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ENDOZOOCORIA ENTRE ORGANISMOS AQUÁTICOS: A IMPORTÂNCIA DAS AVES PARA A DISPERSÃO DE PLANTAS, INVERTEBRADOS E PEIXES

São Leopoldo

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Tese apresentada como requisito para obtenção do título de Doutor em Biologia, pelo Programa de Pós-Graduação em Biologia da Universidade do Vale do Rio dos Sinos – UNISINOS

Área de Concentração: Diversidade e Manejo de Vida Silvestre

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RESUMO: A ampla distribuição geográfica de algumas espécies sesseis ou com baixa capacidade de auto locomoção que habitam sistemas aquáticos continentais isolados sempre foi um enigma ecológico. A dispersão passiva através de um vetor de transporte é a principal explicação para entender a dispersão entre áreas úmidas e neste processo as aves aquáticas desempenham um papel de destaque. Ao contrário de outras regiões biogeográficas, na região Neotropical os estudos sobre a dispersão promovida por aves aquáticas são escassos. Nesta tese são apresentados estudos abordando a importância das aves aquáticas para a dispersão de plantas, invertebrados e peixes na região neotropical. Foram realizadas coletas de dados em campo e experimentos *ex situ*. Em relação aos diásporos de plantas, foi possível identificar que a dispersão depende da interação entre a espécie de ave e a estação do ano. Ainda sobre plantas, comprovou-se que uma angiosperma inteira (*Wolffia columbiana*) sobrevive à passagem pelo trato digestivo de cisnes e marrecas, representando um meio dispersão até então não conhecido para plantas. Em relação aos invertebrados, a dispersão promovida por aves está associada à espécie de ave, não sendo encontrado efeito da estação ou do peso das amostras. Por último, foi comprovado que ovos de peixes-anuais (Rivulidae) continuam seu desenvolvimento e eclodem mesmo após serem ingeridos e expelidos por uma ave, representando uma forma de dispersão desconhecida para vertebrados. Nesta tese é demonstrado que a endozoocoria promovida por aves é fundamental para compreender a dinâmica de dispersão de organismos aquáticos em áreas úmidas neotropicais.

Palavras-chave: Dispersão, Aves aquáticas, Macrófitas, Invertebrados, Peixes, Sementes, Diásporos, Ovos.

ABSTRACT: The wide distribution of freshwater species sessile or with low locomotion capacity inhabitants isolated continental aquatic systems has always been an ecological puzzle. Passive dispersal through a transport vector is the main explanation for understanding the dispersal among these wetlands, and waterbirds play an important role in this process. In the Neotropical region, studies on the dispersal promoted by waterbird are scarce. In this thesis, studies are presented addressing the importance of waterbird for the dispersal of plants, invertebrates and fish in the neotropical region. Field data collections and *ex situ* experiments were carried out. Regarding plant diaspores, it was possible to identify the dispersal depends on the interaction between the bird species and the season. Still on plants, it was proved that an entire angiosperm (*Wolffia columbiana*) survives the passage through the digestive tract of two species of Anatidae, representing a dispersal mode previously unknown. On invertebrates, the dispersal promoted by birds is associated with the bird species, with no effect of the season or sample weight. Finally, it has been proven that killifish (Rivulidae) eggs continue their development and hatch even after being ingested and expelled by a bird, representing an unknown dispersal means to a vertebrate. In this thesis, it is demonstrated that the endozoochory promoted by waterbirds is fundamental to understand the dispersal dynamics of aquatic organisms in neotropical wetlands.

Key words: Dispersal, Waterbird, Macrophytes, Invertebrate, Fish, Seed, Diaspore, Eggs.

SUMÁRIO

APRESENTAÇÃO

Esta tese está estruturada em cinco capítulos e uma seção de conclusão. Os capítulos não estão organizados na ordem cronológica a qual foram desenvolvidos ou publicados, mas sim de uma forma a qual considerou-se mais adequado à fluidez da leitura.

O primeiro capítulo corresponde ao referencial teórico onde foi analisada a bibliografia até então produzida sobre zoocoria, isto é, animais como vetores de dispersão, dando-se ênfase à endozoocoria de organismos aquáticos. Devido à extensa bibliografia encontrada, nesta seção foram citados somente os trabalhos considerados mais relevantes para o entendimento histórico dos avanços sobre o assunto desta tese. A formatação deste capítulo está de acordo com as normas da ABNT (Associação Brasileira de Normas Técnicas).

O segundo capítulo aborda a importância das aves da família Anatidae na dispersão de diásporos de plantas na região neotropical, sendo este um dos capítulos oriundos da concepção original do projeto de tese. Nesta investigação constatou-se que os eventos de dispersão de plantas por endozoocoria na região neotropical dependem da interação entre a espécie de ave e a estação do ano. Este capítulo foi publicado online na forma de artigo científico pela revista Freshwater Biology (Fator de Impacto 3.404) em setembro de 2020 e integrou o volume n° 66 (1) de dezembro do mesmo ano. A versão aqui apresentada está formatada conforme a última versão submetida e aceita pela revista.

O terceiro capítulo consiste em um artigo sobre a dispersão de uma espécie de lentilha-d’água (*Wolffia columbiana*) através do trato digestório de aves, o qual foi publicado na revista Biology Letters (Fator de Impacto 3.323) em dezembro de 2018 e por isso encontra-se no formato desta revista. A ideia deste capítulo surgiu a partir da descoberta de lentilhas-d’água em amostras fecais coletadas em campo. Até onde o conhecimento permitiu chegar, este foi o primeiro relato de uma planta inteira do grupo das angiospermas sobrevivendo à passagem pelo trato digestório de uma ave.

O quarto capítulo aborda a dispersão de invertebrados por endozoocoria. Ele está formatado para futura submissão à revista Hydrobiologia (Fator de Impacto 2.358) e está no formato exigido pela revista. Neste capítulo foi constatado que a dispersão de invertebrados na região neotropical está associada à espécie de ave dispersora, com fraca influência da sazonalidade na composição do que é dispersado.

O quinto capítulo trata da dispersão de ovos de peixes através do trato digestório de cisne-capororoca (*Coscoroba coscoroba*). Este artigo foi publicado na revista Ecology

(Fator de Impacto 4.285) em junho de 2019, estando no formato exigido pela revista. De maneira semelhante ao que ocorreu com a lentilha-d’água, este capítulo se desenvolveu a partir da descoberta de um ovo de peixe-anual em uma amostra fecal de cisne-capororoca coletada em campo, o que originou a ideia de um experimento independente para provar a viabilidade da dispersão de ovos de peixes por endozoocoria. Até onde foi possível pesquisar, esta foi a primeira evidência científica de que ovos de peixes podem eclodir em organismos saudáveis mesmo após passar pelo trato digestório das aves e serem eliminado nos excrementos. Esta descoberta trouxe novas perspectivas sobre o enigma histórico de como peixes conseguem colonizar lagos e poças geologicamente isoladas e distantes entre si.

Por último, é apresentada uma seção com as principais conclusões da tese de doutorado. Aqui procurou-se fazer considerações pontuais dos capítulos encontrados e uma análise geral das pesquisas realizadas, focando nas principais novidades que a tese trouxe a esta área do conhecimento.

1. CAPÍTULO 1: Referencial teórico - Aves como vetores de dispersão de organismos aquáticos

1.1. A dispersão de organismos sesseis ou com baixa capacidade de locomoção entre áreas úmidas

Dispersão é o processo ecológico pelo qual os organismos se deslocam na paisagem, se estabelecendo em habitats distantes de seu local de origem. Este processo é fundamental tanto para a ampliação da distribuição das espécies, através da colonização de novas áreas, quanto para os mecanismos envolvidos na dinâmica de metapopulações (PAKEMAN, 2001; BOWLER; BENTON, 2005). O sucesso da dispersão está associado a fatores como a capacidade de locomoção dos organismos, a distância entre habitats favoráveis ao seu estabelecimento e a permeabilidade da matriz (BROWN; LOMOLINO, 2006; BEGON et al, 2009). Entender os mecanismos de dispersão e qual a frequência que este processo ocorre é fator chave para a compreensão das dinâmicas populacionais e comunitárias (FIGUEROLA; GREEN, 2002; DONATTI et al., 2011; VAN LEEUWEN et al., 2012). O conhecimento dos processos de dispersão pode ser determinante na tomada de decisões de ações que objetivem à conservação, sobretudo em um cenário no qual as interferências antrópicas levam à descaracterização e perda de habitats naturais e a retirada de um elo da cadeia de dispersão pode causar alterações na dinâmica dos ecossistemas (AMEZAGA et al. 2002; BEGON et al., 2006; GALETTI et al., 2013. GREEN et al., 2016).

Habitats aquáticos continentais isolados por matrizes terrestres possuem estruturação paisagística semelhante àquela encontrada no conceito de biogeografia de ilhas oceânicas (FIGUEROLA; GREEN, 2002; GREEN et al., 2016). Para organismos que habitam estes sistemas aquáticos isolados a capacidade de locomoção é determinante nos processos de colonização e recolonização de habitats, sendo que para espécies com baixa ou nenhuma capacidade de locomoção por meios próprios, a dispersão passiva é fundamental para entender seus padrões de distribuição. A dispersão passiva ocorre quando um propágulo ou um organismo inteiro é carregado por um vetor de transporte, sendo os meios mais comuns a anemocoria, hidrocoria e zoocoria (MAGUIRE, 1963; SANTAMARIA, 2002; GREEN et al., 2016). A anemocoria ocorre quando o vento é o agente de dispersão, sendo esse processo mais evidente em algumas espécies de plantas com sementes propriamente adaptadas a este meio de transporte (SOOMERS et al. 2013).

Na hidrocoria, a água atua como agente de dispersão, sendo este processo relevante tanto para organismos inteiros quanto para seus propágulos (DOWNING-KUNZ; STACEY, 2011; SOOMERS et al. 2013). Na zoocoria o vetor de transporte é um animal, sendo esse processo tradicionalmente dividido em sinzoocooria, quando por motivos diversos o vetor intencionalmente transporta o organismo dispersado, epizoocoria (ou ectozoocoria), quando os propágulos ou organismos inteiros são transportados de maneira não intencional, aderidos às partes externas do vetor, e endozoocoria, quando esse processo de dispersão se dá via trato digestório do vetor (FIGUEROLA; GREEN, 2002a; GREEN et al., 2016). Diversas espécies de animais exercem a função de vetores de dispersão passiva em ambientes aquáticos e embora existam evidencias indiretas para anfíbios (BOHONAK; WHITEMAN 1999; VANSCHOENWINKEL et al., 2008a), e diretas para os mamíferos de médio e grande porte (VANSCHOENWINKEL et al., 2008b; VANSCHOENWINKEL et al., 2011), as aves aquáticas possuem relevância especial neste meio de dispersão (GREEN et al., 2016).

1.2.Aves aquáticas como dispersoras de plantas

Em sua obra clássica Darwin (1859) fez considerações sobre como as aves aquáticas podem atuar como agentes de dispersão tanto por epi quanto por endozoocoria. Guppy (1906) relatou ter conseguido germinar sementes de Cyperaceae, *Potamogeton* sp. (Potamogetonaceae) e *Sparganium* sp. (Typhaceae) recuperadas de excremento de pato-real (*Anas platyrhynchos*) os quais ele próprio havia alimentado, enquanto Ridley (1930) descreveu casos em que aves aquáticas efetivamente poderiam dispersar propágulos de plantas. A partir da década de 1960, um século após as observações de Darwin, diversos experimentos foram realizados para ampliar o conhecimento sobre dispersão de sementes por aves aquáticas, investigando quais plantas possuíam sementes aptas a sobreviver à passagem pelo trato digestivo, as taxas de sobrevivência e o tempo de retenção destes propágulos. Neste sentido, os experimentos de Proctor (1961, 1962, 1968), Proctor e Malone (1965), Proctor et al. (1967) e de Vlaming e Proctor (1968) trazem as primeiras informações sistematizadas de sobrevivência e tempo de retenção de sementes de diversas espécies, além de informações sobre a sobrevivência de oogônias de *Chara* (Characeae). Acrescentam-se a estes trabalhos os também precursores estudos de Powers et al. (1978) que identificou a sobrevivência de sementes de cinco espécies de plantas e Agami e Waisel (1986) os quais constataram que 30% das sementes de *Najas marina* (Najadaceae) mantinham-se viáveis após serem ingeridas por pato-real. A partir dos anos 2000 uma série de estudos lançou novas perspectivas sobre o papel das aves na dispersão de plantas, uma vez que também aspectos ecológicos começaram a ser investigados. Neste contexto, informações sobre a frequência da dispersão de sementes (FIGUEROLA; GREEN, 2002b), sazonalidade (FIGUEROLA et al., 2002; BROCHET et al. 2010), de características comportamentais e anatômicas das aves (CHARALAMBIDOU et al., 2005; FIGUEROLA; GREEN, 2005) e os efeitos deste tipo de dispersão para as plantas (SANTAMARIA et al., 2002; CHARALAMBIDOU et al., 2003; FIGUEROLA; GREEN, 2004; ESPINAR et al., 2006; BROCHET et al., 2010a), além da ampliação qualitativa do conhecimento das espécies de plantas passíveis de serem dispersas (FIGUEROLA et al., 2003; GREEN et al., 2008; BROCHET et al., 2009; BROCHET et al., 2010b; BROCHET et al., 2012; GREEN et al., 2013; REYNOALDS; CUMMING, 2016a; VAN LEEUEEN et al., 2017; LOVAS-KISS et al. 2015, 2018; HATTERMANN et al., 2019) evidenciaram a importância e a complexidade da zoocoria promovida por aves aquáticas.

A maior parte dos estudos investigando epi e endozoocoria tem como tema central a dispersão de propágulos dormentes. Poucos estudos abordam a dispersão de plantas inteiras ou fragmentos vegetativos viáveis e as informações existentes provêm de observações ocasionais. Weddel (1849) relata ter observado exemplares da planta *Wolffia brasiliensis* (Araceae) aderidas às penas de anhuma (*Anhima cornuta* – Anhimidae). Darwin (1859) observou plantas chamadas genericamente por ele de lentilhas-d'água sendo transportadas aderidas no dorso de marrecas. Jacobs (1947) encontrou exemplares de *Lemna minor* (Araceae) entre penas de pato-real e Reynolds et al. (2015) fotografou esta mesma espécie de planta aderida à plumagem da marreca *Anas undulata*. Coughlan et al. (2017) confirmaram experimentalmente o potencial de dispersão de plantas inteiras por epizoocoria ao constatarem que *L. minor* e *Azolla filiculoides* (Salvinaceae) eram dispersadas por pato-real entre poças. Denys et al. (2014) encontraram fragmentos da angiosperma *Crassula helmsii* (Crassulaceae) germinando após passagem pelo trato digestivo de gansos enquanto Wilkinson et al. (2017) demonstraram a viabilidade de fragmentos de briófitas em excrementos de pato real. Recentemente Silva et al. (2018 – capítulo 3 desta tese) descobriram que exemplares da angiosperma *Wolffia columbiana* (Araceae) sobrevivem à passagem pelo trato digestório de Anatidae.

1.3. Aves aquáticas como dispersoras de invertebrados

Assim como para plantas, a literatura tratando de aves aquáticas como dispersora de invertebrados nos remete às ponderações de Darwin (1859), o qual considera as aves como potenciais meios de transporte de invertebrados por longas distâncias. Posteriormente, de Guerne (1887, 1888, *apud* GREEN; FIGUEROLA, 2005) relatou a eclosão de efípias de Cladocera e estatoblastos de Briozoa encontrados sobre penas e patas de pato-real, e também Nematoda e Rotiferos a partir de amostras obtidas pela lavagem de patas e bicos de exemplares desta espécie. Brown (1933) comprovou que estatoblástos continuavam viáveis após passarem pelo trato digestivo de pato-real, sendo este o primeiro relato de endozoocoria para estas espécies. Segerstrale (1954) demonsttrou que *Gammarus lacustris* (Amphipoda) conseguiam sobreviver por até duas horas aderidos às penas de patos. Proctor (1964) comprovou que crustáceos também poderiam ser dispersos internamente por pato-real. Malone (1965a; 1965b) confirmou a sobrevivência de ovos de *Artemia* (Crustaceae) e de Gastropoda ao trato digestório, mostrando que estes organismos também poderiam ser dispersos por endozoocoria. Proctor et al. (1967) iniciaram os estudos comparativos de potencial de dispersão em diferentes espécies de aves quando analisaram a sobrevivência de ovos de invertebrados entre pato-real e maçarico (*Charadrius vociferus* - Charadriidae).

A partir dos anos 2000 houve acréscimo considerável no conhecimento sobre a dispersão de invertebrados por aves. Estudos quali-quantitativos e trabalhos comparando a frequência da dispersão e a sobrevivência dos propágulos de invertebrados foram desenvolvidos principalmente na Europa (FIGUEROLA; GREEN, 2002c; CHARALAMBIDOU et al., 2003; FIGUEROLA et al., 2003; FIGUEROLA et al., 2004; CHARALAMBIDOU; SANTAMARIA, 2005; FRISCH et al., 2007; BROCHET et al., 2010). Também é possível encontrar poucos estudos com temas semelhantes para a África (REYNOLDS; CUMMING, 2005 a, b), América do Norte (GREEN et al., 2013) e Oceania (GREEN et al., 2008). Na região neotropical, no entanto, não foram encontrados estudos sobre a endozoocoria de invertebrados.

1.4. Aves aquáticas como dispersoras de ovos peixes

Processos de vicariância são tradicionalmente aceitos como a melhor explicação para a distribuição global das espécies de peixes, mesmo para a ocorrência de espécies em áreas úmidas totalmente isoladas entre si, uma vez que, apesar do atual isolamento, muitos destes ambientes estiveram conectados em tempos passados (YONEKURA et al., 2004; EMMRICH et al., 2014). Porém, para os casos onde as áreas úmidas não possuem um histórico geológico de conexão com outras, como alguns lagos e poças temporárias, a ocorrência de peixes por si só e o compartilhamento de algumas espécies entre áreas úmidas isoladas sempre foi um tema que intrigou pesquisadores ao longo da história (HIRSCH et al., 2018).

Uma das hipóteses mais debatidas que explicaria a dispersão entre áreas isoladas é a possibilidade de que peixes poderiam ser transportados por aves aquáticas através de processos de epizoocoria, quando a ave ao deixar uma área úmida levaria ovos aderidos em suas partes externas, os quais seriam liberados quando a ave pousasse em outra área úmida. Tal possibilidade pode ser encontrada na literatura desde o início do século XIX. Em sua obra sobre História Natural de Aves Gmelin (1809) relata ter observado patos com ovas de peixes aderidos em bicos e pés e que estas aves transportavam os ovos entre áreas úmidas. Darwin (1859) e Wallace (1889) teceram comentários sobre a possibilidade de ovos de peixes serem transportados por meios não usuais, sendo que no caso deste último autor os achados de Gmelin foram citados como exemplo de que aves poderiam exercer a função de dispersoras de peixes. Riehl (1991) fez um relato no qual observou que ovos de peixe-lúcio (*Esox lucius*, Esocidae) encontrados aderidos às patas de patos-real permaneciam viáveis e eclodiam. Loureiro et al. (2011), Bartakova et al. (2013) e Costa (2014) analisando possíveis meios de dispersão de peixes-anuais das famílias Nothobranchiidae e Rivulidae consideraram que o transporte de ovos por aves poderia ser uma alternativa viável para justificar distribuições disformes de populações, porém ponderaram que a probabilidade deste tipo de dispersão deveria ser baixa.

Hirsch et al. (2018) realizaram profunda revisão bibliográfica de publicações científica e não-científicas com o objetivo de sistematizar o conhecimento sobre a possibilidade de dispersão de ovos de peixes por aves. Estes autores concluíram que não haviam publicações científicas robustas que confirmassem a tradicional noção do senso comum de que ovos de peixes poderiam ser dispersados de forma passiva por aves aquáticas. Silva et al. (2019 – Capítulo V deste trabalho) comprovaram que ovos de peixes-anuais das espécies de Rivulidae (*Austrolebias minuano* e *Cynopoecilus fulgens*), sobrevivem à passagem pelo trato digestório de cisne-capororoca. Essa foi a primeira evidência científica de que ovos de peixes podem ser dispersados por aves via endozoocoria, um meio de dispersão até então não cogitado, abrindo novas possibilidades para os estudos de dispersão de ovos de peixes. Recentemente, Lóvas-Kiss et al. (2020) experimentalmente comprovaram que ovos de carpas também podem ser dispersados por endozoocoria de aves aquáticas.

1.5. Fatores que influenciam a dispersão de organismos por aves aquáticas

Muitos são os fatores envolvidos nos processos de dispersão que podem determinar o sucesso da zoocoria e aqui alguns deles são debatidos. Primeiramente, para que a dispersão ocorra é necessário que haja contato entre os organismos dispersores e aqueles a serem dispersados. Apesar de óbvia, essa premissa deve ser considerada principalmente em regiões com marcada sazonalidade, onde existe produção estacional de propágulos a qual precisa ser concomitante com a chegada de aves migratórias (CLAUSEN et al., 2002). Apesar de as aves residentes destes locais exercerem dispersão em escala regional, a ocorrência de aves migratórias pode ampliar a dispersão para longas distâncias (FIGUEROLA; GREEN, 2002a). Uma vez que o contato entre os organismos tenha sido estabelecido é necessário que os propágulos venham a se aderir à parte externa ou sejam ingeridos pelo vetor (CLAUSEN et al., 2002; FIGUEROLA; GREEN, 2002a). Como já relatado aqui, diversos casos comprovaram que essa premissa é atendida e que é relativamente comum no meio natural. Uma vez aderidos ou ingeridos pelos vetores, a resistência dos propágulos passa a ser o fator chave e, como já exemplificado anteriormente, muitos propágulos de plantas e invertebrados podem sobreviver a estes meios de transporte (GREEN et al., 2016).

Apesar de ainda não ser assunto pacificado na literatura há indícios de que a endozoocoria pode ser mais relevante para a dispersão de organismos aquáticos do que a epizoocoria (BROCHET et al. 2010; REYNOLDS; CUMMING, 2016a). Desta forma, a resistência à passagem pelo trato digestório das aves é determinante para a magnitude da dispersão. Experimentos utilizando sementes como modelos trazem resultados contraditórios em relação à resistência e à taxa germinação, indicando que esses índices devem variar entre as espécies dispersadas (FIGUEROLA; GREEN, 2002a). O tempo de retenção também está associado à amplitude da dispersão. Por exemplo, Van Leeuwen et al. (2012) demonstraram que a maior parte dos propágulos ingeridos são expelidos pelas aves no intervalo de até duas horas após a ingestão, porém, mesmo após 5 h ao menos um propágulo viável pode ser encontrado. Considerando uma velocidade média de 75km/h, os autores estimam que em um voo constante a ave pode liberar até cinco propágulos viáveis após terem percorrido mais de 100 km, uma distância pequena para espécies de aves migratórias.

As variações morfológicas e comportamentais também têm influência no potencial de dispersão das espécies de aves aquáticas (GREEN et al., 2016). Os diversos hábitos alimentares e estratégias de forrageio podem levar a diferenças no material que é ingerido pela ave (GREEN, 1998; GREEN et al., 2016). Por exemplo, aves piscívoras dificilmente se alimentarão de sementes, porém, a sugestão de Darwin (1859) de que elas podem atuar como dispersoras secundárias de propágulos ingeridos por peixes que foram predados já foi comprovada tanto para plantas quanto para invertebrados (VAN LEEUWEN et al., 2017). Aves que se alimentam diretamente da vegetação aquática, como as galinhas-d'água (*Fulica* spp) tendem a dispersar diferentes tipos de propágulos quando comparadas com Anatidae filtradores (FIGUEROLA; GREEN, 2002c; GREEN et al., 2016). Dentro da família de Anatidae, as estratégias clássicas de forrageio "dabbling" (na lâmina d'água), "grazzing" (pastejadoras) e "diving" (mergulhadoras) permitem que as aves tenham acesso a diferentes recursos, embora a plasticidade de forrageio nem sempre permita que as diferenças no que é dispersado sejam constatadas facilmente (GREEN et al., 2002; REYNOLDS; CUMMING, 2016b). O tamanho corpóreo é outro fator que também pode influenciar o que é ingerido pelas aves. Aves com tamanhos corpóreos maiores tendem a se alimentar em águas mais profundas (SARRAS et al., 1996; GREEN, 1998; NUDDS et al., 2000) e mesmo nos casos em que as aves se alimentam em conjunto, aquelas com tamanhos maiores tem acesso a recursos diferentes daquelas com tamanhos menores (BAYLEI; BATT, 1974; SARRAS et al., 1996).

Na presente tese alguns dos fatores que influenciam a dispersão por endozoocoria são abordados. Aqui, além de descrever os táxons dispersados por aves aquáticas em uma região biogeográfica pouco estudada, também é investigado como os fatores espécie de ave, sazonalidade, peso das amostras e o tamanho do propágulo (para plantas) influenciam a dispersão de organismos entre áreas úmidas na região neotropical.

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2. CAPÍTULO 2: Seed dispersal by neotropical waterfowl depends on bird species and seasonality

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2.1. Abstract:

1 - Waterbird-mediated endozoochory is an essential mechanism for the dispersal of sessile organisms in freshwater ecosystems. However, in the neotropics there are no previous studies of how different waterbird species vary in the dispersal functions they perform, and how seasonality influences endozoochory. In this study, we identified plant diaspores dispersed in faeces of five South American waterfowl (Brazilian teal *Amazonetta brasiliensis*, yellow-billed teal *Anas flavirostris,* ringed teal *Callonetta leucophrys*, coscoroba swan *Coscoroba coscoroba* and white-faced whistling-duck *Dendrocygna viduata*).

2 - We collected 165 faecal samples from five wetlands in southern Brazil surrounded by pasture and ricefields, then separated and measured intact seeds and other diaspores. Using Generalized Linear Models, we tested how diaspore abundance and taxonomic richness differed among bird species and between cold (April to September) and warm (October to March) periods. We also analysed bird-specific and seasonal variations in diaspore composition through Principal Coordinates Analysis and PERMANOVA. We used Indicator Species Analysis to determine which diaspore species discriminated between bird species and seasons. Finally, we measured diaspore length in order to analyze differences among waterfowl species in the size of diaspores dispersed.

3 - We found 2,066 intact diaspores from 40 different plant taxa, including seeds of 37 angiosperms and diaspores of Lycophyta (*Isoetes* cf. *maxima*), Pterydophyta (*Azolla filiculoides*) and Charophyceae. There was at least one diaspore in 65% of all faecal samples. Diaspores of native amphibious and emergent plants were dominant. We found 1,835 diaspores (from 33 taxa) in the cold period but only 231 (23 taxa) in the warm period. Seeds of the grass *Zizaniopsis bonariensis* and of the sedge *Rynchospora* sp. were the most abundant taxa. A strong interaction between bird species and season was the most important predictor of variation in both taxonomic richness and abundance of diaspores. The taxonomic composition of diaspores differed among waterfowl species and season. Indicator Species Analysis identified 12 plant taxa associated with particular bird species and seasons. Coscoroba swan, the largest bodied species in our study dispersed a higher proportion (8.2%) of large (length >2 mm) seeds.

4 - Despite considerable overlap, there are important differences in the plants dispersed by each species, and the smallest (ringed teal) and largest (coscoroba swan) birds are particularly different. All five waterfowl species are distributed over wide areas of South America and here, we demonstrated that they are likely important plant vectors connecting wetland species at different geographical scales. Many of these plants have previously been assumed to lack mechanisms for long-distance dispersal.

2.2. Key-words: Diaspores; Endozoochory; Waterbird; Wetlands; Neotropics

2.3. Introduction

Dispersal is a key ecological process underlying species distribution (Lomolino et al, 2010). For organisms with little or no locomotion capacity, transport vectors are fundamental to the spread of propagules or mature individuals away from their places of birth or germination (Heleno & Vargas, 2014; Tesson et al. 2015). Many plant species have animals as dispersal vectors, where whole plants or their diaspores are transported on feathers, hair, or skin (epizoochory), or more often inside the digestive tract (endozoochory) (Green et al., 2016; Coughlan et al., 2017). Interactions between vertebrates and plants with fleshy fruits based on endozoochory have been well studied, with many examples from the neotropics (Galetti et al., 2001; Levey et al., 2002; Wenny et al., 2016). Recent studies have shown that endozoochory is also important for a range of aquatic and terrestrial plant species lacking a fleshy fruit, with herbivorous, granivorous and omnivorous waterbirds acting as excellent vectors for long-distance dispersal (Green et al., 2016; Viana et al., 2016; Hattermann et al., 2019).

Waterbird-mediated zoochory allows wetland species to cross the terrestrial matrix between isolated waterbodies (Figuerola & Green, 2002), providing a vital ecological function to freshwater communities (Green & Elmberg 2014). Waterbirds are highly mobile, widely distributed and abundant, making them good dispersal vectors at different spatial scales (Green et al., 2016; Kleyheeg et al., 2019). Amongst the waterbirds, waterfowl (Anatidae – duck, geese and swans) are the best-known dispersers of plant diaspores (Green et al., 2016; Wilkinson et al., 2017; Lovas-Kiss et al., 2018; Silva et al., 2018).

How dispersal ability varies among waterfowl species, and the underlying causes of these differences, remain poorly understood, although bird morphology, foraging behaviour, gut anatomy and movement patterns are all thought to play important roles (Figuerola et al., 2003; van Leeuwen et al., 2012; Green et al., 2016; Reynolds & Cumming, 2016). Seasonality is expected to influence plant dispersal, partly since the phenological match between seed availability and the presence of waterfowl in the area may be crucial to dispersal rates (Clausen, 2002), although studies from the Mediterranean region indicate that seed dispersal can occur at high rates for months after seeds are produced (Figuerola et al., 2003; Brochet et al., 2010).

Since the seminal work by V.W. Proctor and colleagues in the USA (e.g. de Vlaming & Proctor, 1968), studies on endozoochory by waterfowl have predominantly been conducted in Europe (reviewed by Green et al., 2016), but in recent years there have been important studies in Africa (Reynolds & Cumming, 2016), Oceania (Bartel et al., 2018) and North America (Green et al., 2013; Costea et al., 2016, Farmer et al., 2017). In South America, Summers & Grieve (1982) cited upland goose (*Chloephaga picta*) and ruddy-headed goose (*C. rubidiceps* as potential seed dispersers of fleshy-fruit plants in the Falkland Islands. Additionally, Willson et al. (1997) observed seeds of four fleshy-fruit plants in faeces of upland goose and ashy-headed goose (*C. poliocephala*) in Tierra del Fuego. Russo et al. (2020) found evidence that these geese species also disperse mosses by endozoochory. Silva et al. (2018) found whole plantlets of watermeal (*Wolffia columbiana*) surviving in white-faced whistling-duck droppings.

Here, we identified plant diaspores dispersed in faeces of five syntopic South American Anatidae: three Anatinae (Brazilian teal, ringed teal, and yellow-billed teal) an Anserinae (coscoroba swan) and a Dendrocygninae (white-faced whistling-duck). We tested how the abundance, species richness and species composition of plant diaspores dispersed by endozoochory changed among bird species and seasonality. We expected important variation between bird species, reflecting known differences in their morphology and foraging ecology, including their body size (Kear, 2005 a,b). We also expected changes between the warm and cold periods of the year, reflecting differences in the production and availability of diaspores of different plants, and likely seasonal changes in diet (e.g. greater focus on invertebrates during the nesting period, Kear, 2005 a,b).

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2.4. Material and methods

Sampling procedures

Faecal samples were collected in Santa Vitória do Palmar, in the Coastal Plain of southern Brazil (Figure S1), a region composed of a mosaic of permanent and temporary wetlands mixed with native grassland, livestock grazing, commercial forests and ricefields. All five wetlands sampled were surrounded (within less than 50 m) by ricefields and livestock farms. The percentage of each land use (rice or livestock) changes annually according to the rice cycle and actions taken by landowners to expand pastures. During the warm period, the water surface area is reduced, leading the study wetlands to be totally isolated from ricefields. During the cold period, higher water levels lead to hydrological connectivity between the study wetlands and rice fields. The estimated flooded area of each wetland varied between 0 and 0.04 ha during the warm period, and between 0.3 to 3.9 ha during the cold period. The wetlands of this region are important to resident waterbird species as well as austral and boreal migrants that winter or breed in the region (Belton, 1994; Sick, 1997, Guadagnin & Maltchik 2007, Guadagnin et al. 2009, Guadagnin et al. 2012, Maltchik et al. 2011). Temperature ranges from 16 °C ± 2 to 26 °C ± 3 in the warm period (October to March), and from 10 °C ± 2 to 19 °C ± 2 in the cold period (April to September; Rio Grande do Sul, 2019). Although precipitation is similar in both periods (warm: 117 mm ± 15 mm; cold: 123 mm ± 8mm), differences in evapotranspiration rates (warm: 103 mm ± 29 mm; cold: 41 mm ± 14 mm) make the warm period drier.

We collected 165 droppings from five waterfowl species (Brazilian teal, n= 40; coscoroba swan, n= 22; ringed teal, n= 31; white-faced whistling-duck, n = 40; yellow-billed teal, n= 32) in five wetlands located 1–9 km apart, during seven field trips lasting from four to seven days each. Three trips were carried out between October 2017 and January 2018, in the warm period, and four in August 2017, April, May and June 2018, during the cold period. We grouped samples collected during the austral autumn and winter as the cold period, and the austral spring and summer as the warm period. The warm period (spring and summer) is the main breeding season in the study region, except for Brazilian teal which reproduces year-round, and for occasional breeding of resident coscoroba swan during the cold period. All bird species studied can nest in the early warm period, and raise their offspring before the end of the warm period (Belton, 1994; Calabuig et al., 2010; Dias & Fontana, 2002; Mauricio et al., 2013). At the end of the warm period, many coscoroba swans migrate from this region to Argentina (Calabuig et al., 2010), and only a few pairs or family groups remain. The other species studied stay in the region year-round, forming flocks of tens or hundreds of individuals in the cold period, although ringed teal and white-faced whistling-duck were sometimes absent in the study wetlands.

The foraging behaviours of the waterfowl (assigned as dabbling, diving, grazing) were observed in the field over the sampling period (about 200 hours of sampling effort in total). Coscoroba swan (easily the largest species) often fed in the deepest water (1-1.5 m) with head or neck partially submerged, usually apart from the other species, and also grazed alone around lake edges. Brazilian teal, white-faced whistling-duck and yellow-billed teal fed in mixed flocks in the water column at depths of up to 0.5 m, and ringed teal joined these species mainly when feeding at shallower depths. Brazilian teal, ringed teal and yellow-billed teal mainly fed by dabbling at the water surface, and Brazilian teal were also observed up-ending (see Green, 1998). White-faced whistling-duck fed on vegetation by submerging their head in the water, or by grazing around lake edges. No species was seen diving.

We located monospecific groups resting or feeding around lake edges and collected fresh droppings from the grass, with a minimum distance of one meter between samples to prevent resampling of the same individuals. We closely inspected all droppings to avoid contamination from the substrate, then stored them individually in plastic tubes. As our study area is up to 500 km from the laboratory, and each trip lasted from four to ten days, we froze samples (- 4 °C) to avoid fungal infestation. Therefore, we did not test the germinability of seeds. However, previous work has firmly established the quantification of intact seeds recovered from faeces as an adequate proxy for dispersal rate (van Leeuwen et al., 2012; Green et al., 2016).

The samples were weighed and washed in tap water using a sieve (53 μm) in the laboratory at UNISINOS University. We used a Bogorov chamber in a stereomicroscope (1.6 x to 5 x magnification) to separate the diaspores from the other materials. We initially grouped the diaspores by morphotypes, then used literature to identify them to the lowest possible taxonomic level (see References in Supplementary material). We only counted intact diaspores, discarding broken or empty ones.

Data analyses

We analyzed the effects of bird species, seasons and faecal weight on the richness and abundance of diaspores using Generalized Linear Models (GLMs). Model parameters were estimated by maximum likelihood (Laplace approximation). All models fitted best with a negative binomial error distribution, which showed less overdispersion than a Poisson error distribution. In the model of diaspores abundance, we excluded one outlier to improve the model fit, this being a yellow-billed teal sample with 489 seeds of *Rynchospora* sp. We tested for main effects and interactions. We compared these effects against null models (intercept only) and performed model selection using the Akaike information criterion (AIC) (Burnham & Anderson, 2002), retaining only models with delta AIC < 2.0 for further inference. We fitted all the GLMs in the R statistical environment v. 3.6.1 using the function *glm.nb* of the package *lme4* (R Development Core Team, 2019).

In order to assess diaspores composition variation among bird species and seasons we used Principal Coordinates Analysis (PCoA) and Permutational Multivariate Analysis of Variance (PERMANOVA) using the Bray-Curtis distance matrix and 9999 permutations to validate the model significance of PERMANOVA. We ran pairwise tests for multiple comparisons of diaspores composition variation among bird species. Principal coordinates analysis (PCoA, also called metric multidimensional scaling) is a multivariate method (unconstrained ordination) that attempts to summarize (dis-) similarity among a set of samples in a few dimensions. This analysis produces a set of orthogonal axes whose importance is measured by eigenvalues (Lepš & Šmilauer, 2003; Borcard et al., 2018). To calculate PCoA and to plot the ordination, we use the R functions *cmdscale* and *ordiplot* from package vegan in the R statistical environment v. 3.6.1 (R Development Core Team, 2019).

We used Indicator Species Analysis (Dufrêne & Legendre, 1997) to determine which diaspores species discriminated between bird species and seasons. The significance of the observed maximum indicator value (IV) for species was derived from 9999 permutations in R. Finally, we measured diaspore length and classified them into six categories (≤0.5 mm, 0.51-1 mm, 1. 1-1.5 mm, 1.51-2 mm, 2.1-2.5 mm, >2.5 mm) in order to analyze differences among waterfowl species in the size of diaspores dispersed.

We measured five diaspores from each taxon (or all of them when n<5), and used mean values.

2.5. Results

Plant taxa dispersed by endozoochory

We found 2,066 intact diaspores from 40 plant taxa, 31 of which were identified to species level, five to genus and four to family (Table S1). We found diaspores of 37 angiosperms, plus megaspores (Lycophyta - *Isoetes* cf. *maxima*), sporocarps (Pterydophyta - *Azolla filiculoides*) and oogonia (Charophyceae). Twenty-nine of the identified species are native to Brazil and two are alien but considered naturalized (*Echinochloa cruss-galli* and *Salicornia fruticosa*). Five species (*Cyperus difformis, E. cruss-galli, Heteranthera reniformis, Ludwigia erecta* and *Panicum dichotomiflorum*) are rice weeds (Table S1). Eight species are terrestrial, nine amphibious and 15 are aquatic plants (Table S1). Habitat classification of the other eight taxa was not possible (Table S1). Of the aquatic and amphibious plants (24 spp), 15 were emergent, four fixed-floating, one free-floating (*A. filiculoides*) and four submergent (Table S1).

In 65% of all faecal samples, there was at least one diaspore (77% of samples in the cold period and 51% in the warm period). Each sample contained a median of two diaspores in the cold period (Interquartile range - IQR- of 10, 25), ranging from samples without diaspores to a sample from yellow-billed teal that contained 489 seeds of *Rynchospora* sp. In the warm period, the median number of diaspores per sample was one (IQR: 3, 0), with a range of 0-33 diaspores. Cyperaceae (12 taxa) and Poaceae (8) were the most diverse families. We found 1,835 diaspores (from 33 taxa) in the cold period and 231 (23 taxa) in the warm period. Seventeen taxa (42.5% of the total) were recorded only in the cold period, and seven (17.5%) only in the warm period, with 16 (40%) common to both periods. The grass *Zizaniopsis bonariensis* and the sedge *Rynchospora* sp. were the most abundant taxa recorded, representing 50% of all diaspores (Table S1). *Z. bonariensis* was also the most frequently recorded species (20% of samples), followed by *Oldenlandia salzmannii* (17%), *Eleocharis minima* (12%) and *Nymphoides indica* (12%). *Z. bonariensis* was present in samples obtained in three wetlands in the cold period, and four wetlands in the warm period. *Rynchospora* sp., was recorded only during the cold period, in samples from two wetlands.

Effects of bird species, sample weight and seasonality on plant taxon richness and diaspore abundance

The best fit models considered all variables tested and showed that the interaction between bird species and season was the most important effect explaining taxonomic richness and abundance of diaspores ($P<0.05$) (Table 1). In the case of diaspore abundance, there were two other models with ΔAIC < 2 (Table S3).

Table 1. Analysis of Deviance of the best fitted model with the effects of the factors Bird species, Season and Sample Weight, demonstrating that interaction between Species and Seasons had a particularly strong effect on the Richness and Abundance of diaspores dispersed by waterfowl.

Variable	Factor	LR Chisq	df	*P*-value
Richness	Bird	10.712	4	0.030
	Season	5.628	1	0.018
	Weight	6.905	1	0.009
	Bird*Season	23.044	4	<0.001
Abundance				
	Bird	44.821	4	<0.001
	Season	20.273	1	<0.001
	Weight	2.809	1	0.094
	Bird*Season	18.920	4	<0.001

The model fitted through GLM analysis for both Richness and Abundance included all factors investigated: Richness (or Abundance) ~ Bird + Season + Weight + Bird*Season. See Table S2 for further details.

In the warm period, Brazilian teal dispersed a significantly lower richness of diaspores than all other birds ($P<0.05$), except the coscoroba swan ($P>0.05$) (Figure 1, Table S4). In the cold period, white-faced whistling-duck dispersed a lower richness than Brazilian teal, ringed teal and yellow-billed teal ($P<0.05$). In the warm period, Brazilian teal and coscoroba swan dispersed significantly fewer seeds diaspores per sample than other bird species ($P<0.05$), with no difference between them ($P>0.05$) (Figure 2, Table S4). In the cold period, ringed teal dispersed more diaspores per sample than other bird species, and yellow-billed teal dispersed more diaspores than Brazilian teal and white-faced whistling-duck (P<0.05).

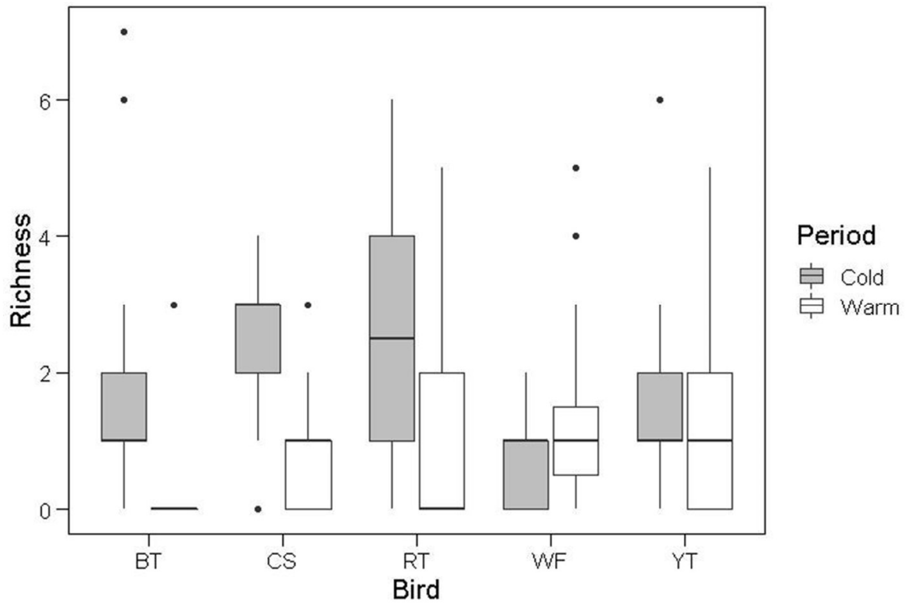


Figure 1. Seasonal variation in taxonomic richness of diaspores dispersed by waterfowl species. BT - Brazilian teal, YT - yellow-billed teal, RT - ringed teal, CS - coscoroba swan and WF - white-faced whistling-duck. Boxes represent the range of quartiles Q2 and Q3 separated by the median (bold horizontal lines). Vertical lines indicate maximum and minimum limits of Q1 and Q4, while dots represent outliers.

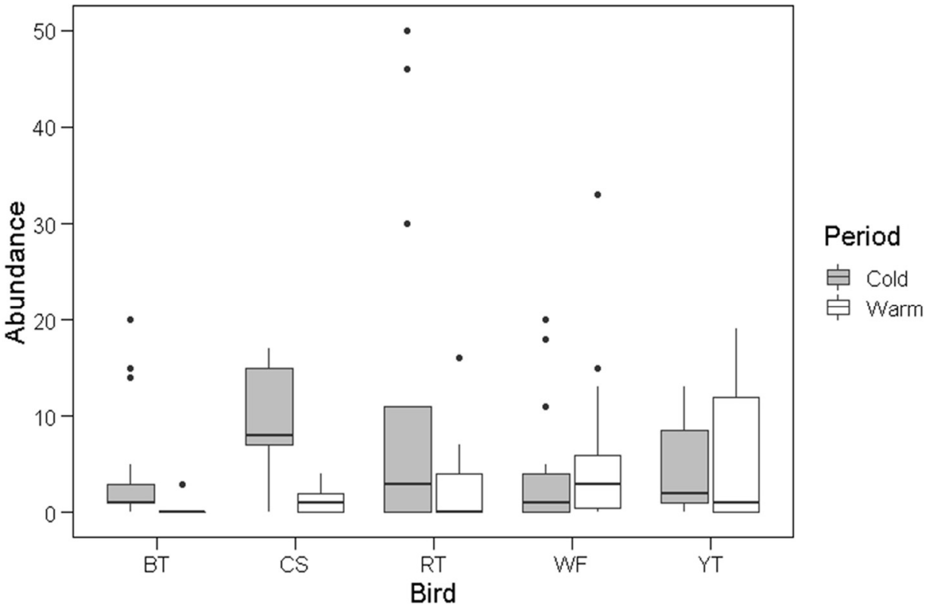
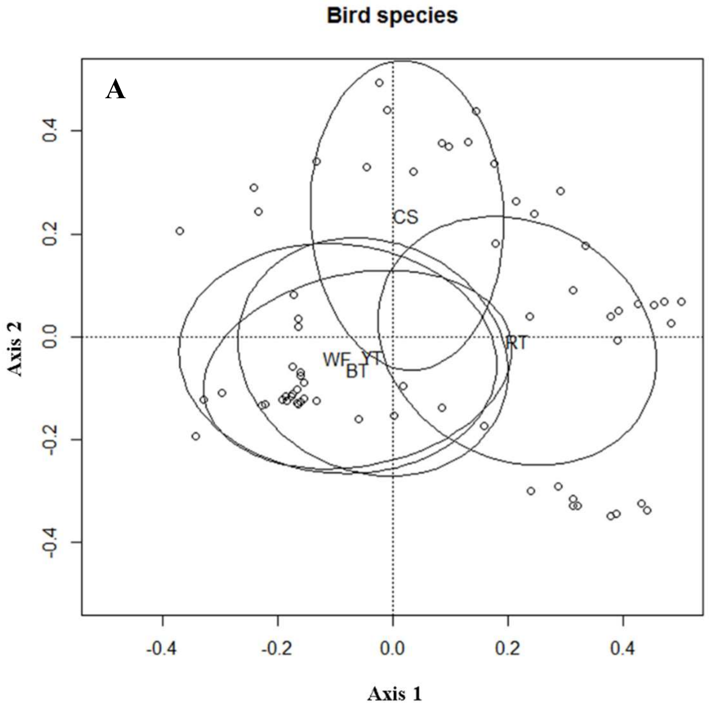


Figure 2. Seasonal variation in abundance of diaspores among waterfowl species. BT - Brazilian teal, YT - yellow-billed teal, RT - ringed teal, CS - coscoroba swan and WF - white-faced whistling-duck. Boxes represent the range of quartiles Q2 and Q3 separated by the median (bold horizontal lines). Vertical lines indicate maximum and minimum limits of Q1 and Q4, while dots represent outliers. We excluded outliers greater than 50 diaspores per sample (five of ringed teal and one of yellow-billed teal) to improve the visualization of differences between species.

Variation in the taxonomic composition of diaspores between vectors

The taxonomic composition of diaspores dispersed differed significantly among waterfowl species ($R^2=0.10$, $F_{4,102}=2.874$, $P<0.05$) and seasons ($R^2=0.02$, $F_{1,105}=2.829$, $P= P<0.05$) (Figure 3 a-b). Diaspore composition varied significantly between coscoroba swan and ringed teal, and between them and all other species. The composition of diaspores dispersed by Brazilian teal, white-faced whistling-duck and yellow-billed teal was not significantly different (Table S5). Hence the largest (coscoroba swan) and smallest (ringed teal) birds were different to those of intermediate size (Figure 3a).



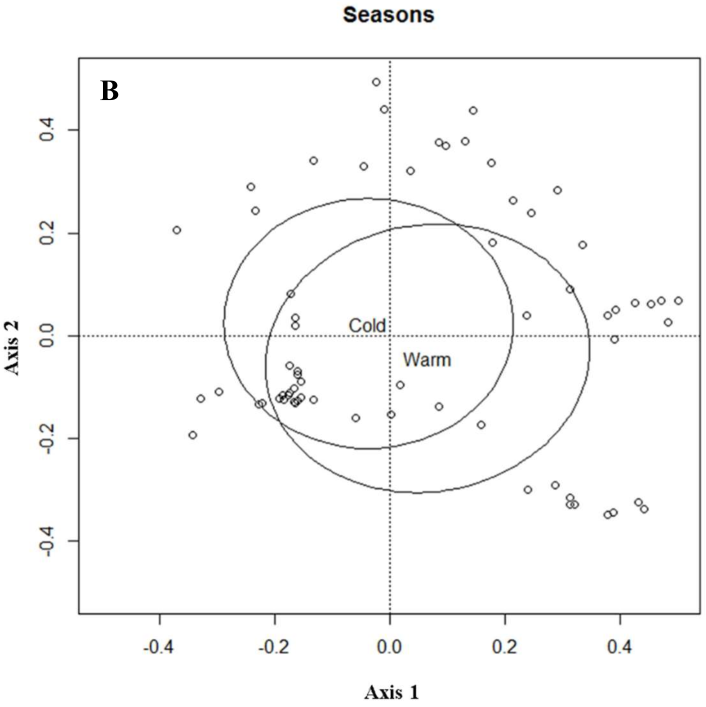


Figure 3. Ordination of taxonomic composition of diaspores, and how it varied among (A) bird species and (B) Cold and Warm periods. The total of variation explained by the axes was 32% (Axis 1 = 17% and Axis 2 = 15%). Bird species are represented by the initials BT - Brazilian teal, YT - yellow-billed teal, RT - ringed teal, CS - coscoroba swan and WF - white-faced whistling-duck.

Dispersal interactions between particular plant and bird species

Seeds of *Echinochloa crussgalli* (IV = 0.200), *Eleocharis flavescens* (IV = 0.220) and *Panicum germinatum* (IV = 0,150) were associated with Brazilian teal (P<0.05). Seeds of *Hydrocleys nymphoides* and those from fleshy fruits of Rubiaceae spp. were associated with coscoroba swan (IV = 0.368 and IV=0.160, respectively; P<0.05). Apocynaceae spp. (IV = 0.197; P<0.05) was associated with white-faced whistling-duck and *Potamogeton pusillus* (IV = 0.237; P<0.05) with yellow-billed teal. Seeds of *Oldenlandia salzmannii* (IV = 0.487), *Eleocharis minima* (IV = 0.451), *Zizaniopsis bonariensis* (IV = 0.439), *Kyllinga odorata* (IV = 0.200) and *E. bonariensis* (IV = 0.225) were more prevalent and abundant in samples from ringed teal (P<0.05). Apocynaceae spp. (IV= 0.165; P<0.05) was mainly dispersed in the warm period and *E. flavescens* (IV= 0.130; P<0.05) in the cold period.

The average length of diaspores dispersed by waterfowl was 1.30 mm ± 0.74 mm. Diaspores with length up to 1.5 mm accounted for 94.3% of the diaspores recorded and were predominant in all bird species (Table S6, Figure 4). Only white-faced whistling-duck and coscoroba swan dispersed diaspores from all length categories, with coscoroba swan having the highest value for size categories above 2 mm (8.2%).

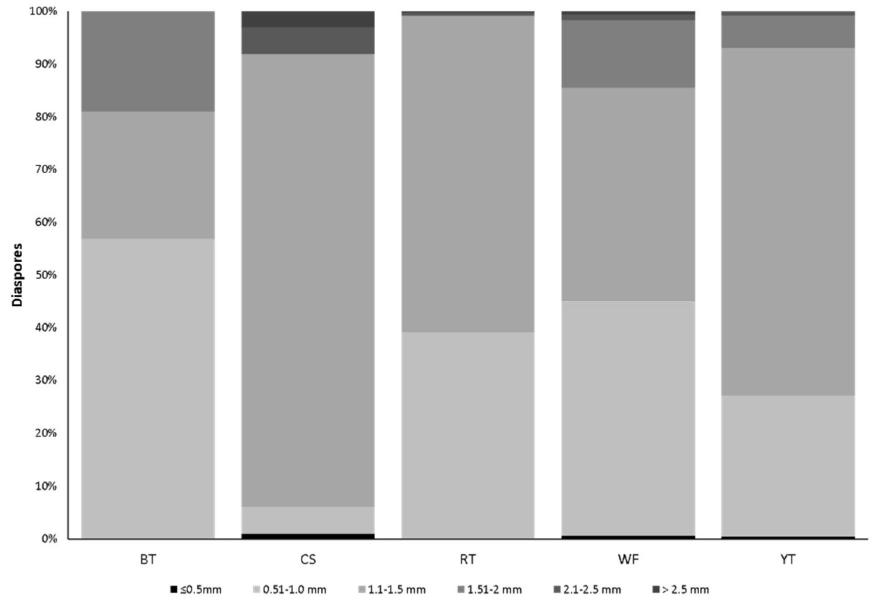


Figure 4. Percentage composition of diaspores per waterfowl species. BT - Brazilian Teal, YT - yellow-billed teal, RT - ringed teal, CS - coscoroba swan and WF - white-faced whistling-duck.

2.6. Discussion

To our knowledge, we have conducted the most detailed study to date of endozoochory by any animal in the neotropics from outside forested environments, and the first study comparing endozoochory by different waterbirds in neotropical wetlands. Our results demonstrate that all five neotropical waterfowl species are important plant vectors, dispersing at least 40 different plant species.

What plants are dispersed?

The most frequently dispersed seeds were of *Z. bonariensis*, an emergent giant grass considered "Vulnerable" in Brazil due to habitat loss. *Z. bonariensis* occurs in isolated populations in southeast and southern Brazil, Argentina, Paraguay and Uruguay and is considered to disperse primarily by anemochory and vegetative propagation (Ferreira et al., 2009; CNCFlora, 2012). Our results suggest that endozoochory by waterfowl has an important but previously unrecognized role in the distribution of *Z. bonariensis*. The second most dispersed plant genus, *Rynchospora* sp., is widely distributed in the region, notably *R. barrosiana*, *R. brittonii* and *R. tenuis* (Weber, 2014), and this may be related to their capacity for frequent dispersal by waterfowl. The plant families Poaceae and Cyperaceae had the highest taxonomic richness, following a pattern reported in previous studies on Anatidae in other continents (de Vlaming & Proctor 1968; Green et al., 2016; Reynolds & Cumming, 2016).

The diaspores dispersed by waterfowl varied from strictly aquatic plants (e.g. *Ruppia maritima*) to amphibious (interface between aquatic and terrestrial, e.g. *E. flavescens*) and terrestrial (e.g. *Solanum americanum*) plants. This is consistent with recent studies in Europe showing that ducks and shorebirds are important vectors for both aquatic and terrestrial plants (Soons et al., 2016; Lovas-Kiss et al., 2018; Lovas-Kiss et al., 2019). The dominant diaspores were those of angiosperms lacking a fleshy fruit, and most of these angiosperm species are widely considered to be self-dispersed or dispersed by water (hydrochory), and hence to have no mechanisms for dispersal between isolated wetlands. Because they lack a fleshy fruit, the importance of endozoochory for these plants is consistently overlooked in the literature and in plant trait databases (Soons et al., 2016; Costea et al., 2019). We did record two plant taxa with fleshy fruits (*S. americanum* and Rubiaceae spp.) which are therefore considered to have an "endozoochory syndrome". These findings reinforce the important function of waterfowl endozoochory in the dispersal of a broad taxonomic range of plants, in line with studies from other continents (Green et al., 2016; Reynolds & Cumming, 2016; Lovas-Kiss et al., 2018).

We also found quillwort megaspores (*Isoetes* cf. *maxima*) and sporocarps of floating ferns (*Azolla filiculoides*) dispersed by waterfowls. Dispersal by endozoochory has previously been proposed as an explanation for the distribution of quillworts (Brunton & Britton, 1999; Troia, 2016), but this has not previously been demonstrated. The fern *A.*

filiculoides is native in our study area and an invasive alien species in other continents (Hussner, 2012; Hill et al., 2020), and it has often been suggested that it may spread by zoochory (Reynolds et al., 2015; Lovas-Kiss et al., 2018). Coughlan et al. (2018) demonstrated experimentally that *A. filiculoides* can survive external transport by waterfowl. Green et al. (2008) found reproductive tissue in the faeces of Australian waterbirds, highlighting the possibility of internal transport. Our results showed intact diaspores of *A. filiculoides* after waterfowl gut passage, confirming its dispersal by endozoochory.

Dispersal depends on seasonality and bird species

The interaction between bird species and season explained the most variance in plant taxonomic richness and diaspore abundance in our study. Figuerola et al. (2003) found a similar result for endozoochory by wintering waterbirds in Spain. These authors argued that changes in the richness and abundance of diaspores dispersed were related to species-specific seasonal variations in diet, feeding behaviour and digestive processing of food. Our findings are likely to have similar explanations, but there is a general lack of detailed studies of diet and feeding behavior in neotropical waterfowl. In one exception, Madriz (1983) found seasonal variation in the frequency of different seeds observed in oesophagi of Brazilian teal in Venezuela, with increased seed ingestion in the rainy season and a decrease in the dry season.

The diaspore composition of coscoroba swan and ringed teal faeces was different from that of the other three species, although the proportion of the variation explained by species was relatively low, suggesting there is much overlap as also reported for Europe (Figuerola et al., 2003; Green et al., 2016). The variation we recorded may be related to the differences in the body size and its influence on foraging behaviour. Coscoroba swan is larger species (~3.500 g) and ringed teal smaller (~350 g) than the other species (of between 500 and 800 g; Kear, 2005 a,b). These extremes in body size and associated differences in access to different depths for feeding may lead to greater habitat segregation, and hence access to diaspores of different plants (Pöysä, 1983; Green, 1998; Ntiamoa-Baidu et al., 1998; Guillemain et al., 2002). In our study, we observed coscoroba swan feeding in the deepest areas, ringed teal in the shallowest areas, and the other three species usually feeding together elsewhere. These field observations suggest that body size may be determinant of niche diaspores segregation. In future, the influence of body size on diaspore dispersal should be further investigated in neotropical waterbirds, with the inclusion of more bird species with different body sizes, and detailed field observations of feeding behaviour and plant ecology (in a manner paralleling the many detailed studies of frugivore behaviour in neotropical forests).

Coscoroba swan dispersed the largest proportion, 8.2%, of large seeds (i.e. >2 mm). This could potentially be because large seeds were less likely to be destroyed in the larger birds (García-Alvarez et al. 2015), but may alternatively be due to a negative relation between body size and the density of lamellae in the bill which have a key role in food processing (Gurd, 2008). High lamellae density facilitates selection of smaller seeds, and this explains for example why the Eurasian teal *Anas crecca* ingests smaller seeds than larger dabbling ducks in Europe (Guillemain et al., 2002; Green et al. 2016). However, we lack data on lamellar density in our study species to test this hypothesis.

The phenology of seed availability may lead to differences in richness and abundance of dispersed diaspores in temperate waterbirds (Clausen et al., 2002; Green et al., 2002; Lovas-Kiss et al. 2019). A lack of information on plant phenology in our study area prevented us from making a detailed analysis of relationships between seasonal variation in seed production and seed dispersal. However, data for 15 plant species dispersed in our study, but obtained previously in similar regions of the extreme south of Brazil, show that some plants produce seeds exclusively in the cold or the warm period and others in both periods (Trevisan, 2005; Giehl, 2012). These data confirm that some species were dispersed exclusively in the period when they would be producing seeds, e.g. *Nymphoides indica* in the cold period and *Hydrocleys nymphoides* in the warm period. However, other plants such as *Salicornia fruticosa* (dispersed in the warm period) and *Ludwigia erecta* (dispersed in both periods) were dispersed in a different season to those when seeds are produced (according to the above literature). Thus, future research into seed production and seed availability in the water column and in sediments is needed to understand how they influence dispersal rates in neotropical wetlands.

Pathways for plant dispersal in the southern half of South America

Information about flight patterns, migration routes, population size and distributions of the study waterfowl species is limited compared with North America or Europe.

However, the available information gives us some indication about the relationships between functional and behavioural variations and the Dispersal Potential (DP, *sensu* Coughlan et al., 2019) of each bird species. Experimental studies have shown that seeds are typically retained in waterfowl guts for at least 4-8 h and often for much longer, with maxima frequently exceeding 24 h (García-Alvarez et al., 2015, Reynolds & Cummings, 2016; Lovas-Kiss et al., 2020). Given flight speeds of 50-78 km/h (Welham, 1994), this enables seed dispersal process by endozoochory over tens or hundreds of km during daily or migratory flights, greatly exceeding the maximum dispersal distances obtained by wind or water dispersal for most plant species, which rarely reach 1 km (Bullock et al., 2017).

Coscoroba swan and white-faced whistling duck are the most migratory species, ringed teal and yellow-billed teal are residents with occasional regional movements and Brazilian teal are typical residents (Belton, 1994, Calabuig et al, 2010; Don Pablo Research Team, 2012; Maurício et al., 2013). In this sense, Coscoroba swan has the higher potential to disperse plants over the greatest distances among the study species, mainly in an east-west direction. This swan has an estimated population of 10,000 - 25,000 individuals distributed across a range of 4,250,000 km^2 (BirdLife International, 2020, Wetlands International, 2020). In southern Brazil, the most recent census was of 1,622 individuals (Dias & Fontana, 2001) with a partially migratory population, most of which leaves the study area in the warm period in an east-west route towards Argentina, moving up to 1,700 km, and then returning in the cold period (Calabuig et al., 2010). Nevertheless, coscoroba swan is also likely to disperse plants in a north-south direction, inside the continent, since part of its population is migratory both in the northern (Pantanal and Chaco to Pampa) and in the southern (Patagonia to Pampa) parts of its range (Carboneras et al., 2019).

White-faced whistling is partially migratory in the neotropics, with an estimated population of 1,000,000 individuals distributed over up to 10,000,000 km^2 (BirdLife International, 2020, Wetlands International, 2020). Southern Brazil has a partially migratory population of about 100,000 individuals (Menegheti & Dotto, 2005) that use the east-west route. In central South America white-faced whistling migrates along a north-south flyway along the Paraná-Paraguay rivers (Blanco et al., 2020). Satellite tracking data from birds fitted with transmitters in Argentina found them to move up to >600 km away from the capture site, with individuals having daily average movements of 0.1 - 23 km (mean of 4 km; Don Pablo Research Team, 2012). There was great individual variability in the timing and direction of movements, with some individuals moving to Brazil, Uruguay or Paraguay.

Some ringed teal make regional north-south seasonal movements between southern Brazil and Uruguay, with irregular routes, and there are also resident populations along this route (Belton, 1994; Maurício et al., 2013, Carboneras et al., 2020). The range of ringed teal is 2,270,000 km^2 with an estimated population of 25,000 - 100,000 (BirdLife International, 2020, Wetlands International, 2020). Satellite tracking data from birds fitted with transmitters in Argentina (Don Pablo Team, 2017) showed that ringed teal moved to locations an average of 238 km from their capture site (min: 9 km, max: 423 km).

Brazilian teal and yellow-billed teal are thought to be resident species that disperse mainly between local wetlands, but there are few data on their movements (Nascimento et al., 2005; Carboneras et al., 2020). They are both abundant (about 1,000,000 individuals per species) with a similar range size (about 12,500,000 km^2), but contrasting distributions (yellow-billed teal from central to southern South America and Brazilian teal from the central to the north).

The DP of each species depends on both the number of diaspores dispersed per individual and the population size (Coughlan et al., 2019), and our results show it also varies seasonally. In the cold season, the greater abundance of diaspores per bird suggests that ringed teal has the highest DP at a local scale, although we are lacking precise population estimates for our study area. At the continental scale, its high population size and intermediate number of diaspores per individual suggest that yellow-billed teal has the highest DP.

2.7. Conclusion

We demonstrated that the five waterfowl species studied play an important role in the dispersal of a broad variety of plants, ranging from strictly aquatic to terrestrial species, and including both native and exotic plants. The interaction between bird species and seasonality explained the most variation in diaspores dispersed. The composition of diaspores dispersed varied between seasons and between three groups of bird species. The scarcity of studies on the phenology of plants and waterfowl movements currently limits our understanding of spatial and temporal patterns of endozoochory in the neotropical region. However, our study demonstrates that this process is important at different spatial scales, and is likely to be central to the maintenance of plant metacommunities and to changes in plant distributions in the face of global change.

2.8. Data availability statement

A summary of the data used in this paper is available as supplementary information.

2.9. Acknowledgments

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2.10. Conflict of interest

The authors declare no conflict of interest.

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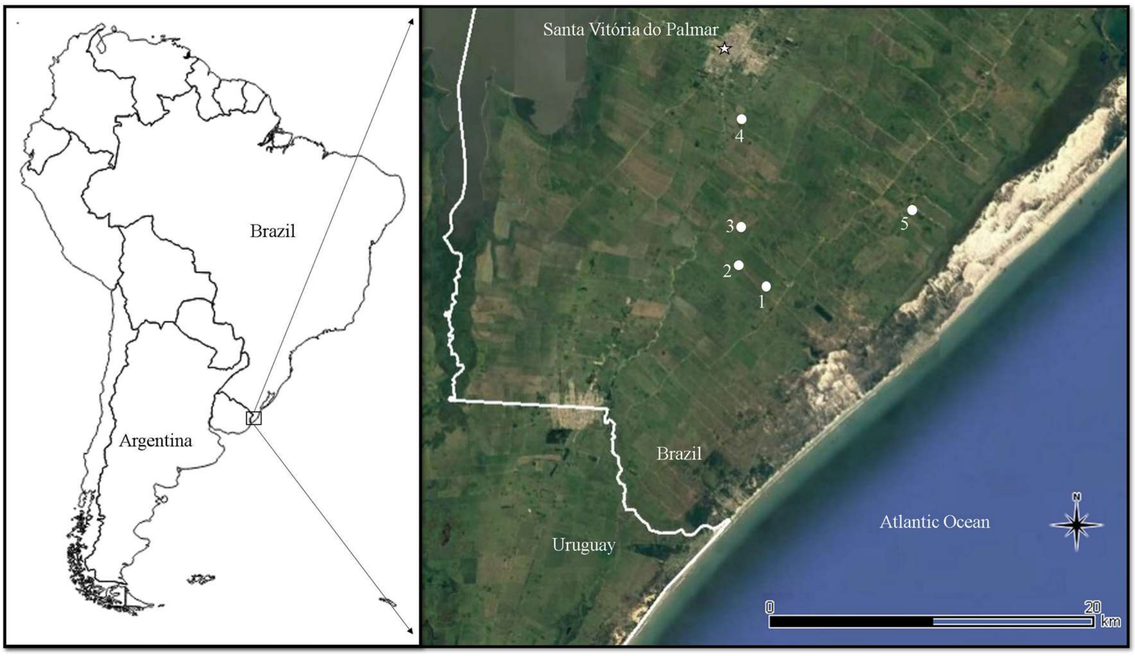

Figure S1. Study area in southern Brazil where samples of the five waterfowl species were collected in five wetlands (white spots).

2.12. Supplementary material

Seed dispersal by neotropical waterfowl depends on bird species and seasonality

Giliandro G. Silva; Andy J. Green; Pedro Hoffman; Vinícius Weber Cristina Stenert; Ádám Lovas-Kiss; Leonardo Maltchik

Table S1. Intact diaspores dispersed by five waterfowl species via endozoochory in southern Brazil in cold and warm periods. Numbers in parentheses are N samples with at least one diaspore / N total samples. Sws = N samples in which at least one seed was recorded.

					Brazilian teal				Coscoroba swan				Ringed teal				White-faced whistling-duck				Yellow-billed teal				Total	
					cold		warm		cold		warm		cold		warm		cold		warm		cold		warm			
					(n=19/25)		(n=1/15)		(n=8/9)		(n=7/13)		(n=14/18)		(n=6/13)		(n=11/17)		(n=17/23)		(n=17/21)		(n=7/11)			
Family	Taxa	Habitat	Habit	Length (mm)	seed	sws	seed	sws	seed	sws	seed	sws	seed	sws	seed	sws	seed	sws	seed	sws	seed	sws	seed	sws	seed	sws
Acanthaceae	*Hygrophila* sp.	-	-	0.45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	1
Alismataceae	*Hydrocleys nymphoides*	*Aquatic*	*Floating*	1.16 ± 0.47	3	1	-	-	34	6	-	-	-	-	-	-	-	-	-	-	1	1	-	-	38	8
Amaranthaceae	*Salicornia fruticosa*	*Terrestrial*	-	0.86	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	1	1
Apocynaceae	*Apocynaceae* spp	-	-	0.51 ± 0.01	5	2	-	-	-	-	-	-	-	-	-	-	-	-	39	6	3	2	-	-	47	10
Charophyceae	*Charophyceae* spp[1]	*Aquatic*	*Submerse*	0.65 ± 0.11	3	2	-	-	-	-	-	-	-	-	1	1	-	-	-	-	184	3	1	1	189	7
Cyperaceae	*Androtrichum trigynum*	*Terrestrial*	-	3.96 ± 0.04	-	-	-	-	-	-	-	-	-	-	1	1	-	-	1	1	-	-	-	-	2	2
Cyperaceae	*Cyperus diformiss*	*Terrestrial*	-	1.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	1	1
Cyperaceae	*Eleocharis radicans*	*Anphibia*	*Emergent*	1.14	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Cyperaceae	*Eleocharis maculosa*	*Aquatic*	*Emergent*	1.30 ± 0.06	-	-	-	-	-	-	-	-	5	2	-	-	-	-	10	2	-	-	-	-	15	4
Cyperaceae	*Eleocharis bonariensis*	*Aquatic*	*Emergent*	1.12 ± 0.13	1	1	1	1	-	-	1	1	19	6	-	-	-	-	3	2	-	-	1	1	26	12
Cyperaceae	*Eleocharis flavescens*	*Aquatic*	*Emergent*	0.97 ± 0.12	38	5	-	-	-	-	-	-	-	-	-	-	6	3	-	-	1	1	-	-	45	9
Cyperaceae	*Eleocharis rabenii*	*Aquatic*	*Emergent*	1.03 ± 0.06	5	2	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	1	1	7	4
Cyperaceae	*Eleocharis minima*	*Anphibia*	*Emergent*	0.97 ± 0.06	2	1	-	-	-	-	3	3	162	8	3	2	1	1	7	3	7	2	-	-	185	20
Cyperaceae	*Fuirena incompleta*	*Anphibia*	*Emergent*	1.62	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Cyperaceae	*Kyllinga odorata*	*Terrestrial*	-	1.06 ± 0.04	-	-	-	-	-	-	-	-	10	3	8	1	-	-	-	-	-	-	-	-	18	4
Cyperaceae	*Rynchospora* sp.	-	-	1.22 ± 0.03	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	492	2	-	-	493	3
Cyperaceae	*Scirpus* sp.	-	-	0.66± 0.06	-	-	-	-	1	1	-	-	-	-	-	-	-	-	2	2	-	-	2	1	5	4
Hydrocharitaceae	*Najas guadalupensis*	*Aquatic*	*Submerse*	1.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	1	1
Isoetaceae	*Isoetes* cf. *maxima*[2]	*Aquatic*	*Emergent*	0.37 ± 0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	1	1	2	2
Juncaceae	*Juncus cf. Bufonius*	*Anphibia*	*Emergent*	0.37	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Juncaceae	*Juncus* sp.	-	-	0.48 ± 0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	-	2	1
Menyanthaceae	*Nymphoides indica*	*Aquatic*	*Floating*	1.33 ± 0.11	1	1	-	-	24	5	-	-	4	3	2	1	35	5	-	-	3	2	30	3	99	20
Onagraceae	*Ludwigia erecta*	*Terrestrial*	-	0.57 ± 0.12	-	-	-	-	-	-	-	-	1	1	1	1	-	-	1	1	-	-	-	-	3	3
Poaceae	*Axonopus purpusii*	*Terrestrial*	-	1.81 ± 0.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	-	2	1
Poaceae	*Echinochloa cruss-galli*	*Anphibia*	*Emergent*	1.73 ± 0.15	5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	4

					Brazilian teal				Coscoroba swan				Ringed teal				White-faced whistling-duck				Yellow-billed teal				Total	
					cold		warm		cold		warm		cold		warm		cold		warm		cold		warm			
					(n=19/25)		(n=1/15)		(n=8/9)		(n=7/13)		(n=14/18)		(n=6/13)		(n=11/17)		(n=17/23)		(n=17/21)		(n=7/11)			
Family	Taxa	Habitat	Habit	Length (mm)	seed	sws	seed	sws	seed	sws	seed	sws	seed	sws	seed	sws	seed	sws	seed	sws	seed	sws	seed	sws	seed	sws
Poaceae	*Poaceae* spp	-	-	0.75	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Poaceae	*Panicum dichotomiflorum*	*Anphibia*	*Emergent*	1.72 ± 0.03	6	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	2
Poaceae	*Panicum germinatum*	*Terrestrial*	-	1.72 ± 0.06	4	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	3
Poaceae	*Paspalum repens*	*Aquatic*	*Emergent*	2.48 ± 0.04	-	-	-	-	1	1	-	-	-	-	-	-	-	-	1	1	-	-	-	-	2	2
Poaceae	*Paspalum* sp.	-	-	1.61 ± 0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	-	2	1
Poaceae	*Zizaniopsis bonariensis*	*Anphibia*	*Emergent*	1.34 ± 0.21	10	4	1	1	17	5	7	4	474	8	6	2	1	1	23	3	3	3	4	2	546	33
Polygonaceae	*Polygonum punctatum*	*Anphibia*	*Emergent*	2.40 ± 0.14	-	-	-	-	4	3	-	-	5	2	-	-	-	-	1	1	1	1	-	-	11	7
Pontederacae	*Heteranthera reniformis*	*Aquatic*	*Floating*	0.84 ± 0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	1	1
Potamogetonaceae	*Potamogeton pusillus*	*Aquatic*	*Submerse*	1.87 ± 0.11	1	1	-	-	-	-	-	-	1	1	-	-	-	-	-	-	46	6	-	-	48	8
Ranunculaceae	*Ranunculus flagelliformis*	*Aquatic*	*Floating*	1.6	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Rubiaceae	*Rubiaceae* spp.[3]	-	-	2.83 ± 0.94	-	-	-	-	1	1	2	2	1	1	-	-	-	-	-	-	-	-	-	-	4	4
Rubiaceae	*Oldenlandia salzmannii*	*Anphibia*	*Emergent*	0.66 ± 0.09	4	4	1	1	-	-	1	1	151	9	9	3	1	1	22	4	1	1	16	5	206	29
Ruppiaceae	*Ruppia maritima*	*Aquatic*	*Submerse*	2.17 ± 0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	1	5	1
Salviniaceae	*Azolla filiculoides*[4]	*Aquatic*	-	0.52 ± 0.07	-	-	-	-	-	-	-	-	9	1	6	1	-	-	1	1	-	-	-	-	16	3
Solanaceae	*Solanum americanum*	*Terrestrial*	-	1.53 ± 0.07	-	-	-	-	-	-	-	-	-	-	-	-	23	2	-	-	-	-	-	-	23	2
Total					92		3		83		15		843		38		67		113		750		62		2066	

[1]Oogone, [2]Megaspore and [3]Sporocarp.

Table S2. Summary outputs of the best model fitted in GLM analysis: Diaspores (Richness or Abundance) ~ Bird species + Season + Sample Weight + Bird species*Season. The intercept corresponds to Brazilian teal and cold period.

	Richness				**Abundance**			
	Estimate	Std. Error	z value	Pr(>|z|)	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	0.157	0.209	0.749	0.454	1.034	0.354	2.919	0,003
Coscoroba swan	-0.313	0.467	-0.671	0.502	-0.116	0.815	-0.143	0,886
Ringed teal	0.487	0.268	1.816	0.069	2.433	0.486	5.007	<0.001
White-faced whistling-duck	-0.808	0.361	-2.240	0.025	0.063	0.517	-0.124	0.901
Yellow-billed teal	-0.002	0.287	-0.009	0.993	1.389	0.475	2.926	0.003
Warm	-1.968	0.620	-3.173	0.001	-2.867	0.766	-3.740	<0.001
Weight	0.073	0.027	2.701	0.007	0.088	0.048	1.813	0.069
Coscoroba swan:Warm	1.197	0.749	1.598	0.110	1.117	1.162	1.051	0.293
Ringed teal: Warm	1.165	0.714	1.632	0.103	0.160	0.096	0.166	0.868
White-faced whistling-duck: Warm	2.676	0.723	3.700	<0.001	3.176	0.929	3.416	<0.001
Yellow-billed teal: Warm	2.065	0.715	2.888	0.004	1.902	0.968	1.966	0.049

Table S3. Summary of the models tested in the GLM analyses through Akaike information criterion (AIC). The best models selected for diaspore richness and abundance considered all variables tested and showed that the interaction between bird species and season was the most important effect explaining taxonomic richness and abundance of diaspores. For diaspore abundance, two other models had ΔAIC<2.

Model tested		**AIC**	**ΔAIC**	**df**
Richness	~Bird + Season + Weight + Bird*Season	506.27		12
	~Bird + Season + Bird*Season	510.94	4.7	11
	~Bird*Season	510.94	4.7	11
	~Bird + Season + Weight	519.98	13.7	8
	~Bird + Season	523.53	17.3	7
	~Season	524.00	17.7	3
	~Bird	528.88	22.6	6
	~1	529.25	23.0	2
Abundance	~Bird + Season + Weight + Bird*Season	855.34		12
	~Bird + Season + Bird*Season	856.12	0.8	11
	~Bird*Season	856.12	0.8	11
	~Bird + Season + Weight	865.17	9.8	8
	~Bird + Season	866.37	11.0	7
	~Bird	883.39	28.0	6
	~Season	890.50	35.2	3
	~1	911.80	56.5	2

Table S4. Summary outputs of the best model fitted in GLM analysis: Diaspores (Richness or Abundance) ~ Bird species + Season + Sample Weight + Bird species*Season changing the intercept to perform a pairwise comparison.

	Richness								**Abundance**							
	Cold period				**Warm period**				**Cold period**				**Warm period**			
Waterfowl	Estimate	Std. Error	z value	Pr(>|z|)	Estimate	Std. Error	z value	Pr(>|z|)	Estimate	Std. Error	z value	Pr(>|z|)	Estimate	Std. Error	z value	Pr(>|z|)
BT-CS	-0.313	0.467	-0.671	0.502	-0.884	0.725	-1.219	0.223	0.116	0.815	0.143	0.886	-1.000	0.952	-1.050	0.293
BT-RT	-0.488	0.268	-1.816	0.069	-1.652	0.661	-2.496	**0.012**	-2.433	0.486	-5.007	**<0.001**	-2.593	0.831	-3.120	**0.002**
BT-WF	0.809	0.361	2.240	**0.025**	-1.867	0.626	-2.981	**0.002**	0.064	0.517	0.124	0.902	-3.112	0.771	-4.036	**<0.001**
BT-YT	0.002	0.287	0.009	0.993	-2.062	0.655	-3.147	**0.002**	-1.390	0.475	-2.926	**0.003**	-3.293	0.845	-3.898	**<0.001**
CS-RT	-0.800	0.448	-1.786	0.074	-0.769	0.510	-1.507	0.132	-2.550	0.806	-3.163	**0.002**	-1.592	0.790	-2.014	**0.044**
CS-WF	0.495	0.485	1.022	0.307	-0.984	0.473	-2.079	**0.038**	0.523	0.780	0.070	0.946	-2.112	0.740	-2.853	**0.004**
CS-YT	0.311	0.490	0.634	0.526	-1.179	0.517	-2.282	**0.022**	-1.506	0.850	-1.772	0.076	-2.292	0.825	-2.779	**0.005**
RT-WF	1.296	0.355	3.648	**<0.001**	-0.215	0.366	-0.587	0.557	2.498	0.535	4.668	**<0.001**	-0.519	0.564	-0.920	0.357
RT-YT	0.489	0.286	1.709	0.087	-0.410	0.414	-0.990	0.322	1.044	0.507	2.060	**0.039**	-0.700	0.662	-1.058	0.290
WF-YT	-0.806	0.377	-2.140	**0.032**	-0.195	0.654	-0.551	0.581	-1.454	0.541	-2.687	**0.007**	-0.180	0.583	-0.309	0.757

BT - Brazilian teal, YT - yellow-billed teal, RT - ringed teal, CS - coscoroba swan and WF - white-faced whistling-duck.

Table S5. Comparison of diaspores composition among waterfowl species. Statically significant values are in bold.

Pairs	F.Model	R2	p.value	p.adjusted
RT - WF	3.185	0.06	0.001	**0.01**
RT- YT	3.005	0.07	0.001	**0.01**
RT- BT	3.544	0.08	0.001	**0.01**
RT- CS	3.855	0.10	0.001	**0.01**
WF- YT	1.998	0.04	0.013	0.13
WF- BT	1.991	0.04	0.024	0.24
WF- CS	3.596	0.08	0.001	**0.01**
YT- BT	1.739	0.04	0.043	0.43
YT- CS	3.406	0.08	0.001	**0.01**
BT- CS	3.518	0.10	0.001	**0.01**

BT - Brazilian teal, YT - yellow-billed teal, RT - ringed teal, CS - coscoroba swan and WF - white-faced whistling-duck.

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Table S6. Diaspores dispersed among the five waterfowl species according to their length (mm), considering the Total values, the percentage (%) and the number of taxa (Taxa) in which the values are distributed per length class.

		Brazilian teal			**Coscoroba swan**			**Ringed teal**			**White-faced whistling-duck**			**Yellow-billed teal**		
Length	n	Total	%	Taxa	Total	%	Taxa	Total	%	Taxa	Total	%	Taxa	Total	%	Taxa
≤0.5	4	0	0	0	1	1.0	1	0	0	0	1	0.6	1	4	0.5	1
0.51-1.0	11	54	57	6	5	5.1	3	344	39.0	6	80	44.4	7	216	26.6	7
1.1-1.5	11	23	24	6	84	85.7	5	529	60.0	6	73	40.6	5	536	66.0	7
1.51-2	9	18	19	6	0	0	0	1	0.1	1	23	12.8	1	50	6.2	3
2.1-2.5	3	0	0	0	5	5.1	2	5	0.6	1	2	1.1	2	6	0.7	2
> 2.5	2	0	0	0	3	3.1	1	2	0.2	1	1	0.6	1	0	0	0

Suplementary Referencial: Literature used to identify seeds and other diaspores found in waterfowl droppings.

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3. CAPÍTULO 3: Whole angiosperms *Wolffia columbiana* disperse by gut passage through wildfowl in South America.

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3.1. Abstract

For the first time, we demonstrate that whole angiosperm individuals can survive gut passage through birds, and that this occurs in the field. Floating plants of the genus *Wolffia* are the smallest of all flowering plants. Fresh droppings of white-faced whistling duck *Dendrocygna viduata* (n = 49) and coscoroba swan *Coscoroba coscoroba* (n = 22) were collected from Brazilian wetlands. Intact *Wolffia columbiana* were recovered from 16% of *D. viduata* and 32% of Coscoroba samples (total = 164 plantlets). Viability of plants was tested, and asexual reproduction was confirmed. *Wolffia columbiana* is an expanding alien in Europe. Avian endozoochory of asexual angiosperm propagules may be an important, overlooked dispersal means for aquatic plants, and may contribute to the invasive character of alien species.

3.2. Keywords: Anatidae, avian vectors, duckweed, endozoochory, plant dispersal, vegetative propagule

3.3. Introduction

The dispersal of viable plant units is recognized as a vital ecosystem service provided by birds, but the great majority of literature focuses on dispersal of seeds by frugivorous birds [1]. It is widely assumed that only plants with a fleshy fruit are adapted for endozoochory, i.e. dispersal through the gut passage of animals [2]. However, studies of waterbirds as plant vectors bring into question these assumptions, and show that endozoochory by non-frugivorous birds is important. Wildfowl (Anseriformes: ducks, geese, swans and screamers) disperse seeds of many angiosperms lacking a fleshy fruit [3], are excellent vectors for long-distance dispersal [4] and have been recently shown to disperse viable moss fragments and fern spores in their guts [5,6]. Here we demonstrate they can disperse entire angiosperms by endozoochory.

The floating, rootless plants of the genus *Wolffia* (Araceae, Lemnoideae) are the world's smallest flowering plants [7]. Like their relatives the duckweeds *Lemna*, they are widely assumed to disperse via waterbirds, but by epizoochory (i.e. attaching on the outside). Darwin [8] observed that when a duck emerges from a pond, whole *Lemna* plantlets can adhere to their feathers, and there is experimental evidence to support this [9]. Even before Darwin [8], Weddell [10] described *Wolffia brasiliensis* from plants he found on the feathers of Brazilian screamer *Anhima cornuta. Wolffia columbiana* has a similar native range to *W. brasiliensis* in freshwater wetlands across temperate and tropical regions from Argentina to Canada [7]. It is also alien and spreading in Europe, where it threatens native *W. arrhiza* [11].

Waterbirds can disperse plants to new habitats they cannot reach by other means [4]. The distributions of *Wolffia* in their native and introduced ranges [11,12] indicates they are effective dispersers, even though epizoochory events may be rare and constrained by desiccation of plants on plumage [13]. It has become clear that endozoochory is more frequent than epizoochory for seed dispersal by waterbirds [4,14]. Wildfowl actively feed on Lemnoideae and disperse viable *Lemna* seeds by endozoochory [4,6,15], although asexual *Lemna* somehow also disperse readily [9]. This raises the question whether endozoochory of vegetative propagules, such as *Wolffia* or *Lemna* plantlets, occurs in nature.

3.4. Material and methods

Fresh droppings of *Dendrocygna viduata* (n = 49) and *Coscoroba coscoroba* (n = 22) were collected between August 2017 and July 2018 (Appendix S1) in five temporary wetlands of Santa Vitória do Palmar, in southern Brazil (Figure 1a; Appendix S2 and S3). These wetlands are situated amongst ricefields and cattle-grazed grasslands. They contain numerous species of emergent and floating aquatic plants [19,20]. *Wolffia columbiana* is common and widespread in permanent and temporary ponds, lakes and water courses. Droppings were collected from grass close to the shoreline, were not in contact with soil or water and were immediately inspected for contamination. We observed *D. viduata* and *C. coscoroba* resting close to the droppings, and given their numbers, each sample is likely to be from a different individual. *Coscoroba* droppings had a distinctive colour, size and texture different from any other waterbird in the area. *Dendrocygna* droppings were collected from monospecific groups.

Samples were stored in separate tubes. In the laboratory, 34 *Dendrocygna* droppings and all *Coscoroba* droppings were frozen until inspection. Fifteen droppings of *D. viduata* were kept at 4°C in the fridge until a viability experiment. All samples were carefully examined under a stereomicroscope, initially to confirm the absence of any plant propagules adhered to the exterior. Frozen faeces were then defrosted in water and examined under the stereomicroscope to separate whole plants. The 15 unfrozen droppings were processed similarly later (just before the viability experiment). Only intact *Wolffia* plants resembling live plants (i.e. with a bright green colour and integral structure – Figure 1b) were counted and removed from the samples. Fragments were also observed in some samples, but were not quantified.

Intact *W. columbiana* plants removed from three fresh *D. viduata* droppings were counted and placed in five Petri dishes. The dropping with more plants (A) was separated into three dishes (A1, A2, A3) to facilitate the counting of new plants produced by asexual reproduction. Plants from the other two droppings (B, C) were placed in separate dishes. All dishes were filled with filtered water from the wetland where droppings were collected. The dishes were placed in a growth chamber (12h dark at 16°C ± 2°C, 12h light at 26°C ± 2°C). The number of living and dead individuals were counted after 7 and 14 days. An increase in the number of plants was considered to demonstrate asexual reproduction, confirming viability.

3.5. Results

A total of 164 intact *W. columbiana* were observed in faecal samples (Figure 1, Appendix S1). Whole plants were observed in seven *Coscoroba* droppings (86 plants, frequency of occurrence = 31.8%, 4–24 plants per sample), and eight *D. viduata* droppings (78 plants, frequency = 16.3%, 1–31 plants per sample).

Intact *W. columbiana* were removed from three of 15 unfrozen *D. viduata* samples, and placed in Petri dishes. After 14 days, we detected vegetative reproduction in four of five dishes (with plants from two droppings). The number of living plantlets increased by 89% (Figure 1, Table 1), with variation among droppings (A=93.3%, B=150%, C=0%).

3.6. Discussion

Our study provides field evidence that vegetative angiosperm propagules can be dispersed by avian endozoochory. Whole *Wolffia* plants were dispersed over an unknown distance between aquatic feeding sites and terrestrial loafing sites. Previously. asexual angiosperm propagules have only been reported from external parts of waterbirds [4,8,9]. Zoochory of asexual propagules allows dispersal outside the period of seed production and availability, e.g. facilitating the colonization of temporary wetlands after heavy rainfall.

Dendrocygna viduata is widespread in Central and South America with an estimated population of one million [17]. Individuals fly an average of up to four kilometres daily between different wetlands [18], making it an ideal plant vector. *Coscoroba coscoroba* is restricted to southern South America with a population of 10,000-25,000 [17]. It is partially migrant with movements of up to 1700 km [19]. Hence, endozoochory of *W. columbiana* by *D. viduata* may be a more frequent process, although *C. coscoroba* may be important for long-distance dispersal.

The high abundance and frequency of intact *W. columbiana* in faeces, and their high viability, suggest this floating plant has a high capacity to survive gut passage. Endozoochory may to be more important for *W. columbiana* than epizoochory. The average retention time for wildfowl faeces is several hours [4], suggesting that wildfowl regularly disperse *Wolffia* over several km during their daily movements [18]. Despite anecdotal support for epizoochory, it is unclear that floating plants both remain attached to birds and resist desiccation during extended flights. There is no risk of desiccation during endozoochory, which may provide a longer maximum retention time.

The particularly small size and simple morphology of *Wolffia* may promote endozoochory. Seeds with a smaller size and round shape are more likely to survive gut passage [4]. More research is needed to establish which angiosperm taxa can survive gut passage as whole plants or as viable fragments (as recently shown for bryophytes [5]). Experimental evidence suggests fragments of the invasive amphibious *Crassula helmsii* may disperse inside wildfowl guts [20]. Given that production of asexual vegetative propagules and an ability to grow from fragments is widespread in plants [21], dispersal of such vegetative propagules (e.g. fragments of grasses or pondweeds, or whole floating plants) by endozoochory may be an important and overlooked process. Clonality is more common in plants that establish readily outside of their native range [21], and the ability to disperse as vegetative propagules by endozoochory may increase their invasiveness. Greater resistance to desiccation has been suggested as key to effective dispersal of plantlets or stem fragments on the outside of animals or on boats or fishing gear [13]. Perhaps the invasive character of some species (e.g. *W. columbiana* or *Lemna minuta*) is more related to greater resistance to gut passage.

3.7. Ethics. This work was authorized by the Brazilian agency SISBIO (n°59225-1).

3.8. Data accessibility. Datasets supporting this article have been uploaded as supplementary material.

3.9. Authors' contribution. G.G.S, A.J.G, V.W, P.H and A.L.K designed this study. G.G.S, and A.J.G led the writing and the others authors contributed equally with the text. G.G.S, V.W and P.H collected and analysed the data. C.S and L.M helped in all steps and acted in coordination. All authors read and approved the final manuscript and agree to be accountable for all the content here presented.

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3.14. Figure and table legends

Table 1. Asexual reproduction of *W. columbiana* recovered from three *D. viduata* droppings, showing changes in the cumulative number of live and dead plants after 7 and 14 days.

	Initial	**Day 7**				**Day 14**			
Sample ID	**Apparently Alive**	**New plants**	**Dead plants**	**Alive plants**	**Total**	**New Plants**	**Dead Plants**	**Alive plants**	**Total**
A1	5	5	-	10	10	10	1	14	15
A2	5	7	3	9	12	8	3	10	13
A3	5	-	3	2	5	3	3	5	8
B	2	2	1	3	4	5	2	5	7
C	1	-	1	-	1	-	1	-	1
Total	18	14	8	24	32	26	10	34	44

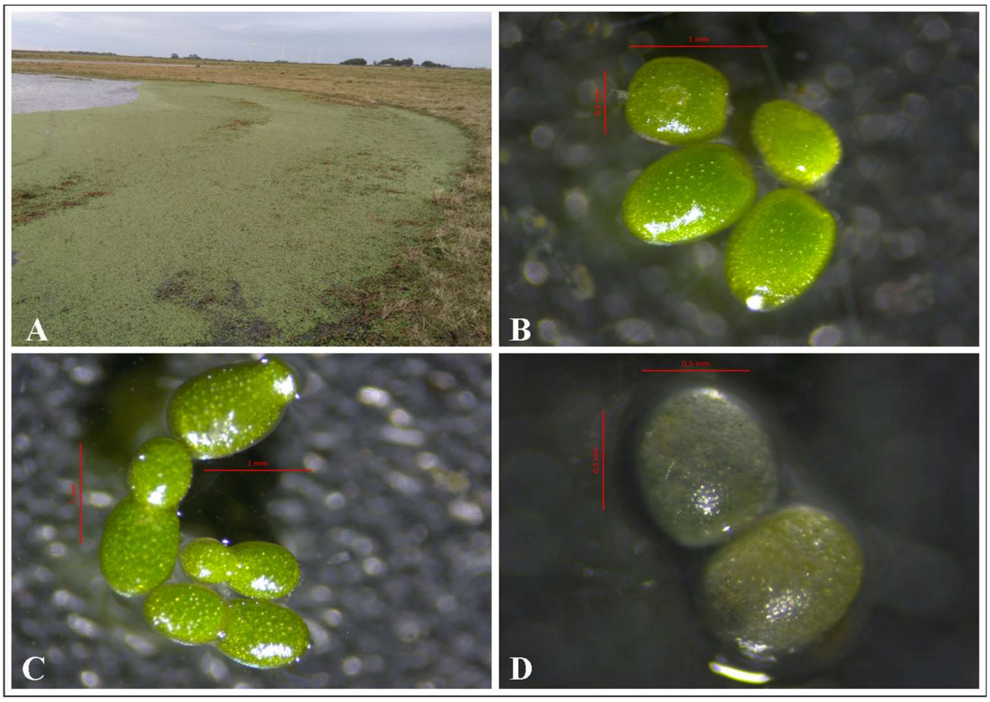

Figure 1. (A) Wetland where faeces of *D. viduata* with *W. columbiana* were collected. B) Intact plantlets obtained from faeces, showing a healthy appearance (bright green colour and integral structure). C) Seven plantlets observed after 7 experimental days, confirming asexual reproduction. D) Plants that died during the experiment lost their colour.

3.15. Supplementary Appendix

Whole angiosperms *Wolffia columbiana* disperse by gut passage through wildfowl in South America.

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Appendix S1

Date, total number of faeces collected, number of samples with *Wolffia columbiana* for each waterbird species from five wetlands, and the number of intact *Wolffia* recovered. The wetland numbers correspond to the locations given in appendix S2 and S3.

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Species	**Number of samples**	**Samples with intact *Wolffia***	**Number of intact *Wolffia***	**Wetland**	**Sampling date**
Dendrocygna viduata	6			2	21/10/2017
Dendrocygna viduata	10			1	18/11/2017
Dendrocygna viduata	7			3	10/02/2018
Dendrocygna viduata	11	5	60	4	25/04/2018
Dendrocygna viduata	15*	3	18	5	27/07/2018
Coscoroba coscoroba	2			2	24/08/2017
Coscoroba coscoroba	13			3	18/11/2017
Coscoroba coscoroba	7	7	86	4	25/04/2018

* These were the samples used for the viability experiment

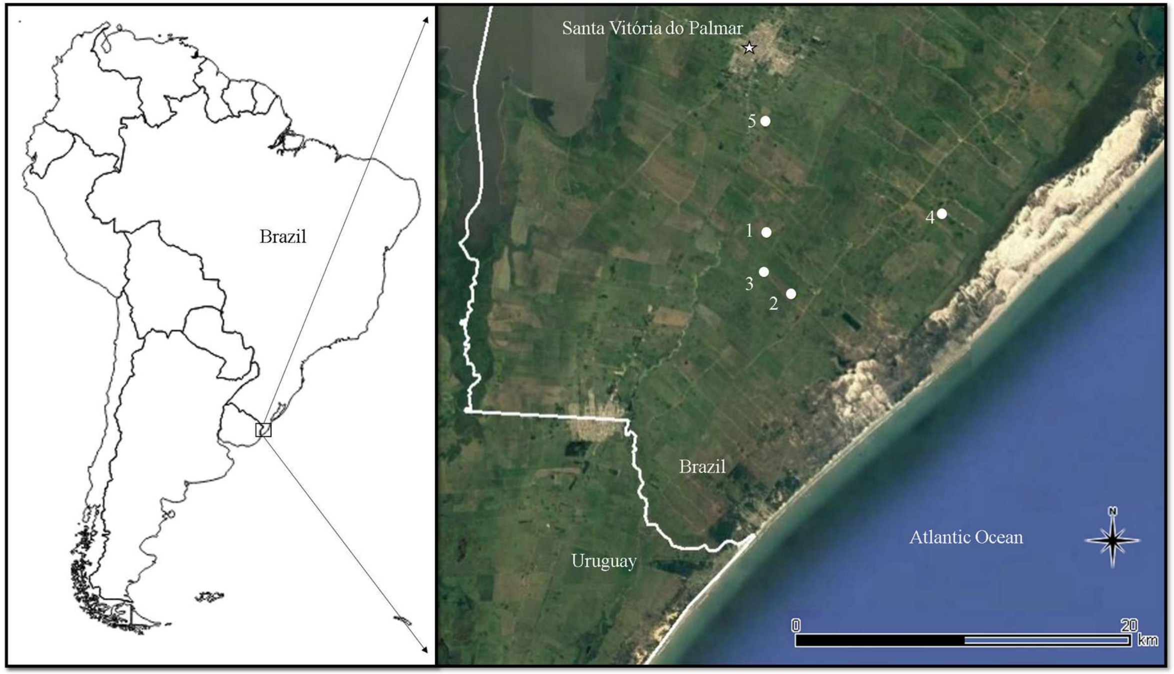
Appendix S2

Coordinates of wetlands where fresh faeces of *C. coscoroba* and *D. viduata* were collected in southern Brazil between August 2017 and June 2018.

Wetland	**Lat°**	**Long°**
1	33°36'33.20"S	53°21'40.00" W
2	33°38'13.10"S	53°20'49.52" W
3	33°37'37.95"S	53°21'47.43" W
4	33°35'57.50"S	53°15'38.67" W
5	33°33'25.45"S	53°21'36.82"W

Appendix S3

Map with location of the study area, in Santa Vitória do Palmar municipality, in the Coastal Plain of southern Brazil. Dots indicate the five wetlands where faeces of *C. coscoroba* and *D. viduata* were collected. Satellite image adapted from Google Earth.



4. CAPÍTULO 4: Endozoochory of aquatic invertebrates in neotropical region by a variety of waterbird species

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4.1. Abstract: Endozoochory promoted by waterbirds is particularly relevant to the dispersal of non-flying aquatic invertebrates. In this study, we identified propagules of invertebrates in faeces of 14 syntopic South American waterbirds, and hatched additional invertebrates from remaining faeces. We tested whether the abundance, species richness and invertebrate composition varied among bird species, and between the cold and warm seasons. We found 172 invertebrate propagules in faecal samples from seven different waterbirds species, including eggs of the Temnocephalida and Notonectidae, statoblasts of *Plumatella* sp. ephippia of Cladocera and 64 eggs from five other unidentified morphotypes). Ciliates (*Paramecium* sp., Litostomatea and non-identified), Nematodes and Rotifers (*Adineta* sp. and Nottomatidae) hatched from samples. Our models suggest that richness and abundance are associated to bird species and not affected by the seasonality. The dispersal by endozoochory is important to a broad variety of invertebrates, being promoted by bird with different ecological and anatomic characteristics, and these factors likely drives the dispersal of invertebrates in the neotropical wetlands.

4.2. Key-words: Endozoochory; waterbird; aquatic invertebrates; propagules; neotropical region.

4.3. Introduction

The wide geographic distribution of some non-flying freshwater invertebrate species is an intriguing ecological issue. Passive dispersal through vectors such as wind, water and animals are keys to understanding the distribution of species with low locomotion capacity (Bohonak & Jenkins, 2003; Green & Figuerola, 2005; Vanschoenwinkel et al., 2011). Zoochory, when an animal is the dispersal vector, is considered the most important way of passive dispersal through long-distances (Frisch et al., 2007; Brochet et al., 2010; Okamura et al. 2019). Endozoochory, when the dispersal occurs internally, inside of the digestive tract of the animal, is fundamental to the dispersal of many aquatic invertebrates between isolated wetlands (Figuerola & Green, 2002; Bohonak & Jenkins, 2003; Vanschoenwinkel et al., 2011).

Waterbirds are particularly relevant to the dispersal of aquatic organisms due to their abundance, geographical range and high locomotion capacity (Brochet et al., 2010; Green et al., 2016, Silva et al. 2020). Darwin (1859) made observations regarding the potential of waterbirds to carry invertebrates such as snails on their feet or feathers (ecto or epizoochory). Brown (1933) demonstrated the viability of the endozoochory in invertebrates, showing that bryozoan statoblasts remains viable after going through the digestive tract of mallard (*Anas platyrhynchos*). Since then, endozoochory was confirmed for a wide spectrum of invertebrates, including crustaceans (notably branchiopoda and ostracoda), rotifers, nematodes, dipteran larvae, beetle eggs, leech eggs, and snails (Proctor, 1964; Malone, 1965a, 1965b; Green & Figuerola, 2005, Brochet et al. 2010; Laux and Kolsch 2014, Rogers, 2014; Simonová et al. 2016, Lovas-Kiss et al. 2018, Moreno et al. 2019).

In this study, we identified and removed propagules of aquatic invertebrates in faeces of 14 syntopic South American waterbirds, and hatched additional invertebrates from remaining faeces. We tested whether the abundance, species richness and invertebrate composition varied among bird species, and between the cold and warm seasons. We expected important variation among bird species (ranging from ducks to herons and ibis), due to their morphological differences and foraging ecology. We also expected seasonal changes in dispersal reflecting differences in the availability of invertebrate propagules commonly found in the wetlands of the region (Stenert et al., 2008), as also recorded for plant propagules (Silva et al. 2020).

4.4. Material and methods

Sampled area

This study was conducted in southern Brazil (Figure 1), considered one of the most important regions for waterbird conservation in South America (Belton, 1994; Silva et al., 2020). We collected 208 faecal samples of 14 waterbird species in five different wetland sites separated from each other by 100-600 km (Figure 1, Table 1). From August 2017 to December 2019, eight collecting events were made, four in the cold and four in the warm period (Supplementary material). We grouped samples collected during the austral autumn and winter as the cold period (April to September), and the austral spring and summer (October to March) as the warm period. The warm period (spring and summer) is the main breeding season in the study region, except for some occasional breeding during the cold period. All bird species studied can nest in the early warm period, and raise their offspring before the end of the warm period (Calabuig et al., 2010; Dias & Fontana, 2002; Mauricio et al., 2013; Silva et al., 2020). Except for coscoroba swan (*Coscoroba coscoroba*) population, many of which migrate from this region to Argentina in the end of the warm period (Antas, 1994; Calabuig et al., 2010), these waterbird species studied stay in the region year-round, although their frequency and abundance vary according to the hydric regime.

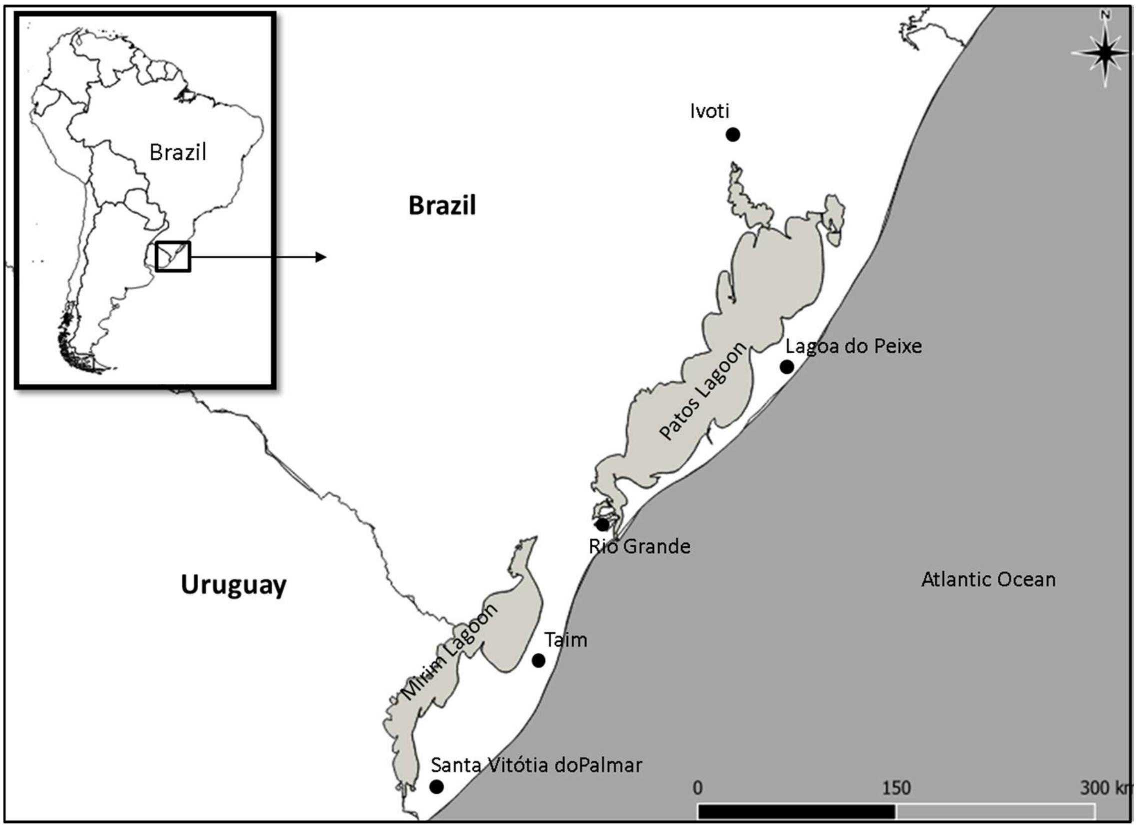

Figure 1 Study area in the coastal plain of southern Brazil with the general localization of the five sampling sites (black dots). Lagoa do Peixe, Taim and Santa Vitória do Palmar were shallower freshwater wetlands. In Ivoti the site was a dormitory of black-crowned night-heron (*Nycticorax nycticorax*), while in Rio Grande it included dormitories of roseate spoon-bill (*Platalea ajaja*) and herons (*Egretta* spp).

Sampling procedures

We obtained 209 faecal samples from two distinct methods (Table S1). Firstly, we located monospecific waterbird groups resting or feeding around lake edges and collected fresh droppings over the grass. Through this method, we collected samples from the six Anatidae (Brazilian teal *Amazonetta brasiliensis*, yellow-billed teal *Anas flavirostris,* silver teal *Anas versicolor*, ringed teal *Callonetta leucophrys*, coscoroba swan *Coscoroba coscoroba* and white-faced whistling-duck *Dendrocygna viduata*) and also southern screamer (*Chauna torquata*), red-gartered coot (*Fulica armillata*), limpkin (*Aramus guarauna*), buff-necked ibis and plumbeous ibis (*Theristicus caudatus* and *T. caerulescens*). Secondly, we collected samples of black-crowned night-heron (*Nycticorax nycticorax*), roseate spoon-bill (*Platalea ajaja*) and *Egretta* spp. (indistinguishable faeces of a mixed group of little blue heron *Egretta caerulea* and snowy egret *E. thula*). After identifying roosts of these species, we used tweezers to scrape faeces from branches, avoiding any contact with soil. In both methods samples were collected with a minimum distance of one meter apart to prevent resampling of the same individual. The samples were stored individually in plastic tubes and frozen (- 4 °C) to avoid fungal infestation prior to arrival at the laboratory.

Laboratory procedures

The samples were defrosted, weighed and washed in tap water using a sieve (53 μm) in the laboratory at UNISINOS University. We used a Bogorov chamber in a stereomicroscope (10x to 1.6x - 5 x of total magnification) to separate the visible propagules from other materials. We only considered intact propagules, discarding broken ones. The eggs and statoblasts found were allocated in plastic tubes with 10 ml of deionised water (pH 7.5) and maintained for 21 days in a controlled chamber (12 h dark at 16ºC + 2ºC, 12 h light at 26ºC + 2ºC). The remaining organic material of each sample were separately hydrated with 100 ml of deionised water and placed in a air- tight plastic box to later observe hatching of invertebrates whose propagules were not detected during the above separation process (mainly because they were very small). Then, these boxes were also maintained for 21 days in the above controlled chamber. Both propagules and pots with organic material were inspected each three days in order to analyse eventual hatchings. When invertebrates were found, they were inspected under the microscope (10x to 10x - 100x of total magnification). Invertebrates were identified according to specimens from the LECEA collection.

Data analyses

We analyzed effects of bird species and sample (faecal) weight on the total richness and abundance of invertebrates using Generalized Linear Mixed Models (GLMMs) with the sampled area as a random factor of four levels. We ran a separate GLMM test to analyze the influence of the season using only samples of five Anatidae species (excluding silver teal), since the number of samples and the adequate temporal repetition was only possible for these five species. Model parameters were estimated by maximum likelihood (Laplace approximation). All models fitted best with a negative binomial error distribution, which showed less overdispersion than a Poisson error distribution. We tested for main effects and interactions. We compared these effects against null models (intercept only) and performed model selection using the Akaike information criterion (AIC) (Burnham & Anderson, 2002), retaining only models with delta AIC < 2.0 for further inference. We fitted all the GLMMs in the R statistical environment v. 3.6.1 using the function *glmer.nb* of the package *lme4* (R Development Core Team, 2019).

We used data of the propagules found in faeces in order to assess invertebrate composition variation among bird species and seasons through Principal Coordinates Analysis (PCoA) and Permutational Multivariate Analysis of Variance (PERMANOVA) using the Bray-Curtis distance matrix and 9999 permutations to validate the model significance of PERMANOVA. For tests of seasonal effects, we used only data from Anatidae, excluding Brazilian teal (no propagules recorded) and silver teal (no temporal replicates). Finally, we calculated the frequency of occurrence of hatchings of the samples with remaining organic material. We did not combine the hatchlings and propagules in a shared analysis because of their different nature. No propagules hatched, but they were countable (unlike hatchlings).

4.5. Results

Invertebrate propagules found in the faecal samples

We found 164 invertebrate propagules in faecal samples from seven different waterbird taxa (Table 1). Eggs of the Platyhelminthes Temnocephalida (n=62) were the most abundant, following by the non-identified morphotype I (n = 58). We also found 32 Notonectidae eggs (Insecta), eight statoblasts of *Plumatella* sp. (Bryozoa), one ephippia of Cladocera and three eggs from four other unidentified morphotypes. Invertebrate propagules were recorded in 16.7% of the faecal samples, with a higher frequency in droppings of coscoroba swan (81.5%), following by yellow-billed teal (23.5%). None of these propagules hatched, probably because they had all been frozen.

Non-identified ciliates were the most frequent taxa hatched, found in samples of all bird species except silver teal and roseate spoonbill (Table 2). We also found *Paramecium* sp. and Litostomatea ciliates. Nematodes hatched from samples of Brazilian teal and buff-necked ibis and Rotifera (*Adineta* sp. and Nottomatidae) emerged from samples of four waterbird species. No invertebrates (whether propagules or hatchlings) were found in the three samples of limpkin or in the four samples of roseate spoonbill.

Table 1. Invertebrates propagules found in samples of waterbird. n = Total of samples. A= Abundance of eggs found in the samples. The Frequency of Occurrence (%) of the propagules in faeces are shown as the sum of all propagules (T.F.O) and individually by invertebrate taxa (F.O)

			YT		RT		SS		CS		WF		ES		BI	
			n = 17		n = 29		n = 18		n = 27		n = 34		n = 6		n = 14	
			T.F.O (23.5)		T.F.O (6.9)		T.F.O (16.7)		T.F.O (81.5)		T.F.O (20.6)		T.F.O (16.7)		T.F.O (14.3)	
			A	F.O	A	F.O	A	F.O	A	F.O	A	F.O	A	F.O	A	F.O
Cladocera																
	Daphnia sp.	Ephippia							1	(3.7)						
Insecta																
	Notonectidae	Egg	9	(23.5)			6	(16.7)	10	(11.1)	5	(14.7)			2	(7.1)
Bryozoa																
	Plumatella sp.	Statoblast			2	(6.9)			2	(7.4)	2	(5.9)	1	(16.7)	1	(7.1)
Platyhelminthes																
	Temnocephala	Egg							62	(29.6)						
Non identifyed																
	Morphotype 1								58	(3.7)						
	Morphotype 2	Egg							1	(3.7)						
	Morphotype 3	Egg							1	(3.7)						
	Morphotype 4	Egg							1	(3.7)						

Bird species are represented by initials: CS = coscoroba swan, RT = ringed teal, WF = white-faced whistling-duck, YT= yellow-billed teal, SS = southern screamer, ES = *Egretta* spp, and BI = buff-necked ibis.

Table 2. Frequency of invertebrate hatchings from organic material remaining from waterbird faeces in Southern Brazil, after sieving and after intact propagules seen under a binocular microscope had been removed.

Taxa	BT	YT	ST	RT	SS	CS	WF	ES	RC	BH	PI	BI
	n = 28	n = 17	n = 2	n = 29	n = 18	n = 27	n = 34	n = 6	n = 7	n = 13	n = 2	n = 14
Ciliophora												
Paramecium sp.	0.0	0.0	50.0	3.4	0.0	3.7	2.9	33.3	0.0	0.0	0.0	14.3
Litostomatea	0.0	11.8	0.0	3.4	0.0	3.7	0.0	0.0	0.0	0.0	0.0	7.1
Non-identified	17.9	41.2	0.0	17.2	27.7	14.8	23.5	33.3	71.4	30.8	50.0	35.7
Nematoda												
Non-identified	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	21.4
Rotifera												
Adineta sp.	3.6	0.0	0.0	3.4	0.0	7.4	0.0	0.0	0.0	0.0	0.0	14.3
Nottomatidae	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Bird species are represented by initials: BT = Brazilian teal, ST = Silver teal, CS = coscoroba swan, RT = ringed teal, WF = white-faced whistling-duck, YT= yellow-billed teal, SS = southern screamer, ES = *Egretta* spp, RC = red-gartered coot, BH = black-crowned night-heron, PI = plumbeous ibis and BI = buff-necked ibis.

Effects of bird species, sample weight and seasonality on invertebrate taxon richness and abundance

The best fitted models considering bird species and sample weight (including all waterbird species) showed that invertebrate richness varied according to bird species but was not affected by the sample weight ($P<0.05$) (Table 3). Similarly, the best fitted models for invertebrate abundance included bird species and sample weight, but only bird species showed a significant effect (Table 3). For abundance, a second model that only included the factor bird species also showed ΔAIC < 2 (Table 3).

Table 3. Summary of the best fitted models explaining invertebrate Richness and Abundance found in faeces of waterbirds in southern Brazil. Both variables were affected by the bird species factor and not by sample weight. Only models with ΔAIC < 2 were represented in the table. The more complex models tested were Variable (Richness or Abundance)~Bird + Weight + (1 | Wetland).

Variable	**Model**	**ΔAIC**	**Weight (AIC)**	**Factor**	***df***	**AIC**	**LR Chisq**	***P*-value**
Richness								
	~Birds	0.0	0.49					
				Bird	13	218.13	45.671	<0.001
Abundance								
	~Birds + Weight	0.0	0.29					
				Bird	13	320.80	54.963	<0.001
				Weight	1	292.95	3.116	0.077
	~Birds	0.7	0.21					
				Bird	13	364.1	97.599	<0.001

In the model selection to analyse the seasonality effect, considering only Anatidae species, we found three models with ΔAIC < 2 but no factor had a significant effect ($P>0.05$). The simplest model with lower ΔAIC < 2 considered the factor Bird species, sample Weight and the interaction between this factor (Table 4)

Table 4. Summary of the best fitted models explaining invertebrate Richness and Abundance found in faeces of waterbirds in southern Brazil using only Anatidae species with adequate temporal repetition in order to analyse the effect of the seasonality. For abundance, only the factor sample weight was relevant in the selected model, while in the model for richness, no factor was had a significant partial effect. The more complex models tested were Variable (Richness or Abundance)~Bird + Season + Weight + Bird*Season + (1 | Wetland).

Variable	**Model**	**ΔAIC**	**Weight (AIC)**	**Factor**	***df***	**AIC**	**LR Chisq**	***P*-value**
Richness								
	~ Bird+Season +Weight +Bird *Season	0.0	0.24					
				Bird	13	143.87	6.262	0.184
Abundance								
	~ Bird+Season +Weight +Bird *Season	0.0	0.46					
				Weight	1	229.21	5.792	0.016
				Bird * Season	4	225.24	7.816	0.098

Variation in the taxonomic composition of invertebrate propagules among waterbird species and seasons

Invertebrate composition differed among waterbirds considering all species ($R^2=0.35$, F=2.396, P<0.05) (Figure 2). There were differences in invertebrate composition according to seasonality, considering only data from Anatidae species ($R^2=0.08$, F=2.911, P= 0.05) (Figure 3).

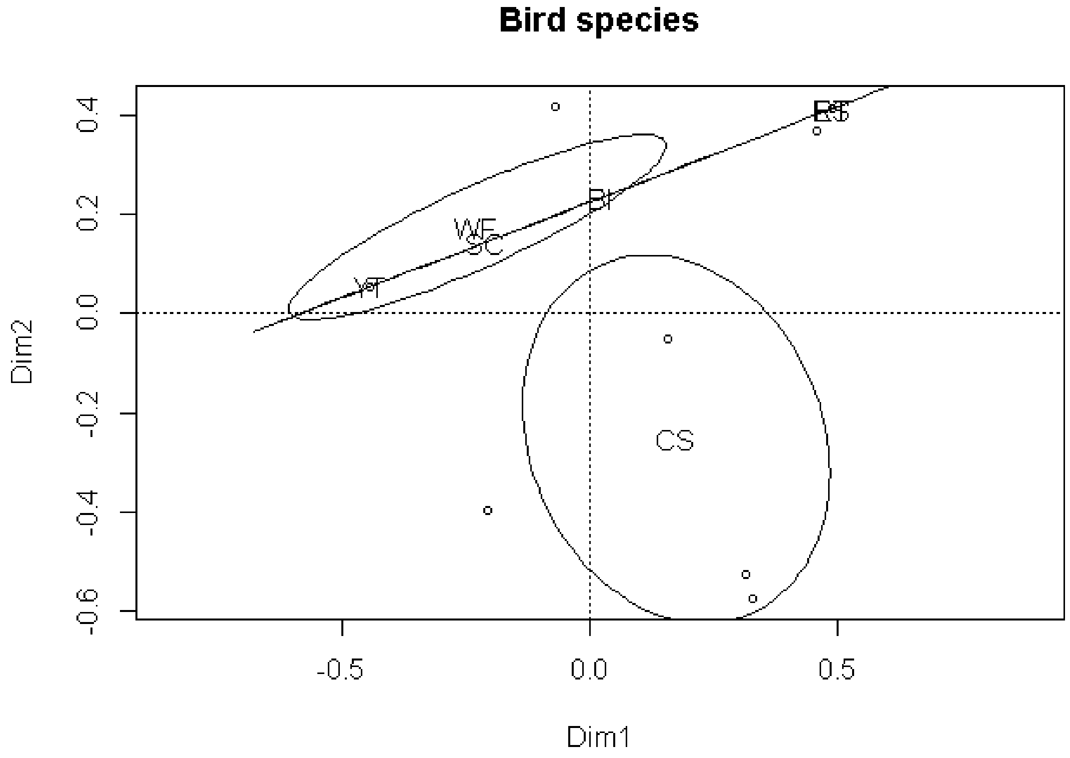

Figure 2. Ordination of taxonomic composition of macroinvertebrate propagules and how it varied among bird species. The combined amount of variation explained by the axes was 88% (Axis 1 = 51% and Axis 2 = 37). Bird species are represented by initials: CS = coscoroba swan, RT = ringed teal, WF = white-faced whistling-duck, YT= yellow-billed teal, SC = southern screamer, ES = *Egretta* spp, and BI = buff-necked ibis.

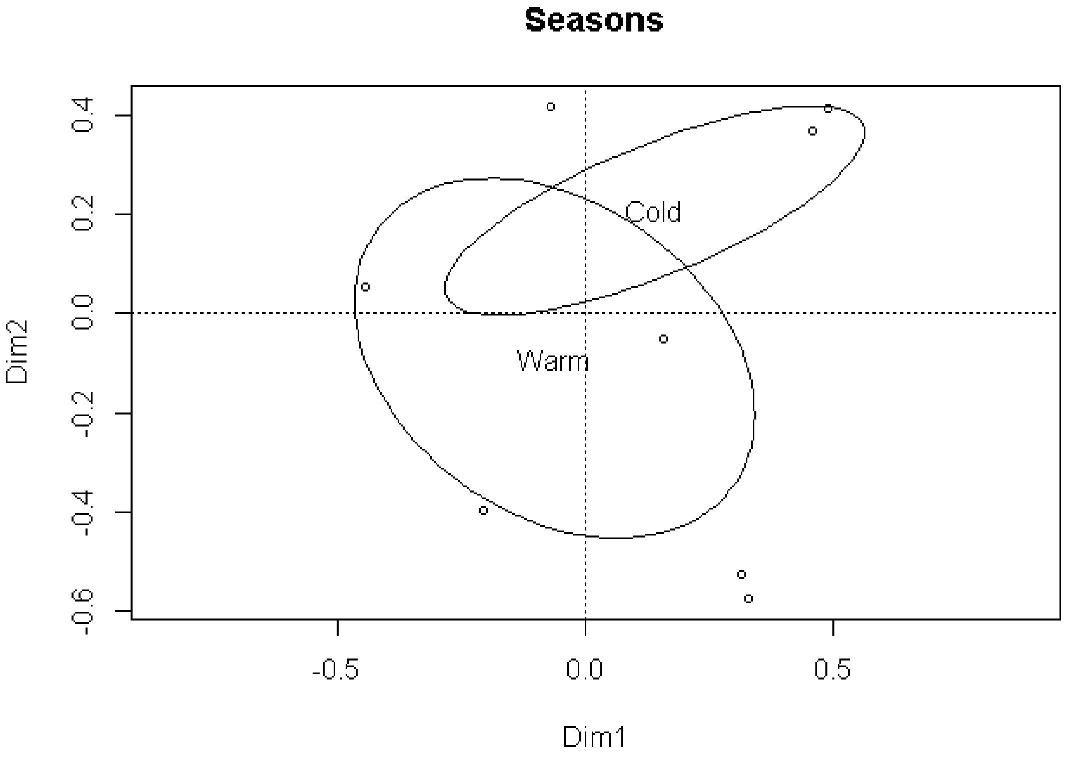

Figure 3. Ordination of taxonomic composition of invertebrate propagules and how it varied among Cold and Warm periods, considering only Anatidae species. The total of variation explained by the axes was 88% (Axis 1 = 51% and Axis 2 = 37).

4.6. Discussion

As far as we know, our work is the first to address the invertebrate dispersal through waterbird endozoochory in the neotropical region. We demonstrated ciliates, nematodes and rotifers emerging from faeces, and described the presence of nine different invertebrate propagules in droppings. Only limpkin and roseate spoonbill showed no propagules or hatching in their faeces. Dispersal of rotifers, nematodes and ciliates by waterbirds already was demonstrated (Frisch et al., 2007; Green et al., 2008). In our study we found ciliates dispersed by 12 from the 14 species analysed, As the samples of the hatch experiments were in closed plastic recipients that were opened only during the inspection in the stereomicroscope, we think it unlikely that the arrival of propagules by air could have contaminated the samples. We did not find references of waterbird endozoochoric dispersal of the ciliates *Paramecium* sp. and Litostomatea, and the rotifers *Adineta* sp. and Notommatidae, and our study is likely the first to find such evidence.

This is despite the fact that a variety of rotifers are dispersed by waterbirds in Europe (Conde-Porcuna et al. 2018, Moreno et al. 2019).

The most abundant propagule was eggs of the Platyhelminthes Temnocephalida, This taxa were found in faeces of coscoroba swan among uncountable fragments of snails (Hydrobiidae and Planorbidae). Temnocephalida species were recognized to be ectosymbionts of snails and others aquatic organisms (Amato & Amato, 2005; Seixas et al., 2010) and the presence of these eggs in the samples is likely due to accidental ingestion, when birds were feeding on snails. We did not find previous references of Temnocephalida eggs dispersed by endozoochory, thus the viability of these eggs needs to be appropriately tested to confirm that they can disperse by endozoochory.

Bryozoans have been shown to be dispersed by waterbirds in several continents, but ours are the first record of this zoochory from the neotropics (Okamura et al. 2019). *Plumatella* sp. statoblasts were found in faeces of coscoroba swan, ringed teal, white-faced whistling-duck, *Egretta* spp, and buff-necked ibis. Few studies have been conducted with neotropical bryozoans and a lot needs to be done to understand the ecology and distribution of neotropical species (Wood & Okamura, 2017; Wood & Liebbe, 2020). Considering that bryozoans statoblasts survive passage through waterbird digestive systems and that they can stay inside the vector for hours (Brown, 1933; Charalambidou et al., 2003), our findings indicates that bryozoans may be dispersed via endozoochory across the neotropical wetlands. Our findings that *Plumatella* sp. statoblasts can be transported inside the bodies of egrets and buff-necked ibis demonstrated that even species considered resident may be important to bryozoan dispersal, since the samples of these birds were collected in their roosts, located usually several kilometres from their feeding sites. *Plumatella* sp. statoblasts were previously recorded in faeces of glossy ibis (Macias et al. 2004).

The presence of Notonectidae (Heteroptera) eggs in samples of coscoroba swan, white-faced whistling-duck, yellow-billed teal, southern screamer and buff-necked ibis suggests that backswimmer species are potentially dispersed by endozoochory, but this requires further investigation. Similar Corixidae (Heteroptera) eggs have been recorded from Anatidae faeces in Europe (Figuerola et al. 2003). Carbonell et al., 2020 found that eggs of boatman (*Trichocorixa verticalis,* Corixidae) showed low resistance to chemical treatment and no resistance to scarification in simulated digestion, and were unable to hatch a number of intact eggs recovered from Eurasian coot (*Fulica atra*) droppings.

Given that we froze samples in the field, and our field sites are not subjected to low temperatures, it is no surprise that we failed to hatch any of the propagules we extracted in the laboratory.

The best fitted models showed invertebrate richness and abundance varying according to bird species and not affected by the sample weight or seasonality. The variation we recorded is likely associated to intrinsic characteristics of bird species not addressed in our study. For example, the foraging strategy and the body size may lead to differences in access to alimentary resources and habitat segregation, and result in differences in diaspores dispersal (Ntiamoa-Baidu et al., 1998; Pöysä, 1983; Green, 1998; Guillemain et al., 2002; Silva et al., 2020). The lack of seasonal effect described to abundance and richness, and the weak effect in the composition needs to be investigated. General seasonal patterns of invertebrate endozoochory are inconsistent in the literature. For example, Sanchez et al. (2007) found that rates of *Artemia* cysts were high in both spring and autumn, and almost inexistent in midwinter. In other hand, Brochet et al. (2010) reported a lack of seasonal pattern in endozoochory of branchiopods, ostracods and bryozoans.

4.7. Conclusion

We demonstrated that 12 of the 14 waterfowl species studied are important to a broad variety of invertebrates. The birds studied vary from resident to migratory and have different feeding strategies and anatomic characteristics, suggesting the endozoochory of invertebrates as important at different spatial scales, and that this process is promoted by a broad variety of waterbird species. The lack of regional studies on invertebrate and bird ecology limits our understanding of the magnitude of this dispersal, and studies addressing these themes can elucidate the patterns of endozoochory of invertebrates in the neotropical region

4.8. Acknowledgments

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4.10. Supplementary material

Table S1 Total of samples collected by waterbirds in cold (c) and warm (w) periods in five localities of the Coastal Plain of Southern Brazil. In the sample sites LP (Lagoa do Peixe), TA (Taim) and SV (Santa Vitória do Palmar) the collected occurred in shallow freshwaters. In RG (Rio Grande) and IV (Ivoti) faecal samples were collected in bushes of bird dormitories.

Family	**Waterbird**	**Samples**	**PLP**		**EET**		**SVP**		**RG**		**IVT**	
		Total	**c**	**w**	**c**	**w**	**c**	**w**	**c**	**w**	**c**	**w**
Anatidae	Brazilian teal	28					12	16				
	Coscoroba swan	27	5	3			7	12				
	Silver teal	3					3					
	Ringed teal	31	2	1			13	15				
	White-faced whistling-duck	35					11	24				
	Yellow-billed teal	18					7	11				
Anhimidae	Southern screamer	18			6	12						
Ardeidae	Black-crowned night-heron	13									1	12
	Egretta spp	6								6		
Aramidae	Limpkin	3					3					
Rallidae	Red-gartered coot	7										
Threskiornithidae	Buff-necked ibis	14	14									
	Plumbeous ibis	2					2					
	Roseate spoonbill	4								4		

5. CAPÍTULO 5: Killifish eggs can disperse via gut passage through waterfowl

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5.1. Main text

The presence of freshwater fish in remote lakes and pools has led to much speculation about how they arrived. It has often been suggested that fish egg dispersal is facilitated by waterbirds by attaching to their feet, feathers or bills (a form of ectozoochory). However, a recent thorough review failed to provide solid empirical evidence that such dispersal occurs (Hirsch et al. 2018). We discovered an alternative mode to waterbird-facilitated dispersal of fish eggs – inside the alimentary system of coscoroba swan (form of endozoochory), and experimentally demonstrated that killifish eggs from bird faeces were capable of continuing their development after spending over 30 h inside a swan (Fig. 1).

Killifish are cyprinodontiform fishes that specialize in marginal habitats, from isolated desert pools to shallow mangrove swamps, including temporary ponds. Some species of killifish independently evolved dry-resistant eggs that are adapted to survive in desiccated pool substrate (Furness et al. 2015). Dry-resistant eggs are crucial for persistence of fish populations in isolated habitats that desiccate annually. Unusually large flooding events are considered the main dispersal strategy of annual killifish across landscapes, though presence of remote populations in high altitude areas and population genetic patterns provide indirect evidence for unusual dispersal events (Bartáková et al. 2013), and suggest the existence of alternative dispersal modes.

While investigating endozoochory of plants and invertebrates in southern Brazil (Parna Lagoa do Peixe and Santa Vitória do Palma), we discovered one egg of an annual killifish (*Austrolebias* sp.) and six fish egg envelopes (chorions) in two of 27 coscoroba swan (*Coscoroba coscoroba*) (Fig. 1A) faecal samples collected in the field (Appendix S1: Table S1). The faeces were collected fresh from grass and were not in contact with soil or water. The egg was apparently intact and contained a well-developed embryo (Fig. 1B, Appendix S1: Fig. S1), but our samples were frozen prior to inspection so egg viability could not be tested. Hence, we experimentally tested whether killifish eggs can survive waterfowl (Anatidae) gut passage and if endozoochory can be a mode of fish egg dispersal.

We used 650 killifish eggs from captive breeding (Appendix S1) of two annual killifish species (350 *Austrolebias minuano,* Fig. 1C and 300 *Cynopoecilus fulgens,* Fig. 1D) that are not closely related but coexist in the Coastal Plain of Rio Grande do Sul, Brazil (31°17' S; 51° 5' W) where droppings with a fish egg and chorions were previously found. The experimental eggs were in diapause I (600 eggs) or diapause II (50 eggs of *A. minuano*) developmental stages (Podrabsky et al. 2017) that are the most frequent for eggs collected in the field (Reichard and Polačik 2019). We mixed the eggs into a standard 120 g ration of corn-based food provided to three captive coscoroba swans in the Parque Zoológico da Fundação Zoobotânica do Rio Grande do Sul (PZFZB) and allowed them to feed on it during a period of 4 hours (07:00–11:00 AM). The ration was then removed, but birds could still feed on aquatic macrophytes present in their enclosure. The following days, the swans were submitted to a normal feeding routine, identical to the experimental day except that no eggs were mixed into their morning ration. At six time intervals (0, 6, 20, 24, 30 and 48 h) after removal of the mixed ration, we collected a total of 55 droppings (mean ± standard deviation weight: 33.13 g ± 14.03 g) and analysed their content for presence of eggs (Appendix S1).

We recovered five viable killifish eggs in four droppings (Appendix S1: Table S2). Three eggs were of *A. minuano* (Fig. 1E-G) and two of *C. fulgens* (Fig. 1H-I). Two *A. minuano* eggs (in one dropping) were collected immediately after ration withdrawal (time: 0 h) and must have gone through the swan digestive system within 4 hours. A single egg of *C. fulgens* was collected after 20h. Two eggs, one of each species, were collected 48 hours after ration withdrawal and must have been in the digestive system for at least 30 hours. We also collected 15 chorions in nine droppings (Appendix S1: Table S2). The experimental procedures were authorized by the UNISINOS Ethic Committee (PPECEUA03.2018) and Brazilian Agency – ICMBio (Sisbio n° 63602-1) and supervised by a veterinarian and biologists from PZFZB.

After their removal from droppings, we observed embryonic development in three of the five eggs recovered, and one of these hatched. One egg (*Aust* 3, *A. minuano*) was recovered in Diapause II stage and developed to Diapause III in 35 days (Appendix S1: Fig. S2), then successfully hatched 49 days after removal from the dropping (Fig. 1J, Appendix S1: Video S1). The embryo hatched in 54 min after immersion in water. Two eggs (*Aust* 2 and *Cyno* 1) were recovered in Diapause I stage and developed beyond Diapause II (Appendix S1: Table S2) but later died from fungal infections. The egg *Aust* 2 died at ready-to-hatch post-diapause III stage following 63 days of development (Appendix S1: Fig. S3). The egg *Cyno*1 died at post-diapause II stage following 49 days of development (Appendix S1: Fig. S4). The other two eggs (*Aust* 1 and *Cyno* 2) died from fungal infection within the first week of incubation (Appendix S1: Table S3). Mortality of eggs from fungal infection is common in the laboratory and unlikely to be due to ingestion by waterfowl. We recovered 48 eggs of *A. minuano* and 65 of *C. fulgens* from the remains of the mixed ration that had not been ingested by waterfowl (5.629 g). The proportion of live eggs egested by birds corresponded to 0.93% of the ingested eggs.

Dispersal of other aquatic taxa that typically coexist with fish in small lakes is well described, with abundant evidence of avian endozoochory of specialized dispersal stages such as seeds, resting eggs of zooplankton and statoblasts of bryozoans (Green et al. 2016; Lovas-Kiss et al. 2018). However, propagules of other organisms lacking obvious adaptations for dispersal can also be spread by endozoochory, with reports including midge larvae, beetle eggs, leech egg cocoons, live plantlets and land snails (Green and Sanchez 2006, Laux and Kolsch 2014, Simonová et al. 2016, Silva et al. 2018). The low proportion of viable fish eggs we detected in experimental droppings is similar to survival rates described for other aquatic propagules ingested by waterfowl (Laux and Kolsch 2014). The annual killifish cycle coincides with peak waterfowl presence in the study region between July and October. Like other waterfowl, coscoroba swans move daily between different waterbodies separated by several km to feed and rest, as well as making much longer movements during seasonal migrations (Silva et al. 2018). The retention times recorded suggest killifish eggs could be dispersed over 2000 km during a non-stop flight (van Leeuwen et al. 2012). Coscoroba swans are typical omnivorous waterfowl that feed within wetland sediments, ingesting a range of items such as macrophytes, seeds and invertebrates.

Our study is the first to our knowledge that demonstrate that a vertebrate egg can survive passage through a digestive tract. The eggs of annual killifish, including our study species, are well adapted to harsh conditions (Reichard and Polačik 2019). The life cycle of annual killifish includes a period when the entire population resides in desiccated sediment of their natal pools as embryos protected in the eggs with a thick chorion (Podrabsky et al. 2017). At this stage, the embryos are protected in diapause II (Reichard and Polačik 2019) with their metabolic rates reduced by 90%, and are insensitive to a harsh external environment (Podrabsky and Hand 1999). In addition, the outer layer of the chorion has complex filaments (Podrabsky and Hand 1999) that may further increase egg protection. Further research should identify how the chorion protects killifish embryos from protease digestion and the acidic environment in the waterfowl stomach.

Dispersal of killifish eggs through gut passage can explain the presence of annual killifish in isolated ephemeral pools that are not subject to flooding, and re-establishment of killifish populations after pools remain dry over multiple years (Reichard 2015). We provide evidence of endozoochory in two annual killifish species, raising the possibility that other fish species might also disperse this way. The extent to which fish species disperse by extraordinary modes such as avian gut passage is expected to be low, but our work suggests it has been overlooked and requires focused research. Current evidence of ectozoochory is based on a single anecdotal report, where viable eggs of pike (*Esox lucius*) were found attached to the feet of mallards (*Anas platyrhynchos*) (Riehl 1991). Hence, as well as being important over evolutionary time scales, endozoochory may also be significant over ecological time scales, and explain surprising records of fish in isolated lakes and pools.

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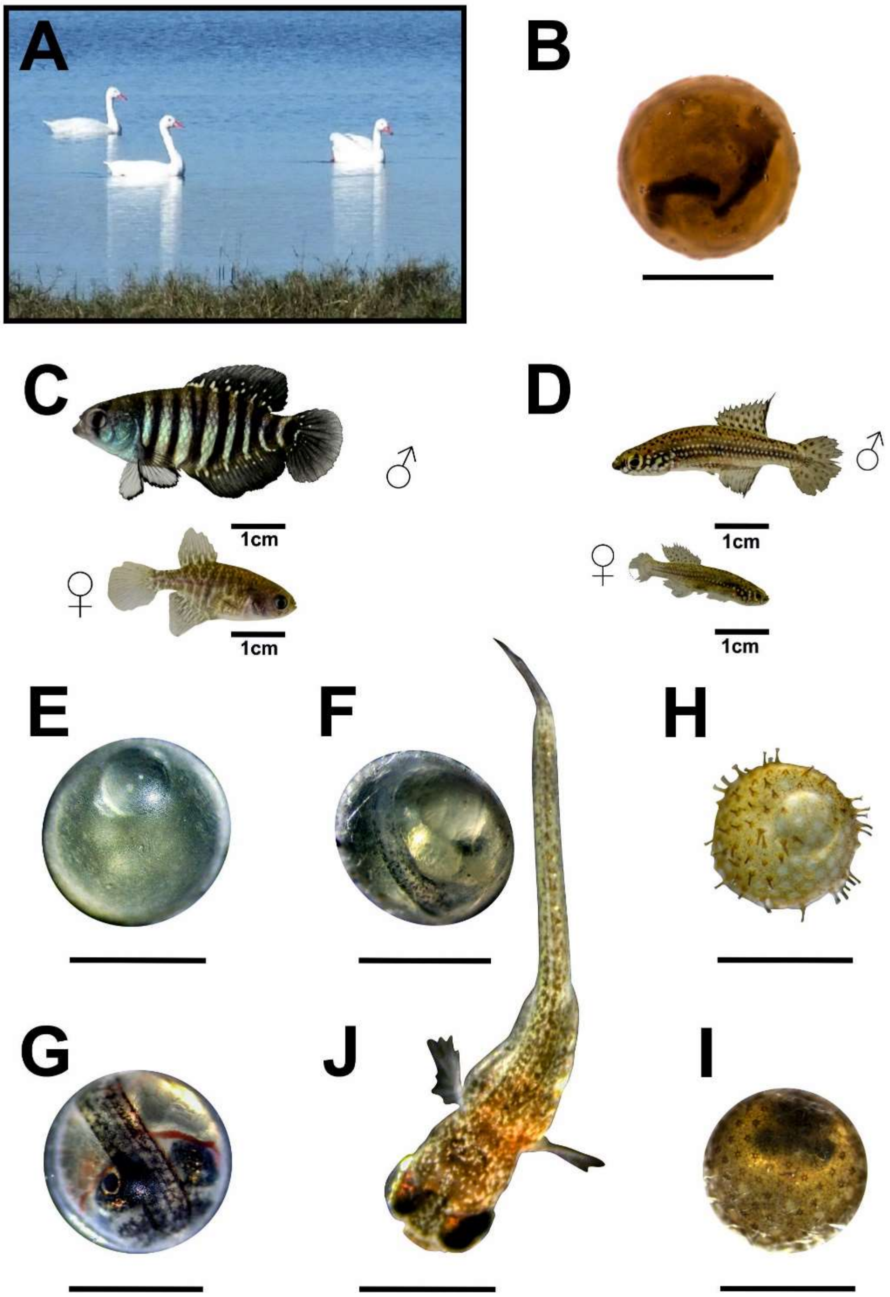

FIG. 1. Study animals and eggs that survived waterfowl gut passage. (A) Coscoroba swans in the Lagoa do Peixe; (B) egg of *Austrolebias* sp. recovered from swan faeces collected in the field; male and female (C) *Austrolebias minuano* and (D) *Cynopoecilus*

fulgens. Egg *Aust* 2 (*A. minuano*) when recovered from an experimental dropping at Diapause I (E), and after its development to Diapause II (F) and Diapause III (G). Egg *Cyno* 1 (*C. fulgens*) when recovered from an experimental dropping in Diapause I (H) and between stages Diapause I and II (I). Freshly hatched juvenile *A. minuano*, after developing from egg *Aust* 3 that survived passage through the coscoroba swan digestive tract (J). Scale bars refers to 1 mm to eggs and hatched fish and 1 cm to adult fish.

5.4. Appendix S1

Supporting Information for G. G. Silva, V. Weber, A. J. Green; P. Hoffmann; V. S. Silva; M. Volcan; L. E. K. Lanés; C. Stenert; M. Reichard and L. Maltchik. 2019. Killifish eggs can disperse via gut passage through waterfowl. *Ecology.*

MATERIALS AND METHODS

Field sampling and procedures

Aiming primarily to investigate endozoochory of plants and invertebrates, we collected 27 droppings of coscoroba swan (*Coscoroba coscoroba*) at Coastal Plain of Rio Grande do Sul, southern Brazil, a region composed of a mosaic of permanent and temporary pools mixed with cattle ranches and rice fields. Two collection sites were located in the region of National Park of Lagoa do Peixe (Parna Lagoa do Peixe) and another four sites in Santa Vitória do Palmar municipality (Table S1). Field sampling was completed from August 2017 to May 2018. The droppings were collected fresh from grass and were not in contact with soil or water. The samples were immediately inspected to avoid any contamination, stored in tubes, and then frozen within six hours of collection. In the laboratory, the droppings were defrosted in deionized water, placed separately in plastic pots and examined under the stereomicroscope in Bogorov chambers. All the content was separated and classified to the lowest taxonomic level possible. From two of the droppings collected we identified one egg of annual killifish (*Austrolebias* sp.) and six fish egg envelopes (chorions). The egg was apparently intact and contained a well-developed embryo (Fig. S1), but egg viability could not be tested because our samples were frozen prior to inspection.

Captive experiment

The experiment to test whether killifish eggs survive gut passage was conducted from May to December 2018 in the Laboratory of Ecology and Conservation of Aquatic Ecosystems of UNISINOS (LECEA) and in the Zoological Park of Fundação Zoobotânica of Rio Grande do Sul (PZFZB). We collected 10 male and 30 female *Austolebias minuano* and 10 male and 30 female *Cynopoecilus fulgens* in three natural ponds in the Coastal Plain (31°17' S; 51° 5' W) and transported them to LECEA. These two annual killifish species are not closely related (Costa 1998) and coexist in the region where coscoroba droppings with fish egg and chorions were previously found (Lanés et al. 2016). In the laboratory, fish were housed in groups of one male and three females in a set of 20 aquaria (30 x 30 x 30 cm). The aquaria had coconut fiber substrate on the bottom where eggs were regularly deposited. The eggs were collected weekly over five months and kept in semi-dry coconut fiber substrate (to imitate natural conditions) until their use in the experiment. Eggs of annual killifish develop via three diapause stages and embryo development is halted for an extended period of time in diapause (Podrabsky et al. 2017). In the experiment, we only used undamaged developing eggs, as confirmed by a characteristic perivitelline space

(Fonseca et al. 2018) and clear signs of embryo development (Furness et al. 2016; Podrabsky et al. 2017). All procedures involving fish and their eggs were authorized by the UNISINOS Ethic Committee (PPECEUA03.2018) and Brazilian Agency – ICMBio (Sisbio n° 63602-1).

The experiment with birds was performed in the PZFZB in November 2018. Three coscoroba swans born in captivity were kept jointly in their home enclosure (3 x 6 m, with sand and concrete substrate). The standard feeding procedure for coscoroba swans in the PZFZB involves a ration of corn-based diet offered once a day, in the morning. During the rest of the day birds feed on aquatic macrophytes supplied from an adjacent pond. We adapted our experimental design to this usual routine and mixed 650 killifish eggs into 120 g of ration. The eggs consisted of 300 diapause I eggs of *A. minuano,* 300 diapause I eggs of *C. fulgens* and 50 diapause II eggs of *A. minuano*. Diapause I is a developmental arrest stage occurring early in the development, at the stage of dispersed blastomeres (Wourms 1972, Podrabsky et al. 2017), and is associated with egg retention in hypoxic pool sediment (Domínguez-Castanedo et al. 2013, Reichard and Polačik 2019). Diapause II occurs at mid-somitogenesis (with a beating heart), is associated with the dry phase of the habitat and is especially resistant to desiccation (Wourms 1972, Domínguez-Castanedo et al. 2013, Reichard and Polačik 2019). Those two embryo developmental stages are most likely to be ingested by waterfowl foraging in submersed vegetation and sediments.

The ration with eggs was available to swans from 7:00 to 11:00 AM, without any intervention such as force-feeding. The ration with eggs was available only on the first experimental day. Left-over food was inspected under a stereomicroscope to quantify the number of eggs that were not ingested. In consecutive days of the experiment, the birds were submitted to their normal routine, fed with identical rations except they contained no fish eggs. No contamination of swan droppings from freshly laid eggs was possible; killifish are not present in the PZFZB and killifish do not lay eggs outside water. The experiment with swans was supervised by a veterinarian and biologists from PZFZB and was authorized by the UNISINOS Ethic Committee (PPECEUA03.2018) and Brazilian Agency – ICMBio (Sisbio n° 63602-1).

Over 48 h following experimental feeding, we collected 55 droppings (mean ± standard deviation weight: 33.131g ± 14.025g) at six time intervals (0, 6, 20, 24, 30 and 48 hours after ration removal). Immediately after each collection, we sifted droppings in tap water and analyzed their contents under a stereomicroscope. To evaluate the development of embryos, we followed the protocol of Fonseca et al. (2018). In brief, we kept the eggs in Petri dishes with Yamamoto solution (NaCl, 0.75%, KCl, 0.02%, CaCl2.02 %) in complete darkness, at room temperature (23°C) and exposed them to air and light only at the time of observation. Eggs considered viable were those in development, confirmed by a characteristic perivitelline space or embryo development. Developmental stage was recorded according to Wourms (1972) and Podrabsky et al. (2017). When the embryo was ready to hatch (post-Diapasue III stage), we immersed it in plain water (~20-22°C; pH 7.2). Once the embryo hatched, it was kept in the tank.

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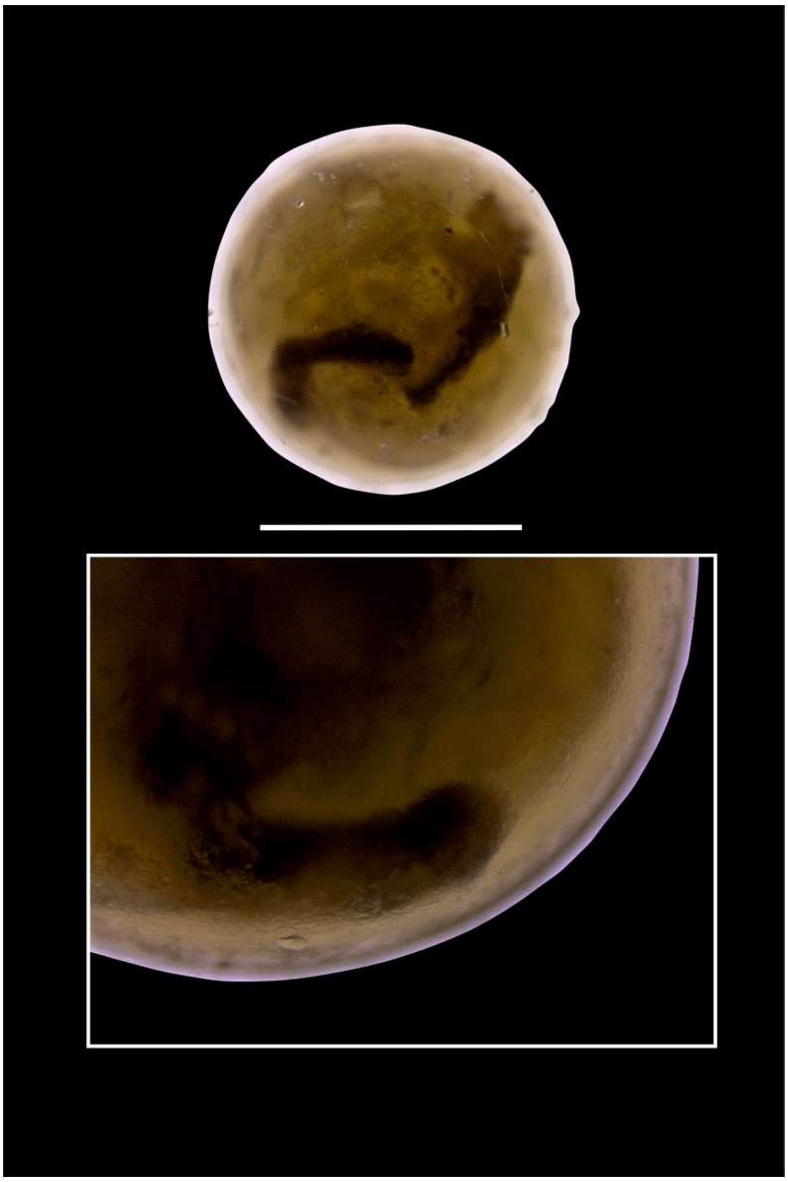


Figure S1. Egg of *Austrolebias* sp. recovered from a dropping of coscoroba swan collected in the field in the Parna Lagoa do Peixe in southern Brazil (A). (B) Detailed illustration of chorion surface and the embryo. Bar = 1 mm.

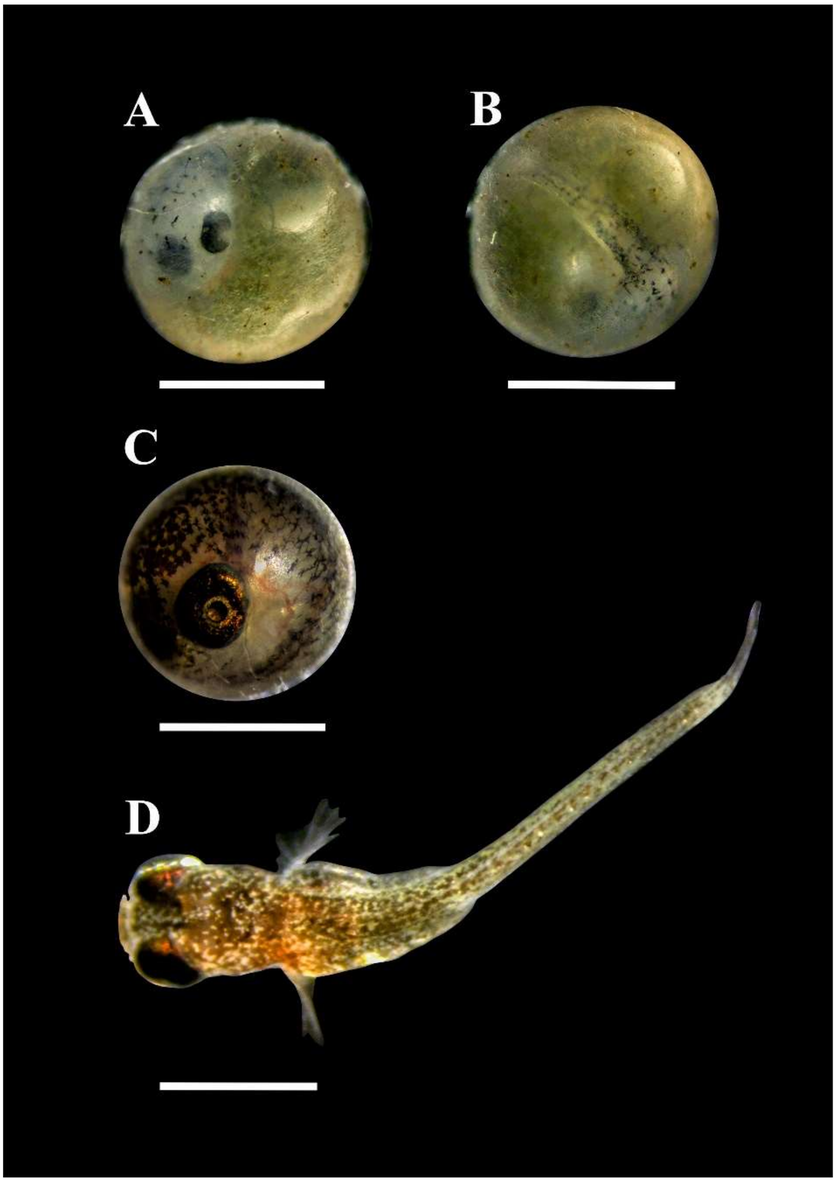


Figure S2. Egg *Aust* 3 of *Austrolebias minuano* that hatched after gut passage from coscoroba swan. (A) Embryo in Diapause II at the time of recovery of swan faeces; (B) developing between Diapause II and III (B) 21 days after recovering from faeces; (C) Diapause III after 35 days and (D) live juvenile fish after hatching (Video S1). The egg was recovered from faeces at least 48 hours after its ingestion by the bird. Bar = 1 mm.

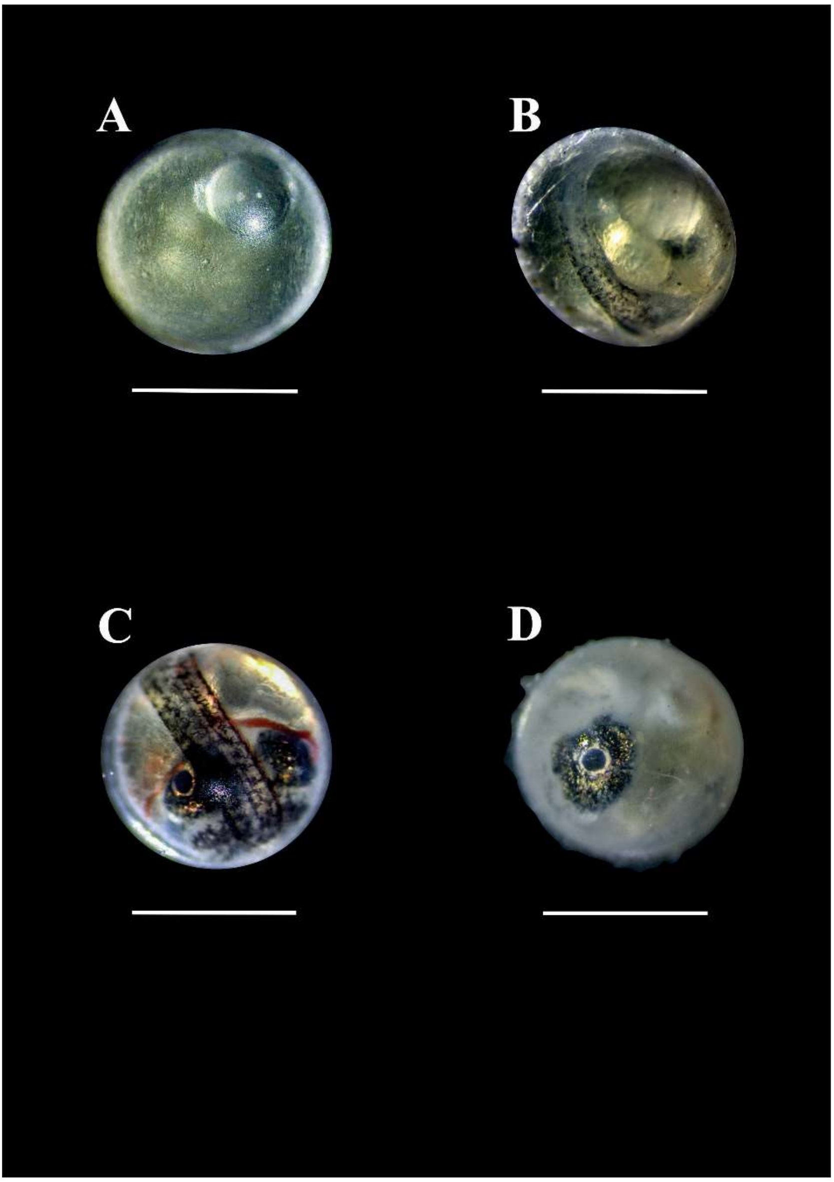
‘ 

Figure S3. Egg *Aust* 2 of *Austrolebias minuano* recovered from coscoroba swan faeces after gut passage as Diapause I. (A) at Diapause I when recovered from faeces; (B) at Diapause II after 28 days of development; (C) at Diapause III after 49 days of development and (D) dead after 63 days of development. Bar = 1 mm.

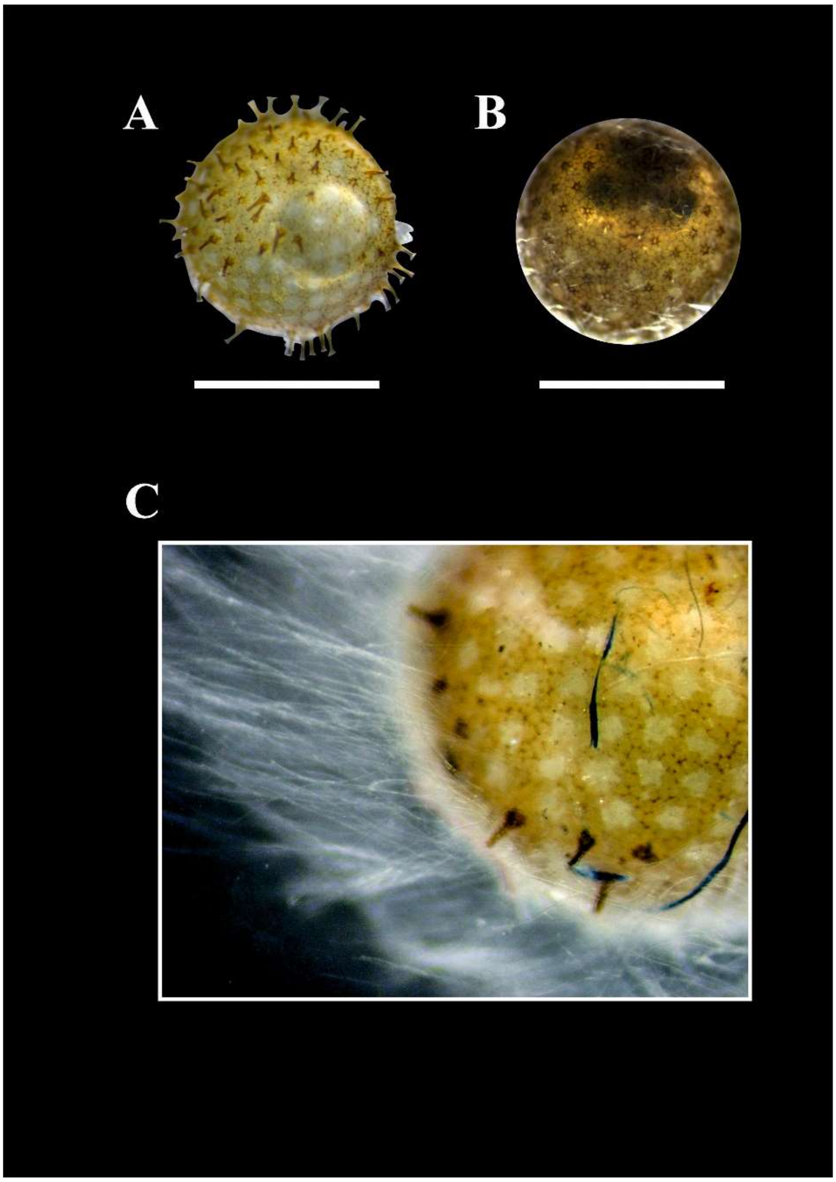


Figure S4. Egg *Cyno* 1 of *Cynopoecilus fulgens* recovered from coscoroba swan faeces after gut passage in Diapause I (A) and during its development between Diapause I and II stages. (B) Fungal infection 49 days later that led to embryo death (C). Bar = 1 mm.

Table S1. Details of coscoroba swan droppings collected in the wild in southern Brazil between August 2017 and May 2018. Two samples collected in the austral winter in a site inside Parna Lagoa do Peixe (PNLP) had fish eggs or chorions. During visits in other seasons, we did not find coscoroba faeces in site PLP 1 or its proximity.

Sample	Site	Fish egg	Chorions	Date	Locality	Coordinates
Winter 1	PLP 1			08/18/2017	Tavares (PNLP)	31°15'54.53"S / 50°57'48.40"W
Winter 2	PLP 1	1	2	08/18/2017	Tavares (PNLP)	31°15'54.53"S / 50°57'48.40"W
Winter 3	PLP 1			08/18/2017	Tavares (PNLP)	31°15'54.53"S / 50°57'48.40"W
Winter 4	PLP 1		4	24/08/2017	Tavares (PNLP)	31°15'54.53"S / 50°57'48.40"W
Spring 1	SVP 4			11/18/2017	Santa Vitória do Palmar	33°35'57.50"S / 53°15'38.67"W
Spring 2	SVP 3			11/18/2017	Santa Vitória do Palmar	33°37'37.95"S / 53°21'47.43"W
Spring 3	SVP 3			11/18/2017	Santa Vitória do Palmar	33°37'37.95"S / 53°21'47.43"W
Spring 4	SVP 3			11/18/2017	Santa Vitória do Palmar	33°37'37.95"S / 53°21'47.43"W
Spring 5	SVP 3			11/18/2017	Santa Vitória do Palmar	33°37'37.95"S / 53°21'47.43"W
Spring 6	SVP 3			11/18/2017	Santa Vitória do Palmar	33°37'37.95"S / 53°21'47.43"W
Summer 1	SVP 1			02/10/2018	Santa Vitória do Palmar	33°36'33.20"S / 53°21'40.00"W
Summer 2	SVP 1			02/10/2018	Santa Vitória do Palmar	33°36'33.20"S / 53°21'40.00"W

Summer 3	SVP 1	02/10/2018	Santa Vitória do Palmar	33°36'33.20"S / 53°21'40.00"W
Summer 4	SVP 1	02/10/2018	Santa Vitória do Palmar	33°36'33.20"S / 53°21'40.00"W
Summer 5	SVP 1	02/10/2018	Santa Vitória do Palmar	33°36'33.20"S / 53°21'40.00"W
Summer 6	SVP 1	02/10/2018	Santa Vitória do Palmar	33°36'33.20"S / 53°21'40.00"W
Summer 7	SVP 1	02/10/2018	Santa Vitória do Palmar	33°36'33.20"S / 53°21'40.00"W
Autumn 1	SVP 2	04/25/2018	Santa Vitória do Palmar	33°38'13.10"S / 53°20'49.52"W
Autumn 2	SVP 2	04/25/2018	Santa Vitória do Palmar	33°38'13.10"S / 53°20'49.52"W
Autumn 3	SVP 2	04/25/2018	Santa Vitória do Palmar	33°38'13.10"S / 53°20'49.52"W
Autumn 4	SVP 2	04/25/2018	Santa Vitória do Palmar	33°38'13.10"S / 53°20'49.52"W
Autumn 5	SVP 2	04/25/2018	Santa Vitória do Palmar	33°38'13.10"S / 53°20'49.52"W
Autumn 6	SVP 2	04/25/2018	Santa Vitória do Palmar	33°38'13.10"S / 53°20'49.52"W
Autumn 7	SVP 2	04/25/2018	Santa Vitória do Palmar	33°38'13.10"S / 53°20'49.52"W
Autumn 8	MST 1	05/13/2018	Mostardas	31° 2'38.37"S / 50°57'42.33"W
Autumn 9	MST 1	05/13/2018	Mostardas	31° 2'38.37"S / 50°57'42.33"W
Autumn 10	MST 1	05/13/2018	Mostardas	31° 2'38.37"S / 50°57'42.33"W

Table S2. Killifish eggs recovered from four *C. coscoroba* faeces from the *ex situ* experiment. The number of eggs (with the number of faeces with eggs in parentheses) is shown for each time of collection. Fifteen chorions were empty and had a small crevice.

Collection time	Viable eggs *A. minuano*	Viable eggs *C. fulgens*	Chorions *A. minuano*
0h	2 (1)		7 (4)
6h			2 (1)
20h		1 (1)	3 (1)
24h			2 (2)
30h			1 (1)
48h	1 (1)	1(1)	
Total	3 (2)	2 (2)	15 (9)

Table S3. Developmental stages of *Austrolebias minuano* (Aust) and *Cynopoecilus fulgens* (Cyno) eggs over consequent days following gut passage in coscoroba swans, recorded until their death or hatching. Roman numbers I, II and III indicate diapause stages.

	Days									
Egg ID*	0	7	14	21	28	35	42	49	56	63
Aust 1	I	Dead								
Aust 2	I	I	I	I	II	II	II	III	III	Dead
Cyno 1	I	I	I	I	I	II	II	Dead		
Aust 3	II	II	II	II	II	III	III	Hatched		
Cyno 2	I	Dead								

*The eggs are listed in order of the timing of their recovery from faeces.

6. CONCLUSÕES

- Nesta tese foi comprovado que aves aquáticas atuam como agentes de dispersão de variados tipos de organismos, de plantas a invertebrados e peixes, em ambientes aquáticos neotropicais.
- A presença de diásporos de angiospermas, licófitas, pteridófitas e carofíceas , com espécies variando de hábitos estritamente aquáticos a terrestres, demonstram que as aves aquáticas são importantes dispersoras de uma ampla variedade de plantas entre as áreas úmidas e suas adjacências. Fatores ecológicos e anatômicos intrínsecos aos organismos envolvidos devem ser estudados para melhor compreender as variações específicas e sazonais da dinâmica de dispersão.
- A endozoocoria, de plantas inteiras que possuem capacidade de reprodução assexuada é um fator relevante que deve ser considerado para entender a ampla distribuição geográfica de algumas espécies e o caráter invasivo de outras.
- Os padrões de dispersão de invertebrados através da endozoocoria de aves deve ser melhor investigado na região neotropical. Os resultados apresentados nesta tese sugerem que uma ampla variedade de organismos (aves e invertebrados) pode estar envolvida na cadeia de dispersão e que fatores ecológicos intrínsecos às espécies podem explicar como a dispersão ocorre na região.
- A inédita comprovação da possibilidade de dispersão de ovos por endozoocoria pode explicar a presença de peixes em locais isolados e em áreas efêmeras. A importância e a magnitude desta descoberta ainda está por ser aprofundada, sobretudo porque novos estudos deverão ampliar o número de espécies de peixes que podem ser dispersadas por este caminho.
- O transporte passivo de organismos por endozoocoria promovida por aves é um dos principais meio de dispersão de espécies aquáticas com baixa de capacidade de locomoção, possibilitando a manutenção das conexões biológicas entre áreas úmidas isoladas. Nesta tese ficou comprovado a importância das aves aquáticas para a dispersão de organismos na região neotropical, uma função ecológica fundamental para a manutenção da biodiversidade, dos processos biogeográficos e dos processos ecológicos de ecossistemas aquáticos.