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INSTITUTO DE OCEANOGRAFIA
PROGRAMA DE PÓS-GRADUAÇÃO EM OCEANOGRAFIA BIOLÓGICA – PPGOB

ASPECTOS ECOLÓGICOS, GENÉTICOS E DA DISTRIBUIÇÃO ESPACIAL DA INVASÃO DO CARANGUEJO *RHITHROPANOPEUS HARRISII* (GOULD, 1841) NA LAGOA DOS PATOS, SUL DO BRASIL

RENAN C. MACHADO

Tese apresentada ao Programa de Pós-graduação em Oceanografia Biológica da Universidade Federal do Rio Grande - FURG, como requisito parcial à obtenção do título de DOUTOR.

Orientador: Prof. Dr. Luiz Felipe Cestari Dumont

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IO – INSTITUTO DE OCEANOGRAFIA
PROGRAMA DE PÓS-GRADUAÇÃO EM OCEANOGRAFIA BIOLÓGICA
E-mail: ccpgob@furg.br – home-page: www.ppgocbio.furg.br

ATA DE TESE DE DOUTORADO – 05/2026

Às oito horas e trinta minutos do dia vinte e um de agosto do ano de dois mil e vinte e seis, por vídeo conferência, sala: https://conferenciaweb.rnp.br/sala/silvina-silvina-botta, Presencial: Auditório João Rocha – IO – FURG/Carreiros, reuniu-se a Comissão Examinadora de Tese de Doutorado do Acad. **Renan Costa Machado**, composta pelos seguintes membros: Prof. Dr. Luiz Felipe Cestari Dumont - Orientador – (PPGOB -IO/FURG); Prof. Dr. Alexandre Miranda Garcia - (PPGOB-IO/FURG); Prof. Dr. André Colling - (PPGOB-IO/FURG); Prof. Dr. Maximilano Maronna - (UFPEL); Profa. Dra. Andrea S. Freire - (UFSC); Prof. Dr. Fernando Mantelatto - (USP). Título da Dissertação: "ASPECTOS ECOLÓGICOS, GENÉTICOS E DA DISTRIBUIÇÃO ESPACIAL DA INVASÃO DO CARANGUEJO RHITHROPANOPEUS HARRISII (GOULD, 1841) NA LAGOA DOS PATOS". Dando início à reunião, a Coordenadora do PPGOB, Profa. Dra. Silvina Botta, agradeceu a presença de todos e fez a apresentação da Comissão Examinadora. Logo após, explicou que o candidato teria um tempo de 45 a 60 min. para explanação do tema, e cada membro da Comissão, um máximo de 30 min. para perguntas. Dando prosseguimento, passou a palavra ao candidato que apresentou o tema e respondeu às perguntas formuladas. Após explanação, a Comissão aprovou o que segue: As sugestões de todos os membros da Comissão Examinadora, que seguem em pareceres em anexo, foram aceitas pelo orientador/candidato para incorporação na versão final. Foi atribuída a seguinte classificação ao candidato: Prof. Dr. André Colling - Classificação: Aprovado; Prof. Dr. Alexandre Miranda Garcia - Classificação: Aprovado; Prof. Dr. Maximiliano Maronna - Classificação: Aprovado; Profa. Dra. Andréa S. Freire - Classificação: Aprovado e Prof. Dr. Fernando Montelatto - Classificação: Aprovado. O candidato foi considerado APROVADO por UNANIMIDADE. Nada mais havendo a tratar eu, Clabisnei Moura de Melo – Secretário do PPGOB, lavro a presente ata que após lida e aprovada, será assinada pela Comissão Examinadora, pelo aluno e pela Coordenadora do PPGOB.

Prof. Dr. Luiz Felipe Cestari Dumont

Prof. André Colling

Prof. Dr. Alexandre Miranda Garcia

Prof. Dr. Maximiliano Maronna

Profa. Dra. Andrea S. Freire

Prof. Dr. Fernando Mantelatto

Acad. Renan Costa Machado

Profa. Dra. Silvina Botta
Coordenadora PPGOB

"Sonhos não envelhecem"
(Clube da Esquina Nº 2)

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RESUMO

As invasões biológicas representam uma das principais ameaças à biodiversidade em ecossistemas aquáticos, especialmente em ambientes estuarinos, sujeitos à intensa influência antrópica. O caranguejo invasor *Rhithropanopeus harrisii*, nativo da costa atlântica da América do Norte, foi registrado na Lagoa dos Patos, sul do Brasil, na década de 1980, sendo considerado uma espécie invasora de sucesso devido à sua elevada plasticidade ecológica, ampla tolerância ambiental e hábito alimentar generalista. Nesse contexto, esta tese teve como objetivo investigar diferentes aspectos ecológicos, genéticos e espaciais associados ao processo de invasão de *R. harrisii* na Lagoa dos Patos, integrando abordagens de genética populacional, ecologia trófica e modelagem ecológica de distribuição de espécies. As análises genéticas indicaram que a população invasora presente no sul do Brasil é provavelmente originária da costa leste dos Estados Unidos, especialmente na região da Carolina do Norte, reforçando a importância da água de lastro como principal vetor de introdução da espécie. Além disso, os resultados evidenciaram baixa diversidade genética na população brasileira, sugerindo um evento de introdução recente associado a efeito fundador. A modelagem ecológica de distribuição potencial revelou ampla adequabilidade ambiental para a espécie na Lagoa dos Patos, especialmente em regiões estuarinas e setores intermediários do sistema lagunar. Os modelos indicaram que matéria orgânica sedimentar, salinidade e profundidade constituem os principais fatores associados à distribuição da espécie. Os resultados também demonstraram elevado potencial de expansão de *R. harrisii* para áreas oligohalinas e de água doce da lagoa, sugerindo sua elevada tolerância ambiental. A avaliação da ecologia alimentar demonstrou que *R. harrisii* apresenta elevada plasticidade trófica e hábito alimentar generalista ao longo do gradiente salino estuarino. Apesar disso, a sobreposição de nicho trófico com as quatro espécies nativas de caranguejos analisadas foi relativamente baixa, indicando possíveis mecanismos de diferenciação de nicho e partilha de recursos que favorecem a coexistência do invasor com potenciais espécies competidoras nativas da Lagoa dos Patos. Em conjunto, os resultados desta tese demonstram que o sucesso invasivo de *R. harrisii* na

Lagoa dos Patos está associado à elevada plasticidade ecológica, ampla tolerância fisiológica, plasticidade trófica, dispersão mediada por atividades humanas, e ampla disponibilidade de recursos tróficos e de habitat para a espécie. Além disso, o estudo contribui para o entendimento dos processos de invasão biológica em ecossistemas estuarinos subtropicais, fornecendo subsídios relevantes para monitoramento ambiental, conservação da biodiversidade e manejo de espécies invasoras em ambientes aquáticos.

Palavras-chave: ecologia estuarina, espécie invasora, genética populacional, Lagoa dos Patos, modelagem ecológica, nicho trófico

ABSTRACT

Biological invasions represent one of the major threats to biodiversity in aquatic ecosystems, particularly in estuarine environments, which are subject to intense anthropogenic influence. The invasive crab *Rhithropanopeus harrisii*, native to the Atlantic coast of North America, was first recorded in the Patos Lagoon, southern Brazil, during the 1980s and is considered a successful invader due to its high ecological plasticity, broad environmental tolerance, and generalist feeding habits. In this context, this thesis aimed to investigate different ecological, genetic, and spatial aspects associated with the invasion process of *R. harrisii* in the Patos Lagoon, integrating approaches from population genetics, trophic ecology, and species distribution modeling. Genetic analyses indicated that the invasive population established in southern Brazil most likely originated from the east coast of the United States, particularly the North Carolina region, reinforcing the importance of ballast water as the primary vector responsible for the species' introduction. Furthermore, the results revealed low genetic diversity within the Brazilian population, suggesting a recent introduction event associated with a founder effect. Ecological niche modeling revealed extensive environmental suitability for the species throughout the Patos Lagoon, particularly in estuarine and intermediate sectors of the lagoon system. The models identified sediment organic matter, salinity, and depth as the main environmental factors associated with the species' distribution. The results also demonstrated a high potential for the expansion of *R. harrisii* into oligohaline and freshwater areas of the lagoon, confirming its broad environmental tolerance. The assessment of feeding ecology demonstrated that *R. harrisii* exhibits high trophic plasticity and generalist feeding habits along the estuarine salinity gradient. Nevertheless, trophic niche overlap with native crab species was relatively low, suggesting potential niche differentiation and resource partitioning mechanisms that may facilitate the coexistence of the invader with native competitor species in the Patos Lagoon. Taken together, the results of this thesis demonstrate that the invasion success of *R. harrisii* in the Patos Lagoon is associated with its high ecological plasticity, broad physiological tolerance, trophic plasticity, human-mediated dispersal, and

the wide availability of trophic and habitat resources. Furthermore, this study contributes to the understanding of biological invasion processes in subtropical estuarine ecosystems, providing relevant information for environmental monitoring, biodiversity conservation, and the management of invasive species in aquatic environments.

Keywords: ecological modeling, estuarine ecology, invasive species, Patos Lagoon, population genetics, trophic niche

PREFÁCIO

Os ecossistemas estuarinos estão entre os ambientes mais dinâmicos e produtivos do planeta, desempenhando papel fundamental na manutenção da biodiversidade, na ciclagem de nutrientes e no suporte às atividades humanas. Entretanto, esses ambientes também figuram entre os mais vulneráveis às alterações antrópicas, especialmente à introdução de espécies exóticas invasoras. O aumento do transporte marítimo global, associado à intensificação das atividades portuárias e às mudanças ambientais, tem favorecido a dispersão de organismos para regiões fora de suas áreas naturais de ocorrência, promovendo impactos ecológicos ainda pouco compreendidos em muitos sistemas costeiros.

Nesse contexto, o caranguejo invasor *Rhithropanopeus harrisii* destaca-se como uma espécie com elevada capacidade adaptativa e ampla tolerância ambiental. Nativo da costa Atlântica da América do Norte, esse caranguejo expandiu sua distribuição para diferentes regiões do mundo ao longo do último século, sendo registrado no Estuário da Lagoa dos Patos, sul do Brasil, na década de 1980. Desde então, pouco se conhecia sobre sua ecologia, origem populacional, potencial de expansão e possíveis impactos sobre as comunidades nativas da região.

Esta tese foi estruturada em cinco capítulos, incluindo uma introdução (Capítulo I) e uma conclusão geral (Capítulo V) redigidas em português, além de três capítulos apresentados em formato de artigo científico. O Capítulo II aborda a origem da invasão de *R. harrisii* na costa sudoeste do Atlântico por meio de análises da região do DNA mitocondrial citocromo oxidase 1 (COI), buscando identificar possíveis populações fonte e compreender os vetores associados ao processo de introdução da espécie na Lagoa dos Patos. Este capítulo foi publicado no periódico *Estuarine, Coastal and Shelf Science*.

O Capítulo III (manuscrito submetido para o periódico *Regional Studies in Marine Science*) teve como objetivo avaliar a distribuição potencial e os padrões espaciais de expansão de *R. harrisii* na Lagoa dos Patos, utilizando modelos ecológicos de distribuição de espécies associados a variáveis ambientais (Maxent e GAM) ao longo do gradiente

lagunar-estuarino. Por fim, o Capítulo IV investigou a ecologia alimentar e a sobreposição de nicho trófico entre *R. harrisii* e espécies nativas de caranguejos ao longo do gradiente salino estuarino, buscando compreender possíveis interações ecológicas e o potencial de competição alimentar entre o invasor e a fauna nativa. Este capítulo foi publicado no periódico *Marine Ecology*.

Esta tese é resultado de cinco anos de pesquisa desenvolvidos no Programa de Pós-Graduação em Oceanografia Biológica da Universidade Federal do Rio Grande – FURG.

CAPÍTULO I

INTRODUÇÃO GERAL

As invasões biológicas constituem uma das principais ameaças à biodiversidade e ao funcionamento dos ecossistemas aquáticos em escala global, sendo particularmente relevantes em ambientes costeiros e estuarinos sujeitos à intensa atividade antrópica. Embora as distribuições geográficas das espécies sejam naturalmente dinâmicas (Lodge 1993), a disseminação mediada pelas atividades humanas ocorre de maneira muito mais rápida do que os processos naturais de expansão (Ruiz et al. 2000; Rius et al. 2015; Essl et al. 2020). A introdução de espécies exóticas geralmente está vinculada a atividades de interesse econômico, como o transporte marítimo e fluvial, que transporta organismos por meio da água de lastro e bioincrustações em estruturas navais, além da aquicultura, aquariofilia e rejeitos sólidos flutuantes (Mineur et al. 2012; Peters et al. 2014; Carlton & Fowler, 2018). Esses vetores tornam ambientes costeiros, estuarinos e lagunares particularmente suscetíveis à introdução e ao estabelecimento de espécies não nativas, sobretudo em sistemas com elevada conectividade hidrológica e intensa atividade antrópica.

A introdução e expansão de espécies invasoras representam atualmente um dos principais desafios ecológicos em escala global, causando impactos significativos sobre a biodiversidade, o funcionamento dos ecossistemas e diferentes atividades econômicas (Magliozzi et al. 2020; Polce et al. 2023). Quando introduzidas fora de sua área natural de ocorrência, seja por transporte acidental, comércio internacional, atividades aquícolas/agropecuárias ou mudanças climáticas, espécies não nativas frequentemente encontram condições favoráveis para seu estabelecimento e rápida dispersão, podendo estabelecer populações persistentes e ampliar progressivamente sua área de distribuição, com potenciais consequências para a estrutura e o funcionamento dos ecossistemas receptores (Seebens et al. 2017; Essl et al. 2020). A propagação contínua dessas espécies em diferentes ecossistemas tem contribuído significativamente para a perda de biodiversidade, uma vez que organismos invasores frequentemente apresentam elevada

plasticidade ecológica e alta capacidade competitiva em relação às espécies nativas (Meyerson et al. 2019; Pyšek et al. 2020; Crystal-Ornelas & Lockwood 2020). O sucesso invasivo dessas espécies está frequentemente associado à ausência de predadores naturais, competidores eficientes e agentes patogênicos que, em suas regiões de origem, atuavam regulando suas populações, hipótese conhecida como “Hipótese da Liberação do Inimigo” (Torchin et al. 2003). De acordo com essa hipótese, durante o processo de introdução, as espécies invasoras podem deixar para trás parte de seus inimigos naturais, particularmente aqueles com relações altamente específicas com o hospedeiro, reduzindo a pressão de predação, parasitismo e patogenicidade sobre suas populações (Torchin et al. 2003). Essa redução da pressão biótica pode favorecer maior sobrevivência, crescimento e reprodução, contribuindo para o estabelecimento e a expansão das populações introduzidas (Torchin et al. 2003). Dessa forma, espécies invasoras podem explorar intensamente os recursos disponíveis, competir com organismos nativos por alimento e habitat, alterar processos ecológicos e modificar a estrutura das comunidades locais e das teias alimentares (Guy-Haim et al. 2018; Ricciardi et al. 2021). Essas alterações podem afetar a ciclagem de nutrientes, reduzir a abundância e distribuição de espécies nativas e, em casos mais severos, provocar declínios populacionais ou extinções locais (Bellard et al. 2016). Além dos impactos ecológicos, invasões biológicas também representam elevados custos econômicos e sociais, devido à necessidade contínua de monitoramento, manejo e mitigação, bem como aos prejuízos causados às atividades humanas, recursos naturais e saúde pública (Diagne et al. 2021; Bailey et al. 2020).

Entre os organismos invasores associados a ambientes aquáticos, os crustáceos decápodes destacam-se devido à elevada plasticidade fisiológica, ampla tolerância ambiental e elevado potencial de impacto sobre comunidades nativas e processos ecossistêmicos (Laughlin & French 1989; Costlow et al. 1966; Fowler et al. 2013; Forsström et al. 2015; Machado et al. 2026). O sucesso de introdução, estabelecimento e dispersão dessas espécies depende de diferentes características relacionadas à história de vida e à fisiologia, como elevada fecundidade, rápido crescimento populacional, presença de estágios larvais planctônicos e ampla capacidade de tolerância a variações ambientais

(Bock et al. 2015). Além disso, fatores genéticos associados à variabilidade populacional e à adaptação rápida podem influenciar diretamente o sucesso invasivo, aumentando a capacidade das populações introduzidas de persistirem e se adaptarem a novas condições ambientais (Roman & Darling 2007; Dlugosch et al. 2015). Em conjunto, essas características favorecem o estabelecimento de populações viáveis e ampliam o potencial de expansão geográfica de espécies invasoras em ambientes aquáticos.

Neste contexto, o uso da genômica populacional tem contribuído significativamente para a compreensão dos processos de invasão biológica, permitindo inferir rotas de introdução, identificar populações-fonte, detectar eventos de introduções múltiplas e avaliar padrões de expansão demográfica e conectividade populacional (Handley et al. 2011; Viard et al. 2016). Durante o processo de introdução, as populações invasoras podem passar por um efeito fundador, no qual um pequeno número de indivíduos estabelece uma nova população, podendo resultar em redução da variabilidade genética e da diversidade haplotípica em relação às populações de origem (Dlugosch & Parker 2008; Estoup et al. 2016). A avaliação da diversidade haplotípica, portanto, constitui uma importante ferramenta para caracterizar a estrutura genética das populações introduzidas e pode fornecer evidências sobre padrões de colonização, expansão e origem das populações invasoras (Estoup & Guillemaud 2010). Por outro lado, múltiplas introduções provenientes de diferentes populações-fonte podem aumentar a diversidade genética e haplotípica das populações invasoras, potencialmente reduzindo os efeitos da deriva genética associados ao efeito fundador e ampliando seu potencial adaptativo (Roman & Darling 2007; Dlugosch & Parker 2008). Além disso, processos de “bridgehead effect”, nos quais populações invasoras secundárias derivam de populações invasoras previamente estabelecidas, podem contribuir para a disseminação da espécie e para a formação de novas populações geneticamente diferenciadas (Handley et al. 2011; Estoup et al. 2016). Dessa forma, a identificação das rotas de invasão e das populações-fonte representa uma etapa fundamental para compreender os mecanismos associados ao sucesso invasivo, além de subsidiar estratégias de monitoramento, conservação e manejo de espécies nativas e não nativas (Bock et al. 2015; McGaughran et al. 2024).

Outro aspecto essencial para compreender o sucesso invasivo está relacionado ao nicho ecológico das espécies introduzidas, especialmente as suas interações tróficas. O fluxo de energia entre os diferentes níveis tróficos, bem como as relações de competição e predação, desempenha papel fundamental na organização das comunidades e no funcionamento dos ecossistemas (Begon et al. 2006; Brose & Hillebrand 2016). Assim, compreender os fatores que estruturam o nicho trófico de uma espécie é fundamental para compreender e avaliar os mecanismos de coexistência entre espécies morfologicamente e filogeneticamente semelhantes (Schoener 1974; Vollrath et al. 2021). A sobreposição trófica ocorre quando diferentes espécies exploram os mesmos recursos alimentares, podendo resultar em competição interespecífica, especialmente em ambientes onde os recursos são limitados (Pianka 1974; Letten et al. 2017). Em situações em que espécies invasoras apresentam maior eficiência competitiva ou elevada plasticidade alimentar, pode ocorrer exclusão competitiva de espécies nativas, reduzindo suas populações e promovendo alterações na estrutura funcional e nas redes tróficas das comunidades locais (David et al. 2017; García et al. 2020).

Os estuários estão entre os ecossistemas aquáticos mais suscetíveis às invasões biológicas devido à intensa influência antrópica associada ao transporte marítimo, atividades portuárias, aquicultura e modificações ambientais decorrentes da ocupação costeira (Magoro et al. 2015; Ojaveer et al. 2018). Esses ambientes apresentam elevada produtividade biológica, forte conectividade entre sistemas continentais e marinhos, e grande heterogeneidade ambiental, fatores que favorecem tanto o estabelecimento quanto a dispersão de espécies exóticas. Além disso, desempenham importante função ecológica como áreas de alimentação, crescimento, reprodução e migração para diferentes espécies de peixes, crustáceos e outros organismos aquáticos (Barletta et al. 2010).

A Lagoa dos Patos é a maior laguna do tipo “estrangulada” do mundo, com aproximadamente 10.360 km², sendo sua região estuarina correspondente a cerca de 10% da área total (Figura 1). A conexão entre a lagoa e o Oceano Atlântico ocorre por meio de um canal com aproximadamente 20 km de comprimento e largura variando entre 0,5 e 3,0 km (Asmus 1998). No estuário, a influência das marés é reduzida, apresentando amplitude

média de 0,5 m, enquanto a circulação hidrodinâmica estuarina é predominantemente controlada pelo regime de ventos especialmente dos quadrantes nordeste-sudoeste (NE–SO) e pelas descargas fluviais da bacia de drenagem (Möller et al. 2001). Esses ventos, associados às descargas fluviais, regulam a distribuição da salinidade e os níveis da água no sistema estuarino (Garcia 1998; Möller et al. 2001).

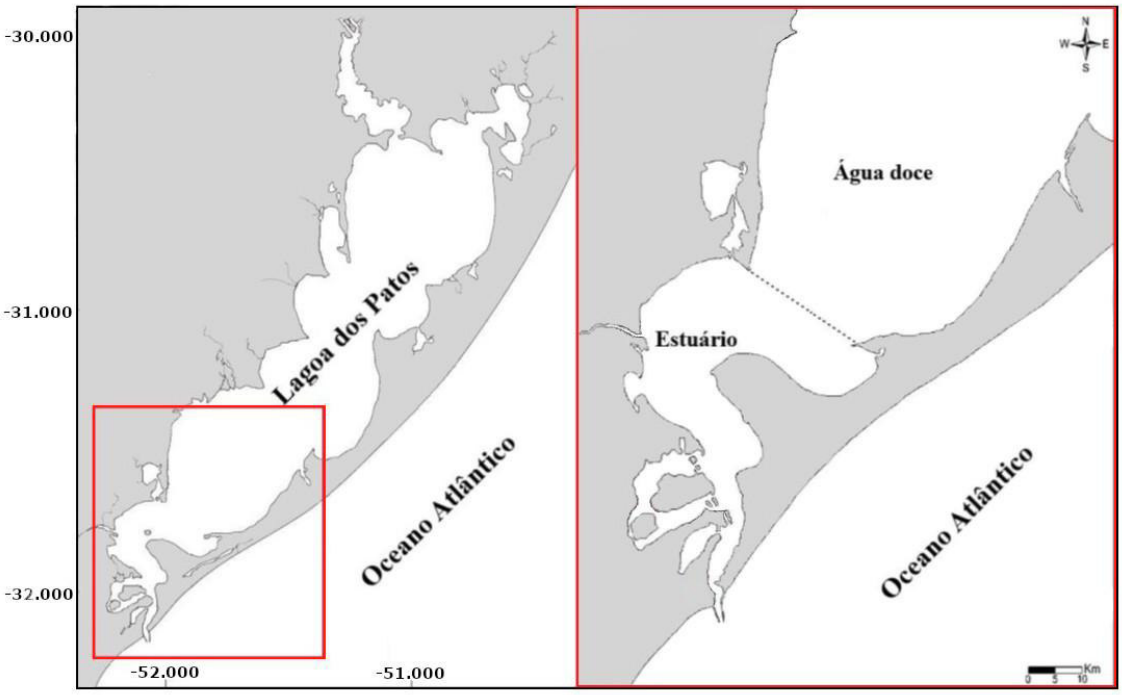


Figura 1. Mapa da Lagoa dos Patos, sul do Brasil. O painel à esquerda mostra toda a extensão da Lagoa e o painel à direita destaca a região do estudo.

A dinâmica hidrológica do Estuário da Lagoa dos Patos exerce forte influência sobre a composição, distribuição, abundância e dominância das comunidades biológicas, moldando a estrutura da fauna e a flora ao longo dos gradientes ambientais do sistema (Odebrecht et al. 2010). Além disso, o sistema apresenta elevada heterogeneidade ambiental, com extensas áreas rasas (<1,5 m) predominantemente arenosas e regiões mais profundas do canal compostas por sedimentos de areia, silte e argila (Calliari et al. 1977; Ortega et al. 2020). As margens da lagoa são ocupadas por extensas áreas de marisma, dominadas por macrófitas halófitas que desempenham papel fundamental na estruturação

dos habitats costeiros (Copertino & Seeliger 2010). Esses ambientes fornecem abrigo, alimento e áreas de reprodução para diversas espécies bentônicas (e.g., *Acantholobulus bermudensis*, *Callinectes sapidus, Cyrtograpsus angulatus, Neohelice granulata* e *Panopeus austrobesus*) e nectônicas, além de sustentarem elevadas densidades de caranguejos braquiúros (Barutot et al. 2011). Essa diversidade de hábitats, associada às variações espaciais e temporais de salinidade, profundidade e características sedimentares, favorece a ocorrência de diferentes espécies e amplia a disponibilidade de nichos ecológicos, contribuindo para a elevada diversidade biológica observada no sistema.

Um fator que contribui para a vulnerabilidade do Estuário da Lagoa dos Patos é a presença do Porto de Rio Grande em suas águas. A intensa atividade portuária representa um importante vetor potencial para a introdução de organismos não nativos. Estima-se que cerca de 80 a 90% do comércio mundial seja realizado por transporte marítimo, favorecendo o deslocamento de espécies entre diferentes regiões biogeográficas através da água de lastro e bioincrustações em embarcações (Bailey 2015; UNCTAD 2023). O transporte marítimo é reconhecido como uma das principais vias de invasão biológica em ambientes costeiros e estuarinos, possibilitando a transferência de larvas, juvenis, adultos e microrganismos entre ecossistemas geograficamente isolados (Bailey 2015; Ojaveer et al. 2018). Como consequência, espécies exóticas podem se estabelecer em novas regiões e causar impactos ecológicos significativos, incluindo alterações nas teias alimentares, competição com espécies nativas, modificações nos processos ecossistêmicos e disseminação de patógenos e parasitas (Wasson et al. 2001; Ojaveer et al. 2018).

Entre os crustáceos invasores associados a ambientes estuarinos destaca-se o caranguejo *Rhithropanopeus harrisii* (Gould, 1841) (Figura 2), conhecido popularmente como “caranguejo-da-lama”. A espécie é nativa da costa atlântica da América do Norte e parte do Golfo do México, ocorrendo desde o Golfo de Saint Lawrence, no Canadá até Veracruz no México, nos Estados Unidos, onde habita principalmente ambientes estuarinos e águas salobras, embora também possa ocorrer em sistemas de água doce (Williams 1984). O primeiro registro da espécie fora de sua área de distribuição nativa ocorreu no estuário de Zuiderzee, na Holanda em 1874, sendo considerado um dos primeiros registros de

invasão de um decápode marinho em águas europeias (Wolf 1954). Ao longo do século XX, *R. harrisii* expandiu-se amplamente por estuários e ambientes costeiros da Europa (Alemanha, Bélgica, Estônia, Finlândia, França, Noruega e Reino Unido), incluindo mares interiores como o Báltico, Negro e Cáspio (Petersen 2006; Simakova et al. 2017; GBIF.org 2026). Posteriormente, populações invasoras foram registradas em outras regiões do mundo, incluindo a Ásia (Japão e Sri Lanka), oriente médio (Irã), Américas do Norte, do Sul e Central (i.e., Brasil, Colômbia, Uruguai, costa oeste dos Estados Unidos, parte do Golfo do México, Panamá e Venezuela) (Iseda et al. 2007; Machado et al. 2024; GBIF.org, 2026) (Figura 3). Atualmente, *R. harrisii* encontra-se estabelecido em mais de 30 países distribuídos por quatro continentes, sendo considerado um dos caranguejos estuarinos invasores de maior distribuição global, cobrindo aproximadamente 45 graus de latitude, provavelmente em decorrência do transporte mediado por atividades humanas, especialmente navegação comercial e transporte marítimo internacional (Roche & Torchin 2007; GBIF.org 2026).



Figura 2. Espécime de *Rhithropanopeus harrisii* (macho) coletado no Estuário da Lagoa dos Patos. Foto: Renan C. Machado.

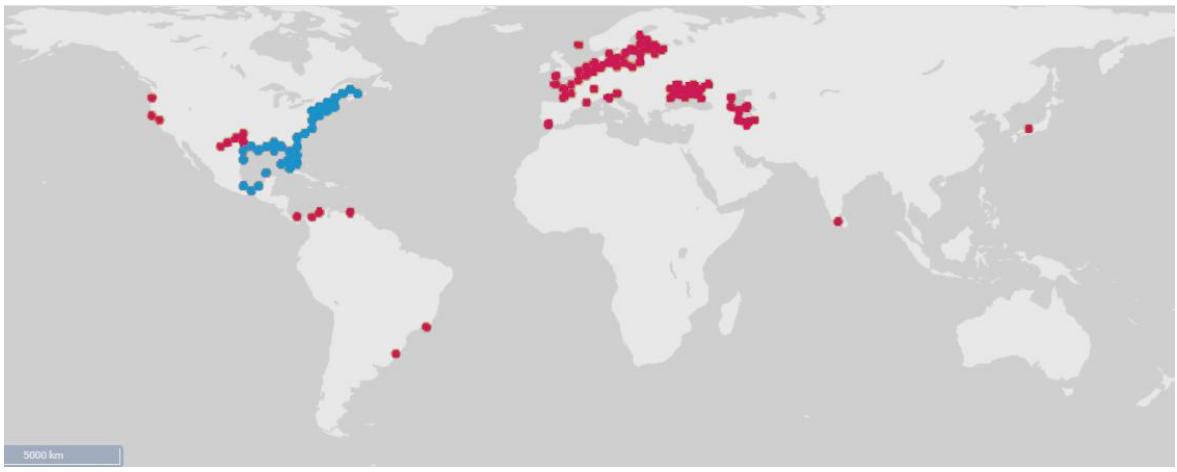


Figura 3. Distribuição global dos registros de ocorrência de *Rhithropanopeus harrisii* segundo o portal Global Biodiversity Facility (GBIF). Pontos azuis representam registros na área de distribuição nativa da espécie, enquanto os pontos vermelhos indicam registros em áreas onde a espécie é considerada não nativa ou invasora. Adaptado de Global Biodiversity Facility (GBIF; https://www.gbif.org/).

No Brasil, o primeiro registro de *R. harrisii* ocorreu no estuário da Lagoa dos Patos, no sul do país, na década de 1980 (D'Incao & Martins 1998). Posteriormente, a espécie foi registrada em outras localidades da costa brasileira, incluindo a Lagoa do Peixe, no Rio Grande do Sul, e no estado de São Paulo, indicando uma ampliação de sua distribuição geográfica ao longo da costa do Atlântico Sul (Loebmann & Vieira 2005; Coelho et al. 2008). Registros adicionais associados ao estado de Alagoas também foram identificados, embora essa ocorrência ainda necessite de confirmação quanto à identificação da espécie e à origem do registro (Coelho et al. 2008). A via exata de introdução de *R. harrisii* no Brasil permanece desconhecida, mas a dispersão por embarcações comerciais é considerada uma das principais hipóteses, especialmente por meio da água de lastro e de bioincrustações (D'Incao & Martins 1998; Roche & Torchin 2007). Nesse contexto, o Porto do Rio Grande-

RS, que recebe embarcações provenientes da Ásia, Europa e Américas e apresenta histórico de trocas de água de lastro nas proximidades do estuário da Lagoa dos Patos, constitui um importante ponto potencial de entrada de organismos não nativos no sul do Brasil (SUPRG/FURG 2016). Uma vez introduzida, a elevada heterogeneidade ambiental da Lagoa dos Patos, caracterizada por amplos gradientes de salinidade, profundidade, composição sedimentar e disponibilidade de matéria orgânica, associada à elevada produtividade biológica do estuário, pode favorecer o estabelecimento e a expansão de espécies invasoras com ampla tolerância ambiental e elevada plasticidade ecológica, como *R. harrisii* (Calliari et al. 1977; Odebrecht et al. 2010; Ortega et al. 2020).

Diversas características tornam *R. harrisii* um invasor potencialmente bem-sucedido, incluindo elevada fecundidade, desenvolvimento larval planctônico com duração variável entre aproximadamente sete e 26 dias (dependendo da temperatura da água), ampla tolerância à salinidade e temperatura e hábitos alimentares generalistas (Costlow et al. 1966; Turoboyski 1973; Laughlin & French 1989; Hegele-Drywa & Normant 2014). A espécie apresenta elevado grau de generalismo alimentar e plasticidade trófica, sendo capaz de ajustar sua dieta conforme a disponibilidade de recursos no ambiente (Turoboyski 1973; Hegele-Drywa & Normant 2009). Estudos realizados em populações invasoras demonstram que sua alimentação inclui detritos, macroalgas, poliquetas, anfípodes, ostracodes, gastrópodes, bivalves, outros crustáceos e material vegetal, evidenciando comportamento onívoro oportunista e ampla flexibilidade alimentar (Hegele-Drywa & Normant 2009; Machado et al. 2026). Essa plasticidade pode contribuir significativamente para o sucesso invasivo da espécie, permitindo sua exploração eficiente de diferentes recursos e nichos ecológicos em ambientes colonizados.

Além disso, *R. harrisii* ocupa uma ampla variedade de hábitats estuarinos, incluindo sedimentos inconsolidados, bancos de macroalgas, pradarias de fanerógamas marinhas, estruturas vegetadas e áreas com detritos orgânicos acumulados (Fowler et al. 2013; Hegele-Drywa & Normant 2014). A espécie apresenta elevada tolerância a variações ambientais, ocorrendo principalmente em águas salobras, mas podendo estabelecer populações em ambientes com salinidades muito baixas e até próximos à água doce

(Laughlin & French 1989; Fowler et al. 2013; Hegele-Drywa & Normant 2014). Ainda, *R. harrisii* possui hábito críptico, utilizando conchas vazias, galhos, vegetação morta, bancos de ostras e pequenos refúgios associados ao sedimento como abrigo (Turoboyski 1973; Williams 1984). Essa característica pode favorecer interações competitivas com espécies nativas de hábitos semelhantes, incluindo outros caranguejos da família Panopeidae, que apresentam pequeno a médio porte e carapaça geralmente oval ou hexagonal (Schubart et al. 2000). Além disso, a elevada plasticidade ecológica e alimentar de *R. harrisii* pode favorecer seu estabelecimento e persistência em diferentes condições ambientais, bem como ampliar sua capacidade de explorar uma variedade de recursos alimentares (Forsström et al. 2015; Kotta et al. 2018). Uma vez estabelecida, a espécie pode exercer efeitos sobre as comunidades bentônicas por meio de mecanismos como predação, bioturbação e competição, além de desencadear efeitos tróficos indiretos que podem modificar as interações entre os componentes da comunidade (Kotta et al. 2018: Lokko et al. 2018).

Apesar do registro consolidado de *R. harrisii* na Lagoa dos Patos, ainda existem lacunas importantes relacionadas à origem e estrutura genética das populações invasoras, às possíveis rotas de introdução, aos fatores ambientais associados à sua distribuição e aos possíveis impactos ecológicos sobre espécies nativas de crustáceos. Considerando que os estuários atuam como importantes ambientes de dispersão, conectividade ecológica e diferenciação populacional (Lacerda et al. 2016; Machado et al. 2020), compreender os processos ecológicos e biogeográficos associados à invasão de *R. harrisii* torna-se essencial para subsidiar estratégias de monitoramento e manejo.

Assim, esta tese pretende contribuir para o entendimento dos mecanismos envolvidos no estabelecimento e expansão de espécies invasoras em ambientes estuarinos subtropicais, fornecendo subsídios científicos relevantes para o monitoramento, manejo e conservação da biodiversidade no estuário da Lagoa dos Patos, e potencialmente auxiliar estratégias de conservação em outros estuários subtropicais e temperados.

HIPÓTESES

Neste contexto e considerando as informações abordadas ao longo da introdução, foram formuladas as seguintes hipóteses para cada um dos capítulos da tese:

Capítulo II

- Considerando o histórico de introdução de *R. harrisii* no estuário da Lagoa dos Patos desde a década de 1980, espera-se que a população brasileira apresente origem associada às populações nativas da costa atlântica da América do Norte, com baixa diversidade haplotípica decorrente de efeito fundador e introdução mediada por água de lastro.

Capítulo III

- Embora *R. harrisii* seja amplamente distribuído no Estuário da Lagoa dos Patos, sua ocorrência em áreas além desse ambiente nunca foi avaliada. Dada sua elevada plasticidade ecológica e ampla tolerância fisiológica, espera-se que a espécie esteja presente em todo o gradiente salino da Lagoa dos Patos.

Capítulo IV

- Devido ao histórico de sucesso invasivo de *R. harrisii*, a espécie apresenta elevada plasticidade e generalismo alimentar, utilizando recursos tróficos semelhantes aos das espécies nativas de caranguejos e potencialmente promovendo sobreposição de nicho trófico.

OBJETIVOS

Esta tese tem por objetivo principal compreender os processos ecológicos e biogeográficos associados à invasão do caranguejo *R. harrisii* na Lagoa dos Patos, por meio da avaliação de sua origem molecular, distribuição potencial em diferentes gradientes ambientais e interações tróficas com espécies nativas de caranguejos.

Os objetivos específicos estão organizados conforme os capítulos da tese:

Capítulo II

- Inferir possíveis rotas de invasão e vetores de introdução da espécie no sul do Brasil.
- Caracterizar a diversidade genética e haplotípica da população invasora de *R. harrisii* na Lagoa dos Patos.
- Comparar geneticamente a população brasileira com populações nativas e invasoras registradas em outras regiões do mundo.

Capítulo III

- Avaliar a distribuição espacial e a extensão potencial de ocorrência de *R. harrisii* ao longo do gradiente salino da Lagoa dos Patos.
- Identificar os principais fatores ambientais associados à ocorrência da espécie, considerando salinidade, temperatura da água, profundidade e matéria orgânica no substrato.

Capítulo IV

- Descrever a dieta natural de *R. harrisii* e das espécies nativas de caranguejos e siris que compartilham o mesmo habitat.
- Avaliar o grau de sobreposição de nicho trófico entre a espécie invasora e as espécies nativas.
- Identificar a amplitude de nicho e a plasticidade alimentar de *R. harrisii* ao longo dos diferentes ambientes amostrados.

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CAPÍTULO II

Origin of *Rhithropanopeus harrisii* (Crustacea: Panopeidae) invasion on the Southwest Atlantic coast

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Renan C. Machado [a], Maria C. S. Cortinhas [b], Bianca Possamai [c], Luiz Felipe C. Dumont [a]

a Programa de Pós-graduação em Oceanografia Biológica, Instituto de Oceanografia, Universidade Federal do Rio, Grande-FURG, Av. Itália, Km 8, s/n, Rio Grande, RS, 96203-900, Brasil.

b Instituto de Oceanografia, Universidade Federal do Rio Grande-FURG, Av. Itália, Km 8, s/n, Rio Grande, RS, 96203-900, Brasil.

c University of Vermont, Rubenstein Ecosystem Science Laboratory, Burlington, VT, United States.

Autor correspondente: Renan C. Machado

e-mail: rcmkssino@hotmail.com

Abstract

Exotic species can have large impacts on the communities and ecosystems where they are established. Understanding the invasion routes and the processes that allow the establishment of these exotic species is necessary to minimize their impacts. Genetic tools allow the comprehension of dispersion patterns, which is fundamental to forecasting the propagation of invasive species. The mud crab *Rhithropanopeus harrisii* is native to North America but has invaded different water bodies around the globe in the past century. To clarify the invasion routes and the population source of the mud crab in Lagoa dos Patos (Brazil), a partial region of CO1 was used and compared to sequences extracted from individuals from Europe and North America. The haplotype diversity found in Brazilian, European, and western North American samples was lower than that in the Gulf of Mexico and eastern North America (native areas). The genetic distances of the 11 analyzed populations were high (Phi-st > 0.8), and the shortest genetic distance was found between southern Brazil and North Carolina (eastern United States). Our results suggest that the population from North Carolina was the source of the Brazilian population, and the ballast water of ships that arrived at Rio Grande Port was likely the vector of this invasion.

Keywords: Ballast water, DNA, Ecological genetics, Exotic species, Invasive species, Mud crab

1. Introduction

The invasion of exotic species is a phenomenon that has exponentially grown in a globalized world. Although species occurrence ranges are naturally dynamic (Lodge, 1993), the dissemination of species mediated by human activities in both marine and terrestrial systems is much faster than their natural expansion (Ruiz et al., 2000; Rius et al., 2015). Activities such as maritime and river transportation, aquariums, aquaculture, and even marine litter are the major routes of invasive species introduction in aquatic systems. The continuous propagation of these species is contributing to biodiversity loss in many ecosystems worldwide, considering that they may be able to outcompete native species (Tavares and Mendonça Jr, 2004; Mineur et al., 2012; Peters et al., 2014; Bellard et al., 2016; Meyerson et al., 2019). In this sense, understanding the biology of an invasive species, the ecological niche that it may occupy, the origin and routes of its invasion, the establishment process, and its demographic history and growth are crucial for adequate management that aims to mitigate the impacts of invasive species on native species, as well as forecast and prevent future invasions.

The success of a new species introduction depends on the life history and physiological features of the species, such as high fecundity and a planktonic larval stage; its establishment and long-term success depend on genetic characteristics that determine the adaptability and plasticity of the species (Bock et al., 2015). Frequently, obtaining reliable information about the origin and dynamics of the invasion process is challenging because new species can be introduced from several geographically distant populations. Moreover, they can originate from secondary introductions that possibly went through sequential bottlenecks during the first introduction and establishment, creating unequally

sized populations that were partially isolated (Rius et al., 2015). Molecular genetics offers unique perceptions of the origin, routes, and mechanisms of propagation that complement observational records of invasive species (Handley et al., 2011). This tool has shown that multiple introductions are common during the process of colonization and demographic expansion, explaining how the populations overcome the founder effects associated with colonization (Handley et al., 2011).

Among the aquatic environments more susceptible to biological invasions, estuaries stand out due to the heavy impact by human activities, such as aquaculture and port activity (Wasserman et al., 2011; Magoro et al., 2015). Moreover, estuaries are transitional areas between marine and freshwater environments, furnishing a variety of essential conditions for the establishment of different species, such as the diversity of habitats and food resources, allowing them to function as ‘steppingstones’ for dispersion and migration among different latitudes (Lacerda et al., 2016). Therefore, estuaries are important systems for understanding the mechanisms involved in genetic differentiation and population and species evolution because they are discrete and isolated by physical and/or ecophysiological barriers that prevent or limit gene flow (Machado et al., 2020), which is especially important concerning biological invasions, considering that in many cases, it is possible to follow their entire evolutionary history.

Port activity in estuaries is one of the major factors responsible for the introduction of nonnative species around the globe (Hewitt et al., 2009; Bumbeer and Rocha, 2016). Ballast water and biofouling of marine structures are among the main vehicles of aquatic exotic species, considering that 80% of consumer goods are transported by ships through the global ocean (Wonham et al., 2001; Bradie et al., 2023). Microorganisms, larvae, and

adults of aquatic species are transferred across the oceans or within regions of the same country in ballast water (Wasson et al., 2001; Bilgin, 2022). The transportation of exotic organisms through ballast water is a current concern and may have serious consequences to native species, mainly due to competition for similar ecological niches, and disease transmission (Briski et al., 2012; Bradie et al., 2023). This was the most likely vector that introduced the mud crab *Rhithropanopeus harrisii* (Gould, 1841) in the Lagoa dos Patos Estuary, Brazil (D'Incao and Martins, 1998), considering that this estuary harbors one of the major ports of the country, the Port of Rio Grande, receiving ships from Asia, Europe, and the Americas, and with records of ballast water exchanges in the surroundings of Lagoa dos Patos Estuary (SUPRG/FURG, 2016).

The mud crab *R. harrisii* originally inhabited brackish and freshwater on the Atlantic Coast of North America (Williams, 1984). In 1874, the species was documented for the first time outside of its native area, in the Netherlands (Wolf, 1954). Between the 1930s and 1950s, the mud crab spread along the European coast, including in inland seas (Baltic, Black, and Caspian Seas) (Petersen, 2006; Simakova et al., 2017). In South America, the first and only record occurred in the 1990s in the Lagoa dos Patos Estuary (D'Incao and Martins, 1998), and more recently, this mud crab was also found in Japan (Iseda et al., 2007). In the last century, *R. harrisii* invaded more than 20 countries on four continents, covering an area of 45 degrees of latitude, most likely due to human mediation (Roche and Torchin, 2007). Many characteristics make *R. harrisii* a successful invader, including high fecundity, a planktonic larval stage with a duration of 7–26 days depending on the water temperature, tolerance to a large range of salinities and temperatures, and omnivory (Costlow et al., 1966; Turoboyski, 1973; Laughlin and French, 1989).

Although *R. harrisii* was registered for the first time in Brazilian waters in 1990 (D'Incao and Martins, 1998), no other reports occurred in the last 30 years in other estuaries in Brazil or South America. Therefore, understanding not only the origin of the invasion but also the status of the current population established in the Lagoa dos Patos Estuary is important for advancing the field of biological invasions. In this context, the objective of our study was to identify the possible routes of the *R. harrisii* invasion of the Lagoa dos Patos Estuary and detect the status of population expansion/retraction to obtain a better standing of the biological invasion processes driven by ballast water.

2.Materials and methods

2.1. Sample collection and processing

Individuals of *R. harrisii* were collected at three sites in the Lagoa dos Patos Estuary (Fig. 1) between June 2021 and January 2022 using two traps submerged at a depth of 1.5 m. The traps were filled with empty gastropod shells, which served as a refuge and attracted the mud crab due to its cryptic habit (Turoboyski, 1973). The traps were checked every 30 days, the animals were collected, and the traps were redeployed at the same location. The collected animals were kept on ice in the field and preserved in 90% ethanol in the laboratory.

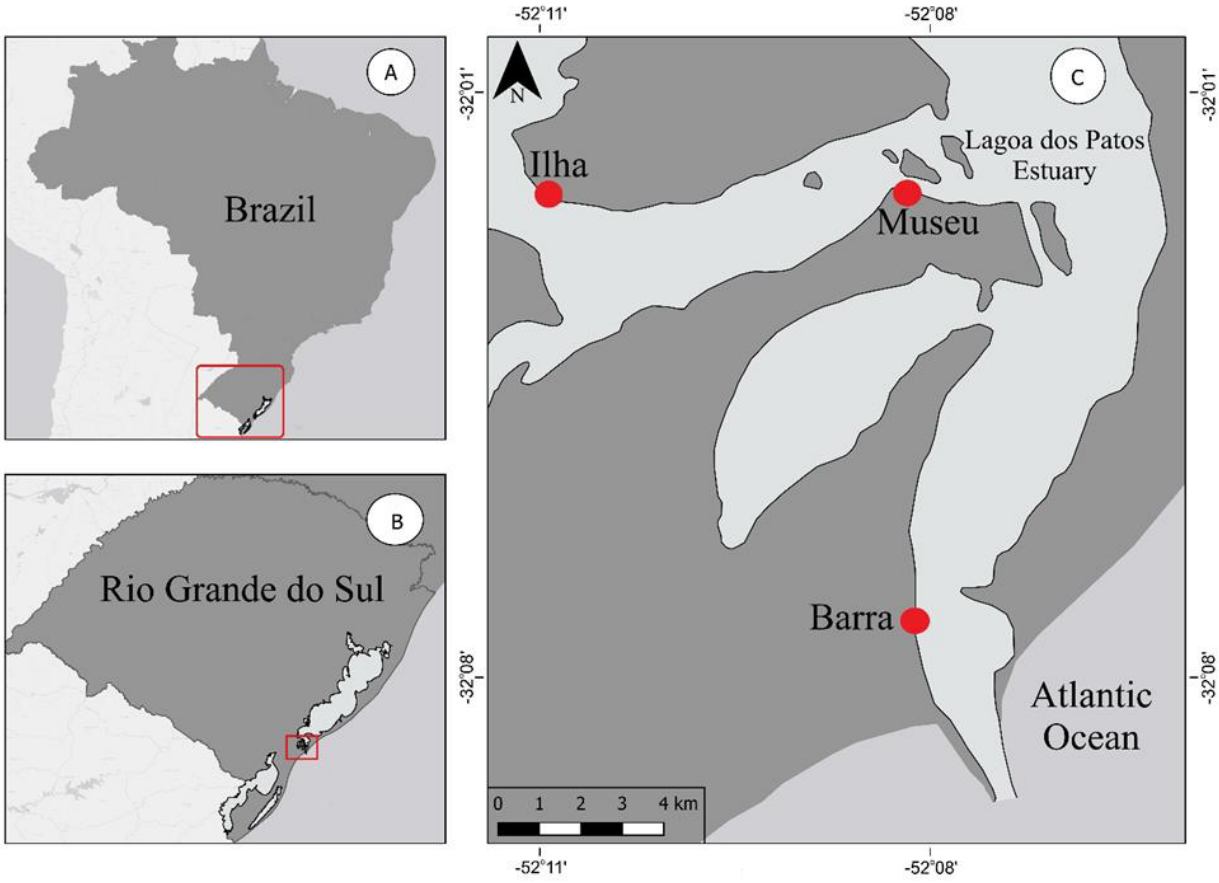


Fig. 1. Sampling sites in the Lagoa dos Patos Estuary. A) Map of Brazil with a red square highlighting Rio Grande do Sul State; B) Map of Rio Grande do Sul State with a red square highlighting the portion of Lagoa dos Patos sampled; C) Lagoa dos Patos Estuary. The red dots represent the three sampling sites. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

2.2. DNA extraction, amplification, and purification

Genomic DNA was extracted from mud crab legs using a modified phenol/chloroform method (Sambrook et al., 1989; Da Silva Cortinhas et al., 2010). DNA integrity and concentration were visually evaluated by electrophoresis in 1% agarose gels and quantified by spectrometry using a Biodrop® 2000. The amplification of 580 bp of

mitochondrial cytochrome *c* oxidase subunit 1 (COI) was performed by using specific primers for the target species. The primers Rhf - forward 5′ GGAGCATGAGCTGGTATAGTAGG 3′ and Rhr - reverse 5′CGACCCTGCTGGAGGAGGAGACCC 3’ were designed using the DNA sequences available in GenBank (National Center Biotechnology Information) for *R. harrisii* from the same region as the model (Projecto-Garcia et al., 2010; Boyle et al., 2010). OligoAnalyzer (PrimerQuest program, accessed on July 23, 2021) was used to verify the efficiency of those primers. The PCR was carried out in a final volume of 25 µL with the following components: buffer 1 X (10 mM, Tris-HCl, pH 8.5 and 50 mM KCl), 3 mM MgCl2, 0.4 mM dNTP, each primer at 0.2 pmol, 2.5 U of Taq DNA polymerase, and 40 ng of DNA. The amplification of the COI region was performed following Da Silva Cortinhas et al. (2022): 5 min of initial denaturation at 94 °C, 35 1-min cycles at 94 °C, 1 min at 64 °C, and 2 min at 72 °C, with a final extension at 72 °C for 5 min. The amplifications were performed using a Veriti™ Thermal Cycler thermocycler.

The products of the amplification were dyed with GelRed (0.001%) and run in 1% agarose gel immersed in Tris/borate/EDTA (TBE) buffer to evaluate the efficiency of the fragment amplification. A molecular weight marker (100 bp or 1 kb) was used to estimate the total size of the amplified fragments, and the results were visualized with a UVPR M20 UV transilluminator. The products with an adequate concentration were purified by precipitation with 20% polyethylene glycol (PEG) 8000 (Schmitz and Riesner, 2006) and resuspended in TE buffer (pH 7). After verification in agarose gel, the purified products were sequenced in both directions by the dideoxynucleotide chain-terminating technique

through capillary sequencing by the company ACTGene (https://actgene.com.br). The sequences are available from GenBank (access codes OR067777-OR067807).

2.3. Obtaining global sequences

Several sequences extracted from GenBank were used in the analysis to identify the origin of the invasion of *R. harrisii* in the Lagoa dos Patos Estuary. The locations and number of samples are provided in Table S1.

2.4. Data analysis

The 31 sequences obtained from individuals from the Lagoa dos Patos Estuary were manually edited using BioEdit 7.2.5 software (Hall, 1999), and the consensus sequence was obtained by aligning both forward and reverse sequences in Clustal W (Thompson et al., 1994), contained in the same software. These sequences were aligned and analyzed with another 200 sequences obtained from Projecto-Garcia et al. (2010), Boyle et al. (2010), and Petersen (2006), totaling 231 sequences with 523 bp (base pairs). The genetic diversity of each population (Phi-st), the number of polymorphic sites (S), the number of haplotypes (H), haplotype diversity (h), and nucleotide diversity (π) were evaluated using Arlequin 3.5 software (Excoffier and Lischer, 2010). Moreover, we performed an analysis of molecular variance (AMOVA) to detect population differentiation, calculated Tajima's D and Fu's Fs to verify the neutrality of the molecular markers and demographic processes such as expansion and bottlenecks (where high negative values indicate demographic expansion), and calculated the raggedness index (Harpending, 1994) and the sum of squared deviations (SSD) between the observed and expected mismatch distributions to validate the estimated expansion model (Schneider and Excoffier, 1999) using Arlequin 3.5 software (Excoffier and Lischer, 2010). According to Harpending (1994), the number of observed differences

among haplotype pairs is usually multimodal in populations in demographic equilibrium (i.e., stable populations, high raggedness index) and unimodal in populations that underwent recent demographic expansion (low raggedness index). The genetic structure among the groups of *R. harrisii* from the 11 analyzed localities was estimated by Phi-st (Φst) using the Tamura–Nei model of nucleotide substitution (Tamura and Nei, 1993), which was selected by jModelTest 2.1.10 (Darriba et al., 2012) as the best-fitting model for our data. The interpretation of Phi-st values was based on Wright (1978), considering that the populations have low differentiation when Phi-st ≤ 0.05, moderate differentiation when Phi-st is between 0.05 and 0.15, high differentiation when the values are between 0.15 and 0.25, and very high differentiation when Phi-st >0.25. A haplotypic network was built using PopART software with the median joining method (Bandelt et al., 1999).

3. Results

The 231 analyzed sequences presented a total of 64 haplotypes defined by 469 monomorphic sites and 54 polymorphic sites (36 parsimony-informative and 18 autapomorphic). The haplotype diversity was low in samples from Brazil, Europe (Bulgaria, Spain, the Netherlands, Poland, and Portugal), and the West Coast of the United States (California/Oregon), while samples from the East Coast of the United States and Gulf of Mexico (North Carolina, New Jersey, Texas) presented high haplotype diversity (Table 1). Nucleotide diversity presented similarly low values in the 11 studied populations (Table 1). The variance analysis showed that the populations were significantly different (p

= 0.00). The differences between populations were higher (58%) than those within populations (42%).

Table 1 Diversity of the mitochondrial DNA – region COI (523 bp) of the mud crab *Rhithropanopeus harrisii* in different locations. N: number of individuals; H: number of haplotypes; S: number of polymorphic sites; h: haplotype diversity; π: nucleotide diversity.

Locations	N	H	S	h	π
Brazil	31	3	2	0.333 ± 0.095	0.0006 ± 0.0007
Bulgaria	20	2	1	0.189 ± 0.108	0.0003 ± 0.0005
Netherlands	16	6	9	0.783 ± 0.072	0.0063 ± 0.0030
Poland	20	3	5	0.563 ± 0.063	0.0017 ± 0.0014
Portugal	20	3	5	0.573 ± 0.055	0.0042 ± 0.0027
Spain	20	6	7	0.794 ± 0.065	0.0053 ± 0.0032
USA - California/Oregon	25	5	9	0.470 ± 0.112	0.0049 ± 0.0030
USA - Louisiana	20	9	10	0.800 ± 0.072	0.0028 ± 0.0019
USA - New Jersey	19	6	5	0.725 ± 0.082	0.0026 ± 0.0019
USA - North Carolina	28	28	29	1.000 ± 0.009	0.0090 ± 0.0050
USA - Texas	12	12	9	1.000 ± 0.034	0.0063 ± 0.0039

The evaluation of the Phi-st values revealed that most of the 11 analyzed populations showed high differentiation. The pairwise test showed Phi-st > 0.80 for Brazil vs. Bulgaria, Brazil vs. Louisiana, Brazil vs. New Jersey, Brazil vs. Texas, Bulgaria vs. Louisiana, Bulgaria vs. New Jersey, Bulgaria vs. Texas, Louisiana vs. Poland, and Louisiana vs. New Jersey (p = 0.00) (Table 2), suggesting that these populations may be going through a speciation process. Short genetic distances were found between the populations of the Netherlands and Portugal (Phi-st = 0.03; p = 0.16) and the Netherlands and Spain (Phi-st = 0.05; p = 0.07) (Table 2). The remaining populations presented Phi-st

values between 0.05 and 0.80, indicating moderate to long genetic distances (p = 0.05) (Table 2).

Table 2 Pairwise Phi-st values for the *Rhithropanopeus harrisii* populations from Brazil, Europe, and the United States.

Locations	1	2	3	4	5	6	7	8	9	10	11
1 - Brazil	0.00										
2 - Bulgaria	0.87	0.00									
3 - Netherlands	0.62	0.49	0.00								
4 - Poland	0.69	0.25	0.33	0.00							
5 - Portugal	0.64	0.63	0.03	0.42	0.00						
6 - Spain	0.55	0.27	0.05	0.10	0.13	0.00					
7 - USA (California/Oregon)	0.45	0.61	0.43	0.48	0.45	0.39	0.00				
8 - USA (Louisiana)	0.89	0.84	0.68	0.79	0.75	0.68	0.76	0.00			
9 - USA (New Jersey)	0.81	0.81	0.14	0.67	0.12	0.35	0.59	0.81	0.00		
10 - USA (North Carolina)	0.25	0.44	0.39	0.34	0.40	0.34	0.09	0.67	0.53	0.00	
11 - USA (Texas)	0.85	0.81	0.63	0.76	0.71	0.64	0.72	0.26	0.77	0.63	0.00

The raggedness indices of Brazil, Bulgaria, Spain, the Netherlands, Poland, Portugal, and California/Oregon showed values higher than 0.1, suggesting that these populations are in demographic equilibrium (stable over time). The populations of North Carolina, Louisiana, New Jersey, and Texas presented low raggedness indices (between 0.01 and 0.06), suggesting that they recently underwent demographic expansion (Table 3).

Table 3 Parameters of the sudden population growth model, goodness-of-fit, and Raggedness index (RI) of the mud crab *Rhithropanopeus harrisii* based on COI region of the mitochondrial DNA. θ0 is the population size before expansion; θ1 is the population size after expansion; and τ is the expansion parameter. SSD is the sum of square deviations of the goodness-of-fit model and the respective p- values.

Locations	population growth model			goodness-of-fit model		Raggedness index	
	Theta 0 (θ_0)	Theta 1 (θ_1)	Thau (τ)	SSD	P	RI	P

Brazil	0.500	0.387	0.412	0.230	0.16	0.215	0.22
Bulgaria	0.281	0.280	0.201	0.015	0.25	0.421	0.45
Netherlands	2.712	3.341	4.852	0.066	0.13	0.105	0.27
Poland	1.409	0.921	0.743	0.036	0.12	0.220	0.16
Portugal	1.409	2.242	4.488	0.273	0.12	0.675	0.01
Spain	1.973	2.773	3.912	0.050	0.23	0.101	0.34
USA - California/Oregon	2.383	2.613	6.727	0.293	0.00	0.389	0.97
USA - Louisiana	2.818	1.468	1.546	0.001	0.86	0.059	0.52
USA - New Jersey	1.430	1.403	0.676	0.009	0.50	0.062	0.71
USA - North Carolina	7.452	4.732	4.115	0.001	0.85	0.019	0.66
USA - Texas	2.980	3.303	3.621	0.011	0.43	0.053	0.48

The pairwise analyses of mismatch distributions among haplotypes showed multimodal distributions for the populations of Brazil, Bulgaria, Spain, the Netherlands, Poland, Portugal, and California/Oregon (populations in equilibrium). However, the populations of North Carolina, Louisiana, New Jersey, and Texas showed unimodal distributions, representing demographic expansion (Fig. 2). Neutrality tests corroborated the previous results, showing lower values (highly negative) for the populations that presented demographic expansion and higher values for the populations in equilibrium (Fig. 2). The distribution models were accepted on the basis of the pSSD values (Table 3)

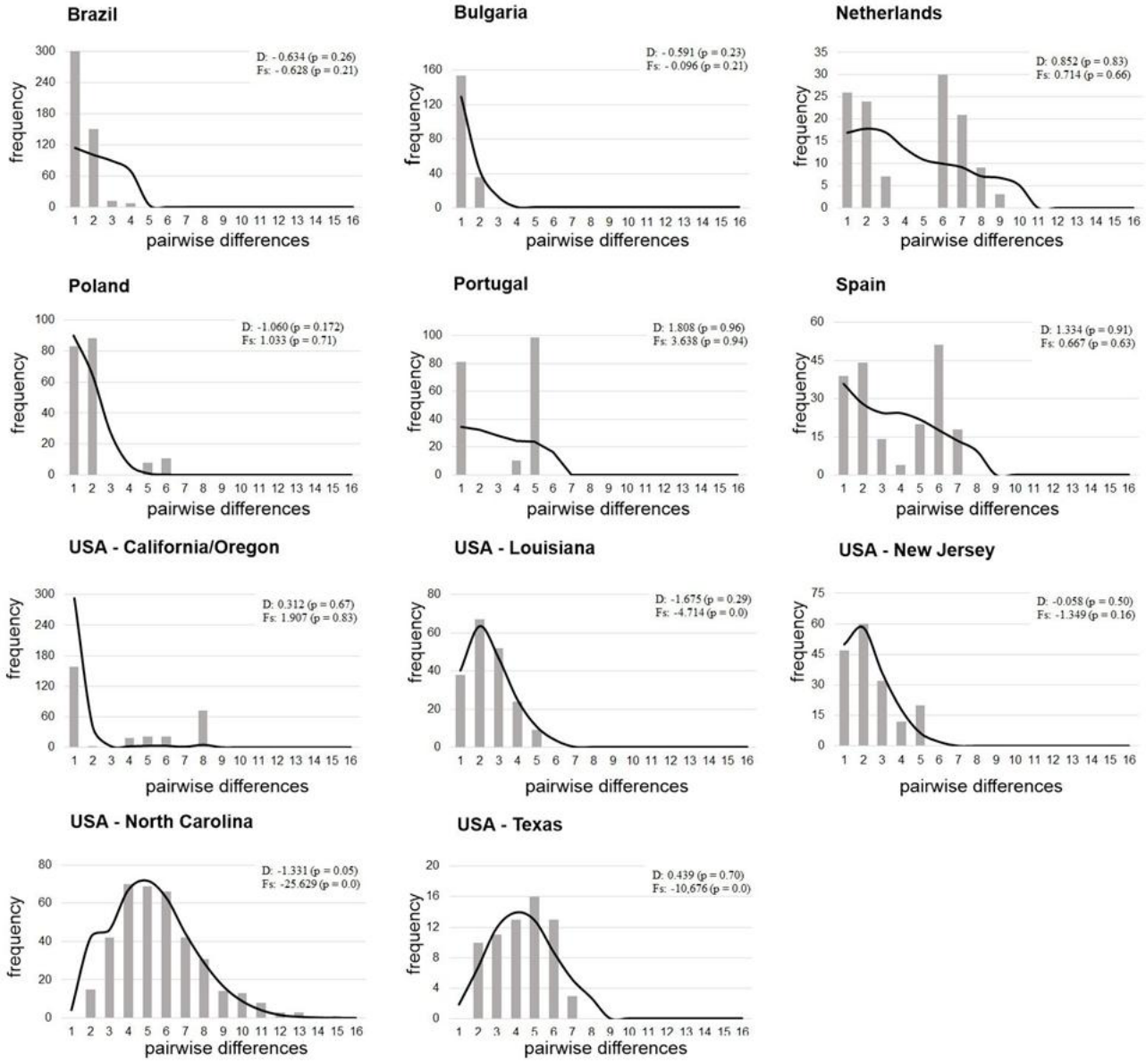


Fig. 2. Pairwise mismatch distributions (bars), sudden growth model (black line), and Tajima's D and Fu's Fs test values with associated probabilities for each studied population of the mud crab *Rhithropanopeus harrisii* based on the partial region of COI from mitochondrial DNA.

The haplotypic network (Fig. 3) showed that populations from Texas and Louisiana (United States) formed a group at one extreme end of the network by sharing two haplotypes (Haps 18 and 19) and presenting exclusive haplotypes in each population. At the other extreme of the network, there was a second group formed by haplotypes from North Carolina (United States), with some shared with California/Oregon (Hap 39), Europe (Bulgaria, the Netherlands, Poland, and Spain) (Hap 4) and Brazil (Hap 1). The third group was formed by the Brazilian population, with two exclusive haplotypes and one shared with North Carolina (Hap 1). The New Jersey haplotypes formed a fourth group, sharing haplotypes with California/Oregon (United States) (Haps 6 and 8) and the European populations from the Netherlands, Poland, Portugal, and Spain (Hap 8 and 9). All populations had exclusive haplotypes, except that of Poland, which presented three haplotypes shared with populations from the United States (California/Oregon, New Jersey, and North Carolina) and Europe (Bulgaria, the Netherlands, Spain, and Portugal) (Haps 4, 6 and 8) (Fig. 3). The network patterns suggested that these populations were structured into 4 groups: Group 1, Brazil; Group 2, Europe and New Jersey; Group 3, Gulf of Mexico (Louisiana and Texas); and Group 4, North Carolina and California/Oregon. Based on these results, the dispersion routes of *R. harrisii* are proposed in Fig. 4.

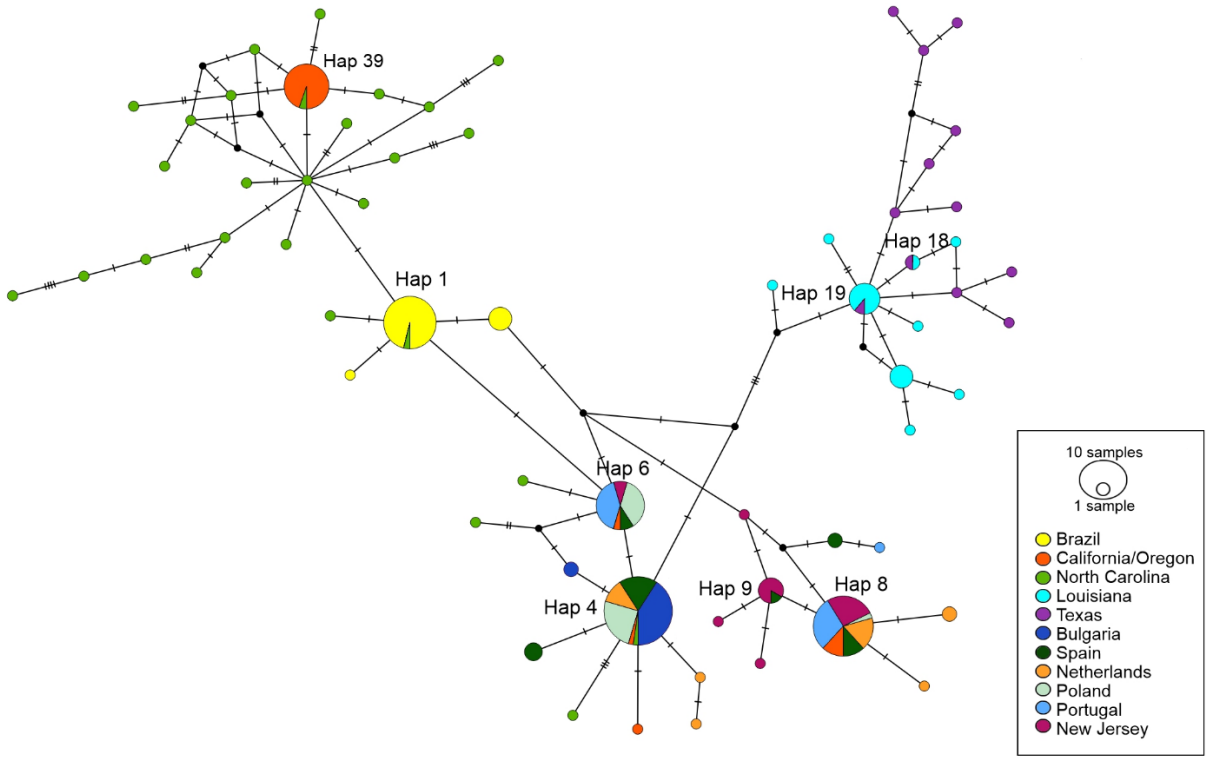


Fig. 3. Median joining haplotype network based on 523 bp from the COI region of mitochondrial DNA of the mud crab *Rhithropanopeus harrisii* sampled in 11 localities (see Table S1 for more information). Circles represent haplotypes, and the size of the circle is proportional to its frequency of occurrence. Different colors represent different localities, and lines denote the putative mutational steps between haplotypes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

4. Discussion

Biological invasions are a frequent threat to local biodiversity and to ecosystems and their services. Identifying the origin and routes of biological invasions in aquatic systems is fundamental for appropriate species management and environmental policy-making.

Here, we identified the probable origin of the mud crab *R. harrisii* in the Lagoa dos Patos Estuary, as well as the growth status of the established population. We found that the population of *R. harrisii* in the Lagoa dos Patos Estuary has lower genetic diversity than the populations in the native region and invasive populations in Europe (except for the Bulgarian population, which presented the lowest genetic diversity among the analyzed populations). Populations founded by a small number of individuals have low genetic diversity and a smaller pool of haplotypes than their source population (McCartney et al., 2019). However, colonizers can still establish growing populations that can persist in the long term regardless of the expected reductions in genetic diversity (McCartney et al., 2019). Therefore, it is likely that a small number of founder individuals were established in the Lagoa dos Patos Estuary, and due to the large geographic distance, there has been no flow with other populations, culminating in low genetic diversity.

The population of *R. harrisii* in the Lagoa dos Patos Estuary presented a smaller number of haplotypes than populations from the United States and Europe, except Bulgaria. These results are likely due to genetic drift, such as a persistent bottleneck effect due to the small population size during the colonization process (Tajima, 1989), or due to the introduction of fewer individuals representing the original population (founder effect) in the colonized areas, leading to low genetic variability. Although this is expected for individuals from recently founded populations, considerable variation in diversity was observed in populations from the Netherlands and Spain, where the haplotypic variability was highly similar to that of the New Jersey (United States) population, with some haplotypes shared between these three regions. The haplotypic pattern found in the Dutch and Spanish populations could be due to several colonization events, more colonizers, or

bottleneck events during the colonization of other European populations (Bulgaria, Portugal, and Poland) (Projecto-Garcia et al., 2010). The remaining European populations, as well as the population from the West Coast of the United States (California/Oregon), also presented fewer haplotypes than the native population from the East Coast of North America. The populations of the native region – the Gulf of Mexico (Louisiana and Texas, United States) – presented high haplotype diversity, being isolated among themselves and genetically distinct from the European populations, likely not having contributed to their colonization.

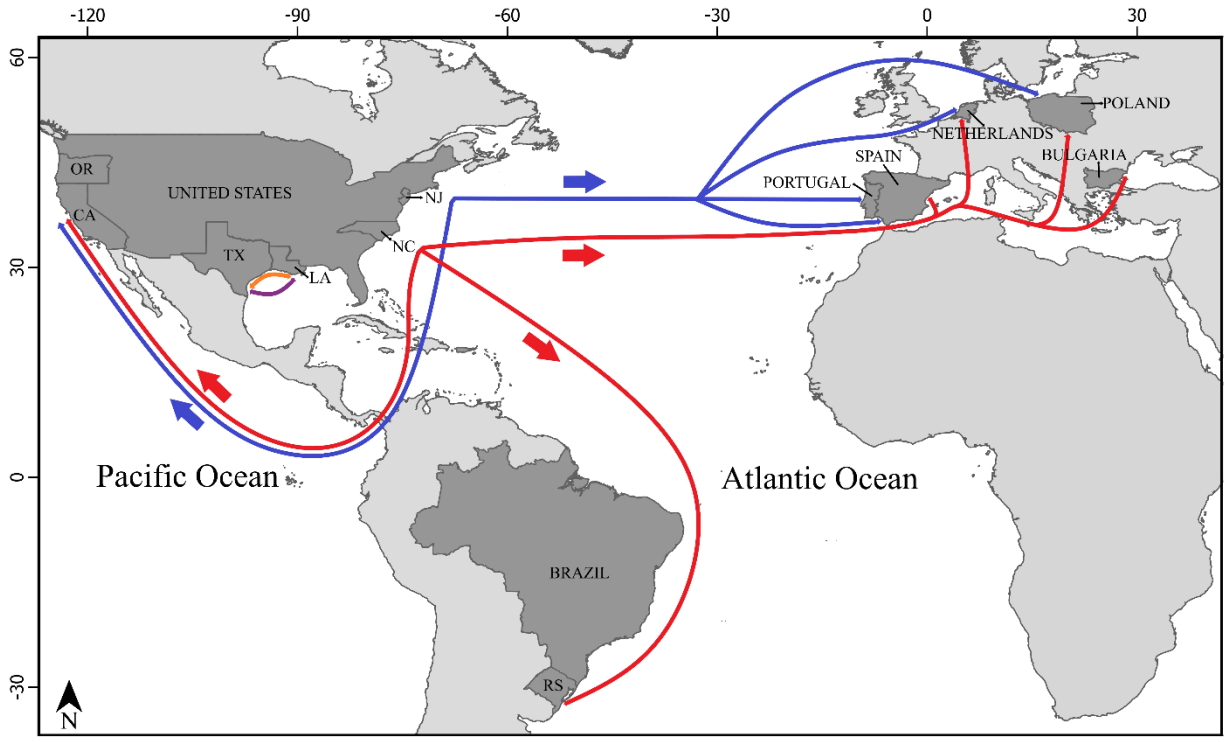


Fig. 4. Representation of the dispersion history of *Rhithropanopeus harrisii* among the studied regions. Blue lines indicate that the original region of the invasion was New Jersey (NJ); red lines indicate populations originating from individuals from North Carolina (NC); orange and purple lines indicate the restricted movement of *R. harrisii* between Louisiana (LA) and Texas (TX) (Gulf of Mexico population). The arrows indicate the direction of the

movement. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

Concerning nucleotide diversity, the values were considered low ($\pi = 0$) in all populations that we analyzed, including the native ones. Considering that the COI gene in crustaceans presents an average mutation rate of 1.40–2.60% per million years (Knowlton et al., 1993; Schubart et al., 1998), the probability of mutation during colonization is low. Moreover, it is possible that the number of individuals analyzed was not sufficient to represent all the existing genetic variability in the regions; thus, a larger number of specimens from the founder population is needed. Low raggedness index values were found in populations from the native areas (Louisiana, New Jersey, Texas, and North Carolina), which indicates that these populations recently underwent demographic growth, while the remaining populations (i.e., invasive populations) presented higher values representing equilibrium (stability). These results agreed with the pairwise mismatch distributions among haplotypes (in that populations undergoing expansion presented a unimodal distribution and stable populations presented a multimodal distribution), the results of Tajima's D and Fu's Fs neutrality tests, and the low nucleotide diversity found. This low nucleotide diversity found in the Lagoa dos Patos Estuary population may indicate that this population is experiencing a bottleneck effect or that the founders are still becoming established. However, it is unlikely that a bottleneck is responsible for this low diversity in the Lagoa dos Patos Estuary, considering that the first occurrence of the species in the estuary was detected only in the 1990s (D'Incao and Martins, 1998), and this population still has not reached a large size. Considering that *R. harrisii* was likely introduced to the

Lagoa dos Patos Estuary through ballast water, a ‘founder effect’ is the most likely explanation. The ‘founder effect’ is generally observed in species with introduced populations (Fu, 1997; Excoffier et al., 2005), such as Brazilian and European populations. Moreover, the Phi-st values showed that the Brazilian population is isolated, not exchanging genes with any of the populations analyzed in the present work.

The haplotype network showed eight haplotypes that were shared among all the analyzed samples, and the population structure considering the frequency and composition of haplotypes showed 4 defined groups (Brazil, Europe + New Jersey, Gulf of Mexico, and North Carolina + California/Oregon). The Lagoa dos Patos Estuary shares only one haplotype with North Carolina, and all other haplotypes are exclusive to Brazil. This fact is likely due to geographic isolation, as the North American and European populations are more than 8000 km away from the Brazilian population. The one haplotype that is shared with North Carolina suggests that this was the source population for the invasion of the Lagoa dos Patos Estuary, and considering that this estuary harbors a large port, ballast water and/or biofouling on ship hulls are the most likely routes. The second group is formed by populations from all European countries that we analyzed and individuals from both coasts of the United States (New Jersey, North Carolina, and California/Oregon), with several haplotypes shared among these populations. The short distance between European regions and the fact that the European continent started global navigation in the XV century facilitated the dispersion of the species and increased the chances of population establishment, which in turn increased the gene flow among populations and then haplotype diversity. In contrast, the population from the Lagoa dos Patos Estuary is young (~30 years), making it the least diverse region examined in this work. The Gulf of Mexico regions

(Louisiana and Texas) formed an isolated group, sharing haplotypes. These isolation patterns can be explained by the proximity of these sampled regions or by the phylogeographic rupture between the Atlantic Coast of North America and the Gulf of Mexico (Avise, 1992). This differentiation among all the groups may be an indicator of the different demographic histories of *R. harrisii* (Boyle et al., 2010). The last group is formed by haplotypes exclusive to North Carolina, one haplotype shared with California/Oregon and another shared with Brazil, which may indicate that the origin of the *R. harrisii* population of the western North American coast is the same as that of the Brazilian population: the North Carolina population. The populations of North Carolina and New Jersey shared the greatest number of haplotypes among all the studied populations. In total, these regions shared 6 haplotypes, where North Carolina shared haplotypes with Brazil, California/Oregon, and Europe, while New Jersey shared haplotypes with Europe and California/Oregon. Due to this pattern, we inferred that the North Carolina population is the source population of the Brazilian and California/Oregon populations, and the New Jersey, North Carolina, and California/Oregon populations may be the source populations of the European populations, which probably went through more than one colonization event. This would also explain the high sharing and variability of haplotypes in the European region.

The Lagoa dos Patos Estuary harbors the Port of Rio Grande, which is the fifth-largest port in Brazil in terms of cargo movement (Bueno, 2023). The vessel traffic in and out of the port of Rio Grande coming from all continents started to intensify at the beginning of the XX century after the construction of the breakwaters at the entrance of the estuary and the increase in channel draft to access the port (Neves, 2008). With the increase

in ship flow, we can assume that the amount of ballast water dumped into the Port of Rio Grande surroundings increased proportionally to this increase in movement, which is mainly responsible for the introduction of exotic species in aquatic systems (Werschkun et al., 2012; Katsanevakis et al., 2014). Some estimates indicate that more than 12 billion tons of ballast water are transported yearly around the world, with approximately 7000 species carried together with the water daily (IMO/MEPC, 1998; Carlton, 2001). Based on the invasion history of several crustacean species on the Brazilian coast (Moraes et al., 2022; Rosa et al., 2019; Rodrigues et al., 2014; Tavares and Mendonca Jr, 2011), we can assume that ballast water, or as a more remote possibility, fouling communities brought on the ships' hulls, was the vector of *R. harrisii* invasion in the Lagoa dos Patos Estuary. Alternatively, the dissemination of the mud crab on the European continent and to the West Coast of North America occurred through the oyster trade, as a regular trade route between New England, the western American coast, and Europe has been active since the XVII century (Projecto-Garcia et al., 2010). This was concluded due to the first record of *R. harrisii* on the European continent being in 1874 in the Netherlands (Wolf, 1954), a period when no ballast water was used in vessels. However, the first record of the mud crab in Brazil occurred only in the 1990s (D'Incao and Martins, 1998), and the use of ballast water was already established.

The main objective of the present work was to identify the origin of the invasion of the mud crab *R. harrisii* in the Lagoa dos Patos Estuary. The analysis of the mitochondrial COI gene (523 bp) showed the shortest genetic distance (Phi-st) and the most frequent haplotype sharing between Brazil and North Carolina (United States). Therefore, we suggest that North Carolina individuals were the founders of the Brazilian population, and

at least one female of the colonizer population in the single colonization event had this shared haplotype. The mutation rate for the analyzed region of the DNA is low, and the reduced genetic diversity that we found suggests that the introduction of *R. harrisii* in the Lagoa dos Patos Estuary occurred recently through ballast water from the East Coast of the United States.

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CAPÍTULO III

Environmental drivers of potential distribution and expansion of the invasive mud crab *Rhithropanopeus harrisii* (Gould, 1841) in a subtropical lagoon in southern Brazil

Artigo submetido no periódico ***Regional Studies in Marine Science***

Renan C. Machado [a*], Bianca Possamai [b], Caroline V. Bernabé [a], Felipe C. Dumont [a]

a Programa de Pós-Graduação em Oceanografia Biológica, Instituto de Oceanografia, Universidade Federal do Rio Grande-FURG, Av. Itália, Km 8, s/n, Rio Grande, RS 96203-900, Brazil.

b University of Vermont, Rubenstein Ecosystem Science Laboratory, Burlington, VT, United States.

* Corresponding author: E-mail: rcmkssino@hotmail.com.

Bianca Possamai: biancapossamai@hotmail.com

Caroline V. Bernabé: vettorazzicarol@gmail.com

Luiz Felipe Cestari Dumont: felipecdumont@gmail.com.

Abstract

The mud crab *Rhithropanopeus harrisii*, native to North America, was first recorded in estuarine waters of Southern Brazil in 1980. Thirty-five years have passed since its first detection, and no assessment regarding the species distribution, expansion, and population status has been made. This study evaluated the potential distribution and expansion patterns of the invasive mud crab in Patos Lagoon, south of Brazil, using ecological modeling such as MaxEnt and GAMs. Results show that this invader has a wide range of adequate habitats in this lagoon system, especially in the estuarine zones, but also can use freshwater areas. Mud crab distribution is strongly influenced by organic matter in the sediment, salinity, and depth. The high ecological plasticity and physiological tolerance of *R. harrisii* favor its expansion into habitats with distinct environmental conditions, including oligohaline zones and freshwater. The concordance between MaxEnt and GAM models supported the robustness of the observed ecological patterns, indicating that the sedimentary and hydrological gradients play a central role in the habitat suitability of this species. We demonstrated, for the first time, that *R. harrisii* expanded its range to freshwater portions of Patos Lagoon. Although our models were limited by the low number of occurrence reports, the results corroborated the elevated invasive potential of *R. harrisii* in the Patos Lagoon and highlighted the importance of continuous monitoring programs and ecological modeling as tools to understand biological invasion processes and to support management strategies for ecosystem conservation.

Keywords: estuarine ecology, invasive species, MaxEnt, mud crab, Species Distribution Modeling (SDM)

1. Introduction

The expansion and distribution of invasive species are one of the main ecological challenges nowadays, deeply affecting biodiversity, ecosystem functioning, and economic activities (Magliozzi et al. 2020; Polce et al. 2023). When introduced to a system where they do not naturally occur, whether by accidental transport, international trade, farming, or climate change, invasive species frequently find suitable conditions for establishment and rapid population growth (Peterson 2003). The expansion of invaders is usually facilitated due to the absence of natural predators, efficient competitors, or diseases that maintained their populations under control in their original habitats (Torchin et al. 2003). Consequently, these invasive species can explore local resources in a very efficient manner, competing with native species for food and habitat, changing ecological cycles, and, in many cases, declining native population and even leading to local extinctions (Guy-Haim et al. 2018). Moreover, climate change may broadens suitable areas for survival and dispersion of these organisms, allowing for their expansion (Stachowicz et al. 2002; Bellard et al. 2018). Therefore, the evaluation of the availability of suitable habitats to potential invaders is fundamental for the decision-making process of management actions prioritizing the conservation of native species.

Species Distribution Models (SDMs) are an important tool to understand the relationship between organisms and environmental variables, allowing the identification of potential habitats, prediction of occurrence, and supporting conservation and management plans (Elith and Leathwick, 2009). In estuarine and lacunar systems, there are relatively few studies using this approach, although correlative models such as Maximum Entropy (MaxEnt) stand out due to their high prediction power, ability to use presence-only data, and efficiency in

representing non-linear relationships between species and environmental factors (Phillips et al. 2006; Elith et al. 2011; Merow et al. 2013). Moreover, the integration of physical, chemical, and nutritional variables make these models suitable for benthic organisms, allowing the identification of the main factors associated with species distribution, and also to predict scenarios of expansion, establishment, or conservation status (Robinson et al. 2011; Becker et al. 2020). Generalized Additive Models (GAMs) provide a complementary approach by allowing the assessment of potentially non-linear relationships between species occurrence and environmental variables through flexible smooth functions, helping to identify ecological responses and environmental conditions associated with species distribution (Hastie and Tibshirani 1990; Wood 2017). Therefore, SDMs and algorithms such as MaxEnt and Generalized Additive Models (GAMs) have been widely used to understand ecological patterns and support management and monitoring actions of native and invasive species in aquatic systems (Guisan and Zimmermann 2000; Elith and Leathwick 2009; Becker et al. 2020).

In aquatic systems, invasive species are mainly introduced via ballast water and ship hull fouling; larvae are more frequently transported by the former, and juvenile and adult stages by the latter (Carlton and Cohen, 2003; Costello et al. 2007). This transportation allows eggs, larvae, juveniles and adults to travel across oceans and regions in the same territory (Wasson et al. 2001). Ballast water was likely the vessel of mud crab *Rhithropanopeus harrisii* (Gould 1841) introduction in the Patos Lagoon estuary, Brazil (D'Incao and Martins 1998, Machado et al. 2025), a native species of brackish to freshwater of North America Atlantic coast (Williams 1984). Many attributes contribute to *R. harrisii* invasive success, including high fecundity, planktonic larval stage with duration between 7 and 26 days (depending on water

temperature), wide salinity and temperature tolerance (euryhaline and eurythermal species), and omnivorous habits (Costlow et al. 1966; Turoboyski 1973; Laughlin and French 1989; Machado et al. 2026). This mud crab is a generalist species with high trophic plasticity, which allows it to adjust its diet to the resource availability (Turoboyski 1973; Hegele-Drywa and Normant 2009; Machado et al. 2026), and also to occupy a wide range of habitats, from soft to rocky bottoms with macroalgal communities in salinities from 1 to 25 (Fowler et al. 2013). Therefore, ecological plasticity favored the dispersion and invasion of *R. harrisii* to a diversity of estuarine systems around the world. The first record of the mud crab outside its native range occurred in 1874 in the Netherlands (Wolf 1954). In the XX century this species spread to more than 20 countries in the European coast and its inland seas (Baltic, Black, and Caspian), Central and South America, and Japan (Petersen 2006; Iseda et al. 2007; Roche and Torchin 2007; Simakova et al. 2017). In South America, the first record occurred in 1990 in the Patos Lagoon estuary (D'Incao and Martins, 1998). Currently, this mud crab has records of established populations in geographic, demographic and genetic expansion in Europe, Asia, Pacific coast of North America, and South America (Iseda et al. 2007; Projecto-Garcia et al. 2010; Fowler et al. 2013, Machado et al. 2025).

Patos Lagoon is the largest lagoon in South America, harboring a port in its estuarine zone with intense trade activity, which enables species invasions (Machado et al. 2025, 2026). The estuary has high benthic diversity, supporting many socioeconomic activities, especially artisanal fisheries (Vieira and Rangel 1988; Seeliger and Odebrecht 2010). Therefore, the introduction of exotic species such as *R. harrisii* represents a potential threat to the ecological equilibrium of this ecosystem, as biological invasions can alter benthic community structure, affecting native species, especially bivalves and small crustaceans (Hegele-Drywa and

Normant 2009; Aarnio et al. 2015). Considering the ecological and socioeconomic relevance of Patos Lagoon and the ecological plasticity of *R. harrisii*, the comprehension of the potential distribution and expansion of this invader in estuarine and freshwater portions of the lagoon is essential. This study aimed to evaluate the current distribution of *R. harrisii* and its spatial colonization patterns along the salinity gradient of Patos Lagoon, identifying the main drivers associated with this species occurrence.

2. Material and Methods

2.1. Study area

Sampling sites were distributed in freshwater and estuarine zones of Patos Lagoon (Fig. 1). Patos Lagoon extends over approximately 10,360 km² and is recognized as the world's largest strangled lagoon, forming an important estuarine system in southern Brazil. The estuarine portion of the lagoon is located in its southern sector (31°–32° S; 51°–52° W) and accounts for approximately 10% of the total lagoon area. The Patos Lagoon system exhibits a salinity gradient strongly influenced by freshwater discharge from the Guaíba, Camaquã, and Jacuí rivers in the northern portion of the lagoon and by seawater intrusion through the Barra channel in Rio Grande (Garcia 1998). This dynamic creates spatial and temporal variation in environmental conditions, making this estuary a very heterogeneous environment (Seeliger and Odebrecht 2010).

2.2. Data collection and sample processing

Biological samples were collected using traps made of plastic crates (30 × 22 × 15 cm; 15 liters volume) filled with gastropods and bivalve shells (for more details see Machado et al. 2026). Five sites along the salinity gradient were sampled (‘Barra’ close to the estuary mouth, ‘Museu’, ‘Ilha’ in the intermediate sectors of the estuary, and ‘Pelotas’ and ‘São Lourenço do Sul’ in the freshwater section of the lagoon; Fig. 1). At each site, one trap was attached to a pier and deployed at shallow waters (Ilha and São Lourenço do Sul at 1.0 m, Museu at 1.5 m, and Barra and Pelotas at 2.0 m depth), ensuring the traps were submersed at all times. Traps were checked once a month from March 2022 to February 2023. After retrieving the traps, shells were inspected, and all crabs removed from shells and traps, stored in plastic bags, and kept on ice. Shells were then washed with field water, placed inside the traps, and the traps were redeployed at the same site and depth.

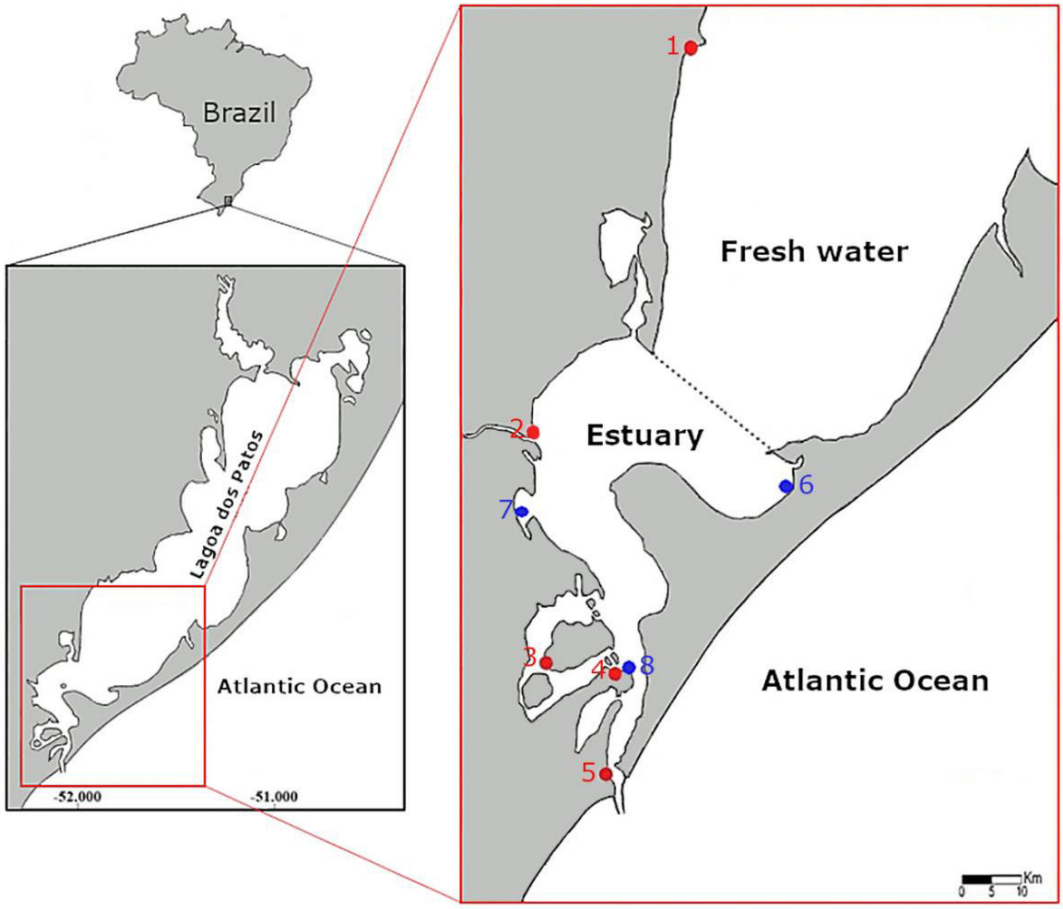


Figure 1. Location of Patos Lagoon in Brazil. The insert on the left shows the portion of the lagoon where samples were collected. The right panel shows sampling sites in red **(**1- São Lourenço do Sul, 2- Pelotas, 3- Ilha, 4- Museu, 5- Barra); blue points represent *Rhithropanopeus harrisii* occurrence retrieved from the GBIF database (6- Justino, 7- Saco do Silveira e 8- Porto Novo).

Sampled organisms were preserved in 4% formalin for 24h, and after this period they were stored in 70% ethanol. The specimens were processed in laboratory where they had sex identified, followed by measurements of carapace width (mm), abdomen width (mm), and total weight (g). All data was stored at Global Biodiversity Information Facility - GBIF

(Machado and Dumont 2025). Species occurrence data was also checked in the GBIF (*Rhithropanopeus harrisii* (Gould, 1841) in GBIF), and three other sites of occurrence were detected and included in our models (Justino, Saco do Silveira, and Porto Novo; Fig. 1).

2.3. Environmental variables

To evaluate the distribution patterns of *R. harrisii* in Patos Lagoon, we used abiotic variables collected during field surveys, including sediment organic matter (OM, %), water depth (m), salinity, and water temperature (°C). These variables were selected based on their ecological relevance to R. harrisii, as salinity and temperature can influence its development and distribution, while depth and sediment characteristics are associated with the availability and suitability of benthic habitats (Costlow 1966; Fowler et al. 2013), as well as on their availability for standardized measurement during field surveys. Water temperature and salinity were measured *in situ* during each sampling event using a multiparameter probe (YSI ProQuatro®). Water depth was measured using a graduated rope equipped with a weighted end, which was lowered vertically until it reached the sediment surface. Sediment organic matter was estimated from samples collected at the same locations as the biological surveys. Approximately 30 g of surface sediment (upper 5 cm) were collected and dried at 50°C for 24 h. Subsequently, a 10 g subsample was combusted in a muffle furnace at 600 °C for 30 min. After combustion, samples were cooled in a desiccator for 30 min and reweighed. Organic matter content (%) was determined using the loss-on-ignition (LOI) method as the difference between dry weight and post-combustion weight (Dean 1974). Mean values of OM, temperature, salinity, and depth recorded at each sampling site were used in the

distribution models. For occurrence sites obtained from GBIF, environmental information available in the database was complemented with historical records from the Laboratório de Ecologia e Pesca de Crustáceos (LEPEC; unpublished data). Mean values of temperature, salinity, depth, and sediment organic matter for each site were used as environmental predictors in the models (Table 1).

Table 1. Average ± standard deviation of abiotic variables used in the distribution models for *Rhithropanopeus harrisii* in the Patos Lagoon. OM is organic matter in the sediment.

Site	lon	lat	Temperature (°C)	Salinity	Depth (m)	OM (%)
Barra	-52.1040	-32.1343	20.0 ± 4.6	19.5 ± 9.6	2.0	0.479 ± 0.26
Porto Novo	-52.0917	-32.0298	20.5 ± 4.5	18.5 ± 9.5	2.0	0.925 ± 0.21
Museu	-52.1054	-32.0260	20.4 ± 4.8	17.2 ± 9.7	1.5	1.293 ± 0.20
Ilha	-52.1981	-32.0262	21.8 ± 5.8	15.9 ± 9.7	1.0	0.464 ± 0.03
Saco do Silveira	-52.2428	-31.8681	21.2 ± 5.6	13.4 ± 6.5	2.5	0.598 ± 0.29
Pelotas	-52.2352	-31.7860	19.9 ± 6.1	7.0 ± 6.3	2.0	0.603 ± 0.16
Justino	-51.8167	-31.8167	20.1 ± 5.1	5.4 ± 5.1	2.5	0.721 ± 0.30
São Lourenço do Sul	-51.9659	-31.3759	20.5 ± 5.7	3.6 ± 3.7	1.0	0.608 ± 0.35

2.4.Data analysis

2.4.1. Spatial interpolation

Because environmental variables were available only for the sampling sites, continuous spatial data were interpolated using the Inverse Distance Weighting (IDW) method (Li and Heap 2008), which gives proportionally stronger influence to the neighboring sites than to the locations spatially distant for the interpolated values. For each environmental variable, a unique continuous raster with 0.01-degree resolution was built, and later all raster layers were

stacked into a single set of environmental predictors used in the modeling (Li and Heap 2008). We generated 5,000 random points of pseudo-absence (background points) distributed inside the study area (Phillips et al. 2009) to represent the habitat available for the species, which were used by the model to compare environmental conditions in sites with species occurrence and the conditions available in the landscape. Part of the southeastern area of the shapefile used (Lagoa Mirim) does not represent a suitable habitat for the species and was removed from the spatial analysis to avoid ecologically unreliable extrapolations. Spatial raster and vector data were manipulated using 'terra' (Hijmans et al. 2026) and 'sf' (Pebesma 2018) packages, respectively. Data cleaning was performed with 'dplyr' (Wickham and François 2015) and 'tidyr' (Wickham et al. 2026) packages. All analyses were performed in R 4.5.3 (R Core Team 2026).

2.4.2. Potential distribution modeling – MaxEnt

The potential distribution of *R. harrisii* in Patos Lagoon was estimated with the Maximum Entropy (MaxEnt) algorithm using 'ENMeval' (Kass et al. 2021) and 'maxnet' (Phillips 2023) packages. MaxEnt estimates the maximum entropy based on the restrictions of the environmental variables (physicochemical factors) observed in the occurrence locations (Phillips et al. 2006; Phillips and Dudík, 2008). Different combinations of parameters were tested for the model complexity, including Feature classes (FC) - Linear (L), Linear + Quadratic (LQ), Hinge (H), Linear + Quadratic + Hinge (LQH), and Regularization multiplier (RM) (Merow et al. 2013; Elith et al. 2011). Model calibration was performed with 'ENMeval' using jackknife cross validation, which is appropriated for small datasets (Kass

et al. 2021). Model selection was based on AICc (Akaike Information Criterion corrected for small samples), ΔAICc, Akaike weights (wAIC), training AUC (Area Under the Curve), average-validation AUC, ΔAUC (difference between training and validation AUC and the 10% omission rate) (Warren and Seifert 2011). Model performance was evaluated by ROC (Receiver Operating Characteristic), and AUC metrics, calculated from the probabilities of species occurrence and the background points using 'pROC' (Robin et al. 2011) package. Performance was classified when AUC = 0.5 as random, 0.7 – 0.8 acceptable, 0.8 – 0.9 good, and ≥ 0.9 excellent (Swets 1988; Fielding and Bell 1997).

The contribution of environmental variables was evaluated by the Percent Contribution (how much the variable helps to improve the model) and Permutation Importance (loss of model performance after permutation of the variable) outputs from MaxEnt (Phillips et al. 2006; Phillips and Dudík 2008; Elith et al. 2011).

2.4.3. Continuous map and occupation estimates

A continuous map was designed to represent spatial distribution and environmental suitability overlapping study area boundaries and species occurrence points using the 'ggplot2' package (Wickham 2016). Occupied area was estimated from a binary raster generated using the 10^{th} percentile training presence threshold (10PTP), a conservative threshold commonly applied in species distribution models that excludes areas with the lowest predicted suitability and provides a more conservative estimate of species occupancy (Phillips et al. 2006; Elith et al. 2011). The binary raster was projected in SIRGAS 2000/UTM zone 22S (EPSG:31982), allowing area calculations in metric units. Total occupied area was

obtained by multiplying the number of pixels classified as suitable by the area of each pixel, and the result was expressed in km² (Wickham 2016; Pebesma 2018; Hijmans et al. 2026).

2.4.4. Environmental response curves

To evaluate the influence of environmental variables on the occurrence of *R. harrisii*, Generalized Additive Models (GAMs) were fitted using the mgcv package in R. Presence records obtained during field surveys were combined with background points generated throughout the study area, resulting in a binary response variable (presence = 1; background = 0). The environmental predictors included water temperature (°C), salinity, depth (m), and sediment organic matter content (%) (Hastie and Tibshirani 1990). Relationships between species occurrence and environmental variables were modeled using penalized regression splines, allowing for potential nonlinear responses. The GAM was fitted assuming a binomial error distribution with a logit link function and parameter estimation by Restricted Maximum Likelihood (REML) (Wood 2011). The significance of smooth terms was assessed using approximate significance tests provided by the model summary. Model performance was evaluated based on the percentage of deviance explained. Partial effect plots with 95% confidence intervals were generated to visualize the influence of each environmental predictor on the probability of occurrence of *R. harrisii* while controlling for the effects of the remaining variables (Wood 2011; Wood 2017).

3. Results

3.1.Selection and evaluation of model performance

The most parsimonious models presented FC = H and LQH, both with RM = 5 and ncoef = 2. The model training stage had an acceptable performance, with AUC_{train} = 0.77 and null omission rate (OR10 = 0), indicating a good representation of known occurrences. However, low AUC_{val} values of ≈ 0.45 suggest limited prediction power during model validation. The remaining models had higher AICc and lower statistical power, indicating lower performance (Table 2). Additional ROC analysis yielded an AUC = 0.77, indicating moderate discriminatory ability to distinguish suitable from unsuitable areas for the occurrence of the species. These results suggest that the environmental variables used in the model have an influence on *R. harrisii* spatial distribution, but the model's capacity for spatial generalization is intermediate. The low validation AUC is likely related to the reduced number of occurrence records available for model calibration (n = 8), which may increase uncertainty during cross-validation.

Table 2. Performance of Feature Classes (FC) combinations and Regularization Multiplier (RM) tested by MaxEnt algorithm. Feature Classes are: linear (L), linear + quadratic (LQ), hinge (H), linear + quadratic + hinge (LQH). Number of model's coefficients (ncoef), Corrected Akaike for small samples (AICc), difference from the best model (ΔAICc), Akaike weights (wAIC), area under the ROC curve for training (AUCtrain) and validation (AUCval), and omission rate after removing points with suitability in the lowest 10 percentile among all points (OR10).

FC	RM	ncoef	AICc	ΔAICc	wAIC	AUCtrain	AUCval	OR10
H	5.0	2	182.70	0.00	0.15	0.77	0.45	0.00

LQH	5.0	2	182.70	0.00	0.15	0.77	0.45	0.00
LQ	0.5	2	185.20	2.50	0.04	0.68	0.23	0.50
LQ	1.0	1	186.35	3.65	0.02	0.59	0.21	0.50
L	0.5	0	186.36	3.66	0.02	0.50	0.19	0.50
L	1.0	0	186.36	3.66	0.02	0.50	0.31	0.37
L	1.5	0	186.36	3.66	0.02	0.50	0.50	0.00
LQ	1.5	0	186.36	3.66	0.02	0.50	0.32	0.25
L	2.0	0	186.36	3.66	0.02	0.50	0.50	0.00
LQ	2.0	0	186.36	3.66	0.02	0.50	0.50	0.00

3.2.Environmental variables contribution

Organic matter in sediments (MO) presented the higher relative importance to the model (20%), followed by salinity and depth (14% and 12% importance, respectively). Temperature did not show influence on *R. harrisii* distribution, with the lower prediction power (2%) compared to all variables (Figure 2).

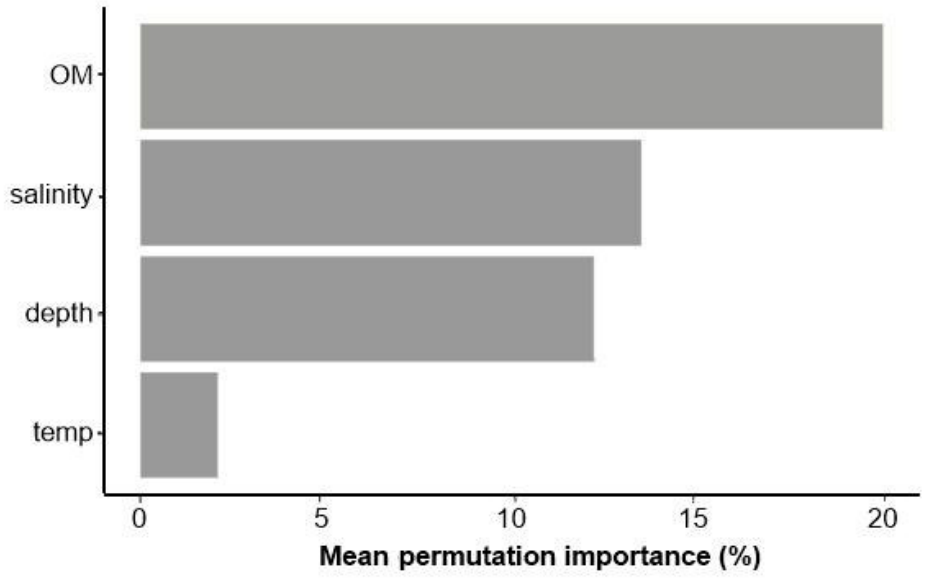


Figure 2. Average variable importance across MaxEnt models for *Rhithropanopeus harrisii* distribution in Patos Lagoon estimated by the permutation importance (%). OM is organic matter in sediments, temp is temperature.

3.3.Potential distribution area

The potential distribution model indicated spatial heterogeneity in the environmental suitability across the study area. Higher probabilities of *R. harrisii* occurrence were observed in the estuarine portion of the Patos Lagoon and central regions, shaping continuous areas of habitat suitability (Figure 3). Areas with high environmental suitability for the species had values closer to the maximum predictive gradient (1), represented by warm colors in Figure 3, while intermediate regions north of the lagoon presented lower probabilities of mud crab occurrence (cold colors indicate values closer to 0; Figure 3).

Occurrence areas estimated by modeling agreed with the higher average of suitability, indicating consistency between occurrence reports and model prediction. Contour lines evidenced the relatively continuous spatial gradients of habitat suitability, allowing the identification of transition zones between more and less favorable areas for the occurrence of *R. harrisii* (Figure 3).

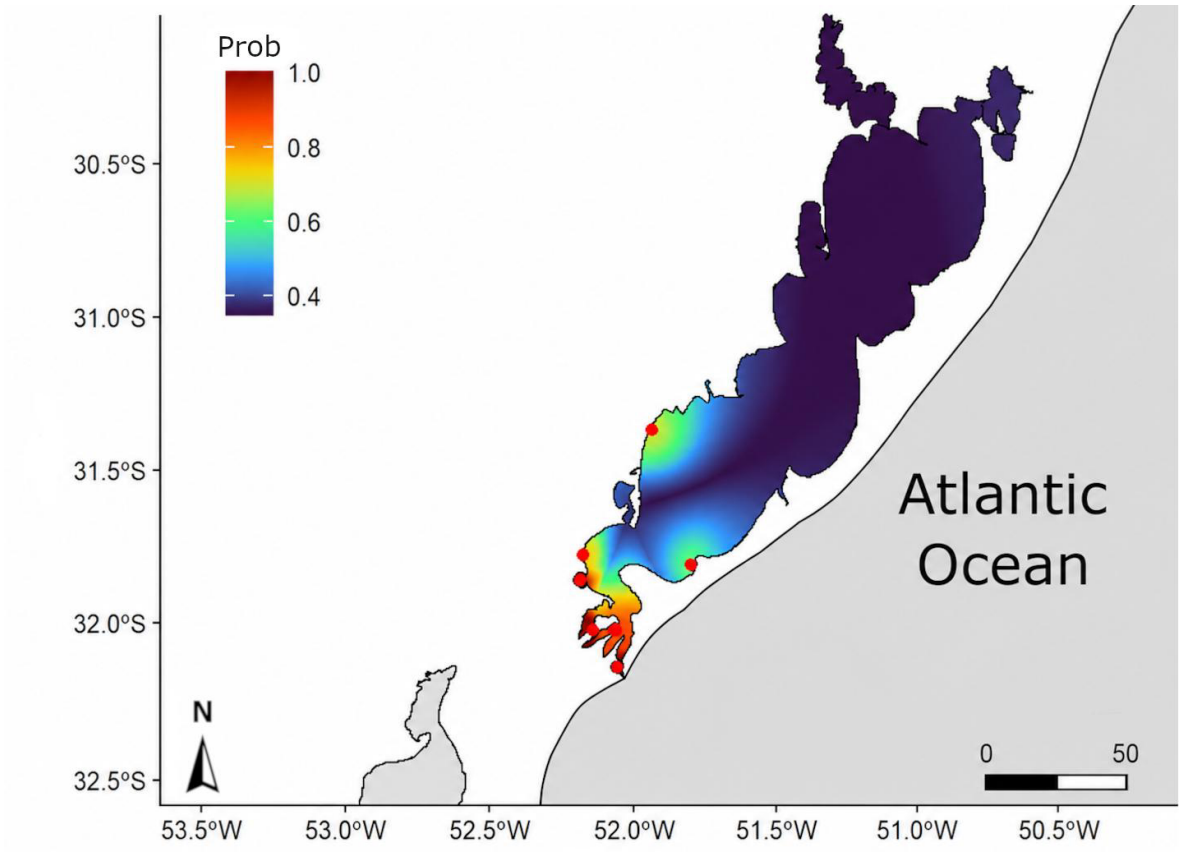


Figure 3. Potential spatial distribution of *Rhithropanopeus harrisii* in Patos Lagoon estimated by MaxEnt modeling. Continuous gradient represents predicted habitat suitability for the species, varying from low (cold colors) to high habitat suitability (warm colors). Red dots show occurrence reports used in the model, and the contour lines show spatial variation in environmental suitability.

The area potentially suitable for *R. harrisii* was estimated at 6,480.93 km², representing 64% of the total area of Patos Lagoon, with more favorable habitat mainly concentrated in the estuarine zone. The estimation of occupied area (km²) and the total area of the suitable

polygon allowed the spatial quantification of potential expansion of the species in the lagoon.

3.4.Environmental response

The best model included all the evaluated variables (global model; Table 3). All the other models with exclusion of one or more variables had substantially higher AICc, which evidences lower explanatory power. Although temperature had low permutation importance (Figure 2), its inclusion in the GAM notably increased model fitness (Table 3).

Table 3. Comparison among Generalized Additive Models (GAMs) fitted to explain the distribution of *Rhithropanopeus harrisii* in Patos Lagoon. Explanatory variables are temperature (temp), salinity (sal), depth, and organic matter in the sediments (OM).

Model	df	AICc	ΔAIC	Weight
temp + sal + depth + OM	10.941	24.898	0.000	0.50
sal + depth + OM	8.902	61.596	36.698	5.37^{-09}
temp + depth + OM	8.655	63.117	38.219	2.51^{-09}
depth + OM	7.277	67.466	42.568	2.85^{-10}
temp + sal + OM	8.609	71.121	46.223	4.59^{-11}
temp + OM	6.395	78.153	53.255	1.36^{-12}
sal + depth	5.922	78.213	53.314	1.32^{-12}
temp + sal + depth	6.216	78.548	53.650	1.12^{-12}
sal + OM	5.467	81.953	57.055	2.04^{-13}

Temperature did not show influence *R. harrisii* distribution (GAM smooth: $x^2 = 0.062$, df = 1.00, p = 0.802; Figure 4A), agreeing with MaxEnt (permutation importance, Figure 2). Salinity was important in describing the distribution, with decreasing the probability of mud crab occupation in higher salinities (GAM smooth: $x^2= 2.025$, df = 1.67, p = 0.026; Figure

4B). The probability of occurrence was higher in shallow habitats, particularly between 1 and 2.5 m depth (GAM smooth: $x^2 = 10.760$, df = 1.00, p = 0.001; Figure 4C), and content of organic matter in the sediments was also important in describing *R. harrisii* distribution, with areas with increased OM presenting higher probability of occupation (GAM smooth: $x^2 = 14.105$, df = 3.65, p = 0.007; Figure 4D).

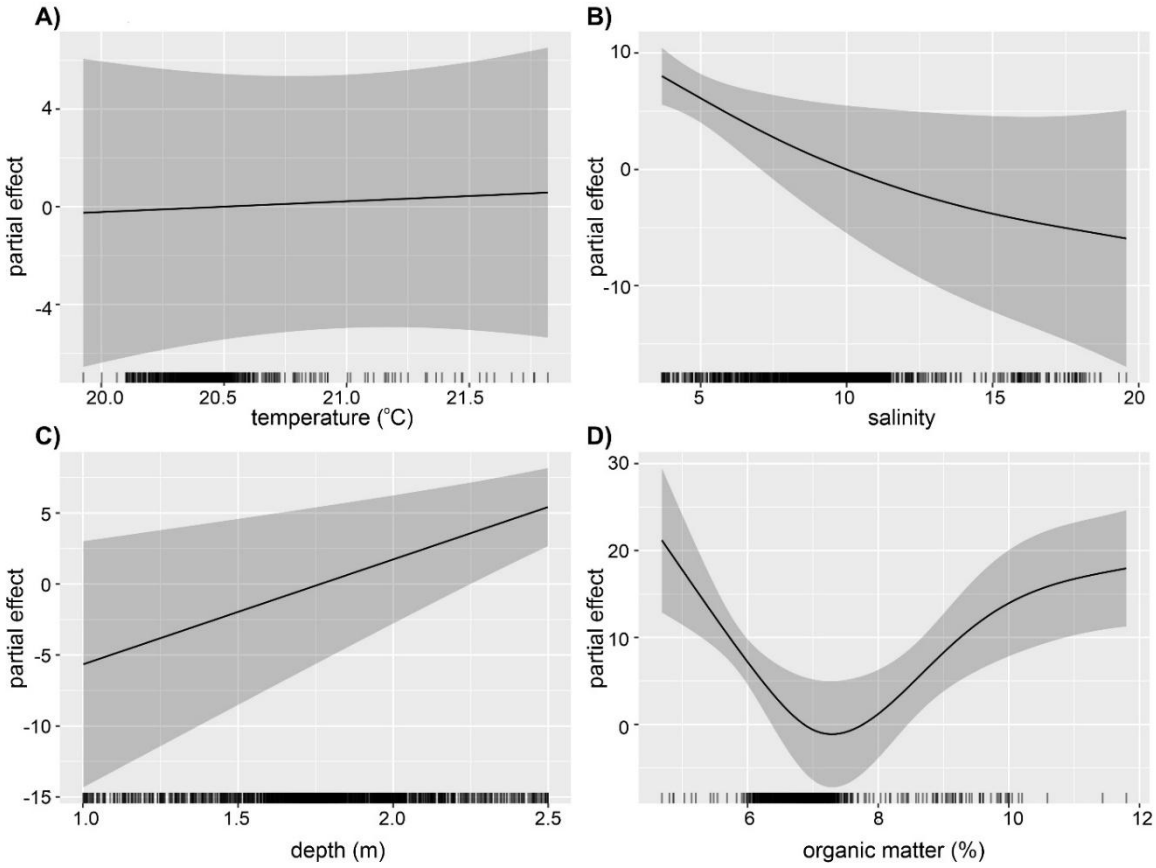


Figure 4. Partial effects of selected variables by the Generalized Additive Model (GAM) on the probability of *Rhithropanopeus harrisii* occurrence in Patos lagoon, Brazil. A) temperature influence, B) salinity influence, C) depth influence, and D) organic matter in sediments. The shaded areas indicate the 95% confidence intervals.

4. Discussion

Our results showed that *Rhithropanopeus harrisii* potential distribution is highly structured by environmental gradients in Patos Lagoon, especially driven by organic matter contents in sediments, salinity and depth. MaxEnt model indicated high environmental suitability in estuarine regions and intermediate sector of the lagoon (salinities 5 – 20), while GAM corroborated the importance of salinity, depth and organic matter in the potential distribution of this mud crab species. These results suggest that the expansion of *R. harrisii* in Patos Lagoon is not random, following specific environmental conditions that favors population establishment.

4.1. Species expansion in Patos Lagoon

The wide area of potentially suitability predicted by the model suggests high potential expansion of this invasive species in the lagoon. The occurrence reports of *R. harrisi*i in different sectors of Patos Lagoon, which includes interior areas with low salinity (< 1) indicates that this species finds suitable areas for colonization beyond the estuarine portion originally invaded and currently occupied. This pattern is related to the high ecological plasticity of *R. harrisii*, also described for other populations of the species around the world (Turoboyski 1973; Iseda et al. 2007; Projecto-Garcia et al. 2010; Fowler et al. 2013; Simakova et al. 2017; Machado et al. 2026), and that allows for its high invasive success. Reproductive population of mud crab has been established in freshwater reservoirs in Texas and Oklahoma, reinforcing the high adaptability and physiological tolerance of this species

(Howells 1998). The occurrence of juveniles and ovigerous females in extremely low salinities indicates that *R. harrisii* is able to complete its life cycle in freshwater, contrasting laboratory studies that show low larval survival in such conditions (Costlow et al. 1966; Laughlin and French 1989). These contrasting results suggest possible larval adaptation and/or geographical variation in population salinity tolerance (Boyle et al. 2010; Projecto-Garcia et al. 2010). This high plasticity and capacity in persisting and reproducing in a wide range of saline conditions explains *R. harrisii* expansion to interior areas of the lagoon, increasing its invasive potential inside Patos Lagoon.

4.2. Habitat suitability

Organic matter in sediments made a high contribution to the ecological models, demonstrating its influence on the potential distribution of *R. harrisii*. These results indicate a strong association between species' occurrence and organically enriched sediments. Sediments that are rich in organic matter present high food availability, microbiota biomass, and so, an abundance of benthic invertebrates, which favors generalist species with detritivore and omnivore habits (Turoboyski 1973; Tsikopoulou et al. 2021). *Rhithropanopeus harrisii* is described as a benthivorous species, including detritus, polychaets, mollusks, small crustaceans, and organic matter deposits in sediments in its diet (Turoboyski 1973; Hegele-Drywa and Normant 2009). At Patos Lagoon, the species also presented opportunistic feeding behavior with a high abundance of organic matter and debris in its diet, demonstrating high trophic plasticity, which favors the occupation of a diversity of estuarine microhabitats (Machado et al. 2026).

Beyond food availability, sediments with high organic content generally occur in areas with low hydrodynamics, favoring higher sediment deposition, which are preferred conditions for the establishment of euryhaline benthic species with invasive potential (Snelgrove and Butman 1994). In Patos Lagoon estuary, the spatial distribution of organic matter is related to the hydro-sedimentary dynamics of the system, which is mostly driven by fluvial discharge, estuarine circulation, and local biological productivity (Rosa and Bemvenuti 2006; Bemvenuti and Colling 2010). Shallow vegetated areas frequently accumulate organic debris and act as important feeding and shelter zones for a diversity of estuarine decapods (Bemvenuti and Netto 1998; Rosa and Bemvenuti 2007; Pinotti 2010). These conditions favor high benthic biomass and continuous trophic resources availability, contributing to the establishment and population growth of *R. harrisii* in the region.

Depth was another important ecological descriptor, indicating that bathymetric gradients influence environmental suitability for the establishment of the species. Zones between 1 and 2.5 m had high influence on species occurrence, which slight increase in the probability of occurrence in areas around 2 m depth. No occurrences of mud crabs in deeper zones of the estuary were detected, so the depth is likely a limitation for the species' habitat use. This use of shallow areas is likely associated with the lower hydrodynamics that favor fine sediments and higher organic matter deposition and benthic community productivity (Rosa and Bemvenuti 2006; Rosa and Bemvenuti 2007). Shallow waters also present more environmental heterogeneity, shelter, and connectivity among estuarine systems than deeper zones, factors that benefit plastic species such as *R. harrisii* (Hegele-Drywa and Normant 2009).

Probability of mud crab occurrence was also strongly influenced by salinity. The species tolerates a wide salinity gradient, but the highest environmental suitability occurred at intermediate salinities, with decreasing environmental suitability with the increase in salinity, reinforcing that salinity is one of the main ecological filters in estuarine systems (Menegotto et al. 2019). *Rhithropanopeus harrisii* is known by its wide osmotic tolerance, being able to occupy brackish and oligohaline systems (Costlow et al. 1966; Laughlin and French 1989; Hegele-Drywa and Normant, 2009). However, even with this high physiological plasticity, salinity extremes may limit/impair osmoregulation, metabolism, reproduction, and larval development of the mud crab (Costlow et al. 1966; Normant and Gibowicz, 2008). The strong space-time heterogeneity in salinity gradients of Patos Lagoon is mostly driven to the fluvial discharge, wind, and marine intrusion (Marques et al. 2009; Bitencourt et al. 2020), directly influencing the benthic assemblage composition and abundance (Bemvenuti and Netto 1998; Pinotti 2010). Therefore, intermediate salinities may offer more favorable conditions to osmotic balance, feeding activity, and establishment of the species. The consolidated occurrence of *R. harrisii* in Patos Lagoon since the 1980's (D'Incao and Martins 1998) supports this idea of high tolerance and adaptation of dynamic conditions of this estuarine-lagoon system.

Temperature can affect metabolic rates, growth, locomotion, reproduction and larval development in decapods, also interacting with hydrological factors such as salinity, and dissolved oxygen (Anger 2003; O'Connor et al. 2007; Madeira et al. 2018; Baudet et al. 2022). In Patos Lagoon, temperature presents low spatial variation, with seasonality being the main factor for temperature variation (Pereira et al. 2012). Therefore, the lack of

relationship between temperature and mud crab habitat suitability shows that temperature is likely to act more as a physiological modulator than as an ecological filter.

4.3. MaxEnt vs. GAM performance

The concordance between MaxEnt and GAM models demonstrates the robustness of observed ecological patterns. Although the algorithms have different approaches, both indicate the importance of sedimentary and hydrological gradients in the potential spatial distribution of the mud crab. The combined use of both approaches allowed us to integrate the predictive spatial capacity of distribution models with the statistical interpretation of ecological responses along the environmental gradients, increasing the reliability of ecological inferences (Phillips et al. 2006; Elith and Leathwick 2009). MaxEnt is based on maximum entropy and presence and pseudo-presence data, being very efficient in spatially identifying suitable areas for species occurrence (Phillips et al. 2006; Elith and Leathwick 2009). Contrasting, GAM allowed the interpretation of environmental gradients and the analysis of non-linear patterns, enabling better comprehension of ecological factors associated with the species distribution (Hastie and Tibshirani 1990; Wood 2017). In both models, organic matter, salinity and depth had strong influence on the habitat suitability for *R. harrisii*, suggesting that these factors are environmental filters for species establishment.

Although both models generated similar conclusions, they presented small differences. Temperature had a low permutation importance in MaxEnt, however its inclusion in GAM significantly improved model fitness, suggesting a complementary effect or indirect interaction with other environmental variables. Temperature, therefore, is not an

environmental filter in Patos Lagoon, but likely acts in physiological and metabolic processes, influencing reproduction and growth of *R. harrisii* (Anger 2003; Madeira et al. 2018).

Low AUC validation values derived from MaxEnt indicate a limitation in the spatial prediction power, which is likely associated with the low number of sites with occurrence reports. Small sample sizes tend to increase spatial uncertainty and overfitting in distribution models based on presence data, especially when multiple environmental predictors are included in the model (Morales et al. 2017; Tessarolo et al. 2021). Moreover, the inherent environmental variability of Patos Lagoon may compromise the proper representation of its ecological heterogeneity when using a limited number of samples (i.e., sampling sites), and therefore, the predictions should be interpreted with caution. However, the convergence of both approaches increased the reliability of our ecological inferences, showing that both models are complementary and have great power to predict the responses of the invader *R. harrisii* to environmental gradients.

The combination of MaxEnt and GAM strengthens the inferences about the factors that influence the potential distribution of the mud crab in Patos Lagoon. To increase the robustness of the predictions and improve model performance, future sampling campaigns should include other sectors of the lagoon and consider seasonality. Moreover, including other environmental variables, such as sediment granulometry, dissolved oxygen, and vegetation coverage, for example, may broaden our understanding of the ecological mechanisms associated with the species distribution.

5. Conclusion

Our results show that the potential spatial distribution of *R. harrisii* in Patos Lagoon is strongly structured by environmental gradients, in special by organic matter, depth, and salinity. The alignment between MaxEnt and GAM supports the consistency of observed patterns, indicating that this species occupies shallow areas, with salinity between 1 and 20, and sediments organically enriched. We showed, for the first time, that *R. harrisii* is using the freshwater portion of Patos Lagoon instead of being restricted to the estuarine areas, as described before. With these results, the elevated ecological plasticity of *R. harrisii* was evident, a characteristic that favors the species colonization and expansion in estuarine systems. Although the predictive performance during validation was limited, the consistency between MaxEnt and GAM results and the ecological plausibility of the identified environmental drivers support the reliability of the inferred distribution patterns. The broad area predicted as potentially suitable for the mud crab in the lagoon suggests high potential for expansion inside Patos Lagoon system, possibly representing a threat to native benthic communities due to the opportunistic behavior and trophic plasticity (see Machado et al. 2026) of the species. The integrated approach with spatial modelling and ecological inferences and interpretation contributes to the understanding of the biological invasion process in subtropical systems, providing relevant insights for monitoring, management and species conservation.

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CAPÍTULO IV

Trophic niche overlap between the invasive crab *Rhithropanopeus harrisii* (Gould, 1841) and native crab species in the Southwest Atlantic coast

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Renan C. Machado [a*], Bianca Possamai [b], Luiz Felipe Cestari Dumont [a]

a Programa de Pós-graduação em Oceanografia Biológica, Instituto de Oceanografia, Universidade Federal do Rio, Grande-FURG, Av. Itália, Km 8, s/n, Rio Grande, RS, 96203-900, Brasil.

b Instituto de Oceanografia, Universidade Federal do Rio Grande-FURG, Av. Itália, Km 8, s/n, Rio Grande, RS, 96203-900, Brasil.

Autor correspondente: Renan C. Machado

e-mail: rcmkssino@hotmail.com

Abstract

The introduction of exotic species is one of the main drivers of biodiversity loss in aquatic ecosystems. The Harris mud crab *Rhithropanopeus harrisii*, native to the Atlantic coast of North America, was recorded in the Patos Lagoon estuary (southern Brazil) in the 1980s, likely introduced via ballast water. However, the effects of this invader on the native fauna were never evaluated. This study assessed the natural diet and trophic niche overlap between the invader and four native crab species (*Cyrtograpsus angulatus*, *Callinectes sapidus*, *Neohelice granulata*, and *Panopeus austrobesus*) along a salinity gradient in the estuary. Multivariate analyses were performed to identify dietary differences among species and sites, and the Morisita–Horn index was applied to estimate trophic overlap. *Callinectes sapidus*, *Panopeus austrobesus*, and *Rhithropanopeus harrisii* exhibited generalist feeding habits, consuming mollusks, crustaceans, polychaetas, plant material, and detritus, whereas *Neohelice granulata* showed a predominantly herbivorous diet, and *Cyrtograpsus angulatus* displayed trophic plasticity by alternating between animal and plant items. Significant differences in diet composition were observed among species, with low overall trophic overlap. The highest overlap was observed between *Callinectes sapidus* and *Panopeus austrobesus*, whereas the lowest overlap occurred between *Neohelice granulata* and *Rhithropanopeus harrisii*. Despite its generalist feeding behavior, *Rhithropanopeus harrisii* appears not to compete strongly with native species, likely exploiting unoccupied niches or using shared resources differently. The invader's high trophic plasticity may enhance its establishment in the estuary, while low overlap suggests niche differentiation or resource partitioning with native crabs.

Keywords: ballast water, competition, feeding ecology, invasive species, mud crab, trophic plasticity

1. Introduction

Exotic species invasion is a phenomenon that exponentially grows in our globalized world causing significant impacts such as biodiversity loss in many ecosystems worldwide (Courchamp et al. 2017; Meyerson et al. 2019). The introduction of exotic species is usually linked to economic activities, such as aquaculture, aquarium hobby, marine and freshwater transportation that carries species in ballast water and/ or as biofouling in ship structures, and through floating solid waste (Tavares and Mendonça 2004; Mineur et al. 2012; Peters et al. 2014; Bailey et al. 2020). Although species occurrence ranges are naturally dynamic (Lodge 1993) and especially intensified with climate change and tropicalization (Vergés et al. 2019; Schuster et al. 2022), the spread driven by human activities in terrestrial and aquatic systems is much faster than the natural expansion of species (Ruiz et al. 2000; Rius et al. 2015). Invasive species can reduce the abundance and presence of native species due to predation and competition, altering food webs and community structure, consequently changing the nutrient cycling and so harming ecosystems, habitats, and populations (Molnar et al. 2008; Bellard et al. 2016; Possamai et al. 2026).

A fundamental step to understanding and mitigating the invasive process is to assess the ecological niche of the invader and how this niche can be adapted to a new environment. Among the many dimensions of ecological niche, the trophic niche is one of the most relevant because the energy flow among trophic levels in a biological community, as well as the interaction among species such as predation and competition, influences the community structure determining the ecosystem functioning (Begon et al. 2006; Possamai et al. 2026). Therefore, it is crucial to understand the processes that regulate trophic niche

to clarify the mechanisms of coexistence between morphologically or phylogenetically similar species (Schoener 1974; González-Ortegón et al. 2010; Vollrath et al. 2021), and to evaluate possible consequences of an invasive species over native ones (Córdova-Tapia et al. 2015). Trophic niche overlap occurs when two or more species use the same feeding resource. However, depending on the availability of the resources in the ecosystem, this overlap may not translate into conflict, allowing for coexistence without competition (Pianka 1974; Vollrath et al. 2021). On the other hand, if resources are limiting and the invasive species have a competitive advantage, a competitive exclusion of the native species may occur, resulting in population-size reduction of the native species, and in extreme cases, local extinction (García et al. 2020). The knowledge of the natural diet of invasive species is essential for management efforts and mitigation of possible environmental disruptions caused by non-native species.

One of the aquatic systems most vulnerable to biological invasion is the estuary because they are exploited intensively by human populations for aquaculture, fisheries, and port activities (Wasserman et al. 2011; Magoro et al. 2015). Estuaries are essential for many crustacean and fish species, developing crucial roles in organisms' life cycle during reproduction, growth, feeding, and migration processes (Barletta et al. 2010). Considering their importance in coastal systems, it is fundamental to understand the ecological function of estuaries and lagoons to provide basal information for adequate coastal management decisions (Magoro et al. 2019). Port activities promote ways for biological invasion, with ballast water and biofouling among the main vectors of transportation of aquatic invasive species (Wonham et al. 2001). Throughout this transport, microorganisms, larvae, and adults of aquatic species are transferred across the oceans or between regions of the same

country (Lavoie et al. 1999; Wasson et al. 2001). Ballast water was the probable vector of the introduction of the mud crab *Rhithropanopeus harrisii* (Gould 1841) in the Lagoa dos Patos estuary, Brazil (D'Incao and Martins 1998), bringing a population from the North Carolina coast, in the United States, to the Brazilian waters (Machado et al. 2025).

The mud crab *R. harrisii* originally inhabits brackish and freshwaters in the Atlantic coast of North America (A. B. Williams 1984). In 1874, the mud crab was first recorded outside its native range in the Netherlands (Wolf 1954), and between 1930 and 1950 the species spread throughout all European coasts, including the inland seas (i.e., Baltic, Black, and Caspian Seas) (Petersen 2006; Simakova et al. 2017). In South America the first record of the mud crab was in 1980 in the Lagoa dos Patos estuary, Brazil (D'Incao and Martins 1998), and, as a more recent invasion, the mud crab was found in 2006 on the Pacific coast of Japan (Iseda et al. 2007).

Rhithropanopeus harrisii belongs to the Panopeidae family, and is an omnivorous species, highly generalist which confers high trophic plasticity, being able to adapt its diet to the resources availability (Turoboyski 1973; Hegele-Drywa and Normant 2009). Invasive species with high trophic plasticity usually have negative impacts on native species and communities, decreasing diversity and abundance of local species (Forsström et al. 2015). The Lagoa dos Patos estuary harbors approximately 50 species of crabs, and amongst them, nine are from the Panopeidae family (Melo 1999; Vieira 2006), which likely had part of their niches used by the invader mud crab due to their close relationship (i.e., same family), with unknown consequences for these native populations.

Despite the presence of *R. harrisii* in the Lagoa dos Patos estuary since the 1980s (D'Incao and Martins 1998), there are no long-term standardized assessments of crab

population abundance before and after the invasion. Existing studies in the system are mostly punctual and do not allow the detection of population trends attributable to the invasive species (D'Incao et al. 1990; Garcia et al. 1996; Colling et al. 2007; Barutot et al. 2011). However, evidence from other estuaries worldwide invaded by *R. harrisii* suggests that this species has significant trophic effects in native communities through high dietary plasticity and resource overlap with native benthic decapods, potentially leading to indirect or sublethal impacts that precede detectable population declines (Turoboyski 1973; Hegele-Drywa and Normant 2009; Simakova et al. 2017). In this context, trophic niche analyses represent a valuable approach to identify early warning signals of ecological impacts caused by invasive species.

Aiming to advance our knowledge about the feeding habits and the possible impacts of the mud crab on the native crab species in the Lagoa dos Patos, we evaluate the natural diet and the trophic niche overlap between native and invasive species in a saline gradient of the lagoon. Salinity is expected to influence prey availability and feeding strategies, so due to the high trophic plasticity of *R. harrisii*, a comprehension of the species' diet in the saline gradient of the estuary is important to understand the mud crab's ability to adapt to new environments. We hypothesize that due to the invasive success of *Rhithropanopeus harrisii*, the species is highly generalist and overlaps the trophic niche of all native species, especially that of native Panopeidae due to their taxonomic similarity. We expect that our results will contribute to the field of invasive biology and provide information on how native species deal with external stressors in a scenario where biological invasions are each time more frequent and intense.

2. Materials and Methods

Lagoa dos Patos is the largest choked lagoon in the world (10,227 km2) and its estuarine region is about 10% of the total area (Figure 1) (Seeliger and Odebrecht 2010). Tides have low influence on Lagoa dos Patos estuary (~0.5 m), while NE–SW winds and rainfall are the main drivers for estuarine circulation (Möller et al. 2001). These parameters define salinity distribution and water levels (Garcia 1998), which largely influence the dominance and composition of faunal and floristic species in the estuarine community (Seeliger and Odebrecht 2010). In the estuarine portion of the Patos Lagoon, salt marshes (flooded vegetated zones) are dominated by grasses and rushes adapted to brackish conditions and shallow waters. The most common species include *Ruppia maritima*, *Spartina* spp., *Scirpus* spp., and *Juncus* spp. Shallow freshwater areas (< 1.5 m) are covered by dense stands of *Scirpus giganteus*, *Schoenoplectus californicus*, and *Typha* sp. (Costa 1997; Copertino and Seeliger 2010). The bottom of shallow waters in the estuary is composed of sand, and the deeper channel bottom is a mix of sand, silt, and clay (Calliari et al. 1977; Ortega et al. 2020).

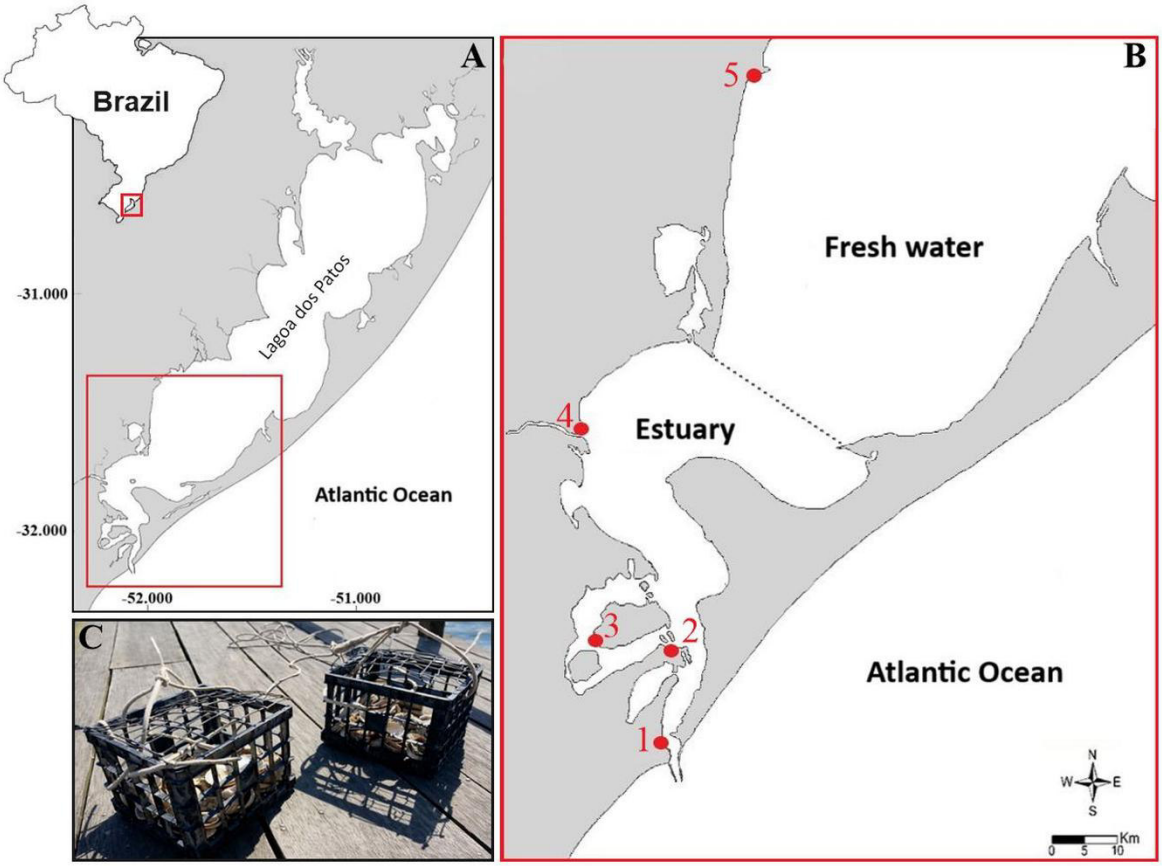


FIGURE 1 Lagoa dos Patos in South of Brazil (A). Location of the sampling sites (red dots): 1 Barra, 2—Museu, 3—Ilha, 4—Pelotas, and 5—São Lourenço do Sul. Dashed line shows the limit of the estuarine portion of the lagoon (B). In (C) is a picture of traps used for the collection of organisms.

2.1. Fieldwork and Sample Processing

Sample collection was conducted monthly between March 2022 and February 2023 at five sites along the western margin of Lagoa dos Patos, ranging from freshwater areas to the estuarine portion, in order to cover the lagoon's salinity gradient (Figure 1). Salinity was measured during all sampling campaigns, and the annual average (±SD) of each site

was: São Lourenço do Sul (SLS, salinity 3.66 ± 3.74), Pelotas (locally known as Pontal da Barra, 7.00 ± 6.38), Ilha (locally known as Ilha dos Marinheiros, 15.91 ± 9.78), Museu (locally known as Museu Oceanográfico, 17.25 ± 9.77), and Barra (19.58 ± 9.62) (Figure 1). Biological samples were collected using unbaited traps made from plastic boxes with perforated sides (60 cm × 22 cm × 15 cm; volume of 30 L), filled with empty mollusk shells (mostly the gastropod *Pachycymbiola brasiliana*), which served as shelter for organisms (Roche et al. 2009; Machado et al. 2025). The traps were covered on the top with a plastic mesh with 3 × 3 cm openings to prevent shell loss but allow crustaceans' colonization. After each sampling event, shells were removed, cleaned (estuarine water), and placed again inside the traps, and the traps were redeployed. One trap was used at each site, installed directly on the substrate and attached to fixed structures such as piers. The predominant substrate at the sampled sites was fine sand to mud, and the traps remained submerged for approximately 30 days during each sampling period.

Individuals were preserved in 4% formalin immediately after collection (approximately 5 min later) in order to halt the digestive process. After 24 h in formalin, the specimens were transferred to 70% ethanol for subsequent processing (> 2 months after collection). Carapace length and width (mm) and total body weight (g) were measured for each individual. Subsequently, the digestive tract was removed and dissected under a stereomicroscope at magnifications of up to 40×. Gut content samples were processed following the methodologies described by M. J. Williams (1981), Barutot et al. (2011), and Bas et al. (2013). Food items were identified to the lowest possible taxonomic level and subsequently grouped into broader taxonomic categories for further analyses. The area

occupied by each item was estimated based on its coverage observed using a Sedgewick-Rafter counting chamber (mm2) and converted into percentage cover relative to the total area of all items recorded in the gut contents.

2.2. ***Data Analyses***

Diet was analyzed with frequency of occurrence (FO) and frequence in area (FA). The FO was calculated by the number of guts that presented prey *i* divided by the total of guts analyzed in each species. FA was calculated by the sum of the area of item *i* in all guts divided by the sum of area of all items (M. J. Williams) 1981. These two frequencies were used to calculate the Feeding Index (IAi), following the equation $IAi = \frac{FOi*FAi}{\sum(FOi*FAi)}$ (Kawakami and Vazzoler 1980).

To compare the diet among species and habitats, we selected the five species most abundant in all sites: the invasive *Rhithropanopeus harrisii* and the native species *Panopeus austrobesus*, *Neohelice granulata*, *Cyrtograpsus angulatus*, and *Callinectes sapidus*. The possible differences in species diets among sites, species, and the interaction of these terms were evaluated with a PERMANOVA in the vegan R package (Oksanen et al. 2025) using 9999 permutations and the Bray–Curtis dissimilarity to build the distance matrix. “Betadisper” function was used to evaluate homogeneity of dispersion ($p = 0.001$, Figure S1). Because PERMANOVA is still a robust analysis even under heterogeneity in dispersions, performing superior to ANOSIM and Mantel tests (Anderson and Walsh 2013), we interpreted the PERMANOVA results. Multilevel pairwise comparisons were performed as a posthoc using the “pairwiseAdonis” package in R. To detect the items responsible for differences and the food item most representative of each species, the

Indicator Species Analysis (IndVal) was employed using the "indicspecies" R package, with 999 permutations (De Cáceres and Legendre 2009). The % in area of each item in the stomachs was used as response for the multivariate analyses.

Trophic niche overlaps were calculated using the niche.overlap function from "spaa" package (Zhang 2025). We calculated the Morisita–Horn Index (Horn 1966) using a matrix with FA values, following the equation: $\hat{C}_H = \frac{2\Sigma_i^n P_{ij} P_{ik}}{\Sigma_i^n P_{ij}^2 + \Sigma_i^n P_{ik}^2}$, where $\hat{C}_H$ is the Morisita-Horn Similarity Index, p_{ij} is the proportion of resource *i* in the total of resources used by the species *j*, p_{ik} is the proportion of resource *i* in the total of resources used by the species *k*, *n* is the number of total resources used. This index varies from 0 to 1, in that 0 indicates absence of overlap and 1 total trophic niche overlap (Krebs 1998). Some food items were grouped to deflate the number of "zeros" in our analyses totalizing 17 items (Acarii, Amphipoda, Bivalve, Brachyura, Bryozoa, Copepoda, Crustacea, Gastropoda, Hydrozoa, Misis, Nematoida, Ostracoda, Polichaeta, Shells remains, Cirripedia, Plant matter, and Tanaidacea). The visualization of groups formed by the overlap analysis was generated by permutational analysis of the multivariate homogeneity of species dispersions using "betadisper" function (Anderson 2006) from "vegan" package (Oksanen et al. 2025). This analysis was performed with the same data as PERMANOVA analysis, also using a distance matrix based on a Bray–Curtis dissimilarity. All analyses were performed in R 4.4.1 software (R Development Core Team 2024), considering $\alpha = 0.05$.

3. Results

3.1 Structure and General Crab Diets

A total of 624 individuals were analyzed, distributed among the five species and the five study sites (Table 1). Average size and weight of individuals were 19.5 ± 13.7 mm and 3.8 ± 6.6 g (Table 1). *Cyrtograpsus angulatus* and *Panopeus austrobesus* were collected only at sites influenced by saline water (Barra, Ilha, and Museu), whereas *Neohelice granulata* was present exclusively at the saltmarsh site (Ilha; Table 1). The other two species, the blue crab *Callinectes sapidus* and the invasive species *Rhithropanopeus harrisii*, were found at all five sampling sites (Table 1).

TABLE 1 Summary of crabs' biometrics by species and site of Lagoa dos Patos, Brazil. Minimum (min), maximum (max), and average ± standard deviation of carapace length (Lt) and animal wet weight (Wt).

Species/site	***n***	**Min Lt (mm)**	**Max Lt (mm)**	**Lt ± SD (mm)**	**Min Wt (g)**	**Max Wt (g)**	**Wt ± SD (g)**
Callinectes sapidus							
Total	87	16.5	104.5	43.9 ± 17.7	0.3	70.5	7.2 ± 9.9
SLS	24	27.1	61.3	37.2 ± 8.5	1.04	6.3	3.3 ± 2.0
Pelotas	32	19.3	72.3	41.8 ± 13.7	0.5	21.8	5.4 ± 4.2
Ilha	21	16.5	104.5	54.4 ± 17.7	0.3	70.5	12.5 ± 14.9
Museu	5	24.5	43.1	32.9 ± 7.7	0.6	9.8	3.5 ± 3.7
Barra	5	38.6	88.7	43.7 ± 5.3	2.8	35.4	10.6 ± 13.9
Cyrtograpsus angulatus							
Total	25	8.3	33.2	20.5 ± 7.6	0.3	12.6	3.7 ± 3.4
SLS	0	0	0	0	0	0	0
Pelotas	0	0	0	0	0	0	0
Ilha	17	16.9	29.2	24.3 ± 4.5	1.6	8.0	4.7 ± 2.2
Museu	2	20.5	33.2	26.8 ± 6.3	2.6	12.6	7.6 ± 5.0

Barra	6	8.3	18.1	12.7 ± 4.0	0.3	2.1	0.9 ± 0.7
Neohelice granulata							
Total	41	21	32	24.6 ± 2.6	4.5	20.8	8 ± 3.3
SLS	0	0	0	0	0	0	0
Pelotas	0	0	0	0	0	0	0
Ilha	41	21	32	24.6 ± 2.6	4.5	20.8	8 ± 3.3
Museu	0	0	0	0	0	0	0
Barra	0	0	0	0	0	0	0
Panopeus austrobesus							
Total	191	6.9	50.2	22.1 ± 11.4	0.1	43.5	6.6 ± 8.4
SLS	0	0	0	0	0	0	0
Pelotas	0	0	0	0	0	0	0
Ilha	46	8.5	35.9	13.6 ± 4.1	0.1	13.5	1.1 ± 1.3
Museu	78	6.9	50.2	24.9 ± 14.1	0.1	43.5	9.5 ± 10.4
Barra	67	9.6	44.6	23.7 ± 9.5	0.3	25.4	6.5 ± 6.6
Rhithropanopeus harrisii							
Total	280	5.6	21.2	10.6 ± 2.5	0.1	2.6	0.5 ± 0.4
SLS	35	5.7	18.7	11.3 ± 3.3	0.1	2.3	0.6 ± 0.5
Pelotas	79	5.6	17.6	10.1 ± 2.4	0.1	2.1	0.4 ± 0.3
Ilha	110	7.4	21.2	11.1 ± 1.9	0.1	2.5	0.5 ± 0.3
Museu	44	5.7	15.1	9.4 ± 2.1	0.1	1.2	0.3 ± 0.2

Barra	12	7.5	20.5	11.7 ± 4.3	0.1	2.6	0.7 ± 0.8

Of all analyzed guts, 504 (80.7%) contained at least one food item, whereas 120 (19.3%) were empty and were therefore excluded from the analyses. The frequency of occurrence of food items shows that *C. sapidus*, *C. angulatus*, *P. austrobesus*, and *R. harrisii* feed mainly on Crustacea (Ostracoda, Amphipod, Isopoda, Cirripedia, Tanaidacea, and Decapoda) and Mollusca (Bivalvia, Gastropoda, and the species *Heleobia australis*; Table 2, Figure 2). Organic matter + sediments, together with the above-mentioned, are the items with higher frequency of occurrence (Figure 2). Crustacea and Mollusca together represent more than 35% (accumulated percentage) of the identified items. When adding organic matter + sediments, the combined percentual increases to 70% of total items consumed by these crabs.

The species *N. granulata* presented the highest consumption of plant matter (~50%), indicating an herbivorous habit for the species (Figure 2). Organic matter + sediments is the only item present in high frequency in all species and sites.

TABLE 2 Frequency of occurrence (%) of all items identified in the stomach of the five crab species of Lagoa dos Patos between 2022 and 2023. C. ang, *Cyrtograpsus angulatus*; C. sap, *Callinectes sapidus*; N. gra, *Neohelice granulata*; P. aus, *Panopeus austrobesus*; R. har, *Rithropanopeus harrisii*. Column group shows the respective group to which item was assigned for analyses.

Group	**Item**	**C. ang** n = 23	**C. sap** n = 63	**N. gra** n = 35	**P. aus** n = 148	**R. har** n = 235
Amphipoda	Amphipoda n.i.				2.03	2.05
	Caprella sp.					2.34
	Gammaridae				2.56	2.01
Cirripedia	*Lepas* sp.		3.13			2.86

	Balanus sp.	11.27	24.98	2.44	13.07	1.27
Crustacea	Calanoida					2.86
	Mysis (Penaeidae larvae)				1.49	
	Brachyura n.i.		4.02	2.44	4.16	2.53
	Ostracoda				1.28	
	Tanaidacea				1.49	
	Exoskeleton remains		16.09	2.44	20.83	38.87
H. australis	*Heleobia australis*	5.88	17.23	2.44		
Mollusca	Bivalvia n.i.				4.32	
	Gastropoda n.i.				1.73	3.86
	Shell remains		19.95		8.48	0.91
Polychaeta	Polychaeta		4.76		3.93	10.80
Others	Acarii				1.49	2.86
	Bryozoa				1.73	
	Hydrozoa					2.95
	Nematoda				2.13	
	Nematomorpha				1.39	
Plant matter	Plant material	16.67	11.46	36.59	8.45	32.49
OM + sediment	Organic matter	74.51	63.24	73.17	20.55	11.83
	Organic matter + sediments	19.61	9.23	12.20	41.03	73.14
	Sediment grains				3.67	2.27

Abbreviation: n.i., non-identified items.

The item with the most volume inside the stomachs of all species was organic matter + sediments. Cirripedia and Mollusca also were present in high volume in *C. sapidus* (Figure 3). Plant material was the second most important item in volume for *C. angulatus* and *N. granulata*, while *P. austrobesus* and *R. harrisii* showed higher variability in their items, as well as similarity in the diet composition, with Crustacea (non-identified), Cirripedia, Mollusca, and Plant material presenting similar importance for both species (Figure 3).

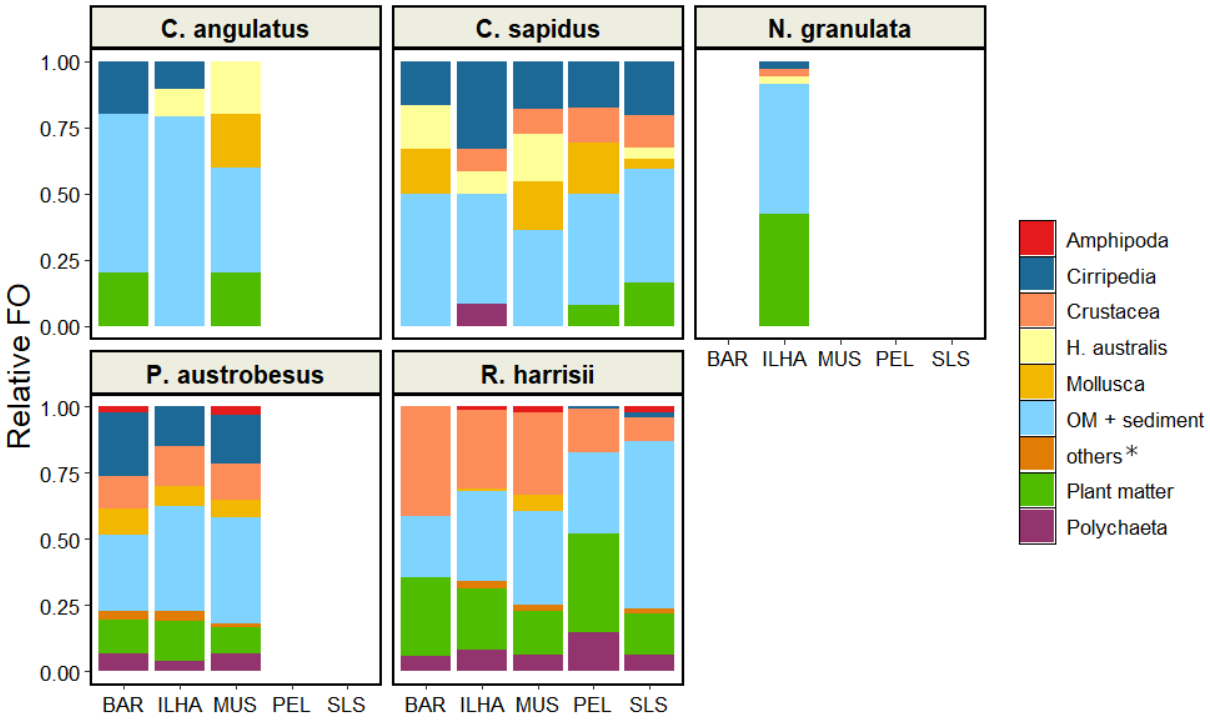


FIGURE 2 Frequency of occurrence (FO) of feeding itens in the crab species *Callinectes sapidus*, *Cyrtograpsus angulatus*, *Neohelice granulata*, *Panopeus austrobesus* and *Rhithropanopeus harrisii* in each sampling site (BAR—Barra, ILHA Ilha, MUS—Museu, PEL—Pelotas, SLS—São Lourenço do Sul) of Lagoa dos Patos, Brazil. Sites are ordered from more saline to less (left to right). Samples were collected between 2022 and 2023. *"others" include, Acarii, Bryozoa, Copepod, Nematoda, and Hydrozoa.

Organic matter + sediments was excluded from the IAi analysis because this item represents remains of non-identified animals and debris mixed with sedimentary matter, which, due to its high frequency, hinders the relative importance of the other items that were identified in the stomachs, also contributing to standardizing the diets and increasing the similarity. Evaluating the relative importance of each item, plant matter and the gastropod *H. australis* dominated the diet of *C. angulatus*. The blue crab *C. sapidus* had a

more diverse diet among locations, including high proportions of Cirripedia, Mollusca, Crustacea (non-identified), and plant matter (Figure 4). Among all the analyzed species, *N. granulata* had the most specialized diet, consuming almost exclusively plant matter. The diet of *P. austrobesus* was dominated by non-identified Crustacea and Cirripedia, while *R. harrisii* had high consumption of non-identified Crustacea (no Cirripedia) and plant matter, the latter especially important in the freshwater sites (Figure 4).

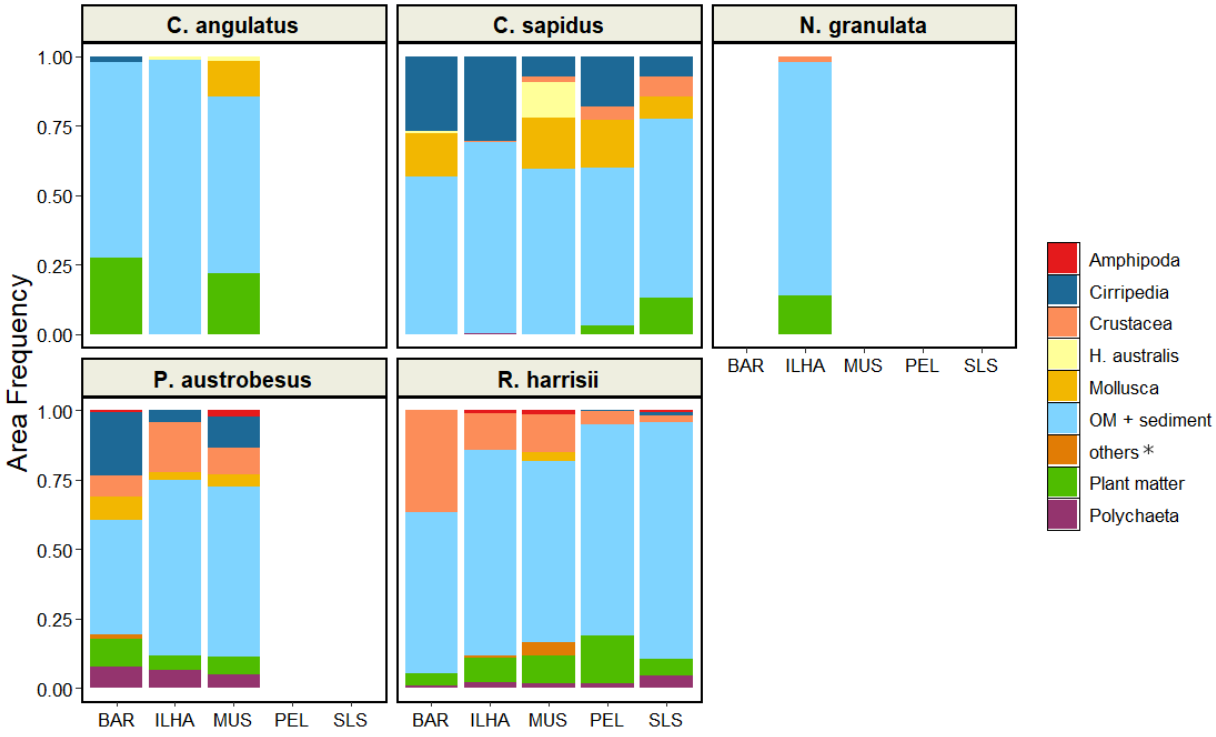


FIGURE 3 Area Frequency of the different items in gut contents of *Callinectes sapidus*, *Cyrtograpsus angulatus*, *Neohelice granulata*, *Panopeus austrobesus* and *Rhithropanopeus harrisii* in five locations (BAR—Barra, ILHA—Ilha, MUS—Museu, PEL—Pelotas, SLS—São Lourenço do Sul) of Lagoa dos Patos, Brazil, sampled between

2022 and 2023. Sites are ordered from more saline to less (left to right). *"others" include, Acarii, Bryozoa, Copepod, Nematoda, and Hydrozoa.

3.2 Natural Diets Comparison Among Species and Sites

Diets differed among species ($F = 8.533$; $df = 4$; $p < 0.001$) and among sites ($F = 1.812$; $df = 4$; $p = 0.014$); however, the species × site interaction was not significant ($F = 2.912$; $df = 7$; $p = 0.053$). These results indicate that the differences observed among sites are mainly due to the absence of certain species at some sampling points. Pairwise comparisons revealed that the diet of *Neohelice granulata* differs from that of most species, except *Cyrtograpsus angulatus*, whereas the diet of *Rhithropanopeus harrisii* differs significantly from the diets of all other species (Table 3). When evaluating which food items were most associated with each crab species, gastropods were associated with *Cyrtograpsus angulatus* (IndVal = 0.441, p = 0.048), Cirripedia (IndVal = 0.556, p = 0.028), and bivalves (IndVal = 0.526, p = 0.031) were significant of *C. sapidus* diet, plant matter was associated with *N. granulata* (IndVal = 0.751, p = 0.001), and crustacea (non-Cirripedia) were associated with *P. austrobesus* and *R. harrisii* (IndVal = 0.772, p = 0.001). For localities of the salinity gradient, Nematoda was associated with the saline site Barra (IndVal = 0.253, p = 0.028), and gastropods were associated with the Museu (IndVal = 0.313, p = 0.016).

Although species diet did not vary statistically across sites, it was possible to notice *C. angulatus* incorporating more gastropods in their diet with the decrease in salinity to compensate for the decrease in Cirripedia (Figure 4). Similarly, *C. sapidus* decreased the consumption of Cirripedia towards freshwater, increasing the consumption of Mollusca in

brackish sites, and other small Crustacea groups in freshwater (Figure 4). *R. harrisii* showed a very clear shift from animal to plant-based diet towards freshwater, evidence of their opportunistic habit and trophic plasticity to shift diets. *P. austrobesus* was the only species that had very similar diets in all sites where they occurred, with a high diversity of items (Figure 4).

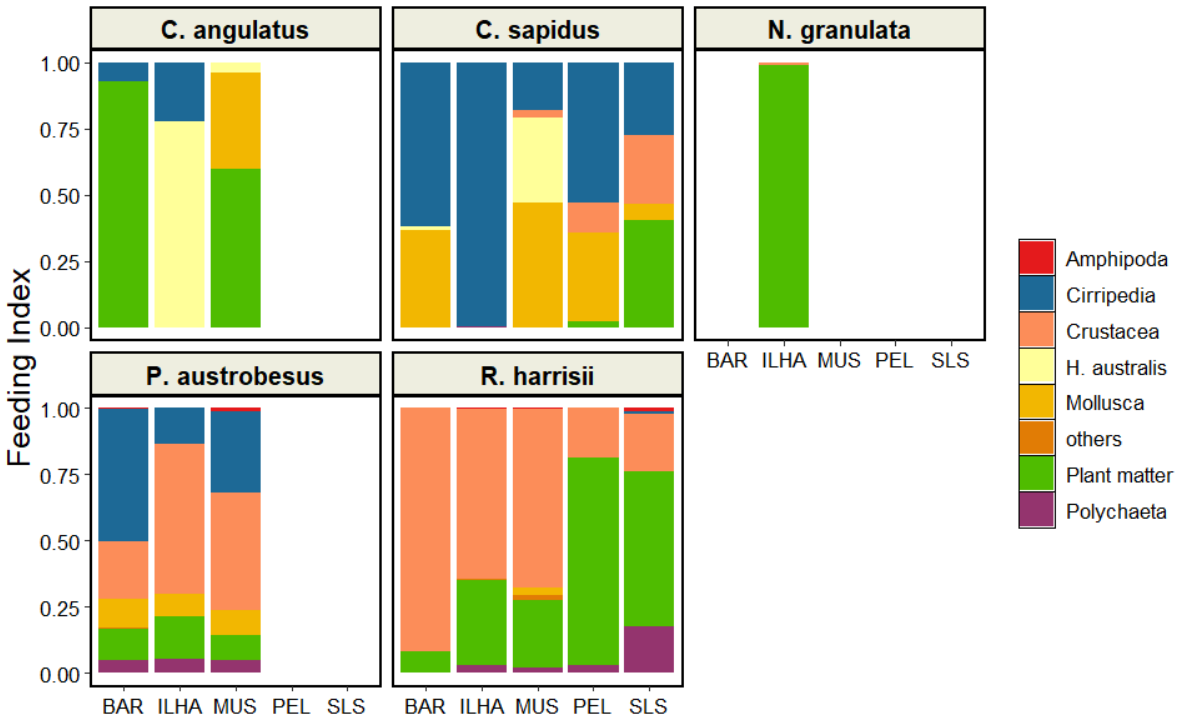


FIGURE 4 Natural diet of *Callinectes sapidus*, *Cyrtograpsus angulatus*, *Neohelice granulata*, *Panopeus austrobesus*, and *Rhithropanopeus harrisii* in the different sites (BAR—Barra, ILHA—Ilha, MUS—Museu, PEL—Pelotas, SLS—São Lourenço do Sul) of Lagoa dos Patos, Brazil expressed by the Feeding Index (IAi). Sites are ordered from more saline to less (left to right). Samples were collected between 2022 and 2023.

3.3 Trophic Niche Overlaps

The Morisita–Horn index indicated low quantitative dietary overlap among species, reflecting differences in the relative importance of consumed items. The highest similarities were observed between *C. sapidus* and *P. austrobesus*, and between *C. angulatus* and *N. granulata* (Table 4), whereas the lowest overlap occurred between *N. granulata* and the invader *R. harrisii* (Table 4, Figure 5).

TABLE 3 Pairwise comparison (PERMANOVA) among crab species diets of Lagoa dos Patos, Brazil, collected between 2022 and 2023. "p.adj" is the p-value adjusted for multiple comparisons using the Bonferroni/Holm correction.

Pairwise	Df	SS	F	R^2	p	p.adj
C. angulatus - *C. sapidus*	1	0.598	1.659	0.062	0.139	1.00
N. granulata - *C. sapidus*	1	3.608	13.439	0.271	0.001	**0.01**
P. austrobesus - *C. sapidus*	1	0.544	1.407	0.013	0.157	1.00
R. harrisii - *C. sapidus*	1	2.817	9.653	0.051	0.001	**0.01**
N. granulata - *C. angulatus*	1	0.906	4.917	0.224	0.010	0.06
P. austrobesus - *C. angulatus*	1	0.755	1.905	0.022	0.052	0.47
R. harrisii - *C. angulatus*	1	0.987	3.456	0.021	0.006	**0.05**
P. austrobesus - *N. granulata*	1	4.538	12.741	0.121	0.001	**0.01**
R. harrisii-N. granulata	1	2.564	9.471	0.053	0.001	**0.01**
R. harrisii-P. austrobesus	1	4.688	14.605	0.059	0.001	**0.01**

The graphical permutational analysis revealed a clear separation among all species' diets, except for *P. austrobesus* and *C. sapidus* (Figure 5). These two species showed overlap in their consumed items, but slightly different centroids (Figure 5), indicating that they exploit a similar set of food items, albeit in different proportions (Figure 4). Samples of *R. harrisii* and *P. austrobesus* showed greater dispersion and low overlap (Figure 5), suggesting higher dietary variability. *N. granulata* had the smallest niche and lowest overlaps.

TABLE 4 Morisita–Horn similarity index for the diets of the five crab species, *Callinectes sapidus*, *Cyrtograpsus angulatus*, *Neohelice granulata*, *Panopeus austrobesus*, and *Rhithropanopeus harrisii* collected in Lagoa dos Patos, Brazil, among 2022 and 2023.

	C. sapidus	***C. angulatus***	***N. granulata***	***P. austrobesus***	***R. harrisii***
C. sapidus	1	0	0	0	0
C. angulatus	0.33	1	0	0	0
N. granulata	0.21	0.40	1	0	0
P. austrobesus	0.42	0.16	0.17	1	0
R. harrisii	0.13	0.13	0.10	0.32	1

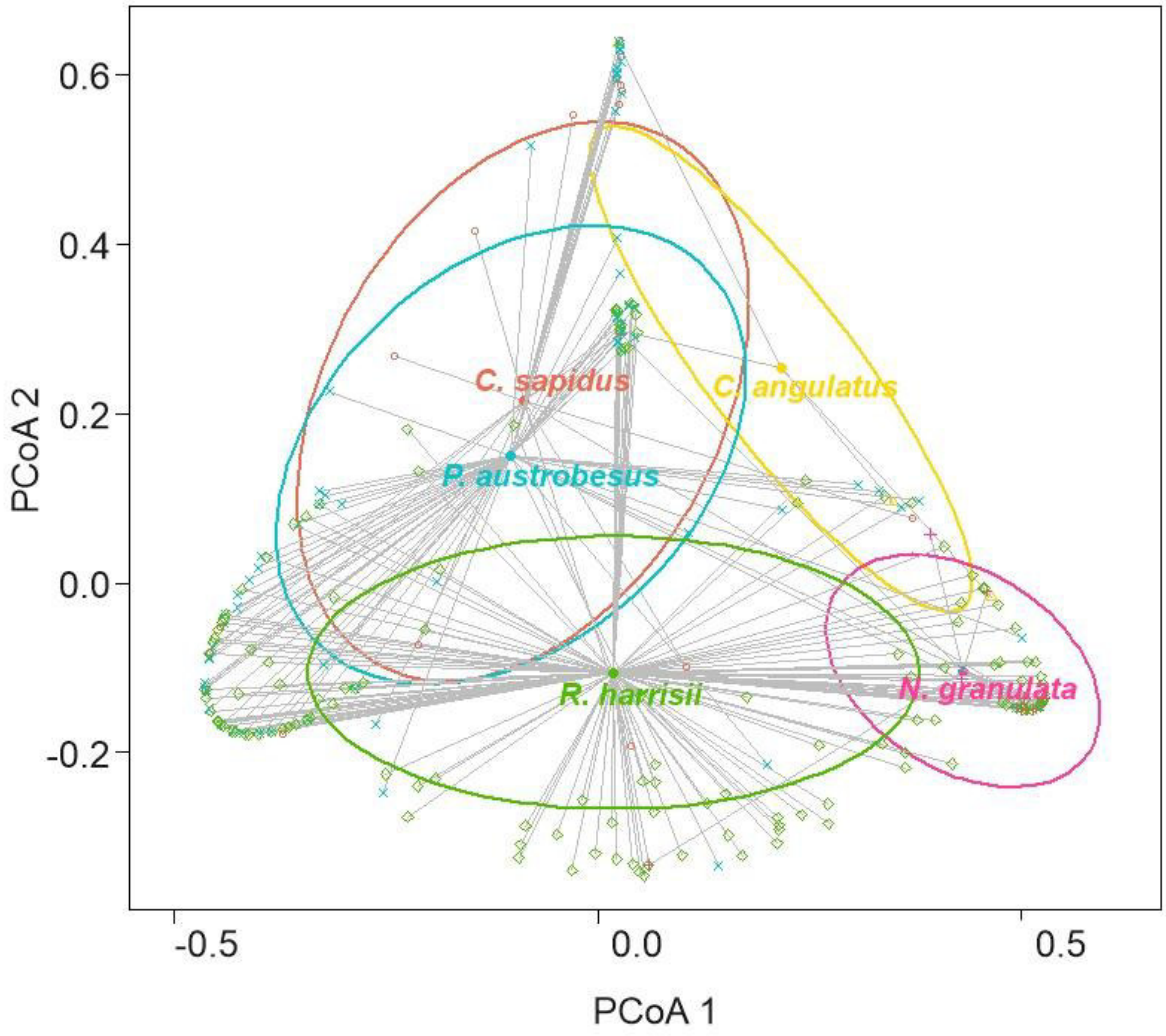


FIGURE 5 Multivariate dispersion plot for the five crab species diet based on the Bray–Curtis dissimilarity matrix. Each point represents a sample, and ellipses were built with one standard deviation from the centroid. Colors indicate the species: *Callinectes sapidus* (red), *Cyrtograpsus angulatus* (yellow), *Neohelice granulata* (pink), *Panopeus austrobesus* (blue), and *Rhithropanopeus harrisii* (green). The relative positions among clusters indicate differences or similarities in diet composition among species.

4. Discussion

The diet analyses employed in this study, which included different metrics to evaluate diet composition, preference, and overlap, revealed a relatively diverse diet among the five species, with differences in the number of consumed items, also allowing the evaluation of the specialization or generalist habit of the studied species. Our results corroborated the hypothesis of generalist feeding habits for the invasive mud crab but rejected the second hypothesis of high niche overlap between the invasive species and other Panopeidae species. Despite similarities in the consumption of some food items, resource partitioning and morphological differences among species likely reduce direct competition. In addition, ecological and behavioral traits, such as differential use of microhabitats, feeding activity periods, and specific trophic preferences, may contribute to the lack of dietary niche overlap. All five species had “organic matter + sediments” as the more abundant item and occupying the most space in the stomach. The high consumption of debris and sediments is a common habit reported for all five species—*Cyrtograpsus angulatus*, *Callinectes sapidus*, *Neohelice granulata*, *Panopeus austrobesus*, and *Rhithropanopeus harrisii* (Turoboyski 1973; Bas et al. 2013; Ortega-Jiménez et al. 2024; Öndes et al. 2025), and the sediment ingestion is associated with the consumption of

mollusks, benthic crustaceans, and plant matter, all resources deeply associated with the bottom (Rakocinski et al. 2003; Bas et al. 2013).

4.1 Trophic Plasticity and Feeding Habits of Crab Species

The invader *R. harrisii*, and natives *C. sapidus* and *P. austrobesus* showed diverse diets, composed of 14, 10, and 18 food items, respectively, much more diverse than *C. angulatus* and *N. granulata*, with 5 and 7 items, respectively. Therefore, our findings corroborate our initial hypothesis that *R. harrisii* would be a very generalist feeder, adapting its diet depending on the availability of resources in each habitat.

Cyrtograpsus angulatus and *N. granulata* showed a strong preference for vegetation, as plant matter was the main item composing their diets. This selectivity in items consumed by these species may limit the distribution and occupation of habitats by these species. *N. granulata* is closely linked to marsh systems, using vegetation for both shelter and consumption, and so any events that reduce marsh habitats will likely result in a decrease in the *N. granulata* population. *C. angulatus*, on the other hand, presented an omnivore and more plastic diet, including almost exclusively animal items in its diet when in the same environment as *N. granulata*. This behavior may indicate that *C. angulatus* has higher adaptability in the use of resources compared to *N. granulata*, and in order to avoid competition, the species feeds on different resources available in the habitat, such as the gastropod *Heleobia australis*, without overlapping *N. granulate* niche. The fact of *H. australis* be the most abundant gastropod in the Lagoa dos Patos estuary (Colling et al. 2007) reinforces this idea.

Contrasting to *N. granulata* and *C. angulatus*, the other species, *R. harrisii*, *C. sapidus*, and *P. austrobesus* showed a high degree of generalism, feeding on a wide variety of food items, such as mollusks, polychaetes, crustaceans, debris, and algae. This trophic plasticity allows them to explore different trophic niches, adapting to environmental variability and abrupt changes in resource availability. As a successful invader, *R. harrisii* confirmed its opportunistic behavior, adjusting its diet to the resource availability which allows using new estuarine habitats (D'Incao and Martins 1998; Hegele-Drywa and Normant 2009). The mud crab was mostly feeding on benthic crustaceans in the saltwater sites of the salinity gradient and switched to heavily relying on plants in the freshwater sites, likely due to the lower abundance of benthic crustaceans in low-salinity habitats (Udalov et al. 2005; Uwadiae 2009). Trophic plasticity was also observed in *C. sapidus*. This species is broadly studied and known as an omnivore and scavenger, showing ontogenetic shifts in its diet and seasonality in feeding resources, acting as an important regulator of benthic communities (Ryer 1987; Oliveira et al. 2006; Encarnação et al. 2025). A much less well-known species, *P. austrobesus*, shares similar feeding patterns to other Panopeidae, feeding on prey with hard shells such as bivalves and gastropods (Meyer 1994; Milke and Kennedy 2001), organisms broadly available in estuarine bottoms (Udalov et al. 2005; Colling et al. 2007; Uwadiae 2009). However, *P. austrobesus* is limited to high salinity habitats, and was not found in salinities below 10, differently from the invader Panopeidae, *R. harrisii*. This trophic plasticity can favor the colonization and persistence of both species in different habitats of Lagoa dos Patos, including areas with high anthropogenic pressure or low availability of specific feeding resources.

4.2 Spatial Variability in Crab Diets

The PERMANOVA indicated differences in food items consumed among sites; however, this was not observed within each species' diet along the salinity gradient, which maintained consistency as shown by the interactive term of the analysis. This uniformity of consumption within the species suggests that the food resources used by these crabs are available in all salinity gradients, but in variable proportions, and so the species showed flexibility in their trophic strategies, using opportunistically the most available resource in each habitat. In the estuarine portion of the lagoon, the macroinvertebrate and vegetation communities are well-described, being composed of different species of amphipods, barnacles, isopods, tanaids, ostracods, mollusks, polychaetes, phanerogams, and macroalgae (Capitoli et al. 1978; Bemvenuti 1987, 1997; Copertino and Seeliger 2010). In the freshwater portion (São Lourenço do Sul site) and transition zone (Pelotas site), we found representatives of similar groups in gut contents, namely amphipods, barnacles, other crustaceans, mollusks, and polychaetes. This similarity in group composition allows for euryhaline crabs to inhabit these different habitats of the lagoon.

Although similar groups of food items were found from salinity 0 to 30, it is worth noticing that in more saline sites (Barra to Museu) the crab species tended to have higher consumption of barnacles and gastropods, while in freshwater (Pelotas and SLS) these items were less found in the guts, with an increase in plants and other smaller crustaceans (most likely amphipods). This shift is expected as the barnacles do not tolerate low salinities (Foster 2018). The structure of the traps, as well as the pier where traps were deployed offers broad substrate for barnacles to attach, and so an abundant resource for

these crab species to feed on in saltwater. On the other hand, freshwater sites allow for different aquatic vegetation to grow, attracting different crustaceans, such as amphipods, that live closely associated with vegetation. We highlight that the p-value of our analysis was marginally significant ($p = 0.053$), therefore we can posit that the lack of statistical response in the spatial variation may be due to our small sample size for some crab species in freshwater sites. C. sapidus was found in all five sites and presented a very similar diet in all habitats, which may be due to their efficient swimming capacity (Spirito 1972), allowing them to explore more habitats than the other species, and move between salt and freshwater in a quick period of time. Because this was one of the few species found in all sites, any spatial differences may be masked by the C. sapidus diet.

4.3 Among-Species Diet Variation and Niche Overlap

The pairwise analysis showed that *N. granulata*, as the only herbivore, differs in its diet from all the other species but *C. angulatus*. These differences in feeding habits likely derive from morphological variation in structures of capture and manipulation of food, besides ecological and behavioral peculiarities, as the differential use of microhabitats, feeding period, or specific feeding preferences. *C. angulatus* is classified as an herbivore/opportunistic species that adapts its diet to the resource availability (Capitoli and Ortega 1993). This opportunistic habit was evident in our work, where *C. angulatus* presented an herbivore diet at Barra, a strictly carnivore diet at Ilha, and omnivore carnivore-herbivore at Museu. Contrasting, *N. granulata* feeds predominantly on plant matter decaying on sediments, and by actively cutting marsh leaves such as *Spartina* sp., which is widely available in the estuary. The herbivory and detritivore behavior is well

documented for *N. granulata* in the Lagoa dos Patos estuary and Argentinean coast (D'Incao et al. 1990; Barutot et al. 2011; Bas et al. 2013). Specifically, about 70% of *N. granulata* diet was composed of plant matter and sediments, and the remaining 30% included polychaeta bristles, copepods, bryozoa, and nematoids (D'Incao et al. 1990; Barutot et al. 2011; Bas et al. 2013), similarly to our findings, where 75% of the diet was composed of plant matter + sediments and 25% of animal remains.

Another pattern identified by the pairwise comparisons was that *R. harrisii*, the invader, showed a distinct diet from all species. As an omnivore/generalist species (Turoboyski 1973; Hegele-Drywa and Normant 2009), we expected a wide range of items consumed by the species, overlapping with other carnivore species. In Lagoa dos Patos, 66% of *R. harrisii* diet was composed of organic matter, sediments and plant matter, and the remaining included mollusks, crustaceans and polychaetas, very similar to what was found in the Baltic Sea (Poland), where 61% was “detritus” and the remaining animal remains, algae and organic matter (Czerniejewski and Rybczyk 2008). The Panopeidae family is known for a generalist diet focusing on small invertebrate prey associated with soft sediments or hard structures, being classified as omnivore predators (Bosa and Masunari 2002; Silliman et al. 2004). However, *R. harrissii* and *P. austrobesus* showed distinct diets, against our initial hypothesis. Both species consumed similar items, as evident in Table 1, but the proportion of each item consumed differs. *P. austrobesus* showed a preference for barnacles and mollusks, likely due to its robust claws that allow breaking hard shells, while *R. harrisii* has smaller claws, making it more difficult to prey on these organisms, and therefore, a higher consumption of debris and plants was observed by this

species. This preference of Panopeidae crabs and the blue crab for small animals is very distinct from the feeding habits of *C. angulatus* and *N. granulata*, which presented an herbivore diet.

The niche overlap analysis (Morisita–Horn Index) agreed with the pairwise analyses, showing low overlap among all species, and again not supporting our initial hypothesis. The lower overlap was found between *N. granulata* and *R. harrisii* (CH = 0.099), herbivore versus generalist. Because the majority of the species are omnivores, generalists, and opportunistic species (Turoboyski 1973; Laughlin 1982; Capitoli and Ortega 1993; Silliman et al. 2004; Oliveira et al. 2006; Barutot et al. 2011; Bas et al. 2013), and the Lagoa dos Patos estuary provides an abundance of animal and plant resources (Bemvenuti 1987, 1997; Copertino and Seeliger 2010), the estuary warrants diversity of niches, with the possibility of resources partitioning without compromising the coexistence of all the species. In Guadalquivir estuary (Spain), a study evaluating niche overlap (Morisita's Index) between a native and an invasive shrimp found high trophic niche overlap, which would suggest competition for food resources among these species; however the invader showed high tolerance to anoxic condition than the native species, and so the authors suggest these species partitioning of other dimensions of niche, such as habitat (González-Ortegón et al. 2010), showing the diversity of niches available in estuaries that can support new species colonization. Although different diets, the five crab species shared similar items, likely due to the high abundance and availability of these resources in the lagoon.

5. Conclusions

Our study presents new information about the feeding ecology of *C. sapidus*, *C. angulatus*, *N. granulata*, *P. austrobesus*, and *R. harrisii* in the Lagoa dos Patos estuary and freshwater portion, indicating that their natural diets are composed majority of benthic prey from hard and soft substrate. Species presented both similarities and differences in their diets. Although many species presented similar items composing their diets, the dominance of these items varied, resulting in low trophic niche overlap among species and a certain degree of selectivity. This pattern indicates low/absence of interspecific competition, with resource partitioning, likely associated with differential periods of foraging or use of distinct microhabitats. The use of different resources across different habitats highlights the generalist and opportunistic habits of most of these crab species, efficiently adapting to the local conditions and exploring available resources along the salinity gradient of the lagoon.

The invasive mud crab *Rhithropanopeus harrisii* does not appear to negatively affect native species populations, based on the differences observed between the diets and trophic niche of native species and the invasive species. However, this interpretation should be approached with caution. *R. harrisii* was first recorded in the Lagoa dos Patos estuary in 1980, and since then, no studies have described community structure, population dynamics, or feeding habits of native species prior to the invasion. The absence of such historical baseline data limits a direct assessment of potential invasion-induced changes in the native community. After more than four decades since *R. harrisii* first record, it is plausible that native populations are currently in homeostasis with the invader. In this context, the low trophic niche overlap observed in this study suggests that the mud crab may have occupied a previously underutilized niche, or that native species have adopted resource-partitioning

strategies throughout the invasion process. From a management perspective, these results indicate that long-established invasive species may become functionally integrated into local food webs, reinforcing the importance of early monitoring and the acquisition of long-term baseline data for detecting and mitigating ecological impacts during the initial stages of biological invasions.

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CAPÍTULO V

Conclusões

Com base nos capítulos apresentados, esta tese demonstrou que o caranguejo invasor *Rhithropanopeus harrisii* possui elevada capacidade de estabelecimento, expansão e adaptação ecológica na Lagoa dos Patos, consolidando-se como uma espécie invasora amplamente distribuída ao longo do gradiente salino do sistema lagunar. Ainda, vale ressaltar que esse trabalho traz o primeiro registro da espécie para além da região estuarina da Lagoa dos Patos, demonstrando que a espécie está bem estabelecida no estuário, e iniciou a colonização de novos habitats na região doce da lagoa. A integração entre abordagens ecológicas, tróficas, genéticas e de modelagem espacial permitiu compreender diferentes dimensões do processo invasivo, fornecendo um panorama abrangente sobre os mecanismos que favorecem o sucesso da espécie em ambientes subtropicais.

Sob a perspectiva genética, os resultados indicaram que a população invasora presente na Lagoa dos Patos provavelmente teve origem na costa leste dos Estados Unidos, especialmente da região da Carolina do Norte, em que a água de lastro de embarcações foi o vetor mais provável da introdução da espécie no sul do Brasil. A baixa diversidade haplotípica observada na população brasileira sugere um evento de introdução relativamente recente associado ao efeito fundador, e, mesmo sob essa condição, a espécie demonstrou elevada capacidade de estabelecimento com reduzida variabilidade genética. Esses resultados reforçam o papel das atividades portuárias como importantes vetores de

invasões biológicas em ambientes estuarinos e evidenciam a necessidade de maior controle sobre o manejo de água de lastro e bioincrustação em sistemas costeiros.

Os resultados da modelagem de distribuição potencial evidenciaram que a ocorrência de *R. harrisii* está fortemente associada a gradientes ambientais relacionados principalmente à matéria orgânica sedimentar, salinidade e profundidade. A ampla adequabilidade ambiental observada em diferentes setores da Lagoa dos Patos demonstra que a espécie apresenta elevada plasticidade ecológica e tolerância fisiológica, permitindo sua permanência tanto em áreas estuarinas quanto em regiões oligohalinas e de água doce. A convergência entre os modelos Maxent e GAM reforçou a consistência ecológica dos padrões encontrados, mesmo diante das limitações associadas ao reduzido número de registros de ocorrência utilizados nas análises. Do ponto de vista ecológico, os resultados indicam que a invasão de *R. harrisii* não depende exclusivamente de condições ambientais restritas, mas sim da combinação entre elevada tolerância fisiológica, comportamento oportunista e capacidade de explorar diferentes tipos de habitat. Essa versatilidade favorece sua expansão espacial e aumenta o potencial de colonização de novas áreas da Lagoa dos Patos e possivelmente de outros sistemas costeiros sul-americanos. Além disso, os modelos preditivos evidenciaram regiões ambientalmente adequadas que ainda possuem baixa amostragem, destacando a importância de programas contínuos de monitoramento para acompanhar a dinâmica populacional da espécie.

As análises tróficas demonstraram que *R. harrisii* apresenta hábito alimentar generalista e elevada plasticidade alimentar, consumindo diferentes grupos de invertebrados bentônicos, detritos e material vegetal conforme a disponibilidade de

recursos ao longo do gradiente salino. Apesar dessa plasticidade trófica, os resultados indicaram baixa sobreposição alimentar com a maioria das espécies nativas analisadas, sugerindo que a coexistência atual pode estar sendo favorecida por diferenciação de nicho e partilha de recursos. Entretanto, a elevada capacidade adaptativa observada sugere que possíveis alterações ambientais futuras, especialmente relacionadas a mudanças hidrológicas e climáticas, podem modificar essas relações ecológicas e potencializar impactos sobre a fauna bentônica nativa.

Em conjunto, os resultados desta tese evidenciam que o sucesso invasivo de *R. harrisii* na Lagoa dos Patos resulta da combinação entre elevada plasticidade ecológica, ampla tolerância ambiental, plasticidade trófica e capacidade de dispersão mediada por atividades humanas. Essas características tornam a espécie um invasor altamente eficiente, capaz de explorar rapidamente ambientes heterogêneos e responder positivamente a diferentes condições ambientais. Dessa forma, esta tese contribui significativamente para o entendimento dos processos de invasão biológica em ecossistemas estuarinos subtropicais. Os resultados fornecem informações relevantes para o monitoramento ambiental, conservação da biodiversidade e manejo de espécies invasoras. Além disso, o estudo reforça a importância da integração entre ferramentas de modelagem ecológica, genética populacional e ecologia trófica para compreender os mecanismos associados à introdução, estabelecimento e expansão de espécies exóticas em ambientes aquáticos.

Os resultados também apresentam implicações diretas para o monitoramento da invasão. O registro de *R. harrisii* além da região estuarina, associado à identificação de áreas ambientalmente adequadas ainda pouco amostradas, indica a necessidade de ampliar

os levantamentos para setores internos da Lagoa dos Patos e para sistemas costeiros adjacentes. Nesse contexto, os modelos de distribuição podem ser utilizados como ferramenta de apoio para direcionar amostragens a áreas com maior potencial de ocorrência, enquanto os resultados genéticos podem auxiliar no acompanhamento da conectividade entre populações e na identificação de novos eventos de introdução. A manutenção de programas de monitoramento espacial e temporal, associada à vigilância dos principais vetores antropogênicos, poderá contribuir para a detecção precoce de novas frentes de expansão. Do ponto de vista da conservação, a baixa sobreposição alimentar observada nas condições avaliadas sugere que não há evidências de forte competição trófica com a maioria das espécies analisadas; entretanto, o acompanhamento das comunidades bentônicas permanece importante, uma vez que alterações ambientais podem modificar essas interações.

Ao mesmo tempo, os resultados obtidos levantam novas questões sobre a trajetória da invasão. Entre as principais estão: qual é a atual extensão da distribuição de *R. harrisii* na Lagoa dos Patos e qual a velocidade de sua expansão em direção às regiões de menor salinidade? a população brasileira resulta de um único evento de introdução ou de múltiplas introduções? qual é o grau de conectividade entre as populações da Lagoa dos Patos e aquelas presentes em outros estuários brasileiros? e quais fatores ambientais e antrópicos determinam o estabelecimento de novos focos de ocorrência? Essas questões poderão ser investigadas por meio da ampliação das amostragens espaciais e temporais, do emprego de marcadores genéticos de maior resolução e da integração de dados ambientais, hidrológicos e relacionados à atividade portuária.

Além disso, experimentos de tolerância fisiológica poderão esclarecer os mecanismos que permitem a ocupação de ambientes oligohalinos e de água doce, enquanto experimentos de exclusão e manipulação de recursos poderão testar diretamente os efeitos da espécie sobre organismos nativos e comunidades bentônicas. A incorporação de interações bióticas, séries temporais ambientais e cenários de mudanças climáticas aos modelos de distribuição também permitirá avaliar possíveis alterações futuras na área de ocorrência da espécie. Dessa forma, esta tese não encerra as questões relacionadas à invasão de *R. harrisii*, mas estabelece uma base para uma agenda integrada de pesquisa, monitoramento e avaliação de impactos, contribuindo para antecipar possíveis novas frentes de expansão e subsidiar estratégias de prevenção e manejo da espécie na Lagoa dos Patos e em outros sistemas costeiros subtropicais.