

**RESPOSTAS DO CARANGUEJO-FANTASMA DO ATLÂNTICO**  
*Ocypode quadrata* (FABRICIUS, 1787) A IMPACTOS  
**ANTROPOGÊNICOS: MÉTRICAS DIRETAS E INDIRETAS**

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SETEMBRO DE 2019

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“Tese apresentada ao Centro de Biociências e Biotecnologia da Universidade Estadual do Norte Fluminense Darcy Ribeiro, como parte das exigências para obtenção do título de Doutor em Ecologia e Recursos Naturais.”

Orientadora: Prof.<sup>a</sup> Dr.<sup>a</sup> Ilana Rosental Zalmon

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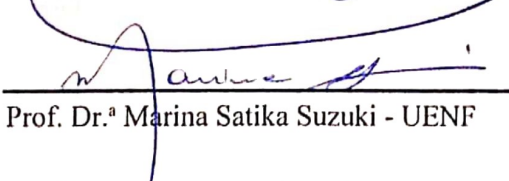
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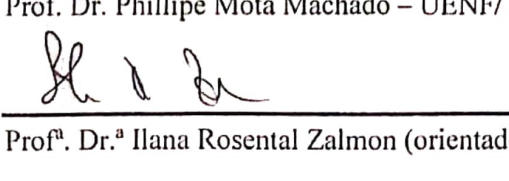
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## LISTA DE ARTIGOS

Esta tese está redigida em formato de artigos científicos, referenciados no texto por meio dos seguintes capítulos em numeração romana:

- I. Costa, L.L., Zalmon, I.R. Multiple metrics of the ghost crab *Ocypode quadrata* (Fabricius, 1787) for impact assessments on sandy beaches. *Estuarine, Coastal and Shelf Science*, 218, 237-245.
- II. Costa, L.L., Madureira, J.F., Zalmon, I.R. Changes in the behaviour of *Ocypode quadrata* (Fabricius, 1787) after experimental trampling. *Journal of the Marine Biological Association of the United Kingdom*, 99 (5), 1135-1140.
- III. Costa, L.L., Arueira, V.F., Da Costa, M.F., Di Benedetto, A.P.M., Zalmon, I.R. Can the Atlantic ghost crab be a potential biomonitor of microplastic pollution of sandy beaches sediment? *Marine Pollution Bulletin*, 145, 5-13.
- IV. Costa, L.L., Secco, H., Arueira, V.F., Zalmon, I.R. Mortality of the Atlantic ghost crab *Ocypode quadrata* (Fabricius, 1787) due to vehicle traffic on sandy beaches: a road ecology approach. Submetido.

O autor principal foi responsável pelo planejamento do estudo, coleta e análise dos dados e redação de todos os tópicos dos capítulos em formato de artigos. Todos os coautores participaram ativamente da coleta de dados e/ou contribuíram intelectualmente no trabalho e estão cientes da publicação ou submissão dos artigos. A contribuição detalhada de cada coautor está disponível no material suplementar ao final da tese.

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## LISTA DE ABREVIATURAS

**AIC** - Akaike's information criterion  
**BD**- Burrow depth  
**BS**- Burrow size  
**D**- Dry Season  
**DB**- Diurnal burrow  
**ET**- Emersion time  
**EF**- Emersion Frequency  
**FC**- Foraging crabs  
**GBA**- Grussaí Beach Arc  
**HIA**- High-Impact A  
**HIB**- High-Impact B  
**IA**- Intermediate beach A  
**IB**- Intermediate beach B  
**LIA**- Low-Impact A  
**LIB**- Low-Impact B  
**MP**- Microplastic  
**MIA**- Medium-Impact A  
**MIB**- Medium-Impact B  
**NB**- Nocturnal burrow  
**nMDS**- Non-Metric Multidimensional Scalling  
**NUA**- Non-urbanized beach A  
**NUB**- Non-urbanized beach B  
**OB**- Occupied burrow  
**PERMANOVA**- Permutational Analysis of Variance  
**R**- Rainy Season  
**SIMPER**- Similarity Percentage Analysis  
**TD**- Tolerance distance  
**UA**- Urbanized beach A  
**UB**- Urbanized beach B  
**VIF**- Variation Inflation Factor



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## RESUMO

Praias arenosas são ecossistemas sob intensa pressão humana em todo o mundo. Caranguejos-fantasma (Crustacea: Ocypodidae) têm sido utilizados como indicadores da urbanização de praias, mas os mecanismos por trás da redução na sua abundância e tamanho corporal em áreas perturbadas ainda são pouco conhecidos. O objetivo desse estudo foi avaliar as respostas do caranguejo fantasma do Atlântico *Ocypode quadrata* (Fabricius, 1787) a múltiplos impactos humanos, incluindo urbanização, pisoteio, poluição por microplásticos e tráfego de veículos. O estudo foi conduzido em áreas com diferentes níveis de perturbações humanas no Arco Praial de Grussaí, sudeste do Brasil. Quatro hipóteses foram testadas: (i) A densidade e o diâmetro das tocas têm relação negativa com a urbanização, mas não são métricas tão eficientes como o número de indivíduos da espécie forrageando à noite como indicador ecológico; (ii) o pisoteio experimental exerce efeitos de curto-prazo na densidade de tocas e no comportamento da espécie; (iii) o caranguejo-fantasma do Atlântico ingere mais microplásticos quando este poluente é mais abundante no sedimento; e (iv) a incidência de caranguejos atropelados por veículos é explicado pela intensidade do tráfego e/ou nível de urbanização. Embora a densidade e o diâmetro das tocas tenham sido negativamente relacionados à urbanização ( $R^2 > 0.55$ ;  $p < 0.01$ ), o número de caranguejos forrageando no período noturno foi a variável que mais explicou (57%) a dissimilaridade de múltiplas métricas diretas e indiretas entre praias com diferentes níveis de urbanização. Portanto, a contagem noturna de caranguejos forrageando é um método mais confiável para as avaliações de impacto, utilizando o caranguejo-fantasma como indicador. O caranguejo-fantasma evitou as áreas mais pisoteadas (3000 x) 24 horas após o experimento ( $p_{\text{permanova}} = 0,036$ ), mas tais áreas foram recolonizadas nos dias seguintes. O tempo de emergência das tocas e a distância de escape de humanos foram reduzidos ( $p_{\text{teste-T}} < 0,001$ ) nas áreas mais pisoteadas, sugerindo uma menor atividade superficial e evasão de áreas com a superfície irregular do sedimento (elevado micro-relevo) pela espécie. A prevalência de microplásticos no trato digestivo dos caranguejos (~37%) não foi relacionada à densidade desse poluente em praias com diferentes níveis de urbanização ( $R^2 < 0,01$ ). Isso indica que a elevada plasticidade trófica de *O. quadrata* e as múltiplas fontes de contaminação podem dificultar o uso da espécie como biomonitor da poluição por microplásticos. Modelos Lineares Generalizados como distribuição binomial evidenciaram que o número de marcas de pneus na areia (indicador do tráfego de veículos) foi positivamente relacionado ao número de caranguejos atropelados na praia ( $p < 0,001$ ), principalmente em áreas não urbanizadas, onde a espécie é mais ativa e abundante. Caranguejos atropelados também foram encontrados na estrada de barro que corta áreas não-urbanizadas, demonstrando uma preocupação sobre estratégias de conservação de praias “além da areia”. O pisoteio e poluição por microplásticos não parecem ser as principais causas de curto-prazo da redução do tamanho da população do caranguejo-fantasma do Atlântico no Arco Praial de Grussaí, enquanto o tráfego de veículos exerce elevada mortalidade direta nas praias. Portanto, o tráfego de veículos deve ser controlado como medida de manejo prioritária na região.

## ABSTRACT

Sandy beaches are ecosystems under intense human pressure worldwide. Ghost crabs (Crustacea: Ocypodidae) have been used as indicator of beach urbanization, but the mechanisms behind the reduction in their abundance and size in disturbed areas are still poorly known. The objective of this study was to assess the responses of the Atlantic ghost crab *Ocypode quadrata* (Fabricius, 1787) to multiple human impacts, including urbanization, trampling, microplastic pollution and vehicle traffic. The study was conducted in areas with distinct levels of human disturbances on the Grussaí Beach Arc, Southeastern, Brazil. Four hypotheses were tested: (i) burrow density and size are negatively related with urbanization, but they are not as efficient as the number of foraging crabs at night as ecological indicator. (ii) experimental trampling exerts short-term effects on the burrow abundance and behaviour of the ghost crab; (iii) the ghost crab ingests more microplastics when this pollutant is more abundant in the sediment; and (iv) the incidence of ghost crabs killed by vehicles is explained by traffic intensity and/or urbanization level. Although both burrow density and size had been negatively related to urbanization ( $R^2 > 0,55$ ;  $p < 0.01$ ), the number of foraging crabs at night was the variable that most explained (57%) the dissimilarity of multiple direct and indirect metrics among beaches with distinct urbanization levels. Thus, the nocturnal counting of foraging crabs is a more reliable method in impact assessments, using the ghost crab as indicator. The ghost crab avoided the heavily trampled (3000x) plots for 24h after the experiment ( $p_{\text{permanova}} = 0.036$ ), but these plots were recolonized over the following days. The emersion time and escape distance from humans were delayed ( $p_{\text{t-test}} < 0.001$ ) in the most trampled plots, suggesting a lower surface activity and an avoidance of irregular (i.e. high micro-relief) sediment surfaces by the species. The prevalence of microplastic in the gut content of the crabs (~37%) was not related to the density of this pollutant on beaches with distinct urbanization levels, according to regression analysis ( $R^2 < 0.01$ ). This indicates that high trophic plasticity of *O. quadrata* and the multiple-sources of contamination may impair its use as a biomonitor of microplastic pollution. Finally, generalized linear models with binomial distributions showed that the number of tire tracks (proxy of vehicle traffic) was positively related with ghost crab car-kills on the beach ( $p < 0.001$ ), mainly in non-urbanized areas, where the species are more active and abundant, confirming the fourth hypothesis. Ghost crabs killed by vehicles were also found on the dirt road crossing low-urbanized areas, showing a concern about beach conservation strategies “beyond the sand”. Trampling and microplastic ingestion seems to be not the main short-term causes of reduction of ghost crabs population size on the Grussaí Beach Arc, while vehicle traffic exerts high direct mortality on the beaches. Therefore, vehicle traffic must be severely controlled as the priority mitigation action in the region.



## 1. INTRODUÇÃO GERAL

Aproximadamente 70% das zonas costeiras do planeta são compostas por praias arenosas (Mclachlan e Defeo, 2017). As praias são importantes áreas de nidificação e alimentação de espécies marinhas ameaçadas (tartarugas e aves costeiras), berçário para recursos pesqueiros, proteção da linha costeira contra eventos extremos e processos erosivos, além de contribuir com a produção secundária e ciclagem de nutrientes por invertebrados endêmicos (Mclachlan e Defeo, 2017). Ainda que sua importância ecológica seja evidente, as praias são ambientes reconhecidos pela população, inclusive por tomadores de decisão, quase exclusivamente pelo seu valor turístico e recreacional (Schlacher *et al.*, 2008). Como consequência do investimento prioritário em infraestrutura para atender a economia do turismo, a despeito das funções ecológicas e da biodiversidade, as praias têm sofrido com múltiplas perturbações, como urbanização e impactos associados (luz artificial, limpeza de praia e supressão de dunas e vegetação), pisoteio, poluição por lixo (macro e microplásticos) e tráfego de veículos (Defeo *et al.*, 2009).

A crescente perturbação humana em praias demanda estratégias de monitoramento, manejo e conservação. Investigações ecológicas de longo prazo são desafiantes, em razão da natureza dinâmica do ecossistema, necessidade por decisões rápidas e limitação de recursos financeiros (Caro, 2010). Assim, o direcionamento de esforços para o manejo de poucas espécies como atalho para diagnósticos ambientais amplos (de todo o ecossistema) tem sido uma estratégia adotada em vários ecossistemas (Simberloff, 1998; Caro, 2010;). Espécies indicadoras, por exemplo, têm sido utilizadas para sinalizar impactos humanos em praias (Costa e Zalmon, 2019a); são espécies fáceis de monitorar e que respondem previsivelmente a alterações ambientais (Market *et al.*, 2003). Critérios para a seleção de espécies indicadoras incluem, além da facilidade de amostragem e sensibilidade ao impacto, a abundância local e o conhecimento acerca da biologia da espécie-alvo (Sidding *et al.*, 2017).

Caranguejos-fantasma (Crustacea: Ocypodidae) são animais endêmicos, normalmente abundantes e amplamente estudados em praias arenosas (Lucrezi e Schlacher, 2014; Schlacher *et al.*, 2016a). A espécie *Ocypode quadrata* (Fabricius, 1787) é a única espécie de caranguejo-fantasma da costa Atlântica. A característica comum a todas as espécies dessa família é a construção de tocas semipermanentes

nas zonas provisória ou permanentemente emersas da praia (Lucrezi e Schlacher, 2014). Apoiados por Warren (1990), que encontrou forte correlação ( $r= 0,84$ ) entre o número de tocas e o número de indivíduos de *Heloccius eordiformis* (Crustacea: Ocypodidae) em um manguezal na Austrália, diversos autores têm recomendado a densidade de tocas de caranguejos-fantasma como indicador de impactos humanos em praias arenosas (Barros, 2001; Neves e Benvenuti, 2006; Magalhães *et al.*, 2009; Gül e Griffen, 2018).

As tocas têm sido utilizadas, portanto, como ferramenta simples e de baixo custo para estimar o tamanho das populações ou determinar variações na abundância e tamanho corporal de caranguejos-fantasma em resposta a condições naturais e induzidas pela ação humana (Hill e Hunter, 1973; Schlacher *et al.*, 2016a). Estima-se que mais de 90% dos estudos sobre o potencial indicador de caranguejos-fantasma no mundo baseiam-se somente no uso de tocas (Costa e Zalmon, 2019a). Os resultados desses estudos em escala global demonstram que, em geral, praias impactadas possuem menores densidades e diâmetro de tocas comparadas às praias pouco-impactadas (Schlacher *et al.*, 2016b). Alguns autores defendem que, mesmo que o número de tocas não reflita o tamanho das populações, a diferença na densidade das mesmas entre praias sob diferentes níveis de impacto revela no mínimo mudanças comportamentais, que ainda são úteis como indicadores ecológicos (Barros, 2001).

De fato, em geral há mais tocas do que indivíduos de caranguejos-fantasma nas praias, logo a contagem de tocas superestima o tamanho das populações (Pombo e Turra, 2013; Silva e Calado, 2013; Schlacher *et al.*, 2016b). Múltiplos fatores abióticos (inundação, vento, umidade, mobilidade do sedimento) e bióticos (territorialismo, manutenção de mais de uma toca, invasão de intrusos) determinam a ocupação e/ou atividade de construção de tocas pelos caranguejos-fantasma (Pombo *et al.*, 2017b). Ainda, a proporção de tocas não ocupadas parece ser maior em praias pouco perturbadas comparadas às praias sob intensa pressão humana (Pombo e Turra, 2019). Assim, espera-se que avaliações de impacto baseadas somente na contagem de tocas sem critérios para reduzir os erros associados a ocupação ou não das mesmas, imponha riscos de superestimação não só do tamanho das populações, mas também do próprio impacto (Pombo e Turra, 2013; 2019). Ainda, a taxa de ocupação de tocas é considerada dinâmica espaço-temporalmente, de modo que fatores de conversão não devem ser usados para extrapolações em ampla escala

(Schlacher *et al.*, 2016c). Por outro lado, previsões do tamanho corporal de caranguejos-fantasma a partir da largura da entrada das tocas são mais precisas (Oliveira *et al.*, 2016; Schlacher *et al.*, 2016c).

Devido a facilidade de amostragem, estudos de comparação da densidade e diâmetro de tocas entre praias sob diferentes níveis de urbanização disseminou-se em todo o mundo, sem que os mecanismos responsáveis por esses padrões fossem claramente revelados (Schlacher *et al.*, 2016b). A urbanização de praias normalmente culmina na ocorrência de múltiplos impactos simultaneamente e, se cada impacto não for estudado isoladamente, a identificação das relações de causa e efeito torna-se improvável, assim como a elaboração de estratégias prioritárias de manejo para a conservação (Lucrezi *et al.*, 2009a; Schlacher *et al.*, 2016b). Embora caranguejos-fantasma sejam considerados excelentes indicadores de impactos humanos e, portanto, atalhos acessíveis para diagnósticos ambientais em ampla escala, seu uso efetivo ainda requer novas abordagens, incluindo estudos comportamentais e de resposta a impactos isolados além da contagem de tocas, para entender os mecanismos letais e subletais responsáveis pela redução do tamanho das populações em escala global (Schlacher *et al.*, 2016b).

Dentre os impactos citados como responsáveis pela letalidade de caranguejos-fantasma em áreas impactadas, inclui-se o pisoteio, a ingestão de lixo e atropelamento por veículos na praia (Schlacher *et al.*, 2007; Lucrezi *et al.*, 2009a; Suciú *et al.*, 2018). O pisoteio afeta os invertebrados das praias diretamente (esmagamento) ou indiretamente, em razão da compactação do sedimento, que por sua vez, dificulta a atividade escavadora e/ou alterações microclimáticas (granulometria, temperatura, umidade e micro relevo) (Moffett *et al.*, 1998; Sheppard *et al.*, 2009). Os invertebrados estão expostos à ingestão de lixo acidentalmente (por filtração e ingestão de sedimentos ou restos animais e vegetais), por confusão (odores, forma e textura similar aos alimentos naturais) ou por ingestão secundária de presas contaminadas, principalmente por microplásticos (plástico < 0,5 mm) (Iannilli *et al.*, 2018; Costa *et al.*, 2019; Horn *et al.*, 2019). Isso porque o sedimento de praias é um depósito de plásticos que, por sua vez, sofrem fragmentação acelerada pela ação da radiação solar e das ondas (Barnes *et al.*, 2009; Cole *et al.*, 2011). Invertebrados que possuem atividade superficial, como caranguejos-fantasma, estão também expostos ao atropelamento por veículos (Schlacher *et al.*, 2007; Davies *et al.*, 2016). Mesmo enterrados, espécies

podem também sofrer danos morfológicos letais em razão do pisoteio e tráfego de veículos (Moffett *et al.*, 1998; Schlacher *et al.*, 2007).

O Arco Praial de Grussaí, localizado no município de São João da Barra, RJ, tem sido utilizado como modelo de estudos sobre impactos humanos na biodiversidade de praias há quase uma década (2012-2019) (Machado *et al.*, 2016, 2017, 2019; Suciú *et al.*, 2018; Costa e Zalmon, 2019a). Pisoteio (> 200 pessoas/100 m<sup>-1</sup> na zona entremarés), tráfego de veículos (~2 veículos/hora/100 m<sup>-1</sup>) e poluição por plásticos (~ 4 itens/m<sup>2</sup>) são impactos recorrentes, principalmente nas praias urbanizadas durante a alta temporada turística, de janeiro a março (Machado *et al.*, 2017; Suciú *et al.*, 2017). O caranguejo-fantasma do Atlântico é uma espécie abundante nessas praias, considerado o melhor indicador local de urbanização (Costa e Zalmon, 2019a; Suciú *et al.*, 2018). A espécie é conhecida como “espera-maré” na região e parece ter elevado potencial de espécie-bandeira (ícone em programas de conservação), dando inclusive o nome de origem tupi ao distrito de Grussaí (gûarusá= caranguejo). Há relatos de moradores e visitantes de que, com a urbanização do distrito de Grussaí e de parte das praias (construção de um polo gastronômico, pavimentação da orla e realização de shows de verão), o caranguejo-fantasma do Atlântico, antes encontrado inclusive nas casas próximas à praia, tenha praticamente desaparecido (comunicação pessoal). Portanto, tanto a área de estudo, quanto a espécie-alvo são modelos interessantes para uma avaliação de impacto que subsidie projetos de conservação piloto das praias da região norte do estado do Rio de Janeiro.

O objetivo geral do presente estudo foi avaliar as respostas do caranguejo-fantasma do Atlântico *Ocypode quadrata* a múltiplos impactos humanos, incluindo urbanização, pisoteio, poluição por microplásticos e tráfego de veículos. A tese foi dividida em quatro capítulos, apresentados em formato de artigos científicos. O primeiro capítulo objetivou definir os padrões de resposta do caranguejo-fantasma à urbanização, incluindo métricas tradicionais baseadas em tocas (densidade, diâmetro e profundidade) e métricas diretas, baseadas em aspectos comportamentais (forrageio noturno, emersão das tocas e distância de escape). A principal hipótese é que (i) a densidade e o diâmetro das tocas (principais indicadores em nível global) não são métricas tão eficientes comparados ao número de indivíduos da espécie forrageando à noite, como indicadores de urbanização.

Os capítulos 2, 3 e 4 buscaram avaliar a resposta do caranguejo-fantasma ao pisoteio, microplásticos e tráfego de veículos respectivamente, para entender quais

destes impactos são responsáveis pelos padrões observados no capítulo 1, particularmente a densidade populacional e tamanho corporal da espécie. Foram testadas, portanto, mais três hipóteses gerais, como segue: (ii) o pisoteio experimental exerce efeitos de curto-prazo na densidade de tocas e no comportamento do caranguejo-fantasma do Atlântico; (iii) o caranguejo-fantasma do Atlântico ingere mais microplásticos quando este poluente é mais abundante no sedimento; e (iv) a incidência de caranguejos atropelados por veículos é explicado pela intensidade do tráfego e/ou pelo nível de urbanização.

## CAPÍTULO I

### MULTIPLE METRICS OF THE GHOST CRAB *Ocypode quadrata* (FABRICIUS, 1787) FOR IMPACT ASSESSMENTS ON SANDY BEACHES

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#### **Abstract**

Our objective was to compare the efficiency of indirect (burrow density, size, depth and occupation rate) and direct (emersion from burrows, tolerance distance from humans and number of foraging crabs) metrics of the ghost crab *Ocypode quadrata* for urbanization impact assessments. We sampled six beaches during high and low tourism seasons along a gradient of human pressure: high impact (urbanized), medium impact (intermediate) and low impact (non-urbanized). We found that the urbanization index and temperature explained the burrow density and size regardless of the bias related to the burrow occupation rate (~60%) and counting period (diurnal or nocturnal). Moreover, the number of nocturnal foraging crabs explained most (57%) of the dissimilarity among the beaches under different levels of human impact. Thus, the counting of foraging crabs at night was found to be a relevant metric for beach impact evaluation. We strongly recommend integrating the counting foraging crabs with traditional burrow measurements (burrow density and size) in impact assessments of sandy beaches.

**Keywords:** Beach monitoring; behaviour; burrow; indicator; human impact; Ocypodidae.

#### 1. INTRODUCTION

Ghost crabs (Ocypodidae) are practical indicators of human impacts on sandy beaches worldwide, particularly when using active burrow density and size as low-cost and easy sampling metrics (Lucrezi *et al.*, 2009b; Pombo e Turra, 2013). However, the

signals of recent activity (i.e., excavated sand, fresh tracks and internal perimeter definition) surrounding burrows are not always related to occupation (Pombo e Turra, 2013; Pombo *et al.*, 2017b). In addition, studies have suggested that acute human trampling inhibits the restoration of disrupted burrows, which may also induce density underestimation during daylight counting (Lucrezi *et al.*, 2009a). Thus, diurnal burrow counting alone might not be a reliable method to estimate the population density of these crabs and conduct effective impact assessments.

Indirect metrics using ghost crab burrows, such as density, size, depth and occupation rate, can be inconstant over time and can be unfeasible for impact assessments because they are naturally influenced by both biotic and abiotic variables (Pombo *et al.*, 2017b). The loss of the activity signals of occupied burrows by wind or tides, the abandonment of burrows or even the maintenance of more than one burrow by a single crab are possible sources of bias when estimating density from burrow counting (Pombo e Turra, 2013). Other examples of bias from burrow estimates are (1) constant changes over time in burrow size in response to recruitment periods and zonation patterns of young and adult crabs; (2) the influence of temperature, sediment moisture and wind on burrow depth, although there is evidence that deep burrows provide crabs protection from human activities (Lucrezi *et al.*, 2010; Lucrezi e Schlacher, 2014; Schlacher *et al.*, 2007); and (3) occupation rate variation mainly due to hydrodynamic conditions (Pombo *et al.*, 2017b).

Direct metrics related to foraging (Schlacher e Lucrezi, 2010; Tewfik *et al.*, 2016) and associative learning (Evans *et al.*, 1976; Costa *et al.*, 2018b) indicate the complex behaviour of ghost crabs. They can be scavengers or predators since their foraging behaviour and trophic position depend on prey availability or even on ontogenetic dietary shifts (Lucrezi e Schlacher, 2014; Tewfik *et al.*, 2016). Stranded plants and animals surrounding burrows provide important feeding opportunities. However, even though ghost crabs can detect and memorize the placement of detritus (Evans *et al.*, 1976), constant sediment vibration impairs both emersion from their burrows and their foraging behaviour (Lucrezi e Schlacher, 2014). For these reasons, emersion from burrows and foraging activity probably depend on the frequency of human presence. Arguably, the behaviour characteristics of beach biota provide important sublethal measurements of human pressure (Schlacher e Lucrezi, 2010) and can indicate several changes in environmental conditions (Wong e Candolin, 2015; Costa e Zalmon, 2017). Moreover, these metrics can be better indicators of impacts than

dynamic indirect metrics, such as burrow density, size, depth and occupation rate. The behavioural aspects of ghost crabs have rarely been studied, so the feasibility of these measurements as indicators of human impacts is not well known.

The use of multiple indexes instead of a single metric has been suggested by many authors to better support planning and the monitoring of the environmental quality of sandy beaches (González *et al.*, 2014; Suciú *et al.*, 2017, for instance). However, most studies with bioindicators have focused only on comparing the abundance of beach organisms among impacted and non-impacted areas (“compare and contrast” approach) as a single ecological indicator (Schlacher *et al.*, 2016b). Specifically, for ghost crabs, burrow density and size have been applied as the main indicators of human impacts on sandy beaches (Aheto *et al.*, 2011; Schlacher *et al.*, 2016b; de Souza *et al.*, 2017). The use of multiple metrics requires other sampling designs but can provide novel indicators that are able to respond constantly to human pressure, regardless of other environmental influences.

The objective of the present study was to compare the efficiency of indirect (burrow density, size, depth and occupation rate) and direct (emersion from burrows, tolerance distance from humans and number of foraging crabs) metrics of the ghost crab *O. quadrata* for the impact assessment of an extensive beach arc in southeastern Brazil. The prediction is that traditional burrow measurements (1-4) and novel direct metrics (5-8) are affected by urbanization as follows: (1) decreasing burrow density (diurnal, nocturnal and occupied) (reviewed by Schlacher *et al.* 2016b); (2) increasing burrow occupation rate (Pombo *et al.*, 2017b); (3) decreasing burrow size (e.g., Aheto *et al.*, 2011); (4) increasing burrow depth (Schlacher *et al.*, 2007); (5) increasing tolerance distance; (6) increasing emersion time; (7) decreasing emersion frequency; and (8) decreasing number of foraging crabs at night. We hypothesize that the number of foraging crabs at night is a more efficient indicator of human impacts than burrow measurements, since the latter can be biased by the burrow occupation rate (e.g., multiple burrows of a single crab) and sedimentary dynamics (e.g., collapsed burrows by winds).

## 2. METHODS

### 2.1. Study site



Six beaches with distinct impact levels along the Grussaí Beach Arc in southeastern Brazil were chosen to compare several metrics of the ghost crab *O. quadrata*. The predominant wind direction in the region is northeast (de Sousa *et al.*, 2004). Grussaí Beach Arc has a set of intermediate morphodynamic beaches with distinct urbanization degrees, including both highly visited and low-pressure areas (Suciu *et al.*, 2017). We scored three impact levels according to an urbanization index adapted from González *et al.* (2014) for impact assessments of sandy beaches: high impact (U: urbanized areas), medium impact (I: intermediate) and low impact (NU: non-urbanized) (Fig. 1, Tab. 1). We did not consider the category “quality of night sky” for the calculation of the urbanization index (see González *et al.*, 2014) since the entire beach arc has a wide supralittoral zone (>200 m), so the light poles when present are far from the beach and do not have major effects on beach fauna (Costa *et al.*, 2017a).

The urbanized beaches are located near urban settlements, have tourist infrastructure (e.g., paved roads, parking and easy access, such as walkways for disabled people) and, consequently, are preferred areas for beachgoers (Suciu *et al.*, 2017). After Grussaí Lagoon (i.e., approximately 1 km from the most urbanized beach) (see Fig. 1), the medium-impacted beaches are near several houses, but there is no parking, kiosks or paved access, although recreational activities also occur in these areas. Approximately 4 kilometres from the urban areas, there are low-impacted beaches with preserved dune vegetation and a low human presence (Suciu *et al.*, 2017).

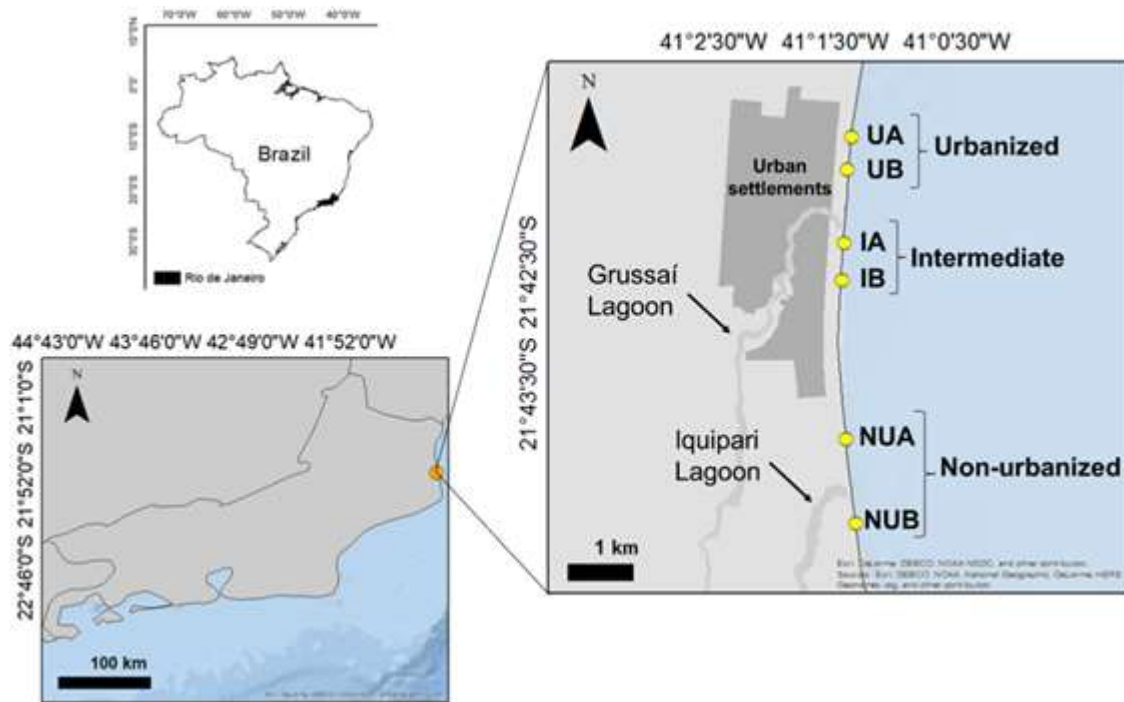


Figure 1. Map of the study area showing the beaches with distinct levels of human impact (high impact, medium impact and low impact) along the Grussaí Beach Arc in southeastern Brazil.

Table 1. Urbanization index of the studied beaches along the Grussaí Beach Arc, southeastern Brazil. The categories “proximity to urban centre,” “building on the sand,” “beach cleaning,” “solid waste on the sand,” “vehicle traffic on the sand” and “frequency of visitors” were scored from 0 to 5 according to Gonzáles et al. (2014). The urbanization index was calculated using the method of Gover (Legendre e Legendre, 1998):  $X = \sum((X-X_{min})/(X_{max}-X_{min}))$ , where x is the score of each category and  $X_{min}$ - $X_{max}$  are the extreme values of the range (0–5).

Category	High-impact		Medium-impact		Low-impact	
	UA	UB	IA	IB	NUA	NUB
Proximity to urban center (0–5)	5	5	5	2	0	0
Building on the sand (0–5)	3	3	3	2	0	0
Beach cleaning (0–5)	1	1	1	1	0	0
Solid waste on the sand (0–5)	5	5	4	5	2	2
Vehicle traffic on the sand (0–5)	5	5	4	3	2	1
Frequency of visitors (0–5)	4	5	4	2	0	0
<b>Urbanization index</b>	<b>0.77</b>	<b>0.80</b>	<b>0.7</b>	<b>0.50</b>	<b>0.13</b>	<b>0.10</b>

For each impact level, we selected two beaches at least 600 m apart from each other (Fig. 2). Although there are few studies about the home ranges of ghost crabs, it was suggested that these crabs can cover distances up to 300 m (Wolcott, 1978; Schlacher e Lucrezi, 2010). Thus, we assume that our beaches are independent replications of urbanization degree, and the populations are probably connected only by larval dispersion. All the beaches were sampled twice in the low tourism season (June to October 2017) and twice in the high tourism season (February and March 2018).

## 2.2. Experimental design

### 2.2.1. Indirect metrics

The indirect metrics included the density of active and open burrows (i.e., diurnal, nocturnal and occupied), burrow size and burrow depth and were compared among the beaches with distinct impact levels. We collected these data from 30 quadrats (4 m<sup>2</sup>) along two transects parallel to the water line: one above and the other below the high tide mark (Fig. 2). Both of the transects were arranged in the beach strata with the highest burrow aggregation (visually determined) to ensure that we always sampled the areas with the highest abundance, regardless of the tide influence and zonation pattern.

After counting, the burrows were carefully covered with sediment and flagged 'daytime'. We also measured the burrow size and depth with a caliper rule and graduated steel cable, respectively. When there were less than 10 burrows in the quadrats, we chose the maximum number of burrows along the transects for size and depth measurements. After 12 hours, we counted the non-flagged burrows (i.e., the nocturnal burrows) in the quadrats, and the reopened flagged burrows were considered occupied.

### 2.2.2. Direct metrics

The following direct metrics were compared among all the beaches: (1) the frequency of crabs that emerged from the burrows (%); (2) the emergence time (s); (3) the tolerance distance from humans (m); and (4) the number of foraging crabs at night. In the morning (up to 08:00), some bait (i.e., canned fish) was placed in the burrow entrance, and we moved 10 metres away, since ghost crabs can perceive the presence

of humans up to this distance (Lucrezi e Schlacher, 2014). We waited no more than 10 minutes for a ghost crab to emerge. Soon after a crab emerged, we carefully walked towards the crab until it returned to its burrow and then measured this distance. The tolerance distance from humans was calculated as follows:

$$TD = MD - ED$$

where TD is the tolerance distance; MD represents the maximum distance from the burrow (10 m) before emergence; and ED is the distance that the crab returned to its burrow.

We counted the number of foraging crabs in the swash zone three times at night (19:30, 20:30 and 21:30) in front of our sampling transects (i.e., 100 m long). The counting was always performed by the same researcher before all other field activity (e.g., burrow counting in the quadrats) to avoid chasing the crabs away to the sea or their burrows in response to human presence.

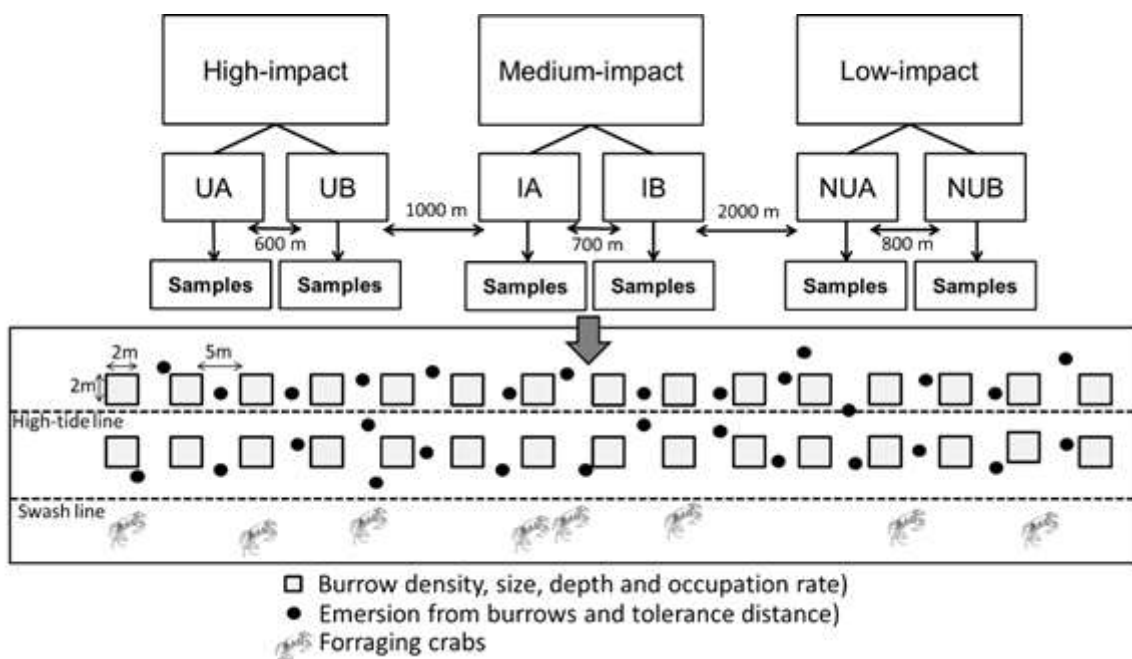


Figure 2. Experimental design of multiple indirect (burrow density, size, depth and occupation rate) and direct (emersion from burrows, tolerance distance and the abundance of foraging crabs) metrics of the ghost crab *Ocyropsis quadrata* on the Grussaí Beach Arc.

### 2.2.3. Potential predictors

The urbanization index was tested as the main anthropogenic predictor of ghost crab metrics; however, we also measured several environmental variables that could potentially explain differences in such metrics in time and space. Thus, we included sediment temperature, wind speed, the percentage of fine sediment and the abundance of potential prey as natural predictive variables.

The temperature of the sediment surface and the maximum wind speed were measured with a portable soil thermometer (Instrutherm TE-400) and an anemometer (Instrutherm AD-250), respectively, as soon as we started the survey (approximately 07:00) and at the end of data collection during the day (approximately 09:00). We repeated this step during the night survey (from 19:00 to 20:00). While temperature directly influences the surface activities of ghost crabs (Haley, 1972; Pombo *et al.*, 2017a), wind can obscure burrow entrances with loose sediment and exert bias on burrow measurements (Lucrezi *et al.*, 2009a). For the sediment analysis, we randomly collected 10 sediment aliquots from five quadrats per transect in a strata 10 cm deep. At the laboratory, the sediment was dried for 24 hours (60°C) and manually sieved in 2 mm, 1 mm and 0.5 mm mesh size to determine the percentage of fine grains (<0.5 mm).

In front of the ghost crab transects, 10 random sediment and pitfall samples were made in the swash and retention zones, respectively, to determine the potential prey abundance on all the beaches and survey dates. The swash zone samplings were performed with a corer (20 x 20 cm). The sediment was sieved with 1 mm mesh, and we counted all the organisms that were screened for 10 minutes. The pitfalls (2 L) with water were arranged in the areas with the highest superficial activity (i.e., retention zone) of the sandhopper *Atlantorchestoidea brasiliensis*, a potential prey item of ghost crabs (Tewfik *et al.*, 2016). After 3 hours, we removed the pitfalls from the sand and counted all the organisms prior to releasing the organisms back onto the beach. Invertebrate saturation in pitfalls is usually reached after approximately six hours (Schlacher *et al.*, 2016a), but we arranged the traps in the main area of human occurrence and tide influence. We had to reduce the waiting time to avoid losing samples.

### 2.3. Data analysis

To search for urbanization or natural influences on the indirect and direct metrics (response variables) of the ghost crab *O. quadrata*, we conducted a multiple regression analysis. We confirmed the normality of the model residuals, including all the predictors for each response variable, using the Shapiro-Wilk test and a visual inspection of the distribution of the residuals with histograms. The homogeneity of the variances was ensured by Cochran's C test. We did not need to transform any of the response variables since all the models were normal ( $p_{\text{Shapiro-test}} > 0.05$ ) and homoscedastic ( $p_{\text{Cochran-test}} > 0.05$ ). The response and predictive variables were averaged for each survey date as sampling units (N= 24). Correlation among the predictors was tested with the Pearson correlation coefficient (R). Model selection was based on the lowest Akaike's information criterion (AIC) values for the possible combination of predictors (excluding correlated predictive variables), which was done using the MumIM package in the software R.

The best models for each response variable included the following predictive variables: (1) density of diurnal burrows (sediment temperature + urbanization index); (2) density of nocturnal burrows (sediment temperature + urbanization index); (3) density of occupied burrows (sediment temperature + urbanization index); (4) occupation rate (null model); (5) burrow size (sediment temperature + urbanization index); (6) burrow depth (sediment temperature); (7) tolerance distance (food abundance + sediment temperature + urbanization index); (8) emersion time (food abundance + urbanization index); (9) emersion frequency (null model); and (10) number of foraging crabs (urbanization index).

Non-metric multidimensional scaling (nMDS) was performed to provide an integrative impact assessment on the Grussaí Beach Arc, including the metrics significantly related to the urbanization index (the density of diurnal, nocturnal and occupied burrows, the burrow size and the number of foraging crabs). The multivariate data matrix was  $\log x + 1$  transformed and based on the Bray-Curtis similarity coefficients. A SIMPER analysis was performed to determine the relevance of the single metrics applied to the impact assessment, i.e., which of these metrics contribute most to the dissimilarity among the beaches with distinct levels of urbanization.

### 3. RESULTS

The mean sediment temperature ranged from 19 to 30°C during the sampling period, < 25°C in the winter and > 27°C in the summer. The percentage of fine sediment was usually higher on the medium-impacted beaches (~45%), followed by the high-impacted (~39%) and low-impacted sites (~32%). The temperature and percentage of fine sediment were negatively correlated ( $p= 0.03$ ;  $R_{\text{Pearson}}= -0.44$ ). The mean wind speed ranged from 6 to 29 km/h, with no clear spatial-temporal patterns. The pitfall samples had a greater sandhopper abundance on the medium-impacted (~71 individuals) and high-impacted (~64) beaches compared to the low-impacted beaches (~18), particularly in the winter. The corer samples did not reveal distinct prey abundance among the high- (~19 individuals), medium- (~16 individuals) and low-impacted (~13) beaches (Appendix A).

The low-impacted beaches had a higher density of diurnal (DB), nocturnal (NB) and occupied burrows (OB), a higher mean burrow size (BS) and a higher number of foraging crabs (FC) at night compared to the high- and medium-impacted beaches (Appendix B). Burrows over 50 mm wide were rarely found on the high-impacted beaches (Appendix C). All of these metrics were significantly ( $p < 0.001$ ) and negatively related to the urbanization index (Tab. 2, Fig. 3). The best regression models for DB, NB, OB, BS and FC always explained more than 55% of the metric variability (Tab. 2). In addition to the urbanization influence, the sediment temperature was also related ( $p < 0.02$ ) to DB, NB and BS (Tab. 2).

The occupation rate ranged from 51 to 68% along the Grussaí Beach Arc. The burrow depth (BD), tolerance distance from humans (TD) and emersion time from the burrows (ET) were usually lower, while the emersion frequency (EF) was higher on at least one low-impacted beach compared to the high-impacted beaches (Appendix B). However, none of these metrics were related to the urbanization index (Tab. 2, Fig. 3). Actually, the TD and ET were affected by sediment temperature (negative influence) and food abundance (positive influence), respectively (Tab. 2). The burrow occupation rate (OC), BD and EF were not explained by any natural variables or the urbanization index (Tab. 2, Fig. 3).

Table 2. Multiple regression models with the lowest AIC values of each metric of the ghost crab *Ocypode quadrata* related to the urbanization index and natural variables on the Grussaí Beach Arc. Significant predictions are marked in bold. The signal of the estimate values indicates the positive or negative (-) influences of the predictors. The best model for the burrow occupation rate and emergence frequency did not include any predictive variables (null models).

<b>Density of diurnal burrows (R<sup>2</sup>= 0.84)</b>	<b>Estimate</b>	<b>Std. Error</b>	<b>t-value</b>	<b>p-value</b>
(Intercept)	2.73	0.37	7.39	<b>&lt;0.00001</b>
Urbanization index	-1.56	0.14	-10.79	<b>&lt;0.00001</b>
Sediment temperature	-0.05	0.01	-3.92	<b>0.00077</b>
<b>Density of nocturnal burrows (R<sup>2</sup>= 0.57)</b>	<b>Estimate</b>	<b>Std. Error</b>	<b>t-value</b>	<b>p-value</b>
(Intercept)	1.83	0.41	4.45	<b>0.00022</b>
Urbanization index	-0.87	0.16	-5.36	<b>0.00003</b>
Sediment temperature	-0.04	0.01	-2.98	<b>0.00715</b>
<b>Density of occupied burrows (R<sup>2</sup>= 0.73)</b>	<b>Estimate</b>	<b>Std. Error</b>	<b>t-value</b>	<b>p-value</b>
(Intercept)	1.21	0.27	4.52	<b>0.00019</b>
Urbanization index	-0.83	0.10	-7.97	<b>&lt;0.00010</b>
Sediment temperature	-0.02	0.01	-1.89	0.07218
<b>Burrow size (R<sup>2</sup>= 0.55)</b>	<b>Estimate</b>	<b>Std. Error</b>	<b>t-value</b>	<b>p-value</b>
(Intercept)	13.51	10.98	1.23	0.23219
Urbanization index	-18.64	4.31	-4.33	<b>0.00029</b>
Sediment temperature	0.10	0.41	2.46	<b>0.02278</b>
<b>Burrow depth (R<sup>2</sup>= 0.12)</b>	<b>Estimate</b>	<b>Std. Error</b>	<b>t-value</b>	<b>p-value</b>
(Intercept)	13.98	19.86	0.70	0.48880
Sediment temperature	1.59	0.77	2.04	0.05350
<b>Tolerance distance (R<sup>2</sup>= 0.32)</b>	<b>Estimate</b>	<b>Std. Error</b>	<b>t-value</b>	<b>p-value</b>
(Intercept)	10.17	2.06	4.95	0.00008
Urbanization index	1.63	0.82	1.99	0.05980
Food abundance	-0.01	0.01	-1.46	0.15850
Sediment temperature	-0.22	0.07	-2.95	<b>0.00790</b>
<b>Emergence time from burrows (R<sup>2</sup>= 0.47)</b>	<b>Estimate</b>	<b>Std. Error</b>	<b>t-value</b>	<b>p-value</b>
(Intercept)	2.17	0.61	3.53	<b>0.00200</b>
Urbanization index	1.56	1.14	1.37	0.18470
Food abundance	0.03	0.01	3.57	<b>0.00180</b>
<b>Number of foraging crabs (R<sup>2</sup>=0.78)</b>	<b>Estimate</b>	<b>Std. Error</b>	<b>t-value</b>	<b>p-value</b>
(Intercept)	24.79	2.04	12.14	<b>&lt;0.00001</b>
Urbanization index	-32.84	3.63	-9.04	<b>&lt;0.00001</b>



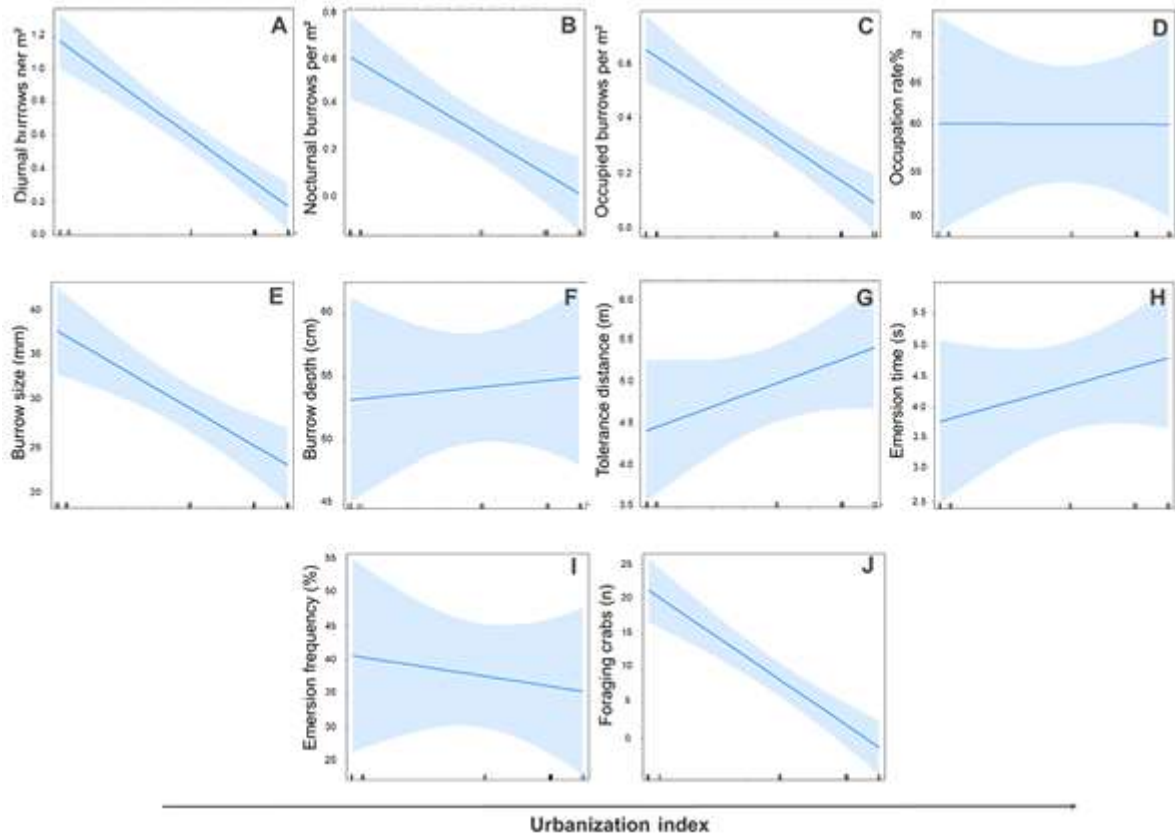


Figure 3. Indirect (A: density of diurnal burrows; B: density of nocturnal burrows; C: density of occupied burrows; D: burrow occupation rate; E: burrow size; and F: burrow depth) and direct (G: tolerance distance from humans; H: emergence time from burrows; I: number of emerged crabs; and J: abundance of foraging crabs at night) metrics of the ghost crab *Ocyropsis quadrata* related to the urbanization index of beaches with distinct impact levels on the Grussaí Beach Arc.

The nMDS showed that the surveys on the low-impacted beaches were clustered in a single group (93% similarity) apart from the medium- and high-impacted beaches (85% similarity) (Fig. 4). Among the metrics related to the urbanization index and included in the multivariate matrix (DB, NB, OB, BS and FC), the FC contributed 57% to the dissimilarity (30%) between the low-impacted beaches and the other beaches, followed by the DB (13%), BS (11%) and NB (9%).

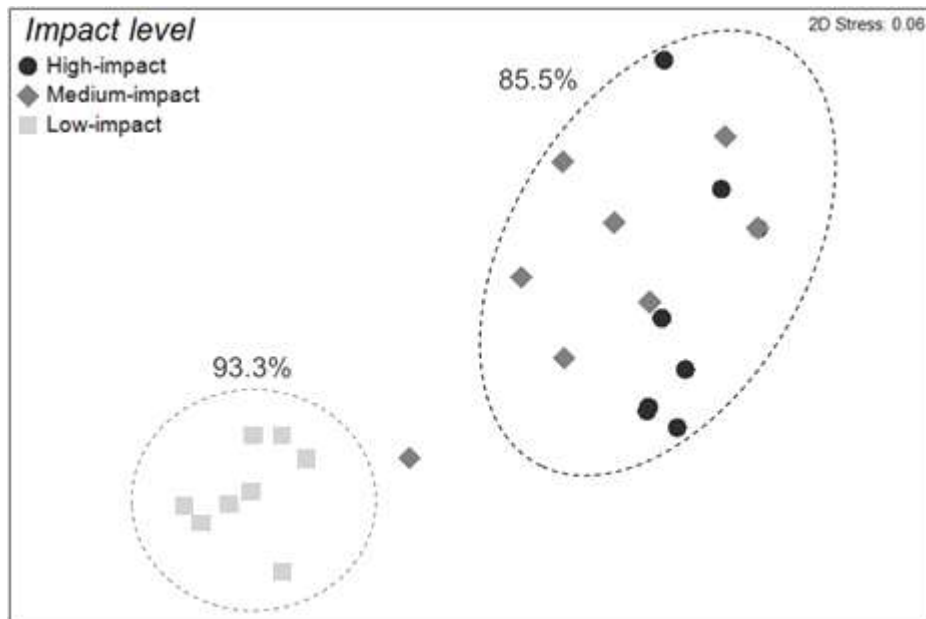


Figure 4. nMDS ordination including the metrics of the ghost crab *Ocypode quadrata* (number of diurnal, nocturnal and occupied burrows per m<sup>2</sup>, burrow size and number of foraging crabs) significantly related to the urbanization index in the regression analysis. Each symbol unit (■●◆) represents a survey date.

#### 4. DISCUSSION

Among the 10 metrics testing the response to a gradient of urbanization, five were negatively related to human impacts, confirming several of our main predictions. These metrics included traditional burrow measurements (burrow density and size), even though they have been contested by some authors (e.g., Pombo e Turra, 2013; Silva e Calado, 2013). Although the indirect metrics had not been biased by occupation rate or sedimentary dynamics, a direct metric (i.e., counting foraging crabs at night) was more related to urbanization levels than indirect metrics as an indicator of human pressure, confirming our work hypothesis and enhancing its use as a rapid, low-cost and non-destructive tool for impact assessment.

Burrow density was negatively related to the urbanization index, regardless of the survey period (i.e., diurnal or nocturnal samplings). Although burrow counting naturally overestimated the density of the ghost crab *O. quadrata* (as observed by Pombo e Turra, 2013), it did not have an influence on the impact assessment efficiency on the Grussaí Beach Arc since the burrow occupation rate (~60%) was not related to the urbanization level. In addition, we did not underestimate the burrow density on the

high-impacted beaches because ghost crabs chronically stay in collapsed burrows without reopening them during daylight (Neves e Benvenuti, 2006; Lucrezi *et al.*, 2009a). Actually, density of nocturnal burrows were also negatively related to the urbanization index, such as diurnal and occupied burrows. Thus, the higher burrow density on the low-impacted beaches does not indicate differences in burrow building activity among the beaches. Instead, this resulted from the actual higher ghost crab density on the low-impacted beaches.

The lower density of burrows on the high-impacted beaches compared to the low-impacted beaches possibly resulted from multiple sources of mortality, including (1) crushing by vehicle traffic or human trampling (Schlacher *et al.*, 2007); (2) ingestion of plastics (Costa *et al.*, 2010; Ugolini *et al.*, 2013); (3) diseases caused by sediment pollution (Suciu *et al.*, 2017); (4) low-energy input because of beach cleaning (Noriega *et al.*, 2012; Stelling-wood *et al.*, 2016); (5) physiological stress caused by intermittent human presence (Schlacher *et al.*, 2011); and (6) the reduction of the crabs resilience to extreme events as a result of dune vegetation suppression (Lucrezi *et al.*, 2010). All of these stressors probably occur synergistically to impair ghost crab populations, but it is only possible to confirm the intensity of each impact with specific experimental designs (Schlacher *et al.*, 2016b).

Burrow size, which is related to the carapace size of crabs (Oliveira *et al.*, 2016; Schlacher *et al.*, 2016c), was negatively influenced by the urbanization level, as observed in other studies (Yong e Lim, 2009; Aheto *et al.*, 2011;). Indeed, we rarely found burrows over 50 mm wide on the impacted beaches (see appendix C). Smaller crabs on impacted beaches can reflect earlier sexual maturity, a lower investment in body growth and/or a higher mortality of juveniles (prior to sexual maturity). In this case, ghost crab populations on urbanized beaches are not able to have a constant reproduction rate, being maintained as a metapopulation by larval dispersion from surrounding beaches. Ghost crabs could also have a lower energy input on impacted beaches and, consequently, slow body growth, but studies have suggested that ghost crabs opportunistically feed on human food scraps (Schlacher *et al.* 2011; Stelling-Wood *et al.* 2016). Despite these mechanisms, burrow size was undoubtedly a good metric for beach impact assessment.

Temperature affected the density of diurnal, nocturnal and occupied burrows together with the urbanization index. Temperature has already been reported as an important driver of ghost crab activity; burrow building usually intensifies during the

warmest days (Haley, 1972; Lucrezi e Schlacher, 2014). This phenomenon seems to be more conspicuous for adult crabs, since temperature was also positively related to the mean burrow size. Temperature was correlated with the lowest percentage of fine sediment, which can also contribute to burrow excavation due to lower sediment compactness and, consequently, higher burrow densities. Otherwise, the FC was a better metric for impact assessments because it concurrently indicates several responses of the ghost crab to human impacts, such as lower density, lower foraging activity and the predominance of juveniles on impacted beaches, regardless of temperature or other natural drivers. In addition to juveniles being mainly diurnal detritus feeders and not preying on swash zone macroinvertebrates at night as adult crabs do (Strachan *et al.*, 1999), it is more difficult to visually detect juveniles during nocturnal counting. Thus, our study showed that counting the number of adult ghost crabs foraging at night in the swash zone is a novel, efficient, rapid and low-cost tool for impact assessments on sandy beaches.

Initially, we expected to find other direct metrics, such as tolerance distance (TD) from humans, emersion time (ET) and emersion frequency (EF) from burrows, as good indicators of beach urbanization impact. Our results showed an increasing trend for the TD and ET as a function of the urbanization index, but it was not statistically significant. The TD may increase on impacted beaches because the visual perception of potential predators by the crabs may decrease in trampled areas due to the irregular sediment surface (i.e., high microrelief) (Costa *et al.*, 2018a). The reaction of the crabs to humans increased (i.e., lower tolerance) with temperature, which is also evidence of high crab activity during the warmest days. Even with the predominance of juveniles, which are usually more active during diurnal periods than adults, the emergence from the burrows was delayed in the impacted areas because the intermittent presence of humans was perceived by the buried crabs (Lucrezi e Schlacher, 2014). Our results showed that food abundance was crucial to crab emersion time, since well-fed ghost crabs probably do not need to emerge quickly from burrows to search for food. This means that compared to low-impacted and high-abundance-prey areas, the ghost crabs on impacted beaches could be more exposed to vehicle traffic and human trampling on the sediment surface of beaches with scarce prey abundance.

Among the indirect metrics, the burrow depth and occupation rate did not respond predictably to the urbanization index, so we do not recommend using these metrics as ecological indicators of human impact. Actually, the two metrics are

simultaneously influenced by multiple abiotic factors, such as sediment moisture, beach slope, storm periods and tide reach (Pombo *et al.*, 2017b). While increasing sediment moisture requires less deep burrows, storm frequency and intensity, beach slope and tide synergistically influence occupation rate due to burrow inundation (Pombo *et al.*, 2017b; Campagnoli *et al.*, 2018;). For these reasons, to evaluate whether burrow depth and occupation rate are influenced by the urbanization impact, it is necessary to control all of these abiotic variables.

In conclusion, except for the number of nocturnal foraging crabs, the direct metrics were not affected by urbanization, and show that they are unreliable indicators for impact assessments. Our results also point out that indirect metrics are still useful tools for impact assessments. Even if they overestimate or underestimate the population size, the burrow occupation rate does not seem to differ among beaches with distinct levels of urbanization on the same beach arc. Although the indicator efficiency of the burrow measurement was not biased by the occupation rate, it was simultaneously influenced by temperature (and/or sediment grain size), which should always be considered in impact monitoring. We strongly recommend coupling traditional burrow measurements (burrow density and size) with counting foraging crabs at night in impact assessments on sandy beaches, especially in areas with well-known nocturnal activity of ghost crabs.

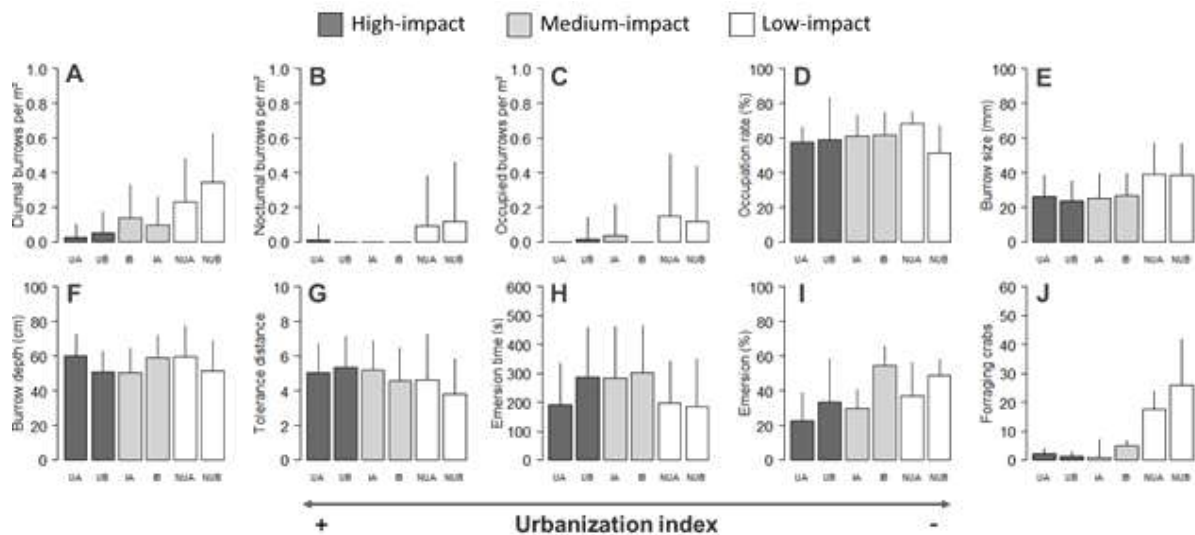
## 5. APPENDIX

Appendix A. Mean values of the environmental variables on the Grussaí Beach Arc.

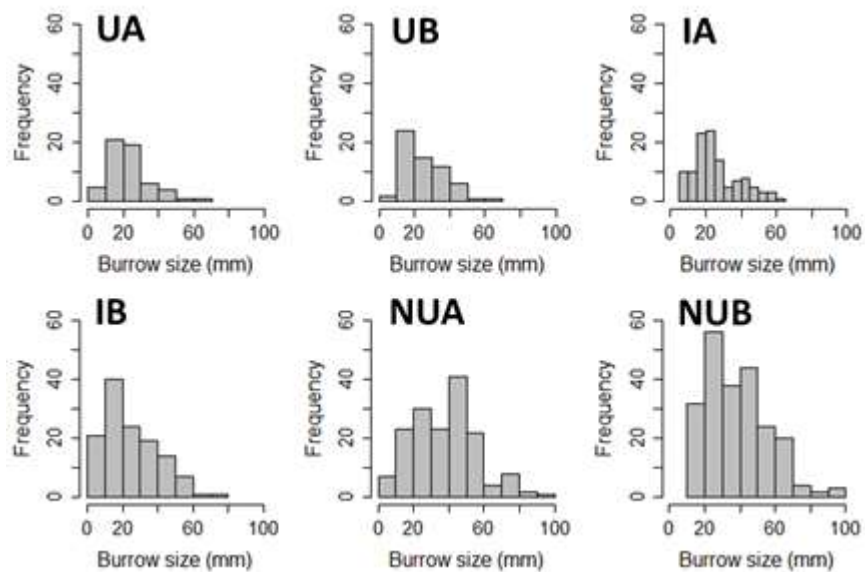
T= temperature; WS= wind speed; PA= prey abundance; and FS= fine sediment.

<b>Beach</b>	<b>Survey</b>	<b>T (°C)</b>	<b>WS (km/h)</b>	<b>PA</b>	<b>FS (%)</b>
UA	Summer I	25.40	5.40	21.60	37.55
	Summer II	27.08	6.20	92.40	37.28
	Winter I	20.99	17.71	90.22	27.50
	Winter II	22.84	23.44	50.30	54.90
UB	Summer I	26.29	9.30	13.10	29.24
	Summer II	28.60	6.43	55.58	33.84
	Winter I	22.05	8.60	1.70	53.25
	Winter II	25.69	29.13	116.89	39.35
IA	Summer I	27.50	26.33	3.89	50.78
	Summer II	27.70	26.30	45.70	36.96
	Winter I	20.79	8.38	94.30	51.80
	Winter II	24.47	20.38	81.11	28.30
IB	Summer I	28.77	20.30	4.70	47.94
	Summer II	28.03	20.23	74.20	41.49
	Winter I	19.11	14.07	71.40	55.11
	Winter II	23.94	6.47	38.40	44.35
NUA	Summer I	30.30	17.27	2.00	13.50
	Summer II	28.25	9.03	38.80	55.47
	Winter I	22.41	12.05	3.00	34.24
	Winter II	23.40	31.50	19.80	55.17
NUB	Summer I	26.90	5.90	59.38	5.76
	Summer II	29.30	17.85	5.25	11.86
	Winter I	23.33	21.81	44.40	45.95
	Winter II	25.25	15.97	9.30	37.82

Appendix B. Comparison of the indirect (A: density of diurnal burrows; B: density of nocturnal burrows; C: density of occupied burrows; D: burrow occupation rate; E: burrow size; and F: burrow depth) and direct (G: tolerance distance from humans; H: emersion time from burrows; I: number of emerged crabs; and J: abundance of foraging crabs at night) metrics of the ghost crab *Ocypode quadrata* among the beaches with distinct impact levels on the Grussaí Beach Arc.



Appendix C. Absolute frequency of burrow size classes in urbanized (UA and UB), intermediate (IA and IB) and non-urbanized (NUA and NUB) beaches, southeastern Brazil.



## CAPÍTULO II

### CHANGES IN THE BEHAVIOUR OF *Ocypode quadrata* (FABRICIUS, 1787) AFTER EXPERIMENTAL TRAMPLING

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#### **Abstract**

The effects of trampling are usually confounded by the diffuse impacts of the urbanization of sandy beaches. We performed a controlled experiment on a beach with low visitation rates to test the hypothesis that ghost crabs avoid building their burrows on impacted plots as a result of the compacted sediment, and they migrate to non-trampled areas. The sampling design encompassed 11 survey quadrats (6 × 6 m) above the strandline, including five trampled plots (100, 300, 900, 1500 and 3000 steps) and six non-trampled plots. The plots were sampled before and after 24, 48 and 72 hours of experimental trampling. We found that the ghost crabs avoided building their burrows in only the 1500x and 3000x trampled plots after 24 hours, but the avoidance was not related to sediment compactness. Additionally, the emersion time and escape distance from humans were significantly delayed in the most trampled plots, suggesting a lower surface activity and an avoidance of irregular (i.e., high micro-relief) sediment surfaces by ghost crabs, which might reduce their ability to perceive potential predators.

**Keywords:** Experimental approach; human impact; sandy beach; ghost crab.

#### 1. INTRODUCTION

Human trampling is considered a near-ubiquitous impact of recreation on urbanized sandy beaches (Schlacher *et al.*, 2016a). In addition to a direct lethality, trampling can hinder the excavation activity of macroinvertebrates due to higher sediment compaction and long-term changes in sediment properties, such as moisture, slope and micro-relief (Lim *et al.*, 2011). However, trampling impacts are usually



confounded with other stressors (e.g., vehicle traffic and beach cleaning), which challenges beach ecologists to distinguish trampling effects from other broader urbanization impacts (Schlacher e Thompson, 2012). Thus, trampling effects on beach biota should be singly assessed (Lucrezi *et al.* 2009a).

Sandy beaches are dynamic ecosystems, and the experimental approaches used to study them are challenging. Worldwide, studies assessing the effects of experimental trampling on beach macrofauna are scarce (Moffett *et al.*, 1998; Schlacher *et al.*, 2016a). In an attempt to determine the effects of human trampling using a snapshot experiment on an intertidal macrobenthic community, Moffett *et al.* (1998) noted that only morphologically delicate macroinvertebrates were crushed by the impact. Similarly, Schlacher *et al.* (2016a) found a negative effect on surface-active sandhoppers. Nevertheless, Jaramillo *et al.* (1996) found no significant changes in macrofauna density after isolating an area from human trampling.

Ghost crabs occur on a broad range of sandy beaches, building their burrows and maintaining territories from the upper intertidal zone to the dune vegetation (Pombo e Turra, 2013). Burrow counting is a non-destructive and inexpensive method to assess population abundance and evaluate the impact of humans on sandy beaches (Schlacher *et al.*, 2016c). Although burrow counting does not always provide a reliable estimate of ghost crab abundance (Lucrezi *et al.*, 2009a; Silva e Calado, 2013;), some criteria might reduce possible bias (i.e., over- or underestimation), such as identifying activity signals (e.g., presence of replaced sediment around the crab burrows), measuring the burrow depth and determining the occupation rate (Pombo e Turra, 2013). Thus, burrow abundance might indicate the abundance of a ghost crab population (Oliveira *et al.*, 2016; Pombo e Turra, 2013) or other behavioural features (Costa *et al.* 2018b) if used with caution.

As a result of studies using burrow counting on sandy beaches with distinct human pressure, the ghost crab *Ocyroide quadrata* has been considered the main bioindicator of the human impact on Atlantic beaches, and trampling is an usual ascribed source of disturbance (Schlacher *et al.*, 2016b). However, only a single study assessed the short-term effects of acute trampling on ghost crabs burrow density (Lucrezi *et al.* 2009a). It is important to note that this controlled experiment dealt with trampling impacts on an urbanized beach that was chronically exposed to several diffuse impacts (e.g., trampling, beach cleaning, vehicle traffic and others). Thus, the

mechanisms responsible for burrow decline still need to be studied in more detail so effective management actions can be recommended.

We aimed to assess the effects of acute trampling on the burrow abundance and behaviour of *O. quadrata*. In addition to evaluating whether a decrease in burrow abundance is associated with higher human trampling and sediment compactness, we tested whether the ghost crab escapes more slowly from humans and/or delays emergence from its burrows in such impacted areas. Ghost crabs are highly motile invertebrates, covering distances up to 300 m, and can avoid trampled areas and compacted sediment (Schlacher e Lucrezi, 2010). In addition, they recognise human presence and sediment vibrations, so behavioural changes are expected to occur in plots with higher human trampling (Lucrezi e Schlacher, 2014)

## 2. METHODS

### 2.1. Study site

The study was performed on a microtidal intermediate sandy beach (Grussaí Beach) in southeastern Brazil (21°44'44.21"S 41° 1'18.28"W). Grussaí Beach is the main destination for local and regional visitors, especially from urbanized areas where there is easy access and good infrastructure (Suciu *et al.*, 2017). On the same beach arc, there are non-urbanized areas that are difficult to access and have low visitation rates (Suciu *et al.*, 2017). To perform a snapshot experiment, we chose the hard-to-reach and non-trampled areas of the beach to avoid the influence of other anthropogenic disturbances, such as beach cleaning, pollution and vehicle traffic. The survey plots were located 1 kilometre, by foot, from the beach access (Fig. 1).

### 2.2. Field experiments

We placed 11 plots (6 × 6 m) parallel to the water line in the interface between the high tide mark and the dune vegetation to avoid the experiment from becoming inundated by the tide. Due to the distribution of transects in the supralittoral zone, some plots were randomly covered by vegetation. We assigned distinct trampling intensities (100, 300, 900, 1500 and 3000 steps) to each impacted plot, and a trampled plot was always followed by a non-trampled plot (Fig. 1). The trampling was performed by two people who weighed approximately 80 kg walking barefoot inside the plots until the required number of steps was reached. We replicated this experimental layout for two

other sites that were 50 m apart (Fig. 1). The experimental trampling was performed on the first day, and the plots were isolated from vehicle traffic or beachgoers using flags and monitored for 24, 48 and 72 hours after the impact. The experimental layout was adapted from Schlacher *et al.* (2016a).

For all isolated plots, only the active ghost crab burrows (Pombo e Turra, 2013) that occurred before the impact and at 24, 48 and 72 hours (sunny days) after trampling were counted. Sediment compactness was measured before trampling and during the following experimental days by dropping a graduated steel cable three times in each plot. We visually estimated the percentage of vegetation cover of the plots. Sediment surface temperature and wind speed were also measured on each survey date (at 09h:00) before the burrows were counted. Sediment moisture was not measured because trampling is expected to exert a significant influence on burrow abundance, irrespective of the possible changes in the evaporation rates or water retention in the sediment following this acute disturbance (Lucrezi *et al.* 2009a).

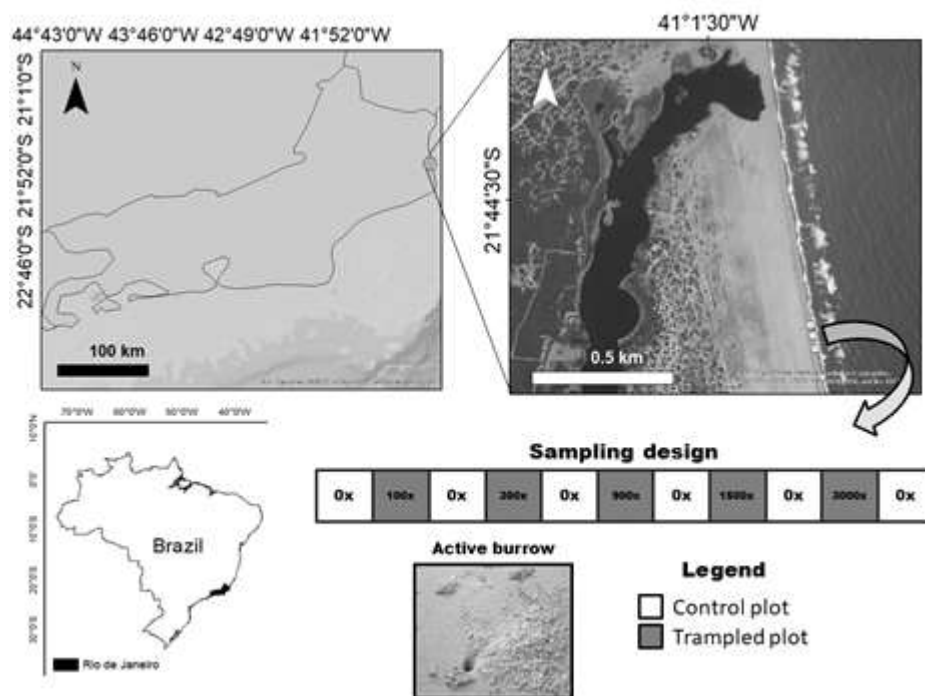


Figure 1. Map of the study site and the experimental sampling design at Grussaí Beach, southeastern Brazil. Each yellow point represents a pseudo-replication of the sampling design composed of five trampled plots and six control plots.

Immediately after completing the experimental trampling on the first day, we performed a second experiment in the control plots and the plots with 3000 steps to assess the emergence time of the ghost crabs from their burrows by placing bait (i.e., canned fish) at the burrow entrance. We also assessed the escape distance (in metres), i.e., the distance between a crab and a human at which the crab returns to its burrow. After the bait was placed at the burrow entrance, we moved 10 metres away since ghost crabs can perceive human presence up to such a distance (Lucrezi e Schlacher, 2014). We waited no more than 10 minutes for the ghost crab to emerge. Soon after a crab emerged, we carefully walked towards it until it returned to its burrow, and we then measured the distance between the observer and the crab at the time the crab retreated to its burrow. The experiment proceeded until 10 ghost crabs emerged from their burrows in each treatment (control × trampled plots).

### 2.3. Data analysis

The effect of experimental trampling on the abundance of ghost crab burrows was analysed according to the BACI (before-after-control-impact) design (Underwood, 1992). We counted the number of active burrows before and after trampling in the impacted (100x, 300x, 900x, 1500x and 3000x steps) and control (0x steps) plots. A permutational analysis of variance (PERMANOVA) based on Euclidian distance was performed to compare burrow abundance among the following fixed factors: (1) time (24 hours, 48 hours and 72 hours) and (2) trampling intensity (0x, 100x, 300x, 900x, 1500x and 3000x). A posteriori PERMANOVA pair-wise test was used to compare the differences in burrow abundance before and after the impact of each trampling intensity (from control to 3000x steps). Because distinct burrows were used for each treatment, the independent-samples t-test was used to compare the ghost crab emergence times and escape distances between the control and trampled (3000 steps) plots. The most important variables affecting the abundance of ghost crab burrows during the experiment were assessed using Poisson Generalized Linear Models (GLMs). Initially, we applied a principal component analysis (PCA) with potential predictors to search for covariation. We did not find correlated ( $R > 0.4$ ) variables. Second, we ran distinct models using all possible combinations of at least three predictors ( $n = 15$ ). The model performance was evaluated using the corrected Akaike Information Criterion (AIC) based on the combined predictors (Burnham e Anderson, 2002). The model with the lowest AIC score included “sediment

penetrability” + “sediment temperature” + “vegetation cover” and was considered in the GLM analysis.

### 3. RESULTS

In general, the sediment penetrability was lower (i.e., higher compactness) in the trampled plots ( $\approx 11$  cm) compared to the control plots ( $\approx 13$  cm). After the impact, there was no significant difference in the sediment compactness among the distinct trampling intensities. The sediment temperature ranged from 25.8°C before trampling to 29.1°C on the last survey date. The wind speed ranged from 14.0 to 17.8 km/hour during the sampling campaigns. The vegetation cover ranged from 0 to 90% in each plot.

The abundance of ghost crab burrows increased in the control plots 24 hours after the experimental trampling, but it was not significant (Fig. 2, Tab. 1). Only the plot with 3000x steps had significantly fewer burrow openings 24 hours after trampling, with a decline of 50% compared to the pre-impact sampling (Fig. 2, Tab. 2). After 72 hours, there was an increase in the abundance of burrows in almost all the plots up to 900 steps, with a significant difference only in the control areas (Fig. 2, Tab. 2) ( $p=0.01$ ;  $t=36.30$ ).

Table 1. Permutational analysis of variance (PERMANOVA) and pair-wise test comparing the abundance of ghost crab burrows among time (before and after trampling) and trampling intensities (0x, 100x, 300x, 900x, 1500x and 3000x). Significant values ( $p<0.05$ ) are marked in bold.

Source	df	SS	MS	Pseudo-F	P(perm)	Perms
Time (Ti)	3	1196	398.8	1.735	0.155	999
Trampling intensity (Tr)	5	869	173.8	0.756	0.608	998
Ti x Tr	15	2893	192.9	0.839	0.641	998
Residuals	108	24825	229.9			
Total	131	30947				
Pair-wise test	Before x 24 hours		Before x 48 hours		Before x 72 hours	
	t	p	t	p	T	P
Control plots	0.915	0.365	9.909	0.172	36.302	<b>0.001</b>
100x	0.769	0.903	1.069	0.403	0.390	0.667
300x	0.966	0.509	531,73	1.000	0.285	0.908
900x	0.912	0.700	1.069	0.486	10.621	0.322
1500x	1.064	0.492	0.699	0.705	0.292	1.000
3000x	13.069	<b>0.036</b>	0.810	0.611	0.102	1.000

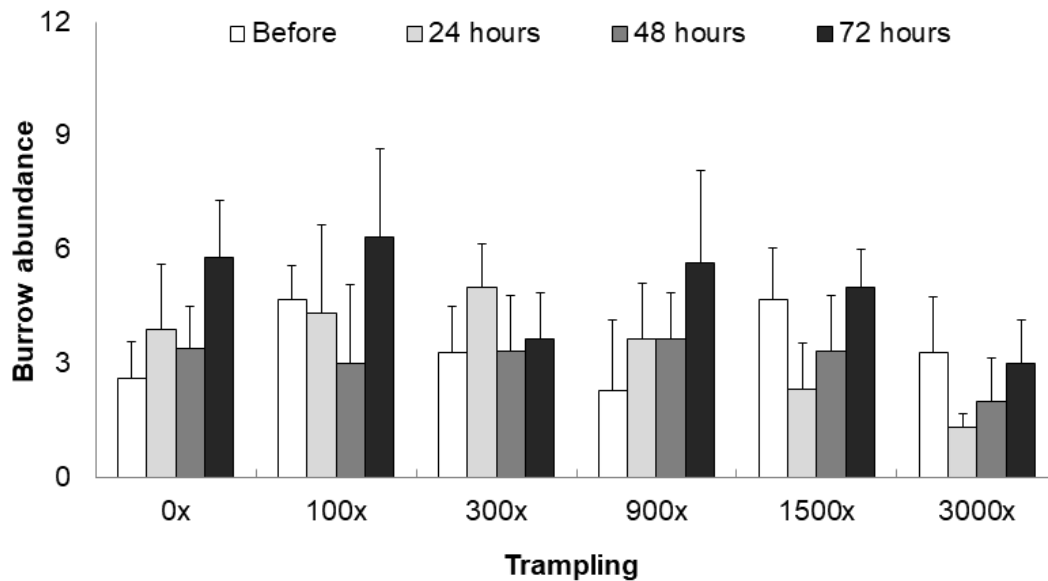


Figure 2. Average number of burrows before and after (24 hours, 48 hours and 72 hours) the trampling impacts with distinct intensities (100, 300, 900, 1500 and 3000 steps).

The burrow abundance was negatively associated with sediment penetrability, but such a predictor was not significant in the GLM (Tab. 2). In fact, only the environmental variables “vegetation cover” and “sediment temperature” were significantly ( $p < 0.01$ ) associated with burrow abundance (Tab. 2). In general, the highest burrow abundance was found in the plots with the least vegetation and on the warmest days (Fig. 3).

Table 2. Model predictors of the Poisson generalized linear models for burrow abundance (response variable) as functions of potential predictors (sediment temperature, vegetation cover and sediment penetrability). Significant values ( $p < 0.05$ ) are marked in bold.

Model predictors	Estimate	Std. error	z-value	p-value
Intercept	-3.070	1.237	-2.481	<b>0.013</b>
Sediment penetrability	0.042	0.002	1,759	0.078
Sediment temperature	0.149	0.038	3.884	<b>&lt;0.001</b>
Vegetation cover	-0.011	0.002	-4.405	<b>&lt;0.001</b>

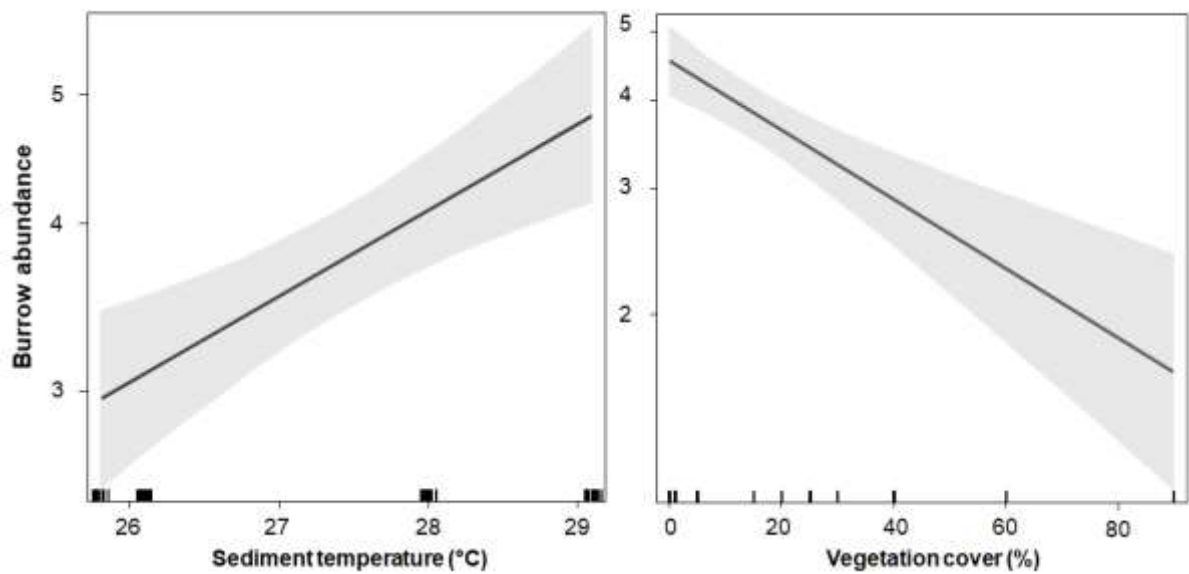


Figure 3. Generalized linear model predictions of burrow abundance as a function of sediment penetrability, sediment temperature, trampling intensity, vegetation cover and wind speed measured during a snapshot experiment. Shaded areas indicate 95% confidence bands. Only sediment temperature and vegetation cover were significant predictors of burrow abundance.

The emergence time of the ghost crab was significantly ( $p < 0.01$ ) lower in the control plots compared to the trampled plots (Fig. 4). The escape distance was also significantly delayed ( $p < 0.05$ ) in the plots with 3000 steps compared to the non-trampled plots (Fig. 4).

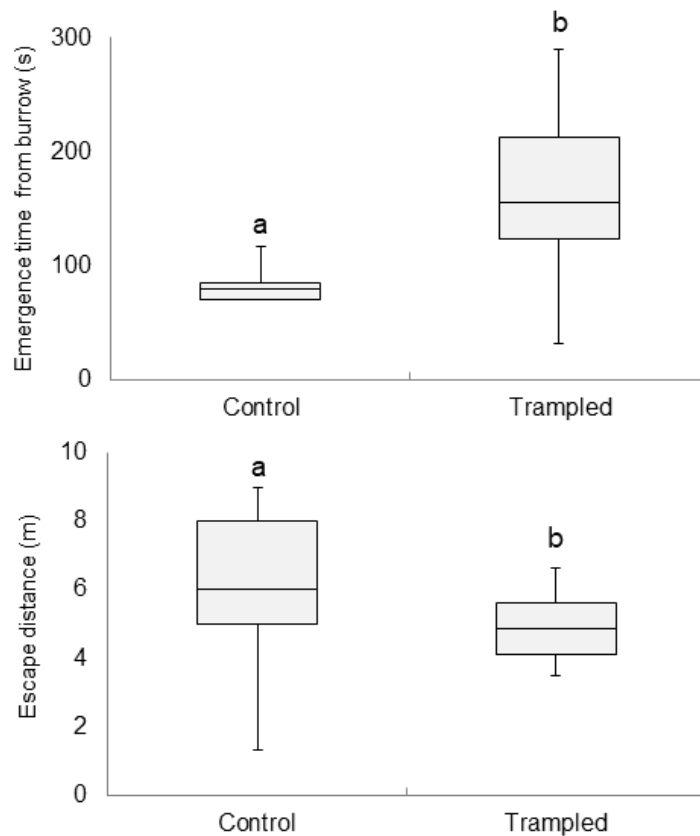


Figure 4. Boxplot of the emergence time (A) and escape distance (B) of the ghost crab *O. quadrata* in the control and trampled (3000 steps) plots. The black line and boxes represent the median values and interquartile range, respectively. The line bars are the highest and lowest values in the dataset (non-outliers).

#### 4. DISCUSSION

This study is one of the few attempts to experimentally understand how trampling, apart from other diffuse impacts of urbanization (e.g., beach cleaning and vehicle traffic; Schlacher *et al.*, 2007; Stelling-wood *et al.*, 2016), can affect the abundance and behaviour of ghost crabs (Lucrezi *et al.*, 2009a). Most of the “compare and contrast” designs attribute the decline of the ghost crab burrows on urbanized beaches to disturbances caused by intermittent human trampling, but they did not individually test the effects of this stressor (Schlacher e Thompson, 2012; Schlacher *et al.*, 2016b). Indeed, our snapshot experiment showed that the most trampled areas (3000x steps) had significantly fewer burrow openings, but after two days, these sites were occupied again. We assume that the acute trampling performed in the present study does not reflect the reality of urbanized sandy beaches, which are impacted by



chronic trampling. In such areas, ghost crabs seem to restore their burrow openings overnight (Lucrezi *et al.* 2009a). Thus, ghost crabs could be more resilient in areas with chronic urbanization impacts, unless trampling is caused by unusually large crowds during holidays and vacation periods.

The reduction in burrow abundance in the most trampled plots occurred simultaneously with an increase in this value in the control and less trampled plots (300 and 900x). This suggests that disturbed ghost crabs migrated to less trampled sites and/or that other crabs preferred to build their burrows on non-trampled areas. Burrow construction and maintenance requires high energy demands and metabolic costs, which are likely immediately experienced by the ghost crab following heavy and acute disturbances (Lucrezi e Schlacher, 2010). Heavy trampling may enhance water evaporation on the surface, decreasing sediment moisture (Lucrezi *et al.* 2009a) and affecting burrow construction and maintenance. However, if sediment moisture was crucial to burrow construction after trampling, we would not have found higher burrow abundances on the warmest days and in the low-vegetated plots because taller vegetation and mild temperatures may prevent high evaporation, providing more shade and moist areas for ghost crabs (Schlacher *et al.* 2011).

Ghost crabs are unlikely to be directly crushed by acute human trampling when compared with less mobile organisms (Moffet *et al.*, 1998) since their burrows can reach more than one meter deep, suggesting that the crabs may be partially protected (Pombo e Turra, 2013). As empirical evidence of burrow importance, Schlacher *et al.* (2007) found that burrows protect crabs against cars, but vehicles killed the shallowly buried individuals (5 to 30 cm). Studies have demonstrated that human disturbance influences burrow depth, creating deeper burrows as a behavioural protection mechanism of the crab (Lucrezi *et al.*, 2010; Schlacher e Lucrezi, 2010b). Additionally, ghost crabs can perceive sounds, vibrations and human presence at distances of up to 10 metres and turn back to their burrows (Lucrezi e Schlacher, 2014). Even on urbanized sandy beaches, chronic trampling occurs mainly during diurnal periods, while *O. quadrata* is usually a nocturnal forager (Milne e Milne, 1946; Abarcana-Arenas *et al.* 2007).

The sediment compactness was higher in the trampled plots compared to the control plots, but there was no significant difference among the distinct trampling intensities over time. Thus, the reduction of the burrow abundance in the 1500x and 3000x trampled plots 24 hours after the experiment may be attributed not to the

hindering of excavation activity but to the recovery from a stressful situation since the longer the trampling period, the more time under stress. Indeed, we did not observe a significant relationship between sediment penetrability and burrow abundance. However, the recently trampled plots were completely disturbed. Although ghost crabs can visually detect objects or predators near them (Lucrezi e Schlacher, 2014), this ability is limited (Lucrezi e Schlacher, 2014). Thus, the irregular ground (i.e., high micro-relief) of the sediment surface probably impairs the visual detection of potential predators and humans. In fact, we found that both the crab escape response and the emergence time were delayed in the plots with 3000 steps, suggesting a possible sublethal effect (e.g., surface activity and predator perception reduction) of trampling and a mechanism responsible for the burrow decline in trampled beaches. If ghost crabs did not die after the experimental trampling, the occupied burrows should have been restored at night and recounted in the following days, even in the most trampled plots, as observed by Lucrezi *et al.* (2009a). However, according to Neves e Benvenuti (2006), ghost crabs may not reopen their trampled burrows to protect themselves from the impact. Additionally, we considered only active burrows in a dry region of the beach (see methods). According to Pombo e Turra (2013), signs of activity on abandoned burrows soon disappear in this beach stratum. Thus, the inactive burrows were considered unoccupied, and they were not recounted.

If ghost crabs are not crushed by trampling, what are the mechanisms responsible for the reduction in the abundance of burrows on urbanized sandy beaches? Answering this question requires a careful assessment, including other experimental approaches. In the case of chronic human trampling, the collapse of part of a burrow requires that it should be-reopened and, consequently, creates a high energy cost. As a result, crabs can migrate to undisturbed areas, but when trampling is recurrent and non-trampled areas are rare, the crabs may stay in their collapsed burrows without reopening them (Neves e Benvenuti, 2006). We suggest that intermittent human presence on urbanized beaches causes intense stress and inhibits vital ghost crab behaviours, including diurnal surface activities, especially for juvenile crabs, nocturnal foraging and the need for moist gills; this results in a reduction in the health and growth of this species. Chronic trampling is also expected to have long-term effects, such as the removal of food from the burrow vicinity (Stelling-wood *et al.*, 2016). To reduce the speculation about these mechanisms, experimental and behavioural studies should be prioritized, since indirect and “compare and contrast”

studies observing the human impact on ghost crab burrows are abundant (Schlacher e Lucrezi, 2010; Schlacher *et al.*, 2016b;).

We found an influence of natural predictors on ghost crab abundance in this short-term survey. Ghost crabs avoided building their burrows in the plots with the densest vegetation because the root system likely prevented proper burrowing (Schlacher *et al.* 2016). In some cases, when the root system is not overly extensive, it is also possible that vegetation indirectly influences vigilance capacity and increases the predation risk for ghost crabs, as observed for shorebirds (Metcalf, 1984). In addition, the predation of ghost crabs by owls, for instance, may be more intense in dune vegetation (Blankensteyn, 2006a; Branco *et al.*, 2010). Indeed, ghost crabs seem to prefer building their burrows in the unvegetated and wettest parts (e.g., upper intertidal and subterrestrial fringe) of sandy beaches (Turra *et al.* 2007). An increase in the burrow abundance was also observed in all plots 72 hours after the experiment, which is related to an increase in the sediment temperature. It is well accepted that temperature determines the active levels of ghost crabs, but it seems to be related to regional patterns (Valero-Pacheco *et al.* 2007; Pombo *et al.* 2018). Other studies reported that warmer days may also be the most active days for *O. quadrata* (Haley, 1972; Lucrezi e Schlacher, 2014). It has been suggested that wind can obscure the burrow entrance on the upper shore, where there is loose sand (Lucrezi e Schlacher, 2009; Pombo e Turra, 2013), but we did not find a significant influence of wind speed. Similarly, sediment compactness seemed to influence burrow morphology and depth (Lim *et al.*, 2011), but it was not related to burrow abundance during our experimental trampling.

In conclusion, ghost crabs avoided heavily trampled plots for 24 hours after the experiment, but these plots were recolonized over the following days. There was not a significant linear relationship of ghost crab abundance and trampling intensity or sediment compactness because only the 1500x and 3000x trampled plots were avoided during the first recount, probably due to the inability of the crabs to perceive potential predators. Therefore, the hypothesis that increased sediment compactness would cause avoidance of the recently trampled plots by the ghost crabs was not promptly supported. Here, we suggest that burrow counting should not be performed immediately after events with unusual crowds, even on urbanized sandy beaches; ghost crabs can avoid building burrows in these places, so their abundance may be underestimated in such cases.

## CAPÍTULO III

### CAN THE ATLANTIC GHOST CRAB BE A POTENTIAL BIOMONITOR OF MICROPLASTIC POLLUTION OF SANDY BEACHES SEDIMENT?

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#### **Abstract**

The objective of the present study was to test whether the Atlantic ghost crab *Ocypode quadrata* is a reliable biomonitor of microplastic (MP) pollution of beach sediments. To test the hypothesis (H1) that the sediment is the main source of MP ingestion, the proportion of MP types (hard plastic, microfibers, pellet, soft plastic, and extruded polystyrene foam) in the gut content was compared with that on the strandline. The types of MPs in the gut content and sediment had similar proportions; black (~49%) and blue (~45%) microfibers were responsible for this similarity (55%), hence confirming H1. However, the second hypothesis (H2) that prevalence of MP in the gut content is related to its density on beach with distinct urbanization degree was not accepted. These results indicate that high trophic plasticity of the ghost crab and, consequently, multiple-sources of contamination may interfere with its use as a biomonitor of MP pollution.

**Keywords:** Biomonitoring; Marine debris; Marine litter; Microfiber; *Ocypode*; Plastic.

#### 1. INTRODUCTION

Ghost crabs (Crustacea: Ocypodidae) are useful bioindicators of the condition and changes of sandy beaches owing to their sensitivity to human impacts, wide range of distribution, and easy sampling (Schlacher *et al.*, 2016b). They usually respond predictably to human impacts mainly by reduced burrow abundances and size, but they are still assumed to be good ecological indicators for impact assessments (Schlacher *et al.*, 2016b; Costa e Zalmon, 2019b). However, ghost crab species have not been tested as possible biomonitors of any pollutant worldwide, and they are the most conspicuous invertebrate of beach ecosystems (Schlacher *et al.*, 2016b). It is surprising, since sandy beaches have been increasingly facing a threat due to several pollutants such as light, noise, oil, microorganisms, and plastics (Corcoran *et al.*, 2009; Bejarano e Michel, 2016; Duarte *et al.*, 2016; Cabrini *et al.*, 2017).

Marine debris are deposited on both pristine and modified sandy beaches and transported by tides, currents, wind, rivers, and beach users (Corcoran *et al.*, 2009). Plastics constitute the majority (80-85%) of marine debris worldwide (Auta *et al.*, 2017). Fragmentation of plastics into small particles occurs effectively because of constant wave abrasion, UV light, oxygen availability, and turbulence (Barnes *et al.*, 2009; Cole *et al.*, 2011). This breakdown continues until the fragments become secondary microplastics (MP) (<5 mm). MP from primary sources enters into marine environments as virgin pellets from cosmetic products and industrial applications (*e.g.*, abrasives) (Cole *et al.*, 2011). MP can readily adsorb hydrophobic organic compounds and be easily ingested by infaunal invertebrates such as ghost crabs (Ivar Do Sul e Costa, 2014; Egbeocha *et al.*, 2018).

MP ingestion by aquatic organisms generally depends on the feeding behavior and distance from urban areas (Peters e Bratton, 2016; Setälä *et al.*, 2016; Welden e Cowie, 2016). However, MP uptake by wild-caught beach invertebrates has been rarely studied (*e.g.*, Gusmão *et al.*, 2016; Iannilli *et al.*, 2018), although sandy beaches are one of the major hotspots for marine debris accumulation. Overall, filter feeder mussels have been widely suggested as indicators of MP pollution of coastline and beach sediments, even if they are not endemic inhabitants of sandy beach sediments (Fossi *et al.*, 2018; Li *et al.*, 2019).

Facility for sampling, high local abundance, and wide range of distribution are recommended criteria to consider a species as good bioindicators and/or biomonitors. Ghost crab species fulfill all of these criteria, but the relationship between the pollutant content (including MP) in the species and that in the environment, which is supposed

to be an important feature of effective biomonitors (Croteau *et al.*, 1998), has never been reported worldwide. Additionally, plastic ingestion by ghost crabs has not yet been reported, but because of their high trophic plasticity (Tewfik *et al.*, 2016), they are susceptible to ingest MP indirectly from multiple sources including sediment (Costa *et al.*, 2010), wrack (Goss *et al.*, 2018), carrion (e.g., dead fish), other contaminated prey (Watts *et al.*, 2014) or even directly (Costa *et al.*, 2019). Accumulation of natural debris (food source) and MP occurs predominantly near to upper beach strandlines (Costa *et al.*, 2010), where ghost crabs usually search for food and construct their burrows (Lucrezi e Schlacher, 2014; Gül e Griffen, 2018).

The objective of the present study was to test whether the Atlantic ghost crab *Ocypode quadrata* (Fabricius, 1787) can be a useful biomonitor of MP pollution of beach sediment (upper strandline). For this purpose, we compared the proportion of distinct MP types in the ghost crab gut content with the proportion in the one on the upper strandline, to test the hypothesis (H1) that sediment from this beach zone is an important source of MP ingestion. In addition, we tested the hypothesis (H2) that the prevalence of MP in the gut content of this species is related to the level of sediment pollution on beaches with distinct urbanization levels, regardless of potential food availability.

## 2. METHODS

### 2.1. Study site

The study was conducted on the Grussaí Beach Arch (GBA) in southeastern Brazil (between 21°41'21"S/41°1'21"W and 21°44'24"/41°1'21"). The GBA has semidiurnal microtide beaches with intermediate morphodynamics and different levels of human impact. Six beaches along the GBA were categorized into three impact levels according to urbanization index (UI): "low-impact" (UI < 0.2), "medium-impact" (0.3 > UI < 0.7), and "high-impact" (González *et al.*, 2014; Costa e Zalmon, 2019b). High-impact beaches (urbanized) include areas with the highest density of macrodebris (mean > 2.5 items/m<sup>2</sup>) mainly from recreational sources, related to local human pressure (>200 people/100 m<sup>-1</sup> in the intertidal zone) (Suciu *et al.*, 2017). Medium- (intermediate) and low-impact (non-urbanized) beaches also receive marine debris from recreational sources transported by beachgoers, wind, and currents but usually have lower density (~1 item/m<sup>2</sup>) than high-impact beaches (Suciu *et al.*, 2017).

The whole beach arc is under similar influence of the Paraíba do Sul River plume, a medium-sized river (~1500 Km long and 55400 Km<sup>2</sup> of area) that crosses the most industrialized states of Brazil, that is, Rio de Janeiro, São Paulo, and Minas Gerais (Carvalho, 2016). Thus, distance from the river mouth does not represent a source of bias related to spatial distribution of the sampling areas according to urbanization levels and marine debris input. The outflow discharge into the ocean is usually higher in the rainy season (~780 m<sup>3</sup> s<sup>-1</sup>) than in the the dry season (~330 m<sup>3</sup> s<sup>-1</sup>) (Souza *et al.*, 2010).

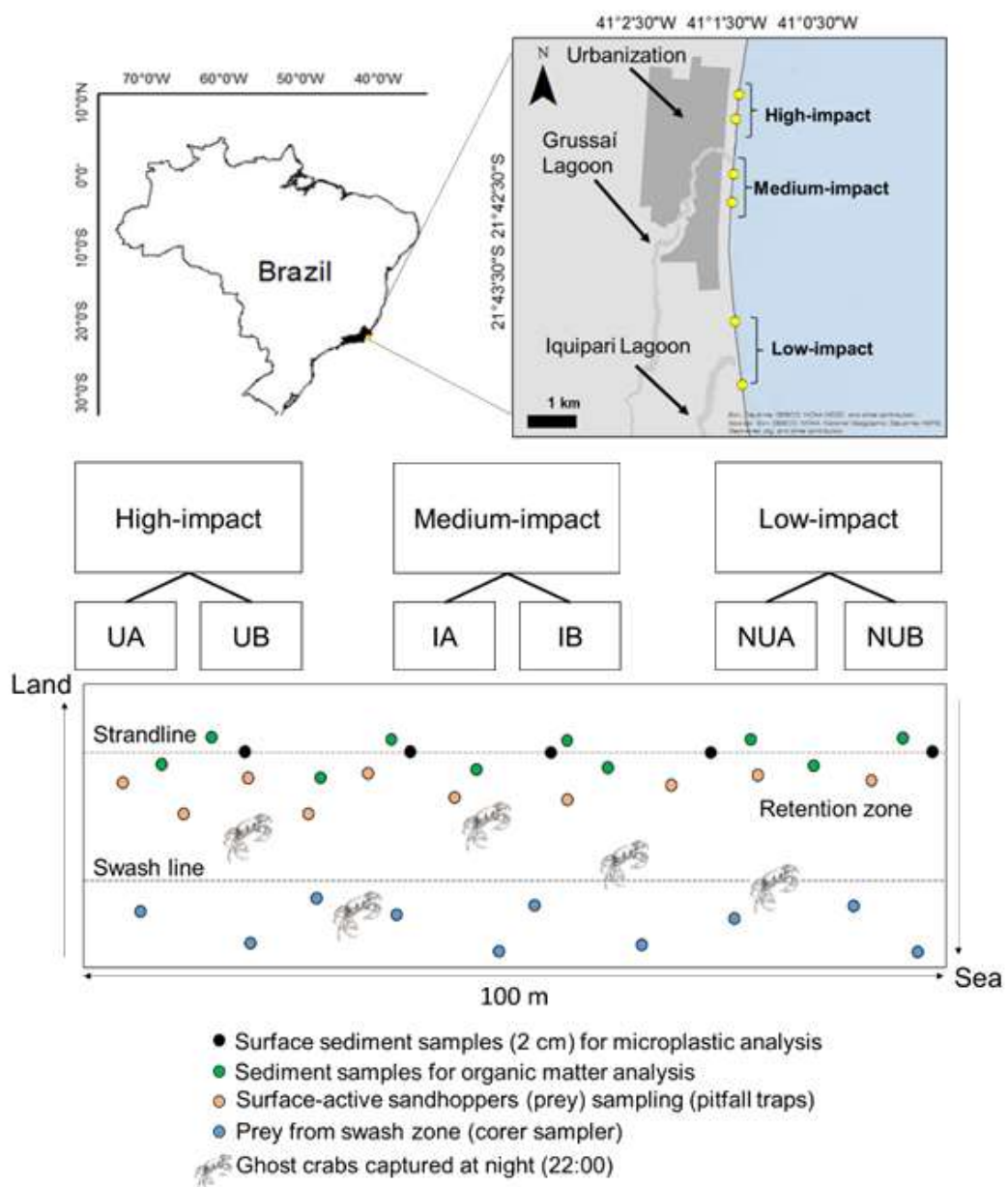


Figure 1. Map of the study site showing the Grussaí Beach Arch, in southeastern Brazil, and the sampling design.

## 2.2. Field and laboratory procedures

Beaches were sampled twice in the dry season (July-October 2017) and twice in the rainy season (February-March 2018). For each survey, five to ten ghost crabs, depending on their local abundance on the sediment surface ( $n_{\text{total}}= 132$ ), were randomly sampled at night (~22:00h) along a transect parallel to the water line in the intertidal zone (100 m length) (Fig. 1). The collection was performed before the crabs returned back to the sea or to their burrows, to avoid MP ingestion induced by the capture. Each ghost crab was captured by hand and euthanized on ice before dissection (Tewfik *et al.* 2016).

In the laboratory, the dorsal carapace was removed and the gut content was inspected under a stereomicroscope (10-40x magnification) to identify MP. The guts were flushed into a petri dish with filtered (glass microfiber filter Whatman G/F) distilled water to ensure that the detectability of MP was not impaired by food volume. Before each gut inspection, all the materials (e.g., petri dishes and tweezers) and the laboratory bench were cleaned with 95% alcohol using paper towels soaked with alcohol. In addition, alcohol, water, and petri dishes were previously inspected under the stereomicroscope (five control samples) to avoid bias related to contamination by clothes (white cotton lab coats), airborne, and/or laboratory bench fibers (Hidalgo-Ruz *et al.*, 2012).

The MP (0.3-5 mm) and macroplastics found in the gut of the crabs were visually sorted and classified by types (hard plastic fragments, microfibers, virgin pellets, soft plastic fragments, and extruded polystyrene foam) and colors (blue, black, red, transparent, yellow, and others). Visual sorting and identification of large MP (between 1 and 5 mm) offer an easy, simple, and fast method for both experts and trained nonprofessionals, as most of them are usually identifiable by microscopy (Shim *et al.*, 2016). Pieces of plastics were distinguished from sand, shells, and organic debris based on the following criteria suggested by Hidalgo-Ruz *et al.* (2012): (1) no organic structures are visible, (2) microfibers should be equally thick throughout their length, and (3) particles have clear and homogeneous colors. Sand particles were identified as a nonplastic item owing to their geometric or tetrahedral appearance (Davidson e Dudas, 2016). The particles were also prodded with a needle as an additional test to identify MP (Shim *et al.*, 2017).



The sediment from the strandline of the six beaches was sampled by scraping the first 2 cm of sand from five random positions along the same transect of the ghost crab sampling (Hidalgo-Ruz *et al.*, 2012; Besley *et al.*, 2017) Besley *et al.*, 2017 (Fig. 1). Ghost crabs are predominantly scavengers, and most of their potential food resources (carrion, wood detritus, and wrack) accumulate on the strandline surface (Lucrezi e Schlacher, 2014). In the laboratory, the sediment samples were weighed and examined under a stereomicroscope (10-40x magnification). After dry sorting, sediment samples were directly filtered (glass microfiber filter Whatman G/F), and the filter was inspected under a stereomicroscope. The same laboratory procedures (visual sorting and control samples) performed in the crab gut samples were applied for sediment screening to avoid sample contamination.

### 2.3. Other predictive variables

To evaluate whether MP ingestion by the ghost crab is related to food availability, macroinvertebrates and organic matter content in the sediment were assessed. Potential prey from the swash zone were collected in 10 random sediment samples with a corer measuring 20 cm in diameter and depth. The sediment was sieved using a 1-mm mesh, and all individuals were counted in the field during 10 minutes of screening per sample. To sample the surface-active sandhopper *Atlantorchestoidea brasiliensis* (other potential prey of the ghost crab according to Tewfik *et al.* 2016), 10 random pitfall samples were made in the retention zone (Fanini e Lowry, 2016). After 3 hours, we removed the pitfalls from the sand because of the rising tide and human disturbances and counted all organisms before releasing them back to the beach. The organic matter content from 10 sediment samples close to the strandline (up to 5 m depth) was determined based on the loss-on-ignition method, calculating the difference between freeze-dried and incinerated sediment at 350°C for 15 h (Goldin, 1987).

### 2.4. Data analysis

To test the hypothesis (H1) that sediment from the high-tide mark is the main source of MP ingestion by the Atlantic ghost crab, the proportion of MP types and colors in their gut content and in the sediment was compared using the permutational analysis of variance (PERMANOVA). The PERMANOVA design included two fixed

factors: impact level (low-impact, medium-impact, and high-impact) and location (gut content vs. sediment from the strandline). The multivariate data matrix with the distinct MP types and colors (variables) was based on the Bray-Curtis similarity coefficient. Significant similarity between location factors ( $p > 0.05$ ) indicates that sediment from the strandline is an important source of MP ingestion by the ghost crab (Costa *et al.*, 2018b; Grant *et al.*, 2018). The Similarity Percentage Analysis (SIMPER) was used to determine the contribution of each MP type and color for the similarity between gut content of the Atlantic ghost crab and the content in sediment samples.

A linear regression analysis was conducted to test the hypothesis (H2) that the prevalence of MP in the gut content of the Atlantic ghost crab is related to the level of sediment pollution (strandline) in the beaches with distinct urbanization levels, regardless of food availability. The frequency of crabs ingesting MP and the abundance of MP per individual were significantly correlated ( $R = 0.86$ ); thus, only the frequency of crabs ingesting MP was included as a response variable in the regression analysis. Sediment pollution was calculated as the product of the density (MP/g of sediment from the strandline) and the frequency of occurrence of MP in each sampling campaign.

To ensure that food volume in the gut content and crab size did not bias the MP inspection, we performed a generalized linear model (GLM) with binomial distribution, including the MP presence or absence as a function of the gut repletion and carapace length. We also included in the binomial models the predictive variables urbanization level and food availability (organic matter percentage in the sediment and prey abundance). Multicollinearity was inspected by the variation inflation factor (VIF); predictive variables were removed from the model in a stepwise manner until all variables had  $VIF < 3$ , indicating the absence of multicollinearity (Zuur *et al.*, 2010). Our data did not have any correlated variable. The best combination of the predictive variables (urbanization level, organic matter, prey abundance, carapace length, and gut repletion) was selected according to the lowest AIC (Akaike information criterion) values (Zuur *et al.*, 2010). The best model included prey abundance and gut repletion as the predictive variables of MP ingestion. The analyses were conducted using the "car" and "MuMIn" packages in R version 3.4.3 (Fox e Weisberg, 2011; Barton, 2018).

### 3. RESULTS

#### 3.1. MP ingestion by the Atlantic ghost crab

Except for a low-impact (LIA) beach (54%), crabs from areas of distinct urbanization levels (medium-impact MIA and MIB; high-impact beaches: HIA and HIB) had similar mean frequency of crabs with MP in their gut contents (LIB= 34%; MIA= 30%; MIB= 33%; HIA= 30% and HIB= 42%) (Fig. 3A). This frequency varied from 0% to 100% in all the beaches, regardless of season (Fig. 4A).

MP abundance in the gut contents ranged from 1 to 158 items/individual (Fig. 2). Microfibers (93%) were the most frequently ingested MP type, followed by hard plastic fragments (2.5%), soft plastic fragments (1.9%), and extruded polystyrene foam (1.7%). Most of these microfibers were black (39%), blue (38%), and transparent (7%) (Fig. 3A). Virgin plastic pellets (n= 158) were found in only one crab in the MIA beach. Ingestion of the macrodebris “twine” (n=1 crab) and “bait elastic” (n= 2 crabs) was also found.

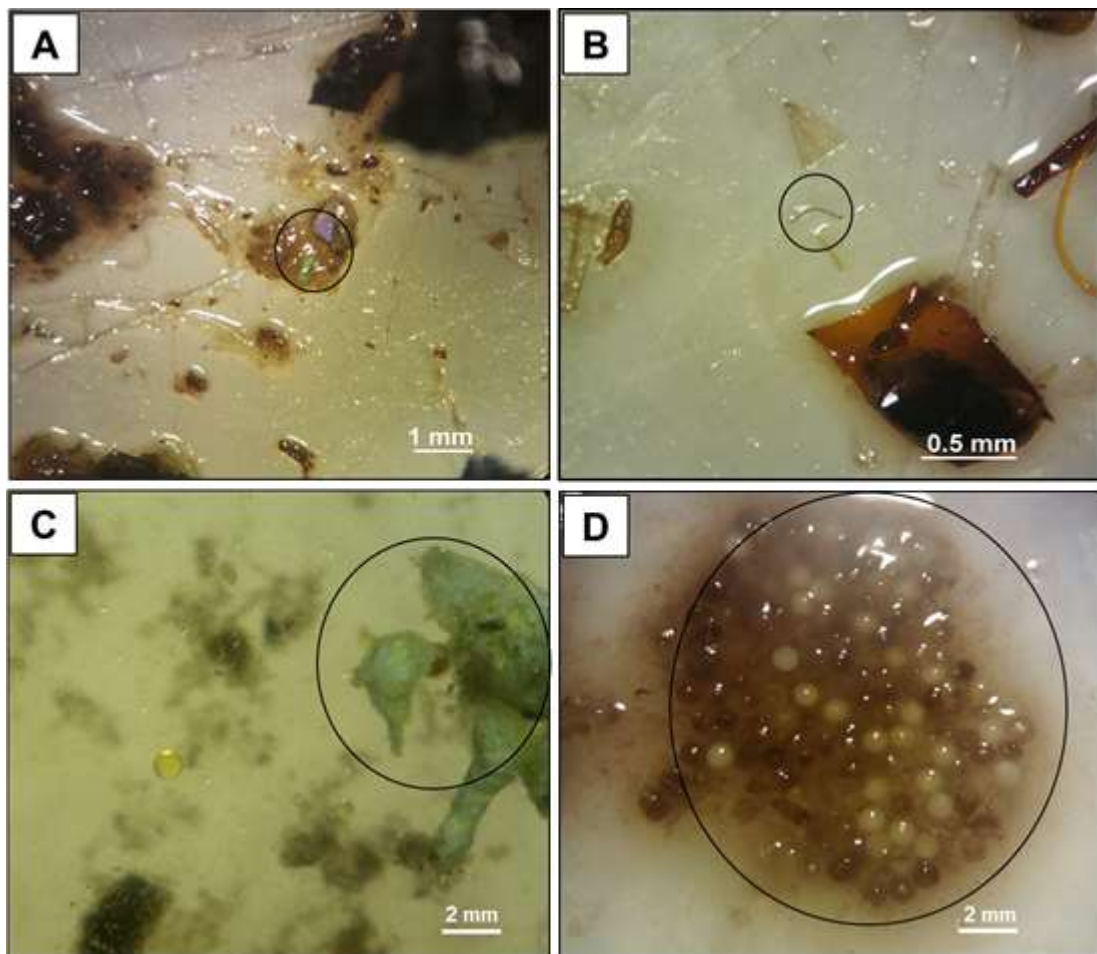


Figure 2. Microplastics in the gut content of the Atlantic ghost crab *Ocypode quadrata*. A= hard plastic; B= microfiber; C= extruded polystyrene foam; D= virgin plastic pellets.

### 3.2.MP in the sediment

The mean MP density in the sediment was higher on the LIA beach (0.4 items/g sediment) than on other beaches, which had values ranging from 0.1 to 0.2 items/g of sediment during the study period (Fig. 3B). Microfibers were the most representative MP type (90%) in the sediment from strandline, followed by soft plastic fragments (4%), extruded polystyrene foam (3%) and hard plastic fragments (2%) (Fig. 3B). Most of microfibers in this compartment were black (44%), blue (40%), and transparent (3%) (Fig. 3B). Virgin plastic pellets were not found on the strandline.

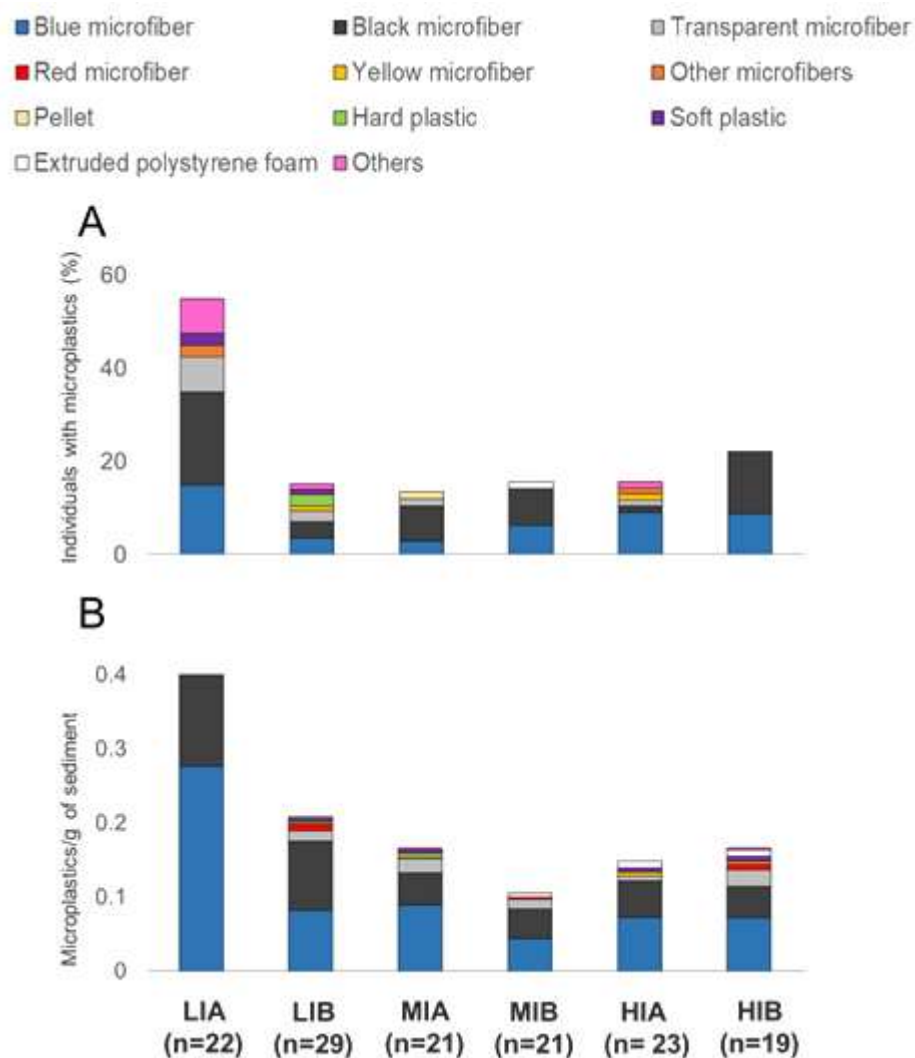


Figure 3. Frequency of the Atlantic ghost crab *Ocyropsis quadrata* with distinct microplastic types in their gut contents (A) (n=132) and microplastic density in the sediment from the strandline (B) on the Grussaí Beach Arch, southeastern Brazil. Low-

impact beaches (LIA and LIB); medium-impact beaches (MIA and MIB); high-impact beaches (HIA and HIB).

### 3.3. Relationship of MP in ghost crab gut and in sediments

MP types and colors from gut contents and sediment samples had similar proportions (pseudo-F= 1.58;  $p_{\text{permanova}}$ = 0.18); black (~49%) and blue microfibers (~45%) were mainly responsible for this similarity (55%) according to SIMPER analysis. The prevalence of MP in the gut content of the ghost crab (Fig. 4A) was not related to beach sediment pollution across distinct urbanization levels and seasons (Fig. 4B).

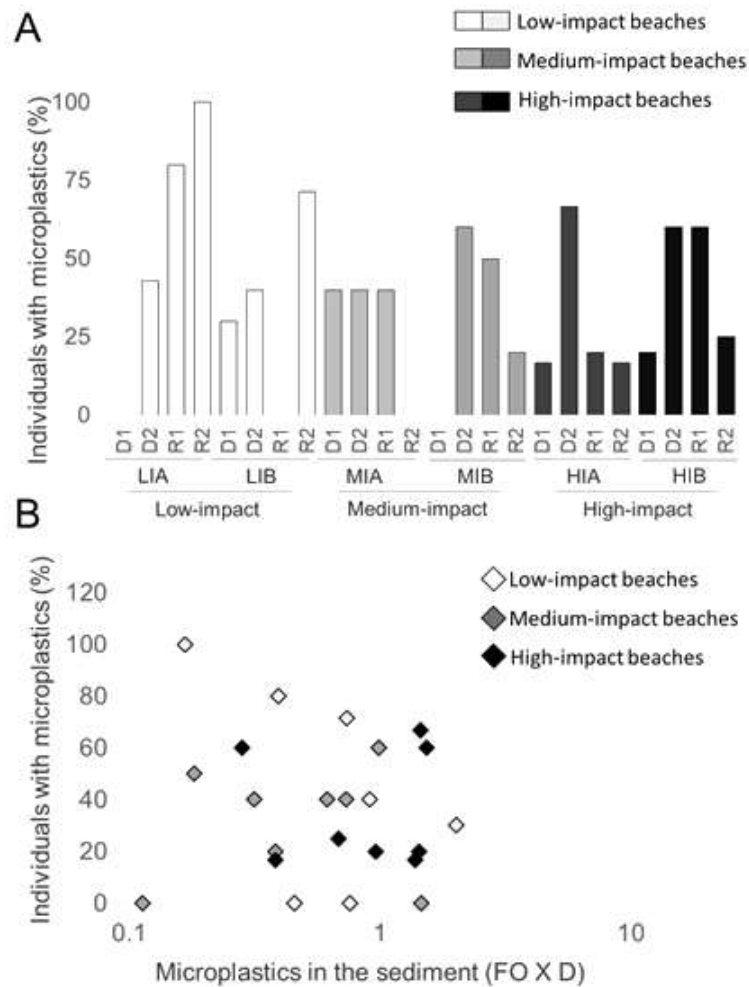


Figure 4. Frequency of the Atlantic ghost crab *Ocypode quadrata* with microplastics in their gut contents (n= 132) (A) and the relationship between these contaminated crabs and the amount of MP (D: density x FO: frequency of occurrence) on strandline (sediment) on the Grussaí Beach Arch, southeastern Brazil (B). D= dry season; R= rainy season. LIA and LIB= low-impact beaches; MIA and MIB= medium-impact beaches; HIA and HIB= high impact beaches.

The likelihood of occurrence of MP in gut contents was positively related ( $z= 3.02$ ;  $p= 0.002$ ) to gut repletion, that is, higher food volume = higher MP occurrence. In the model including all the predictive variables, carapace length was not related to MP ingestion ( $z= 1.44$ ;  $p= 0.149$ ) (Table 1). Similarly, in the best model according to the AIC values, MP ingestion by the Atlantic ghost crab was not related to food availability (organic matter and prey abundance) and urbanization level (Table 1).

Table 1. Generalized linear model with binomial distribution including the MP presence or absence as a function of urbanization degree, organic matter, prey abundance, gut repletion, and carapace length. The best model was ranked by the Akaike information criterion (AIC). Significant predictors ( $p < 0.05$ ) are marked in bold. (+): positive effect.

Global model (AIC= 174.22)	Estimate	Std. Error	Z-value	p-value
Intercept	-2.98	1.34	-2.23	<b>0.025</b>
Urbanization level	0.25	0.72	0.34	0.733
Organic matter	0.22	0.73	0.30	0.763
Prey abundance	-0.01	0.01	-1.43	0.151
Gut repletion (+)	0.41	0.13	3.21	<b>0.001</b>
Carapace length	0.04	0.03	1.37	0.171
Best model (AIC= 168.64)	Estimate	Std. Error	Z-value	p-value
Intercept	-1.24	0.48	-2.57	<b>0.010</b>
Prey abundance	-0.01	0.01	-1.64	0.102
Gut repletion (+)	0.36	0.12	3.02	<b>0.002</b>

#### 4. DISCUSSION

The present study reported, for the first time, the plastic ingestion by a ghost crab species. The Atlantic ghost crab *O. quadrata* is another species to be included in the list of the increasing number of marine species ingesting marine debris (reviewed by Laist, 1987; Gall e Thompson, 2015;). Unfortunately, this result is not surprising because of the ubiquitous presence of MP on beach sediment, including microfibers (Gago *et al.*, 2018), the most prevalent MP in the gut content of the Atlantic ghost crab. Even though inspection under stereomicroscopy, as a single method, imposes risks of producing false-negative or -positive results for small MP (<1 mm), visual sorting is still reliable for large MP, particularly for easy and fast monitoring (Shim *et al.*, 2017). Here, we tested the Atlantic ghost crab as a possible biomonitor, assuming that the proportion of large MP on sediment and digestive tract is a proxy of beach pollution by MP of different sizes. On the other hand, macrodebris (e.g., extruded polystyrene foam, twine, and bait elastics) were not as prevalent in the gut contents of the Atlantic ghost crab as MP. Therefore, although previous studies showed that this species interacts with macrodebris stimulated by potential food odours, ingestion seems to be avoided in natural contexts (Costa *et al.*, 2018b, 2019).

Our results are similar to global patterns indicating microfibers as the most predominant MP in the marine environment presently (Barrows *et al.*, 2018; Gago *et al.*, 2018; Barletta *et al.*, 2019). Microfiber occurrence in the water and in the sediment

has been attributed mainly to the release of synthetic fibers from garments during washing and fragmentation of fishing materials (e.g., trawl nets and ropes) (Cole, 2016). Predominance of black and blue microfibers is a further evidence that microfibers originate from nylon of fishing activities, boat ropes, and clothing fibers from wastewater (Browne *et al.*, 2011; Chubarenko *et al.*, 2016). The possible sources of MP in the beach sediment are extensive. The GBA is located in the vicinity of the most important river discharge of southeastern Brazil (Paraíba do Sul) (Almeida *et al.*, 2007) and three coastal lagoons (Grussaí, Iquipari, and Açú) (de Sousa *et al.*, 2004). Thus, these environments are chronic sources of wastewater, with microfibers and fishing gear brought by wind and currents to all the beach arc, regardless of urbanization (Welden e Cowie, 2016; Rochman, 2018).

The present data confirmed recent findings that plastics enter sandy beach food webs (Lourenço *et al.*, 2017). Gusmão *et al.* (2016) reported the ingestion of microfibers by *Saccocirrus* spp. (Annelida: Polychaeta) in Brazil, and Iannilli *et al.* (2018) found MP in the beachhopper *Talitrus saltator* (Crustacea: Talitridae) in Italy, showing that typical transient predators of sandy beaches (e.g., fish and shorebirds) are exposed to ingest MP from their prey (Lourenço *et al.*, 2017). Specifically, ghost crabs may play an important role in redistributing MP and/or with sorbed contaminants (e.g., POPs: persistent organic pollutants) from ocean through sediment, prey, and carrion to inland predators (e.g., birds of prey and possums) (Clark *et al.*, 2016). It has been shown that beach crustaceans can accumulate POPs that is probably sourced from MP ingestion (Chua *et al.*, 2014; Ungherese *et al.*, 2016).

The risks that MP ingestion poses on vital rates (e.g., consumption, growth, reproduction, and survival) are still not clear for benthic invertebrates (Foley *et al.*, 2018). If vital rates are being negatively affected by MP ingestion, then the ghost crab (or other beach invertebrates) population might decline, affecting important trophic links for the ecosystem functioning. Although a long-term monitoring of both population size and MP ingestion is necessary to ensure about this causality relationship, we found that ingestion occurred in low-impact beaches in similar proportions (or even higher) as those of most urbanized areas. However, population size is approximately four times higher in the former than in the latter (Suciu *et al.*, 2018; Costa e Zalmon, 2019b), suggesting that MP ingestion is not the main mechanism responsible for decline in ghost crab population. Thus, MP ingestion possibly does not affect the ghost crabs' ability to prey or ingest enough calories from detritus and/or escape from



predators and, consequently, might have constant reproduction and survival rates (Foley *et al.*, 2018). Indeed, our data showed that MP presence in the gut content of the ghost crab was not negatively related to either size or food volume. This result differs from that of an experimental approach with beachhoppers (Crustacea: Talitridae), showing that their survival and mobility were negatively affected by MP ingestion (Tosetto *et al.*, 2016).

The predominance of black and blue microfibers and a similar proportion of other MP or macrodebris types (e.g., hard plastics, soft plastics, and extruded polystyrene foam) in the gut content of the Atlantic ghost crab and sediment corroborate our first hypothesis that sediment from strandline is an important source of MP to the Atlantic ghost crab. This suggests that the species accidentally ingests the most common MP during their feeding on detritus on the sediment surface, as observed with other scavenger crustaceans (Welden e Cowie, 2016). Microfibers associated with sediment and algae fragments in the strandline are small enough to be easily fit into the ghost crab mouth, which are probably unable to prevent ingestion of these inconspicuous particles together with food pieces. It has been suggested that even macroalgae and seagrass usually stranded on sandy beach strandlines and consumed by ghost crabs are potential vectors for incorporating MP into marine food webs (Goss *et al.*, 2018).

Although the most abundant MP types in the sediment were the main ingested ones, there was no relationship between the prevalence of MP in the gut contents of the Atlantic ghost crab and the pollution level by MP in the sediment; thus, our second hypothesis was not accepted. Although the frequency of contaminated crabs ranged from 0 to 100% from urbanized to nonurbanized beaches, the density of MP in the sediment remained usually constant with time, regardless of urbanization levels and season (0.1 to 0.2 MP/g sediment). Thus, neither urbanization nor sediment dynamics had clear effects on MP deposition on the upper strandline.

The complex behavior of ghost crab is probably driving the MP prevalence in their gut contents. One possibility is that ghost crabs may retain microfibers in their gut (~20 days) (Watts *et al.*, 2014) in distinct rates, compared with the rates at which MP from land or ocean deposits in the sediment. Opportunistic feeding on punctual sources of food instead of detritus from the strandlines (Schlacher *et al.*, 2013), or foraging on other beach zones (e.g., inside burrow, supralittoral, and surrounding lagoons) can also exert bias in monitoring the sediment pollution levels using only the

ghost crab as biomonitor. Indeed, ghost crabs can use all the beach strata during foraging activity (Schlacher e Lucrezi, 2010). Similarly, Iannilli *et al.* (2018) suggested that differences in MP composition ingested by the scavenger *T. saltator* and found in the sediment were related to selectivity of foraging patches in the intertidal zone.

The unreliability of deposit feeders and scavenger as biomonitors of MP pollution is similar to findings by other authors. Deposit feeders can preferentially ingest MP on the basis of size or select particles (including MP) that exhibit biofilms as organic matter source (Hurley *et al.*, 2017). Similarly, ghost crabs also feed on sediment and may present particles' selectivity (Branco *et al.*, 2010; Lim *et al.*, 2016). Opportunistic scavengers and facultative predators, such as ghost crabs, probably ingest MP from many sources compared to filter feeders (Welden e Cowie, 2016). Indeed, filter feeders, mostly mussels, usually have higher MP prevalence and concentration in their body than other trophic guilds (Setälä *et al.*, 2016) and are suggested as global biomonitors of MP pollution in the ocean (Wesch *et al.*, 2016; Li *et al.*, 2019). The filter feeders are predicted to encounter the most MP because this feeding strategy concentrates food from large volumes of water with this kind of debris (Desforges *et al.*, 2015).

The binomial models showed that the ingestion of MP by the ghost crab was not predicted by urbanization level, organic matter, prey abundance, and carapace length. These results are further evidence that factors such as broad spectrum of activities, a wide home range, and a complex interaction of ghost crabs with the dynamic beach substrate make difficult to find a direct relationship between MP ingestion and MP amount in a single beach zone. Although large volumes of plastics (microplastics and macroplastics) are deposited in the upper strandline, which is also a beach zone with concentration of natural debris and feeding opportunities, ghost crabs have clear preference by punctual and high-calorific value source of food (e.g., fish) (Harris *et al.*, 2019). Urbanization level or availability of typical food (e.g., macroinvertebrates) on the beach seems not to disrupt this opportunistic behavior of the Atlantic ghost crab and to drive the MP uptake (Costa *et al.*, 2019). Therefore, monitoring of the indicator potential of MP pollution using ghost crabs requires the measurement of the availability of this punctual source of food, as well the quantification of MP in several beach strata (e.g., inside burrow and supralittoral areas).

Our results agree with the assumption that the biomonitoring of plastics in marine ecosystems is complex and should rely on the combination of several indicator

species with distinct characteristics, particularly feeding habitats and/or mobility (Bonanno e Orlando-Bonaca, 2018). Filter feeders are global biomonitors of MP pollution because they ingest MP types in a similar proportion as those found in their environment (H1). In addition, they usually provide a direct relationship between its pollutant content and the pollution level of the environment (H2) (Desforges *et al.*, 2015; Waite *et al.*, 2018; Li *et al.*, 2019), in a contrasting manner to that of scavengers or deposit-feeder species (Hurley *et al.*, 2017; Iannilli *et al.*, 2018). Thus, although invertebrates can be useful biomonitors at local scales, their feeding habits need to be considered before biomonitor selection. The Atlantic ghost crab properly indicated most of MP deposited in the strandline in a qualitative way (MP types). However, the prevalence of MP in the gut content of the Atlantic ghost crab did not follow the constant density of MP in the sediment at our spatial-temporal scales.

In conclusion, the Atlantic ghost crab ingests plastics (microplastics and macroplastics), and the sediment from strandline is an important source (but not the only one) of debris, but prevalence of MP in the gut content of this species did not provide a direct relationship with the pollution level in the sediment from strandline. Therefore, this species was not a good biomonitor (quantitative indicator) of MP pollution in our spatial-temporal scale (six beaches; during one year). Dynamic inputs of carrion from the ocean are probably a driver of feeding preferences and MP ingestion by the Atlantic ghost crab instead of available resources on the strandline and typical food availability. Future studies must prioritize to assess the biomonitor potential of other beach species, particularly filter feeders (*Donax* spp. and *Emerita* spp.) together with ghost crabs, to identify reliable indicators of MP pollution in this hotspot of marine debris accumulation worldwide.

## CAPÍTULO IV

### MORTALITY OF THE ATLANTIC GHOST CRAB *Ocypode quadrata* (FABRICIUS, 1787) DUE TO VEHICLE TRAFFIC ON SANDY BEACHES: A ROAD ECOLOGY APPROACH

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#### **Abstract**

Sandy beaches are not roads, but they have been used as such worldwide, threatening endemic fauna such as ghost crabs (Crustacea: Ocypodidae). The objective of the present study was to identify the spatial factors influencing the incidence of ghost crabs (*Ocypode quadrata*) killed by vehicles. This study included a systematic study of carcasses with clear signals of crushing by cars on beaches with distinct urbanization levels and on the dirt road crossing low-urbanized beach stretches. Predictive variables (e.g., tyre tracks on the sand, proxies of urbanization, distance from coastal lagoons, beach width, wind speed and air temperature) were obtained for the kill points and random points. Generalized linear models with binomial distributions showed that the number of tyre tracks nearby (positive correlation) and indicators of urbanization in the environment (negative correlation) were the main variables explaining the ghost crab kills on the beach. Similarly, the likelihood of finding crabs killed by vehicles on the dirt road was associated with the areas with the densest ghost crab populations (low-urbanized areas). Therefore, vehicle traffic must be severely controlled mainly in low-urbanized beaches, both on the sand and dirt roads crossing natural beach vegetation, as an important conservation strategy and mitigation action.

**Keywords:** Human impact; Ocypodidae; ORV-vehicles; road-kill; sandy beach, coastal ecology.

## 1. INTRODUCTION

Roads exert important sources of disturbance for land and semi-aquatic wildlife, creating barriers to animal movement, fragmenting habitats and causing direct mortality from animal-vehicle collisions (Forman e Alexander, 1998; Clevenger *et al.*, 2001). Several taxonomic groups have been killed by vehicles, including vertebrates such as amphibians, reptiles, birds and mammals (Glista *et al.*, 2007; Fahrig e Rytwinski, 2009), and even invertebrates such as insects (Baxter-Gilbert *et al.*, 2015). Counts of dead animals can be useful for evaluating the magnitude of the impact but are insufficient for understanding the spatial factors influencing this source of mortality (Clevenger *et al.*, 2003). There are many factors explaining the spatial patterns of road kills, including taxon-specific factors (e.g., abundance, mobility, reproductive periods and dispersion patterns), road characteristics (e.g., traffic volume) and landscape features (e.g., land use and native vegetation conservation) (Gunson *et al.*, 2011; Sillero *et al.*, 2019). If these factors are well known, transportation planning projects can contemplate specific environmental mitigation actions (Malo *et al.*, 2004; Gunson *et al.*, 2011).

Due to an obvious reason, direct kills by vehicles are rarely assessed for marine organisms (e.g., Schlacher *et al.*, 2007). Sandy beaches are a marine ecosystem and are not roads, but sand from beaches has been used worldwide (Petch *et al.*, 2018). Vehicle traffic on sandy beaches has negative effects on the physical features of the beach (e.g., compactness, moisture, erosion and vegetation cover) (Schlacher *et al.*, 2008a), the survival of beach biodiversity (Schlacher *et al.*, 2007; Knisley *et al.*, 2018; Schlacher *et al.*, 2008b) and behaviour of endemic organisms (Schlacher e Lucrezi, 2010; Merwe *et al.*, 2012). Endemic macroinvertebrates are less abundant in areas with intense vehicle traffic compared to pristine beaches; however, because this impact is usually correlated with urbanization and, consequently, synergistic disturbances (e.g., trampling and beach cleaning), causality relationships (e.g., animal/vehicle collisions) are still not clear (Schlacher *et al.*, 2016b).

Among beach macroinvertebrates, ghost crabs (Crustacea: Ocypodidae) are the main indicators of the impacts of vehicle traffic (Schlacher *et al.*, 2016b; Costa e Zalmon, 2019a). Ghost crabs occur worldwide, with distribution from tropical to temperate latitudes, and their presence is perceived mainly by their prominent burrows in the sand (Lucrezi e Schlacher, 2014). Consequences from vehicle traffic include changes to burrow architecture (Schlacher and Lucrezi, 2010), reduction in home

range (Schlacher e Lucrezi, 2010) and direct mortality (Schlacher *et al.*, 2007). However, the spatial patterns and factors that are not species-specific (e.g., burrow depth) and directly affect mortality are unknown.

The objective of the present study was to identify the spatial factors influencing the incidence of ghost crabs (*Ocypode quadrata*) killed by vehicles. This study included a systematic record of ghost crab carcasses with crushing signals on the sand of beaches with distinct urbanization levels and on a dirt road crossing a beach stretch. The null hypothesis is that the incidence of ghost crabs killed by vehicles is random (not explained by any factor) both on the sand and on the dirt road. This is the first study to demonstrate the death of crabs due to vehicle collisions in America.

## 2. METHODS

### 2.1. Study site

An extensive beach arc in southeastern Brazil (21°S, 41°W) was chosen as a study model to apply the road ecology methodology to assess the direct impact of vehicle traffic on the Atlantic ghost crab. The Grussaí Beach Arc (GBA) has a set of microtidal beaches with intermediate morphodynamics and areas with distinct visitation and vehicle traffic rates.

The beaches close to walkway accesses, paved streets and restaurant centres (urbanized beaches) have higher visitation rates ( $> 200$  people/100 m<sup>-1</sup> in the intertidal zone) and frequent diurnal vehicle traffic on the sand ( $\sim 2$  vehicles/hour/100 m<sup>-1</sup>), mainly during the high tourist season (January to March) and holidays (Machado *et al.*, 2017). These human activities in urbanized areas impair both diurnal and nocturnal surface activity (e.g., foraging) of the remaining ghost crabs ( $<1$  burrows/100 m<sup>2</sup>), which are mostly juveniles (burrows  $<2$  cm) (Appendix 1).

Between the Grussaí and Iquipari Lagoons (see Fig. 1), beaches are in front of several houses, but there is no parking, commercial kiosks or paved access, and vehicles are less frequently used on the sand ( $\sim 1$  vehicle/day/100 m<sup>-1</sup>) by anglers and by Açú Port for monitoring marine turtle nests (Machado *et al.*, 2017; Costa e Zalmon, 2019b). In this beach stretch (intermediate beach in relation to urbanization level – item 2.2 of Material and methods), foraging ghost crabs are found crossing the beach vegetation via the dirt road at night. Intermediate beaches are also found 500 m north

of the “walkway access 3” site (see Fig. 1), where there is a paved road, but beach dunes and vegetation are preserved in this area, and there are few visitors.

To the south of the Iquipari Lagoon (non-urbanized beaches in relation to urbanization level), vehicles are predominantly used for monitoring turtle nests, and low-human pressure (0 people/m<sup>2</sup>) allows diurnal and nocturnal surface activity of the ghost crab, which is at least four times (~16 occupied burrows/100 m<sup>2</sup>) more abundant in this area compared with the other urbanized beaches (Appendix 1).

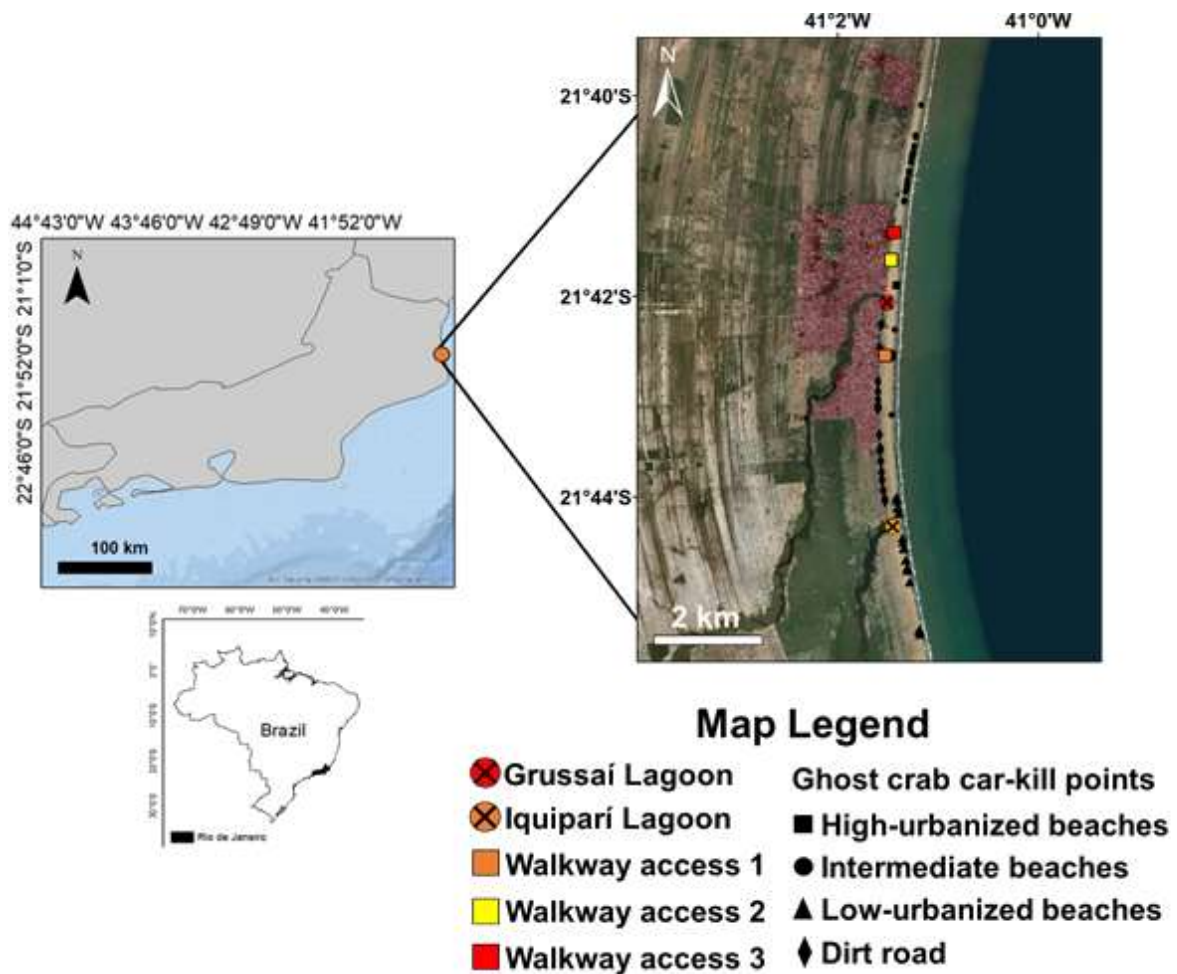


Figure 13. Grussaí Beach Arc (GBA) with areas under distinct urbanization levels along the southeastern coast of Brazil. Black symbols (■●▲◆) represent ghost crabs killed by vehicles on the sand (n = 47) and on the dirt road (n = 20) crossing the beach vegetation. The red-shaded area represents urban settlements.

## 2.2. Ghost crabs killed by vehicles on the beaches

To search for the ghost crabs killed by vehicles on the beach, the areas with tyre tracks on the sand were inspected during six sampling campaigns (September, October and November 2017 and 2018) during the low tourism season and six campaigns during the high tourist season (January, February and March 2018 and 2019). These periods usually have intense beach use and vehicle traffic. The crabs with evidence that were killed by (crushed dorsal carapace under tyre tracks) were searched for over 10 km in the zone between the high-tide mark and 10 metres above the high-tide mark (Fig. 1), which is the beach strata with the highest burrow aggregation on the GBA (Costa e Zalmon, 2019b). This ~10 km long stretch was inspected by foot by two observers, and the samplings were conducted after the peak of visitation and vehicle traffic on the beach (after 16:00). The ghost crab kill locations on the beach sand were obtained by the GPSMAP Garmin (62sc) with at least 5 m accuracy.

For each record of ghost crabs killed by vehicles, a random point was randomly selected to measure the proxy of traffic intensity (number of tyre tracks on the sand), landscape and beach features as predictive variables. For both the vehicle-killed and random points, the numbers of ghost crab burrow openings and vehicle tyre tracks were counted in quadrats (10 x 10 m) starting at the high-tide mark. The National Institute for Meteorology (INMET) provided mean daily air temperature and wind speed from the Campos dos Goytacazes municipality (30 km from Grussaí Beach Arch). The beach width (distance from the water line to the beginning of vegetation) and shortest distances from (1) urban settlements; (2) coastal lagoons; and (3) walkway accesses were obtained from Google Earth images (04/01/2019). The urbanization index and recreational potential were calculated according to González *et al.* (2014) and Mclachlan *et al.* (2013), respectively.

### 2.3. Ghost crabs killed by vehicles on the dirt road

The ghost crab road kills were also searched for along the beachfront between the Grussaí and Iquipari Lagoons (Fig. 1), where individuals forage at night in the dirt road crossing the beach vegetation. This ~4 km long stretch was inspected at night in a vehicle at 20 km/h with two observers on each beach survey date. After georeferencing the road-kill and random points (see the above section), the light pollution was measured with a digital luximeter (Minipa MLM-1011). Two researchers visually counted the number of foraging crabs at night. The number of vehicles parked



at the end of the road during the peak of beach visitation (01:00 pm, Machado *et al.* 2017) was used as a proxy of vehicle traffic rates on the road. Air temperature and wind speed were provided by INMET, and beach width and distance from urban settlements and coastal lagoons were obtained from Google Earth images (04/01/2019).



Figure 2. Vehicle tracks on the sand (A), a ghost crab killed by a vehicle on the sand (B), the dirt road crossing the beach vegetation and a foraging ghost crab at night (C) and a ghost crab road-killed by a vehicle (D).

#### 2.4. Data analysis

Principal component analysis (PCA) was performed and included all the predictive variables to explore the covariance structure in the dataset. Pearson correlation analysis was used to assess the correlation coefficient (R-value) between predictive variables.

Generalized linear models (GLMs) were employed to test the null hypothesis that the patterns of ghost crabs killed by vehicles on the sand and on the dirt road are

random. The models were constructed through the presence/absence of ghost crabs killed by vehicles as a response variable with binomial distribution represented by crushed crabs = 1 or random points = 0 among the total number of records, and logit as a link-function.

For the vehicle-killed and random points on the beach, distinct GLMs were performed with non-correlated variables ( $n = 7$  models) and with all the predictive variables (global model). The absence of multicollinearity in each model was confirmed using the variance inflation factor (VIF), deleting each variable step-by-step with  $VIF > 3$ , until the VIFs of all predictive variables were less than 3 (Zuur *et al.*, 2010). Model selection (global and excluding correlated variables) was based on the lowest Akaike's information criterion (AIC) values for all possible combinations of predictive variables. The same procedures were used to select the best models ( $n = 7$  models with non-correlated predictive variables) to explain the factors that determined the mortality of the Atlantic ghost crab on the dirt road. The analyses were performed using "car" and "MuMIn" packages in R version 3.4.3. (Fox e Weisberg, 2011; Barton, 2018).

### 3. RESULTS

#### 3.1. Predictive variables on the beaches

The first and the second principal components of the PCA explained 55% and 16% of the total variance in the dataset from the beach, respectively (Fig. 3A). The urbanization index was positively correlated with recreational potential ( $R = 0.93$ ) and beach width ( $R = 0.84$ ) and negatively correlated with burrow abundance ( $R = -0.65$ ), distance from urban settlements ( $R = -0.76$ ), distance from walkway accesses ( $R = -0.84$ ) and air temperature ( $R = -0.59$ ) (Fig. 3A). All of these variables were collinear ( $R > |0.5|$ ). The number of tyre tracks on the sand, distance from coastal lagoons and wind speed showed no marked correlations with any predictive variable (Fig. 3A).

#### 3.2. Ghost crabs killed by vehicles on the beaches

A total of 47 ghost crabs killed by vehicles were found on the sand during the 12 sampling campaigns (0.39 individuals/km/day of sampling), 33 in intermediate areas ( $UI = 0.2- 0.7$ ), 13 in non-urbanized areas ( $UI < 0.2$ ) and only one in the most urbanized areas ( $UI > 0.7$ ) (Fig. 1). Among the crabs killed by vehicles on intermediate

beaches, 74% (n= 26) were found during a single survey during the high tourism season (February 2018).

All binomial GLMs showed that the ghost crabs killed by vehicles were positively related to the number of tyre tracks on the sand ( $p < 0.00001$ ;  $z\text{-value} \geq 4.5$ ) (Tab. 1). In addition, the predictive variables related to urbanization (distance from walkway accesses, recreational potential, beach width, distance from urban settlements and urbanization index) also predicted the crabs killed by vehicles (Tab. 1).

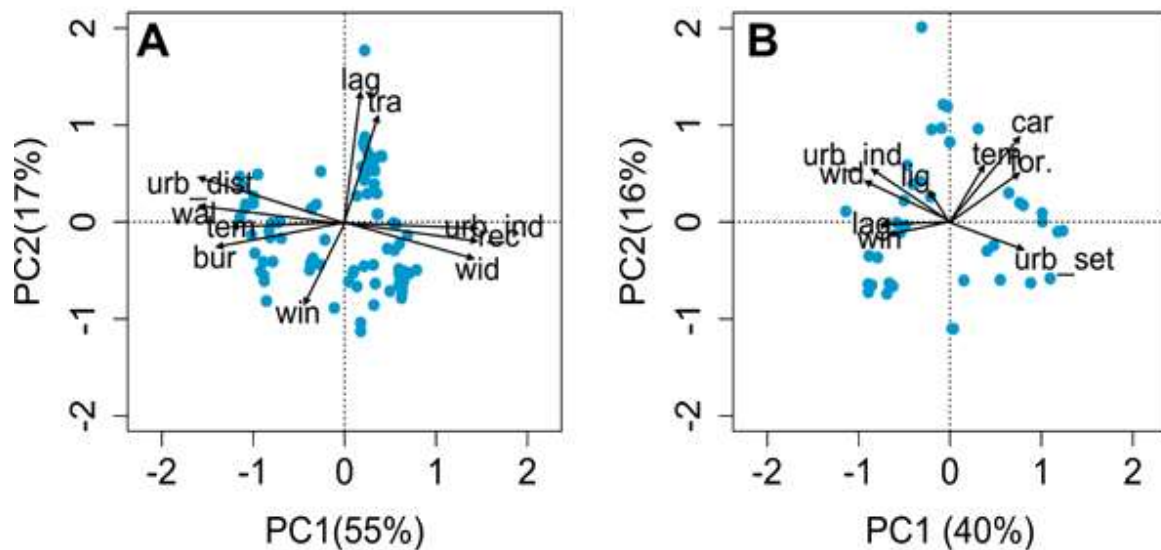


Figure 3. Principal component analysis showing the relationship of the predictive variables on the beach (A) and on the road (B). Blue dots indicate observations. Abbreviations indicate the number of tyre tracks on the sand (tra), number of ghost crab burrow openings/100 m<sup>2</sup> (bur), air temperature (tem), wind speed (win), urbanization index (urb\_ind), distance from urban settlements (urb\_dist), distance from coastal lagoons (lag), distance from walkway accesses (wal), beach width (wid), parked vehicles (car), light pollution (lig) and number of foraging crabs at night (for).

The crabs killed by vehicles on the sand were positively related to the distance from walkway accesses ( $p = 0.0001$ ;  $z\text{-value} = 3.31$ ) and urban settlements ( $p = 0.0041$ ;  $z\text{-value} = 2.87$ ) and negatively related to beach width ( $p = 0.0022$ ;  $z\text{-value} = -3.06$ ), recreational potential ( $p = 0.0019$ ;  $z\text{-value} = -3.11$ ) and urbanization index ( $p = 0.0042$ ;  $z\text{-value} = -2.86$ ) (Tab. 2; Fig. 4). Not all models showed statistical support

for the influence of wind speed and distance from coastal lagoons on the ghost crabs killed by vehicles on the beach (Tab. 1).

Table 1. Generalized linear models (GLMs) with a binomial distribution. The vehicle-killed (1) and random (0) points on the beach represent the response variable. The best models were ranked by the Akaike information criterion (AIC). Significant predictors of the models are marked in bold.

<b>Best beach models</b>				
Model 1 (AIC= 77.1)	Estimate	Std. Error	z-value	p-value
(Intercept)	-7.809	1.618	-4.826	<b>1.4 x 10<sup>-6</sup>*</b>
Distance from coastal lagoons	0.000	0.000	1.836	0.0663
Distance from walkway accesses	0.000	0.000	3.314	<b>0.0001*</b>
Number of tire tracks	0.646	0.143	4.490	<b>7.17 x 10<sup>-6</sup>*</b>
Wind speed	0.414	0.184	2.245	<b>0.0248*</b>
Model 2 (AIC= 78.8)	Estimate	Std. Error	z-value	p-value
(Intercept)	-4.153	1.162	-3.571	<b>0.0003*</b>
Distance from coastal lagoons	0.001	0.000	1.836	0.0663
Number of tire tracks	0.626	0.138	4.527	<b>5.99x 10<sup>-6</sup>*</b>
Recreational potential	-0.44	0.142	-3.112	<b>0.0019*</b>
Wind speed	0.366	0.177	2.060	<b>0.0394*</b>
Model 3 (AIC= 79.8)	Estimate	Std. Error	z-value	p-value
(Intercept)	-3.199	1.008	-3.174	<b>0.0015*</b>
Beach width	-0.031	0.010	-3.059	<b>0.0022*</b>
Number of tire tracks	0.616	0.129	4.750	<b>2.04 x 10<sup>-6</sup>*</b>
Wind speed	0.235	0.167	1.409	0.1589
Model 4 (AIC= 81.1)	Estimate	Std. Error	z-value	p-value
(Intercept)	-6.958	1.429	-4.867	<b>1.13 x 10<sup>-6</sup>*</b>
Distance from coastal lagoons	0.000	0.000	1.356	0.1751
Distance from urban settlements	0.001	0.000	2.867	<b>0.0041*</b>
Number of tire tracks	0.613	0.135	4.550	<b>5.38x 10<sup>-6</sup>*</b>
Wind speed	0.421	0.174	2.419	<b>0.0155*</b>
Model 5 (AIC= 81.1)	Estimate	Std. Error	z-value	p-value
(Intercept)	-4.025	1.119	-3.595	<b>0.0003*</b>
Distance from coastal lagoons	0.001	0.000	2.097	<b>0.0360*</b>
Number of tire tracks	0.553	0.123	4.498	<b>6.86x 10<sup>-6</sup>*</b>
Urbanization index	-4.326	1.513	-2.859	<b>0.0042*</b>
Wind speed	0.286	0.169	1.694	0.0903

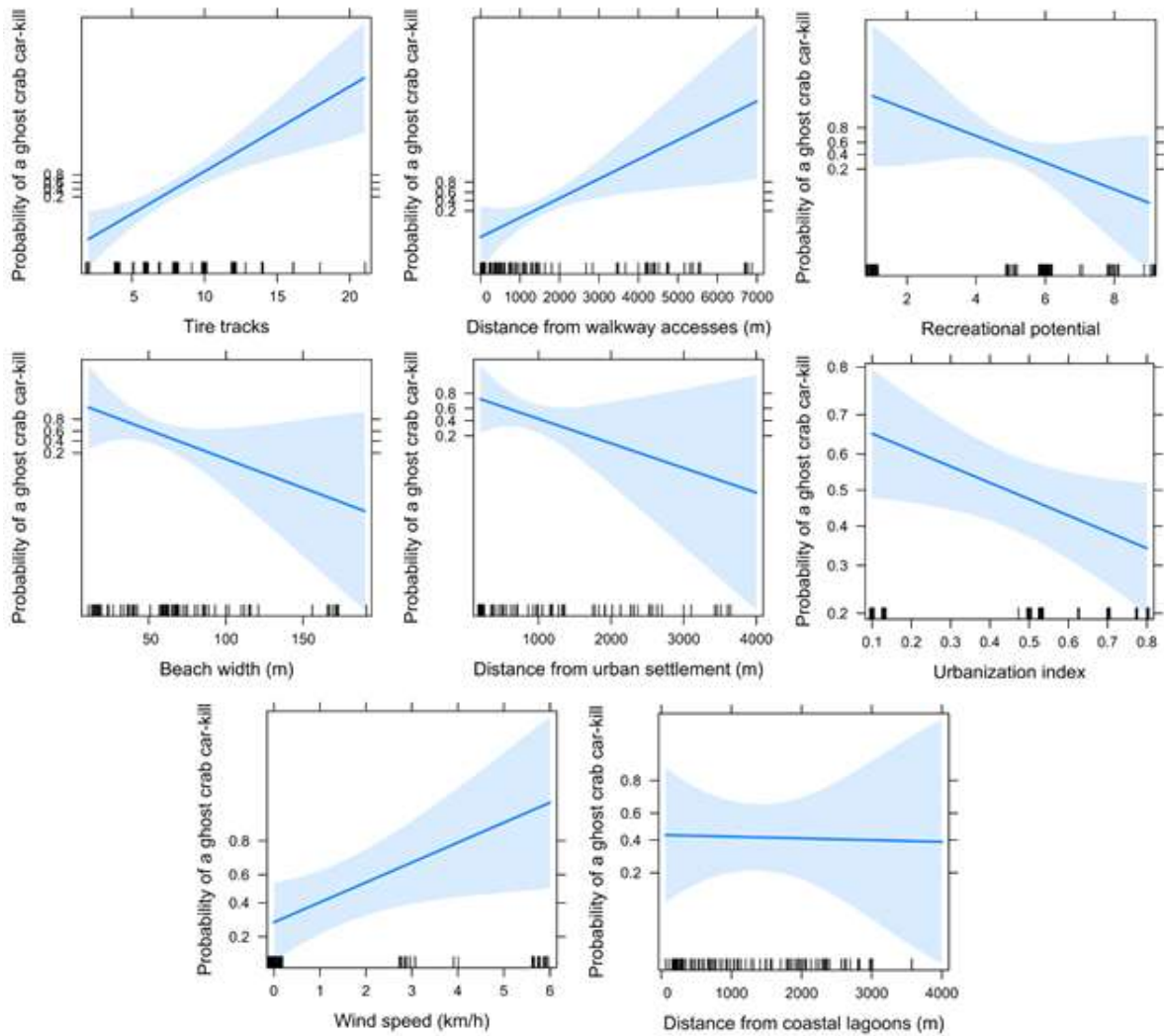


Figure 4. Probability of finding an Atlantic ghost crab (*Ocypode quadrata*) killed by a vehicle on the sand of the Grussaí Beach Arc related to the number of tyre tracks on sand (a proxy of vehicle traffic), distance from walkway accesses, recreational potential, beach width, distance from urban settlements and urbanization index. The shaded area indicates 95% the confidence interval.

### 3.3. Predictive variables on the dirt road

The first and second principal components of the PCA explained, respectively, 40% and 16% of the total variance in the dataset from the road (Fig. 3B). The urbanization index in this area was positively correlated with the beach width ( $R = 0.70$ ) and negatively correlated with the distance from urban settlements ( $R = -0.77$ ) (Fig. 3B). The number of vehicles parked at the end of the road was positively correlated

(not necessarily with a cause-effect relationship) with air temperature (warmest days with more vehicles) ( $R = 0.53$ ) and the number of nocturnal foraging crabs on the road ( $R = 0.59$ ) (Fig. 3B). Artificial light, distance from coastal lagoons and wind speed showed no marked correlations ( $R < |0.5|$ ) (Fig. 3B).

### 3.4. Ghost crabs killed by vehicles on the dirt road

A total of 20 ghost crabs killed by vehicles were found on the dirt road during 12 sampling campaigns (0.42 individuals/km/day of sampling), mainly during the low tourism season surveys (90%,  $n = 18$ ). The best binomial GLM (AIC = 53) showed that the number of ghost crabs killed by vehicles increased with decreasing beach width ( $p = 0.0158$ ;  $z$ -value = -2.41) (Tab. 2). Other candidate models sorted by AIC did not show a clear influence of other predictive variables (Tab. 2). The effect plots for the number of foraging crabs and the urbanization index were plotted due to the marginality of the  $p$ -values for these predictive variables (Tab. 2) and small number of samples ( $n = 20$ ). This result showed a tendency for an increasing number of crabs killed by vehicles with increasing numbers of foraging crabs at night and decreasing urbanization (Fig. 5).

Table 2. Generalized linear models (GLMs) with a binomial distribution. The vehicle-killed (1) and random (0) points on the dirt road crossing the beach vegetation represent the response variable. The best models were ranked by the Akaike information criterion (AIC). Significant predictors of the models are marked in bold.

<b>Best road models</b>				
Model 1 (AIC= 53.5)	Estimate	Std. Error	z-value	p-value
(Intercept)	3.365	1.601	2.096	<b>0.0361*</b>
Beach width	-0.08	0.03	-2.413	<b>0.0158*</b>
Distance from coastal lagoon	0.000	0.000	1.762	0.0781
Model 2 (AIC= 55.3)				
(Intercept)	0.115	0.951	0.121	0.904
Number of foraging crabs at night	0.113	0.075	1.497	0.135
Urbanization index	-2.437	1.683	-1.448	0.148
Model 3 (AIC= 55.5)				
(Intercept)	-0.968	0.602	-1.606	0.1083
Number of foraging crabs at night	0.137	0.071	1.917	0.0552
Model 4 (AIC= 55.5)				
(Intercept)	1.129	0.688	1.641	0.1007
Urbanization index	-3.051	1.628	-1.874	0.0609

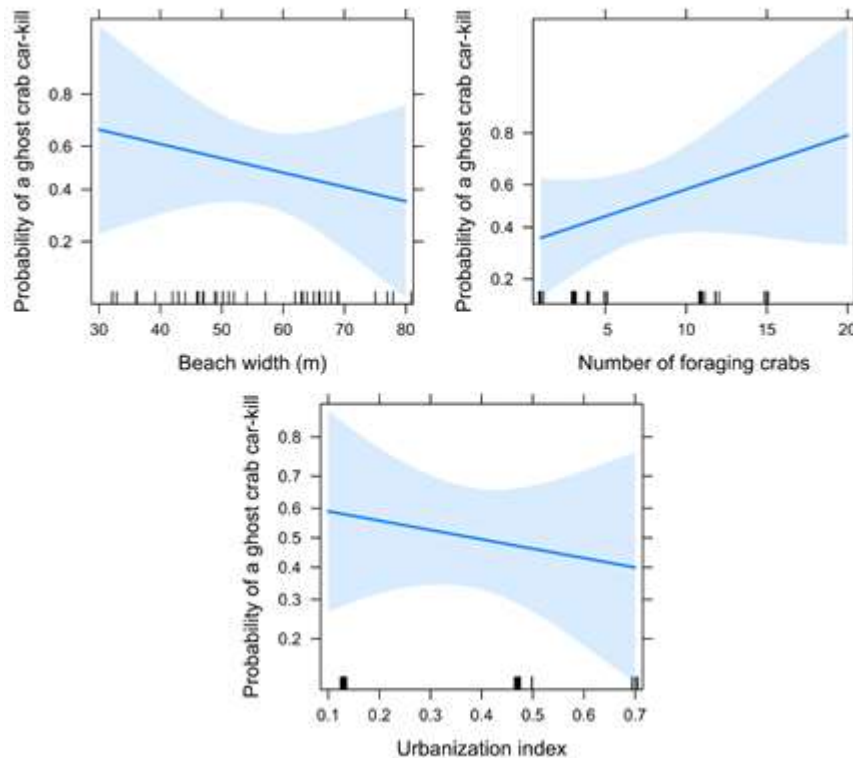


Figure 5. Probability of finding an Atlantic ghost crab (*Ocypode quadrata*) killed by a vehicle on the road crossing the beach vegetation on the Grussaí Beach Arc related to the beach width, number of foraging crabs at night and urbanization index. The shaded area indicates the 95% confidence interval.

#### 4. DISCUSSION

The null hypothesis that ghost crabs are randomly killed by vehicles on the beach sand was not accepted. Urbanization level (and the other correlated variables) and vehicle traffic intensity (tyre tracks as a proxy) were the main predictors of crabs killed by vehicles. Most of the crabs killed by vehicles were found on intermediate and non-urbanized beaches, where they are more abundant, active and consequently exposed on the sediment surface, compared to urbanized areas (Costa e Zalmon, 2019b). Low-urbanized beaches also have more dense vegetation (lower beach width) than urbanized areas, resulting in vehicles travelling mainly in the supralittoral fringe and high intertidal zones, where the ghost crabs are predominantly distributed on the GBA (Costa e Zalmon, 2019b). These results highlight the harmful and acute impact of vehicles on pristine beaches, which are usually motivated by angling activity and/or

the search for quiet and remote landscapes (Petch *et al.*, 2018), or even by environmental monitoring purposes in our study site (Costa e Zalmon, 2019b).

Most of the crabs killed by vehicles (76%) on the intermediate beaches occurred in a single survey during the high tourism season when there was an increased number of tyre tracks on the sand. This increase resulted from extensive vehicle traffic at night on the beach sand due to a traffic jam on the nearby paved road caused by an increased number of local tourists going to summer shows. Consequently, four-wheel vehicle (4x4) owners used the beach at night to more quickly arrive at these summer events. Schlacher *et al.* (2007) stressed that vehicle traffic at night is disastrous; a single vehicle can crush almost 1% of a ghost crab population in a single day, including shallowly buried individuals (< 30 cm). Intermediate beaches at the GBA have an estimated population of approximately six crabs per 100 m<sup>2</sup> (Costa e Zalmon, 2019b). Assuming that this area with high mortality stretches approximately 18,500 m<sup>2</sup> (theoretically ~1110 crabs), this single vehicle traffic event may have been responsible for the mortality of at least 2.3% of the ghost crab population (surface-active crabs).

It is possible that the number of ghost crabs killed by vehicles was underestimated in the present study because only crabs at the sediment surface were inspected instead of searching for dead crabs inside burrows (Schlacher *et al.*, 2007). This phenomenon occurred mainly in non-urbanized areas, where crab carcasses probably persist for less time than on urbanized beaches due to the increased abundance of scavengers at night, including the ghost crab itself. Nevertheless, more crabs killed by vehicles were actually found in the low-urbanized areas, indicating that some crabs are also crushed during daylight. Indeed, diurnal emersion from burrows (stimulated by a food resource) of the ghost crab is more frequent on intermediate (~41%) and non-urbanized (~37%) beaches than urbanized beaches (~29%) (Costa e Zalmon, 2019b).

Apart from the crabs killed by vehicles on the beach, the present study reported the incidence of ghost crabs killed by vehicles on a dirt road crossing the beach vegetation for the first time. This raises one more concern about beach conservation strategies “beyond the sand”. Ghost crabs have a wide home range (~300 m), mainly in non-disturbed areas, and can use beach dunes and vegetation, mainly during foraging periods (Lucrezi and Schlacher, 2014; Schlacher e Lucrezi, 2010). Worryingly, Brazilian sandy beaches are usually crossed by paved and non-paved roads (Amaral *et al.*, 2016), so it is likely that this anthropogenic source of mortality for ghost crab



populations is not restricted to a local case study and deserves further investigation. Most of the GBA has beach vegetation as the only natural barrier to hinder ghost crab migrations from the beach to the road because high dunes are naturally absent in the area where we searched for crushed crabs.

The low number of crabs killed by vehicles ( $n= 20$ ) made it difficult to find predictive variables to explain this mortality (which is also influenced by the smaller sampling area length  $\sim 4$  km). Nevertheless, some predictive models indicated the beach width, number of foraging crabs and urbanization index as possible predictors. With decreasing beach width (longer beach vegetation) and urbanization and increasing number of foraging crabs on the road at night, the number of crushed crabs was usually higher. These areas probably have the densest crab populations on the beach, including foraging crabs at night (Costa e Zalmon, 2019b).

Particularly during the low tourism season (winter and autumn), when we found 90% of the crabs killed by vehicles, more ghost crabs ( $n_{\text{total}}= 271$  crabs; 96%) were found foraging on the dirt road at night and were exposed to crushing by vehicles. The distribution of the crabs far from ( $\sim 200$  m) the beach crossing the dirt road during the winter and autumn seasons may be related to reproductive behaviour. For example, the crabs killed by vehicles that had their gender identified ( $n= 5$ ) were all females during this period. However, this zonation pattern needs further investigation because the spatial distribution of ghost crabs on sandy beaches can be influenced by multiple factors (e.g., extreme events, human disturbance and physical environment) (Gül e Griffen, 2018; Machado *et al.*, 2019).

Vehicle traffic is considered the main cause of the decline in ghost crab populations worldwide (Costa e Zalmon, 2019a; Schlacher *et al.*, 2016b). On the other hand, there are still scarce studies with novel approaches, including manipulative experiments (Schlacher *et al.* 2007), behavioural studies (Schlacher e Lucrezi, 2010) and assessments of direct impacts of vehicle traffic in natural contexts (as in the present study) that identify the mechanistic causes of the contrasts between low and high-disturbed areas (Schlacher *et al.*, 2016b). Our sampling design was adapted from typical "road ecology" studies that aim to evaluate the spatial-temporal patterns and factors influencing the mortality (road kills) of several animals (Rosa e Bager, 2012; Secco *et al.*, 2017). This approach was reliable for assessing the effects of vehicle traffic on beach biodiversity and identifying priority areas for coastal environments and ghost crab conservation.

At a local scale, the non-urbanized beaches on the GBA have dense populations of the Atlantic ghost crab ( $\sim 16$  crabs/100 m<sup>2</sup>) compared to urbanized beaches ( $\sim 1$  crabs/100 m<sup>2</sup>) (Suciu *et al.*, 2018; Costa e Zalmon, 2019b; Machado *et al.*, 2019;), even though we found more crabs killed by vehicles than on urbanized beaches. However, this relationship must be evaluated with caution. Vehicle traffic is more intense on urbanized beaches and probably already has chronic effects, so larval settlement and juvenile survival, as well as surface activity, become difficult in these disturbed areas (Costa e Zalmon, 2019b). With low abundance and low number of surface-active crabs, the probability of an individual being crushed by a vehicle at the sediment surface on urbanized beaches is low, but this does not mean that vehicle traffic does not have other indirect impacts (e.g., sediment compaction) that impair the species settlement and survival (Schlacher e Lucrezi, 2010).

At first, acute mortality on low-urbanized beaches does not seem to have major effects on the local ghost crab population size. However, we assume (based on previous surveys, Machado *et al.* 2017; Suciu *et al.* 2018) that the vehicle traffic intensity is low ( $\sim 1$  vehicle/day) on this beach stretch and usually restricted to anglers or employees of environmental monitoring programmes focused on turtle nests (Costa e Zalmon, 2019b). However, our data clearly show that if this traffic intensity (diurnal and nocturnal) increases (tyre tracks as a proxy), mortality may overcome the settlement rates and will have medium- to long-term chronic effects on the ghost crab population size in the GBA, as is the apparent scenario on most urbanized beaches.

In conclusion, vehicle traffic imposes mortality of the Atlantic ghost crab, even at low intensities. Acute impacts on the GBA were found mainly in low-urbanized areas, where the Atlantic ghost crab is still abundant and active at the surface. Based on our case study, vehicle traffic frequency must be reduced, mainly on low-urbanized beaches and during nocturnal periods, both in sand and on dirt roads crossing beach vegetation. Surveillance and punishment policies may be important mitigation actions to prevent vehicle traffic on the beach. Due to the difficulty in maintaining surveillance, natural obstructions (e.g., trunks) to reduce the average traffic speed could be an effective solution. On the dirt road, we suggest speed monitoring and signs indicating the presence of ghost crabs as initial management actions. Ultimately, the “road ecology” approach was reliable to identify the acute effects of vehicle traffic on the beach and adjacent areas, and it is recommended as a novel sampling design for future studies in coastal areas with similar characteristics.

## 2. DISCUSSÃO GERAL

As praias urbanizadas do Arco Praial de Grussaí possuem menores densidade e diâmetro de tocas em relação às praias menos perturbadas, seguindo o padrão global de indicação de impacto por caranguejos-fantasma (Schlacher *et al.*, 2016b). O tamanho corporal reduzido e a baixa densidade populacional nas áreas urbanizadas culminam no menor número de caranguejos forrageando no período noturno. Além da relação proporcional entre o tamanho da população e o número de indivíduos que emergem das suas tocas para se alimentar à noite, sabe-se que o caranguejo-fantasma do Atlântico tende a ter atividade noturna mais proeminente entre os adultos. Embora variações regionais ocorram em razão principalmente da temperatura (Pombo *et al.*, 2017a), os juvenis tendem a ter atividade diurna para evitar canibalismo (Lucrezi e Schlacher, 2014). Portanto, o menor número de caranguejos forrageando no período noturno sugere que as praias urbanizadas do Arco Praial de Grussaí, de fato, abrigam populações pouco abundantes e dominadas por juvenis (ver capítulo I). Assim, o menor número de tocas nas praias urbanas não reflete apenas mudanças no comportamento de construção de tocas. Da mesma forma, o menor diâmetro médio das tocas não resulta da construção de tocas menores pelos caranguejos, como estratégia para economizar energia diante da perturbação, como sugerido por Gül e Griffen, (2018).

O número de caranguejos forrageando à noite respondeu mais previsivelmente ao nível de urbanização do que as métricas baseadas em tocas (ver capítulo I). Isso confirma a primeira hipótese do presente estudo, de que a densidade e o diâmetro das tocas não são métricas tão eficientes comparados ao número de indivíduos da espécie forrageando à noite, como indicadores de urbanização. Por outro lado, o censo visual pode subestimar o tamanho das populações de caranguejos-fantasma, uma vez que os indivíduos podem se manter dormentes em suas tocas em períodos reprodutivos e/ou sob temperaturas estressantes (Schlacher e Lucrezi, 2010; Yong e Lim, 2019). Portanto, o censo visual do caranguejo-fantasma do Atlântico é eficiente como indicador de impacto (Wolcott e Wolcott, 1984), mas também deve ser utilizado com cautela em estimativas do tamanho das populações, assim como a contagem de tocas (Yong e Lim, 2019).

O pisoteio isolado não teve efeito letal para o caranguejo-fantasma do Atlântico, similar aos resultados de outros autores (Lucrezi *et al.*, 2009a). O presente estudo

confirmou a hipótese de que o pisoteio experimental exerce efeitos negativos de curto-prazo na densidade de tocas, mas os efeitos negativos não se prolongaram por mais de 24 horas, indicando baixa ou nenhuma letalidade induzida pelo pisoteio agudo (Lucrezi *et al.*, 2009a). Os indivíduos evitam sair das tocas no período diurno devido ao pisoteio e possivelmente têm sua capacidade de perceber predadores reduzida em razão da irregularidade do sedimento (micro-relevo causado pelas pegadas) (ver capítulo II). Entretanto, é improvável que tais mudanças comportamentais sejam suficientes para manter o tamanho da população nas praias urbanas do Arco Praial de Grussaí cinco vezes menor do que nas áreas pouco visitadas. A presença de visitantes é reportada, inclusive, por favorecer caranguejos-fantasma pelos restos de alimento deixados na areia da praia (Steiner e Leatherman, 1981; Strachan *et al.*, 1999; Blankensteyn, 2006; Schlacher *et al.*, 2011). Além disso, as praias da região recebem forte aglomeração de pessoas (~200 pessoas/100 m<sup>-1</sup>) exclusivamente no verão (janeiro a março), enquanto a espécie possivelmente se reproduz durante todo o ano na costa brasileira (Alberto e Fontoura, 1999; Blankensteyn, 2006; Oliveira *et al.*, 2016). Portanto, o pisoteio sazonal no Arco Praial de Grussaí, embora tenha efeitos negativos para outras espécies de invertebrados sedentários na região (Machado *et al.*, 2017), possivelmente não tem efeitos isolados de curto-prazo para o caranguejo-fantasma do Atlântico, que possui mobilidade para escapar dos visitantes da praia e se abrigar em suas tocas (Lucrezi *et al.*, 2009a).

O Arco Praial de Grussaí recebe elevada quantidade de lixo (até 4 itens/m<sup>2</sup>, > 80% plástico) principalmente de fonte recreativa nas áreas urbanizadas (Suciu *et al.*, 2017). No mesmo arco praial, o caranguejo-fantasma do Atlântico carrega itens plásticos para a entrada das suas tocas, ou constroem tocas ao redor de tais itens, por motivações ainda pouco discutidas (p. ex. memorização espacial e estabilidade sedimentar) (Costa *et al.*, 2018b). Um dos mecanismos possíveis é a interação por confusão com lixo motivada por estímulos químicos (lixo com odor de potenciais fontes alimentares) (Costa *et al.*, 2019). O presente estudo reportou ingestão principalmente de microplásticos, como microfibras (~ 93%), enquanto poucos indivíduos ingeriram itens plásticos maiores que 1 mm (~ 3%) (ver capítulo III). Portanto, assume-se que a ingestão de microplásticos ocorre acidentalmente, durante a ingestão de sedimentos, macroalgas, carcaças de animais e presas. Devido à elevada plasticidade alimentar (Tewfik *et al.*, 2016; Yong e Lim, 2019) e, portanto, múltiplas fontes de contaminação possíveis, a espécie possivelmente não é um

indicador quantitativo (biomonitor) da poluição de praias por microplásticos (ver capítulo III). Assim, a terceira hipótese de que a ingestão de microplásticos é mais frequente em áreas onde os microplásticos são mais abundantes no sedimento não foi aceita.

Estudos recentes demonstram que a ingestão de microplásticos por invertebrados aquáticos tem efeitos negativos na ingestão de calorias (em razão da falsa sensação de saciedade ou obstrução do trato digestivo), crescimento, reprodução e mobilidade (revisado por Foley *et al.*, 2018). Por outro lado, existem poucas evidências de que a ingestão de microplásticos *per se* tem efeitos imediatos para a sobrevivência em contexto natural e, conseqüentemente promove redução do tamanho das populações (Foley *et al.*, 2018). O presente estudo não avaliou os efeitos da ingestão de microplásticos pelo caranguejo-fantasma do Atlântico em nível de sobrevivência dos indivíduos e tamanho da população. Entretanto, não se encontrou relação entre a presença ou abundância de microplásticos com o peso e tamanho corporal da espécie, assim como a frequência de indivíduos que ingeriram pelo menos um item plástico não foi relacionado ao nível de urbanização (ver capítulo III). Esses resultados sugerem que a ingestão de microplásticos não é letal e responsável pela redução do tamanho populacional e corporal do caranguejo-fantasma do Atlântico no Arco Praial de Grussaí. Entretanto, ressalta-se o potencial de transferência trófica de microplásticos a partir da ingestão por caranguejos-fantasma (para predadores como aves e gambás) e do desconhecimento acerca de efeitos subletais, temas que devem ser priorizados em estudos futuros e divulgados em programas de redução da emissão e descarte inadequado de plásticos (Farrell e Nelson, 2013; Foley *et al.*, 2018).

Diferente do pisoteio e ingestão de microplásticos, que parecem não ter efeitos letais agudos e de curto prazo na densidade populacional e tamanho corporal do caranguejo-fantasma do Atlântico em praias urbanizadas, o tráfego de veículos é letal (Schlacher *et al.*, 2007). Mesmos os indivíduos abrigados no interior das suas tocas podem ser esmagados por veículos, principalmente aqueles abrigados em tocas rasas (< 25 cm) (Schlacher *et al.*, 2007). Verificou-se que a mortalidade do caranguejo-fantasma do Atlântico na superfície do sedimento foi relacionada ao aumento do tráfego na areia da praia e foi mais frequente em áreas pouco urbanizadas, confirmando a quarta hipótese do presente estudo.

Os adultos da população do caranguejo-fantasma do Atlântico têm atividade predominantemente noturna no Arco Praial de Grussaí. Entretanto, não é impossível que algumas carcaças presentes na superfície do sedimento sejam resultados de atropelamentos diurnos, já que é comum em áreas pouco visitadas, a emersão de indivíduos adultos em busca de alimento e/ou para umedecer suas brânquias, comportamento importante para a respiração da espécie (Lucrezi e Schlacher, 2014; Pombo *et al.*, 2017a). Além disso, veículos são utilizados por funcionários do Porto do Açú para monitoramento de ninhos de tartarugas marinhas durante o dia e é uma possível fonte de mortalidade do caranguejo-fantasma do Atlântico nas praias pouco urbanizadas da região.

A ausência de juvenis (atividade diurna) atropelados pode indicar menor vulnerabilidade ao atropelamento, por serem mais ágeis que os adultos (Gül e Griffen, 2018) ou uma limitação da metodologia de busca por carcaças para prever áreas mais sensíveis ao atropelamento, método adaptado dos estudos sobre atropelamento de vertebrados em rodovias (Clevenger *et al.*, 2003). Entretanto, esse fato pode ser também uma evidência de que os atropelamentos ocorrem principalmente à noite, como relatado por outros autores (Schlacher *et al.*, 2007; Gül e Griffen, 2018). Um evento de mortalidade em massa, com estimativa de perda de cerca de 2% da população em apenas um dia, por exemplo, resultou do tráfego noturno na areia da praia de Grussaí; motoristas utilizaram a praia para evitar congestionamentos da estrada de acesso ao Balneário de Atafona, onde ocorreram shows durante o verão de 2018 (ver capítulo IV). Durante a noite, carcaças são encontradas também na estrada de chão que corta a restinga das praias menos visitadas do Arco Praial de Grussaí. Alguns indivíduos forrageiam nessa região durante a noite e estão expostos ao atropelamento. A fiscalização do tráfego de veículos no Arco Praial de Grussaí, entretanto ocorre somente durante o dia, quase que exclusivamente na alta temporada turística e na região próximo ao polo gastronômico, para proteger visitantes da praia dos riscos de acidente (observação pessoal). Isso evidencia que a gestão de praias do município de São João da Barra tem como único foco a economia do turismo, como tem sido relatado em praias de todo o mundo (revisado por Schlacher *et al.*, 2008).

O pisoteio, a poluição por plásticos e o tráfego de veículos não são os únicos impactos antropogênicos para a fauna de praias arenosas em nível global. Particularmente para caranguejos-fantasma, reporta-se também como fontes de

perturbação em praias urbanizadas: (i) a limpeza de praia; (ii) redução da disponibilidade de presas; (iii) supressão da vegetação, (iv) eventos climáticos extremos (ressacas), (v) obras de engenharia; e (vi) captura recreativa. A limpeza de praias pode remover detritos (algas e carcaças) e impactar negativamente presas que são importantes recursos tróficos para caranguejos-fantasma (Oliveira *et al.*, 2016; Stelling-wood *et al.*, 2016). A redução na disponibilidade de presas pode também limitar o crescimento dos indivíduos e contribuir para o menor tamanho corporal em praias urbanizadas (Gül e Griffen, 2018). A vegetação de restinga fornece áreas sombreadas que amenizam a severidade física das praias (insolação, baixa umidade do sedimento), e favorecem a camuflagem e proteção dos caranguejos-fantasma contra predadores (Schlacher *et al.*, 2011). A vegetação pode ainda, ser refúgio dos caranguejos-fantasma durante eventos de ressaca e a sua supressão para construção de estruturas de contenção (enrocamentos) diminuem a resiliência das populações em áreas urbanizadas (Lucrezi *et al.*, 2010; Machado *et al.*, 2019). A captura recreativa de caranguejos-fantasma contribui para a redução do tamanho das populações, particularmente de adultos que são mais facilmente encontrados do que os juvenis (Gül e Griffen, 2018).

Os impactos supracitados são importantes em escala regional e global, mas não explicam a redução na densidade e tamanho corporal do caranguejo-fantasma do Atlântico em escala local. No Arco Praial de Grussaí, as praias urbanizadas recebem limpeza da praia uma vez por dia, entretanto a limpeza é manual e seletiva, o que favorece a manutenção de recursos alimentares para caranguejo-fantasma (Suciu *et al.*, 2017). Da mesma forma, as principais presas do caranguejo-fantasma do Atlântico, como tatuís (*Emerita brasiliensis*), sernambis (*Donax hanleyanus*) e pulgas-da-praia (*Atlantorchestoidea brasiliensis*) e insetos (Branco *et al.*, 2010; Tewfik *et al.*, 2016) persistem nas áreas urbanizadas do Arco Praial de Grussaí (Costa e Zalmon, 2017; Machado *et al.*, 2017). Obras de enrocamento não foram realizadas nas praias estudadas e, embora a vegetação seja menos densa e com a presença de espécies exóticas nas áreas urbanas, a extensa faixa de areia do pós-praia e presença de passarelas de acesso, podem servir como abrigo para o caranguejo-fantasma, além de minimizar possíveis impactos da luz artificial na orla (Costa *et al.*, 2017). A captura de adultos de caranguejos-fantasma não tem sido reportada no Brasil e, possivelmente não contribui para a redução da densidade populacional e tamanho

corporal nas praias da região, como observado na Carolina do Sul, EUA (Gül e Griffen, 2018).

Haja vista que o pisoteio e a poluição por microplásticos possivelmente não tem efeitos letais comprovados, que outros impactos são ausentes ou de baixa intensidade no Arco Praial de Grussaí e que os carros na areia impõem elevada letalidade para a população do caranguejo-fantasma do Atlântico, o controle do tráfego de veículos (diurno e noturno) deve ser priorizado como estratégia de manejo para a conservação da biodiversidade das praias da região. Os indivíduos do caranguejo-fantasma do Atlântico possivelmente não atingem a maturidade sexual, devido à letalidade de recém-recrutas e juvenis e os adultos não encontram facilmente pares reprodutivos em razão da baixa densidade populacional (efeito de Allee, ver Courchamp *et al.* 1999) nas praias urbanizadas. Assim, assume-se que tocas ainda são encontrados nas áreas perturbadas graças a dispersão das larvas oceânicas, com a produção de prole em áreas adjacentes pouco perturbadas, que devem ser restritas ao turismo como zonas de conservação. O tráfego de veículos precisa ser controlado, portanto, ao longo de todas as praias, mas para a conservação da biodiversidade, a fiscalização deve priorizar as praias pouco urbanizadas, onde as populações do caranguejo-fantasma e de outras espécies são abundantes (Costa e Zalmon, 2019b).

A gestão de praias arenosas abrange inúmeros requisitos legais; compete aos órgãos públicos (federais, estaduais e municipais) a implementação de medidas que garantam a qualidade ambiental (Constituição Federal de 1988, no § 4º do seu artigo 225). Recentemente, estabeleceu-se a transferência de gestão de praias urbanas e rurais (TAGP) da União para os municípios (Portaria SPU nº 113/2017, atualizado pela portaria 44/2019). A adesão da gestão de praias não é obrigatória, mas ao assinar o TAGP os municípios garantem autonomia legal para realização de eventos culturais com fins lucrativos (isento de taxas para o governo federal), do ordenamento das praias (p.ex. instalação de barracas de praia, quiosques, vendedores ambulantes) e passam a ter mais responsabilidade na manutenção da qualidade socioambiental (p. ex. contenção de erosão, proteção da biodiversidade e gestão de lixo). Entretanto, o município de São João da Barra, que abrange as praias do distrito de Grussaí, não aderiu até o momento o TAGP, o que pode ser empecilho para implementar medidas de manejo para a conservação na região, incluindo aquelas sugeridas no presente estudo.



Enquanto os órgãos públicos são responsáveis pela implementação de medidas que garantam a sustentabilidade ambiental e econômica do uso das praias e mitiguem os impactos, a Universidade tem o papel de gerar informações científicas que aumentem as chances de sucesso de tais medidas. O engajamento de tomadores de decisão envolvidos na gestão costeira e cientistas, considerada falha em praticamente todo o mundo, é fundamental para implementação de projetos efetivos de conservação (Thomas A Schlacher *et al.*, 2008). Entretanto, existem outras razões, além da falta de comunicação entre ecólogos e gestores, do uso desordenado das praias, como segue: (i) praias arenosas são vistas como ecossistemas unicamente recreativos; (ii) lacuna de conhecimento científico na área de Biologia da Conservação em praias; (iii) falta de padronização de métodos de amostragem da biodiversidade. Por conseguinte, o manejo de praias tem priorizado turismo e erosão costeira (Thomas A Schlacher *et al.*, 2008).

Frente à escassez de recursos financeiros e necessidade por decisões rápidas, recomenda-se o manejo das praias baseado no uso de poucas espécies, visando diagnósticos amplos (atalhos de conservação) e ampla divulgação das praias como sistemas naturais de alto valor ecológico (Caro, 2010; Harris *et al.*, 2014). Os principais atalhos de conservação são (i) espécies indicadoras: utilizadas para avaliação e monitoramento de impactos humanos; (ii) espécies guarda-chuva: espécies com requerimentos de habitat comuns a espécies coexistentes (o manejo de uma espécie guarda-chuva tem potencial de promover conservação em ampla escala); e (iii) espécies-bandeira: espécies com elevado potencial carismático, importantes para a divulgação da ciência e de projetos de conservação em uma linguagem acessível à população (p.ex. redes sociais) e para obtenção de patrocínios. Até o presente, sabe-se somente do potencial indicador dos caranguejos-fantasma. Assim, estudos futuros na costa brasileira devem priorizar o diagnóstico do caranguejo-fantasma do Atlântico ou de outras espécies de fácil amostragem como atalhos de conservação de praias arenosas.

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#### 4. SUGESTÕES DE ESTUDOS FUTUROS

- Identificar a influência de fatores ecológicos dependentes e independentes de densidade na taxa de ocupação de tocas, para propor fatores de conversão e determinar o tamanho das populações;
- Propor métodos simples para determinar taxa de ocupação de tocas;
- Testar a suitability do caranguejo-fantasma como espécie guarda-chuva e espécie-bandeira em projetos de conservação;
- Descrever as consequências subletal e letal da ingestão de plásticos pelo caranguejo-fantasma;

- Identificar variáveis preditivas espaciais e temporais do atropelamento do caranguejo-fantasma em praias arenosas e estradas adjacentes à vegetação e dunas;
- Identificar os mecanismos pré e pós-recrutamento que resultam em menor tamanho populacional do caranguejo-fantasma em praias urbanizadas comparado às praias pristinas;
- Descrever os padrões demográficos das populações do caranguejo-fantasma em praias arenosas com diferentes níveis de urbanização, incluindo atributos da história de vida (fecundidade e sobrevivência em diferentes classes etárias);
- Determinar a importância do caranguejo-fantasma em redes tróficas de diferentes tipos de *habitat* adjacentes às praias arenosas (infralitoral, mediolitoral, supralitoral e dunas/restingas), utilizando análises isotópicas e inspeção do trato digestivo;
- Descrever a dinâmica da população do caranguejo-fantasma antes e após a transposição de indivíduos de praias não-urbanizadas para praias urbanizadas, utilizando o desenho BACI (*before-after-control-impact*).

## 5. APÊNDICES

Apêndice 1. Lista dos coautores dos artigos científicos/manuscritos que compõem a tese de doutorado, com suas respectivas contribuições.

<b>Co-autor</b>	<b>Título</b>	<b>Local</b>	<b>Capítulo</b>	<b>Contribuições</b>
Ana Paula Madeira Di Beneditto	Doutora	UENF	3	Interpretação dos resultados e revisão do manuscrito
Helio Secco	Doutorando	UFRJ/NUPEM	4	Delineamento experimental e revisão do manuscrito
Julyana Figueiredo Madureira	Graduanda	UENF	2	Delineamento experimental, interpretação dos resultados e revisão do manuscrito
Mônica Ferreira da Costa	Doutora	UFPE	3	Delineamento experimental, interpretação dos resultados e revisão do manuscrito
Vitor Figueira Arueira	Graduando	UENF	3 e 4	Delineamento experimental, interpretação dos resultados e revisão do manuscrito
Ilana Rosental Zalmon	Doutora	UENF	1, 2, 3 e 4	Orientação do doutorado, delineamento experimental, interpretação dos resultados e revisão dos manuscritos