In nine ponds (56%), $\hat{\alpha}Emi - NH$ was at least twice as much $\hat{\alpha}_c$. $\hat{\alpha}NH - Emi$ was higher than or equal to twice as much $\hat{\alpha}_c$ in eight ponds (50%) where both species co-occur (Fig. 4c).

DISCUSSION

The richness and composition of aquatic macrophytes found in permanent and intermittent ponds in this study can be considered representatives for southern Brazil (Kafer et al., 2011; Pereira et al., 2012). The most frequent species are also frequent in other wetlands of southern Brazil (Rolon & Maltchik, 2006; Rolon et al., 2010; Oliveira et al., 2019). Although the spatial extent of sampling was relatively small, the diversity of aquatic macrophytes represents the regional flora of wetlands. Hence, the results can be extrapolated to the ecological context of subtropical wetlands.

The species packing was similar between permanent and intermittent ponds according to t-tests. Also, this index did not influence species richness, although it explained a portion that was relatively important for species composition. The species packing index evaluates the mean strength interaction among species in a community (Lombardo & Mjelde,

2014). Although the interactions occur similarly between permanent and intermittent ponds, we verified that this biological feature plays an important role in the assemblage organization of subtropical aquatic macrophytes. Therefore, it deserves to be further investigated in these environments. The percentage not explained of the species composition variation is probably related to abiotic characteristics not evaluated in this study, but those that can influence the composition of aquatic macrophyte assemblages (Bornette & Puijalon, 2011). These abiotic characteristics could be turbidity, water nutrient concentration, altitude, wetland size, and habitat diversity in the environment (Rolon & Maltchik, 2006; Rolon et al., 2008; Kisson et al., 2013).

The depth did not influence species packing. The influence of depth is commonly investigated on other macrophyte assemblage features, like species richness and composition (Casanova & Brock, 2000), abundance (Sousa et al., 2011), and biomass (Chambers & Prepas, 1990). However, at an assemblage level, there are no studies investigating the influence of depth on spatial niche overlap of aquatic macrophytes. At the population level, we can mention Palma-Silva et al. (2005), who verified that *Eleocharis mutata* (L.) Roem. & Schult. disfavors the growth of *Typha dominguensis* Pers. in successive periods of drought, and Yu et al. (2013), who documented ideal depth conditions for the development of an endemic and critically threatened aquatic macrophyte in Taiwan. Although we did not evidence the influence of depth on spatial overlap of aquatic macrophytes, further investigations about which factors can affect their biological interactions are important to enable the proper environmental management for wetlands conservation.

Regarding the paired overlap indexes, we highlight that both interactions between *Luziola peruviana* and *Nymphoides humboldtiana*, and between *Eleocharis minima* and *N. humboldtiana* contributed more than expected for aquatic macrophytes assemblages' interactions in most ponds evaluated. Once the pairs of species that strongly interact in aquatic macrophyte assemblages are recognized, as our study identified, future studies might investigate what types of interactions are occurring between these species. Therefore, besides getting a better understanding of interspecific relationships, decision-makers can evaluate management strategies involving frequent species in the wetlands of southern Brazil.

The interaction between *L. peruviana* and *E. minima* seems more intense than the interaction between *E. minima* and *L. peruviana*. This may indicate that *L. peruviana* dominates over *E. minima*. *L. peruviana* is a very common species in the wetlands of southern Brazil and often has a high percentage of coverage in these environments (Maltchik et al.,

2007; Pereira et al., 2012). This is a perennial, amphibious, rooted caespitose species that can reproduce both by seeds and stolons (Boldrini et al., 2005). *E. minima* is a perennial, amphibious, and caespitose herb that presents the development of vegetative buds in the inflorescence, which can generate new individuals asexually, allowing the formation of homogeneous spots of this species on the margins of the wetlands (Trevisan & Boldrini, 2008). Although both species are caespitose herbs and ordinary in wetlands, *L. peruviana* has greater stature than *E. minima*. The development by stolons of *L. peruviana* allows individuals to follow seasonal changes in the water level (Nieff, 2000). Thus, *L. peruviana* individuals act as floating ones, where the stems develop over the water. Differently, scapes of *E. minima* do not reach more than 20 cm in height (Trevisan & Boldrini, 2008) and usually form upright “carpets” on the substrate, resembling a submerged species. In addition, larger plants have advantages in obtaining light in nutrient-rich environments (Aerts, 1999). Therefore, in periods of flood and conditions with high concentrations of nutrients, *L. peruviana* can obtain advantages over *E. minima*. In this study, we evaluated patches with at least 10 cm of water depth, where this interaction may have been evidenced, although both species also inhabit saturated soils.

The Levin’s and Lombardo and Mjelde’s indexes may reflect competitive interactions *latu sensu* (Lombardo & Mjelde, 2014). Inferring which types of biological interaction (competition or facilitation) actually occur depends on several factors, such as the existence of limiting resources to one of the taxa, greater interspecific competition than intraspecific, different use of resources among potential competitors, and balance between populations and their environment (Connell, 1983; Lombardo & Mjelde, 2014). Such factors are not possible to be empirically verified but rather in experimental manipulative studies through hypothesis tests (Gopal & Goel, 1993). However, identifying strong interactions between taxa in an assemblage, as verified in this study for the pairs *L. peruviana* and *E. minima*, for example, is of great value because it serves as a basis for future investigations of possible interactions between the indicated taxa, especially in permanent ponds. Also, the species packaging indexes found in this study are data that had not yet been measured for subtropical macrophyte assemblages or pairs of species occurring in southern Brazil. Consequently, inferences about the meaning of these values in interspecific interactions are challenging.

CONCLUSION

We concluded that the spatial overlap of aquatic macrophytes was similar between permanent and intermittent ponds. Moreover, the spatial overlap did not influence the richness of species, but it was important in the composition of the assemblages. The depth did not interfere with the spatial overlap of the aquatic macrophytes from the studied ponds. We highlight a possible overlap of *Luziola peruviana* over *Eleocharis minima* due to the structural differences of these species. Since these species co-occur in permanent and intermittent ponds, a symmetrical competition may be occurring between these species and this interaction deserves to be further investigated. Although this study has been one of the first in portraying interspecific interactions (macrophyte-macrophyte) at the assemblage level in natural aquatic environments, future studies must deepen the relationship of spatial overlap and the structure of these assemblages of aquatic environments, as well as the role of the hydroperiod in biological interactions.

Tab. 1 List of aquatic macrophyte species sampled in permanent and intermittent ponds in the extreme of southern Brazil

Family	Species	Abbreviation
Alismataceae	<i>Echinodorus grandiflorus</i> (Cham. & Schltr.) Micheli	EG
Alismataceae	<i>Sagittaria montevidensis</i> Cham. & Schltdl.	Smo
Amaranthaceae	<i>Alternanthera philoxeroides</i> (Mart.) Griseb.	AP
Apiaceae	<i>Centella asiatica</i> (L.) Urb.	CA
Apiaceae	<i>Eryngium eburneum</i> Decne.	EE
Apiaceae	<i>Eryngium chamissonis</i> Urb.	ECh
Apiaceae	<i>Lilaeopsis brasiliensis</i> (Glaz.) Affolter	LB
Araceae	<i>Lemna</i> cf. <i>valdiviana</i> Phil.	L
Araceae	<i>Pistia stratiotes</i> L.	Pst
Araceae	<i>Spirodela intermedia</i> W. Koch	SI
Araceae	<i>Wolffia brasiliensis</i> Wedd.	WB
Araceae	<i>Wolffiella oblonga</i> (Phil.) Hegelm.	WO
Araliaceae	<i>Hydrocotyle ranunculoides</i> L.f.	HR
Araliaceae	<i>Hydrocotyle verticillate</i> Thunb.	HV
Asteraceae	<i>Enydra anagallis</i> Gardner	Ean
Campanulaceae	<i>Lobelia hederacea</i> Cham.	LH
Cyperaceae	<i>Eleocharis maculosa</i> (Vahl) Roem. & Schult.	Ema
Cyperaceae	<i>Eleocharis minima</i> Kunth	Emi
Cyperaceae	<i>Eleocharis obtusetrigona</i> (Lindl. & Nees) Steud.	EO
Cyperaceae	<i>Eleocharis sellowiana</i> Kunth	ES

Cyperaceae	<i>Eleocharis</i> cf. <i>viridans</i> Kük. ex Osten	Evi
Cyperaceae	<i>Eleocharis</i> sp.	Esp
Cyperaceae	<i>Rhynchospora</i> sp.	RS
Cyperaceae	<i>Schoenoplectus californicus</i> (C.A.Mey.) Soják	Sca
Haloragaceae	<i>Myriophyllum aquaticum</i> (Vell.) Verdc.	Maq
Hydrocharitaceae	<i>Limnobium laevigatum</i> (Humb. & Bonpl. ex Willd.) Heine	LL
Juncaginaceae	<i>Triglochin scilloides</i> (Poir.) Mering & Kadereit	Tsci
Lentibulariaceae	<i>Utricularia breviscapa</i> C. Wright ex Griseb.	UB
Lentibulariaceae	<i>Utricularia foliosa</i> L.	UF
Lentibulariaceae	<i>Utricularia gibba</i> L.	UG
Linderniaceae	<i>Micranthemum umbrosum</i> (Walter ex J.F.Gmel.) S.F. Blake	MU
Marsileaceae	<i>Marsilea ancylopoda</i> A.Braun	Man
Marsileaceae	<i>Regnellidium diphyllum</i> Lindm.	RD
Mayacaceae	<i>Mayaca fluviatilis</i> Aubl.	MF
Menyanthaceae	<i>Nymphoides humboldtiana</i> (Kunh) Kuntze	NH
Onagraceae	<i>Ludwigia</i> sp.	LS
Plantaginaceae	<i>Bacopa monnieri</i> (L.) Pennell	BM
Plantaginaceae	<i>Callitriche rimosa</i> Fassett	CR
Plantaginaceae	<i>Gratiola peruviana</i> L.	GP
Poaceae	<i>Luziola peruviana</i> Juss. ex J.F.Gmel.	LP
Poaceae	<i>Oplismenopsis najada</i> (Hack. & Arechav.) Parodi	ON
Poaceae	<i>Paspalum pumilum</i> Nees	Ppum
Poaceae	Poaceae (sp.)	Poa
Poaceae	<i>Trichanthecium schwackeanum</i> (Mez) Zuloaga & Morrone	Tsch
Polygonaceae	<i>Polygonum punctatum</i> Elliott	Ppun
Pontederiaceae	<i>Eichhornia azurea</i> (Sw.) Kunth	Eaz
Pontederiaceae	<i>Eichhornia crassipes</i> (Mart.) Solms	EC
Pontederiaceae	<i>Pontederia cordata</i> L.	PC
Potamogetonaceae	<i>Potamogeton ferrugineus</i> Hagstr.	PF
Potamogetonaceae	<i>Potamogeton</i> sp.	Psp
Ranunculaceae	<i>Ranunculus apiifolius</i> Pers.	RA
Ranunculaceae	<i>Ranunculus flagelliformis</i> Sm.	RF
Ricciaceae	<i>Ricciocarpos natans</i> (L.) Corda	RN
Rubiaceae	<i>Oldenlandia salzmännii</i> (DC.) Benth. & Hook.f. ex B.D.Jacks.	OS
Salviniaceae	<i>Azolla filiculoides</i> Lam.	AF
Salviniaceae	<i>Salvinia biloba</i> Raddi	SB
Salviniaceae	<i>Salvinia minima</i> Baker	Smi
Scrophulariaceae	Scrophulariaceae (sp.1)	S
Solanaceae	<i>Schwenckia curviflora</i> Benth.	Scu
Xyridaceae	<i>Xyris jupicai</i> Rich.	XJ

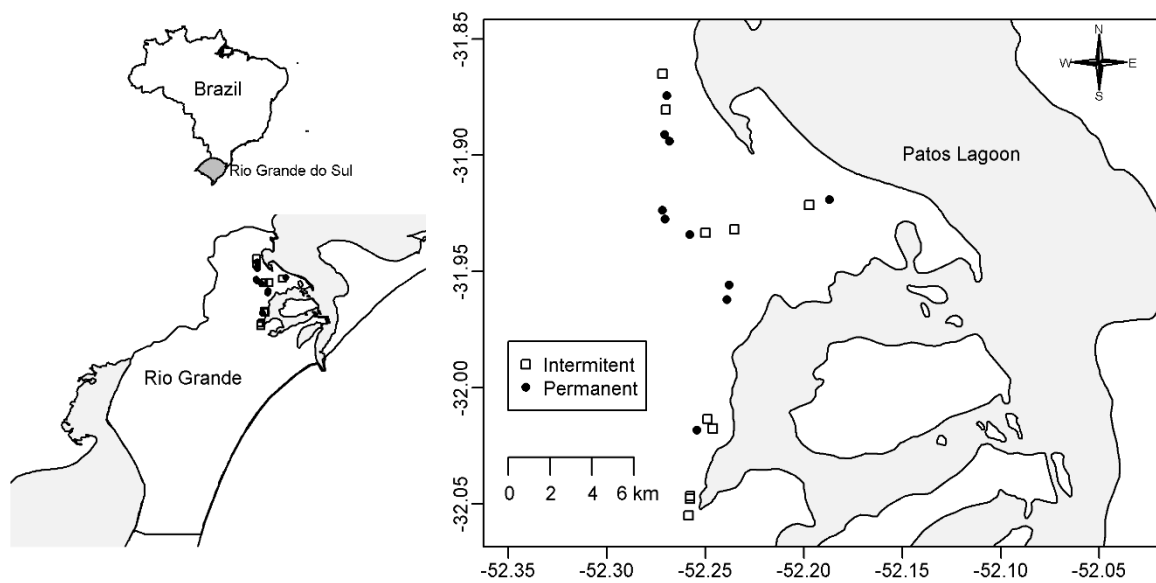


Figure 1. Intermittent and permanent ponds sampled in southern Brazil

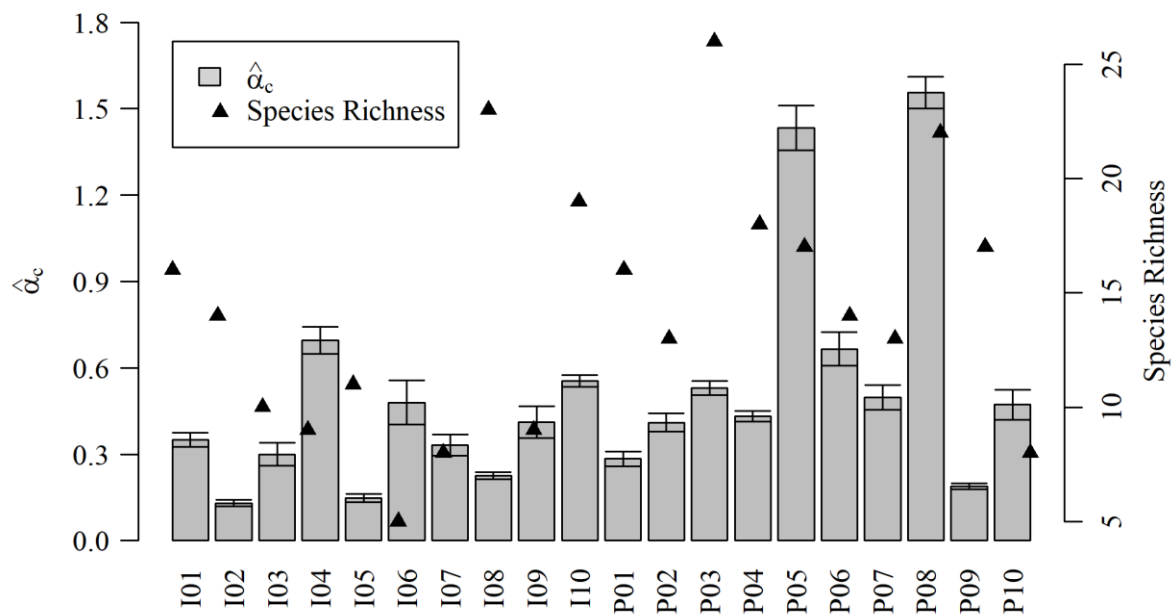


Figure 2. Richness and community indexes of Lombardo and Mjelde $\pm$ standard deviation ($\hat{\alpha}_c \pm SE\hat{\alpha}_c$) of each intermittent (I) and permanent (P) pond sampled in southern Brazil

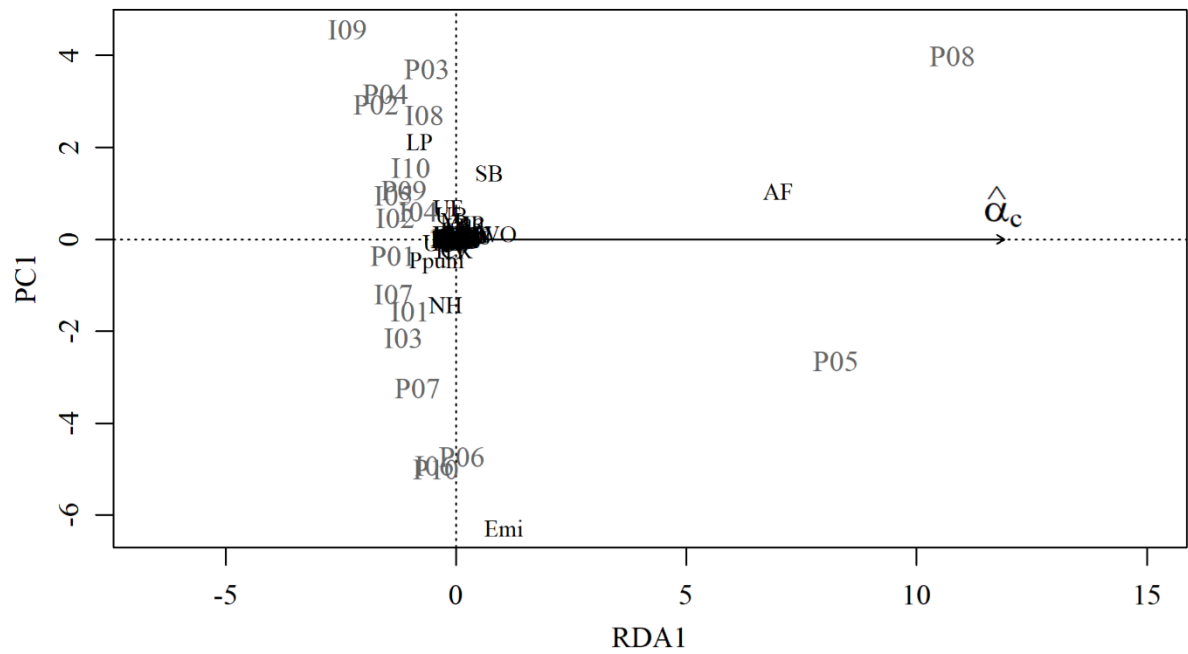


Figure 3. Redundancy analysis between the cover matrix of aquatic macrophyte species sampled in ponds and the spatial overlap index of communities

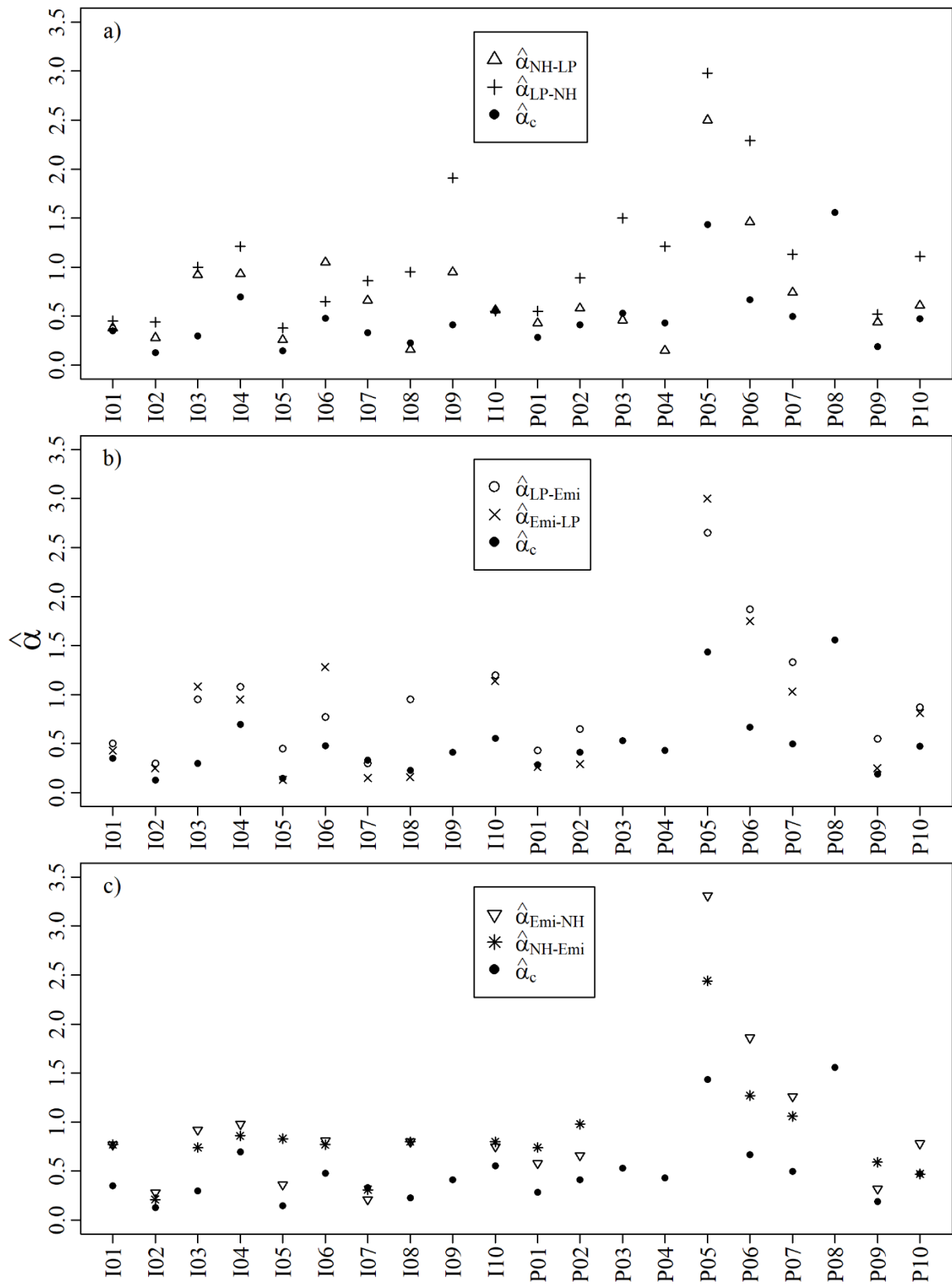


Figure 4. Lombardo and Mjelde indexes ($\hat{\alpha}_{ij}$) among the three most frequent species of aquatic macrophytes sampled in ponds of southern of Brazil. NH: *Nymphoides humboldtiana*, LP:

Luziola peruviana, Emi: *Eleocharis minima*. a) Indexes between NH and LP, b) LP and Emi, and c) Emi and NH

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1 **CAPÍTULO 4 – Aquatic macrophytes of ponds in two physiographic regions of southern**
2 **Brazil: floristic, assemblage structure, and beta diversity.**

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Aquatic macrophytes of ponds in two physiographic regions of southern Brazil: floristic, assemblage structure, and beta diversity.

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ABSTRACT

Aquatic macrophyte assemblages play a fundamental role in pond functioning. Knowing the diversity and distribution of species promotes more effective conservation and management. This study aimed to: 1) survey aquatic macrophytes of ponds in two physiographic regions of southern Brazil (*Encosta do Sudeste* and the *Litoral*); 2) examine the differences in richness, composition, and beta diversity of aquatic macrophytes between these regions; 3) assess whether the structure of functional groups (amphibious, emergent, and hydrophyte) differs between these regions; and 4) identify the pattern of beta diversity generation (turnover or nestedness). To accomplish these aims, we sampled aquatic macrophytes in 40 ponds (20 in each region). 243 Species were recorded, which were distributed between 119 genera and 55 families. Total species richness and composition were different between the physiographic regions. The richness and composition of amphibious species, as well as the composition and beta diversity of emergent species, differed between regions, no differences were found for hydrophyte species. Turnover was the main beta diversity generating pattern. We concluded that these physiographic regions are unique in terms of aquatic macrophytes assemblage structure and ponds of the *Encosta do Sudeste* should receive greater attention to conserve biodiversity.

Keywords: *Encosta do Sudeste*, the *Litoral*, functional groups, species richness, species composition, beta diversity

1 INTRODUCTION

2 Aquatic macrophytes play an important role in the functioning of aquatic ecosystems,
3 promoting high productivity and consequently supporting complex trophic webs, for example,
4 and participating in nutrient cycling (Rejmankova 2011). The neotropical region is the world's
5 largest hotspot for aquatic macrophyte diversity (Chambers et al. 2008; Murphy et al. 2019).
6 Approximately 60% of the aquatic macrophyte species found in the neotropics are endemic to
7 this region (Chambers et al. 2008). Floristic studies on the aquatic flora of Brazil have been
8 carried out in order to contribute to our knowledge of this group of organisms and their
9 conservation (e.g. Pott & Pott 1997, Bove et al. 2003, Pivari et al. 2011, Moura et al. 2015,
10 Pinheiro & Jardim 2015, Ferreira et al. 2017, Oliveira et al. 2019). Such studies are important
11 because, in addition to supporting other ecological studies, they reveal the group's diversity,
12 geographic distribution, and possible patterns about environmental and spatial variables
13 (Moura-Júnior et al. 2013).

14 Assessing beta diversity (heterogeneity among communities) is important for management
15 and conservation. Beta diversity associates the local diversity (alpha diversity) with regional
16 diversity (gamma diversity) of communities (Whittaker 1972). Beta diversity can result from
17 two distinct processes: turnover and nestedness. The turnover component indicates
18 replacement of one or more species between communities (Legendre et al. 2005) while
19 nestedness indicates a change in richness, with loss or gain of species (poorer communities
20 would be subsets of richer ones) (Ulrich & Gotelli 2007). In theory, environmental
21 heterogeneity (environmental filter), competition, and/or historical events can explain
22 turnover patterns, while different species dispersion abilities (spatial filter) can generate
23 nestedness (Hubbell 2001; Leibold et al. 2004; Legendre 2014). Disassociating the
24 components of beta diversity can support an understanding of ecological processes such as the
25 composition, structure, and functioning of communities. Studies on the beta diversity of

1 aquatic macrophyte communities have increased in recent decades (Boschilia et al. 2016;
2 Fernández-Aláez et al. 2020; García-Girón et al. 2020).

3 Wetlands are favorable environments for the development of aquatic macrophytes. They
4 perform various ecosystem services, such as the regulation and water supplementation of
5 aquatic bodies, reducing the risk of flooding, recharging of aquifers and groundwater, water
6 supply for human populations and animals, sediment retention, storage of organic carbon, and
7 ecotourism and recreation (Cunha et al. 2014). However, it is estimated that approximately
8 30% of these areas have been lost to human activities only in 2009 worldwide (Hu et al. 2017)
9 and more than 60% since 1900 AD (Davidson 2014). Coastal wetlands are the most
10 vulnerable areas (Darrah et al. 2019).

11 Southern Brazili wetlands are generally shallow and extensive, near coastal regions, which
12 favor the establishment and coexistence of a high diversity of aquatic macrophytes of
13 different functional groups (Esteves 1998; Chambers et al. 2008). Two regions of southern
14 Brazil, *Encosta do Sudeste* and the *Litoral*, have a large number of wetlands, compared to
15 other regions (Maltchik 2003). Nonetheless, agriculture, mainly of irrigated rice (Magalhães
16 et al. 2004), and forestry of pine (Rolon et al. 2011b) are the main threats to the integrity of
17 the ponds of these regions. These regions have different geological formations (Fortes 1959)
18 which could distinguish macrophyte diversity.

19 Studies on the diversity of aquatic macrophytes of the *Litoral* are more common (e.g. Mauhs
20 et al. 2006; Rolon et al. 2008; Trindade et al. 2018; Bertuzzi et al. 2019), especially at *Lagoa*
21 *do Peixe* (Rolon et al. 2008; Rolon et al. 2011; Rolon et al. 2013), a Ramsar Site, and at
22 *Estação Ecológica do Taim* (Irgang et al. 1984; Ferreira et al. 2009; Pereira 2015). However,
23 in *Encosta do Sudeste*, the few existing studies on aquatic macrophytes are concentrated in
24 the wetlands of Rio Grande Municipality (Kafer et al. 2011; Pereira et al. 2012a; Pereira et al.
25 2012b). However, it is important to note that more wide-reaching studies were carried out by

Irgang & Gastal-Jr (1996), Rolon and Maltchik (2006), Rolon et al. (2010) and Oliveira et al. (2019).

Due to the geomorphological differences between the *Encosta do Sudeste* and the *Litoral*, combined with the lack of knowledge about the structure of aquatic macrophytes of *Encosta do Sudeste*, and due to the importance of understanding aquatic macrophyte diversity in wetlands for biodiversity conservation, the aims of this study were to: 1) survey the aquatic macrophytes in ponds of the two physiographic regions of southern Brazil (*Encosta do Sudeste* and the *Litoral*); 2) evaluate the differences in richness, composition, and beta diversity of aquatic macrophytes between these regions; 3) verify whether the structure of functional groups (amphibious, emergent, and hydrophytes) differs between these regions, since these groups can respond to ecological processes in different ways (Trindade et al. 2018) and consequently have different structuring patterns (Catian et al. 2018); and 4) identify the pattern of beta diversity generation (turnover or nestedness) in assemblages of the physiographic regions and functional groups. We hypothesized that the richness, composition, and beta diversity will differ between these physiographic regions and that the patterns found will differ among functional groups of aquatic macrophytes.

MATERIALS AND METHODS

Study area

The study was carried out in 40 natural freshwater ponds of two physiographic regions of *Rio Grande do Sul* - Brazil: 20 in the *Encosta do Sudeste* and 20 in the *Litoral* (Fortes 1959) (Fig. 1). These regions border two large coastal lagoons (*Lagoa dos Patos* and *Mirim*), where the *Encosta do Sudeste* is located to the west and the *Litoral* to the east of the lagoons. Although they have distinct geological formations, these regions have similar sizes, with *Encosta do Sudeste* occupying 14,981 km² and the *Litoral* 14,905 km², according to Fortes (1959). The

Encosta do Sudeste is mainly composed of a depositional system of alluvial fans that were probably formed between the upper quaternary and tertiary periods, while the *Litoral* region was formed by lagoon-barrier depositional systems within the last 400,000 years (Villwock & Tomazelli 2007). The ground of *Encosta do Sudeste* is mostly flat and the altitude generally remains below 30 m, but there are also rocky spurs reaching up to 200 m (Brena 2002). The ground of the *Litoral* is flat to slightly wavy and the altitude is low (from 5 to 10 m) (Brena 2002).

The ponds were sampled on private properties with the prior authorization of the owners. They were small (< 2 ha; mean, 0.58 ha), shallow (<1.5 m depth), and were distributed at least 10 km from each other. According to (Junk et al. 2014), the ponds can be classified as Coastal wetlands separated from the sea with fluctuating water level, temporary lakes and ponds. The climate in these regions is moist subtropical. The mean annual temperature varies between 16 and 20 °C, the mean warmest month varies between 22 and 26 °C and the mean coldest month varies between 10 and 15 °C (Nimer 1977). Annual rainfall ranges from 1000 to 1500 mm (Villwock & Tomazelli 2007).

Data collection

Aquatic macrophytes were collected from the ponds between January and April 2017. Non-linear time-based transects (3 min each transect) were performed as described by Rolon et al. (2012), traversing the ponds on foot, to record macrophyte presence. The transects followed the variation in the pond's structure (at the border of the pond and from the border towards the center of the ponds). To achieve sampling sufficiency, the number of transects varied according to the size and heterogeneity of the ponds. The sampling was exhaustive, ending after two sequential transects (6 min) without a new species being recorded (Rolon et al. 2012). Our sampling was careful concerning the structural heterogeneity of the environments,

as to the functional groups. We consider that the lack of register of a new occurrence of species in 6 min indicated the sample sufficiency. The number of transects ranged from 5 to 34 transects (mean 11.7). Species were identified in the field and in the laboratory, and then fertile specimens were herborized and included in the HURG herbarium (*Herbário Universidade do Rio Grande*). Specimens of common or rare species were recorded only photographically. The taxonomic framework followed the classification outlined in APG IV (2016) for angiosperms and Smith et al. (2006) for pteridophytes. The scientific names of the species are in agreement with Flora do Brasil (2020). The broad concept of aquatic macrophytes, which includes all occurring plants in wet environments (Cronk & Fennessy 2001), was used to sample the species.

Aquatic plants were classified in three functional groups for data analysis: amphibious, emergent, and hydrophytes (floating and submerged). The classification was based on Irgang & Gastal Jr (1996) and on the biological forms presented in the field for species not listed by these authors. Amphibious are plants that survive in hydromorphic soils and tolerate long periods of drought, they are generally situated at the limits of flooding from water bodies as they cannot withstand long periods of submergence (Cronk & Fennessy 2001). Emergent species are rooted in the substrate and their vegetative and reproductive structures emerge from the water column (Lacoul & Freedman 2006). Epiphytes (which live on other aquatic plants without rooting in the substrate) and lianas (which are rooted in the substrate but need support from other plants) were treated as emergent macrophytes in the data analysis due to a small number of species. Hydrophytes are floating or submerged aquatic plants which are highly dependent on water. Floating plants need water to support their plant structures, and can be free (not rooted in the sediment) or rooted with floating leaves (Cronk & Fennessy 2001). The submerged plants are highly dependent on water, both morphologically and physiologically, since they survive totally immersed (free or rooted), being able to expose

only their reproductive parts to the air (Cronk & Fennessy 2001). In terms of water dependence, there is a gradual increase in degree of dependence for the respective groups (amphibious, emergent, and hydrophytes) if we consider aspects such as species' tolerance to periods of drought, spread of propagules, morphology, and physiology. The term "all macrophytes" refers to the set of all sampled aquatic macrophytes, regardless of functional group.

Data Analysis

To assess differences in the richness of aquatic macrophytes (all macrophytes and functional groups) between the physiographic regions, means comparison tests (parametric and non-parametric, depending on the assumptions of the tests) were used. The assumptions of normality and homoscedasticity of the t-test were verified using the Shapiro-Wilk and Levene tests, respectively. For non-parametric data, the Wilcoxon-Mann-Whitney test was used for independent samples. To verify differences in the composition of aquatic macrophytes (all macrophytes and functional groups) between physiographic regions, Permutational Multivariate Analysis of Variance (PERMANOVA) was performed, using the Jaccard index to calculate the distance matrices between the sampled sites. Multivariate Analysis of Variance tests were performed using the "adonis" function of the "vegan" package in the R environment (Oksanen et al. 2016). To assess the dissimilarity (beta diversity) of species composition between the ponds of the physiographic regions, Multivariate Dispersion Homogeneity Tests were performed (PERMDISP; Anderson et al. 2006), followed by a permutation test with 4999 permutations. This approach has been widely used as a means to assess beta diversity between sample points (Anderson et al. 2006). These analyses were performed using the "betadisper" and "permutest" functions of the "vegan" package in the R environment (Oksanen et al. 2016). The beta diversity was also evaluated using Baselga's

(2010) method, which partitions the overall beta diversity (β_{JAC}) into turnover (β_{SIM}) and nestedness (β_{NES}) components. Partitioned beta diversity allows to test if there was species replacement among communities (turnover) or if the poorer sites are a subset of richer ones (nestedness) (Baselga 2010). This analysis was performed for all the 40 ponds and for each one of the physiographic regions, as well as for all macrophytes, amphibious, emergent and hydrophyte species. The function “beta.multi” and the Jaccard dissimilarity index of the “betapart” package in the R environment were used to partition beta diversity (Baselga 2010). An Analysis of Indicator Species (De Cáceres et al. 2010) was performed using the “multipatt” function of the “indicspecies” package in the R environment (Cáceres & Legendre 2009) to identify possible species of aquatic macrophytes that characterize the ponds of each of the physiographic regions.

The presence and absence data for the species were ordered using Principal Coordinate Analysis (PCoA) to visualize the patterns and amplitudes of species distribution between the physiographic regions and in each physiographic region. The Jaccard similarity coefficient was used to generate distance matrices among all sites for this analysis. For graphic purposes, only the indicator species and those most closely related to the PCoA axes were displayed. All analyses were performed for all macrophytes and for each one of the functional groups (amphibious, emergent, and hydrophytes).

RESULTS

A total of 243 species of aquatic macrophytes were recorded in the ponds of the physiographic regions *Encosta do Sudeste* and the *Litoral*, distributed in 119 genera and 55 families (Tab.1). The most representative families in terms of number of species were the Cyperaceae (53), Poaceae (38), Asteraceae (29), Fabaceae (10), Juncaceae (9) and Apiaceae (7), and 31 were monospecific (13% of species) (Fig. 2). The most representative genera were

1 *Cyperus* (21) and *Eleocharis* (17). Of the total species, 185 were amphibious, 41 were
 2 emergents, and 17 were hydrophytes (Tab. 2). In the *Encosta do Sudeste* region, 221 species
 3 were recorded (mean = 40.7; standard deviation = 13.3) and there were 138 species in the
 4 *Litoral* (mean = 28.8; standard deviation = 8.7) (Tab. 2). 105 Species were exclusive to
 5 *Encosta do Sudeste*, while 22 were exclusive from *Litoral*.
 6 The species richness was different between the physiographic regions ($F = 11.15$; $p = 0.002$),
 7 the greater being found in *Encosta do Sudeste* (Tab. 3). The species composition was also
 8 different ($F = 2.68$; $p = 0.001$), but there was no difference in beta diversity ($F = 0.426$; $p =$
 9 0.533 ; Fig. 3A) between the physiographic regions. Regarding the functional groups, for the
 10 amphibious group, richness and composition differed between physiographic regions,
 11 richness being greater in the *Encosta do Sudeste* (Tab. 3), but beta diversity was similar
 12 between the regions ($F = 0.325$; $p = 0.535$; Fig. 3B). As regards the emergent group,
 13 composition, and beta diversity ($F = 4.40$; $p = 0.043$; Fig. 3C) differed between the regions
 14 (Tab. 3); ponds of *Encosta do Sudeste* showed greater beta diversity. However, for the
 15 hydrophytes group, there were no differences in the richness, composition (Tab. 3), or beta
 16 diversity ($F = 0.335$; $p = 0.565$; Fig. 3D) between the regions.
 17 The range of multivariate dispersion was greater in the *Litoral* assemblages for all
 18 macrophytes, and amphibious and hydrophyte species. The overall beta diversity (β_{JAC}) in all
 19 the 40 ponds was 0.94 for all macrophytes, and amphibious and emergent species, and 0.92
 20 for hydrophytes. The β_{JAC} in *Encosta do Sudeste* was 0.89 for all macrophytes, 0.90 for
 21 amphibious and emergent species, and 0.87 for hydrophytes. The β_{JAC} in the *Litoral* was 0.89
 22 for all macrophytes, 0.89 for amphibious species, and 0.88 for emergent and hydrophyte
 23 species.
 24 The turnover component was greater than nestedness for all analyzed groups (Tab. 4). The
 25 turnover component of beta diversity (β_{SIM}) in all the 40 ponds was 0.92 for all macrophytes,

0.91 for amphibious and emergent species, and 0.85 for hydrophytes. The β_{SIM} in *Encosta do Sudeste* was 0.86 for all macrophytes and the amphibious group, 0.84 for emergent species, and 0.78 for hydrophytes. The β_{SIM} in the *Litoral* was 0.86 for all macrophytes, 0.85 for amphibious and emergent species, and 0.74 for hydrophytes species. The nestedness component of beta diversity (β_{NES}) in all the 40 ponds was 0.02 for all macrophytes, 0.03 for amphibious and emergent species, and 0.07 for hydrophytes. The β_{NES} in *Encosta do Sudeste* was 0.03 for all macrophytes, 0.04 for the amphibious group, 0.06 for emergent species, and 0.09 for hydrophytes. The β_{NES} in the *Litoral* was 0.03 for all macrophytes, 0.04 for amphibious species, 0.03 for emergent species, and 0.14 for hydrophyte species.

The indicator species of the *Encosta do Sudeste* were: *Cyperus lanceolatus* Poir., *Hygrophila costata* Ness, *Fimbristylis complanata*, *Heteranthera reniformis* Ruiz & Pav., *Eryngium chamissonis* Urb., *Setaria parviflora* (Poir.) Kerguelen, *Pluchea sagittalis* (Lam.) Cabrera, *Eragrostis plana* Nees, *Mikania micrantha* Kunth, *Paspalum urvillei* Steud., *Eleocharis montana* (Kunth) Roem. & Schult., *Commelina diffusa* Burm.f., *Borreria palustris* (Cham. & Schltdl.) Bacigalupo & E.L.Cabral, *Hyptis* sp. and *Ludwigia multinervia* (Hook. & Arn.) Ramamoorthy (Anexo 6). The indicator species of the *Litoral* were: *Rhynchospora holoschoenoides* (Rich.) Herter, *Echinodorus grandiflorus* (Cham. & Schltr.) Micheli, *Ranunculus bonariensis* Poir., *Helanthium tenellum* (Martius) Britton and *Eriocaulon modestum* Kunth (Anexo 7).

In addition to the indicator species, the Principal Coordinate Analysis revealed that the species with a close relationship with Axis 1 of the PCoA were: *E. modestum* and *Eleocharis geniculata* Roem. & Schult. (positively related), and *Carex longii* Mackenz. and *Carex bonariensis* Desf. Ex Poir. (negatively related). This axis separated the physiographic regions, with the *Litoral* being positive and the *Encosta do Sudeste* negative.

DISCUSSION

The richness of aquatic macrophytes found in this study can be considered representative for southern Brazil, since the richness found in studies covering wider areas recorded similar numbers: 114 in the *Litoral* (Trindade et al. 2018), 331 species in the Coastal Plain of *Rio Grande do Sul* (Irgang & Gastal-Jr 1996), 153 and 250 in *Rio Grande do Sul* State (Rolon & Maltchik 2006; Rolon et al. 2010, respectively) and 760 in southern Brazil (Oliveira et al. 2019). In addition, six other species (*Callitriche rimosa* Fassett, *Micranthemum umbrosum* (Walter ex J.F.Gmel.) S.F.Blake, *Potamogeton ferrugineus* Hagstr., *Ranunculus apiifolius* Pers., *R. flagelliformis* Sm., and *Triglochin scilloides* (Poir.) Mering & Kadereit) were recorded in the *Litoral* in another study carried out by the authors (KM Pereira unpubl. res.), totaling 249 species, and 144 for this region. Other studies carried out in both physiographic regions (*Encosta do Sudeste* and *Litoral*) on smaller scales have obtained lower richness values: 56 in *Rio dos Sinos* floodplain (Maltchik et al. 2010), 18 in a wetland of *Palmares do Sul* (Mauhs et al. 2006), 43 in six lakes of *Rio Grande* Municipality (Pereira et al. 2012a, Pereira et al. 2012b), 45 in wetlands of *Tapes* Municipality (Rolon et al. 2017), 82 in a wetland of *Rio Grande* Municipality (Kafer et al. 2011), and 105 and 176 in *Lagoa do Peixe* (Rolon et al. 2008; Rolon et al. 2011). However, the local character, the sampling effort, and the types of wetlands sampled should be taken into account when considering these values. The Cyperaceae, Poaceae, and Asteraceae were also representative families in these studies (Mauhs et al. 2006; Maltchik et al. 2010; Rolon et al. 2011; Rolon et al. 2017). *Encosta do Sudeste* and the *Litoral* differed in respect of richness, species composition, and set of indicator species of all aquatic macrophyte species in the ponds. This indicates that these physiographic regions are peculiar in terms of the structure of aquatic macrophyte assemblages. The *Encosta do Sudeste* had greater richness than the *Litoral*, indicating that *Encosta do Sudeste* is a promising region for better understanding the structure of these

1 assemblages, as still little is known about this region. Greater geomorphological age may be
2 one of the causes of the greater species richness of *Encosta do Sudeste* (Villwock &
3 Tomazelli 2007), which can affect the colonization history of species (Alahuhta et al. 2018).
4 However, other environmental factors, like water and sediment characteristics, climate,
5 hydrology, and environmental heterogeneity, may be acting to promote this greater diversity
6 (Lacoul & Freedman 2006, Rolon et al. 2008, Stein et al. 2014, García-Girón et al. 2020).
7 Differences in the structure of functional groups were mainly found for amphibious and
8 emergent species. Brazilian tropical and subtropical wetlands contain a large diversity of
9 amphibious and emergent species (Bove et al. 2003, Cervi et al. 2009, Rolon et al. 2010, Pott
10 et al. 2011), which supports the percentages found for the functional groups of this study. This
11 result is probably related to the characteristics of the ponds analyzed, which are small and
12 shallow, and mostly intermittent, favoring the establishment of amphibious species and
13 limiting the occurrence of hydrophytes (Rolon et al. 2010, Magalhaes et al. 2013). The high
14 diversity of amphibious and emergent groups occurs mainly in the outer zone of the wetlands
15 (Murray-Hudson et al. 2012, Magalhaes et al. 2016), which is extensive in the southern
16 ponds. Besides, beta diversity differed between the physiographic regions concerning the
17 emergent group; the greater beta diversity was observed in *Encosta do Sudeste*.
18 Similar statistical values of beta diversity for aquatic macrophytes (for all macrophytes,
19 amphibious, and emergent species) in the ponds of the physiographic regions indicate that the
20 species in these assemblages disperse/replace species in a similar way. However, even though
21 there are no significant differences in beta diversity between the regions, the greater
22 amplitude of variation in the distances to the group centroid found in the Litoral indicate that
23 the aquatic macrophytes assemblages in that region are more heterogeneous among
24 themselves than the assemblages found in the Encosta do Sudeste. Significant differences in

1 beta diversity are not always observed among assemblages of aquatic macrophytes (Bubíková
2 & Hrivnák 2018).

3 As regards the assemblages of emergent macrophytes (a group that is abundant in the
4 wetlands of southern Brazil), significant differences were found in terms of beta diversity,
5 with the Encosta do Sudeste being more heterogeneous than the Litoral. It is to be expected
6 that there are more conservation facilities in regions with high values of beta diversity to
7 safeguard their greater floristic diversity than those regions with less beta diversity (Felfili &
8 Felfili 2001) but in Encosta do Sudeste there is only the Camaquã State Park. However, there
9 are no published studies on the Park's diversity of aquatic macrophytes already, and neither
10 concrete conservation strategies. Besides, the heterogeneity of emergent species composition
11 could be a result of environmental heterogeneity (Stein et al. 2014), which needs to be
12 investigated and thereafter conserved. In the Litoral, the Taim Ecological Station and the
13 Lagoa do Peixe National Park are the areas that are most well-known by the scientific
14 community (Irgang et al. 1984; Rolon et al. 2011; Rolon et al. 2013).

15 Higher values for the turnover component of beta diversity than for nestedness component
16 indicate that there is species replacement among aquatic macrophyte assemblages in the
17 ponds of these physiographic regions (Baselga 2010). It implies that not only conservation
18 units (as sources of diversity), but also small ponds are important sites for the conservation of
19 aquatic macrophyte species (Deane & He 2018), especially ponds in the analyzed
20 physiographic regions, whose integrity must be maintained. Turnover generally presents
21 higher values than nestedness in the beta diversity of aquatic macrophytes (Boschilia et al.
22 2016; Schneider et al. 2019; Sun et al. 2019; Fernández-Aláez et al. 2020, Nunes et al. 2021).

23 The fact that turnover is the main generator of beta diversity indicates that environmental
24 heterogeneity, competition, or historical events are probably the main influencing factors in
25 the structure of the aquatic macrophyte assemblages in the physiographic regions of this study

(Leibold et al. 2004; Legendre 2014). Physiography explained the differences in species richness and composition, as seen in the results of the means comparison and PERMANOVA tests, reflecting the influence of historical geological formation events on colonization by aquatic macrophyte species (Swanson et al. 1988). Even though environmental factors and interspecific interactions have not been evaluated in this study, they probably influence the structuring of these assemblages. Schneider et al. (2019) found that hydrogeomorphological variables, such as connectivity, distance to the river, lake altitude, and water level were the largest factors influencing the turnover component of beta diversity in aquatic macrophytes of neotropical lakes, while spatial variables exclusively explained the nestedness component. Fernández-Aláez et al. (2020) reported that local environmental conditions, such as ionic content (calcium, alkalinity, conductivity) and soluble reactive phosphorus, made a greater contribution than spatial distances among sites to beta diversity patterns.

As regards the hydrophytes the nestedness portion was relatively higher than the nestedness portion of the other functional groups, which may indicate that a spatial dispersion limitation affects this group. Hydrophyte species disperse their diaspores mainly by hydrocoria while the wind is the main disperser of amphibious and emergent species (Cronk & Fennessy 2001; Soons 2006). Wind dispersion can carry seeds greater distances than water dispersion (Soomers et al. 2013), which can result in hydrophytes being subject to spatial limitation. Nunes et al. (2021) verified that the nestedness portion was important when the life forms of macrophytes was analyzed in coastal rivers, although the turnover was most important for all macrophytes.

Concerning the conservation of aquatic macrophytes, it is important to mention that some of the species found in these regions are on the Red List of Brazil Flora maintained by the *Centro Nacional de Conservação da Flora* – CNCFLORA (Martinelli & Moraes 2013). Among the species of concern are *Thalia multiflora* Horkel ex Körn. and *Pleroma asperior*

(Cham.) Triana, which are categorized as data deficient (DD), *Lobelia hederacea* Cham., classified as near threatened (NT), *Lilaeopsis brasiliensis* (Glaz.) Affolter, considered to be vulnerable (VU), and *Schwenckia curviflora* Benth., which is classified as endangered (EN). In the Zoobotany Foundation of *Rio Grande do Sul*'s Red List of threatened species, *Cyperus berroi* (C.B.Clarke) Barros and *Eriocaulon modestum* Kunth are classified as vulnerable, *T. multiflora* as critically endangered (CR), and *P. asperior* as endangered (Anexo 8). This threat states reinforces the importance of conserving wetlands in these physiographic regions. We can conclude that the ponds of the physiographic regions *Encosta do Sudeste* and *Litoral* are unique in aquatic macrophyte assemblage structure in terms of species richness and composition. However, the physiographic region whose aquatic plant assemblages are still little known (*Encosta do Sudeste*) displayed an absolute richness that was superior to the region that is more widely studied (the *Litoral*) and had higher values of beta diversity for emergent species. This reveals that *Encosta do Sudeste* could be a promising area for the study of aquatic macrophytes and important for the conservation of these species. The amphibious and emergent functional groups were the groups with the highest representation in terms of species richness, due to the ponds of these areas being both small and shallow. These groups showed differences in species richness and composition between the physiographic regions. The main beta diversity generating pattern was turnover. Finally, we conclude that maintaining the integrity of the ponds of these regions, jointly with increasing the number of conservation units, is important for the conservation of aquatic macrophytes, especially those threatened with extinction.

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Table 1 Family, species list, functional groups (Amphibious, Emergent, and Hydrophyte), frequency percentage of the occurrence, and voucher (*Herbarium* HURG) of aquatic macrophytes sampled in subtropical ponds of two physiographic regions. The species with an “*” were collected in a complementary survey.

Family	Taxon	Functional Group	Voucher HURG	Frequency % in Encosta do Sudeste	Frequency % in Litoral
Acanthaceae	<i>Hygrophila costata</i> Ness	Amphibious	6992, 6993	70	25
Alismataceae	<i>Echinodorus grandiflorus</i> (Cham. & Schltr.) Micheli	Emergent	6995	15	55
	<i>Helanthium tenellum</i> (Martius) Britton	Emergent	6994, 6996, 6997	5	40
	<i>Sagittaria montevidensis</i> Cham. & Schltdl.	Emergent	6998, 6999	30	20
Amaranthaceae	<i>Alternanthera philoxeroides</i> (Mart.) Griseb.	Emergent	7000	20	20
Apiaceae	<i>Centella asiatica</i> (L.) Urb.	Amphibious	7002, 7003	95	85
	<i>Eryngium ebracteatum</i> Lam.	Amphibious	7004, 7005	0	10
	<i>Eryngium eburneum</i> Decne.	Amphibious	7006, 7007	5	5
	<i>Eryngium elegans</i> Cham. & Schltdl.	Amphibious	7008	5	0
	<i>Eryngium chamissonis</i> Urb.	Amphibious	7009, 7010	40	0
	<i>Eryngium</i> sp.	Amphibious	7011	35	20
	<i>Lilaeopsis brasiliensis</i> (Glaz.) Affolter	Emergent	7012	15	5
Apocynaceae	<i>Rhabdadenia madida</i> (Vell.) Miers	Emergent	7014	5	0
Araceae	<i>Lemna</i> cf. <i>valdiviana</i> Phil.	Hydrophyte	-	20	30
	<i>Pistia stratiotes</i> L.	Hydrophyte	7016	10	15
	<i>Spirodela intermedia</i> W. Koch	Hydrophyte	-	20	5
	<i>Wolffia brasiliensis</i> Wedd.	Hydrophyte	-	20	15
	<i>Wolffiella oblonga</i> (Phil.) Hegelm.	Hydrophyte	-	20	25
Araliaceae	<i>Hydrocotyle bonariensis</i> Lam.	Amphibious	-	55	35
	<i>Hydrocotyle ranunculoides</i> L.f.	Amphibious	7017, 7018	30	30

Asteraceae	<i>Hydrocotyle verticillata</i> Thunb.	Amphibious	7019, 7020	15	5
	<i>Achyrocline satureioides</i> (Lam.) DC.	Amphibious	7021	5	0
	<i>Aspilia montevidensis</i> (Spreng.) Kuntze	Amphibious	7022	5	0
	<i>Baccharis crispa</i> Spreng.	Amphibious	7001, 7031, 7032	20	5
	<i>Baccharis penningtonii</i> Heering	Amphibious	7033	5	0
	<i>Baccharis sagittalis</i> (Less.) DC.	Amphibious	7034	5	0
	<i>Baccharis</i> sp.	Amphibious	-	0	5
	<i>Chaptalia</i> sp.	Amphibious	7035	5	0
	<i>Chromolaena laevigata</i> (Lam.) R.M.King & H.Rob.	Amphibious	7037	10	0
	<i>Conyza</i> sp.	Amphibious	7038, 7039	20	0
	<i>Eclipta elliptica</i> DC.	Amphibious	7040	5	0
	<i>Eclipta prostrata</i> (L.) L.	Amphibious	7041, 7042	20	5
	<i>Enydra anagallis</i> Gardner	Emergent	7043	35	40
	<i>Gamochaeta</i> sp.	Amphibious	7044, 7045	15	0
	<i>Mikania micrantha</i> Kunth	Emergent	7046, 7047	35	0
	<i>Mikania</i> sp.	Emergent	-	10	0
	<i>Orthopappus angustifolius</i> (Sw.) Gleason	Amphibious	7048	5	0
	<i>Pluchea sagittalis</i> (Lam.) Cabrera	Amphibious	7049, 7050	50	15
	<i>Pterocaulon balansae</i> Chodat	Amphibious	7051, 7052	10	0
	<i>Pterocaulon rugosum</i> (Vahl) Malme	Amphibious	7053	5	0
	<i>Senecio bonariensis</i> Hook. & Arn.	Amphibious	7054, 7055	15	0
	<i>Senecio</i> cf. <i>grisebachii</i> Backer	Amphibious	7028	25	5
	<i>Senecio</i> sp.	Amphibious	7027	5	0
	<i>Solidago chilensis</i> Meyen	Amphibious	7056	5	0
	<i>Stenachaenium</i> sp.	Amphibious	7057, 7058	5	0
	<i>Symphyotrichum squamatum</i> (Spreng.) G.L.Nesom	Amphibious	-	5	0
	Asteraceae (sp. 1)	Amphibious	7023	5	0
	Asteraceae (sp. 2)	Amphibious	7024, 7025	5	0
	Asteraceae (sp. 3)	Amphibious	7026	5	0
	Asteraceae (sp. 4)	Amphibious	7029, 7030	10	0

Begoniaceae	<i>Begonia cucullata</i> Willd.	Amphibious	7059	5	0
Blechnaceae	<i>Blechnum</i> sp.	Amphibious	7060	0	5
Burmanniaceae	<i>Burmannia bicolor</i> Mart.	Amphibious	-	5	0
Cabombaceae	<i>Cabomba caroliniana</i> A.Gray	Submerged	-	75	65
Campanulaceae	<i>Lobelia hederacea</i> Cham.	Amphibious	7061, 7062	30	20
Caryophyllaceae	<i>Drymaria cordata</i> (L.) Willd. ex Roem. & Schult.	Amphibious	7063, 7064	30	0
Commelinaceae	<i>Commelina diffusa</i> Burm.f.	Amphibious	7065, 7066	10	0
	<i>Floscopa glabrata</i> (Kunth) Hassk.	Amphibious	7067, 7068	10	0
Convolvulaceae	<i>Ipomoea</i> sp.	Amphibious	-	10	0
Cyperaceae	<i>Carex bonariensis</i> Desf. Ex Poir.	Amphibious	7313	10	0
	<i>Carex longii</i> Mackenz.	Amphibious	7315, 7316, 7317	5	0
	<i>Carex polysticha</i> Boeckeler	Amphibious	7314	0	10
	<i>Cyperus berroi</i> (C.B.Clarke) Barros	Emergent	6340, 6341, 6342, 6343, 6344	15	5
	<i>Cyperus blepharoleptos</i> Steud.	Amphibious	6345, 6346, 6347, 6348	5	0
	<i>Cyperus brasiliensis</i> (Kunth) Bauters	Emergent	6349	20	0
	<i>Cyperus brevifolius</i> (Rottb.) Endl. ex Hassk.	Amphibious	6350, 6351, 6352	20	5
	<i>Cyperus eragrostis</i> Lam.	Amphibious	6353, 6354, 6355, 6356, 6357, 6358	5	0
	<i>Cyperus esculentus</i> L.	Emergent	-	10	0
	<i>Cyperus giganteus</i> Vahl	Emergent	6359	35	20
	<i>Cyperus haspan</i> L.	Amphibious	6360, 6361	30	5
	<i>Cyperus hermaphroditus</i> (Jacq.) Standl.	Amphibious	6375, 6376	5	0
	<i>Cyperus iria</i> L.	Amphibious	6385, 6386	60	5
	<i>Cyperus lanceolatus</i> Poir.	Amphibious	7070, 7071	20	10
	<i>Cyperus luzulae</i> (L.) Retz.	Amphibious	6387, 6388	50	55
	<i>Cyperus obtusatus</i> (J.Presl & C.Presl) Mattf. & Kük.	Amphibious	6395, 6396	20	5

<i>Cyperus odoratus</i> L.	Amphibious	6413, 6414, 6415	70	55
<i>Cyperus polystachyos</i> Rottb.	Amphibious	7072, 7073	0	5
<i>Cyperus reflexus</i> Vahl	Amphibious	6416	10	10
<i>Cyperus rigens</i> C.Presl	Amphibious	6417, 6418, 6419	0	5
<i>Cyperus rotundus</i> L.	Amphibious	6420	20	5
<i>Cyperus sesquiflorus</i> (Torr.) Mattf. & Kük.	Amphibious	6421, 6422, 6423, 6424	10	5
<i>Cyperus surinamensis</i> Rottb.	Amphibious	6425, 6426, 6427	35	10
<i>Cyperus trigynus</i> Spreng.	Emergent	7069	5	0
<i>Cyperus virens</i> Michx.	Amphibious	6428, 6429	0	15
<i>Eleocharis acutangula</i> (Roxb.) Schult.	Emergent	7074, 7075	10	20
<i>Eleocharis bonariensis</i> Nees	Emergent	7076, 7077	5	0
<i>Eleocharis contracta</i> Maury	Amphibious	7078	0	10
<i>Eleocharis dunensis</i> Kük.	Amphibious	7079, 7080	15	20
<i>Eleocharis flavescens</i> (Poir.) Urb.	Emergent	7081, 7082	0	5
<i>Eleocharis geniculata</i> (L.) Roem. & Schult.	Emergent	7083, 7084	5	0
<i>Eleocharis interstincta</i> (Vahl) Roem. & Schult.	Emergent	7085	35	25
<i>Eleocharis maculosa</i> (Vahl) Roem. & Schult.	Emergent	7086, 7087	45	70
<i>Eleocharis minima</i> Kunth	Emergent	7088, 7089	35	5
<i>Eleocharis montana</i> (Kunth) Roem. & Schult.	Emergent	7090, 7091	45	35
<i>Eleocharis obtusetrigona</i> (Lindl. & Nees) Steud.	Emergent	7092, 7093	5	0
<i>Eleocharis parodii</i> Barros	Emergent	7094	10	20
<i>Eleocharis sellowiana</i> Kunth	Emergent	7095, 7096	5	15
<i>Eleocharis viridans</i> Kük. ex Osten	Amphibious	7100	30	20
<i>Eleocharis</i> sp. 1	Amphibious	7097	5	0
<i>Eleocharis</i> sp. 2	Amphibious	7098	5	0
<i>Eleocharis</i> sp. 3	Amphibious	7099	55	5
<i>Fimbristylis complanata</i> (Retz.) Link	Amphibious	7101, 7102	20	5
<i>Fimbristylis dichotoma</i> (L.) Vahl	Amphibious	7103, 7104	0	5

	<i>Fimbristylis</i> sp.	Amphibious	7105	5	0
	<i>Fuirena robusta</i> Kunth	Emergent	-	15	0
	<i>Rhynchospora corymbosa</i> (L.) Britton	Amphibious	7106, 7107	5	50
	<i>Rhynchospora holoschoenoides</i> (Rich.) Herter	Amphibious	7108, 7109	35	40
	<i>Rhynchospora microcarpa</i> Baldwin	Amphibious	7110, 7111	35	25
	<i>Rhynchospora tenuis</i> Link	Amphibious	7113, 7114	5	0
	<i>Rhynchospora</i> sp.	Amphibious	7112	15	45
	<i>Schoenoplectus californicus</i> (C.A.Mey.) Soják	Emergent	7115, 7116	30	35
	<i>Scleria distans</i> Poir.	Amphibious	7117, 7118	5	15
Droseraceae	<i>Drosera brevifolia</i> Pursh	Amphibious	7119	0	25
Eriocaulaceae	<i>Eriocaulon modestum</i> Kunth	Amphibious	7120, 7121	10	5
Fabaceae	<i>Desmodium tortuosum</i> (Sw.) DC.	Amphibious	7122, 7123	15	0
	<i>Desmodium</i> sp.	Amphibious	-	25	10
	<i>Erythrina crista-galli</i> L.	Amphibious	-	10	10
	<i>Mimosa bimucronata</i> (DC.) Kuntze	Amphibious	-	20	30
	<i>Sesbania punicea</i> (Cav.) Benth.	Amphibious	7128, 7129	10	5
	<i>Vigna longifolia</i> (Benth.) Verdc.	Amphibious	7130, 7131	10	0
	<i>Vigna luteola</i> (Jacq.) Benth.	Amphibious	7132, 7133	10	0
	<i>Vigna</i> sp.	Amphibious	7134, 7135	15	5
	Fabaceae (sp. 1)	Amphibious	7124, 7125	10	0
	Fabaceae (sp. 2)	Amphibious	7126, 7127	40	30
Haloragaceae	<i>Myriophyllum aquaticum</i> (Vell.) Verdc.	Emergent	7136, 7137	20	10
Hydrocharitaceae	<i>Limnobium laevigatum</i> (Humb. & Bonpl. ex Willd.) Heine	Hydrophyte	7138, 7139	0	10
Iridaceae	<i>Sisyrinchium micranthum</i> Cav.	Amphibious	7140, 7141	5	0
Juncaceae	<i>Juncus densiflorus</i> Kunth	Amphibious	7142	5	0
	<i>Juncus effusus</i> L.	Amphibious	7143, 7144	15	5
	<i>Juncus marginatus</i> Rostk.	Amphibious	7145, 7146	50	55
	<i>Juncus microcephalus</i> Kunth	Amphibious	7147, 7148	10	0
	<i>Juncus pallescens</i> Lam.	Amphibious	7149	15	0
	<i>Juncus scirpoides</i> Lam.	Amphibious	7150, 7151	65	40

	<i>Juncus tenuis</i> Willd.	Amphibious	7156, 7157	5	15
	<i>Juncus</i> sp. 1	Amphibious	7152, 7153	5	5
	<i>Juncus</i> sp. 2	Amphibious	7154, 7155	25	0
Lamiaceae	<i>Hyptis</i> sp.	Amphibious	7158, 7159	20	50
Lentibulariaceae	<i>Utricularia gibba</i> L.	Hydrophyte	7160	5	5
	<i>Utricularia tricolor</i> A.St.-Hil.	Emergent	-	25	15
	<i>Utricularia</i> L.	Hydrophyte	-	70	30
Lythraceae	<i>Cuphea carthagenensis</i> (Jacq.) J.Macbr.	Amphibious	7161, 7162	5	0
	<i>Cuphea racemosa</i> (L.f.) Spreng.	Amphibious	7163	5	0
	<i>Heimia apetala</i> (Spreng.) S.A.Graham & Gandhi	Amphibious	7164	5	0
Malvaceae	<i>Hibiscus diversifolius</i> Jacq.	Amphibious	-	0	5
Marantaceae	<i>Thalia multiflora</i> Horkel ex Körn.	Emergent	7165	5	5
Marsileaceae	<i>Marsilea ancylopoda</i> A.Braun	Amphibious	7166, 7310, 7312	40	20
	<i>Regnellidium diphyllum</i> Lindm.	Amphibious	7167, 7168	10	20
Mayacaceae	<i>Mayaca fluviatilis</i> Aubl.	Emergent	7169, 7170	5	0
Melastomataceae	<i>Pleroma asperior</i> (Cham.) Triana	Amphibious	7171	65	80
Menyanthaceae	<i>Nymphoides humboldtiana</i> (Kunh) Kuntze	Amphibious	7172	5	0
Onagraceae	<i>Ludwigia elegans</i> (Cambess.) H.Hara	Amphibious	7173	5	0
	<i>Ludwigia erecta</i> (L.) H.Hara	Amphibious	7174	50	45
	<i>Ludwigia grandiflora</i> (Michx.) Greuter & Burdet	Emergent	7175, 7176	50	50
	<i>Ludwigia hexapetala</i> (Hook. & Arn.) Zardini et al.	Emergent	7177, 7178	25	0
	<i>Ludwigia multinervia</i> (Hook. & Arn.) Ramamoorthy	Amphibious	7180, 7181	35	15
	<i>Ludwigia</i> sp.	Amphibious	7179	5	0
Orchidaceae	<i>Habenaria parviflora</i> Lindl.	Amphibious	-	0	20
	<i>Habenaria</i> sp. 1	Amphibious	7182, 7183	5	0
	<i>Habenaria</i> sp. 2	Amphibious	7184	20	5
Orobanchaceae	<i>Agalinis communis</i> (Cham. & Schltdl.) D'Arcy	Amphibious	7185, 7186	5	0
Passifloraceae	<i>Passiflora</i> sp.	Amphibious	7187, 7188	5	25
Plantaginaceae	<i>Bacopa lanigera</i> (Cham. & Schltdl.) Wettst.	Emergent	7189, 7190	30	35
	<i>Bacopa monnieri</i> (L.) Pennell	Emergent	7191, 7192	20	25

Poaceae	<i>Gratiola peruviana</i> L.	Amphibious	7193, 7194	10	0
	<i>Mecardonia procumbens</i> (Mill.) Small	Amphibious	7195	5	0
	<i>Andropogon arenarius</i> Hack.	Amphibious	7196	10	0
	<i>Andropogon bicornis</i> L.	Amphibious	7197	10	15
	<i>Andropogon lateralis</i> Nees	Amphibious	7198, 7199	5	0
	<i>Andropogon lindmanii</i> Hack.	Amphibious	7200	10	20
	<i>Axonopus compressus</i> (Sw.) P. Beauv.	Amphibious	7201, 7202	20	25
	<i>Axonopus fissifolius</i> (Raddi) Kuhlm.	Amphibious	7203, 7204	5	0
	<i>Calamagrostis viridiflavescens</i> (Poir.) Steud.	Amphibious	7205	10	0
	<i>Cynodon dactylon</i> (L.) Pers.	Amphibious	7206	5	5
	<i>Echinochloa colona</i> (L.) Link	Amphibious	7207, 7208	5	0
	<i>Echinochloa crusgalli</i> (L.) P.Beauv.	Amphibious	7210	10	10
	<i>Eragrostis cataclasta</i> (L.) P.Beauv.	Emergent	7211, 7212	0	5
	<i>Eragrostis hypnoides</i> (Lam.) Britton, Sterns & Poggenb.	Amphibious	7213	40	5
	<i>Eragrostis plana</i> Nees	Amphibious	7214, 7215	5	30
	<i>Ischaemum minus</i> J.Presl	Amphibious	7216, 7217	45	45
	<i>Leersia hexandra</i> Sw.	Amphibious	7218, 7219	95	100
	<i>Luziola peruviana</i> Juss. ex J.F.Gmel.	Amphibious	7220, 7221	0	5
	<i>Oplismenopsis najada</i> (Hack. & Arechav.) Parodi	Emergent	7222, 7223, 7224	0	5
	Paniceae (sp. 1)	Amphibious	7225	25	40
	<i>Panicum dichotomiflorum</i> Michx.	Amphibious	7226, 7227, 7228, 7229	5	0
	<i>Panicum</i> sp. 1	Amphibious	7230	5	0
	<i>Panicum</i> sp. 2	Amphibious	7231	5	0
	<i>Panicum</i> sp. 3	Amphibious	7232	5	0
	<i>Panicum</i> sp. 4	Amphibious	-	5	0
	<i>Paspalum dilatatum</i> Poir.	Amphibious	7233	0	5
	<i>Paspalum durifolium</i> Mez	Amphibious	7234	0	5
	<i>Paspalum maculosum</i> Trin.	Amphibious	7235	0	10
	<i>Paspalum modestum</i> Mez	Amphibious	7236, 7237	5	5

	<i>Paspalum notatum</i> Flügge	Amphibious	7238, 7239	15	15
	<i>Paspalum pumilum</i> Nees	Amphibious	7240, 7241	35	0
	<i>Paspalum urvillei</i> Steud.	Amphibious	7243, 7244	5	5
	<i>Paspalum</i> sp.	Amphibious	7242	5	5
	<i>Sacciolepis vilvoides</i> (Trin.) Chase	Amphibious	7247, 7248	5	0
	<i>Sacciolepis</i> sp.	Amphibious	7245, 7246	40	0
	<i>Setaria parviflora</i> (Poir.) Kerguelen	Amphibious	7249, 7250	5	0
	Stipeae (sp. 1)	Amphibious	7251, 7252	25	10
	<i>Trichanthecium schwackeanum</i> (Mez) Zuloaga & Morrone	Amphibious	7253, 7254	5	0
	<i>Zizaniopsis</i> cf. <i>bonariensis</i> (Balansa & Poitr.) Speg.	Amphibious	-	5	0
	Poaceae (sp. 1)	Amphibious	-	30	35
Polygalaceae	<i>Polygala appendiculata</i> Vell.	Amphibious	7255, 7256	5	0
	<i>Polygala brasiliensis</i> L.	Amphibious	7257	5	0
	<i>Polygala molluginifolia</i> A.St.-Hil. & Moq.	Amphibious	7258	10	5
Polygonaceae	<i>Polygonum acuminatum</i> Kunth	Amphibious	7259, 7260	5	0
	<i>Polygonum ferrugineum</i> Wedd.	Amphibious	7261	5	5
	<i>Polygonum hydropiperoides</i> Michx.	Amphibious	7262, 7263	10	0
	<i>Polygonum meisnerianum</i> Cham.	Amphibious	7264, 7265	65	65
	<i>Polygonum punctatum</i> Elliott	Amphibious	7266, 7267	15	5
	<i>Polygonum</i> sp.	Amphibious	-	5	5
Pontederiaceae	<i>Eichhornia azurea</i> (Sw.) Kunth	Hydrophyte	-	10	5
	<i>Eichhornia crassipes</i> (Mart.) Solms	Hydrophyte	7268	50	10
	<i>Heteranthera reniformis</i> Ruiz & Pav.	Amphibious	7269, 7270	15	0
	<i>Pontederia cordata</i> L.	Emergent	7271	0	15
	<i>Pontederia subovata</i> (Seub.) Lowden	Emergent	7272, 7273	10	0
	<i>Pontederia</i> sp.	Emergent	-	10	0
Pterydaceae	<i>Acrostichum danaeifolium</i> Langsd. & Fisch.	Amphibious	7274	0	40
Ranunculaceae	<i>Ranunculus bonariensis</i> Poir.	Amphibious	7275, 7276	15	35
Ricciaceae	<i>Ricciocarpos natans</i> (L.) Corda	Hydrophyte	7277	25	0

Rubiaceae	<i>Borreria palustris</i> (Cham. & Schltdl.) Bacigalupo & E.L.Cabral	Amphibious	7278, 7279	10	0
	<i>Borreria verticillata</i> (L.) G.Mey.	Amphibious	7280, 7281	20	0
	<i>Cephalanthus glabratus</i> (Spreng.) K.Schum.	Amphibious	7282, 7283	20	0
	<i>Diodia saponariifolia</i> (Cham. & Schltdl.) K.Schum.	Amphibious	7284, 7285	65	65
	<i>Oldenlandia salzmannii</i> (DC.) Benth. & Hook.f. ex B.D.Jacks.	Amphibious	7286, 7287	5	0
Salicaceae	<i>Salix humboldtiana</i> Willd.	Amphibious	7288	60	55
Salviniaceae	<i>Azolla filiculoides</i> Lam.	Hydrophyte	-	5	0
	<i>Salvinia auriculata</i> Aubl.	Hydrophyte	-	15	10
	<i>Salvinia biloba</i> Raddi	Hydrophyte	7289, 7290	25	20
	<i>Salvinia minima</i> Baker	Hydrophyte	-	5	10
	<i>Salvinia</i> sp.	Hydrophyte	-	5	20
Solanaceae	<i>Schwenckia curviflora</i> Benth.	Amphibious	7291, 7292	5	0
	<i>Solanum glaucophyllum</i> Desf.	Amphibious	-	10	30
Sphagnaceae	<i>Sphagnum</i> sp.	Amphibious	7293, 7294	5	0
Thelypteridaceae	<i>Thelypteris</i> sp.	Amphibious	7295	15	0
Typhaceae	<i>Typha domingensis</i> Pers.	Emergent	7296	15	15
Urticaceae	<i>Boehmeria cylindrica</i> (L.) Sw.	Amphibious	7297, 7298	20	0
Verbenaceae	<i>Glandularia</i> sp.	Amphibious	7299, 7300	5	0
	<i>Phyla nodiflora</i> (L.) Greene	Amphibious	-	25	5
	<i>Verbena</i> sp.	Amphibious	7318	25	35
Xyridaceae	<i>Xyris jupicai</i> Rich.	Amphibious	7301, 7302	5	0
	<i>Xyris</i> sp.	Amphibious	-	70	25
Juncaginaceae	<i>Triglochin scilloides</i> (Poir.) Mering & Kadereit	Emergent	7307		*
Linderniaceae	<i>Micranthemum umbrosum</i> (Walter ex J.F.Gmel.) S.F.Blake	Amphibious	7304, 7305		*
Plantaginaceae	<i>Callitriche rimosa</i> Fassett	Emergent	7303		*
Potamogetonaceae	<i>Potamogeton ferrugineus</i> Hagstr.	Hydrophyte	7306		*
Ranunculaceae	<i>Ranunculus apiifolius</i> Pers.	Emergent	7309		*
	<i>Ranunculus flagelliformis</i> Sm.	Emergent	7308		*

Table 2 Total richness and by physiographic region (mean $\pm$ standard deviation) for the species pool and for each functional group of aquatic macrophytes in ponds of southern Brazil.

	All macrophytes	Amphibious	Emergent	Hydrophyte
Total	243 (34.8 $\pm$ 12.7)	185 (24.9 $\pm$ 10.5)	41 (7 $\pm$ 3)	17 (3.8 $\pm$ 2.3)
Encosta do				
Sudeste	221 (40.7 $\pm$ 13.3)	169 (31.1 $\pm$ 10.7)	35 (6.7 $\pm$ 3.6)	17 (4 $\pm$ 2.2)
Litoral	138 (28.8 $\pm$ 8.7)	95 (18.8 $\pm$ 5.9)	28 (7.3 $\pm$ 2.2)	15(3.6 $\pm$ 2.5)

Table 3 W and *p*-values produced by the Wilcoxon-Mann-Whitney tests; F statistics and *p*-values of PERMANOVA tests for the species pool and for the functional groups of aquatic macrophytes in the ponds of the physiographic regions *Encosta do Sudeste* and the *Litoral*.

Attribute		All macrophytes	Amphibious	Emergent	Hydrophyte
Means comparison	W/F	11.15*	20.31*	156.500	146.5
tests	<i>p</i> -value	0.002	0.0001	0.349	0.480
PERMANOVA	F	2.68	3.05	2.16	1.54
	<i>p</i> -value	0.001	0.001	0.004	0.131

Table 4 Components of partitioned analysis of Beta diversity for all the 40 ponds and physiographic regions of southern Brazil for all macrophytes and functional groups.

Groups	Beta diversity	All macrophytes	Amphibious	Emergent	Hydrophytes
40 Ponds	Turnover	0.92 (97.7%)	0.91 (97.1%)	0.91 (97.1%)	0.85 (92.4%)
	Nestedness	0.02 (2.3%)	0.03 (2.9%)	0.03 (2.9%)	0.07 (7.6%)
Encosta do Sudeste	Turnover	0.86 (96.3%)	0.86 (96.0%)	0.84 (93.3%)	0.78 (90.1%)
	Nestedness	0.03 (3.7%)	0.04 (4.0%)	0.06 (6.7%)	0.09 (9.9%)
Litoral	Turnover	0.86 (96.3%)	0.85 (95.9%)	0.85 (96.2%)	0.74 (84.4%)
	Nestedness	0.03 (3.7%)	0.04 (4.1%)	0.03 (3.8%)	0.14 (15.6%)

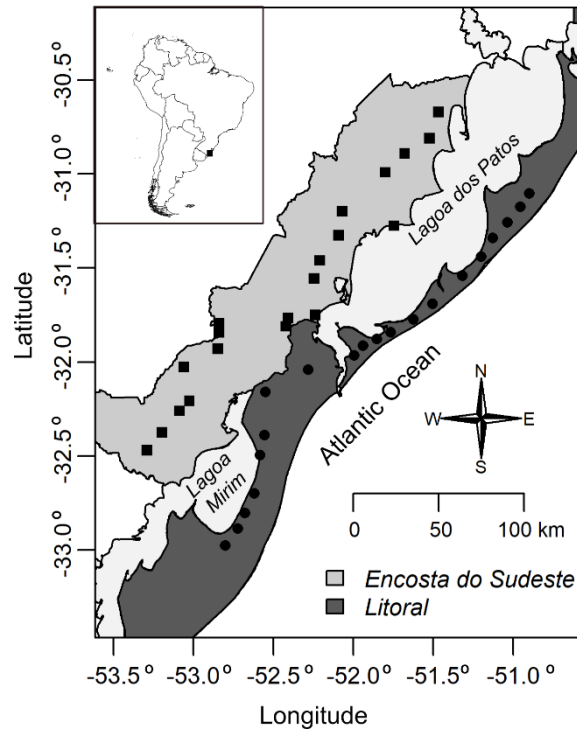


Figure 1 Sites of sampled ponds and the physiographic regions of southern Brazil: *Encosta do Sudeste* (■) and the *Litoral* (●).

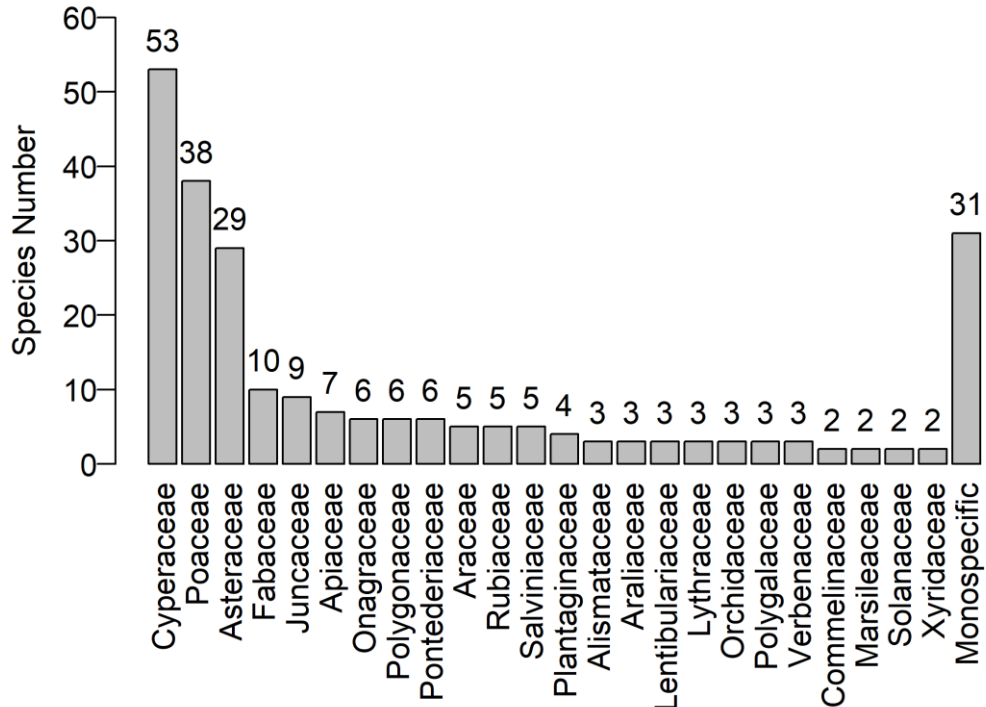


Figure 2 Species number per family of aquatic macrophytes from ponds in the physiographic regions *Encosta do Sudeste* and the *Litoral*, southern Brazil.

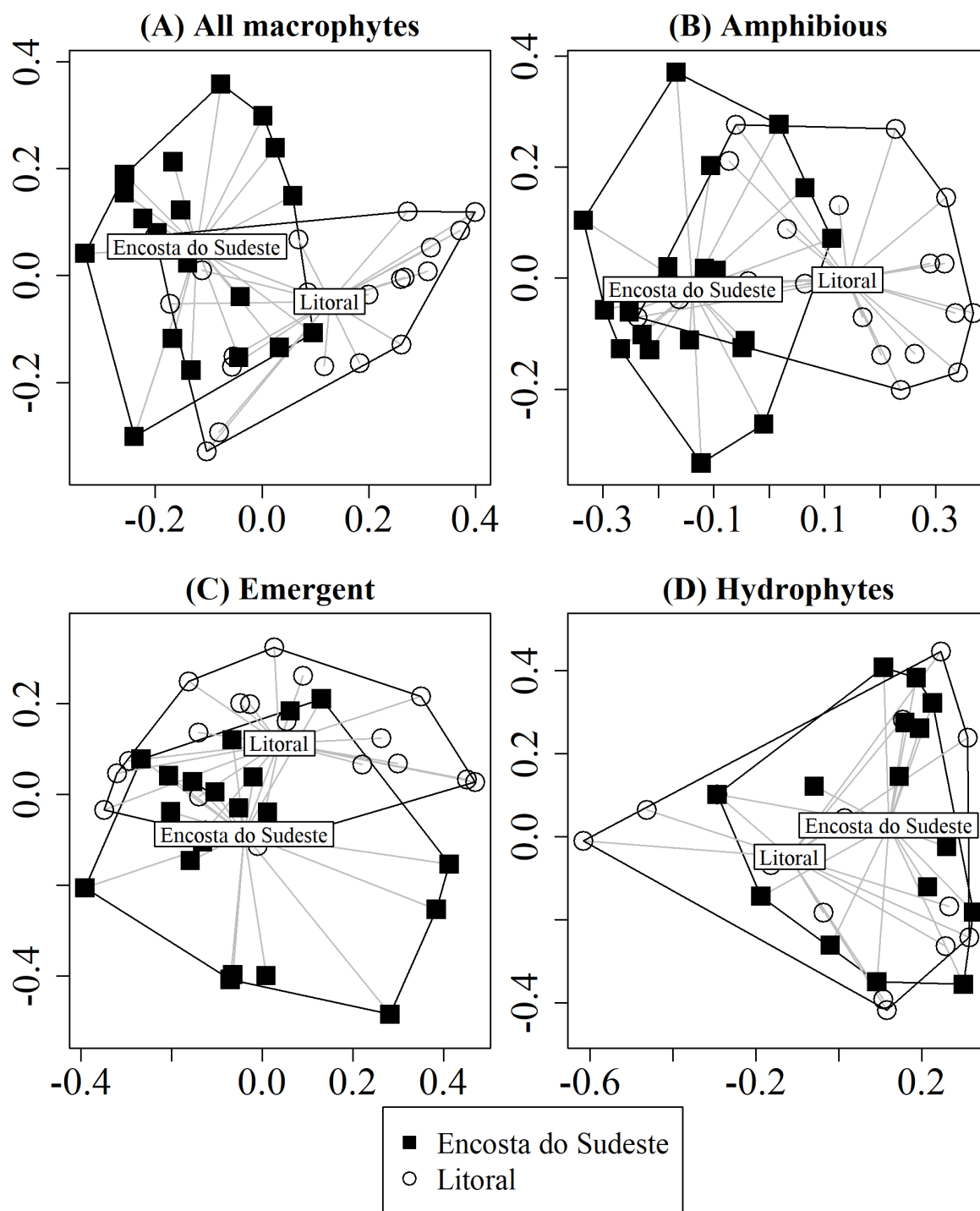


Figure 3 Principal Coordinate Analysis (PCoA) and Multivariate Dispersion Homogeneity Tests (PERMDISP) of the species pool and of the functional groups of aquatic macrophytes in the physiographic regions *Encosta do Sudeste* and the *Litoral*, southern Brazil.

CONSIDERAÇÕES FINAIS E PERSPECTIVAS

O presente estudo corrobora teorias de que para entender a estruturação de comunidades biológicas, é necessária a integração de diversos fatores. Verificou-se que fatores paisagísticos, fisiográficos, climáticos, ambientais locais e interações biológicas são importantes na estruturação de assembleias de macrófitas aquáticas de áreas úmidas subtropicais.

No primeiro capítulo, a tese retratou a relação entre fatores paisagísticos, como a disponibilidade de habitats, isolamento e uso da terra (áreas agrícolas e urbanas); fisiografia através da categorização das áreas úmidas de acordo com a formação geológica (Encosta do Sudeste e Litoral); e climáticos (temperatura e precipitação), e riqueza e composição de assembleias de macrófitas aquáticas. Os resultados obtidos nesse estudo, demonstram a complexidade das inter-relações entre as variáveis (efeitos secundários) no entendimento dos mecanismos que estruturam as assembleias de macrófitas aquáticas. Dentre os resultados encontrados, destacamos a influência positiva que as áreas agrícolas tiveram sobre a riqueza de macrófitas aquáticas, indo de encontro ao esperado nas nossas hipóteses e ao que se encontra em outros estudos. Atribuímos esse resultado à possível influência do cultivo de arroz irrigado, abundante nas áreas agrícolas do sul do Rio Grande do Sul. A partir disso, abre-se perspectivas para investigar mais especificadamente possíveis influências do cultivo de arroz irrigado sobre assembleias de macrófitas aquáticas em nível de paisagem. Para isso, técnicas de sensoriamento remoto que possibilitem a distinção entre o que é orizicultura irrigada, outras áreas agrícolas e áreas úmidas naturais nas imagens de satélite são necessárias. Além disso, destacamos também que relações negativas da disponibilidade de habitats e positivas do isolamento entre a riqueza de macrófitas aquáticas podem ocorrer devido a efeitos secundários, como interações interespecíficas e processos tróficos. A influência da orizicultura também poderia explicar a possível conectividade funcional das assembleias de macrófitas entre as áreas úmidas estudadas, uma vez que muitas áreas úmidas foram transformadas em áreas de cultivo de arroz irrigado, que além de servir de habitat complementar, podem atuar como bancos de sementes de macrófitas. Desse modo, futuros estudos que avaliem esses efeitos conjuntamente com os fatores estudados podem elucidar os mecanismos estruturadores das assembleias de macrófitas aquáticas das áreas úmidas do sul do Brasil.

No segundo capítulo, investigamos a estruturação de macrófitas aquáticas de acordo com a abordagem de metacomunidades, quantificando a influência de fatores espaciais como indicadores (*proxies*) de processos de dispersão e de fatores ambientais como indicadores de

nicho ambiental em duas escalas espaciais. Verificamos que os fatores apresentam importâncias distintas entre os diferentes grupos funcionais de macrófitas aquáticas, assim como, em escalas espaciais distintas. Ao contrário do que esperávamos, macrófitas anfíbias e emergentes são mais influenciadas por fatores espaciais, enquanto hidrófitas são mais influenciadas por fatores ambientais. Isso indica que anfíbias e emergentes apresentam limitação na dispersão e que hidrófitas são mais propensas a impactos ambientais locais. A partir desses resultados, esse estudo pode servir como base para outros que avaliem e proponham estratégias de conservação específicas adequadas a determinados grupos funcionais de macrófitas aquáticas no sul do Brasil. Além disso, esse trabalho contrapõe à ideia de que macrófitas aquáticas são pouco limitadas pela dispersão e corrobora estudos recentes que adicionam a importância de avaliar o papel da dispersão na estruturação dessas assembleias. Esse estudo também corrobora outros estudos que observaram que as respostas das comunidades variam dependendo da escala espacial observada.

No terceiro capítulo, testamos a aplicabilidade de um índice de sobreposição espacial desenvolvido especificamente para avaliar relações interespecíficas de macrófitas aquáticas. Assim, gerou-se resultados que podem servir como base para estudos que pretendam avaliar a sobreposição de nicho espacial de macrófitas aquáticas em geral, tanto de áreas úmidas do sul do Brasil como de outros tipos de ambientes aquáticos e localidades. Dentre esses resultados, podemos destacar a possível competição simétrica de *Luziola peruviana* sobre *Eleocharis minima* devido a diferenças estruturais dessas espécies.

Por último e não menos importante, no quarto capítulo apresentamos o levantamento florístico desenvolvido durante a tese, além de investigar diferenças na riqueza, composição e padrões de diversidade beta entre as regiões fisiográficas Encosta do Sudeste e Litoral. Contribuímos para o conhecimento sobre a flora de macrófitas aquáticas do sul do Brasil, verificando que as regiões fisiográficas são singulares quando a diversidade de macrófitas aquáticas. Destacamos a Encosta do Sudeste como uma região propícia para o desenvolvimento de futuros trabalhos florísticos e ecológicos devido à alta diversidade de macrófitas encontrada nesse trabalho, e que até então era pouco investigada. Além disso, verificamos que os padrões que geram a diversidade beta ocorrem principalmente por turnover, destacando a importância da diversidade e quantidade das áreas úmidas dessas regiões fisiográficas para a conservação da diversidade de macrófitas aquáticas, principalmente das ameaçadas de extinção, e não só das áreas úmidas presentes em unidades de conservação já existentes.

De modo geral, essa tese abre a perspectiva para investigar o papel dos diferentes tipos de cultivos agrícolas, assim como, interações interespecíficas e controle trófico sobre as macrófitas aquáticas em áreas úmidas rasas. É importante, também, que os grupos funcionais de macrófitas continuem sendo abordados de maneira diferenciada nos trabalhos ecológicos, principalmente por apresentarem respostas contrárias ao que esperávamos. Além disso, interações entre *Luziola peruviana* e *Eleocharis minima* precisam ser futuramente testadas, visto que em nosso trabalho verificamos uma possível competição (*latu sensu*) entre essas espécies.

ANEXOS

ANEXO 1 - Material suplementar capítulo 1

Electronic Supplementary Material

Flora

Influences of Landscape and Climatic Factors on Aquatic Macrophyte Richness and Composition in Ponds

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Table S1. Species list, functional groups (Amphibious, Emergent, Floating and Submerged) and frequency of the occurrence of aquatic macrophytes sampled in subtropical ponds.

Family	Taxon	Functional Group	Frequency
Acanthaceae	<i>Hygrophila costata</i> Ness	Amphibious	19
Alismataceae	<i>Echinodorus grandiflorus</i> (Cham. & Schltr.) Micheli	Emergent	14
	<i>Helanthium tenellum</i> (Martius) Britton	Emergent	9
	<i>Sagittaria montevidensis</i> Cham. & Schltdl.	Emergent	10
Amaranthaceae	<i>Alternanthera philoxeroides</i> (Mart.) Griseb.	Emergent	8
Apiaceae	<i>Centella asiatica</i> (L.) Urb.	Amphibious	36

	<i>Eryngium ebracteatum</i> Lam.	Amphibious	2
	<i>Eryngium eburneum</i> Decne.	Amphibious	2
	<i>Eryngium elegans</i> Cham. & Schltdl.	Amphibious	1
	<i>Eryngium chamissonis</i> Urb.	Amphibious	8
	<i>Eryngium</i> sp.	Amphibious	11
	<i>Lilaeopsis brasiliensis</i> (Glaz.) Affolter	Emergent	4
Apocynaceae	<i>Rhabdadenia madida</i> (Vell.) Miers	Emergent	1
Araceae	<i>Lemna</i> cf. <i>valdiviana</i> Phil.	Floating	10
	<i>Pistia stratiotes</i> L.	Floating	5
	<i>Spirodela intermedia</i> W. Koch	Floating	5
	<i>Wolffia brasiliensis</i> Wedd.	Floating	7
	<i>Wolffiella oblonga</i> (Phil.) Hegelm.	Floating	9
Araliaceae	<i>Hydrocotyle bonariensis</i> Lam.	Amphibious	18
	<i>Hydrocotyle ranunculoides</i> L.f.	Amphibious	12
	<i>Hydrocotyle verticillata</i> Thunb.	Amphibious	4
Asteraceae	<i>Achyrocline satureioides</i> (Lam.) DC.	Amphibious	1
	<i>Aspilia montevidensis</i> (Spreng.) Kuntze	Amphibious	1
	<i>Baccharis crispa</i> Spreng.	Amphibious	5
	<i>Baccharis penningtonii</i> Heering	Amphibious	1
	<i>Baccharis sagittalis</i> (Less.) DC.	Amphibious	1
	<i>Baccharis</i> sp.	Amphibious	1
	<i>Chaptalia</i> sp.	Amphibious	1
	<i>Chromolaena laevigata</i> (Lam.) R.M.King & H.Rob.	Amphibious	2
	<i>Conyza</i> sp.	Amphibious	4
	<i>Eclipta elliptica</i> DC.	Amphibious	1
	<i>Eclipta prostrata</i> (L.) L.	Amphibious	5
	<i>Enydra anagallis</i> Gardner	Emergent	15
	<i>Gamochaeta</i> sp.	Amphibious	3
	<i>Mikania micrantha</i> Kunth	Emergent	7

	<i>Mikania</i> sp.	Emergent	2
	<i>Orthopappus angustifolius</i> (Sw.) Gleason	Amphibious	1
	<i>Pluchea sagittalis</i> (Lam.) Cabrera	Amphibious	13
	<i>Pterocaulon balansae</i> Chodat	Amphibious	2
	<i>Pterocaulon rugosum</i> (Vahl) Malme	Amphibious	1
	<i>Senecio bonariensis</i> Hook. & Arn.	Amphibious	3
	<i>Solidago chilensis</i> Meyen	Amphibious	1
	<i>Stenachaenium</i> sp.	Amphibious	2
	<i>Symphyotrichum squamatum</i> (Spreng.) G.L.Nesom.	Amphibious	1
	Asteraceae (sp. 1)	Amphibious	1
	Asteraceae (sp. 2)	Amphibious	1
	Asteraceae (sp. 3)	Amphibious	1
	Asteraceae (sp. 4)	Amphibious	1
	Asteraceae (sp. 5)	Amphibious	1
	Asteraceae (sp. 6)	Amphibious	6
Begoniaceae	<i>Begonia cucullata</i> Willd.	Amphibious	2
Blechnaceae	<i>Blechnum</i> sp.	Amphibious	1
Burmanniaceae	<i>Burmannia bicolor</i> Mart.	Amphibious	1
Cabombaceae	<i>Cabomba caroliniana</i> A.Gray	Submerged	1
Campanulaceae	<i>Lobelia hederacea</i> Cham.	Amphibious	28
Caryophyllaceae	<i>Drymaria cordata</i> (L.) Willd. ex Roem. & Schult.	Amphibious	10
Commelinaceae	<i>Commelina diffusa</i> Burm.f.	Amphibious	6
	<i>Floscopa glabrata</i> (Kunth) Hassk.	Amphibious	2
Convolvulaceae	<i>Ipomoea</i> sp.	Amphibious	2
Cyperaceae	<i>Carex bonariensis</i> Desf. Ex Poir.	Amphibious	1
	<i>Carex longii</i> Mackenz.	Amphibious	2
	<i>Carex polysticha</i> Boeckeler	Amphibious	1
	<i>Cyperus berroi</i> (C.B.Clarke) Barros	Emergent	2
	<i>Cyperus blepharoleptos</i> Steud.	Amphibious	4

<i>Cyperus brasiliensis</i> (Kunth) Bauters	Emergent	1
<i>Cyperus brevifolius</i> (Rottb.) Endl. ex Hassk.	Amphibious	4
<i>Cyperus eragrostis</i> Lam.	Amphibious	5
<i>Cyperus esculentus</i> L.	Emergent	1
<i>Cyperus giganteus</i> Vahl	Emergent	2
<i>Cyperus haspan</i> L.	Amphibious	11
<i>Cyperus hermaphroditus</i> (Jacq.) Standl.	Amphibious	7
<i>Cyperus iria</i> L.	Amphibious	1
<i>Cyperus lanceolatus</i> Poir.	Amphibious	13
<i>Cyperus luzulae</i> (L.) Retz.	Amphibious	6
<i>Cyperus obtusatus</i> (J.Presl & C.Presl) Mattf. & Kük.	Amphibious	21
<i>Cyperus odoratus</i> L.	Amphibious	5
<i>Cyperus polystachyos</i> Rottb.	Amphibious	25
<i>Cyperus reflexus</i> Vahl	Amphibious	1
<i>Cyperus rigens</i> C.Presl	Amphibious	4
<i>Cyperus rotundus</i> L.	Amphibious	1
<i>Cyperus sesquiflorus</i> (Torr.) Mattf. & Kük.	Amphibious	5
<i>Cyperus surinamensis</i> Rottb.	Amphibious	3
<i>Cyperus trigynus</i> Spreng.	Emergent	2
<i>Cyperus virens</i> Michx.	Amphibious	9
<i>Eleocharis acutangula</i> (Roxb.) Schult.	Emergent	3
<i>Eleocharis bonariensis</i> Nees	Emergent	6
<i>Eleocharis contracta</i> Maury	Amphibious	1
<i>Eleocharis dunensis</i> Kük.	Amphibious	2
<i>Eleocharis flavescens</i> (Poir.) Urb.	Emergent	7
<i>Eleocharis geniculata</i> (L.) Roem. & Schult.	Emergent	1
<i>Eleocharis interstincta</i> (Vahl) Roem. & Schult.	Emergent	1
<i>Eleocharis maculosa</i> (Vahl) Roem. & Schult.	Emergent	12
<i>Eleocharis minima</i> Kunth	Emergent	23

	<i>Eleocharis montana</i> (Kunth) Roem. & Schult.	Emergent	8
	<i>Eleocharis obtusetrigona</i> (Lindl. & Nees) Steud.	Emergent	16
	<i>Eleocharis parodii</i> Barros	Emergent	1
	<i>Eleocharis sellowiana</i> Kunth	Emergent	6
	<i>Eleocharis viridans</i> Kük. ex Osten	Amphibious	4
	<i>Eleocharis</i> sp. 1	Amphibious	10
	<i>Eleocharis</i> sp. 2	Amphibious	1
	<i>Eleocharis</i> sp. 3	Amphibious	1
	<i>Fimbristylis complanata</i> (Retz.) Link	Amphibious	12
	<i>Fimbristylis dichotoma</i> (L.) Vahl	Amphibious	5
	<i>Fimbristylis</i> sp.	Amphibious	1
	<i>Fuirena robusta</i> Kunth	Emergent	1
	<i>Rhynchospora corymbosa</i> (L.) Britton	Amphibious	3
	<i>Rhynchospora holoschoenoides</i> (Rich.) Herter	Amphibious	11
	<i>Rhynchospora microcarpa</i> Baldwin	Amphibious	15
	<i>Rhynchospora tenuis</i> Link	Amphibious	12
	<i>Rhynchospora</i> sp.	Amphibious	1
	<i>Schoenoplectus californicus</i> (C.A.Mey.) Soják	Emergent	12
	<i>Scleria distans</i> Poir.	Amphibious	13
Droseraceae	<i>Drosera brevifolia</i> Pursh	Amphibious	4
Eriocaulaceae	<i>Eriocaulon modestum</i> Kunth	Amphibious	5
Fabaceae	<i>Desmodium tortuosum</i> (Sw.) DC.	Amphibious	7
	<i>Desmodium</i> sp.	Amphibious	3
	<i>Erythrina crista-galli</i> L.	Amphibious	3
	<i>Mimosa bimucronata</i> (DC.) Kuntze	Amphibious	4
	<i>Sesbania punicea</i> (Cav.) Benth.	Amphibious	10
	<i>Vigna longifolia</i> (Benth.) Verdc.	Amphibious	3
	<i>Vigna luteola</i> (Jacq.) Benth.	Amphibious	2
	<i>Vigna</i> sp.	Amphibious	2
	Fabaceae (sp. 1)	Amphibious	4

	Fabaceae (sp. 2)	Amphibious	2
Haloragaceae	<i>Myriophyllum aquaticum</i> (Vell.) Verdc.	Emergent	14
Hydrocharitaceae	<i>Limnobiium laevigatum</i> (Humb. & Bonpl. ex Willd.) Heine	Floating	6
Iridaceae	<i>Sisyrinchium micranthum</i> Cav.	Amphibious	2
Juncaceae	<i>Juncus densiflorus</i> Kunth	Amphibious	1
	<i>Juncus effusus</i> L.	Amphibious	1
	<i>Juncus marginatus</i> Rostk.	Amphibious	4
	<i>Juncus microcephalus</i> Kunth	Amphibious	21
	<i>Juncus pallescens</i> Lam.	Amphibious	2
	<i>Juncus scirpoides</i> Lam.	Amphibious	3
	<i>Juncus tenuis</i> Willd.	Amphibious	21
	<i>Juncus</i> sp. 1	Amphibious	4
	<i>Juncus</i> sp. 2	Amphibious	2
Lamiaceae	<i>Hyptis</i> sp.	Amphibious	5
Lentibulariaceae	<i>Utricularia gibba</i> L.	Submerged	14
	<i>Utricularia tricolor</i> A.St.-Hil.	Emergent	2
	<i>Utricularia</i> sp.	Submerged	8
Lythraceae	<i>Cuphea carthagenensis</i> (Jacq.) J.Macbr.	Amphibious	20
	<i>Cuphea racemosa</i> (L.f.) Spreng.	Amphibious	1
	<i>Heimia apetala</i> (Spreng.) S.A.Graham & Gandhi	Amphibious	1
Malvaceae	<i>Hibiscus diversifolius</i> Jacq.	Amphibious	1
Marantaceae	<i>Thalia multiflora</i> Horkel ex Körn.	Emergent	1
Marsileaceae	<i>Marsilea ancylopoda</i> A.Braun	Amphibious	2
	<i>Regnellidium diphyllum</i> Lindm.	Amphibious	12
Mayacaceae	<i>Mayaca fluviatilis</i> Aubl.	Emergent	6
Melastomataceae	<i>Pleroma asperior</i> (Cham.) Triana	Amphibious	1
Menyanthaceae	<i>Nymphoides humboldtiana</i> (Kunh) Kuntze	Amphibious	29
Onagraceae	<i>Ludwigia elegans</i> (Cambess.) H.Hara	Amphibious	1
	<i>Ludwigia erecta</i> (L.) H.Hara	Amphibious	1

	<i>Ludwigia grandiflora</i> (Michx.) Greuter & Burdet	Emergent	19
	<i>Ludwigia hexapetala</i> (Hook. & Arn.) Zardini et al.	Emergent	20
	<i>Ludwigia multinervia</i> (Hook. & Arn.) Ramamoorthy	Amphibious	5
	<i>Ludwigia</i> sp.	Amphibious	10
Orchidaceae	<i>Habenaria parviflora</i> Lindl.	Amphibious	1
	<i>Habenaria</i> sp. 1	Amphibious	4
	<i>Habenaria</i> sp. 2	Amphibious	1
Orobanchaceae	<i>Agalinis communis</i> (Cham. & Schltdl.) D'Arcy	Amphibious	5
Passifloraceae	<i>Passiflora</i> sp.	Amphibious	1
Plantaginaceae	<i>Bacopa lanigera</i> (Cham. & Schltdl.) Wettst.	Emergent	6
	<i>Bacopa monnieri</i> (L.) Pennell	Emergent	13
	<i>Gratiola peruviana</i> L.	Amphibious	9
	<i>Mecardonia procumbens</i> (Mill.) Small	Amphibious	2
Poaceae	<i>Andropogon arenarius</i> Hack.	Amphibious	1
	<i>Andropogon bicornis</i> L.	Amphibious	2
	<i>Andropogon lateralis</i> Nees	Amphibious	5
	<i>Andropogon lindmanii</i> Hack.	Amphibious	1
	<i>Axonopus compressus</i> (Sw.) P. Beauv.	Amphibious	6
	<i>Axonopus fissifolius</i> (Raddi) Kuhlman.	Amphibious	9
	<i>Calamagrostis viridiflavescens</i> (Poir.) Steud.	Amphibious	1
	<i>Cynodon dactylon</i> (L.) Pers.	Amphibious	2
	<i>Echinochloa colona</i> (L.) Link	Amphibious	2
	<i>Echinochloa crusgalli</i> (L.) P.Beauv.	Amphibious	1
	<i>Eragrostis cataclasta</i> (L.) P.Beauv.	Emergent	4
	<i>Eragrostis hypnoides</i> (Lam.) Britton, Sterns & Poggenb.	Amphibious	1
	<i>Eragrostis plana</i> Nees	Amphibious	9
	<i>Ischaemum minus</i> J.Presl	Amphibious	7
	<i>Leersia hexandra</i> Sw.	Amphibious	18

	<i>Luziola peruviana</i> Juss. ex J.F.Gmel.	Amphibious	39
	<i>Oplismenopsis najada</i> (Hack. & Arechav.) Parodi	Emergent	1
	Paniceae (sp. 1)	Amphibious	1
	<i>Panicum dichotomiflorum</i> Michx.	Amphibious	13
	<i>Panicum</i> sp. 1	Amphibious	1
	<i>Panicum</i> sp. 2	Amphibious	1
	<i>Panicum</i> sp. 3	Amphibious	1
	<i>Panicum</i> sp. 4	Amphibious	1
	<i>Paspalum dilatatum</i> Poir.	Amphibious	1
	<i>Paspalum durifolium</i> Mez	Amphibious	1
	<i>Paspalum maculosum</i> Trin.	Amphibious	1
	<i>Paspalum modestum</i> Mez	Amphibious	2
	<i>Paspalum notatum</i> Flügge	Amphibious	2
	<i>Paspalum pumilum</i> Nees	Amphibious	6
	<i>Paspalum urvillei</i> Steud.	Amphibious	7
	<i>Paspalum</i> sp.	Amphibious	2
	<i>Sacciolepis vilvodes</i> (Trin.) Chase	Amphibious	2
	<i>Sacciolepis</i> sp.	Amphibious	1
	<i>Setaria parviflora</i> (Poir.) Kerguelen	Amphibious	8
	Stipeae (sp. 1)	Amphibious	1
	<i>Trichanthecium schwackeanum</i> (Mez) Zuloaga & Morrone	Amphibious	7
	<i>Zizaniopsis</i> cf. <i>bonariensis</i> (Balansa & Poitr) Speg.	Amphibious	1
	Poaceae (sp. 1)	Amphibious	1
Polygalaceae	<i>Polygala appendiculata</i> Vell.	Amphibious	13
	<i>Polygala brasiliensis</i> L.	Amphibious	1
	<i>Polygala molluginifolia</i> A.St.-Hil. & Moq.	Amphibious	1
Polygonaceae	<i>Polygonum acuminatum</i> Kunth	Amphibious	3
	<i>Polygonum ferrugineum</i> Wedd.	Amphibious	1
	<i>Polygonum hydropiperoides</i> Michx.	Amphibious	2

	<i>Polygonum meisnerianum</i> Cham.	Amphibious	2
	<i>Polygonum punctatum</i> Elliott	Amphibious	26
	<i>Polygonum</i> sp.	Amphibious	4
Pontederiaceae	<i>Eichhornia azurea</i> (Sw.) Kunth	Floating	2
	<i>Eichhornia crassipes</i> (Mart.) Solms	Floating	3
	<i>Heteranthera reniformis</i> Ruiz & Pav.	Amphibious	12
	<i>Pontederia cordata</i> L.	Emergent	3
	<i>Pontederia subovata</i> (Seub.) Lowden	Emergent	3
	<i>Pontederia</i> sp.	Emergent	2
Pterydaceae	Pteridaceae (sp. 1)	Amphibious	2
Ranunculaceae	<i>Ranunculus bonariensis</i> Poir.	Amphibious	8
Ricciaceae	<i>Ricciocarpos natans</i> (L.) Corda	Floating	10
Rubiaceae	<i>Borreria palustris</i> (Cham. & Schltdl.) Bacigalupo & E.L.Cabral	Amphibious	5
	<i>Borreria verticillata</i> (L.) G.Mey.	Amphibious	2
	<i>Cephalanthus glabratus</i> (Spreng.) K.Schum.	Amphibious	4
	<i>Diodia saponariifolia</i> (Cham. & Schltdl.) K.Schum.	Amphibious	4
	<i>Oldenlandia salzmannii</i> (DC.) Benth. & Hook.f. ex B.D.Jacks.	Amphibious	26
Salicaceae	<i>Salix humboldtiana</i> Willd.	Amphibious	1
Salviniaceae	<i>Azolla filiculoides</i> Lam.	Floating	23
	<i>Salvinia auriculata</i> Aubl.	Floating	1
	<i>Salvinia biloba</i> Raddi	Floating	5
	<i>Salvinia minima</i> Baker	Floating	9
	<i>Salvinia</i> sp.	Floating	3
Solanaceae	<i>Schwenckia curviflora</i> Benth.	Amphibious	5
	<i>Solanum glaucophyllum</i> Desf.	Amphibious	1
Sphagnaceae	<i>Sphagnum</i> sp.	Amphibious	8
Thelypteridaceae	<i>Thelypteris</i> sp.	Amphibious	1
Typhaceae	<i>Typha domingensis</i> Pers.	Emergent	3

Urticaceae	<i>Boehmeria cylindrica</i> (L.) Sw.	Amphibious	6
Verbenaceae	<i>Glandularia</i> sp.	Amphibious	4
	<i>Phyla nodiflora</i> (L.) Greene	Amphibious	1
	<i>Verbena</i> sp.	Amphibious	6
Xyridaceae	<i>Xyris jupicai</i> Rich.	Amphibious	12
	<i>Xyris</i> sp.	Amphibious	1

ANEXO 2 - Material suplementar capítulo 2

Supplementary Material. The Role of Spatial Factors, Environmental Niche and Spatial Scale on Aquatic Macrophyte Metacommunities in Subtropical Ponds.

Table S1. Correlation coefficients (r) among environmental variables sampled in 40 subtropical ponds of Southern Brazil. Values higher than 0.6 and lower than -0.6 are in bold. See Methods Section for environmental codes.

	W T	p H	O R P	E C	O D	P O D	T D S	D H	M D	A lt	P Si ze	L W A	A gr	U rb	Te m p	T S	MT We Q	M TD Q	MT Wa Q	M TC Q	Pr ec	P S	P W eQ	P D Q	P W aQ	P C Q	Re gio n
WT	1, 0																										
pH	0, 0	1, 0																									
OR P	0, 0	0, 1	1, 0																								
EC	0, 0	0, 2	0, 3	1, 0																							
OD	0, 0	0, 4	0, 2	0, 1	1, 0																						
pO D	0, 0	0, 4	0, 2	0, 1	0, 9	1, 0																					
TD S	0, 0	0, 2	0, 3	1, 0	0, 1	0, 2	1, 0																				
DH	0, 0	0, 1	0, 0	0, 1	0, 0	0, 0	0, 1	1, 0																			
MD	0, 1	0, 1	0, 1	0, 2	0, 0	0, 1	0, 2	0, 6	1, 0																		
Alt	0, 0	0, 3	0, 1	0, 2	0, 1	0, 1	0, 2	0, 0	0, 0	1, 0																	
PSi ze	0, 1	0, 2	0, 2	0, 2	0, 2	0, 2	0, 2	0, 6	0, 4	0, 3	1, 00																
LW A	0, 1	0, 3	0, 1	0, 0	0, 1	0, 1	0, 0	0, 0	0, 1	0, 2	0, 22	1, 00															
Agr	0, 0	0, 1	0, 0	0, 0	0, 0	0, 0	0, 0	0, 1	0, 2	0, 3	0, 27	0, 55	1, 0														
Urb	0, 1	0, 1	0, 2	0, 1	0, 1	0, 1	0, 1	0, 3	0, 4	0, 1	0, 04	0, 07	0, 1	1, 0													
Te mp	0, 2	0, 2	0, 2	0, 8	0, 3	0, 3	0, 9	0, 1	0, 5	0, 6	0, 24	0, 05	0, 5	0, 7	1, 00												
TS	0, 0	0, 3	0, 1	0, 0	0, 0	0, 0	0, 2	0, 8	0, 6	0, 8	0, 18	0, 01	0, 8	0, 3	0, 45	1, 0											
MT We Q	0, 3	0, 1	0, 0	0, 3	0, 5	0, 9	0, 3	0, 8	0, 0	0, 2	0, 27	0, 22	0, 1	0, 4	0, 52	0, 1	1,0 0										
MT DQ	0, 0	0, 0	0, 2	0, 7	0, 2	0, 2	0, 8	0, 0	0, 6	0, 6	0, 02	0, 06	0, 2	0, 9	0, 69	0, 2	0,3 0	1,0 0									

[illegible]

ANEXO 3 – Material suplementar capítulo 3

Spatial niche overlap of aquatic macrophytes in intermittent and permanent ponds

Hydrobiologia

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Lombardo and Mjelde indexes among pairs of aquatic macrophyte species ($\hat{\alpha}_{ij}$) in ponds of the extreme south of Brazil. The abbreviations of the names of the species can be found in Table 1. I01 to I010 represent intermittent ponds, and P01 to P10 permanent ponds.

I01	AP	B M	CA	Em i	HV	LB	LH	LS	LP	M U	NH	OS	Ppu m	Ppu n	PC	UG
<i>Alternanthera philoxeroides</i>	0	0,1 2	NA	0,0 1	0,0 6	NA	NA	NA	0,1 6	NA	NA	0,0 4	NA	NA	NA	NA
<i>Bacopa monnieri</i>	0,4 2	0	0,7 7	0,4 3	0,4	0,7 7	0,6 4	0,6 4	0,3 6	0,0 9	0,2 6	0,3 6	0,78	0,1 6	1,1 4	0,1 5
<i>Centella asiatica</i>	NA	0,2 3	0	0,5 9	0,2 4	2,2 2	NA	NA	0,1 8	0,2 5	NA	0,4 3	0,42	0,1 7	NA	NA
<i>Eleocharis minima</i>	0,0 4	0,6 5	3,0 3	0	0,6 7	3,0 3	1,7 8	1,7 8	0,4 3	1,2 4	0,7 7	0,7	1,58	0,3 1	0,4 4	0,1 5
<i>Hydrocotyle verticillata</i>	0,4	0,8 4	1,6 9	0,9 3	0	1,6 9	1,3 8	1,3 8	0,9 3	1,1 7	0,8 8	1,0 4	1,22	1,4 1	0,5 6	0,7 5
<i>Lilaeopsis brasiliensis</i>	NA	0,2 3	2,2 2	0,5 9	0,2 4	0	NA	NA	0,1 8	0,2 5	NA	0,4 3	0,42	0,1 7	NA	NA
<i>Lobelia hederacea</i>	NA	0,1 9	NA	0,3 5	0,2	NA	0	1,8 2	0,0 6	NA	0,4 9	0,1 5	0,79	NA	NA	0,1 4
<i>Ludwigia</i> sp.	NA	0,1 9	NA	0,3 5	0,2	NA	1,8 2	0	0,0 6	NA	0,4 9	0,1 5	0,79	NA	NA	0,1 4
<i>Luziola peruviana</i>	0,9 5	0,6 3	1,0 6	0,5	0,7 9	1,0 6	0,3 8	0,3 8	0	0,7 1	0,4 5	0,7 9	0,5	0,6 7	0,1 5	0,6 2
<i>Micranthemum umbrosum</i>	NA	0,0 7	0,7 2	0,6 9	0,4 7	0,7 2	NA	NA	0,3 3	0	0,5 5	0,4 1	0,68	0,3 6	NA	0,0 2
<i>Nymphoides humboldtiana</i>	NA	0,3 9	NA	0,7 7	0,6 4	NA	2,4 7	2,4 7	0,3 8	0,9 8	0	0,5 7	1,37	0,3 3	0,4 4	0,5 2
<i>Oldenlandia salzmannii</i>	0,3	0,7 3	2,9 1	0,9 3	1	2,9 1	1,0 4	1,0 4	0,8 9	1	0,7 6	0	1,15	1,1 9	0,4 2	1,0 6
<i>Paspalum pumilum</i>	NA	0,7 8	1,4 3	1,0 5	0,5 9	1,4 3	2,6 7	2,6 7	0,2 8	0,8 1	0,9 1	0,5 7	0	0,1 4	0,4 8	0,2 1
<i>Polygonum punctatum</i>	NA	0,1	0,3 5	0,1 2	0,4	0,3 5	NA	NA	0,2 3	0,2 5	0,1 3	0,3 5	0,08	0	0,1 2	0,1 5
<i>Pontederia cordata</i>	NA	0,3 4	NA	0,0 9	0,0 8	NA	NA	NA	0,0 3	NA	0,0 9	0,0 6	0,14	0,0 6	0	0,0 6
<i>Utricularia gibba</i>	NA	0,0 9	NA	0,0 6	0,2 2	NA	0,2 9	0,2 9	0,2 1	0,0 1	0,2 1	0,3 1	0,12	0,1 5	0,1 2	0

102	CR	CA	Emi	GP	HV	LP	MF	NH	OS	Poa	RS	Scu	UG	XJ
<i>Callitriche rimosa</i>	0	NA	0,05	NA	NA	0,04	NA	0,03	0,09	NA	NA	NA	0,03	NA
<i>Centella asiatica</i>	NA	0	0,06	0,07	0,46	0,13	NA	0,07	0,16	NA	0,23	NA	0,08	NA
<i>Eleocharis minima</i>	0,34	0,13	0	0,35	0,21	0,25	0,41	0,28	0,15	0,32	0,3	0,41	0,28	0,86
<i>Gratiola peruviana</i>	NA	0,09	0,21	0	NA	0,13	NA	0,11	0,09	0,12	0,21	NA	0,2	0,48
<i>Hydrocotyle verticillata</i>	NA	0,15	0,03	NA	0	0,09	NA	0,04	NA	NA	0,23	NA	0,04	NA
<i>Luziola peruviana</i>	0,28	0,34	0,3	0,25	0,66	0	0,34	0,44	0,26	0,31	0,48	0,34	0,36	0,16
<i>Mayaca fluviatilis</i>	NA	NA	0,06	NA	NA	0,04	0	0,04	NA	0,32	NA	0,46	0,04	NA
<i>Nymphoides humboldtiana</i>	0,16	0,11	0,21	0,14	0,2	0,28	0,2	0	0,15	0,18	0,19	0,2	0,27	0,21
<i>Oldenlandia salzmännii</i>	0,22	0,14	0,06	0,06	NA	0,09	NA	0,08	0	0,07	NA	NA	0,09	NA
<i>Poaceae (sp.)</i>	NA	NA	0,09	0,05	NA	0,07	0,55	0,06	0,05	0	NA	0,55	0,08	NA
<i>Rhynchospora sp.</i>	NA	0,15	0,09	0,1	0,46	0,12	NA	0,08	NA	NA	0	NA	0,11	NA
<i>Schwenckia curviflora</i>	NA	NA	0,06	NA	NA	0,04	0,46	0,04	NA	0,32	NA	0	0,04	NA
<i>Utricularia gibba</i>	0,22	0,2	0,32	0,38	0,27	0,35	0,27	0,43	0,27	0,34	0,43	0,27	0	0,28
<i>Xyris jupicai</i>	NA	NA	0,13	0,12	NA	0,02	NA	0,04	NA	NA	NA	NA	0,04	0

I03	Emi	ES	HV	LP	NH	Ppum	Poa	RS	Scu	XJ
<i>Eleocharis minima</i>	0	0,05	2,46	1,08	0,92	0,28	0,19	0,29	0,64	0,03
<i>Eleocharis sellowiana</i>	0,01	0	NA	0,03	0,01	0,49	NA	0,15	NA	NA
<i>Hydrocotyle verticillata</i>	0,44	NA	0	0,39	0,15	0,18	NA	0,25	1,12	NA
<i>Luziola peruviana</i>	0,95	0,14	1,94	0	1	0,26	NA	0,17	0,58	0,06
<i>Nymphoides humboldtiana</i>	0,74	0,05	0,68	0,92	0	0,14	0,04	0,09	0,2	0,02
<i>Paspalum pumilum</i>	0,1	1	0,36	0,11	0,06	0	0,09	0,38	0,18	NA
Poaceae (sp.)	0,03	NA	NA	NA	0,01	0,04	0	0,24	NA	NA
<i>Rhynchospora sp.</i>	0,19	0,55	0,88	0,13	0,07	0,68	0,85	0	0,63	0,25
<i>Schwenckia curviflora</i>	0,23	NA	2,24	0,23	0,09	0,18	NA	0,35	0	0,33
<i>Xyris jupicai</i>	0	NA	NA	0,01	0,01	NA	NA	0,07	0,16	0

I04	AP	AF	Emi	HV	LH	LP	MU	NH	OS
<i>Alternanthera philoxeroides</i>	0	0,15	0,04	NA	NA	0,2	0,06	0,13	0,12
<i>Azolla filiculoides</i>	0,3	0	0,83	0,92	1,83	0,67	0,52	0,82	1
<i>Eleocharis minima</i>	0,13	1,41	0	1,6	1,56	0,95	1,07	0,98	1,37
<i>Hydrocotyle verticillata</i>	NA	0,46	0,47	0	1,83	0,32	0,43	0,57	0,75
<i>Lobelia hederacea</i>	NA	0,46	0,23	0,92	0	0,1	0,21	0,17	0,37
<i>Luziola peruviana</i>	0,78	1,31	1,08	1,23	0,82	0	1,02	1,21	1,14
<i>Micranthemum umbrosum</i>	0,22	0,92	1,11	1,51	1,47	0,92	0	0,96	1,1
<i>Nymphoides humboldtiana</i>	0,38	1,23	0,86	1,7	1,01	0,93	0,81	0	1,26
<i>Oldenlandia salzmannii</i>	0,3	1,25	1	1,88	1,83	0,73	0,78	1,05	0

I05	Emi	Ean	LP	MU	NH	OS	Ppum	Poa	RS	Scu	XJ
<i>Eleocharis minima</i>	0	NA	0,13	2,53	0,36	0,03	NA	0,19	0,08	NA	NA
<i>Enydra anagallis</i>	NA	0	0,02	NA	0,03	0,06	0,32	0,05	0,05	NA	0,32
<i>Luziola peruviana</i>	0,45	0,1	0	1,42	0,38	0,2	0,1	0,19	0,36	0,05	0,1
<i>Micranthemum umbrosum</i>	1,34	NA	0,22	0	0,71	NA	NA	0,31	0,12	NA	NA
<i>Nymphoides humboldtiana</i>	0,83	0,12	0,26	3,1	0	0,06	0,12	0,25	0,26	0,02	0,12
<i>Oldenlandia salzmännii</i>	0,07	0,32	0,15	NA	0,07	0	0,32	0,14	0,16	0,06	0,32
<i>Paspalum pumilum</i>	NA	0,32	0,02	NA	0,03	0,06	0	0,05	0,05	NA	0,32
Poaceae (sp.)	0,44	0,22	0,13	1,32	0,24	0,12	0,22	0	0,16	0,34	0,22
<i>Rhynchospora</i> sp.	0,23	0,24	0,3	0,63	0,32	0,17	0,24	0,2	0	0,16	0,24
<i>Schwenckia curviflora</i>	NA	NA	0,01	NA	0,01	0,02	NA	0,11	0,04	0	NA
<i>Xyris jupicai</i>	NA	0,32	0,02	NA	0,03	0,06	0,32	0,05	0,05	NA	0

I06	CR	Emi	LP	NH	Ppum
<i>Callitriche rimosa</i>	0	0,15	0,02	0,08	0,79
<i>Eleocharis minima</i>	0,87	0	1,28	0,81	0,51
<i>Luziola peruviana</i>	0,09	0,77	0	0,65	0,2
<i>Nymphoides humboldtiana</i>	0,43	0,77	1,05	0	0,97
<i>Paspalum pumilum</i>	1,96	0,22	0,14	0,42	0

107	Emi	Ean	HV	LP	NH	OS	Ppum	XJ
<i>Eleocharis minima</i>	0	0,07	0,35	0,15	0,21	0,07	0,11	0,06
<i>Enydra anagallis</i>	0,06	0	0,19	0,57	0,63	NA	0,48	1,24
<i>Hydrocotyle verticillata</i>	0,31	0,19	0	0,19	NA	NA	0,13	0,4
<i>Luziola peruviana</i>	0,3	1,29	0,43	0	0,86	0,31	0,76	1,11
<i>Nymphoides humboldtiana</i>	0,31	1,09	NA	0,66	0	0,35	0,63	0,47
<i>Oldenlandia salzmannii</i>	0,02	NA	NA	0,05	0,07	0	0,04	NA
<i>Paspalum pumilum</i>	0,21	1,05	0,28	0,74	0,81	0,25	0	1,03
<i>Xyris jupicai</i>	0,03	0,72	0,23	0,28	0,16	NA	0,27	0

108	AP	AF	Emi	Evi	Ean	HR	LL	LH	LS	LP	MU	Maq	NH	Ppum	Pst	Ppun	RF	RD	SB	Smi	S	Tsch	UG
<i>Alternanthera philoxeroides</i>	0	0,09	0,8	NA	NA	NA	0,24	NA	NA	0,16	0,08	NA	0,8	NA	NA	NA	NA	NA	0,04	NA	NA	NA	NA
<i>Azolla filiculoides</i>	0,31	0	0,31	0,25	0,2	0,49	0,24	0,25	0,5	0,49	0,59	0,74	0,31	0,25	NA	0,74	1,72	0,69	0,36	0,32	1,37	0,46	0,92
<i>Eleocharis minima</i>	0,8	0,09	0	NA	NA	NA	0,24	NA	NA	0,16	0,08	NA	0,8	NA	NA	NA	NA	NA	0,04	NA	NA	NA	NA
<i>Eleocharis</i> cf. <i>viridans</i> .	NA	0,07	NA	0	0,17	0,05	0,1	0,64	NA	0,12	NA	NA	NA	0,64	NA	NA	NA	NA	NA	NA	NA	0,32	NA
<i>Enydra anagallis</i>	NA	0,24	NA	0,74	0	0,3	0,47	0,74	0,66	0,51	0,5	0,96	NA	0,74	0,74	0,96	0,29	NA	0,41	0,32	NA	0,99	0,6
<i>Hydrocotyle ranunculoides</i>	NA	0,72	NA	0,27	0,35	0	0,37	0,27	0,54	0,33	0,44	0,79	NA	0,27	0,27	0,79	1,04	0,37	0,57	0,76	0,76	0,5	0,59
<i>Limnobium laevigatum</i>	1,31	0,36	1,31	0,53	0,58	0,39	0	0,53	0,25	0,56	0,45	0,36	1,31	0,53	0,54	0,36	0,11	0,03	0,38	0,35	NA	0,61	0,37
<i>Lobelia hederacea</i>	NA	0,07	NA	0,64	0,17	0,05	0,1	0	NA	0,12	NA	NA	NA	0,64	NA	NA	NA	NA	NA	NA	NA	0,32	NA
<i>Ludwigia</i> sp.	NA	0,28	NA	NA	0,3	0,21	0,09	NA	0	0,11	0,44	1,92	NA	NA	NA	1,92	0,58	NA	0,82	0,64	NA	0,96	0,64
<i>Luziola peruviana</i>	0,95	0,83	0,95	0,76	0,7	0,38	0,62	0,76	0,35	0	0,65	0,51	0,95	0,76	0,76	0,51	1,19	0,4	0,33	0,17	0,95	0,87	0,69
<i>Micranthemum umbrosum</i>	0,42	0,85	0,42	NA	0,58	0,44	0,42	NA	1,14	0,55	0	1,01	0,42	NA	0,34	1,01	1,33	0,49	0,59	0,34	0,98	0,51	0,99
<i>Myriophyllum aquaticum</i>	NA	0,2	NA	NA	0,22	0,15	0,07	NA	0,96	0,08	0,19	0	NA	NA	NA	1,92	0,58	NA	0,6	0,64	NA	0,96	0,64
<i>Nymphoides humboldtiana</i>	0,8	0,09	0,8	NA	NA	NA	0,24	NA	NA	0,16	0,08	NA	0	NA	NA	NA	NA	NA	0,04	NA	NA	NA	NA
<i>Paspalum pumilum</i>	NA	0,07	NA	0,64	0,17	0,05	0,1	0,64	NA	0,12	NA	NA	NA	0	NA	NA	NA	NA	NA	NA	NA	0,32	NA
<i>Pistia stratiotes</i>	NA	NA	NA	NA	0,17	0,05	0,1	NA	NA	0,13	0,07	NA	NA	NA	0	NA	NA	NA	0,06	NA	NA	NA	NA
<i>Polygonum punctatum</i>	NA	0,2	NA	NA	0,22	0,15	0,07	NA	0,96	0,08	0,19	1,92	NA	NA	NA	0	0,58	NA	0,6	0,64	NA	0,96	0,64
<i>Ranunculus flagelliformis</i>	NA	0,83	NA	NA	0,11	0,34	0,03	NA	0,51	0,34	0,44	1,01	NA	NA	NA	1,01	0	0,49	0,25	0,34	0,98	0,51	0,77
<i>Regnellidium diphyllum</i>	NA	0,38	NA	NA	NA	0,14	0,01	NA	NA	0,13	0,19	NA	NA	NA	NA	NA	0,56	0	0,02	0,1	0,81	NA	0,27
<i>Salvinia biloba</i>	0,16	0,41	0,16	NA	0,38	0,45	0,29	NA	1,69	0,22	0,47	2,47	0,16	NA	0,26	2,47	0,6	0,04	0	0,85	0,07	1,23	0,49
<i>Salvinia minima</i>	NA	0,26	NA	NA	0,22	0,44	0,19	NA	0,96	0,08	0,19	1,92	NA	NA	NA	1,92	0,58	0,15	0,62	0	NA	0,96	0,64
Scrophulariaceae (sp.)	NA	0,38	NA	NA	NA	0,15	NA	NA	NA	0,16	0,19	NA	NA	NA	NA	NA	0,56	0,4	0,02	NA	0	NA	0,27
<i>Trichantheum schwackeanum</i>	NA	0,26	NA	0,64	0,45	0,19	0,22	0,64	0,96	0,28	0,19	1,92	NA	0,64	NA	1,92	0,58	NA	0,6	0,64	NA	0	0,64
<i>Utricularia gibba</i>	NA	0,76	NA	NA	0,41	0,34	0,2	NA	0,96	0,34	0,57	1,92	NA	NA	NA	1,92	1,33	0,4	0,36	0,64	0,81	0,96	0

109	BM	Ema	EE	GP	LP	NH	OS	UB	UG
<i>Bacopa monnieri</i>	0	NA	NA	NA	0,01	NA	NA	0,09	NA
<i>Eleocharis maculosa</i>	NA	0	NA	NA	0,56	0,5	2	0,23	1
<i>Eryngium eburneum</i>	NA	NA	0	0,66	0,16	NA	NA	0,07	0,21
<i>Gratiola peruviana</i>	NA	NA	0,33	0	0,06	NA	NA	0,03	NA
<i>Luziola peruviana</i>	0,11	2,25	0,64	0,46	0	1,91	1,96	1,01	1,9
<i>Nymphoides humboldtiana</i>	NA	1	NA	NA	0,95	0	2	0,93	1
<i>Oldenlandia salzmännii</i>	NA	1	NA	NA	0,24	0,5	0	0,19	0,5
<i>Utricularia breviscapa</i>	0,5	0,63	0,2	0,17	0,68	1,27	1,02	0	0,42
<i>Utricularia gibba</i>	NA	2,01	0,43	NA	0,94	1	2	0,31	0

I10	AF	BM	CR	Emi	Esp1	Ean	L	LS	LP	Man	Maq	NH	ON	Pst	RA	Smo	Tsci	UG	WO
<i>Azolla filiculoides</i>	0	1,22	1,03	1,1	1,6	1,16	0,88	1,06	1,07	0,57	0,92	1,03	2,16	1,02	1,04	0,71	1,88	0,91	1,3
<i>Bacopa monnieri</i>	0,91	0	1,05	1,26	1,42	0,72	0,76	0,79	1,16	1,19	1,03	0,87	2,16	0,93	1,06	0,71	1,88	0,88	1,3
<i>Callitriche rimosa</i>	0,77	1,05	0	1,08	1,42	0,93	0,63	0,54	0,96	1,19	0,72	0,89	2,16	1,05	1,06	0,71	NA	0,73	0,4
<i>Eleocharis minima</i>	0,68	1,04	0,9	0	1,82	0,96	0,47	0,46	1,14	0,99	0,61	0,75	3,65	0,88	0,56	0,27	0,72	0,67	0,5
<i>Eleocharis</i> sp.	0,6	0,71	0,71	1,1	0	0,92	0,34	0,53	0,9	0,28	0,3	0,67	2,16	0,8	0,49	NA	NA	0,31	NA
<i>Enydra anagallis</i>	0,43	0,36	0,46	0,58	0,92	0	0,19	0,45	0,44	0,28	0,21	0,49	2,16	0,58	NA	NA	NA	0,4	NA
<i>Lemna</i> cf. <i>valdiviana</i>	0,88	1,02	0,84	0,75	0,9	0,51	0	0,97	0,66	0,69	0,95	1	NA	0,83	1,04	0,71	1,88	0,76	1,3
<i>Ludwigia</i> sp.	0,83	0,82	0,56	0,58	1,09	0,93	0,76	0	0,49	0,43	0,87	0,88	0,37	0,7	0,61	0,28	2,8	0,65	1,46
<i>Luziola peruviana</i>	0,7	1	0,84	1,2	1,56	0,77	0,43	0,41	0	0,65	0,5	0,55	3,77	0,75	0,68	0,38	0,52	0,6	0,53
<i>Marsilea ancylopoda</i>	0,16	0,46	0,46	0,46	0,21	0,21	0,2	0,16	0,29	0	0,4	0,24	0,64	0,46	NA	0,48	NA	0,39	NA
<i>Myriophyllum aquaticum</i>	0,79	1,19	0,83	0,85	0,68	0,49	0,82	0,96	0,66	1,19	0	0,74	0,64	0,7	0,97	0,48	2,49	0,88	1,72
<i>Nymphoides humboldtiana</i>	0,68	0,77	0,79	0,8	1,19	0,86	0,66	0,75	0,56	0,56	0,57	0	0,34	0,87	0,41	0,11	1,31	0,5	0,66
<i>Oplismenopsis najada</i>	0,27	0,36	0,36	0,74	0,72	0,72	NA	0,06	0,72	0,28	0,09	0,06	0	0,36	NA	NA	NA	0,31	NA
<i>Pistia stratiotes</i>	0,76	0,93	1,05	1,06	1,6	1,16	0,62	0,67	0,86	1,19	0,6	0,98	2,16	0	0,49	0,71	NA	0,62	NA
<i>Ranunculus apiifolius</i>	0,33	0,45	0,45	0,29	0,42	NA	0,33	0,25	0,33	NA	0,36	0,2	NA	0,21	0	NA	NA	0,18	0,54
<i>Sagittaria montevidensis</i>	0,09	0,12	0,12	0,05	NA	NA	0,09	0,04	0,07	0,21	0,07	0,02	NA	0,12	NA	0	NA	0,1	NA
<i>Triglochin scilloides</i>	0,24	0,31	NA	0,14	NA	NA	0,24	0,45	0,1	NA	0,36	0,25	NA	NA	NA	NA	0	0,27	0,94
<i>Utricularia gibba</i>	0,8	1,03	0,85	0,95	0,72	0,93	0,67	0,73	0,81	1,19	0,89	0,66	2,16	0,72	0,49	0,71	1,88	0	1,3
<i>Wolffiella oblonga</i>	0,32	0,43	0,13	0,2	NA	NA	0,32	0,47	0,2	NA	0,5	0,25	NA	NA	0,42	NA	1,88	0,37	0

P01	BM	EG	Emi	EO	Esp2	HV	LH	LP	NH	OS	Ppum	Poa	Smi	Sca	Scu	UG
<i>Bacopa monnieri</i>	0	NA	NA	NA	NA	NA	NA	0,11	0,03	NA	NA	NA	NA	0,15	NA	0,02
<i>Echinodorus grandiflorus</i>	NA	0	NA	NA	NA	1,36	0,66	0,46	NA	NA	0,66	NA	NA	NA	NA	0,18
<i>Eleocharis minima</i>	NA	NA	0	0,47	0,4	0,08	0,16	0,26	0,58	0,4	0,07	4,16	0,1	0,07	0,13	0,98
<i>Eleocharis obtusetrigona</i>	NA	NA	0,44	0	NA	NA	NA	0,3	0,88	NA	0,25	4,79	0,33	0,23	NA	0,46
<i>Eleocharis sp.2</i>	NA	NA	0,11	NA	0	0,1	0,21	0,08	0,04	0,64	NA	NA	NA	NA	NA	NA
<i>Hydrocotyle verticillata</i>	NA	2,61	0,04	NA	0,19	0	0,57	0,32	0,02	0,19	0,56	NA	NA	NA	0,12	0,13
<i>Lobelia hederacea</i>	NA	1,98	0,13	NA	0,64	0,9	0	0,34	0,07	0,64	0,69	NA	NA	NA	0,4	0,11
<i>Luziola peruviana</i>	0,71	2,88	0,43	0,54	0,47	1,03	0,71	0	0,55	0,47	0,7	0,66	0,46	0,53	0,07	0,66
<i>Nymphoides humboldtiana</i>	0,16	NA	0,74	1,24	0,21	0,05	0,11	0,43	0	0,21	0,3	5,78	0,2	0,18	0,13	0,65
<i>Oldenlandia salzmännii</i>	NA	NA	0,11	NA	0,64	0,1	0,21	0,08	0,04	0	NA	NA	NA	NA	NA	NA
<i>Paspalum pumilum</i>	NA	1,98	0,05	0,21	NA	0,88	0,69	0,34	0,19	NA	0	NA	NA	NA	0,4	0,18
Poaceae (sp.)	NA	NA	1,1	1,38	NA	NA	NA	0,11	1,18	NA	NA	0	NA	NA	NA	0,69
<i>Salvinia minima</i>	NA	NA	0,03	0,09	NA	NA	NA	0,07	0,04	NA	NA	NA	0	0,43	NA	0,06
<i>Schoenoplectus californicus</i>	0,26	NA	0,03	0,11	NA	NA	NA	0,15	0,06	NA	NA	NA	0,75	0	NA	0,07
<i>Schwenckia curviflora</i>	NA	NA	0,03	NA	NA	0,06	0,13	0,01	0,03	NA	0,13	NA	NA	NA	0	0,01
<i>Utricularia gibba</i>	0,1	0,77	1,14	0,58	NA	0,29	0,17	0,46	0,59	NA	0,26	3,05	0,24	0,18	0,04	0

P02	Emi	EO	LS	LP	MF	MU	NH	PF	RD	SB	Sca	UF	UG
<i>Eleocharis minima</i>	0	0,75	1,11	0,29	NA	2,21	0,66	0,33	NA	0,08	0,36	0,26	0,42
<i>Eleocharis obtusetrigona</i>	0,82	0	1	0,64	NA	1,99	1,2	0,6	NA	0,17	0,38	0,64	0,87
<i>Ludwigia</i> sp.	0,82	0,66	0	0,15	NA	1,99	0,5	0,3	NA	NA	0,27	0,3	NA
<i>Luziola peruviana</i>	0,65	1,33	0,47	0	1	0,94	0,89	0,37	0,38	0,78	0,92	0,86	1,34
<i>Mayaca fluviatilis</i>	NA	NA	NA	0,16	0	NA	NA	NA	NA	NA	0,29	0,44	NA
<i>Micranthemum umbrosum</i>	0,82	0,66	1	0,15	NA	0	0,5	0,3	NA	NA	0,27	0,3	NA
<i>Nymphoides humboldtiana</i>	0,98	1,6	1	0,58	NA	1,99	0	0,6	NA	0,17	0,4	0,64	0,87
<i>Potamogeton ferrugineus</i>	0,32	0,53	0,39	0,16	NA	0,79	0,4	0	0,14	0,23	0,12	0,26	NA
<i>Regnellidium diphyllum</i>	NA	NA	NA	0,06	NA	NA	NA	0,05	0	0,16	NA	0,12	NA
<i>Salvinia biloba</i>	0,16	0,29	NA	0,63	NA	NA	0,22	0,43	0,81	0	0,63	0,4	0,87
<i>Schoenoplectus californicus</i>	0,7	0,66	0,71	0,77	1,5	1,42	0,52	0,23	NA	0,65	0	0,67	0,62
<i>Utricularia foliosa</i>	0,61	1,35	0,93	0,88	2,74	1,86	1,01	0,62	0,76	0,5	0,81	0	0,25
<i>Utricularia gibba</i>	0,16	0,29	NA	0,22	NA	NA	0,22	NA	NA	0,17	0,12	0,04	0

P03	AP	AF	EG	Eaz	EC	EO	Esp 1	Ean	EC h	HR	HV	L	LB	LH	LS	LP	MU	Ma q	NH	Ppu n	PC	RN	SB	S	WB	W O
<i>Alternanthera philoxeroides</i>	0	0,6 8	0,3 2	NA	0,3 1	0,1 3	0,36	2,0 3	4,0 5	NA	0,4	0,6 8	0,3 8	0,3 6	NA	0,3 9	0,3 8	0,0 6	0,1 8	2,03	NA	0,6 8	0,9 8	0,4	0,6 8	0,6 8
<i>Azolla filiculoides</i>	1,1 9	0	NA	3,1 2	2,2 6	1,6 8	NA	2,3 5	2,7 6	2,0 7	NA	2,2 2	NA	NA	1,8 6	1,0 9	NA	1,5 2	0,9 6	2,52	1,9 3	2,2 2	2,2 2	NA	2,2 2	2,2 2
<i>Echinodorus grandiflorus</i>	0,0 9	NA	0	NA	NA	NA	0,16	NA	NA	NA	NA	NA	NA	0,2	NA	0,0 5	NA	0,0 4	NA	NA	NA	NA	NA	NA	NA	NA
<i>Eichhornia azurea</i>	NA	0,5 2	NA	0	0,5 4	NA	NA	NA	NA	1,3 5	NA	0,5 2	NA	NA	1,6 6	0,1 7	NA	0,6 5	NA	NA	NA	0,5 2	0,7 5	NA	0,5 2	0,5 2
<i>Eichhornia crassipes</i>	0,3 4	1,4	NA	2,0 2	0	1,2 9	NA	0,3 9	0,7 8	1,5 8	NA	1,4	NA	NA	1,1 4	0,4	NA	0,7	NA	0,71	NA	1,4	1,5 4	NA	1,4	1,4
<i>Eleocharis obtusetrigona</i>	0,1 2	0,8 4	NA	NA	1,0 4	0	0,21	NA	NA	0,5 2	0,6 3	0,8 4	0,3 2	0,2 6	NA	0,6 3	0,3 2	NA	NA	1,14	NA	0,8 4	1,0 8	0,6 3	0,8 4	0,8 4
<i>Eleocharis</i> sp.	0,3 2	NA	0,5	NA	NA	0,2 1	0	NA	NA	NA	0,6 3	NA	0,5 9	0,5 6	NA	0,2 8	0,5 9	0,1	0,2 8	NA	NA	NA	NA	0,6 3	NA	NA
<i>Enydra anagallis</i>	1,1 9	0,7 8	NA	NA	0,2 1	NA	NA	0	2,7 6	0,3 7	NA	0,7 8	NA	NA	0,4 5	0,6	NA	0,7 8	0,9 6	1,38	1,9 3	0,7 8	0,5 9	NA	0,7 8	0,7 8
<i>Eryngium chamissonis</i>	1,1 9	0,4 6	NA	NA	0,2 1	NA	NA	1,3 8	0	NA	NA	0,4 6	NA	NA	NA	0,1 5	NA	NA	NA	1,38	NA	0,4 6	0,6 6	NA	0,4 6	0,4 6
<i>Hydrocotyle ranunculoides</i>	NA	1,1 7	NA	4,5 8	1,4 4	0,5 9	NA	0,6 2	NA	0	NA	1,1 7	NA	NA	2,0 1	0,5 3	NA	1,3	0,6 2	NA	1,2 4	1,1 7	1,1 7	NA	1,1 7	1,1 7
<i>Hydrocotyle verticillata</i>	0,1 2	NA	NA	NA	NA	0,2 1	0,21	NA	NA	NA	0	NA	0,3 2	0,2 6	NA	0,1 3	0,3 2	NA	NA	NA	NA	NA	NA	0,6 3	NA	NA
<i>Lemna</i> cf. <i>valdiviana</i>	1,1 9	2,2 2	NA	3,1 2	2,2 6	1,6 8	NA	2,3 5	2,7 6	2,0 7	NA	0	NA	NA	1,8 6	1,0 9	NA	1,5 2	0,9 6	2,52	1,9 3	2,2 2	2,2 2	NA	2,2 2	2,2 2
<i>Lilaeopsis brasiliensis</i>	0,2 2	NA	NA	NA	NA	0,2 1	0,39	NA	NA	NA	0,6 3	NA	0	0,3 5	NA	0,2 4	0,5 9	0,0 5	0,2 8	NA	NA	NA	NA	0,6 3	NA	NA
<i>Lobelia hederacea</i>	0,2 9	NA	0,5 5	NA	NA	0,2 3	0,5	NA	NA	NA	0,7	NA	0,4 7	0	NA	0,2 5	0,4 7	0,0 8	0,1 3	NA	NA	NA	NA	0,7	NA	NA
<i>Ludwigia</i> sp.	NA	0,7 9	NA	4,2 3	0,7 8	NA	NA	0,5 7	NA	1,5 1	NA	0,7 9	NA	NA	0	0,3 8	NA	1,2	0,5 7	NA	1,1 4	0,7 9	0,7 1	NA	0,7 9	0,7 9
<i>Luziola peruviana</i>	0,7 4	1,1 7	0,3 3	1,0 8	0,6 9	1,3 6	0,61	1,9 5	0,9 5	1,0 2	0,8 3	1,1 7	0,7 8	0,5 9	0,9 8	0	0,7 8	1	1,5	1,28	2,5 4	1,1 7	0,8 8	0,8 3	1,1 7	1,1 7
<i>Micranthemum umbrosum</i>	0,2 2	NA	NA	NA	NA	0,2 1	0,39	NA	NA	NA	0,6 3	NA	0,5 9	0,3 5	NA	0,2 4	0	0,0 5	0,2 8	NA	NA	NA	NA	0,6 3	NA	NA
<i>Myriophyllum aquaticum</i>	0,0 7	0,9 6	0,1 7	2,4 5	0,7 2	NA	0,12	1,4 7	NA	1,4 4	NA	0,9 6	0,0 9	0,1 1	1,7 8	0,5 8	0,0 9	0	1,0 6	NA	2,9 3	0,9 6	0,5	NA	0,9 6	0,9 6
<i>Nymphoides humboldtiana</i>	0,1	0,3 2	NA	NA	NA	NA	0,18	0,9 6	NA	0,3 7	NA	0,3 2	0,2 8	0,1	0,4 5	0,4 6	0,2 8	0,5 6	0	NA	1,9 3	0,3 2	0,0 2	NA	0,3 2	0,3 2
<i>Polygonum punctatum</i>	1,1 9	0,8 4	NA	NA	0,3 8	0,7 6	NA	1,3 8	2,7 6	NA	NA	0,8 4	NA	NA	NA	0,3 9	NA	NA	NA	0	NA	0,8 4	1,0 8	NA	0,8 4	0,8 4
<i>Pontederia cordata</i>	NA	0,3 2	NA	NA	NA	NA	NA	0,9 6	NA	0,3 7	NA	0,3 2	NA	NA	0,4 5	0,3 9	NA	0,7 8	0,9 6	NA	0	0,3 2	0,0 2	NA	0,3 2	0,3 2
<i>Ricciocarpos natans</i>	1,1 9	2,2 2	NA	3,1 2	2,2 6	1,6 8	NA	2,3 5	2,7 6	2,0 7	NA	2,2 2	NA	NA	1,8 6	1,0 9	NA	1,5 2	0,9 6	2,52	1,9 3	0	2,2 2	NA	2,2 2	2,2 2
<i>Salvinia biloba</i>	1,3 3	1,7 1	NA	3,4 8	1,9 2	1,6 7	NA	1,3 6	3,0 8	1,6	NA	1,7 1	NA	NA	1,2 9	0,6 3	NA	0,6 1	0,0 4	2,5	0,0 8	1,7 1	0	NA	1,7 1	1,7 1

Scrophulariaceae(sp.)	0,1 2	NA	NA	NA	NA	0,2 1	0,21	NA	NA	NA	0,6 3	NA	0,3 2	0,2 6	NA	0,1 3	0,3 2	NA	NA	NA	NA	NA	NA	0	NA	NA
<i>Wolffia brasiliensis</i>	1,1 9	2,2 2	NA	3,1 2	2,2 6	1,6 8	NA	2,3 5	2,7 6	2,0 7	NA	2,2 2	NA	NA	1,8 6	1,0 9	NA	1,5 2	0,9 6	2,52	1,9 3	2,2 2	2,2 2	NA	0	2,2 2
<i>Wolffiella oblonga</i>	1,1 9	2,2 2	NA	3,1 2	2,2 6	1,6 8	NA	2,3 5	2,7 6	2,0 7	NA	2,2 2	NA	NA	1,8 6	1,0 9	NA	1,5 2	0,9 6	2,52	1,9 3	2,2 2	2,2 2	NA	2,2 2	0

P04	AF	BM	EG	EC	EO	Esp1	Ean	HR	LS	LP	Maq	NH	PC	RN	SB	SI	UB	UF
<i>Azolla filiculoides</i>	0	2,47	NA	1,79	0,75	0,82	1,46	2,47	0,28	0,48	0,59	2,47	0,57	0,85	1,07	0,35	0,07	1,46
<i>Bacopa monnieri</i>	0,82	0	NA	1,79	0,39	0,82	1,24	2,47	NA	0,15	0,42	2,47	0,21	0,49	0,62	NA	NA	NA
<i>Echinodorus grandiflorus</i>	NA	NA	0	NA	0,66	0,19	NA	NA	0,21	0,36	0,14	NA	0,31	0,44	0,46	0,91	0,22	NA
<i>Eichhornia crassipes</i>	1,05	3,14	NA	0	0,5	0,57	1,57	3,14	0,02	0,1	0,22	3,14	0,23	0,43	0,79	NA	0,05	0,02
<i>Eleocharis obtusetrigona</i>	1,08	1,68	1,42	1,22	0	0,56	0,99	1,68	0,66	0,89	0,91	1,68	0,64	1,19	1,48	1,36	0,42	1
<i>Eleocharis</i> sp.	0,82	2,47	0,28	0,98	0,39	0	1,24	2,47	NA	0,24	0,24	2,47	0,37	0,54	0,62	NA	0,16	0,04
<i>Enydra anagallis</i>	0,97	2,47	NA	1,79	0,46	0,82	0	2,47	0,08	0,27	0,49	2,47	0,25	0,49	0,62	0,35	0,07	NA
<i>Hydrocotyle ranunculoides</i>	0,82	2,47	NA	1,79	0,39	0,82	1,24	0	NA	0,15	0,42	2,47	0,21	0,49	0,62	NA	NA	NA
<i>Ludwigia</i> sp.	0,48	NA	0,54	0,06	0,78	NA	0,21	NA	0	0,62	0,76	NA	0,55	0,46	0,83	0,72	0,53	0,58
<i>Luziola peruviana</i>	1,27	1,21	1,41	0,47	1,64	0,62	1,05	1,21	0,96	0	0,88	1,21	0,94	1,19	1,57	1,54	0,61	0,7
<i>Myriophyllum aquaticum</i>	1,05	2,21	0,37	0,67	1,12	0,42	1,3	2,21	0,78	0,59	0	2,21	0,53	0,52	0,79	0,82	0,5	0,16
<i>Nymphoides humboldtiana</i>	0,82	2,47	NA	1,79	0,39	0,82	1,24	2,47	NA	0,15	0,42	0	0,21	0,49	0,62	NA	NA	NA
<i>Pontederia cordata</i>	1,29	1,42	1,04	0,88	1,01	0,85	0,83	1,42	0,73	0,82	0,68	1,42	0	1,35	1,19	0,69	0,58	1,28
<i>Ricciocarpos natans</i>	1,42	2,47	1,1	1,22	1,38	0,9	1,23	2,47	0,46	0,76	0,49	2,47	0,99	0	1,52	0,91	0,39	0,98
<i>Salvinia biloba</i>	1,42	2,47	0,91	1,79	1,38	0,82	1,24	2,47	0,65	0,8	0,6	2,47	0,7	1,22	0	0,91	0,18	1,46
<i>Spirodela intermedia</i>	0,24	NA	0,91	NA	0,63	NA	0,35	NA	0,28	0,39	0,31	NA	0,2	0,36	0,46	0	0,25	NA
<i>Utricularia breviscapa</i>	0,12	NA	0,58	0,15	0,51	0,27	0,19	NA	0,55	0,41	0,5	NA	0,45	0,4	0,24	0,64	0	0,1
<i>Utricularia foliosa</i>	0,72	NA	NA	0,02	0,34	0,02	NA	NA	0,17	0,13	0,05	NA	0,28	0,29	0,54	NA	0,03	0

P05	AF	Emi	HR	L	LS	LP	NH	OS	Ppum	Ppun	RD	RN	SB	Sca	UG	WB	WO
<i>Azolla filiculoides</i>	0	2,59	2,69	2,67	2,36	2,02	2,26	1,1	1,1	4	2,92	2,27	2,78	2,19	1,1	2,7	2,53
<i>Eleocharis minima</i>	2,47	0	4,05	3,13	2,85	3	3,31	3,28	3,28	2,53	1,96	2,68	2,27	0,08	3,28	2,83	1,01
<i>Hydrocotyle ranunculoides</i>	0,73	1,15	0	1	1,04	0,62	0,73	NA	NA	NA	0,26	1,75	0,89	NA	NA	1,4	0,27
<i>Lemna cf. valdiviana</i>	2,54	3,11	3,5	0	3,06	2,99	2,97	2,84	2,84	2,86	2,43	3,15	2,5	0,32	2,84	3,34	1,35
<i>Ludwigia sp.</i>	1,68	2,12	2,73	2,29	0	2,1	2,14	NA	NA	3,37	2,05	2,45	2,83	0,91	NA	2,93	1,3
<i>Luziola peruviana</i>	1,7	2,65	1,94	2,65	2,49	0	2,98	4,24	4,24	1,17	2,02	2,31	1,64	1,07	4,24	2,19	1,51
<i>Nymphoides humboldtiana</i>	1,6	2,44	1,91	2,21	2,13	2,5	0	3,43	3,43	1,83	1,25	1,23	1,49	NA	3,43	1,48	0,65
<i>Oldenlandia salzmännii</i>	0,15	0,47	NA	0,41	NA	0,68	0,66	0	2,84	NA	NA	NA	NA	NA	2,84	NA	0,11
<i>Paspalum pumilum</i>	0,15	0,47	NA	0,41	NA	0,68	0,66	2,84	0	NA	NA	NA	NA	NA	2,84	NA	0,11
<i>Polygonum punctatum</i>	0,94	0,62	NA	0,71	1,12	0,33	0,61	NA	NA	0	0,78	NA	1,57	NA	NA	0,99	0,23
<i>Regnellidium diphyllum</i>	2,03	1,42	0,68	1,78	2	1,67	1,23	NA	NA	2,29	0	1,54	2,34	4,67	NA	1,87	3,84
<i>Ricciocarpos natans</i>	1,23	1,52	3,5	1,8	1,87	1,49	0,95	NA	NA	NA	1,2	0	1,28	0,32	NA	2,52	0,91
<i>Salvinia biloba</i>	1,87	1,6	2,2	1,77	2,68	1,31	1,42	NA	NA	4,49	2,27	1,59	0	2,6	NA	2,48	2,27
<i>Schoenoplectus californicus</i>	0,37	0,01	NA	0,06	0,21	0,21	NA	NA	NA	NA	1,12	0,1	0,64	0	NA	0,08	2,01
<i>Utricularia gibba</i>	0,15	0,47	NA	0,41	NA	0,68	0,66	2,84	2,84	NA	NA	NA	NA	NA	0	NA	0,11
<i>Wolffia brasiliensis</i>	1,83	2,01	3,5	2,39	2,8	1,76	1,42	NA	NA	2,86	1,83	3,15	2,5	0,32	NA	0	1,09
<i>Wolffiella oblonga</i>	0,98	0,41	0,39	0,55	0,71	0,69	0,36	0,31	0,31	0,38	2,14	0,64	1,3	4,66	0,31	0,62	0

P06	AF	Emi	GP	HV	LB	LH	LS	LP	MU	NH	OS	Ppum	UG	WB
<i>Azolla filiculoides</i>	0	0,89	1,98	0,34	NA	0,53	2,17	0,99	0,37	0,85	1,14	NA	2,33	1,7
<i>Eleocharis minima</i>	1,36	0	2,61	2,19	1,84	1,69	0,04	1,75	1,91	1,86	2,58	1,58	0,41	0,33
<i>Gratiola peruviana</i>	0,4	0,34	0	NA	NA	NA	NA	0,19	NA	0,2	0,79	NA	NA	NA
<i>Hydrocotyle verticillata</i>	0,19	0,82	NA	0	0,73	1,02	NA	0,59	0,69	0,41	1,16	2,76	NA	NA
<i>Lilaeopsis brasiliensis</i>	NA	0,24	NA	0,26	0	0,35	NA	0,37	1,14	0,2	NA	NA	NA	NA
<i>Lobelia hederacea</i>	0,2	0,43	NA	0,69	0,67	0	NA	0,38	0,39	0,32	0,48	0,57	NA	NA
<i>Ludwigia</i> sp.	0,5	0,01	NA	NA	NA	NA	0	0,13	NA	0,03	NA	NA	0,99	0,76
<i>Luziola peruviana</i>	1,62	1,87	1,55	1,67	2,97	1,61	0,91	0	2,9	2,29	1,52	1,31	1,34	0,91
<i>Micranthemum umbrosum</i>	0,21	0,71	NA	0,69	3,22	0,57	NA	1,01	0	1,02	0,09	0,62	0,3	NA
<i>Nymphoides humboldtiana</i>	0,88	1,27	1,02	0,75	1	0,85	0,13	1,46	1,87	0	0,92	0,44	0,54	0,13
<i>Oldenlandia salzmännii</i>	0,8	1,18	2,74	1,42	NA	0,87	NA	0,65	0,12	0,62	0	1,01	NA	NA
<i>Paspalum pumilum</i>	NA	0,21	NA	0,98	NA	0,3	NA	0,16	0,22	0,09	0,29	0	NA	NA
<i>Utricularia gibba</i>	0,9	0,1	NA	NA	NA	NA	1,66	0,32	0,21	0,2	NA	NA	0	1,07
<i>Wolffia brasiliensis</i>	0,69	0,09	NA	NA	NA	NA	1,33	0,22	NA	0,05	NA	NA	1,12	0

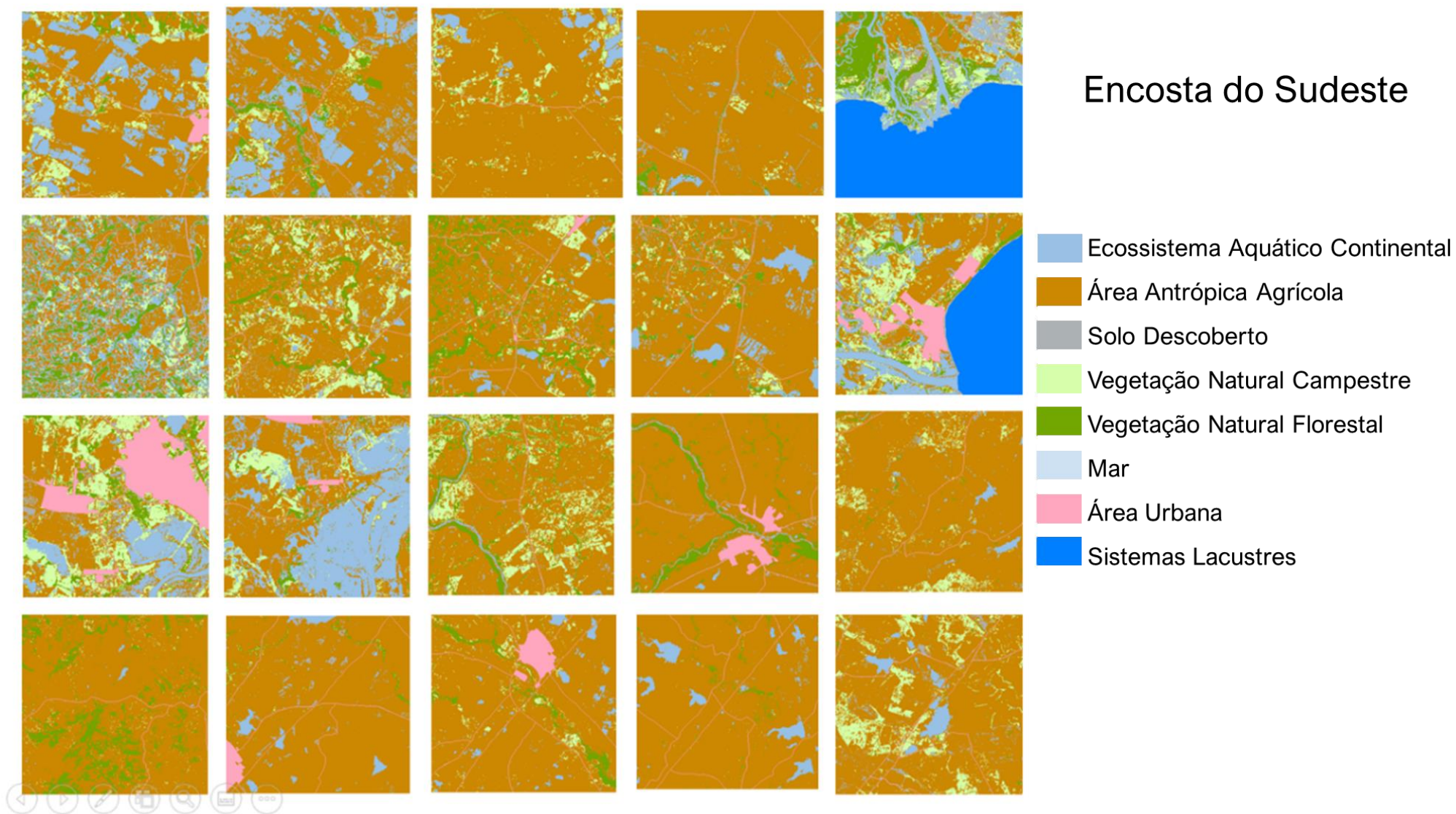
P07	CR	Ema	Emi	EO	GP	LP	MU	Maq	NH	OS	Ppum	Scu	UG
<i>Callitriche rimosa</i>	0	NA	0,63	NA	0,57	0,69	NA	0,54	0,38	NA	0,64	NA	0,37
<i>Eleocharis maculosa</i>	NA	0	1,26	NA	0,28	0,65	NA	NA	1,4	NA	0,93	2,8	1,19
<i>Eleocharis minima</i>	1,33	2,64	0	0,19	0,97	1,03	0,39	0	1,26	0,39	1,59	4,01	1,34
<i>Eleocharis obtusetrigona</i>	NA	NA	0,05	0	NA	0,09	NA	0,05	0,04	NA	NA	NA	0,17
<i>Gratiola peruviana</i>	0,44	0,21	0,36	NA	0	0,33	1,06	NA	0,52	1,06	0,88	NA	0,11
<i>Luziola peruviana</i>	1,87	1,76	1,33	0,41	1,17	0	0,59	0,72	1,13	0,59	1,3	2,08	1,38
<i>Micranthemum umbrosum</i>	NA	NA	0,06	NA	0,46	0,07	0	NA	0,07	0,8	0,27	NA	NA
<i>Myriophyllum aquaticum</i>	0,27	NA	0	0,04	NA	0,13	NA	0	0,03	NA	NA	NA	0,02
<i>Nymphoides humboldtiana</i>	0,66	2,46	1,06	0,11	1,19	0,74	0,37	0,11	0	0,37	1,06	1,29	0,99
<i>Oldenlandia salzmännii</i>	NA	NA	0,06	NA	0,46	0,07	0,8	NA	0,07	0	0,27	NA	NA
<i>Paspalum pumilum</i>	0,64	0,93	0,76	NA	1,15	0,48	0,8	NA	0,61	0,8	0	2,8	0,47
<i>Schwenckia curviflora</i>	NA	0,93	0,64	NA	NA	0,26	NA	NA	0,24	NA	0,93	0	0,47
<i>Utricularia gibba</i>	0,74	2,37	1,28	0,55	0,28	1,02	NA	0,09	1,13	NA	0,93	2,8	0

P08	AF	CR	Eaz	Ean	HR	L	LB	LL	LS	LP	Man	Maq	ON	PC	Psp	RA	RN	SB	Smi	Tsci	WB	WO
<i>Azolla filiculoides</i>	0	3,27	3,03	3,23	3,16	3,39	3,27	3,26	3,28	3,22	3,29	3,18	3,36	2,84	3,42	3,36	3,17	3,19	3,27	3,37	3,27	3,29
<i>Callitriche rimosa</i>	0,37	0	NA	NA	0,16	NA	3,21	0,27	NA	1,07	2,61	0,39	0,44	NA	NA	NA	0,53	NA	NA	NA	0,36	0,3
<i>Eichhornia azurea</i>	0,68	NA	0	NA	1,5	0,72	NA	0,92	0,69	NA	NA	0,37	NA	1,52	NA	NA	1,11	1,56	0,83	NA	0,74	0,92
<i>Enydra anagallis</i>	0,36	NA	NA	0	0,71	NA	NA	0,61	NA	NA	NA	0,38	NA	NA	NA	0,79	NA	0,2	0,4	0,63	0,35	0,57
<i>Hydrocotyle ranunculoides</i>	1,7	0,79	3,62	3,44	0	0,87	0,79	2,48	0,8	0,5	0,68	0,99	0,7	1,65	NA	1,95	1,42	2,74	1,87	1,56	1,75	2,4
<i>Lemna cf. valdiviana</i>	1,89	NA	1,81	NA	0,9	0	NA	1,46	3,01	2,09	0,6	2,07	2,36	1,6	3,35	1,62	2,18	1,56	2,08	1,96	1,85	1,5
<i>Lilaeopsis brasiliensis</i>	0,37	3,21	NA	NA	0,16	NA	0	0,27	NA	1,07	2,61	0,39	0,44	NA	NA	NA	0,53	NA	NA	NA	0,36	0,3
<i>Limnobium laevigatum</i>	2,48	1,84	3,14	4,16	3,49	1,99	1,84	0	1,26	1,81	1,6	1,59	1,94	1,8	1,04	2,58	2,25	3,1	2,54	2,45	2,46	3,1
<i>Ludwigia sp.</i>	1,24	NA	1,16	NA	0,56	2,04	NA	0,63	0	1,2	0,57	1,95	1,67	0,53	2,65	1,53	0,99	1,02	1,39	1,76	1,24	0,88
<i>Luziola peruviana</i>	1,08	3,21	NA	NA	0,31	1,25	3,21	0,8	1,06	0	2,79	1,14	1,86	NA	0,38	0,74	1,58	0,46	0,78	1,25	1,05	0,89
<i>Marsilea ancylopoda</i>	0,54	3,85	NA	NA	0,21	0,18	3,85	0,35	0,25	1,37	0	0,64	0,65	NA	0,21	0,22	0,69	0,02	0,11	0,18	0,53	0,42
<i>Myriophyllum aquaticum</i>	2,17	2,37	1,13	2,34	1,26	2,54	2,37	1,43	3,51	2,33	2,67	0	2,49	2,31	3,88	2,54	1,94	1,34	2,15	2,53	2,18	1,63
<i>Oplismenopsis najada</i>	1,95	2,29	NA	NA	0,75	2,45	2,29	1,48	2,55	3,22	2,3	2,11	0	1,14	2,74	1,78	1,99	1,47	1,85	2,51	1,9	1,56
<i>Pontederia cordata</i>	0,63	NA	1,52	NA	0,69	0,64	NA	0,53	0,31	NA	NA	0,76	0,44	0	1,42	NA	1,04	0,93	0,78	NA	0,7	0,42
<i>Potamogeton sp.</i>	0,94	NA	NA	NA	NA	1,66	NA	0,38	1,94	0,32	0,36	1,57	1,31	1,76	0	0,96	0,75	0,44	1,04	0,94	0,92	0,39
<i>Ranunculus apiifolius</i>	1,5	NA	NA	3,17	1,61	1,29	NA	1,51	1,81	0,98	0,6	1,66	1,38	NA	1,56	0	0,49	1,15	1,65	2,64	1,47	1,64
<i>Ricciocarpus natans</i>	2,12	3,21	3,34	NA	1,77	2,62	3,21	1,98	1,76	3,16	2,79	1,9	2,3	3,13	1,82	0,74	0	2,19	2,02	1,25	2,15	1,92
<i>Salvinia biloba</i>	1,89	NA	4,15	1,04	3,01	1,66	NA	2,42	1,6	0,81	0,09	1,16	1,5	2,46	0,94	1,53	1,93	0	2,19	1,65	1,95	2,37
<i>Salvinia minima</i>	2,92	NA	3,34	3,17	3,1	3,32	NA	2,99	3,29	2,09	0,6	2,82	2,86	3,13	3,35	3,3	2,69	3,3	0	3,3	2,93	2,99
<i>Triglochin scilloides</i>	1,88	NA	NA	3,17	1,61	1,96	NA	1,8	2,59	2,09	0,6	2,07	2,42	NA	1,91	3,3	1,04	1,55	2,06	0	1,83	1,95
<i>Wolffia brasiliensis</i>	3,29	3,21	3,34	3,17	3,26	3,32	3,21	3,26	3,29	3,16	3,23	3,2	3,29	3,13	3,35	3,3	3,23	3,3	3,3	3,3	0	3,29
<i>Wolffiella oblonga</i>	2,68	2,19	3,35	4,2	3,63	2,19	2,19	3,33	1,89	2,16	2,09	1,95	2,19	1,54	1,15	2,99	2,34	3,26	2,73	2,85	2,67	0

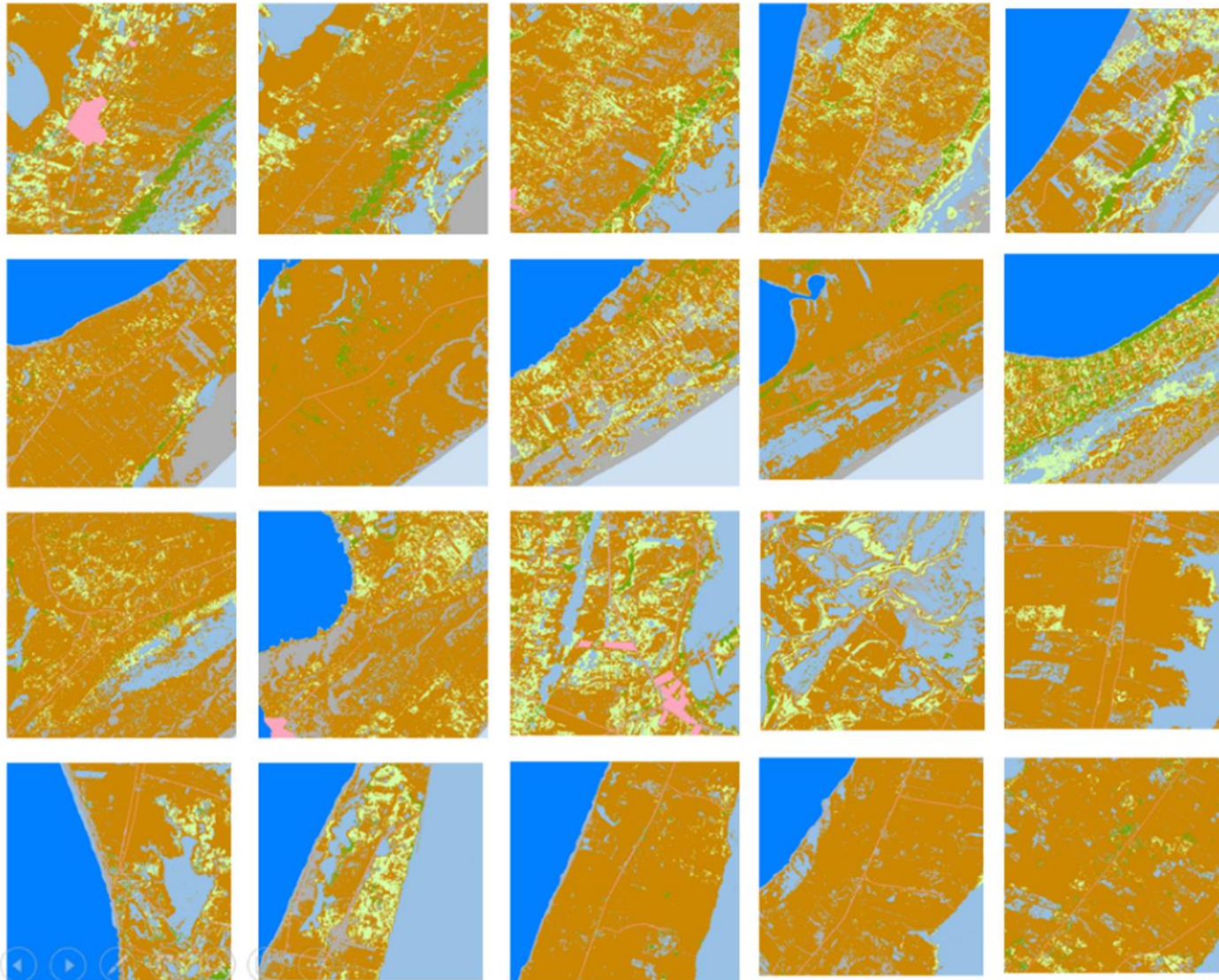
P09	AF	CA	Em i	EO	Ea n	HR	H V	LL	LS	LP	M U	N H	Ppu n	RD	Sm i	U G	W B
<i>Azolla filiculoides</i>	0	N A	0,0 9	0,3 5	0,2 6	N A	N A	0,3 5	0,3	0,1 9	N A	0,1 7	0,1 4	N A	0,3 5	0,2 4	N A
<i>Centella asiatica</i>	N A	0	0,0 3	N A	N A	N A	0,3	N A	N A	0,0 2	N A	0,0 3	NA	N A	N A	0,0 4	N A
<i>Eleocharis minima</i>	0,0 8	0,1	0	0,2 3	0,1 5	0,3 1	0,1	0,0 8	0,2	0,2 5	0,0 7	0,3 2	0,5 3	0,8 4	0,0 7	0,1 5	N A
<i>Eleocharis obtusetrigona</i>	0,3 5	N A	0,2 4	0	0,1 5	N A	N A	0,2 5	0,3 2	0,2 4	N A	0,1 8	0,0 8	0,6 4	0,2 3	0,2 5	N A
<i>Enydra anagallis</i>	0,3 8	N A	0,2 4	0,2 2	0	0,5 1	N A	0,3 4	0,3 3	0,3 4	0,3	0,3 6	0,4 8	N A	0,4 7	0,3 1	0,6 3
<i>Hydrocotyle ranunculoides</i>	N A	N A	0,2 5	N A	0,2 6	0	N A	0,0 9	0,1 8	0,2 3	0,4 8	0,2 7	0,4 5	N A	0,1 1	0,1 2	0,4 4
<i>Hydrocotyle verticillata</i>	N A	0,3	0,0 3	N A	N A	N A	0	N A	N A	0,0 2	N A	0,0 3	NA	N A	N A	0,0 4	N A
<i>Limnobium laevigatum</i>	0,3	N A	0,0 7	0,2 1	0,2	0,1	N A	0	0,1 6	0,1 6	0,3 1	0,1 4	0,0 4	N A	0,1 3	0,1 9	N A
<i>Ludwigia sp.</i>	0,3 4	N A	0,2 5	0,3 7	0,2 6	0,2 8	N A	0,2 2	0	0,3	0,4 3	0,2 9	0,3 3	0,2 5	0,2 9	0,2 6	N A
<i>Luziola peruviana</i>	0,4	0,1 7	0,5 5	0,5	0,4 8	0,6 4	0,1 7	0,4	0,5 5	0	0,5 3	0,5 2	0,6	0,7	0,3 9	0,4 2	0,4 9
<i>Micranthemum umbrosum</i>	N A	N A	0,0 2	N A	0,0 5	0,1 6	N A	0,0 9	0,0 9	0,0 6	0	0,0 5	NA	N A	N A	0,0 6	N A
<i>Nymphoides humboldtiana</i>	0,3	0,2 3	0,5 9	0,3 2	0,4 3	0,6 2	0,2 3	0,3	0,4 5	0,4 4	0,3 6	0	0,7 8	0,4 8	0,2 9	0,3 2	0,3 4
<i>Polygonum punctatum</i>	0,0 9	N A	0,3 4	0,0 5	0,2	0,3 7	N A	0,0 3	0,1 7	0,1 8	N A	0,2 7	0	N A	0,1 2	0,0 6	0,1 5
<i>Regnellidium diphyllum</i>	N A	N A	0,2 2	0,1 6	N A	N A	N A	N A	0,0 5	0,0 8	N A	0,0 7	NA	0	N A	0,0 8	N A
<i>Salvinia minima</i>	0,3 5	N A	0,0 7	0,2 3	0,3 2	0,1 5	N A	0,1 5	0,2 6	0,1 9	N A	0,1 6	0,2	N A	0	0,2 3	0,4 4
<i>Utricularia gibba</i>	0,4 7	0,3	0,3 2	0,5 1	0,4 2	0,3 1	0,3	0,4 4	0,4 6	0,4 1	0,4 8	0,3 7	0,2	0,6 4	0,4 6	0	0,4 4
<i>Wolffia brasiliensis</i>	N A	N A	N A	N A	0,1 1	0,1 5	N A	N A	N A	0,0 6	N A	0,0 5	0,0 6	N A	0,1 1	0,0 6	0

P10	CA	Emi	HV	LS	LP	MU	NH	Ppum
<i>Centella asiatica</i>	0	0,4	NA	NA	0,14	NA	0,24	0,35
<i>Eleocharis minima</i>	2,96	0	1,26	0,01	0,81	0,99	0,78	1,68
<i>Hydrocotyle verticillata</i>	NA	0,77	0	NA	0,65	1,03	0,6	1,36
<i>Ludwigia sp.</i>	NA	0	NA	0	0,03	NA	0,01	NA
<i>Luziola peruviana</i>	1,12	0,87	1,15	0,22	0	1	1,11	1,22
<i>Micranthemum umbrosum</i>	NA	0,54	0,93	NA	0,51	0	0,38	1,01
<i>Nymphoides humboldtiana</i>	1,06	0,47	0,59	0,04	0,61	0,42	0	0,51
<i>Paspalum pumilum</i>	1,49	0,99	1,31	NA	0,66	1,08	0,5	0

ANEXO 4 – Imagens geradas a partir da classificação digital supervisionada das imagens de satélite.

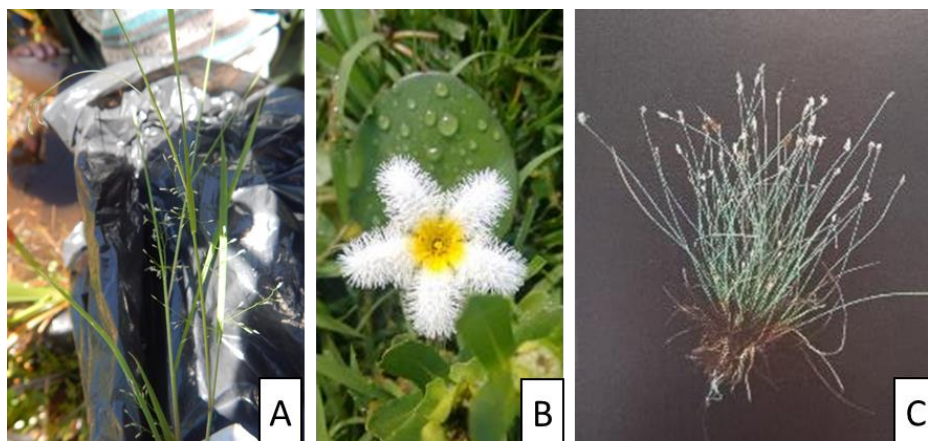


Litoral



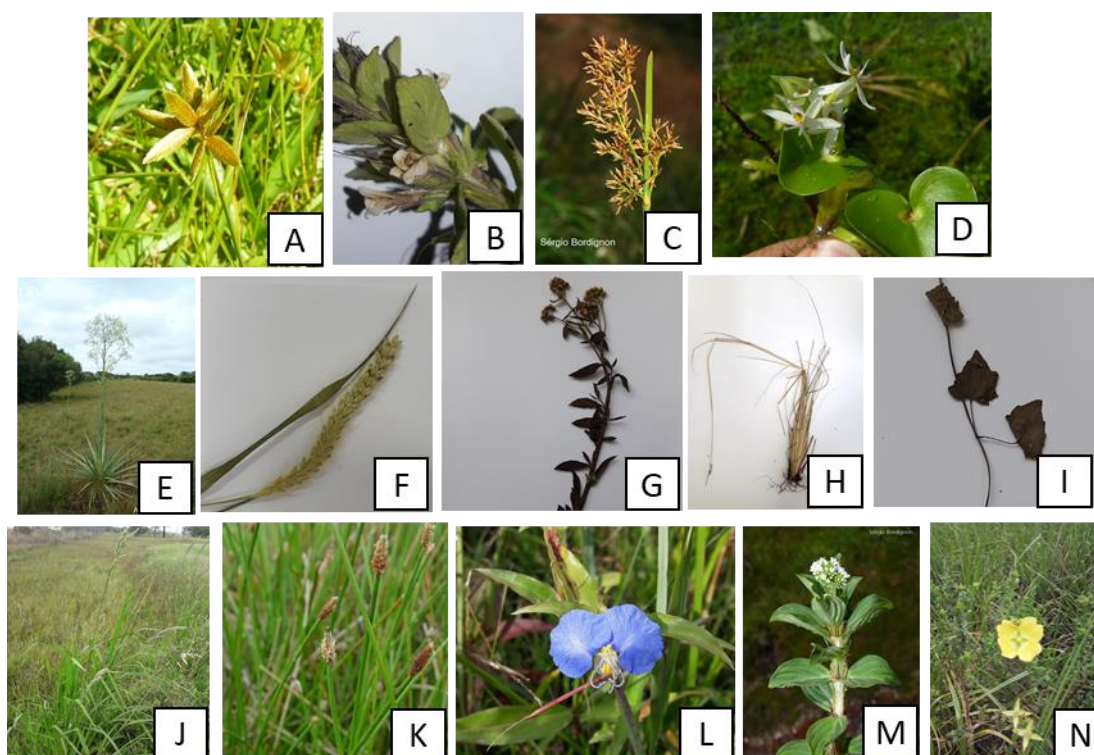
- Ecossistema Aquático Continental
- Área Antrópica Agrícola
- Solo Descoberto
- Vegetação Natural Campestre
- Vegetação Natural Florestal
- Ecossistema Marinho
- Área Antrópica Urbana
- Grandes Lagoas

ANEXO 5 – Espécies mais frequentes de áreas úmidas intermitentes e permanentes de Rio Grande – RS



A) *Luziola peruviana* Juss. ex J.F.Gmel.; B) *Nymphoides humboldtiana* (Kunth) Kuntze; C) *Eleocharis minima* Kunth. **Fonte:** A e B – Karine Massia Pereira; C – Plantas Aquáticas do Pantanal, Pott & Pott (2010).

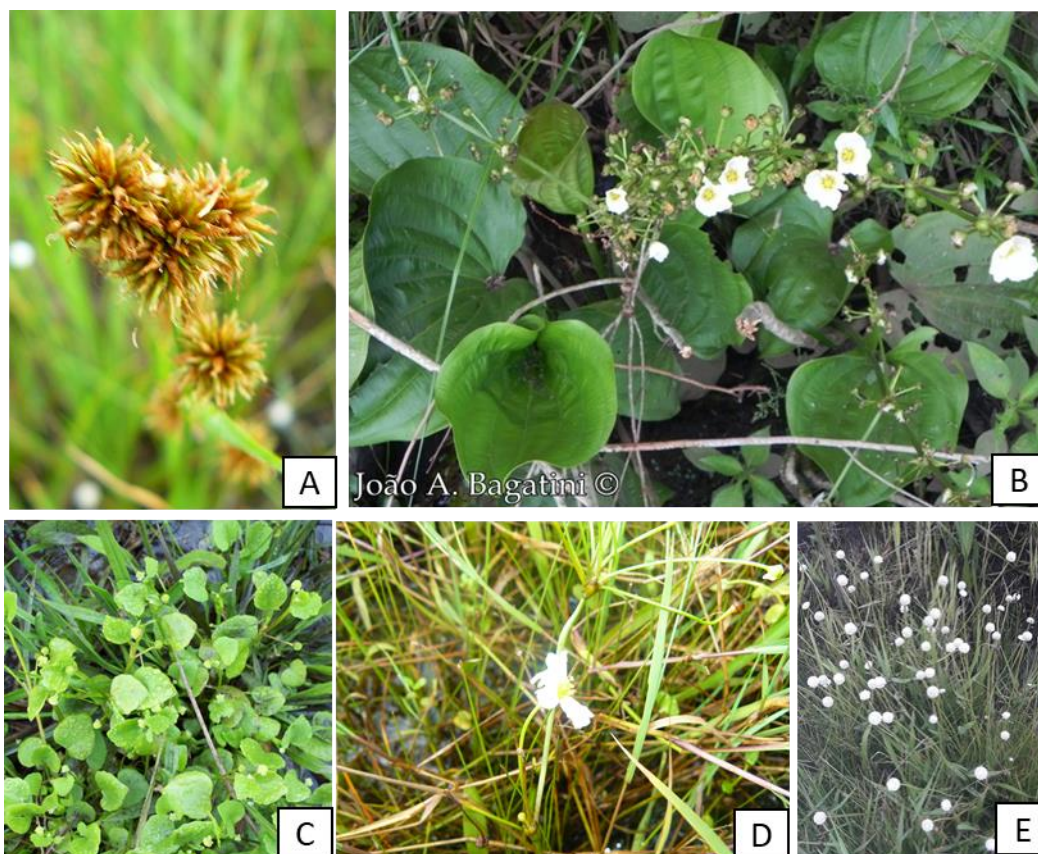
ANEXO 6 – Espécies Indicadoras da Encosta do Sudeste



A) *Cyperus lanceolatus* Poir.; B) *Hygrophila costata* Ness; C) *Fimbristylis complanata*; D) *Heteranthera reniformis* Ruiz & Pav.; E) *Eryngium chamissonis* Urb.; F) *Setaria parviflora* (Poir.) Kerguelen; G) *Pluchea sagittalis* (Lam.) Cabrera; H) *Eragrostis plana* Nees; I) *Mikania micrantha*

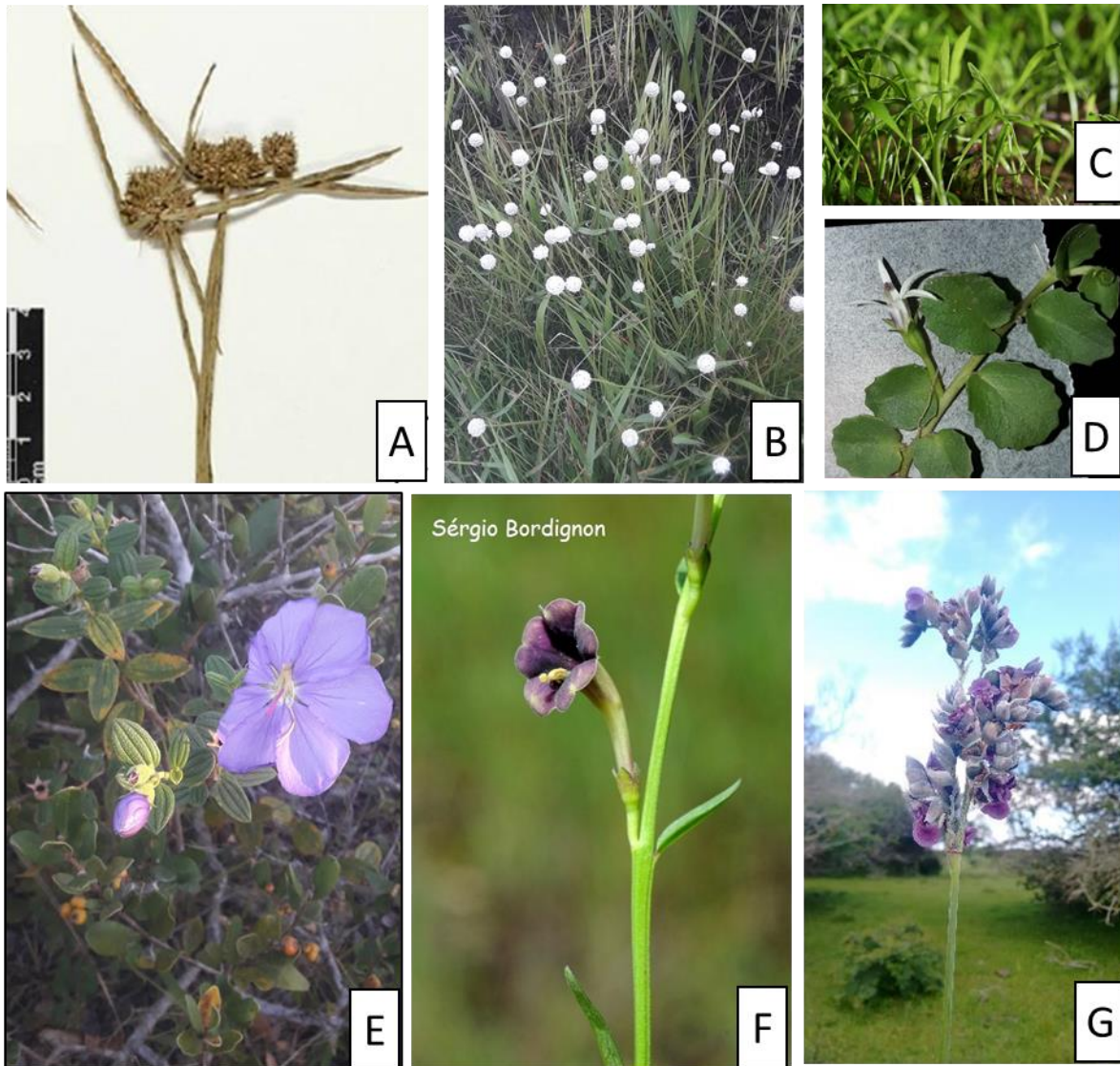
Kunth; J) *Paspalum urvillei* Steud.; K) *Eleocharis montana* (Kunth) Roem. & Schult.; L) *Commelina diffusa* Burm.f.; M) *Borreria palustris* (Cham. & Schltdl.) Bacigalupo & E.L.Cabral; N) *Ludwigia multinervia* (Hook. & Arn.) Ramamoorthy. **Fonte:** A, B, D, E, F, G, H, I, J, L, N – Karine Massia Pereira; C e M – Sérgio Bordignon, Flora Digital do Rio Grande do Sul; K - Angelo A. Schneider, Flora Digital do Rio Grande do Sul.

ANEXO 7 – Espécies indicadoras do Litoral



A) *Rhynchospora holoschoenoides* (Rich.) Herter; B) *Echinodorus grandiflorus* (Cham. & Schltr.) Micheli; C) *Ranunculus bonariensis* Poir.; D) *Helanthium tenellum* (Martius) Britton; E) *Eriocaulon modestum* Kunth. **Fonte:** A, D e E – Karine Massia Pereira; B – João Bagatini, Flora Digital do Rio Grande do Sul; C – Andrés Gonzalez.

ANEXO 8 – Espécies constantes nas listas vermelhas do CNCFLORA e da Fundação Zoobotânica do Rio Grande do Sul



A) *Cyperus berroi* (C.B.Clarke) Barros; B) *Eriocaulon modestum* Kunth; C) *Lilaeopsis brasiliensis* (Glaz.) Affolter; D) *Lobelia hederacea* Cham.; E) *Pleroma asperior* (Cham.) Triana; F) *Schwenckia curviflora* Benth.; G) *Thalia multiflora* Horkel ex Körn. **Fonte:** B, D, E e G – Karine Massia Pereira; A - William Matzenauer; C - Jan Lukavský; F - Sérgio Bordignon – Flora Digital do Rio Grande do Sul

ANEXO 9 - Is the presence of eggs a relevant cue for predators of freshwater chelonian nests?

ANEXO 10 - Ovicidal *in vitro* activity of 2-aryl-3-(2-morpholinoethyl)thiazolidin-4-ones and 2-aryl-3-(3-morpholinopropyl)thiazolidin-4-ones against *Fasciola hepatica* (Linnaeus, 1758)

ANEXO 11 - Ovicidal *in vitro* activity of the fixed oil of *Helianthus annuus* L. and the essential oil of *Cuminum cyminum* L. against *Fasciola hepatica* (Linnaeus, 1758)

ANEXO 12 – Carta de aceite revista Flora

ANEXO 13 – Certificados de revisão profissional do inglês dos capítulos da tese

SHORT COMMUNICATION

Is the presence of eggs a relevant cue for predators of freshwater chelonian nests?

A presença de ovos é uma questão relevante para predadores de ninhos de quelônios de água doce?

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Abstract

Predation is considered a game between two players – predator and prey – where such pressure might affect the interactions, acting on prey and predator distribution and abundance. We evaluate whether the predation level in chelonian nests varies according to the presence of eggs in nests. Our hypothesis is that owing to the clues (visual and olfactory) of upturned soil left in newly constructed nests, predators attack nests regardless of the presence of eggs on nests. We constructed artificial nests organized in two treatments (15 with eggs, and 15 without eggs) in ESEC Taim, southern Brazil, and checked the nests during two consecutive days. We identified the possible predators through photographic traps installed near the nests, associated with the identification of footprints on disturbed nests. We verified high predation rates in both nests, which corroborate our hypothesis. We identified two canids (*Cerdocyon thous* and *Lycalopex gymnocercus*) as predators around the nests. Our results suggest that turtle nests are highly detectable by predators, and the location cues used by pampa fox to find newly constructed nests are related to soil disturbance and not to egg presence on nests.

Keywords: foraging, interspecific interactions, predation rate.

Resumo

A predação é considerada um jogo entre dois jogadores – predador e presa –, no qual tal pressão pode afetar as interações, atuando na distribuição e abundância de presas e predadores. Neste trabalho, avaliamos se a taxa de predação em ninhos artificiais varia em função da presença de ovos. Nossa hipótese é que, devido às pistas (visuais e olfativas) de solo revolvido deixadas em ninhos recém construídos, os predadores atacam ninhos independentemente da presença de ovos. Para testar essa hipótese, utilizamos ovos de codorna e construímos 30 ninhos artificiais divididos em dois tratamentos (15 com ovos e 15 sem ovos) na ESEC Taim, Brasil, e verificamos os ninhos durante dois dias consecutivos. Identificamos possíveis predadores por meio de registro em armadilhas fotográficas instaladas próximas aos ninhos, associadas à identificação das pegadas nos ninhos perturbados. Identificamos altas taxas de predação em ambos os tratamentos, corroborando nossa hipótese. Identificamos *Cerdocyon thous* e *Lycalopex gymnocercus* como predadores mais frequentes. Nossos resultados sugerem que os ninhos de quelônios são altamente detectáveis por predadores e que as pistas de localização usadas pelos graxains para encontrar ninhos recém-construídos estão relacionadas com a perturbação do solo, e não com a presença de ovos nos ninhos.

Palavras-chave: forrageamento, interação interespecífica, taxa de predação.

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Predation is considered a game between two players – predator and prey (Caro, 2005), where such pressure might affect interactions, acting on prey and predator distribution and abundance (Chase *et al.*, 2002; Chesson and Kuang, 2008; Lamarre-DeJesus and Griffin, 2015). Egg predation using artificial nests is a common method to study the predation relationships (Zanette, 2002; Martin and Joron, 2003; Bernstein *et al.*, 2015). These experiments are important to test ecological and behavioral hypothesis related to predation (Oja *et al.*, 2015; Galvao *et al.*, 2018), which can also be used to identify the potential predators and the factors that influences their activity (Roper, 1992; Burkey, 1993). Some predation patterns are not easy to observe in natural nests; therefore, investigations through artificial nests are essential (Major and Kendal, 1996).

Concerning Chelonia, nest predation rates may vary widely according to species and population. Nest predation levels are usually high in turtles (Congdon *et al.*, 1987; Burke *et al.*, 1998; Aresco, 2004; Dawson *et al.*, 2016), which can cause low survival rates in juveniles and embryos (Hamilton *et al.*, 2002). In nests of *Emydoidea blandingii* Holbro ok 1838 (Emyidae) the predation rate was 64% (Congdon *et al.*, 1983), whereas it was 81.9% and 86.5% in nests of *Malaclemys terrapin* Schoepff 1792 (Emyidae) in different years (Butler *et al.*, 2004). In nests of *Chelydra serpentina* Linnaeus 1758 (Chelydridae) the predation rate was wide, varying of 30% to 100% (Congdon *et al.*, 1987), as well as in *Chrysemys picta* Schneider 1783 (Emyidae), the variation of which was 19.7% to 76.2% (Kolbe and Janzen, 2002). Divergent nest predation rate was also found in *Trachemys dorbignyi* Duméril and Bibron 1835 (Emyidae), reaching 98% in a conservation area (Gonçalves *et al.*, 2007) and only 18.1% in anthropic environment (Fagundes *et al.*, 2010). *Phrynops hilarii* Duméril and Bibron 1835 (Chelidae) nest predation also was high (82.35%) in a conservation area (Bujes, 1998). Such intraspecific divergence is possibly related with predator presence, highlighting the importance of predator behavior to comprehend the nest predation rates. Regarding freshwater turtles, the main predators found are foxes, lizards, possums, and hawks (Gonçalves *et al.*, 2007; Dawson *et al.*, 2016), beyond human predation through hunting and habitat destruction (Norris *et al.*, 2018).

The location of chelonian nests by predators might be facilitated by sighting females during the spawning or by olfactory traces associated with females' secretions during the spawning, smell of eggs and/or soil revolved in nest construction (Wilhoft *et al.*, 1979). According to Moll and Legler (1971), the location of nests of *Trachemys scripta* Wied-Neuwied 1839 by lizards and armadillos occurred through the detection of odor of females' urine, but the visual vestiges could be act in the successful nest predation. In human-modified areas, *C. picta* and *C. serpentina* nests were more vulnerable to opportunistic predators,

since nest discovery is facilitated in these environments (Wirsing *et al.*, 2012). Therefore, the causes of high predation rates in chelonian nests remain poorly understood, besides its importance regarding to degraded environments and vulnerable species.

To understand the ecological mechanisms behind the predation in freshwater chelonian nests, it is necessary to understand the predator behavior. Predators can find nest incidentally, during the first day of nest establishment (Wirsing *et al.*, 2012), or a week after the oviposition (Riley and Litzgus, 2014), showing a wide range of behavior according to predator species, prey species, and different environments. They can use the olfactory or visual signals to find the turtle's nests, but these mechanisms remain unclear. Since nests predation levels are usually high in turtles, understanding predator behavior during the nest predation is important especially for management of endangered species.

Possibly, predators attack turtle's nests when they find some signal (olfactory or visual) of upturned soil left in newly constructed nests. For example, goannas and crabs increase nest visitation after a nest had been opened by a goanna or after hatchlings had emerged from the nest (Lei and Booth, 2018). However, it is not clear whether is advantageous for the predators to attack a nest even when there are no eggs, dispensing energy to dig independently of egg presence on nests. To collaborate with the understanding about the predation relationship, we present in this paper a field experiment where we tested the chelonian nest predation, independently of eggs on nests. Our hypothesis is that owing to the clues (visual and olfactory) of upturned soil left in newly constructed nests, predators attack nests regardless of egg presence.

Our experiment was conducted at the Taim Ecological Station (ESEC Taim), in the coastal plain landscape of Rio Grande do Sul, extreme southern Brazil (S32°32'25.4" and W52°32'31.2"). Wide fields associated with wetlands are characteristics of this region, in which the predominant ecosystems are lagoons and wetlands colonized by aquatic macrophytes, sandy beaches, dunes, coastal fields and restinga vegetation (herbaceous vegetation in sandy soil) (Calliari, 1998). The weather is humid subtropical (Cfa, according to Köppen, 1948), with well-defined seasons (warm summers and cold winters).

At least four species of chelonians occur in the study region: *Phrynops hilarii*, *Trachemys dorbignyi*, *Acanthochelys spixii* Spix 1824 and *Hydromedusa tectifera* Cope 1869 (Gomes and Krause, 1982; Bager and Rosado, 2010). The sampling period (April 2016) coincided with the reproductive period of at least two of these species, *P. hilarii* and *A. spixii* (Bujes, 2010). There are some potential predators of the nests of chelonians in this area: Argentine giant tegu (*Tupinambis merianae* Duméril and Bibron 1839), Molina's hog-nosed skunk (*Conepatus chinga* Mo-

lina, 1782), six-banded armadillo (*Euphractus sexcinctus* Linnaeus 1758), southern caracara (*Caracara plancus* Miller 1777) and pampas fox (*Lycalopex gymnocercus* Fisher 1814, *Cerdocyon thous* Linnaeus 1766) (Gonçalves *et al.*, 2007).

We constructed 30 artificial nests at ESEC Taim in April 2016, simulating nests of chelonians, with two treatments: with eggs (n=15) and without eggs (n=15) (Figure 1). We excavate the nests with dimensions of 15 cm (depth) x 20 cm (width) (Bujes, 1998) in regions of low vegetation near water bodies, around 20 m distant from water (Bernstein *et al.*, 2015). We alternate the arrangement of treatments (nests without eggs and nests with eggs); the mean distance between treatment pairs was approximately 20 meters. For the treatment of egg nests, we added three commercially obtained quail eggs (*Coturnix coturnix japonica*). To mimic the water of the bladder thrown by chelonians during the excavation of the nests (Ernst *et al.*, 1994), we used water from a small artificial lake (Tamandaré Square, Rio Grande, RS, Brazil) that is inhabited by freshwater female chelonians *P. hilarii*, *Trachemys elegans* Wied-Neuwied 1839, *T. dorbignyi*, *A. spixii* (Loebmann, personal observation). This water was sprayed into all nests, namely at the bottom of the hole, on the surface of the nest, as well as above eggs when present.

We checked the nests every 24 hours for two consecutive days, always early in the morning, according to the methodology described by Marchand *et al.* (2002). After the first survey, the predated/excavated nests were reconstructed for reassessment after 24 hours. In this way, we work with the average of nests predated in the two surveys. The identification of the predators was done through observation, identification and registration of footprints in the disturbed nests and in one of the nests, we used a photographic trap (TRACE/MCS 12639) to capture images.

Considering that our response variable is qualitative (presence/absence of predation), a Chi-Square test was performed to compare differences between the frequency of predated and non-predated nests in the two treatments (with eggs and without eggs) ($p < 0.05$). Yates' continuity correction was applied, when necessary. We performed all analyses in R environment (R Core Team, 2017).

The rate of predation in both nests was 78% (23.5 ± 4.95 nests) (Figure 2), where 90% of the nests were predated in the first survey (27 nests) and 66.7% in the second survey (20 nests). There were no differences in the number of predated nests between treatments ($\chi^2 = 0.049$; $df = 1$; $p = 0.825$). The predation frequency in nests with eggs was $73\% \pm 9.43\%$ (11 ± 1.41 nests), and in nests without eggs was $83\% \pm 9.43\%$ (12.5 ± 3.54 nests). The photographic

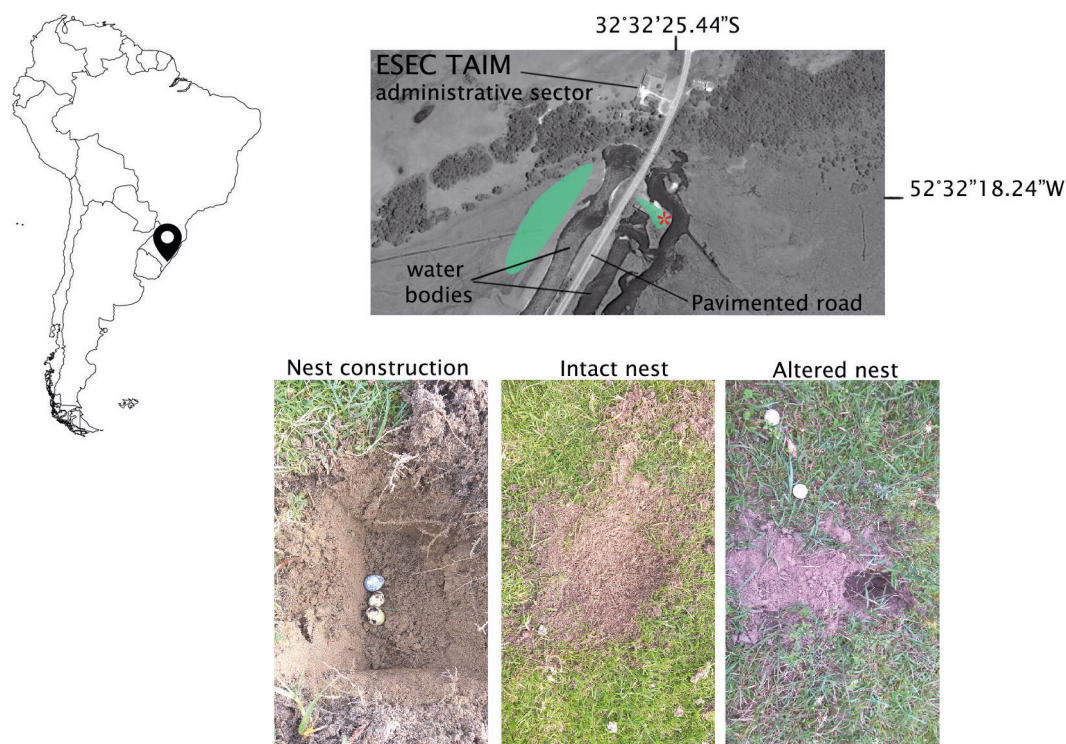


Figure 1. Study area: Taim Ecological Station (ESEC Taim), southern Brazil. Sites of nest construction are highlighted by green polygons. Asterisk indicates the place where the photographic trap was placed.

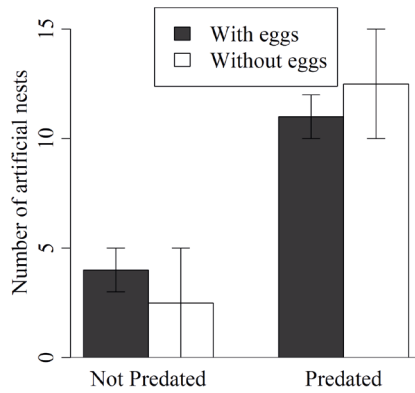


Figure 2. Number of artificial nests predated and not predated between two treatments, at Taim Ecological Station (ESEC Taim), southern Brazil.

trap recorded the presence of *L. gymnocercus* in the artificial nests (Figure 3). Only two species footprints were recorded in some predated nests: *L. gymnocercus* and *C. thous* in nine and 14 predated nests, respectively, and both footprints were recorded simultaneously in five artificial nests.

Turtle nests are highly detectable by predators and mothers have little ability to influence predation risk of their nests (Voves *et al.*, 2016). Probably, predators attack nests when they find any olfactory or visual cues from a possible nest. Olfactory cues thus could be important in locating nests, especially as a result from the female voiding her bladder prior to digging (Burke *et al.*, 2005; Oddie *et al.*, 2015). However, turtle-related olfactory cues are not necessary in nest predation (Wilhoft *et al.*, 1979; Burke *et al.*, 2005; Strickland *et al.*, 2010; Bernstein *et al.*, 2015; Geller, 2015). Other cues could be soil disturbance-related which are inherent in the construction of artificial nests and produces both visual and olfactory signals that have led to difficulties in resolving which are used as nest location cues (Geller, 2015). Our results showed a high predation rate in both artificial nests, with and without eggs, indicating that the presence of eggs was not important for predators. Combined with the high predation rates observed on artificial nests, the present findings indicate that the cues used by nest-foraging pampas fox are olfactory and related to disturbed soil, as this was the only component common to all depredated artificial nests and was responsible for high predation rates regardless of presence of eggs or not.

Revolved soil odor can be a strong draw for predators, since rates of predation in artificial nests in disturbed excavation soils are high, especially in the first 24 hours (Bernstein *et al.*, 2015). We also observed higher predation in the first 24 hours, highlighting the soil disturbance as an important cue used by nest predators. On the other hand,



Figure 3. Photographic trap image of two individuals of *Lycalopex gymnocercus* (pampas fox) near an artificial nest at the Taim Ecological Station (ESEC TAIM), southern Brazil. The arrow indicates the approximate position of the nest.

the visual attraction related with the soil revolved should also be considered (Fagundes *et al.*, 2010). Artificial nests with visual soil disturbance are more likely to be depredated than artificial nests lacking soil disturbance (Strickland *et al.*, 2010; Voves *et al.*, 2016). The absence of olfactory traces, such as the odor of eggs and the metabolites produced by the eggs inside the nests did not interfere in the probability to attract predators. Therefore, soil odor and soil irregularities due to excavation may have been one of the factors responsible for the found of these nests by predators (Bernstein *et al.*, 2015).

Furthermore, identification of predatory species is fundamental to understanding the predator-prey relationship (Gonçalves *et al.*, 2007). However, these authors argue that predators of chelonian nests are most often identified based on evidence and suggest as an alternative the use of photographic traps. According to the same authors, this methodology was appropriate, recording five of the six species that were identified as predators of *T. dorbignii* nests in the study area (Gonçalves *et al.*, 2007). We verified through the analysis of the footprints and with the aid of the images captured by the photographic trap that individuals of *L. gymnocercus* and *C. thous* predated the nests with and without eggs in our study. According to Bernstein *et al.* (2015), turtles are subject to high destruction rates of their nests by mesopredators, such as possums (*Mephitis mephitis* Schreber, 1776), red fox (*Vulpes Vulpes* Linnaeus, 1758) and, mainly, pampas fox, which are the most important.

Our results show that high predation rates in artificial nests were not related to the presence of eggs, and, thus, other factors should be responsible for the attraction and/or predation behavior of predators, corroborating our hypothesis. Then, it is plausible that the attraction of predators to turtle nests occurs due to the odor of upturned soil or even through the visualization of the altered soil. Therefore, we

emphasize the need for further studies on the multiple factors that affect the rates of predation of chelonian nests, to understand the prey-predator relationships and the role of each species in the trophic chains. In addition, we highlight the role of conservation units for the maintenance of biodiversity, which facilitates the verification of ecological relations with the minimum of anthropic intervention.

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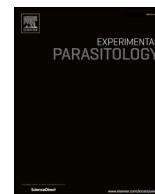
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Ovicidal *in vitro* activity of 2-aryl-3-(2-morpholinoethyl)thiazolidin-4-ones and 2-aryl-3-(3-morpholinopropyl)thiazolidin-4-ones against *Fasciola hepatica* (Linnaeus, 1758)



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ABSTRACT

Although there is a variety of biological activity reports regarding compounds derived from thiazolidin-4-ones, no data related to ovicidal activity against trematodes, particularly *Fasciola hepatica* are available. Since there are reports about anthelmintic resistance in *F. hepatica*, new drugs are required. Thus, this study evaluated ovicidal action *in vitro* against *F. hepatica* eggs in two systematic series of thiazolidin-4-ones: 2-aryl-3-(2-morpholinoethyl)thiazolidin-4-ones (**1a-h**) and 2-aryl-3-(3-morpholinopropyl)thiazolidin-4-ones (**2a-h**) at different concentrations (20, 2, 0.2, 0.02 and 0.002 µg/ml). The egg hatch assay (EHA) was used to evaluate the ovicidal action property of such compounds. In addition, potential negative effects of the compounds on metabolic activity of bovine kidney (MDBK) cells were evaluated by determining mitochondrial dehydrogenase activity. The eggs used in the EHA were obtained from parasites removed from the liver of cattle, which were discarded by slaughtering after sanitary inspection. The results of EHA showed that compounds **2a-h** exhibited ovicidal activity, especially compounds **2b** which showed 90% ovicidal activity and viability of 93% MDBK cells at the concentration of 2 µg/ml; and **2e** with 96–99% ovicidal activity at 0.2 µg/ml, 0.02 µg/ml and 0.002 µg/ml. The results show the potential of compound **2b** to continue the studies in the production of new compounds with anthelmintic action.

1. Introduction

Compounds derived from thiazolidin-4-ones present great scientific interest because of their chemical and biological properties (Jain et al., 2012; Tripathi et al., 2014). Some of their biological activities can be cited as: antiviral (Manvar et al., 2016); antibacterial (Rane et al., 2012); antifibrotic (Kaminsky et al., 2016); anti-inflammatory (Deep et al., 2012); antioxidant (Júnior et al., 2013); antiprotzoal (de Oliveira Filho et al., 2015); and antifungal (Secchi et al., 2016). Although there is a variety of biological activity reports regarding compounds derived from thiazolidin-4-ones, to the best of our knowledge no data

related to the ovicidal activity against *Fasciola hepatica* are available.

The helminth *F. hepatica* belongs to the class Trematoda family and cause fasciolosis, a parasitic disease that affects both animals (sheep, goats, cattle and buffaloes) and humans (Mas-Coma et al., 2009). In animals this disease causes anemia, abortion, progressive loss of physical and developmental conditions leading to loss of milk and beef productivity. The estimated global annual losses are three billion US dollars (Oakley et al., 1979; Aleixo et al., 2015).

Drugs used to control the fluke are faciolicides which act in one or more steps of the trematode (Fairweather and Boray, 1999). They include halogenated phenol, salicylanilide, benzimidazole, sulphonamide

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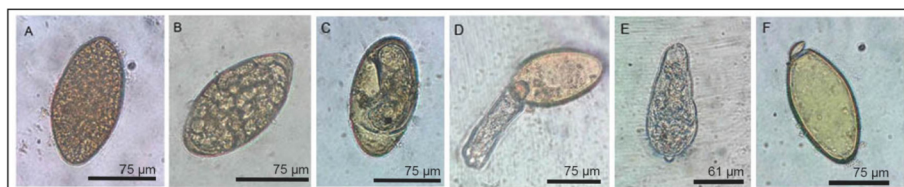
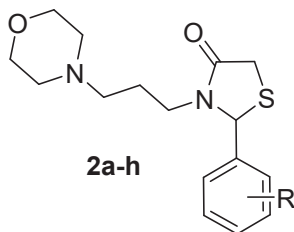


Fig. 1. Different developmental stages of *Fasciola hepatica* eggs in the negative control: (A) eggs on day zero of incubation. (B) Morula stage or cell division observed on the seventh day of incubation. (C) Miracidium observed on the fourteenth day. (D) Moment of egg hatching, output of miracidium. (E) Miracidium. (F) Egg after hatching and egression of the miracidium. Image magnification $\times 150$.

Table 1

Percentage of eggs hatched after compound incubation of *Fasciola hepatica* eggs and the percentage ovicidal action of compounds **2a-h** at different concentrations tested.



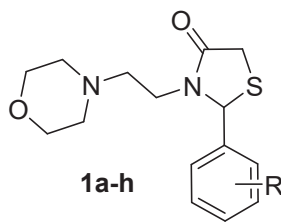
Comp.	R	Eggs hatched after compound incubation $\pm$ S.D. (%)					Ovicidal action (%)					miLogP ^a
		Dose (μ g/ml)					Dose (μ g/ml)					
		20	2	0.2	0.02	0.002	20	2	0.2	0.02	0.002	
2a	4-F	40 $\pm$ 8*	48 $\pm$ 3*	35 $\pm$ 1*	45 $\pm$ 4*	43 $\pm$ 2*	42 $\pm$ 11	32 $\pm$ 5	49 $\pm$ 2	36 $\pm$ 5	39 $\pm$ 3	1.93
2b	2,4-F	5 $\pm$ 2*	4 $\pm$ 1*	7 $\pm$ 2*	3 $\pm$ 0*	7 $\pm$ 1*	97 $\pm$ 2	90 $\pm$ 2	96 $\pm$ 1	90 $\pm$ 1	77 $\pm$ 1	2.02
2c	2,4-Cl	39 $\pm$ 2 ^{ns}	36 $\pm$ 5*	43 $\pm$ 3*	53 $\pm$ 6*	49 $\pm$ 6*	45 $\pm$ 3	49 $\pm$ 5	39 $\pm$ 5	24 $\pm$ 9	30 $\pm$ 9	3.05
2d	2,6-Cl	26 $\pm$ 9*	26 $\pm$ 4*	33 $\pm$ 3*	41 $\pm$ 4*	21 $\pm$ 3*	63 $\pm$ 13	63 $\pm$ 6	53 $\pm$ 3	42 $\pm$ 6	70 $\pm$ 4	3.03
2e	4-Cl	1 $\pm$ 1*	2 $\pm$ 1*	2 $\pm$ 1*	3 $\pm$ 1*	2 $\pm$ 1*	99 $\pm$ 1	98 $\pm$ 1	97 $\pm$ 1	96 $\pm$ 1	97 $\pm$ 1	2.44
2f	4-NO ₂	22 $\pm$ 5*	22 $\pm$ 5*	16 $\pm$ 2*	7 $\pm$ 1*	18 $\pm$ 5*	90 $\pm$ 7	69 $\pm$ 5	68 $\pm$ 7	77 $\pm$ 3	90 $\pm$ 1	1.73
2g	4-OCH ₃	62 $\pm$ 5*	58 $\pm$ 2*	49 $\pm$ 1*	48 $\pm$ 1*	54 $\pm$ 1*	12 $\pm$ 8	18 $\pm$ 2	30 $\pm$ 1	31 $\pm$ 2	23 $\pm$ 2	1.82
2h	2-OCH ₃	6 $\pm$ 1*	8 $\pm$ 1*	21 $\pm$ 4*	6 $\pm$ 1*	12 $\pm$ 1*	91 $\pm$ 1	89 $\pm$ 1	69 $\pm$ 5	91 $\pm$ 1	83 $\pm$ 7	1.77

Significant differences between the control group and the treated group are indicated by *p < 0.05; ^{ns} statistically insignificant. Dunett's test.

^a Theoric calculation from www.molinspiration.com web site.

Table 2

Percentage of eggs hatched after compound incubation of *Fasciola hepatica* eggs and the percent ovicidal action of compounds **1a-h** at different concentrations tested.



Comp.	R	Eggs hatched after compound incubation $\pm$ S.D. (%)					Ovicidal action (%)					miLogP ^a
		Dose (μ g/ml) (%)					Dose (μ g/ml)					
		20	2	0.2	0.02	0.002	20	2	0.2	0.02	0.002	
1a	2-F	42 $\pm$ 4*	40 $\pm$ 3*	54 $\pm$ 5*	56 $\pm$ 8*	47 $\pm$ 9*	40 $\pm$ 6	42 $\pm$ 5	22 $\pm$ 6	20 $\pm$ 10	33 $\pm$ 14	1.61
1b	2,4-F	71 $\pm$ 5 ^{ns}	70 $\pm$ 3 ^{ns}	73 $\pm$ 2 ^{ns}	83 $\pm$ 3 ^{ns}	78 $\pm$ 8 ^{ns}	0	0	0	0	0	1.71
1c	2,4-Cl	62 $\pm$ 2*	66 $\pm$ 6*	79 $\pm$ 2 ^{ns}	78 $\pm$ 2 ^{ns}	85 $\pm$ 3 ^{ns}	11 $\pm$ 3	6 $\pm$ 9	0	0	0	2.78
1d	2,6-Cl	78 $\pm$ 1 ^{ns}	82 $\pm$ 5 ^{ns}	80 $\pm$ 4 ^{ns}	83 $\pm$ 5 ^{ns}	81 $\pm$ 1 ^{ns}	0	0	0	0	0	2.75
1e	4-Cl	70 $\pm$ 5 ^{ns}	68 $\pm$ 5 ^{ns}	66 $\pm$ 5*	72 $\pm$ 1 ^{ns}	76 $\pm$ 1 ^{ns}	0	3 $\pm$ 7	6 $\pm$ 7	0	0	2.17
1f	4-NO ₂	67 $\pm$ 1*	60 $\pm$ 3*	69 $\pm$ 1*	73 $\pm$ 1 ^{ns}	76 $\pm$ 1 ^{ns}	4 $\pm$ 1	14 $\pm$ 4	2 $\pm$ 1	0	0	1.45
1g	4-OCH ₃	72 $\pm$ 5 ^{ns}	70 $\pm$ 3 ^{ns}	66 $\pm$ 1*	71 $\pm$ 4 ^{ns}	49 $\pm$ 2*	0	0	5 $\pm$ 1	0	29 $\pm$ 3	1.55
1h	6-F,2-Cl	42 $\pm$ 4*	40 $\pm$ 3*	54 $\pm$ 4*	56 $\pm$ 7*	47 $\pm$ 9*	40 $\pm$ 6	43 $\pm$ 5	22 $\pm$ 6	20 $\pm$ 9	33 $\pm$ 13	2.24

Significant differences between the control group and the treated group are indicated by *p < 0.05; ^{ns} statistically insignificant. Dunett's test.

^a Theoric calculation from www.molinspiration.com web site.

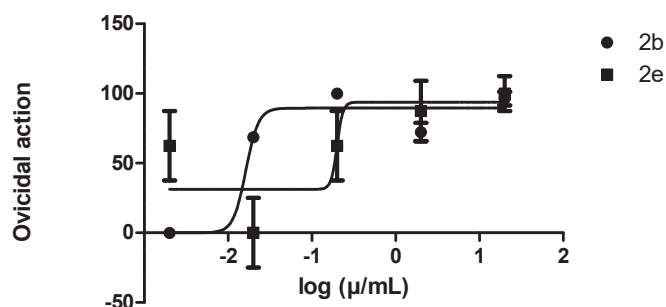


Fig. 2. Graphic efficacy versus log (µg/ml) for compounds **2b** and **2e**.

and phenoxyalkane classes (Fairweather and Boray, 1999). In humans fascioliasis is mainly treated with these drugs: triclabendazole (benzimidazole) and bithionol (halogenated phenol) (Couturier et al., 1999; Ramachandran et al., 2012; Chen et al., 2013).

Since the 1980s resistance of *F. hepatica* against fasciolicides has been reported in many countries: Spain (Alvarez-Sánchez et al., 2006), Argentina (Olaechea et al., 2011; Sanabria et al., 2013), United Kingdom (Coles and Stafford, 2001), Peru (Ortiz et al., 2013), Australia (Overend and Bowen, 1995), Netherlands (Moll et al., 2000) and Northern Ireland (Hanna et al., 2015). There are human cases of fasciolosis that were unresponsive to triclabendazole (Gil et al., 2014).

Therefore, this study evaluates the *in vitro* ovicidal activity using the egg hatch assay (EHA) against *F. hepatica* eggs and *in vitro* evaluating the metabolic function of mitochondrial dehydrogenase activity in two systematic series of thiazolidine-4-ones: 2-aryl-3-(2-morpholinoethyl)thiazolidin-4-ones (**1a-h**) and 2-aryl-3-(3-morpholinopropyl)thiazolidin-4-ones (**2a-h**).

2. Materials and methods

2.1. Synthesis of desired compounds

The compounds were synthesized by the research group of Applied Bioactive Chemistry Laboratory (LaQuiABio) of Federal University of Pelotas, in accordance with its standard procedures described in previous work (Gouvea et al., 2016). The purity of compounds were determined by CG-MS analyses (above 98%).

The tested compounds were solubilized in DMSO in order to obtain a concentration of 20,000 µg/ml. After it was diluted in DMSO to afford stock solutions of 4000 µg/ml, 400 µg/ml, 40 µg/ml, 4 µg/ml and 0.4 µg/ml.

2.2. Isolation of the *F. hepatica* eggs

Livers infected with *F. hepatica* were obtained in slaughterhouses located in the city of Pelotas of Rio Grande do Sul - Brazil. Faced with the permission conceded by the managers, the pieces were obtained

from disposals, directly after sanitary inspection. The samples were stored in labeled plastic bags and sent to the Parasitology Laboratory of the Department of Microbiology and Parasitology Institute of Biology of the Federal University of Pelotas, where they were processed. The *F. hepatica* flukes were removed after cuts in the bile ducts and in the tissue of infected livers.

The living parasites were directly collected and stored in glass vials containing warm saline solution (approximately 30 °C) to shed eggs. After three hours, the parasites were removed and the suspension containing the eggs, was transferred to a beaker for sedimentation. The eggs were washed three times with distilled water, and filtered through a set of four sieves (100, 170, 200 and 250 mesh) size. Directly after that, they were stored in glass containers, properly identified, containing sterile distilled water and were kept refrigerated until preparation of EHA (Ueno and Gonçalves, 1998).

2.3. Egg hatch assay

The EHA for *F. hepatica* was performed based on recently described methodologies (Robles-Pérez et al., 2014; Arafa et al., 2015). Tests were conducted in triplicate plates using a six-well flat bottom cell culture (Mark KASVI, model K12-006). The assay was performed with thiazibendazole 25 µg/ml (Vet Pharma - Handling of Pharmacy RS-Brazil), as positive control. The negative control is a water solution of DMSO at concentration of 0.6%.

To different flasks containing 4.97 mL of sterile distilled water, 30 µL of each stock solutions were added, mixed and transferred to the plates. 1 mL of volume egg suspension containing approximately 100 eggs was added to the plate and the final concentrations were 0.002 µg/ml, 0.02 µg/ml, 0.2 µg/ml, 2.0 µg/ml and 20 µg/ml.

Subsequently the plates were incubated at 28 °C with 80% humidity in a Greenhouse Incubator Type B.O.D. (Eletrólabs brand, model EL 101/03) in a dark environment for 14 days. On the fourteenth day of incubation the plates were exposed to light for 2 h, to stimulate the emergence of miracidia (Robles-Pérez et al., 2014; Arafa et al., 2015).

The counting of hatched and total eggs was performed using an inverted microscope (Brand: Olympus - CKX 41). The results of each treatment were expressed as a percentage using the following formulas: (a) percentage of hatching = (number of hatched eggs/total number of eggs) x 100; (b) ovicidal action (%) = [(% eggs hatched in control - % eggs hatched after compounds incubation)/% eggs hatched in control] x 100 (Canevari et al., 2013).

2.4. Cell viability assay

2.4.1. Madin Darby bovine kidney (MDBK) culture

The cell line was maintained in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics and incubated at 37 °C in a humidified atmosphere containing 5% CO₂.

Table 3
Cell viability in MDBK cells of thiazolidinones **1a-h** and **2a-h**.

Comp.	R	Cell viability (%)					Comp.	R	Cell viability (%)				
		Concentration (µg/ml)							Concentration (µg/ml)				
		20	2	0.2	0.02	0.002			20	2	0.2	0.02	0.002
1a	2-F	62	80	89	98	93	2a	4-F	92	128	132	139	140
1b	2,4-F	55	75	88	92	90	2b	2,4-F	60	93	100	104	111
1c	2,4-Cl	21	68	76	87	93	2c	2,4-Cl	5	38	64	84	101
1d	2,6-Cl	2	12	48	73	89	2d	2,6-Cl	40	83	96	95	105
1e	4-Cl	76	89	91	91	96	2e	4-Cl	61	79	91	92	92
1f	4-NO ₂	87	100	100	100	100	2f	4-NO ₂	62	95	105	105	121
1g	4-OCH ₃	83	97	97	97	100	2g	4-OCH ₃	86	117	134	136	143
1h	6-F,2-Cl	70	80	80	83	90	2h	2-OCH ₃	84	92	103	101	106

2.4.2. Cell viability assay by MTT

To assess cell metabolic function by mitochondrial dehydrogenase activity 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used (Mosmann, 1983). MDCK cells were cultured in 96-well microplates for 24 h in a CO₂ chamber at 37 °C up to the confluence. The tested compounds were initially solubilized in DMSO and subsequently diluted in MEM resulting in 0.002 µg/ml, 0.02 µg/ml, 0.2 µg/ml, 2.0 µg/ml e 20 µg/ml. A cell control without adding compounds was maintained.

Cell viability was assessed 48 h after exposure of the cells to different concentrations of the compounds. After this period, the solution containing the compound was aspirated and added to 50 µL/well of MTT (1 mg/ml) in each well and the plates were maintained in a CO₂ chamber for 4 h. MTT was discarded and added to 50 µL/well of ethyl alcohol and the plates returned to the CO₂ incubator for another 10 min for subsequent reading. Absorption was determined spectrophotometrically at 540 nm. All tests were performed in duplicate (ISO/EN10993-5, 2009).

2.5. Statistical analysis

A one-way analysis of variance was used to evaluate differences among the different treated groups. The multiple Dunnett comparison test was conducted to confirm significant differences between the different groups with the control. Differences were considered significant at $p < 0.05$. The EC₅₀, that is the dose at which 50% of the maximum effect is produced or the concentration of drug at which the drug is half-maximally effective, of the compounds with better ovicidal activity were calculated using four-parameter logistic regression. The data were analyzed using GraphPad PRISM® (version 5.03).

3. Results

3.1. EHA

On the seventh day of incubation, the cell division stage (morula) was observed in both the negative and the vehicle control. Approximately on the eleventh day, the embryonated eggs were observed, i.e. the formation of the miracidium. After light exposure, on the fourteenth day, the eggs with the open operculum were observed indicating the egression of the miracidium from the egg (Fig. 1).

The eggs used in the EHA were susceptible to thiabendazole (25 µg/ml) (positive control) with an average rate of inhibition development of $100 \pm 0\%$. In the negative control and control with distilled water, approximately the same percentage of hatching ($\pm$ SD) with 70% (63–77%) and 71% (66–76%) was observed, respectively. The thiazolidin-4-ones have a point in common with thiabendazole, the presence of a heterocyclic thiazole in its chemical structure. Compounds **2a-h** showed statistically differences ($p < 0.05$) in hatching percentages of all concentrations tested, ranging from 1% to 62% and good ovicidal activity were observed for compounds **2e** and **2f** at the lowest concentration, as shown in Table 1. Some **1a-h** compounds showed a significant statistical difference ($p < 0.5$) in the percentage of hatching between the different concentrations and the negative control. However, ovicidal activities were between zero and 45% (Table 2).

The EC₅₀ for compounds that have ovicidal activity above 95% were calculated. The thiazolidinone **2b** EC₅₀ 0.01631 µg/ml and thiazolidinone **2e** EC₅₀ 0.2 µg/ml (Fig. 2).

3.2. Cell viability

The *in vitro* cell viability assay in MDBK cells for compounds **1a-h** and **2a-h** are shown in Table 3. Results are expressed in percentage of cell viability at different concentrations.

4. Discussion

The objective of this study was to determine if the thiazolidinone derivatives used presented an ovicidal activity *in vitro* in *F. hepatica* eggs, testing the behavior of eggs when incubated in the presence of different concentrations of the compounds discussed.

The observations obtained in the EHA of DMSO negative control and for the embryonic development of *F. hepatica* eggs corroborate those described by Arafa et al. (2015) and (Fairweather et al., 2012). The use of DMSO as solvent at a final concentration of 0.6% did not affect the viability of the eggs, since there were no statistically significant differences in their development.

The results showed that compounds **2a-h** presented better results than **1a-h**, since all showed a statistically significant difference in hatching treatment compared to the negative control ($p < 0.05$). Compounds **2a-h** obtained ovicidal activity above 95% in two compounds tested in **2e** ($99 \pm 1\%$, 20 µg/ml) and **2b** ($97 \pm 2\%$, at a concentration of 20 µg/ml).

Compound **2e** showed a hatching percentage ($1\% \pm 1$, 20 µg/ml) similar to the findings of Fairweather et al. (2012), who used triclabendazole sulphoxide (60 µg/ml) and the hatching percentage was found to be 1–2%. The ovicidal activity remained above 95% at all concentrations tested and their cellular viability at concentrations (0.2 µg/ml, 0.02 µg/ml and 0.002 µg/ml) were above 91%. This compound, having good ovicidal activity and cellular viability, suggests that it is a promising ovicidal for the treatment of *F. hepatica*.

Another relevant point is that, although the ovicidal activity of the other compounds of series 2 has not been as relevant as those reported above, the compounds deserve special attention. According to Arafa et al. (2015), oxcyclozanide *in vitro* did not affect egg development and egg hatch percentages, but presents efficacy *in vivo*.

Previous work suggested that inhibition of hatching depends on the hydrophobic nature of the drug and the increased activity correlates with high lipid solubility (Lacey, 1988). Mottier et al. (2003) have also reported that the lipophilicity of albendazole may facilitate the penetration of parasitic helminth eggs. So, the mLogP of compounds are shown in Tables 1 and 2. Compounds **2a-h**, which have an extra methylene group, have higher mLogP than corresponding **1a-h**, however they are quite similar and no conclusion could be made.

The cell viability assay showed that only two compounds are safe at 20 µg/ml (**1f** and **2a**). Unfortunately, compound **2e** showed only 79% of cell viability at 2 µg/ml and was safe at the same concentration of its EC₅₀ (0.2 µg/ml). It is highlighted that compound **2b** has an EC₅₀ of 0.016 µg/ml and presents cell viability of 93% at 2 µg/ml.

5. Conclusions

It can be concluded that, in general, thiazolidinones **2** present better ovicidal results than thiazolidinones **1**. The best ovicidal activities were observed for compounds **2b** (EC₅₀ 0.01631 µg/ml) and **2e** (EC₅₀ 0.2 µg/ml) which showed a considerable reduction in the hatching of *F. hepatica* eggs. Compound **2b** shows cell viability since 2 µg/ml and compound **2e** since 0.2 µg/ml. These results show that thiazolidinone **2b** was the best compound in our study, suggesting the continuation of studies with this important class of heterocyclic compounds.

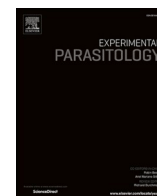
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Ovicidal *in vitro* activity of the fixed oil of *Helianthus annuus* L. and the essential oil of *Cuminum cyminum* L. against *Fasciola hepatica* (Linnaeus, 1758)

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ABSTRACT

The fascioliasis is a parasitic disease of importance in veterinary medicine and public health. For this parasitosis, the treatment by synthetic fasciolicides is used and due to their intense use although they have been shown less effective because of the establishment of resistant *Fasciola hepatica* population to these drugs, with a global concern. The use of derived products of plants with biological activity has been shown promising in the control of parasites. In this context, we evaluated the chemical composition and action of ovicidal *in vitro* fixed oil of *Helianthus annuus* L. (FOH) and essential oil of *Cuminum cyminum* L. (EOC), as well as their combination (FOH + EOC) of *F. hepatica*. In the assay *in vitro* of *F. hepatica* were submitted to different concentrations of oils, such as FOH (2.3 mg/mL + 0,017 mg/mL); EOC (2.07 mg/mL + 0,004 mg/mL) and the combination of (1.15 mg/mL + 1.03 mg/mL to 0,0085 mg/mL + 0,008 mg/mL) as well as a positive control of thiabendazole (0.025 mg/mL) and a negative control with distilled water and tween. The identification of the majority chemical compounds was performed by gas chromatography. The –cell viability of the oils was tested in MDBK cellular line by the MTT method. The majority compounds in the FOH were the linoleic (53.6%) and oleic (35.85%) unsaturated fatty acids, and the majority phytochemicals compounds in the EOC were the Cumaldehyde (26.8%) and the 2-Caren 10-al (22.17%). The EOC and the combination presented effectiveness of 99% (± 1) and of 94% (± 1) in the concentration of 0.03 mg/mL and 0.035 mg/mL+0.03 mg/mL, respectively, and the FOH was insufficiently active as ovicidal. The cell viability at this concentration of EOC was 93%. From the results above we could infer that the EOC is promising as a new alternative for the fascioliasis control.

1. Introduction

The fascioliasis is an important parasitic infection in veterinary medicine and has a zoonotic potential by the direct damages caused in animals and elevated economic loss. It is capable to infest humans characterizing as a global public health problem and most of the time is a disease that is sub diagnosed (Dutra et al., 2010; Gil et al., 2014; Ashrafi et al., 2015; Mas Coma et al., 2018).

There are two species described as the main responsible for the disease; *Fasciola hepatica* predominantly is found in regions of temperate weather and in America, and *Fasciola gigantica* is found in tropical regions of Africa and Asia (Soliman, 2008).

This parasite has a heteroxenous biological cycle, which means that it needs an intermediate host, being Lymnaeidae (Gastropoda), and ideal conditions such as high humidity and temperature, as well as the definitive host presence (mammals in general), who eliminates the eggs

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into the environment. Those are important aspects to consider for the epidemiology and control of fascioliasis (Martins et al., 2014).

However, control is done with the use of synthetic anti-helminthic drugs such as; closantel, nitroxylin, albendazole and triclabendazole, which are the drugs of choice for the treatment of infections caused by liver parasites (Gil et al., 2014; Kelley et al., 2016).

The administration and indiscriminated drug use in inadequate periods originates resistant populations. Anti-helminthic resistance is reported in Australia, Europe and South America (Olaechea et al., 2011; Brockwell et al., 2014; Novobilský et al., 2016). Other important aspect to be aware of is the presence of chemical residuals in derived products such as meat and milk and the environmental impact (Botsoglou and Fletouris, 2001). As an alternative, studies made with derivatives of plants showed direct or indirect negative effects against endoparasites, being sulfides, polyphenolic and terpenoids compounds the responsible for the anti-helminthic activity (Chan-Pérez et al., 2017; Pinto et al., 2019).

The interest in studies with bioactive compounds has been growing highlight. (Sinott et al., 2019). In the parasitology area, studies showed the anti-parasite effect of the essential oils against different parasites (Callander and James, 2012; Remédio et al., 2015; Ferreira et al., 2018). Studies evaluating the synergism between essential oils and antimicrobial agents presented promising results (Rosato et al., 2010).

Reports related to the anti-helminthic action of the *Cuminum cyminum* L. (EOC) and *Helianthus annuus* L. (FOH) against the eggs of the trematode *Fasciola* spp. are scarce. Studies that evaluated the *in vitro* effect of *Artemisia annua* L. extracts, *Asimina triloba* L. and *Artemisia absinthium* L. were performed by Ferreira et al. (2011); and of *Momordica charantia* L. by Pereira et al. (2016). Moreover, semi-synthetic chlorophyllin compound is derived from *Spinacia oleracea* Singh and Singh (2016). The efficacy of essential oils of *Cymbopogon nardus* and *azadirachta indica* has been evaluated *in vitro* on *Fasciola gigantica* (Jeyathilakan et al., 2010).

In this context, the objective of this study was to evaluate the *in vitro* ovicidal activity of the essential oil *Cuminum cyminum* L., of the fixed oil of *Helianthus annuus* L. and the combination of both oils against *F. hepatica* eggs.

2. Materials and methods

2.1. Collect and recuperation of the eggs of *F. hepatica*

The samples of the parasitized bovine livers with *F. hepatica* were obtained from different slaughterhouses, after inspection by the Municipal Inspection Service (MIS) from the city of Pelotas – RS. The livers were stored in plastic bags properly identified and sent to the Laboratory of Parasitology of the DEMP (Department of Microbiology and Parasitology) at IB (Institute of Biology) of the Federal University of Pelotas (UFPEL). The eggs were recovered through the natural egg-laying obtaining technique, described by Ueno and Gonçalves (1998). After the process, they were stored under refrigeration until the conduct of the experiment.

2.2. Fixed oils

The *Helianthus annuus* L. (FOH) was processed in the Laboratory of Lipidomics and Biorganic (LLB) from the Center of Chemical Science, Pharmaceutics and Food (CCQFA) of the Federal University of Pelotas (UFPEL). The extraction of the oil was made from seeds from the producer Argensun in Argentina. The process constituted in the milling in a Willey model B-602 cutting mill, and further drying in an oven at 45 °C, followed extraction, where 50 g of the sample and 300 mL of hexane solvent were collocated in a Soxhlet apparatus at a temperature of 60 °C for a 6-hour period. At the end of the process, the solvent was removed through a rotary vacuum evaporator and the oil storage in an amber bottle under refrigeration.

2.3. Essential oil

The *Cuminum cyminum* L. (EOC) was processed in the Research Laboratory of Natural Products (LPPN) of the CCQFA of UFPEL. The seeds were obtained from the food supplier Luar Sul RS, Brazil. (Cominho semente ET – 006 Rev.: 04 Pág. 1/1 Date 1^a Ed.: 29/03/06 Date Rev.:11/07/2013. The technique used for the oil's collection was based on the drag by water's vapor, using a Clevenger apparatus. Once the volatile oil was obtained, it was separated from the water, dried with Na₂SO₄ sodium sulfate and storage in an amber bottle under refrigeration.

2.4. Chromatographic analyses of the oils

2.4.1. Essential oil

The essential oil identification was done using a gas chromatograph attached to a mass detector, model GC/MS-QP 2010SE (Shimadzu, Japan), equipped with an AOC-20i auto-injector (auto-injector). The separation has occurred in a RTX-5MS (Restek, USA) capillary column, and the quantification was done by standard area and the compounds identification by the mass spectrometer, using the NIST 8 library of the GC/MS. The sample oil was diluted with hexane (analytic degree, ultra pure).

2.4.2. Fixed oil

2.4.2.1. Fatty acid methyl esters preparation. The fatty acids were converted to their methyl esters using the boron trifluoride-methanol (BF₃) method, as shown in the literature (Moss et al., 1974). The resultant mixture of fatty acid methyl esters (FAMES) in hexane/chloroform (4:1, v/v) was subjected to gas chromatography-flame ionization detection (GC-FID).

2.4.2.2. Gas-chromatography analysis. The quantitative GC analyses were performed according to the following conditions using a gas chromatograph GC/FID-2010 with an AOC-20i autosampler (Shimadzu Corporation, Kyoto, Japan) equipped with a fused-silica capillary column (Rtx-WAX, 30 m × 0.25 mm I.D. × 0.25 µm film thickness). Injections were performed with a 1:25 split ratio, and hydrogen was used as the carrier gas under constant flow mode at 1.2 mL/min. The injector was heated to 250 °C, and the flame-ionization detector operated at 250 °C. The initial programmed oven temperature was 100 °C, which was increased by 7 °C/min up to 200 °C, increased by 5 °C/min to 202.6 °C and held isothermal for 2 min at this temperature. It was then increased by 5 °C/min to 222.9 °C and held isothermal for 2 min, and then increased by 5 °C/min to 230 °C and held isothermal for 10 min at 230 °C. The internal standard solution, containing nonadecanoate methyl ester (C19:0 ≥ 99.0%; Sigma-Aldrich, St. Louis, Missouri, USA), was prepared at a concentration of 2 mg/mL by dissolving 20 mg methyl nonadecanoate in 10 mL of n-hexane in a volumetric flask. The analyses were performed at the Federal University of Pelotas (Laboratory of Lipidomics and Biorganic).

2.5. Ovicidal activity assay of the oils against *F. hepatica*

2.5.1. Preparation of the solutions of the oils

The solutions of the oils were based on the methodology described by Freire et al. (2014), with adaptations. Each test solution was prepared individually with sterile distilled water, tween 80¹ and with each oil (FOH or EOC). In sterile tubes was added 45 µL of each oil, 180 µL Tween 80 and water distilled sterile to complete the final volume of 15 mL. The tubes were individually stirred in shaker mark solutions AP 59 (Phoenix

¹ The tween 80 was used to emulsify the oil.

Luferco) for a period of 5 min. Ten concentrations were performed with EOC (0.12%–0.0005%) and eight concentrations with FOH (0.25%–0.002%), using the described method. The solutions of the combinations were prepared using equal proportions of the EOC and FOH test solutions totaling 45 μ L. The combined solutions of oils were distributed in 8 different concentrations (0.25%–0.002%). To express the results in mg/mL, the density of each oil (FOH = 0.92 g/cm³; EOC 0.83 g/cm³) and the equation (density = mass/volume) were used.

2.5.2. Egg's hatching assay (EHA) *in vitro*

The tests were performed based on the methodologies described by Robles-Pérez et al. (2014) and Arafa et al. (2015). Sixteen cell culture plates (KASVI, K12-006) were added 5 mL of oil solution and 1 mL of egg suspension (containing 90–110 eggs) in each well. The plates were stored for 14 days in a BOD incubator (Eletrolab brand, model EL 101/03) with a temperature of 28 °C and humidity of 80%, under the light and with observation every three days to follow the development of the embryo. At the end of the 14 day period, the plates were exposed to light for 2 h to stimulate the miracidid hatch. The counting of hatched and total eggs was performed using an inverted microscope (Brand: Olympus - CKX 41). The negative control was performed with sterile distilled water and tween 80 1%, and the positive control was 0.025 mg/mL thiabendazole (VeT.Pharma - Hearing of Pharmacy RS - Brazil). All assays were performed in triplicate.

The results of each treatment were expressed as a percentage using the following formulas: (a) *percentage of hatching* = (number of hatched eggs/total number of eggs) $\times$ 100; (b) *ovicidal action (%)* = [(% eggs hatched in control - % eggs hatched after solutions of oils incubation)/% eggs hatched in control] $\times$ 100 (Canevari et al., 2013)

2.6. Cell viability assay

For evaluation of cell viability *in vitro* after exposure of the oils, MDBK (Madin-Darby-Bovine-Kidney) line cells were used. The quantitative evaluation of the cellular viability was performed through the MTT method using the MTT dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Mosmann, 1983). The MDBK cells were cultivated in micro plates for 24 h in a CO₂ chamber at 37 °C until confluence. Then the serial dilutions from the different solutions of the oils in MEM were made, resulting in an initial concentration of (IC) = 4.6 mg/mL and a final concentration of (FC) = 0.015 mg/mL for the FOH; EOC(IC) = 4.14 mg/mL and (FC) = 0.015 mg/mL; FOH + EOC (IC) = 2.3 mg/mL + 2.07 mg/mL e (FC) = 0.01 mg/mL + 0.0075 mg/mL.

The cell viability was evaluated after the exposition of the cells in the different concentrations of the oils for 48 h. After this period, the supernatant (MEM + solution of the oil) was aspirated and 50 μ L/well of MTT (1 mg/mL) was added in each well of the cell culture plates of 96 holes and maintained in a CO₂ chamber for 4 h. The MTT was eliminated and 50/well of ethyl alcohol was added, returning the plates to the CO₂ chamber for another ten minutes for further lecture. The absorption at 540 nm was spectrophotometric determined, according to the protocol (ISO/EN10993-5, 2009). All the assays were performed by duplicate.

2.7. Statistical analyses

The analyses of variance ANOVA was used to evaluate differences in the hatching percentage between the concentrations of the oils and the controls. The statistical Dunnett's test (comparing multiple percentages) was performed to confirmed significance differences between the different groups with the control. The differences were considered significant for $p < 0.05$. The software GraphPad PRISM ® (version 5.03) was used to perform the statistical analyses.

3. Results

3.1. Chromatographic analyses

The chromatographic analyzes for the oil of *Cuminum cyminum* L. identified the following compounds: cumaldehyde (26.8%), 2- caren 10-al (22.17%), 1-phenyl-1 butanol (19.47%), gamma-Terpinen (15.24%), beta-pinene (10.75%) and cymene (5.56%).

For the fixed oil of *Helianthus annuus* L. the identified compounds and their respective concentrations were: linoleic acid (C18:2n6c) (53.638%), oleic acid (C18:1n9c) (35.805%), palmitic acid (C16:0) (5.791%), steric acid (C18:0) (4.106%), arachidic acid (C20:0) (0.244%), lignoceric acid (C24:0) (0.228%), gadoleic acid (C20:1n9) (0.096%), behenic acid (C22:0) (0.0866%), palmitoleic acid (C16:1) (0.073%), myristic acid (C14:0) (0.071%), linoleic acid (C18:3n3) (0.043%) and margaric acid (C17:0) (0.039%).

3.2. Egg's hatching assay of oils

It was observed in the negative control (1% tween 80 in distilled water) and in the control with distilled water the cell division (Morula phase) on the fifth day of incubation and between the ninth and the tenth, evolved to the on-site eye phase embryo inside the egg (Fig. 1-a) and on the fourteenth day, they had embryonic eggs containing the miracidium (Fig. 1-b), incubating after exposure to light. In the negative control and control with distilled water, approximately the same hatching percentage ($\pm$ SD) was observed with 87% (81–93%) and 84% (82–88%), respectively.

In the different concentrations tested with FOH, a statistically significant difference ($p < 0.05$) in egg hatching percentages was observed in relation to the data obtained with the negative control. There was an ovicidal action above 50% at doses 2.3 and 1.15 mg/mL of FOH (Table 1). The tested EOC concentrations showed statistically significant differences ($p < 0.05$), with egg percentage hatching below 2% and consequently the percentage of ovicidal activity obtained was between 98 and 100% (Table 2).

The EHA with the FOH and EOC combinations showed ovicidal activity above 90% in seven combinations, only the combination of 0.0085 mg/mL FOH and 0.008 mg/mL EOC showed ovicidal activity of 19%. (Table 3).

3.3. Assessment of cell viability

The highest cellular viabilities were of 100% for the FOH of *H. annuus* L., and 99% for the EOC of *C. cyminum* L. and for the combination (FOH + EOC) in the concentration of 0.015 mg/mL. The lowest viabilities were of 56% for the combination (FOH + EOC) in the concentration of (1.15 mg/mL + 1.03 mg/mL) respectively and 58% for the EOC of *C. cyminum* L. in the concentration of 4.14 mg/mL, as observed in Table 4.

4. Discussion

The chromatographic study of *H. annuus* L. FOH used in this work presented 53.63% of linoleic acid and 35.80% of oleic acid, results close to those found by Fonseca and Gutierrez (1974), Queiroga and Durán (2010) and Etievant et al. (1984) which obtained 56.30%; 52.85% and 71.7% of linoleic acid and 27.68% and 26% and 15.9% of oleic acid., respectively. In the EOC of *C. cyminum* L. the main constituent found was Cumaldehyde (26.8%), which corroborates with the findings of Cunha et al. (2003) and Martinez-Velazquez et al. (2011), which obtained 25–35% and 22.03%, respectively. The small variations may be related to the origin of the seeds used.

The use of the 1% Tween 80 used to emulsify the oil does not affect the development of *F. hepatica* eggs, as there was no statistically significant difference between the percentage of hatching of the negative

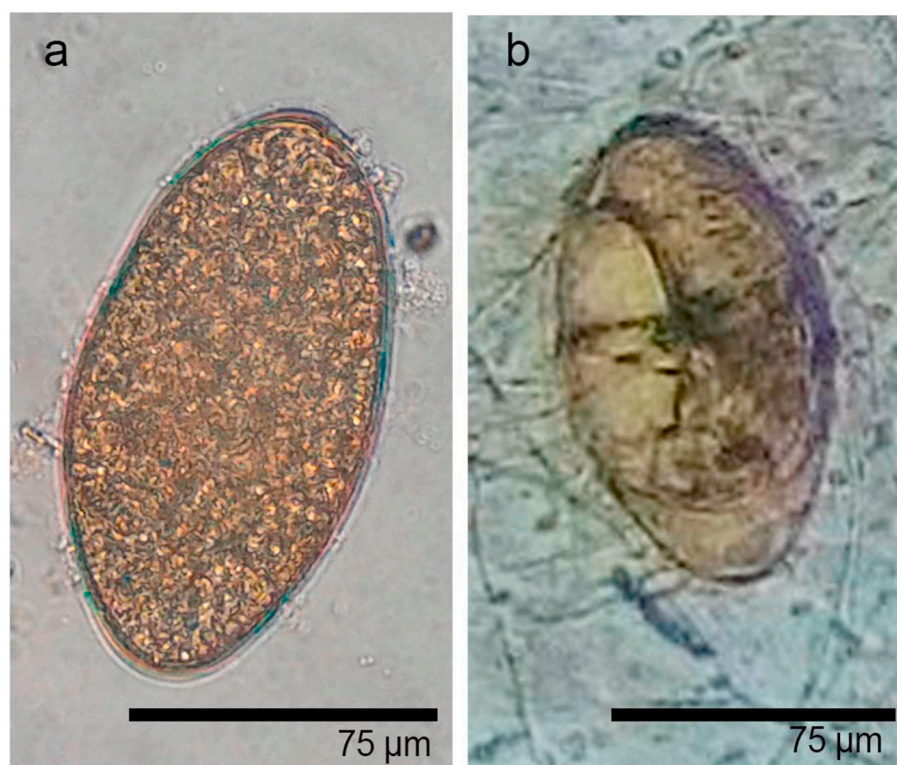


Fig. 1. Phases of *Fasciola hepatica* eggs observed in the negative controls and with distilled water: (a) eggs one day zero of incubation; (b) embryonic egg containing the miracidium.

Table 1

Percentage of eggs hatched after FOH incubation of *Fasciola hepática* eggs and the percentage ovicidal action of FOH at different concentrations tested. All groups tested had significant differences ($p < 0.05$) in relation to the control group. Dunnett's test.

Dose (mg/mL)	2.3	1.15	0.57	0.28	0.14	0.07	0.035	0.017
% Eggs hatched (SD)	42 (1)	32 (4)	46 (1)	59 (5)	62 (3)	62 (1)	64 (1)	82 (1)
% Ovicidal action (SD)	52 (1)	63 (5)	47 (3)	33 (6)	29 (3)	29 (1)	26 (1)	6 (1)

control ($\pm$ SD) 87% (81–93%) and control with distilled water hatching operations and uses 84% distilled water (82–88%).

The essential oil of *C. cyminum* L. presented good ovicidal activity (99% and 97%) and good cell viability (97% and 99%) at low

concentrations of 0.03 mg/mL and 0.016 mg/mL, respectively. The work carried out with essential oils evaluated mainly the anthelmintic potential against nematodes (Sinott et al., 2019) and insecticidal potential against ectoparasites vectors of human and animal diseases (James, 2012; Martinez-Velazquez et al., 2011). In relation to antiparasitic activity against *Fasciola* spp., there are few reports that evaluated the *in vitro* effect, which makes it difficult to compare the results obtained in this study with other scientific reports.

However, resistance in anthelmintics commonly used to control fasciolase is present throughout the world (Brennan et al., 2007; Olachea et al., 2011; Brockwell et al., 2014; Novobilsky et al., 2016), which makes these results promising when there is a search for effective and non-toxic alternatives.

The ovicidal activity in the combination of the FOH and EOC had a similar result with the results obtained only with the EOC, which the ovicidal activities were considerably better than the FOH. For example, in the concentrations of FOH and EOC combinations of 0.07 mg/mL + 0.06 mg/mL, 0.035 mg/mL + 0.03 mg/mL and 0.017 mg/mL + 0.016

Table 2

Percentage of eggs hatched after EOC incubation of *Fasciola hepática* eggs and the percentage ovicidal action of EOC at different concentrations tested.

Dose (mg/mL)	2.06	1.03	0.51	0.25	0.13	0.06	0.03	0.016	0.008	0.004
% Eggs hatched (SD)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.5)	2 (0.5)	44 (3)	52 (6)
% Ovicidal action (SD)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	99 (0.5)	98 (0.5)	49 (3)	39 (7)

All groups tested had significant differences ($p < 0.05$) in relation to the control group. Dunnett's test.

Table 3

Percentage of eggs hatched after incubation of FOH and EOC combinations of hepatic *Fasciola* eggs and percentage of ovicidal action of the different combinations of FOH and EOC tested.

Dose (FOH/EOC, mg/mL)	1.15/1.03	0.57/0.51	0.28/0.25	0.14/0.13	0.07/0.06	0.035/0.03	0.017/0.016	0.0085/0.008
% Eggs hatched (SD)	0 (0)	0 (0)	0 (0)	0 (0)	4 (2)	1.3 (1)	5 (2)	71 (2)
% Ovicidal action (SD)	100 (0)	100 (0)	100 (0)	100 (0)	95 (2)	99 (1)	95 (3)	19 (2)

Table 4

Viability percentage of the MDBK (Madin-Darby-Bovine-Kidney) cells against the different concentrations of the fixed oil (FOH) of *H. annuus* L., essential oil (EOC) of *C. cyminum* L. and the combination of the fixed oil (FOH) of *H. annuus* L. + the essential oil (EOC) of *C. cyminum* L.

FOH(mg/mL)	%	EOC(mg/mL)	%	FOH + EOC(mg/mL)	%
4.6	80	4.14	58	2.3 + 2.07	59
2.3	93	2.07	59	1.15 + 1.03	56
1.15	100	1.03	60	0.57 + 0.51	64
0.57	100	0.51	58	0.28 + 0.25	72
0.28	100	0.25	60	0.14 + 0.12	72
0.14	100	0.12	66	0.07 + 0.06	75
0.07	100	0.06	85	0.03 + 0.03	86
0.03	100	0.03	93	0.015 + 0.015	88
0.015	100	0.016	99	0.0075 + 0.0075	99

mg/mL, the ovicidal activities were 95%, 99% and 95%, respectively, and the ovicidal activities at the corresponding EOC concentrations were 100%, 99% and 98%. As there was no potentiation of the ovicidal effect in the combination of the oils, there is no indication that there was no synergy in the combinations. The EOC had high ovicidal activity, even in comparison to the combination of the oils and the OFH. According to Martinez-Velasquez et al. (2011) (26.8%), 2-Caren 10-al (22.17%), 1-Phenylbutanol (19.47%) and terpenes (gamma- Terpinen - 15.24%) present in the EOC, but that minor components, such as beta-pyrene and Cymene also recorded in the chromatography, may increase the biological activity of this essential oil.

Most volatile oils, also known as essential oils, are composed of phenylpropanoid derivatives or terpenoids (secondary metabolites), with monoterpenes (such as those found in EOC) and sesquiterpenes the compounds responsible for different biological activities of volatile oils (Athanasiadou et al., 2007; Balamurugan and Selvarajan, 2009; Yadav and Tangpu, 2009; Martinez-Velasquez et al., 2011; Hossain et al., 2013) point out some secondary metabolites present in plant derivatives as possibly responsible for the anthelmintic effect, including fasciolytic activity.

Products derived from natural resources are capable of causing lesser side effects to the host and less environmental damage. And because of their secondary metabolites, plants are capable of producing various effects against parasites, such as reduced fertility, disruption of development, and reduced feed (Roy and Saraf, 2006).

5. Conclusions

The best ovicidal activities obtained were with essential oil of *Cuminum cyminum* L. 98% at the concentration of 0.016 mg/mL). When essential oil of *Cuminum cyminum* L. and fixed oil of *Helianthus annuus* L. were combined, no data were found that could suggest synergism, since the results of the ovicidal activities of the combinations coincided with the data obtained with the isolated EOC. The results were promising for the continuity of new studies in the search for new bioactives to be used in the control of *Fasciola hepatic*.

Author statement

As the corresponding author, I declare that the co-authors collaborated with the main author, Maria Antonieta Machado Pereira da Silva, in the research stages as listed below.

- Collect and recuperation of the eggs of *F. hepatica*; Ovicidal activity assay of the oils against *F. hepatica*: Fabiane Knepper Zehetmeyr, Karine Massia Pereira, Rosaria Helena Machado, José Pablo Villarreal Villarreal, Natália Berne Pinto.

- Obtaining and chromatographic analysis of the *Helianthus annuus* L. and *Cuminum cyminum* L.: Bruna Silveira Pacheco and Rogério Antonio Freitas.

- Evaluation of cell viability *in vitro*: Silvia de Oliveira Hubner.

- Project coordinator, advisor and funding: Maria Elisabeth Aires Berne, Patrícia da Silva Nascente.

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28 de março de 2021 16:23

Responder a: Flora <flora@elsevier.com>Para: Karine Massia Pereira <karinemassia@gmail.com>

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Authors: Karine Massia Pereira; Sonia Marisa Hefler; Ana Silvia Rolon

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