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AVALIAÇÃO DAS ALTERAÇÕES MORFOLÓGICAS E EFEITOS GENOTÓXICOS EM
CRUSTÁCEOS DA INFRAORDEM BRACHYURA EXPOSTOS A SEDIMENTOS
CONTAMINADOS POR HIDROCARBONETOS POLICÍCLICOS AROMÁTICOS,
ORGANOCLOREDOS E METAIS TRAÇO EM AMBIENTES RECIFAIS

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RECIFAIS**

Tese apresentada ao Programa de Pós-Graduação
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Área de concentração: Oceanografia Biológica.

Orientador: Prof. Dr. Jessor Fidelis de Souza Filho

Coorientadoras: Prof.^a Dr.^a Eliete Zanardi Lamardo

Prof.^a Dr.^a Tereza Cristina dos Santos Calado

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RESUMO

Os impactos antrópicos podem afetar a saúde dos organismos marinhos, o que ressalta a importância de estudos de monitoramento das espécies e do ambiente. O presente estudo teve como objetivo realizar uma análise comparativa da contaminação do sedimento por hidrocarbonetos policíclicos aromáticos (HPAs), compostos organoclorados (OCPs) e metais, e os efeitos destes compostos na saúde de braquiúros (*Pachygrapsus transversus* e *Eriphia gonagra*) em quatro ambientes recifais de Pernambuco e Alagoas (Pernambuco: Gaibu e Praia dos Carneiros; Alagoas: Ponta Verde e Pontal do Coruripe). Foram considerados as potenciais fontes de estresse ambiental das áreas, como lançamento de esgoto, turismo intenso ou proximidade de rios que cortam regiões com usinas de cana-de-açúcar. Foram realizadas coletas trimestrais, entre fevereiro/2019 e fevereiro/2020. Os indivíduos foram mensurados e as células da hemolinfa foram analisadas quanto a presença de células micronucleadas. Foi realizada a extração e análise de HPAs, OCs e metais das amostras de sedimento. Durante o estudo, a partir do fim do mês de agosto de 2019, foram observadas grandes manchas de óleo em praias e estuários do nordeste e parte do sudeste brasileiro. Assim, a pesquisa apresenta dados anteriores a este evento, na sequência e seis meses após a chegada do óleo na costa. Uma das áreas estudadas (Ponta Verde) não foi impactada por este evento, sendo utilizada como referencial de ambiente não exposto ao óleo em estudo comparativo. Os resultados mostraram alterações populacionais relacionadas a razão sexual da espécie *P. transversus* nas áreas atingidas pelo óleo e no tamanho dos espécimes nas áreas com impactos crônicos de pisoteio (Ponta Verde). Para *E. gonagra*, por outro lado, foram encontrados espécimes oleados e com alterações morfológicas após o derramamento de óleo. As frequências de células micronucleadas de ambas as espécies aumentaram significativamente em todas as áreas afetadas, imediatamente após o desastre. Os dados de contaminação de HPA e OCs estão fortemente relacionados com as alterações genotóxicas que, por sua vez, também estão relacionadas às alterações morfológicas. Os resultados apontam que a presença massiva do óleo, e os impactos crônicos, como aporte de pesticidas organoclorados e metais através de rios, podem causar alterações às espécies que habitam nestes ambientes. Em longo prazo, isso pode se traduzir em mudanças estruturais significativas nas comunidades de caranguejos, eventualmente afetando toda a teia trófica do ecossistema recifal.

Palavras-chave: caranguejo, teste de micronúcleo, estrutura populacional, derramamento de óleo, ambientes costeiros.

ABSTRACT

Anthropogenic impacts can affect marine organisms' health, highlighting the importance of monitoring studies of the species and the environment. The present study aimed to perform a comparative analysis of sediment contamination by polycyclic aromatic hydrocarbons (PAHs), Organochlorine compounds (OCPs), and metals (MTs), and the effects of these compounds on brachyuran (*Pachygrapsus transversus* and *Eriphia gonagra*) in reefs environments from Pernambuco and Alagoas (Pernambuco: Gaibu and Carneiros Beach; Alagoas: Ponta Verde and Pontal do Coruripe). Potential sources of environmental stress of the areas were considered such, as sewage discharge, intense tourism, or proximity of rivers that pass-through regions with sugar cane mills. Quarterly collections were performed between February 2019 and February 2020. The individuals were measured and the hemolymph cells were analyzed for the presence of micronucleated cells, which are indicators of potential genotoxicity. PAHs and OCs and metals were extracted and quantified from the sediment samples. During this study, as of the end of August 2019, slicks of oil reached several beaches and estuaries in the northeast and part of southeastern Brazil. Therefore, this research presents data prior to the oil spill, right after, and six months after this event. One of the studied areas (Ponta Verde) was not impacted by the oil and served for comparative analyses. Results showed population changes related the sex ratio of the species *Pachygrapsus transversus* in areas affected by oil and in the size of specimens in areas with chronic trampling impacts (Ponta Verde). For *Eriphia gonagra*, on the other hand, oiled specimens and specimens with morphological changes were found after the oil spill. The frequencies of micronucleated cells of both species increased significantly in all affected areas. PAH and OC contamination data are strongly related to these genotoxic and morphological alterations. The results point out that the massive presence of oil, and chronic impacts, such as inputs of organochlorine pesticides and metals through rivers, may cause alterations to the species inhabiting these environments. In the long term, this may translate into significant structural changes in crab communities, eventually affecting the entire trophic web of the reef ecosystem.

Keywords: crabs, micronucleus test, population structure, oil spill, coastal environments

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LISTA DE ABREVIATURAS E SIGLAS

Ace	Acenaphthene
Acy	Acenaphtylene
AL	Alagoas
ANOVA	Análise de Variância
Ant	Anthracene
APACC	Área de Proteção Ambiental Costa dos Corais
As	Arsênio
Aug	August
AW	Abdomen Width
BaA	Benzo[a]anthracene
BaP	Benzo[a]pyrene
BbF	Benzo[b]fluoranthene
BghiP	Benzo[ghi]perylene
BkF	Benzo[k]fluoranthene
BOS	Before Oil Spill
CA	Carneiros
CAPES	Conselho Nacional de Desenvolvimento Científico
Chr	Chrysene
CL	Carapace Length
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
CPUE	Catch per unit effort
Cr	Cromo
Cu	Cobre
CV	Coeficiente of variation
CW	Carapace Width
d.w.	Dry weight
DahA	Dibenzo[a,h]anthracene
DBOFB	Dibromooctafluorobiphenyl
DDD	Dicloro-difenil-dicloroetano
DDE	Dicloro-difenil-dicloroeteno

DDT	Dicloro-difenil-tricloroetano
DNA	Ácido Desoxirribonucleico
ENOR	Estaleiro do Nordeste
ERL	Effects range low
ERM	Effects range median
F	Flúor
FACEPE	Fundação de Amparo à Ciência e Tecnologia de Pernambuco
Fe	Ferro
Feb	February
Fl	Fluorene
Flu	Fluoranthene
FS	Frequency of spots
g	Grama
GB	Gaibu
GC-MS	Gas chromatograph coupled to a mass spectrometer
GL	Gonopod Length
HCH	Hexachlorocyclohexan
HCl	Ácido clorídrico
HClO ₄	Ácido perclórico
HF	Ácido fluorídrico
HNO ₃	Ácido nítrico
HPA	Hidrocarboneto Policíclico Aromático
ICP-MS	Inductively coupled plasma mass spectrometry
IP	Indeno[1,2,3-cd]pyrene
IPEA	Instituto de Pesquisa Econômica Aplicada
LOQ	Limit of quantification
M	Machos
Max	Máximo
Mg	Magnésio
Min	Mínimo
Mn	Manganês

M-Nap	Methylnaphthalene
MNF	Micronucleus Frequency
MT	Metais Traço
N	Nitrogênio
n.d.	Not detected
Nap	Naphthalene
ng	Nanogram
Ni	Níquel
NOAA	National Oceanic and Atmospheric Administration
Nov	November
OCP	Organochlorine Pesticides
OM	Organic Matter
PAH	Polycyclic Aromatic Hydrocarbons
Pb	Chumbo
PC	Pontal do Coruripe
PCA	Principal Component Analysis
PE	Pernambuco
PEL	Probable effect level
PELD TAMS	Programa Ecológico de Longa Duração – Tamandaré Sustentável
Phe	Phenanthrene
POC	Pesticida organoclorado
POP	Pesticidas Orgânicos Persistentes
PV	Ponta Verde
Pyr	Pyrene
RAOS	Right after oil spill
REC	Recovery
SD	Standard deviation
SRM	Standard reference material
St	Stations
TCMX	tetrachloro-m-xylene
TEL	Threshold effect level

TM	Trace Metals
Tons	Toneladas
Zn	Zinco

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1 INTRODUÇÃO

1.1 Estrutura da tese

A presente tese realiza um estudo analítico sobre os efeitos da poluição marinha sobre os caranguejos. Com esta finalidade foi feita uma Introdução Geral, falando sobre conceitos básicos de temas chaves dos capítulos seguintes. Também serão apresentados os tópicos 2 e 3 que apresentam hipótese/objetivos e descrição das áreas de estudo, respectivamente. Os resultados da tese, presentes no tópico 4, 5 e 6, são compostos por três artigos:

Artigo 1 (Tópico 4): **“Reef crab population changes after oil spill disaster reach Brazilian tropical environments”** – Este artigo descreve a estrutura da população de *Pachygrapsus transversus* em quatro áreas recifais comparando a proporção sexual, tamanho de maturidade, média de tamanho da população, além de observar potenciais efeitos da presença do óleo na população (principalmente efeitos físicos, de oclusão de tocas ou morte por asfixia de indivíduos) e de outros impactos antrópicos. Este artigo está publicado no periódico **Marine Pollution Bulletin** (<http://doi.org/10.1016/j.marpolbul.2022.114047>) e formatado de acordo com as normas do mesmo.

Artigo 2 (Tópico 5): **“Population structure of *Eriphia gonagra* (Fabricius, 1780) in sandstone reefs of northeastern Brazil with different degrees of impact”** – Este manuscrito descreve a estrutura das populações de *E. gonagra* em quatro áreas recifais comparando a proporção sexual, tamanho de maturidade e média de tamanho da população, estabelecendo correlações com os impactos antrópicos aos quais essas áreas são submetidas. Este manuscrito está formatado de acordo com as normas do periódico **Ocean and Coastal Research**.

Artigo 3 (Tópico 6): **“Genotoxic and morphological damages in two species of reef crabs exposed to sediments contaminated with PAHs, organochlorines and trace metals”** – Este manuscrito analisa efeitos genotóxicos da contaminação ambiental em caranguejos das espécies *Eriphia gonagra* e *Pachygrapsus transversus* coletados em quatro ambientes recifais, contabilizando as células com dano genômico expresso em células micronucleadas. O manuscrito ainda leva em consideração o derramamento de óleo ocorrido em praias do Nordeste do Brasil em 2019. Ele está formatado de acordo com as normas do periódico **Science of the Total Environment**.

As referências de cada artigo estão ao final deles. Por fim, o tópico 7 apresenta as considerações finais deste estudo, seguido pelas referências da parte introdutória e descrição da área de estudo.

1.2 Aspectos gerais

Com o crescimento populacional humano, o aumento desordenado na produção de mundial de resíduos sólidos vem se tornando um problema cada dia mais grave. Além disso, as condições precárias de saneamento básico de muitos países e no tratamento de resíduos resultam no despejo de efluentes nos corpos hídricos, que chegam consequentemente aos oceanos (D'AGOSTINHO; FLUES, 2006; LANDRIGAN et al., 2020). A poluição das águas tem fontes domésticas, comerciais e industriais e pode causar inúmeros danos à fauna e flora dos ecossistemas aquáticos. Esses resíduos antrópicos trazem consigo contaminantes, que podem ser biodegradáveis e serem transformados e/ou decompostos pela ação de bactérias ou fatores químicos, ou, quando persistentes, mantem-se por longo tempo no sedimento e podem bioacumular e, em alguns casos, biomagnificar através dos níveis tróficos (MORAES; JORDÃO, 2002).

Entre os poluentes mais abundantes dos corpos hídricos, podem-se destacar três grupos representativos: os hidrocarbonetos policíclicos aromáticos (HPAs), os compostos organoclorados e os metais traço. Estes três grupos de poluentes são persistentes e possuem como característica geral a resistência à degradação, bioacumulação e alta toxicidade (LEBLANC, 1997, NEWMAN, 2009; AMIARD-TRIQUET et al., 2012). Esses compostos, por serem altamente tóxicos para organismos aquáticos, podem gerar diversos efeitos adversos em vários sistemas fisiológicos e mortalidade de organismos mais susceptíveis (BAPTISTA-NETO et al., 2008; LANDIS et al., 2017).

Os HPAs representam uma família de compostos orgânicos, constituídos de carbono e hidrogênio, contendo dois ou mais anéis aromáticos condensados. São formados principalmente em processos de combustão incompleta de matéria orgânica e encontram-se na natureza como contaminantes de solo, ar, água e alimentos (BÍCEGO et al., 2008; CARUSO; ALABURDA, 2008; ABDEL-SHAIFY et al., 2016). As emissões desses compostos podem ser de fontes naturais, como vulcões e queimadas espontâneas, ou antrópicas, destacando-se derrames de petróleo, a queima de carvão, a fumaça de cigarros e de escapamento de veículos e podem alcançar os ecossistemas aquáticos através da interação oceano-atmosfera ou do descarte de efluentes domésticos e industriais (LAW; BISCAYA, 1994; ABDEL-SHAIFY; MANSOUR,

2016; CABRAL, 2017). Os HPAs compõem menos de 15% do petróleo bruto, e possuem grande potencial carcinogênico e mutagênico, o que levou a Agência de Proteção Ambiental dos EUA (USEPA) a incluir 16 HPAs na lista de poluentes prioritários (SOUZA; CORRÊA, 2016, NCUBE et al., 2017).

A quantidade de HPA nos ecossistemas marinhos costeiros do nordeste do Brasil teve um aumento inesperado. A partir de agosto de 2019, uma grande quantidade de óleo cru começou a aparecer em quase toda a costa nordeste e parte do sudeste brasileiro. Foi registrada a chegada desse óleo em mais de 1000 localidades e coletadas, aproximadamente, 5.000 toneladas deste material (LOURENÇO et al., 2020; SOARES et al., 2020). Os impactos desse derramamento sobre os organismos marinhos têm sido estudados desde o desastre (DISNER; TORRES, 2020; CAMPELO et al., 2021; MAGALHÃES et al., 2022). O contato direto dos animais com esse material pode causar inúmeros danos. Por possuir uma consistência pegajosa, o material pode aderir à superfície de recifes e ficar em contato com o ambiente e a biota por muito tempo (CRAVEIRO et al., 2021; LIRA et al., 2021; GUSMÃO et al., 2021).

Os compostos organoclorados são hidrocarbonetos sintetizados pelo homem, portanto, não ocorrem naturalmente no ambiente (YOGUI, 2002) e são incluídos na categoria de poluentes orgânicos persistentes (POPs). Um exemplo clássico é o diclorodifeniltricloreto (DDT), pesticida organoclorado (POC) muito usado no século XX e um dos mais conhecidos do mundo. Banido no Brasil em 1985 para atividades agrícolas, teve seu uso continuado para medidas de prevenção ao vetor de algumas doenças como malária e doença de Chagas. Uma vez na natureza, o DDT é transformado em seus metabólitos, o diclorodifenildicloreto (DDD) e diclorodifenildicloreto (DDE). As concentrações dos metabólitos no ambiente e as complexidades de sua degradação podem indicar se contaminação é recente: quanto maior a concentração de DDT nas amostras, mais recente é a contaminação. Por outro lado, altas concentrações dos metabólitos DDE e DDD indicam contaminação mais antiga (BAPTISTA-NETO et al., 2008). A presença de DDT e seus congêneres em estudos ambientais recentes, em grandes concentrações, mesmo após o banimento, sugere que os danos na biota aquática, sedimento e seres humanos podem ser maiores do que o esperado (GRISOLIA, 2005; KENGARA et al., 2019; PAUMGARTTEN, 2020; PANIS et al., 2022). Aproximadamente 25% da produção mundial de organoclorados chegam ao oceano e a principal fonte para a contaminação do mesmo é a atmosfera (FLORES et al., 2004). Este transporte atmosférico é conhecido como destilação global, e vem afetando regiões polares que possuem pouca ou

nenhuma atividade agrícola, acumulando DDT e DDE no tecido adiposo de leões marinhos e outros mamíferos aquáticos (RYAN, 2004).

Os metais traço, diferentemente dos organoclorados, estão presentes naturalmente na hidrosfera. No entanto, devido à expansão urbana e atividades metalúrgicas, combustão de combustíveis fósseis, eliminação de resíduos e práticas agrícolas, suas concentrações no ambiente têm aumentado ao longo dos últimos anos (FLORES et al., 2004; HE et al., 2015). O oceano é considerado a última etapa do ciclo hidrológico dos metais, que podem chegar até ele por três vias: drenagem continental dos rios, entrada atmosférica e fontes hidrotermais (SALOMONS; FORSTNER, 1984; GIBBS; GUERRA, 1997; CYRIAC et al., 2021). Os metais pesados são elementos químicos que possuem número atômico superior a 22, alta densidade, são estáveis, não degradáveis e conservativos, e podem estar biodisponíveis, podendo assim se acumular nos organismos (AN et al., 2001; MERFA, 2010). Alguns metais traço, como Cu, Fe, Mg, Mn e Zn são elementos essenciais no crescimento celular e em funções fisiológicas em espécies de decápodes de água doce e marinhos, mas em elevadas concentrações podem ser tóxicos aos organismos (PHILLIPS, 1980; GHERARDI et al., 2012).

Alguns contaminantes, como os HPAs e POCs, são quimicamente estáveis, mas suscetíveis à fotodegradação, portanto, no ar e expostos a luz, as meias vidas desses compostos variam de horas a poucos dias (CARUSO; ALABURDA, 2008). No entanto, as principais vias de degradação são através da biodegradação, catalisada por fungos, bactérias e algas e processos enzimáticos (HARITASH; KAUSHIK, 2009). Assim, esses compostos possuem característica lipofílica e, assim como os metais, podem ser adsorvidos ao sedimento, onde podem resistir por vários meses a muitos anos (FERREIRA et al., 2010). Esses compostos agregados ao sedimento podem ser ressuspensos pela ação das correntes e de organismos bioturbadores e serem ingeridos pelos animais detritívoros e suspensívoros, categorias de hábito alimentar na qual se enquadram diversas espécies de crustáceos (POWER; CHAPMAN, 1992; CHAPMAN et al., 1998; MERFA, 2010; CABRAL, 2017). Além do sedimento ser um item alimentar por ser uma fonte de carbonato de cálcio para a produção de uma nova carapaça, após os eventos de ecdise, alguns grupos de crustáceos, principalmente os da infraordem Brachyura (caranguejos e siris), ingerem sedimento para auxiliar na digestão mecânica e redução das partículas de alimento ingerido (BRANCO; VERANI, 1997; MANTELATTO; CHRISTOFOLETTI, 2001; FERREIRA et al., 2011), o que pode intensificar a absorção desses contaminantes.

Embora crustáceos marinhos e estuarinos estejam em contato constante com contaminantes antrópicos, ainda estão entre os animais mais abundantes desses ambientes, o

que pode refletir uma tolerância a tais compostos (DEPLEDGE; BJERREGAARD, 1989). No entanto, quando expostos a altas concentrações dessas substâncias, os indivíduos podem apresentar algumas alterações comportamentais, fisiológicas, moleculares ou morfológicas. Esses fenômenos também podem ocorrer se compostos essenciais, que são substâncias que fazem parte dos processos fisiológicos, estiverem em excesso (TORREIRO-MELO et al., 2015; MACIEL et al., 2015).

Entre as formas de analisar como essas espécies são afetadas por impactos antrópicos, o teste de micronúcleo é bastante utilizado em organismos marinhos e estuarinos para observar macrolesões no DNA (NUDI et al., 2010; PINHEIRO et al., 2013; CABRAL, 2017; HONG et al., 2018). A formação de células micronucleadas observadas nos granulócitos da hemolinfa ou sangue é proveniente de alterações na divisão celular, que pode formar um núcleo principal e um núcleo de tamanho menor, porém com textura e coloração semelhantes ao núcleo maior. A longo prazo, essas alterações podem causar danos aos animais e muitas vezes foram correlacionadas ao estresse ambiental devido à presença de contaminantes, como HPA, por exemplo (CABRAL, 2017).

O presente estudo se propôs a analisar os contaminantes nos sedimentos em uma área com abertura de galerias de esgoto ativas (Ponta Verde – Maceió/AL), uma área na imediata desembocadura do Rio Coruripe (Pontal de Coruripe - Coruripe/AL) e uma área com intensa atividade turística e proximidade do Porto de Suape (Gaibu – Cabo de Santo Agostinho/PE). Além destas, uma área que está inclusa na Área de Proteção Ambiental Costa dos Corais (APA-CC) foi investigada: Praia dos Carneiros (Tamandaré/PE). Esta área, apesar de estar localizada numa região protegida, sofre com intensa atividade turística e presença de embarcações de lazer no local. As áreas recifais contempladas pertencentes ao estado de Alagoas foram alvos de poucos trabalhos relacionados à poluição marinha e seus efeitos na carcinofauna. As poucas pesquisas realizadas tratam da contaminação de espécies de crustáceos por microplástico (BARROS et al., 2020; SANTANA et al., 2022a). Os resultados da contaminação química no sedimento foram correlacionados às alterações morfológicas no crescimento relativo, presença de manchas e más-formações observadas. Além de verificar alterações morfológicas, os danos genotóxicos também foram mensurados, através da frequência de células micronucleadas na hemolinfa.

Portanto, o presente estudo visa compreender mais sobre a potencial exposição dos caranguejos marinhos aos contaminantes presentes no sedimento de seus habitats, tendo em vista que as pesquisas têm sido realizadas em frequência maior com decápodes estuarinos e

com espécies de importância econômica (PINHEIRO; TOLEDO, 2010; PINHEIRO et al., 2013), além de comparar áreas de potencial turístico, com diferentes níveis de contaminação. Como o derramamento de óleo ocorreu durante a pesquisa, ainda foi possível realizar comparações do ambiente antes e depois do óleo, e entre áreas que foram e não foram atingidas. É importante conhecer os fatores externos que podem influenciar no pleno desenvolvimento das espécies, em todas as suas funções ecológicas que desempenham no ambiente. Para isso, estudos de ecotoxicologia são de fundamental importância, pois avaliam as relações dos poluentes e seus efeitos na biota, constituindo importantes ferramentas para o monitoramento ambiental porque suas informações proporcionam um diagnóstico mais eficaz dos impactos ambientais (LAITANO; RESGALLA, 2002).

2 HIPÓTESE E OBJETIVOS

2.1 Hipótese

Hipótese 1: As populações de *Pachygrapsus transversus* de quatro ambientes recifais expostos a diferentes tipos de impactos (por exemplo, descarga de esgotos, turismo e derramamento de petróleo), sofrem alterações no crescimento relativo, tamanho da maturidade e desequilíbrio na proporção sexual.

Hipótese 2: As populações de *Eriphia gonagra* de quatro ambientes recifais expostos a diferentes tipos de impactos (por exemplo, descarga de esgotos, turismo e derramamento de petróleo), sofrem alterações no crescimento relativo, tamanho da maturidade e desequilíbrio na proporção sexual.

Hipótese 3: Os caranguejos *Pachygrapsus transversus* e *Eriphia gonagra* sofrem alterações e deformidades na carapaça e lesões no DNA relacionadas com a contaminação do sedimento por HPAs, organoclorados e metais.

2.2 Objetivos

2.2.1 Objetivo geral

Realizar um estudo comparativo sobre os efeitos de diferentes níveis de exposição a metais traço, HPAs, OCs e Metais em braquiúros de ambientes recifais de Alagoas e Pernambuco.

2.2.2 Objetivos específicos:

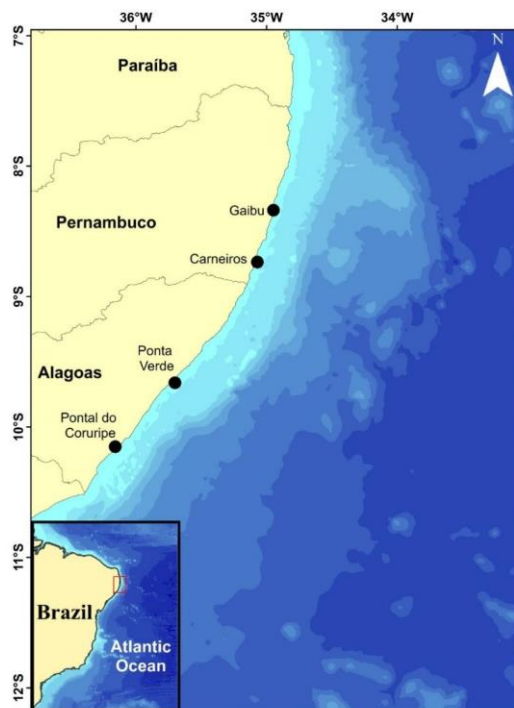
- Verificar se há diferenças no crescimento relativo, maturidade sexual e razão sexual das espécies entre as regiões avaliadas (Artigos 1 e 2);
- Analisar carapaça dos braquiúros para verificar a integridade das estruturas morfo-anatômicas externas (Artigos 1 e 2);
- Avaliar a contaminação dos sedimentos das regiões estudadas em termos de hidrocarbonetos policíclicos aromáticos (HPAs), organoclorados (OCs) e metais traço (MTs) (Artigo 3);
- Investigar se há evidências de genotoxicidade através do teste de micronúcleo na hemolinfa dos caranguejos das áreas de coleta (Artigo 3);

- Investigar se há correlação entre os níveis de contaminação e as diferenças morfológicas observadas (Artigo 3);
- Integrar os resultados químicos e biológicos para entender possíveis correlações de causa e efeito, principalmente entre os períodos pré- e pós-acidente com óleo ocorrido no segundo semestre de 2019 (Artigo 3).

3 ÁREAS DE ESTUDO

As áreas de estudo estão localizadas no nordeste do Brasil: Praia de Gaibu ($8^{\circ} 20' 3''$ S, $34^{\circ} 56' 58''$ O) e Praia dos Carneiros ($8^{\circ} 42' 14''$ S, $35^{\circ} 4' 45''$ O) no estado de Pernambuco e Praia de Ponta Verde ($9^{\circ} 39' 58''$ S $35^{\circ} 41' 32''$ O) e Pontal do Coruripe ($10^{\circ} 9' 20''$ S, $36^{\circ} 7' 55''$ O) no estado de Alagoas (Figura 1). O clima da região é tropical, quente e úmido, com chuvas de outono-inverno (KOTTEK et al., 2006). Todas as áreas possuem recifes areníticos, com cobertura algal e coralínea e que são habitat de diversas outras espécies. Para o estudo foram determinadas quatro estações em cada área (Figuras 2, 5, 8 e 11). Os recifes de Gaibu, Carneiros e Pontal do Coruripe formam barreiras paralelas à costa, que durante a maré baixa formam um canal entre os recifes e a praia. Por outro lado, na Ponta Verde, os recifes são uma continuidade da praia, formação conhecida como ‘recife de franja’ (SALLES, 1995; CORREIA; SOVIERZOSKI, 2008). As quatro áreas de estudo apresentam diferentes fontes de contaminação. Cada uma delas será detalhada nos tópicos seguintes.

Figura 1 - Localização das áreas de estudo no nordeste do Brasil: praias de Gaibu e dos Carneiros em Pernambuco e Ponta Verde e Pontal do Coruripe em Alagoas

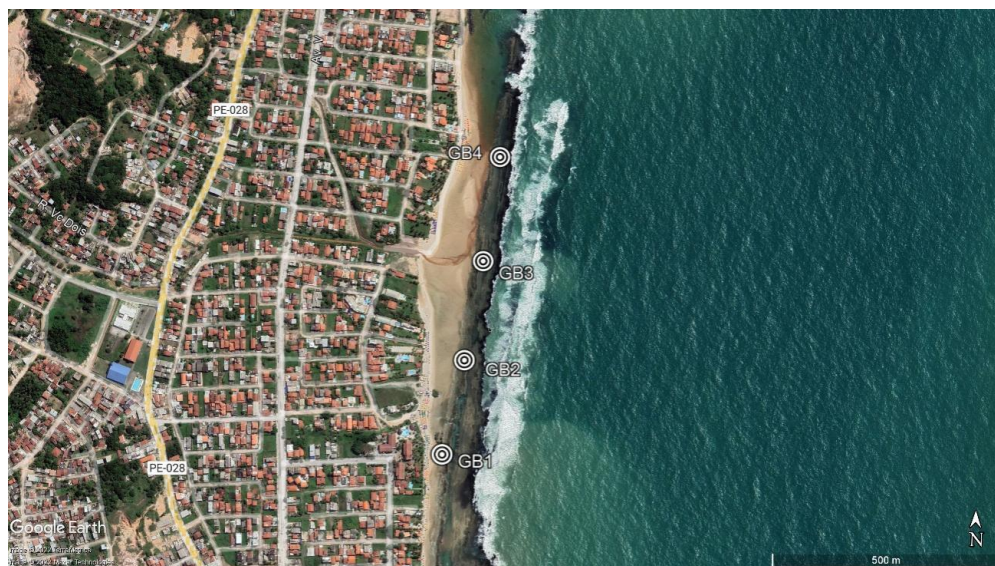


Fonte: A autora, 2022.

3.1 Praia de Gaibu

A Praia de Gaibu fica no litoral central do estado de Pernambuco, no município de Cabo de Santo Agostinho, a cerca de 37 km da capital Recife. Na região há um recife arenítico com cerca de 1.700 metros de comprimento paralelo à praia e sua parte mais larga possui aproximadamente 120 metros (Figuras 2 e 3). A baixa salinidade proporcionada pelo afloramento de águas subterrâneas provenientes do lençol freático que ocorre na área praial e pela presença dos riachos Arrombado e Gaibu (BRAGA et al., 2003; NEGROMONTE et al., 2012), assim como sedimento mais fino, proporcionaram a instalação e crescimento de árvores de mangue em áreas adjacentes e sobre os recifes (MADRUGA-FILHO, 2004) (Figura 4). As praias em torno de Gaibu estão em processo de urbanização (VASCONCELOS et al., 2019). Além da população local residente, a região possui diversos hotéis, pousadas e resorts, o que contribui com sua urbanização e traz uma grande quantidade de turistas, aumentando a produção de efluentes (BRAGA et al., 2003). Esse esgoto *in natura*, ligado clandestinamente aos riachos e galerias de drenagem pluvial, acaba chegando ao oceano e são lançados imediatamente sobre os recifes, podendo carrear diversos contaminantes que podem afetar a saúde da biota marinha (STABILI et al., 2013). A área ainda fica próxima ao Porto de Suape, fato que também pode ser uma fonte de contaminação para esse ambiente recifal a depender das correntes locais.

Figura 2 - Distribuição das estações de coleta (GB1, GB2, GB3 e GB4) sobre os recifes da Praia de Gaibu, Cabo de Santo Agostinho, Pernambuco



Fonte: A autora, 2022

Figura 3 - Recifes areníticos de Gaibu, em Cabo de Santo Agostinho – PE, durante a maré baixa.



Fonte: A autora, 2022

Figura 4 - Árvores de mangue *Laguncularia racemosa* (L.) C. F. Gaertn instaladas próximo e sobre os recifes areníticos de Gaibu, em Cabo de Santo Agostinho, Pernambuco



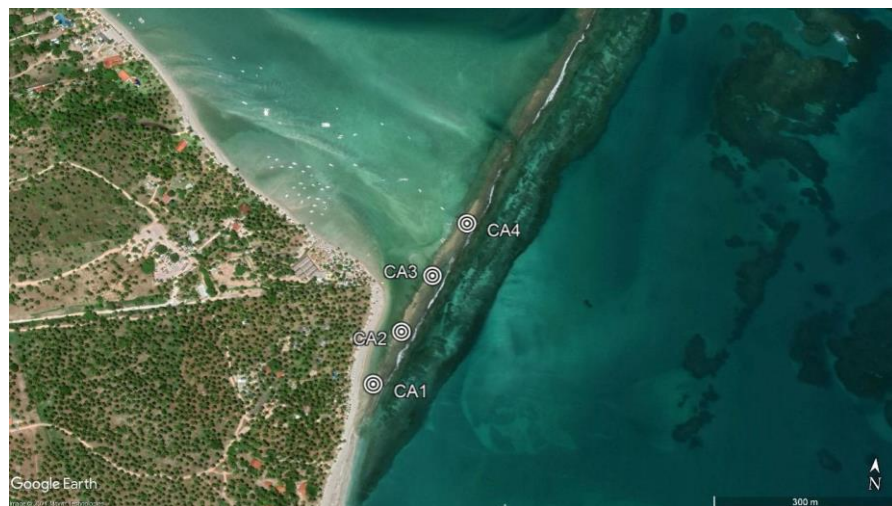
Fonte: A autora, 2022

3.2 Praia dos Carneiros

A Praia dos Carneiros está situada no litoral sul do estado de Pernambuco, no município de Tamandaré, a cerca de 104 km da capital pernambucana, Recife. A praia faz parte da Área de Proteção Ambiental Costa dos Corais (APACC), criada em 1997 (BRASIL, 1997). Nesta

região há várias formações recifais que permanecem quase sempre submersas, mas uma barreira em especial (aproximadamente 2.085 metros de comprimento e 705 metros de largura) (Figura 5), na desembocadura do Rio Formoso, fica exposta durante a baixamar e é de fácil acesso à população sem auxílio de embarcações. No entanto, por ser uma área com grande fluxo turístico, há numerosas embarcações de lazer, como catamarãs e lanchas, e até mesmo barcos de pesca de pequeno porte que ancoram na foz do rio. Essas embarcações podem vazear combustível para o ambiente marinho/estuarino, além serem potenciais agentes danificadores dos recifes devido aos procedimentos de ancoragem (FIRMINO, 2006; LOURENÇO et al., 2015). Além disso, o grande número de turistas acaba acarretando alguns impactos, como o elevado pisoteio do ambiente recifal para acessar as piscinas naturais que se formam entre as estruturas areníticas (STEINER et al., 2006) (Figura 6). Assim como em Gaibu, o afloramento de água subterrânea, e no caso de Carneiros, o aporte de água doce do Rio Formoso, proporcionaram um ambiente ótimo para o desenvolvimento de árvores de mangue próximo aos recifes (Figura 7).

Figura 5 - Distribuição das estações de coleta (CA1, CA2, CA3 e CA4) sobre os recifes da Praia dos Carneiros, Tamandaré, Pernambuco



Fonte: A autora, 2022

Figura 6 - Banhistas caminhando sobre os recifes da Praia dos Carneiros, Tamandaré, Pernambuco, Brasil



Fonte: A autora, 2022

Figura 7 - Árvores de *Laguncularia racemosa* (L.) C. F. Gaertn na Praia dos Carneiros, em Tamandaré, Pernambuco, Brasil



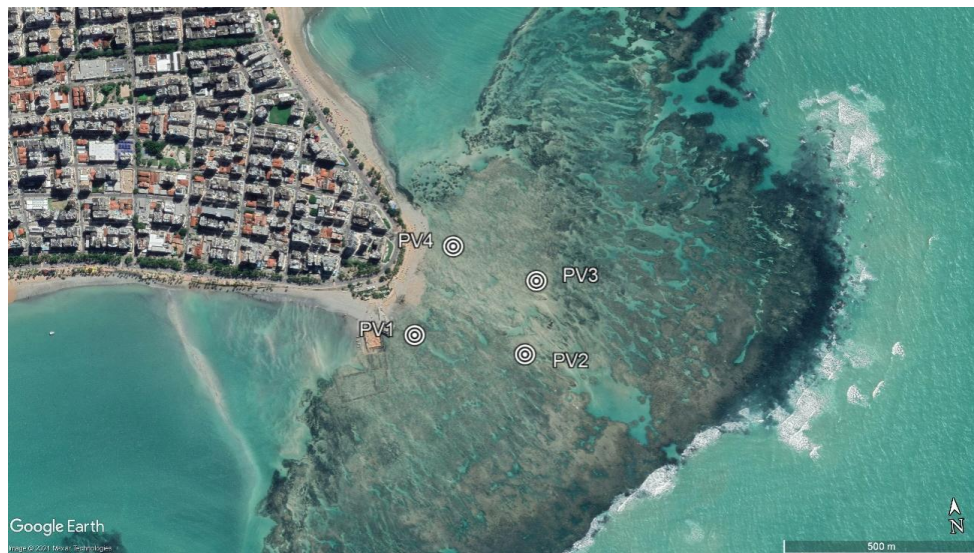
Fonte: Tereza Calado, 2019

3.3 Praia da Ponta Verde

A Praia da Ponta Verde está localizada no litoral central de Alagoas, na capital alagoana Maceió. Nessa região há um recife de franja de, aproximadamente, 2.357 metros de comprimento e 935 metros de largura (Figura 8). Sobre este recife estão instaladas estruturas de pesca chamadas currais e um farol (Figuras 9 e 10), que é um dos pontos turísticos da cidade

e atrai muitos visitantes que, durante as marés baixas, caminham sobre os recifes causando danos na fauna e cobertura algal através do pisoteio frequente. É uma área muito turística, com diversos hotéis e pousadas, e próxima ao centro urbano da cidade. Além disso, a área sofre diretamente com variados impactos, como a presença de galerias de esgoto (PÁDUA et al. 2016; XAVIER, 2020; SANTANA et al., 2022a; 2022b) (Figura 10).

Figura 8 - Distribuição das estações de coleta (PV1, PV2, PV3 e PV4) sobre os recifes da Praia da Ponta Verde, Maceió, Alagoas.



Fonte: A autora, 2022

Figura 9 - Recifes da Praia da Ponta Verde, em Maceió, Alagoas, com destaque para o farol instalado sobre a estrutura recifal



Fonte: Alberis Santos, 2019

Figura 10 - Galerias de escoamento de águas pluviais e esgoto, desaguando sobre a região dos recifes da Praia da Ponta Verde, Maceió, Alagoas



Fonte: A autora, 2022

3.4 Praia do Pontal do Coruripe

A Praia do Pontal do Coruripe fica situada no município de Coruripe, no litoral sul do estado de Alagoas, distante 90 km da capital Maceió. O povoado do Pontal do Coruripe teve seu crescimento acelerado a partir da década de 1950, com comércio de moradores locais, artesanato e casas de veraneio, o que atraiu alguns hotéis e pousadas (SILVA et al., 2018). No entanto, a região recebe turistas em um número menor se comparado às outras três áreas de estudo. Em 2010, havia um planejamento para construir o Estaleiro do Nordeste (ENOR) no Pontal, que seria o maior da América Latina, porém, após alguns entraves no licenciamento ambiental, o projeto foi transferido para o povoado de Miaí de Cima (IPEA, 2014), e posteriormente engavetado por problemas de financiamento da obra. Uma das justificativas para a mudança de local foi a importância das atividades extrativistas no manguezal e da atividade pesqueira local (DÓRIA, 2017). Na região está presente um recife do tipo barreira, com aproximadamente 2 metros de altura, e que mede cerca de 700 metros de comprimento e 29 metros de largura (Figura 11). O Pontal do Coruripe fica próximo à desembocadura do Rio Coruripe, que passa por usinas de cana-de-açúcar e pode trazer contaminantes ao oceano, resultantes dos processos de tratamento da cana-de-açúcar e de esgotos que são lançados no rio e seus afluentes, já que o saneamento básico não abrange toda a cidade (SANTOS, 2017).

Figura 11 - Distribuição das estações de coleta (PC1, PC2, PC3 e PC4) sobre os recifes da Praia de Pontal do Coruripe, Coruripe, Alagoas

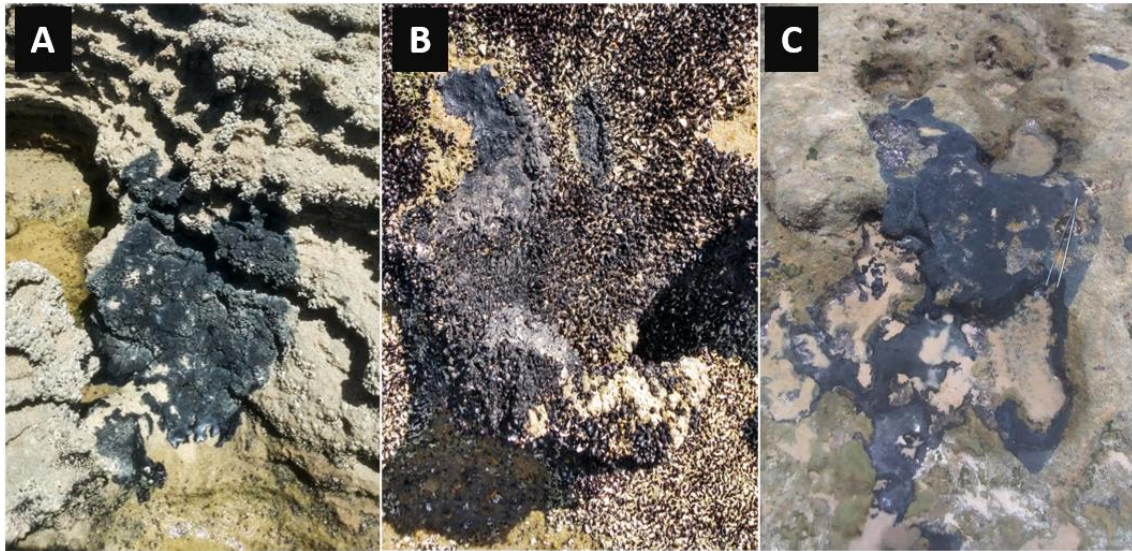


Fonte: A autora, 2022

3.5 Derramamento de óleo nas áreas de estudo

Além de todas essas fontes de impacto que as áreas de estudo estão expostas, entre agosto e setembro de 2019, três das quatro áreas (exceto Ponta Verde) foram atingidas por grandes quantidades de óleo pesado. A resposta ao desastre nas praias, estuários e ambientes recifais costeiros foi bastante rápida e eficiente, principalmente por parte da própria população residente e pescadores das áreas atingidas (SOARES, et al 2020; ARAÚJO et al., 2020), porém restaram algumas manchas que permaneceram aderidas aos arenitos (Figura 12). Foram realizadas duas coletas após o impacto do óleo, uma em novembro de 2019 e outra em fevereiro de 2020. No fim de junho de 2020 houve o reaparecimento de uma quantidade menor de óleo nessas áreas e novamente a Ponta Verde não foi afetada.

Figura 12 - Manchas de óleo aderido aos recifes areníticos de: (A) Gaibu, (B) Carneiros e (C) Pontal do Coruripe, encontradas após o derramamento de óleo que atingiu a costa brasileira



Fonte: (A) e (B) A autora, 2019 e (C) Carlos Eduardo Aragão, 2020

4 ARTIGO 1 - REEF CRAB POPULATION CHANGES AFTER OIL SPILL DISASTER REACH BRAZILIAN TROPICAL ENVIRONMENTS

Abstract: The oil spill that reached Brazilian Coast in 2019 was one of the most extensive disasters and its effects on distinct species are still under investigation. This study evaluated the effects of the oil spill on the crab *Pachygrapsus transversus* in four reef areas in Northeast of Brazil that are also under different levels of chronic anthropogenic impacts. Changes in population aspects were investigated including maturity, sex ratio, and relative growth considering periods before and after the oil spill. An acute decrease in the number of females captured in areas most affected by oil spill was evident and may be associated with the closure of burrows used for protection. Crabs from the most touristic area presented a decrease in the medium size of carapace and maturation compared to crabs from other less visited areas, which highlights the importance of studying the effects of impacts on marine fauna.

Keywords: *Pachygrapsus transversus*, Grapsidae, anthropogenic impacts, petroleum

Natural reef environments are typical of tropical regions and house several species of marine fauna and flora, being a refuge and substrate for the establishment of populations (Villça, 2002; Correia and Sovierzoski, 2005). Coastal reefs are one of the ecosystems most threatened by anthropogenic action (Ferreira and Maida, 2006) since they are easily accessible to people that frequently tramples on corals. This process disturbs the growth of corals and algae and may cause the death of sensitive species (Villça, 2002; Goldberg and Wilkinson, 2004; Leite et al., 2018). In addition, reefs are also impacted by compounds chronically or punctually introduced through sewage, nautical activities, tourism, rivers, leached from contaminated soils, among others (Pádua et al., 2016; Lara-Martín et al., 2020). These chemical contaminants may cause biochemical, morphological, physiological, and behavioural changes in animals, which could lead to the complete disappearance of species (Bigatti et al., 2009; Pinheiro and Toledo, 2010; Jesus et al., 2020).

One of the most representative species in terms of abundance in Brazilian reefs is *Pachygrapsus transversus* (Gibbes, 1850), which commonly inhabits intertidal zones and occupies crevices in rocky shores, sandstone, biogenic reefs, pier pillars and mangrove roots (Abele, 1976; Melo, 1996). This species has a wide distribution (Atlantic boundaries, eastern Pacific coast, and the Mediterranean Sea) and great ecological importance, acting actively in the maintenance of reef cover through their omnivorous feeding habit (Barros et al., 2020).

Additionally, they are a key component of the reef trophic cascade, serving as food for several species of fish, birds and other crustaceans (Christofolletti et al., 2010; Barros et al., 2020). Given its ecological relevance, numerous studies have focused on the ecology of *P. transversus* (Flores and Negreiros-Fransozo, 1998; Flores and Paula, 2002; Oliveira et al., 2015; Araújo et al., 2016; Barros et al., 2020). However, studies evaluating the effects of anthropogenic impacts on the ecology and populations of *P. transversus* are still scarce, making it difficult for a realistic understanding of environmental and animal health. Due to the implications for human health, most studies evaluating human action on crustaceans are related to species of economic value. That is not the case for *P. transversus*, which became the target more frequent of these researches only in recent years (Barros et al., 2020; César-Ribeiro, 2021).

Impacts on reef species such as *P. transversus* are usually chronic since stressors such as tourism and untreated sewage may be constant. Occasionally other pollutant sources can synergistically impact these species, such as oil from ship accidents or leaks in petroleum platforms (Krebs and Burns, 1977; Yim et al., 2020). In August 2019, a large amount of crude oil hit approximately 1000 coastal regions, as mangroves, estuaries and beaches, in the Northeast and part of the Southeast Brazilian coast, and over 5000 tons of oil residues were removed from shores (Lourenço et al., 2020; Soares et al., 2020). Direct contact with this oil could form a sticky layer on animals possibly killing them by asphyxia and change the benthic species composition (Craveiro et al., 2021). In addition, oil composition includes several molecules with varying degrees of toxicity, causing immediate or long-term impacts with sub-lethal or fatal damages. A decrease in the number of individuals of the species *Minuca pugnax* (Smith, 1870) was reported after an oil spill in Wild Harbour (Massachusetts-EUA) (Krebs and Burns, 1977). According to the authors, this reduced crab density may be associated to decrease of juvenile settlement, changes in the sex ratio and behavioral disorders. Several others oil spills effects on populations and species of crustaceans have been already reported (Malan et al., 1988; Poggiale and Dauvin, 2001; Roth and Baltz, 2009; Felder et al., 2014; Frank et al., 2020).

Ecological aspects such as sex ratio and maturity allow an overview of populations in the natural environment. Natural variations in these metrics among individuals of the same species, from different locations, should be expected due to geographical (*i.e.* latitude, temperature and sun exposure) and ecological factors (Hines, 1989; Helmuth et al., 2006; Denny et al., 2011; Gaitán-Espitia et al., 2014; Seabra et al., 2015). On the other hand, anthropogenic

activities may contribute significantly to these variations (Boudaya et al., 2019; Gül and Griffen, 2019). The present study describes population aspects of *P. transversus* species from four tropical reef areas in terms of relative growth, maturity, and sex ratio, which are continuously exposed to different stressors. During the studied period, the 2019 oil spill disaster occurred and each area was affected in a different way, creating the possibility of observing the oil impacts upon this species.

The tropical studied reefs are located in Northeastern Brazil, being 2 in Pernambuco State: Gaibu (8°20'3"S 34°56'58"W) and Carneiros Beach (8°42'14"S 35°4'45"W); and 2 in Alagoas State: Ponta Verde (9°39'58"S 35°41'32"W) and Pontal do Coruripe (10°9'20"S 36°7'55"W) (Figure 1). All of them are located at touristic beaches and undergo other types of threats besides trampling. At Ponta Verde sewage is discharged close to reefs (Pádua et al., 2016) and constant fishing activities over the reefs, where artisanal fishermen installs traps; The reef at Pontal do Coruripe is located close to the mouth of Coruripe River, which carries effluents and debris from sugarcane mills of the region (Souza-Júnior et al., 2001). Besides the smaller sewage galleries around Gaibu reefs, the area is close to Port of Suape, an important harbour that receives cargo and large ships, with port cargo handling about 23 million tons per year (Madruga-Filho, 2004; SUAPE, 2021); The last one, Carneiros reefs, is located inside of the Environmental Protection Area Costa dos Corais (APACC) and it was expected to be very well preserved. Unfortunately, the area receives many leisure vessels such as speedboats and catamarans that can leak fuel or cause damage to the reef structure with the anchoring procedures (Firmino, 2006; Lourenço et al., 2015). In addition to all these impacts, between August and September 2019, 3 out 4 areas (except Ponta Verde) were hit by large amounts of crude oil (Soares et al., 2020; Araújo et al., 2020). By the end of October 2019, approximately 1,018 tons of oil residues were removed from coastal environments in Cabo de Santo Agostinho, the city that Gaibu reefs are located. The second most affected area was Tamandaré region (where Carneiros reefs are located), about 208 tons were collected (Teixeira, 2019). Coruripe reefs were the least affected one, but still 138 tons of oil residue were removed (Carvalho, 2019).

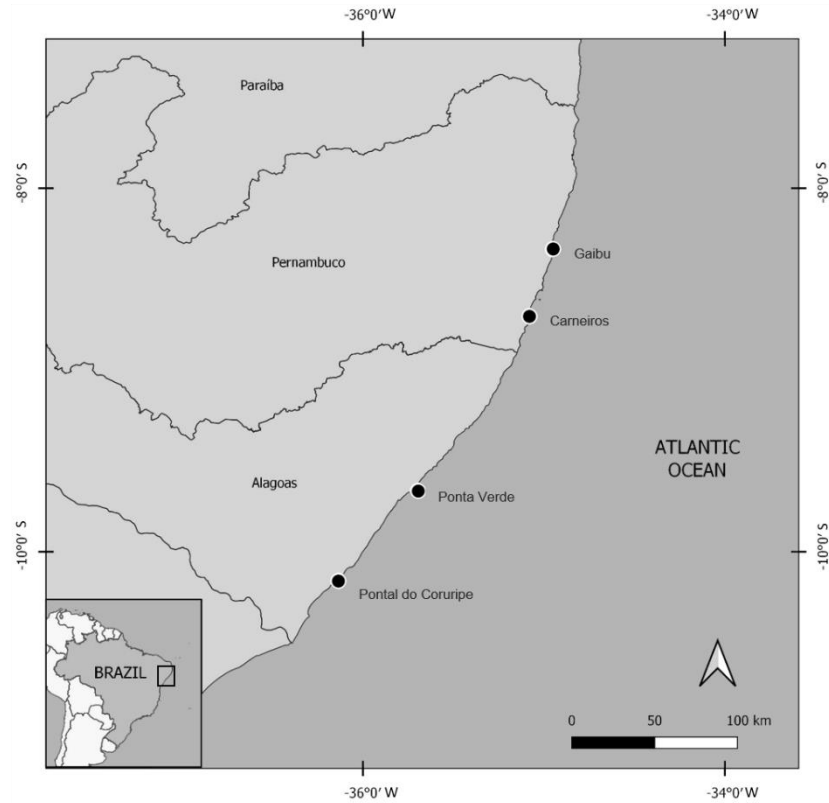


Figure 1. Location of the sampling areas in Brazilian Northeast (states of Pernambuco and Alagoas)

Every three months between February 2019 and February 2020 individuals of *P. transversus* were manually collected from each reef area, during two hours at low tide, by two experienced researchers. Two of these samplings were performed after oil spill (AOS) disaster (November 2019 and February 2020). Salinity was measured *in situ* using a refractometer and water and air temperature with a mercury thermometer. Crabs were stored in plastic bags and transported to the laboratory.

Cephalothorax width (CW) and the abdomen width (AW) were measured in females and CW and the length of the propod of the largest cheliped (CL) in males. The regressions CW vs. AW in the females, and CW vs. CL in the males were adjusted by the potential function ($y = a \cdot x^b$), considering CW as the independent variable (adapted from Huxley, 1950). The size of morphological maturity was determined using a non-hierarchical classification (K-means cluster) procedure to derive one model for the immature phase, and a second model for the adult

phase, for each biometric relationship (adapted from Sampedro et al., 1999; Corgos and Freire, 2006) using the software Past® version 2.17.

After euthanasia by freezing, the individuals were then dissected by a dorsal incision bordering the carapace for the macroscopic analysis of the gonads, based on their coloration and size in comparison with the rest of the viscera and the cephalothoracic cavity (modified from Moura and Coelho, 2004). All stages of gonad maturation were classified according to Moura and Coelho (2004) and Santana et al. (2018) for females, and by Hartnoll (1965) for males. Body size at physiological maturity was determined based on the distribution of the individuals by size class and plotting a logistic curve the estimated size of 50% of the individuals of each sex are physiologically mature, i.e., L50% (Moura and Coelho, 2004). For this analysis was used the sizeMat package (version 1.1.1) in the free statistical program RStudio® (version 1.3.959) (R Core Team, 2020).

The sex ratio was estimated by calculating the percentage of male and female individuals collected in each month of the studied period. Differences among sampled periods and areas as well as before (BOS) and after (AOS) oil spill were evaluated. Chi-square (χ^2) was used to test the significance of observed deviations in the sex ratio (Freitas and Santos, 2002). All analyses were considered $\alpha = 0.05$. The individuals were distributed into size classes using the frequency tables of the BioEstat®. The normality of the data distributions was evaluated using the Kolmogorov-Smirnov test (Zar, 1996; Araújo and Lira, 2012). All areas were compared based on abiotic parameters, CW average, and maturity size using one-way ANOVA and Tukey using the software Past® 2.17 (Underwood, 1997; Diele et al., 2005).

A total of 553 individuals of *P. transversus* (284 males and 269 females, from which 83 were ovigerous females) were captured (Table 1). CW data had a normal distribution. The highest mean size values were observed at Carneiros and Pontal do Coruripe, and the lowest occurred at Ponta Verde, where the average of the individuals was approximately 3 mm smaller than the other areas. There were significant differences in the CW values of the crabs between the study areas (Figure 2). Values of CW of *P. transversus* of both sexes were smaller in Ponta Verde than in Gaibu ($p=0.037$), Carneiros ($p=0.018$) and Pontal do Coruripe ($p=0.016$). There is a tendency for males to be wider than females ($p=0.596$). There was no correlation between temperature, salinity, and measured biological parameters (Supplementary Figure S1).

Table 1. Number of individuals (N), range, mean, standard deviation (SD) of carapace width (in millimeters, mm) of *Pachygrapsus transversus* from sandstone reefs in Northeastern Brazil.

Area	Sex (n° individuals)	CW range (mm)	CW Mean $\pm$ SD (mm)
Gaibu	F (n=72)	3.75 - 18.98	12.15 $\pm$ 3.17
	M (n=98)	6.30 - 18.65	13.68 $\pm$ 3.14
Carneiros	F (n=41)	7.52 - 18.23	13.10 $\pm$ 2.83
	M (n=25)	7.22 - 19.25	14.34 $\pm$ 3.24
Ponta Verde	F (n=76)	5.32 - 12.25	9.25 $\pm$ 1.22
	M (n=80)	5.83 - 12.54	9.33 $\pm$ 1.39
Pontal do Coruripe	F (n=80)	6.60 - 25.02	13.32 $\pm$ 3.27
	M (n=81)	7.56 - 28.28	14.51 $\pm$ 4.26

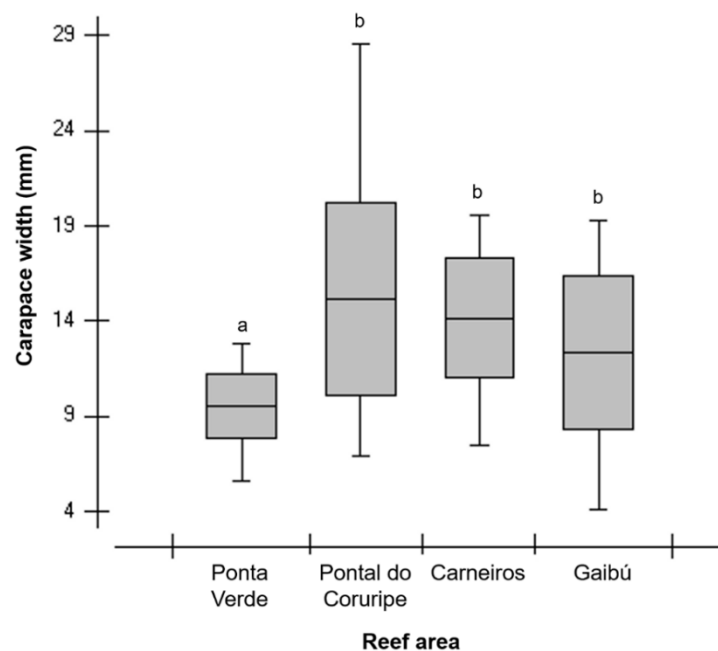


Figure 2. Carapace width (mm) of *Pachygrapsus transversus* from Gaibu, Carneiros, Ponta Verde and Pontal do Coruripe. The upper bar is the maximum value, the lower bar is the minimum value, the top of the box is the upper quartile, the bottom of the box is the lower quartile, and the internal bar is the mean. Different letters (*a* and *b*) indicate differences between the means ($p < 0.05$).

Relative growth of immatures of both sexes was allometric positive in all areas (Figure 3). Immature females from all areas and males from Gaibu, Carneiros, and Ponta Verde had higher relative growth rates than adults. In Pontal do Coruripe, immature males had a lower growth rate than adults. Females of *P. transversus* mature physiologically earlier than males in all areas. On the contrary, morphological maturity firstly occurs in all areas in males. The crabs from Ponta Verde are smaller than those captured in the other areas in both sexes. Also, males and females of *P. transversus* reach morphological maturity in smaller sizes, at least 2 mm earlier than Gaibu females, who have the second lowest value (Table 2). Morphological maturity was obtained later for both sexes in Pontal do Coruripe.

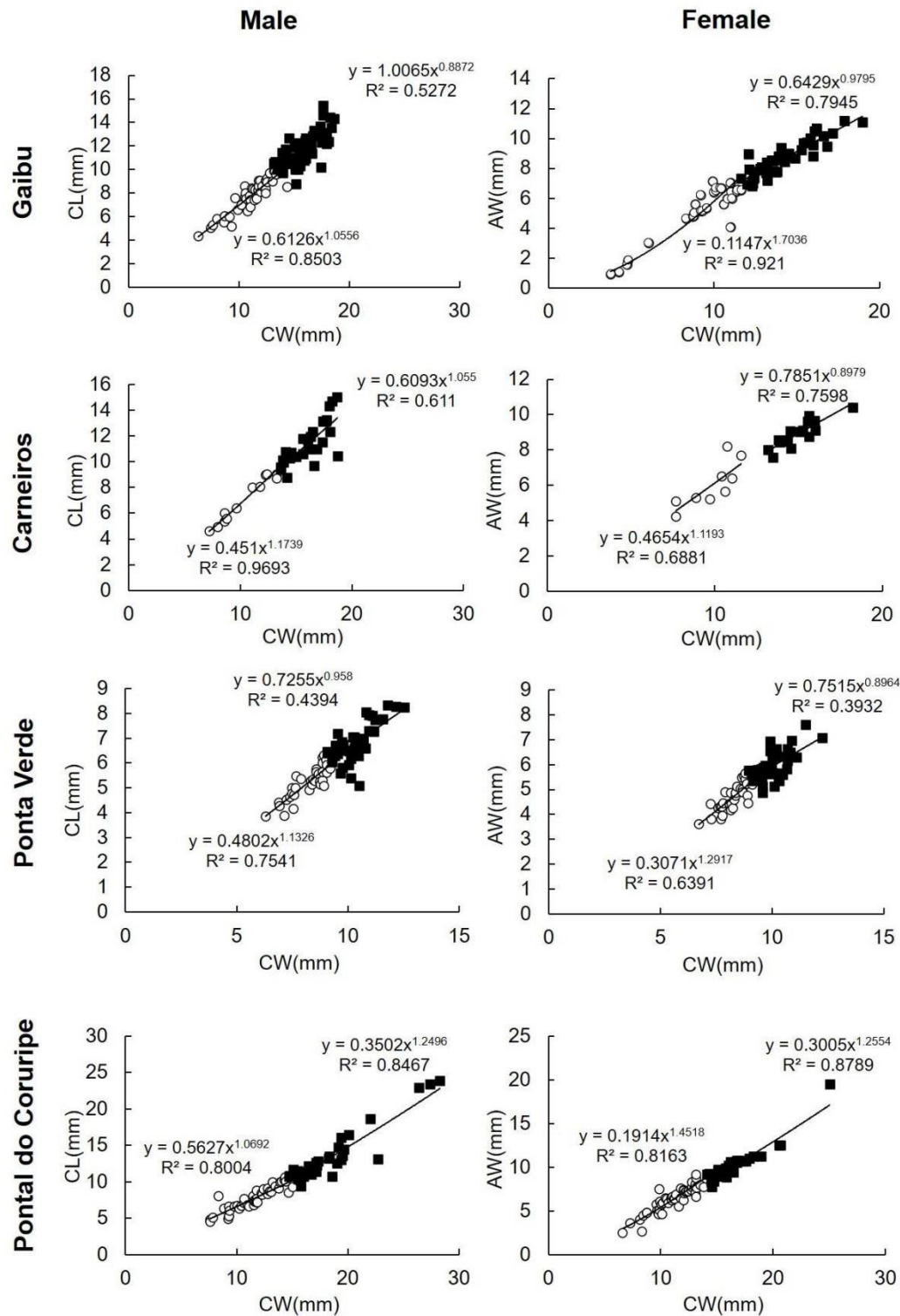


Figure 3. Relative growth between the propodus of major cheliped length (CL, for males) and abdomen width (AW, for females) versus carapace width (CW) in individuals of *Pachygrapsus transversus* from four reefs in Northeastern of Brazil. White circles: Immature; Black squares: Mature.

Table 2. Size of carapace width (mm) (CW) for morphological and physiological maturity (L50) of *Pachygrapsus transversus*, and the smallest ovigerous females in the study area.

	Gaibu		Carneiros		Ponta Verde		Pontal do Coruripe	
	F	M	F	M	F	M	F	M
Morphological	13.15	11.63	13.61	13.18	9.08	8.95	14.69	14.08
Physiological	6.45	10.83	9	9.1	5.03	7.3	7.1	9.98
Smallest ovigerous female	9.18	-	11.03	-	7.8	-	8.13	-

The sex ratio differed significantly from the proposed scientifically 1: 1 (M: F). In Carneiros ratio was 1: 1.64 (female-biased) and in Gaibu was 1:0.73 (male-biased) (Table 3). Except at Gaibu, larger *P. transversus* (> 15 mm) females were more abundant than males. Most individuals were observed in smaller CW sizes, but at Pontal do Coruripe, which presented an even distribution all over the classes. In general, males prevailed at largest size classes. Probably this occurred because after moult puberty, females start to direct energy towards egg production and nutrition, while males develop strength for territorial activities and fighting for females (Araújo et al.2012; Santana et al., 2018). This sexual dimorphism, i.e., males larger than females, is common in grapsoid crabs like *P. transversus* (Flores and Paula, 2002), *Sesarma rectum* Randall, 1840 (Castiglioni et al., 2011), and *Aratus pisonii* H. Milne Edwards, 1837 (Santana et al., 2018). Similar results were reported for the same and other species in Brazil, such as *P. transversus* and *Eriphia gonagra* in Pernambuco (Araújo et al., 2016), *P. transversus* in Ceará (Furtado-Ogawa, 1977), and in São Paulo (Flores and Negreiros-Franozo, 1999a); and around the world, such as in Portugal (Flores and Paula, 2002), and in Lebanon (Arab et al., 2015).

Table 3. Distribution of sex ratio (males: females) in size classes based on CW for *Pachygrapsus transversus*. Specimens were collected at Gaibu, Carneiros, Ponta Verde, and Pontal do Coruripe

Size class of CW (mm)	Gaibu (M:F)	Carneiros (M:F)	Ponta Verde (M:F)	Pontal do Coruripe (M:F)
0.00 — 5.00	0:1*	0:0	0:0	0:0
5.00 — 10.00	1:0.92	1:1.50	1:0.85	1:0.83
10.00 — 15.00	1:1	1:1.30	1:1.17	1:0.79
15.00 — 20.00	1:0.30*	1:2.25*	0:0	1:1.31
20.00 — 25.00	0:0	0:0	0:0	1:1.50
25.00 — 30.00	0:0	0:0	0:0	1:3
Total	1:0.73*	1:1.64*	1:0.95	1:0.98

*proportion differing significantly between the sexes ($p < 0.05$)

The *P. transversus* males were significantly more abundant than females in Gaibu, corroborating previous studies in São Paulo, Brazil (1.24:1; M:F; Flores and Negreiros-Fransozo, 1999b), in the lebanese coast of the Mediterranean Sea (1:0.89; M:F; Arab et al., 2015), and Pernambuco, Brazil (1.31:1; M:F; Araújo et al., 2016). A similar predominance pattern was also observed for other grapsoid crabs (Santana et al., 2018; Rocha et al., 2019). This balance in the proportion M: F was also observed for the congener species *Pachygrapsus marmoratus* (Fabricius, 1787) (Arab et al., 2015), and *Plagusia depressa* in another study in Pontal do Coruripe (Rocha et al., 2019). It is important to mention that the prevalence of males over females could be a consequence of the animal sampling strategy. The females, especially the ovigerous ones, tend to stay longer in the burrows for protection (Abele et al., 1986; Campos and Oshiro, 2001), leading to misinterpretation of data. Gaibu, Carneiros, and Pontal do Coruripe coral reefs were the most impacted by the oil spill among the studied reefs. At these locations, there was a drastic decrease in the abundance of *P. transversus* females sampled after the oil spill (AOS) (Table 4). During sampling several oiled animals were observed inside their crevices (where these animals live) obstructed with oil.

Table 4. Sex ratio of *Pachygrapsus transversus* before (BOS) and after (AOS) the oil spill occurrence between August and September 2019

Size class of CW (mm)	Gaibu		Carneiros		Ponta Verde		Pontal do Coruripe	
	BOS	AOS	BOS	AOS	BOS	AOS	BOS	AOS
0.00 — 5.00	0:1*	0:0	0:0	0:0	0:0	0:0	0:0	0:0

5.00 — 10.00	1:1.25	1:0.4	1:3	1:0	1:0.62	1:1.57	1:0.85	1:0.8
10.00 — 15.00	1:1.19	1:0.7	1:1.75	1:0.6	1:1.28	1:1	1:0.90	1:0.53
15.00 — 20.00	1:0.52	1:0*	1:4*	1:0.5	0:0	0:0	1:1.66	1:0.9
20.00 — 25.00	0:0	0:0	0:0	0:0	0:0	0:0	1:0.5	0:0
25.00 — 30.00	0:0	0:0	0:0	0:0	0:0	0:0	1:3	0:0
Total	1:0.98	1:0.35*	1:2.57*	1:0.45	1:0.78	1:1.34	1:1.09	1:0.78

Note: *proportion differing significantly ($p < 0.05$)

Several factors can affect health and success in the development of crustacean species (Murray et al., 1999; Stevčić et al., 2018). Some species are resilient and can survive in areas highly impacted by different contaminants, but they may undergo physiological and population changes, changing reproductive processes to perpetuate the species (Belgrad and Griffen, 2016). Individuals of *P. transversus* collected in Ponta Verde have reached morphological and physiological maturity in smaller size classes than those from other areas (Table 2), pointing out Ponta Verde as the most impacted area. This finding is corroborated by previous studies, which concluded that this anticipation in sexual maturity may be caused by anthropogenic interferences, forcing precocious maturing and an earlier reproduction (Belgrad and Griffen, 2016; Santana et al., 2018).

The AW approach for females and CL for male crabs are excellent variables to analyse maturity and allometric growth because growth rates usually change after individuals reach the moult puberty estimated (Castiglioni and Coelho 2011; Araújo et al. 2012). The female's abdomen gets wider at maturity, providing a larger incubatory chamber for the eggs (Fernández-Vergaz et al. 2000). In males, chelipeds grow to assist processes of courtship and disputing for territory and females (Warner 1970; Pescinelli et al. 2015). In the present study, the relatively increasing growth of the dependent variable after puberty moult was only observed for males in Pontal do Coruripe. Most areas had negative allometric growth, except for males from Carneiros, which changed to isometry. Besides, females from Pontal do Coruripe, showed a decrease in growth rate, but still remained in positive allometry. Pontal do Coruripe and Carneiros also presented individuals with later morphological and physiological maturities compared to Gaibu and Ponta Verde, which are areas under significant anthropogenic influence. Anticipation of maturity in crustaceans exposed to major environmental changes was previously observed for *Armases rubripes* (Rathbun, 1897) (Lima and Oshiro, 2006).

Ponta Verde, Gaibu, and Carneiros have the highest tourist visitation rates, which could be the main cause for the observed decrease in the number of individuals of *P. transversus* in the larger CW size classes. Pontal do Coruripe, the least visited area, presented an opposite pattern, with individuals evenly distributed in all classes. Ponta Verde and Gaibu reefs are in more urbanized areas (Pádua et al., 2016; Vasconcelos et al., 2019) and, at Ponta Verde, trampling is a frequent impact once visitors are encouraged to walk on the reef as a tourist attraction. Intense trampling in coastal reefs may change the benthic community structure reducing algal and coral cover, disturbing the micro-habitat, decreasing the shelters of some species, reducing top predators, and potentially modifying the composition of the biodiversity of the area (Brossnan and Crumrine, 1994; Stevčić et al., 2018). Larger-sized animals are easily seen and, in places with intense visitation, are probably more captured, decreasing the individuals at the largest CW sizes (Murray et al., 1999). An urbanized tropical beach (Northeast of Brazil) with high visitation rates, presented similar CW distribution for *E. gonagra* and *P. transversus* individuals (Araújo et al., 2016), as observed in the present study. This pattern was also observed for the species *E. verrucosa* (Forskål, 1775) in reef crabs from Spain, where authors observed a decrease in size and population (Stevčić et al., 2018), attributing to anthropogenic interference.

Among the variables that can change the expected sex ratio (1:1), it is important to highlight environmental pressure, food availability, migration, differential behavior between the sexes, and abiotic factors (Wenner, 1972; Góes and Fransozo, 2000). The knowledge of these variables can provide clues to understand the reason that, in general, males are more abundant and larger on Pontal do Coruripe than in other reefs (Table 3). A previous study in this region reported a sex ratio favouring males, investigating the grapsoid *Plagusia depressa* (1.1:1; M: F; Rocha et al., 2019). Among all regions, Pontal do Coruripe has less tourist activity, with subsequent less trampling compared to other studied areas. Once males tend to adventure further away from their shelters (Furtado-Ogawa, 1977; Abele et al., 1986; Arab et al., 2015) they can be easily captured. In the other reefs (Gaibu, Carneiros, and Ponta Verde), the animals were less visible, increasing the sampling effort to capture them in crevices with tweezers. That probably increases the capture of females, which naturally stay more hidden than males. Other crabs caught in rocks, with similar habits, also presented the sex ratio in favour of males as *Menippe nodifrons* in Southeast of Brazil (Rodrigues-Alves et al., 2013).

The decrease in the number of *P. transversus* females observed in samplings after oil spill (Gaibu, Carneiros, and Pontal do Coruripe), is probably due to the oil blocked shelter entrances. Brachyuran females in general remain in crevices longer than males, especially if ovigerous, or in the presence of predators or humans (Abele et al., 1986; Campos and Oshiro, 2001). Behavioural changes were previously observed in crustaceans from oil-impacted areas probably to increased defensive behaviour (Burger et al., 1991), which can accentuate the cryptic habit of this species. Based on these facts, some hypotheses are raised for the lower capture of females after oil spill: (i) females were trapped in the crevices covered by oil and died of asphyxiation; (ii) females were unable to go back to their oiled shelters and ended up preyed upon or migrated to more protected areas; (iii) the two previous hypotheses occurred simultaneously, generating this marked decrease in females capture. This is an acceptable reason and could explain why this decrease was not observed at Ponta Verde, an area where oil did not reach the reefs.

Crabs population differences were observed between the most visited areas and those with lowest incidence of tourists or fisheries activities. Before oil spill, the areas already underwent chronic impacts due to sewage discharges and industrial residues. However, changes due to the oil spill occurred in August and September 2019 were also observed. It is remarkably important that future studies would carry out chemical analysis of the environment and evaluate sublethal effects, which may cause physiological, genotoxic, and behavioral changes in the species that inhabit the affected areas. All this highlights how important is to preserve and monitor coastal and marine environments concerning the impacts to which they are exposed. This monitoring can provide input for creating environmental education strategies and the implementation or expansion of protected areas, which can help to avoid or mitigate anthropogenic impacts on marine communities.

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5 ARTIGO 2 – POPULATION STRUCTURE OF *Eriphia gonagra* (FABRICIUS, 1780) IN SANDSTONE REEFS EXPOSED TO DIFFERENT DEGREES OF IMPACT IN NORTHEASTERN BRAZIL

ABSTRACT

The present study aims to describe and compare the population aspects of the reef crab *Eriphia gonagra* in four reef areas from Northeastern Brazil. We hypothesized that there are population differences between the areas, which may be related to the various types and degrees of anthropogenic impacts that these areas are exposed to, such as trampling and sewage discharge. Moreover, in August 2019, some reef areas of this study were affected by an oil spill that reached beaches on the coast of Brazil. There were manually sampled 222 individuals of *E. gonagra* from February 2019 to February 2020 and taken to the laboratory for measurement and analysis. The periods before and after the oil spill were analyzed in the areas that were affected (Gaibu, Carneiros and Pontal do Coruripe) and there were no influences, but we found some oiled individuals. At Ponta Verde and Carneiros was a decrease in medium size of carapace and maturation, when compared to crabs captured in other less visited areas. The main factors affecting population size are trampling and high visitation rates, common in tourist areas. The impacts of this oil spill and anthropogenic actions on reef benthic crustacean species should continue to be studied to obtain an overview of the health of these environments.

KEYWORDS: ERIPHIIDAE, CRUSTACEAN, OILED MARINE ANIMAL, ANTHROPOGENIC IMPACT, MARINE ENVIRONMENT

INTRODUCTION

The red-finger rubble crab *Eriphia gonagra* (Fabricius, 1780) belongs to the family Eriphiidae MacLeay, 1838 (Ng et al., 2008) and is part of the benthic fauna. *E. gonagra* is distributed across the western Atlantic coast from North Carolina, USA to Santa Catarina, Brazil (Melo, 1996). It inhabits rocky shores and other consolidated marine bottoms and can be found in varied locations, hiding in caves and other places. It also inhabits intertidal environments up to five meters deep in the infralittoral where it is found in rock crevices and natural burrows formed by overlapping rocks (Melo, 1996).

Studies carried out with *E. gonagra* have investigated larval development (Fransozo,

1987), relative growth (Góes and Fransozo, 1997), heterochely (Góes and Fransozo, 1998), sex ratio (Góes and Fransozo, 2000), fertility (Góes et al., 2005), use of habitat (Andrade et al., 2014), population structure (Araújo et al., 2016), reproduction (Teixeira et al., 2017), feeding habits (Rodrigues et al., 2020, Santana et al., 2022a), and gonad development (Rios et al., 2022). Despite not having commercial importance, this species represents an essential link in the food web, being important to the local cycling of nutrients and contaminants (Reynoldson, 1987). Besides, information on population biology is important and necessary for expanding ecological knowledge of the species and its life cycle, and how it is affected by anthropogenic impacts.

Benthic organisms are excellent indicators of environmental health due to their relatively long-life cycles in addition to their sessile or sedentary nature which makes them difficult for escaping from adverse environmental conditions (Blanchet et al., 2008; Boudaya et al., 2019). Moreover, benthic invertebrates have intimate contact with water and sediment which may contain contaminants that act as stressors (Hale and Heltshe, 2008; Boudaya et al., 2019). Benthic crustaceans are among the most abundant animals in reefs with sessile, sedentary and vagile species. This group has structured populations but may be sensitive to natural and anthropogenic environmental changes (Gesteira and Dauvin, 2000; Van Son and Thiel, 2007; Suciú, 2017).

Coastal reef environments are vulnerable to several human impacts, posing a risk to species that grow over them (Ferreira and Maida, 2006). In 2019, during samplings collection of population data for this study, an oil spill hit the coast of Brazil, spreading across 3000 kilometers from northeastern through southeastern localities of the country (Soares et al., 2020). The hypothesis of the present study is that there are a decrease in terms of size, relative growth, size of maturity and unbalance in sex ratio, in the populations of *E. gonagra*, among four reef areas in northeastern Brazil exposed to different types of impacts (e.g. sewage

discharge, tourism and oil spill).

METHODS

The four reefs investigated are located in northeastern Brazil: Gaibu - Cabo de Santo Agostinho ($8^{\circ} 20' 3''$ S, $34^{\circ} 56' 58''$ W), Carneiros – Tamandaré ($8^{\circ} 42' 14''$ S, $35^{\circ} 4' 45''$ W), Ponta Verde – Maceió ($9^{\circ} 39' 58''$ S, $35^{\circ} 41' 32''$ W) and Pontal do Coruripe – Coruripe ($10^{\circ} 9' 20''$ S, $36^{\circ} 7' 55''$ W) (Figure 1).



Figure 1. Geographical location of the study areas in northeastern Brazil.

The four investigated areas are exposed to trampling due to walking of visitors as well as other types of impact. At Ponta Verde, there are sewage outfalls (Pádua et al., 2016) and fishing corrals over the reefs. At Pontal do Coruripe, reefs are near the mouth of Coruripe

Creek that flows through sugarcane mills (Souza-Júnior et al., 2001). At Gaibu, there is small sewage outfalls and the site is close to the Port of Suape (Madruga-Filho, 2004). Finally, Carneiros, despite is located inside the Costa dos Corais marine protected area (CC-MPA), receives numerous recreational vessels such as speedboats and catamarans which might leak fuel or affect the reef structure due to anchoring (Firmino, 2006; Lourenço et al., 2015). In addition to the described chronic impacts, three of the four sites (Ponta Verde is the exception) were hit by and acute oil spill event between August and September 2019 (Soares et al., 2020; Araújo et al., 2020).

FIELD SAMPLING

A total of five quarterly sampling campaigns were carried out at each of the four sites between February 2019 and February 2020. *Eriphia gonagra* specimens were manually and randomly sampled using tweezers and gloves for two hours during low tide. The method of catch per unit effort (CPUE) included two persons. All specimens were placed in a plastic container with water and pebbles for replicating the natural environment and avoiding stress to the animals. Abiotic factors were measured in the field, including salinity (using a refractometer), water and air temperature (using a thermometer).

LABORATORY PROCEDURES AND STATISTICAL ANALYSES

In the laboratory, the specimens were identified, sexed, weighed and sized (with a 0.01 mm precision calliper). In females, measurements included cephalothorax width (CW) and abdomen width (AW) at its widest point in the third abdominal somite. In males, measurements included CW, length of the propod of the greater cheliped (CL) from the tip of dactyl to the articulation with carpus, width of the propod of the major cheliped (PW) and gonopod length (GL). The power function ($y = a.x^b$) was used to adjust the empirical points

of CW vs. CL, CW vs. GL and CW vs. PW regressions in males, and CW vs. AW regression in females. CW was set as the independent variable (adapted from Huxley, 1950). For males, regression with the highest coefficient of determination was used in this study. Isometric growth is denoted by a constant b equals to 1 while positive and negative allometric growths are denoted by $b > 1$ and $b < 1$, respectively.

Animal gonads were macroscopically examined based on their coloration and size in contrast to other viscera tissues and cephalothorax chamber (modified from Moura and Coelho, 2004). In females, four stages of gonad maturation were identified: (1) primary, (2) maturing, (3) mature, and (4) recovering immature (spent). Male gonads were assigned to either (1) immature or (2) mature. Gonad stages were classified according to the method described by Santana et al. (2022) (modified from Hartnoll, 1965 and Moura and Coelho, 2004). The estimated size at which 50% of the individuals of each sex are physiologically mature (L50) was determined based on the distribution of individuals by size class and the plot of a logistic curve (Moura and Coelho, 2004). The softwares Statistica® and RStudio® were employed for the statistical analyses described above.

Sex ratio was calculated using the frequency of males and females collected in each sampling date. Sex ratio was also compared before (BOS) and after (AOS) the oil spill. The chi-square (χ^2) test was performed to determine the significance of observed sex ratio discrepancies (Freitas and Santos, 2002). All analyses were performed setting $\alpha = 0.05$. Individuals were divided into size classes using the free software BioEstat® frequency tables. The Kolmogorov-Smirnov test was used to check normality of data distribution. The four reef sites were compared in terms of abiotic factors, CW average, and maturity size (Underwood, 1997; Diele et al., 2005) using one-way ANOVA followed by Tukey post-hoc test on the free software Past® 2.17.

RESULTS

ABIOTIC DATA

Higher air and water temperatures were recorded in all sites during February 2019, November 2019 and February 2020 (Table 1). Such months match up with dry season in northeastern Brazil. The lower salinities reported at Pontal do Coruripe and Carneiros over the sampling period indicate freshwater inputs from creeks Coruripe and Formoso, respectively. Salinity was lower during the wet season campaigns (May and August).

Table 1. Salinity, air and water temperatures in each sampling campaign at Gaibu, Carneiros, Ponta Verde and Pontal do Coruripe between February 2019 and February 2020.

	Air (°C)				
	Feb 2019	May 2019	Aug 2019	Nov 2019	Feb 2020
Gaibu	28.9	26.9	27.6	28.3	28.5
Carneiros	28.5	27.0	27.8	29.9	28.2
Ponta Verde	27.8	27.0	26.3	29.5	28.2
Pontal de Coruripe	28.2	26.3	27.8	28.0	28.0
	Water (°C)				
	Feb 2019	May 2019	Aug 2019	Nov 2019	Feb 2020
Gaibu	28.3	26.2	28.3	30.0	29.4
Carneiros	28.7	27.3	28.1	32.8	29.3
Ponta Verde	28.1	27.8	26.5	31.5	28.9
Pontal de Coruripe	28.5	26.5	28.2	30.4	29.0
	Salinity				
	Feb 2019	May 2019	Aug 2019	Nov 2019	Feb 2020
Gaibu	35.7	34.7	35.6	35.5	35.1
Carneiros	35.0	34.0	35.5	35.1	35.5
Ponta Verde	35.2	35.7	36.6	36.5	35.9
Pontal de Coruripe	34.7	33.0	34.6	35.5	35.0

ABUNDANCE, SIZE AND RELATIVE GROWTH

A total of 222 individuals of *E. gonagra* (118 males and 104 females, being 31 ovigerous) were captured in all sites (Table 2). The highest number of individuals caught was

recorded at Gaibu. Larger CW averages of *E. gonagra* were found at Pontal do Coruripe and Gaibu while lower CW averages were found at Carneiros and Ponta Verde with a small difference between them. Differences in mean CW between sites were not significant (ANOVA, $df=3$, $F=0.84$, $p > 0.47$) and CW data showed normal distribution (Figure 2). On average, females were larger than males in two of the four study sites (Carneiros and Pontal do Coruripe).

Table 2. Number of individuals (N), minimum (Min.), maximum (Max.), average, standard deviation (SD) of carapace width (CW) in millimeters (mm) of *Eriphia gonagra* from sandstone reefs in northeastern Brazil.

Area	Gaibu		Carneiros		Ponta Verde		Pontal do Coruripe	
Sex	F	M	F	M	F	M	F	M
N	44	40	35	40	16	17	9	21
CW Min. (mm)	14.65	16.65	15.37	12.44	19.78	18.45	19.75	12.67
CW Max. (mm)	35.84	39.36	34.76	38.57	34.79	35.29	34.87	40.37
CW Mean (mm)	27.60	27.97	26.45	26.25	26.52	26.85	28.56	25.64
CW SD (mm)	4.35	5.57	4.65	4.93	5.10	5.65	5.17	9.01

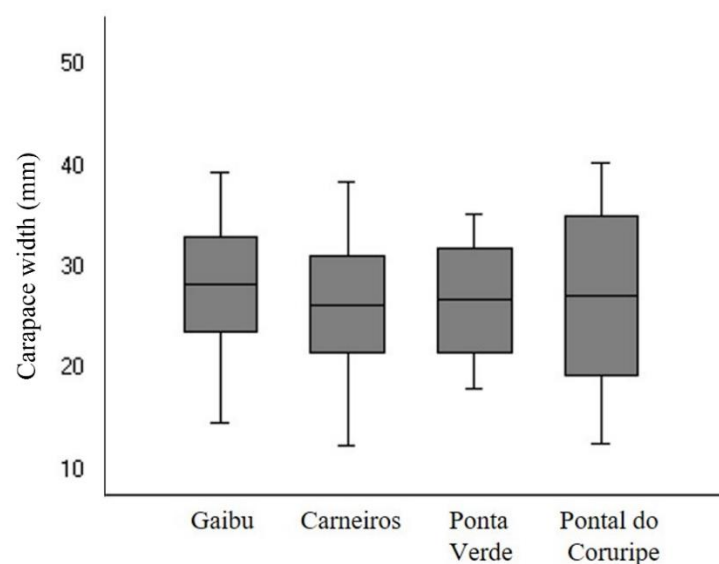


Figure 2. Box plot of carapace width (mm) of *Eriphia gonagra* from Gaibu, Carneiros,

Ponta Verde and Pontal do Coruripe. Upper bar is the maximum value, lower bar is the minimum value, top of box is the upper quartile, bottom of box is the lower quartile, and inner bar in the box is the average.

For males, the best growth model was fitted for GL vs. CW (Figure 3). In juvenile and adult females of *E. gonagra*, abdomen growth was allometric positive, except for juveniles from Pontal do Coruripe ($b = 0.2535$), but this is totally biased since the model was fitted with just two points.. In males, on the other hand, the gonopod length showed negative allometric growth in both life stages at Pontal do Coruripe and Gaibu with faster growth rates in juveniles than adults (again except for Pontal do Coruripe). Juveniles of both sexes from Gaibu, Carneiros and Ponta Verde exhibit faster growth rates than adult individuals.

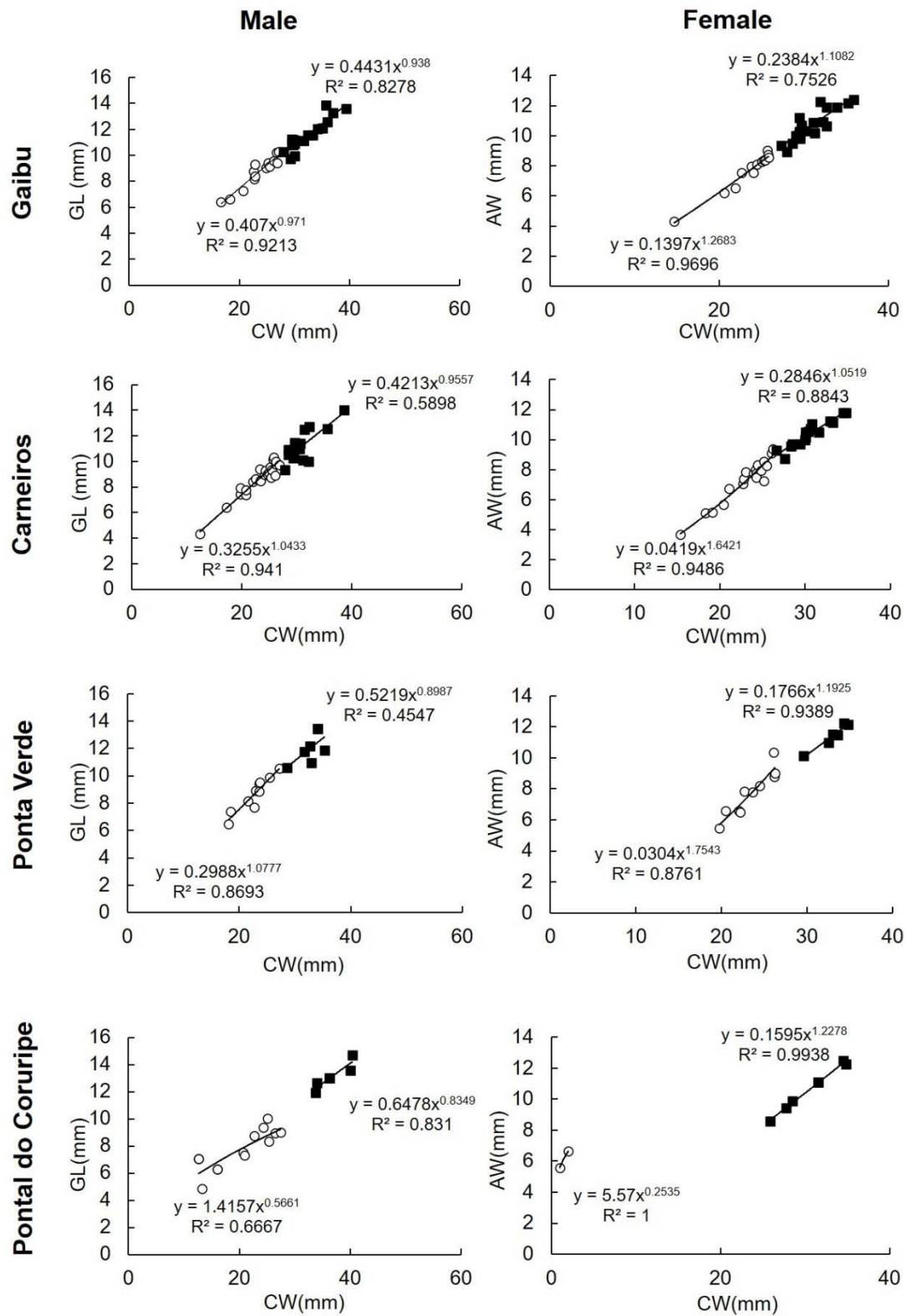


Figure 3. Relative growth models for juvenile (open circles) and adult (closed squares) individuals of *Eriphia gonagra* grouped according to sex (male and female) and sampling site (Gaibu, Carneiros, Ponta Verde and Pontal do Coruripe). For males, the model is fitted

between carapace width (CW) and length of the propodus of major gonopod (GL). For females, the model is fitted between carapace width (CW) and abdomen width (AW).

PHYSIOLOGICAL MATURITY

In the four sites, males of *E. gonagra* matured physiologically earlier than females. The size of the smallest ovigerous females also followed this pattern, the smallest being found at Carneiros and the largest at Ponta Verde. The physiological maturity of males was reached earlier at Gaibu and later at Pontal de Coruripe (Figure 4A). For females, the size of first physiological maturity occurred earlier at Carneiros and later at Pontal do Coruripe (Figure 4B).

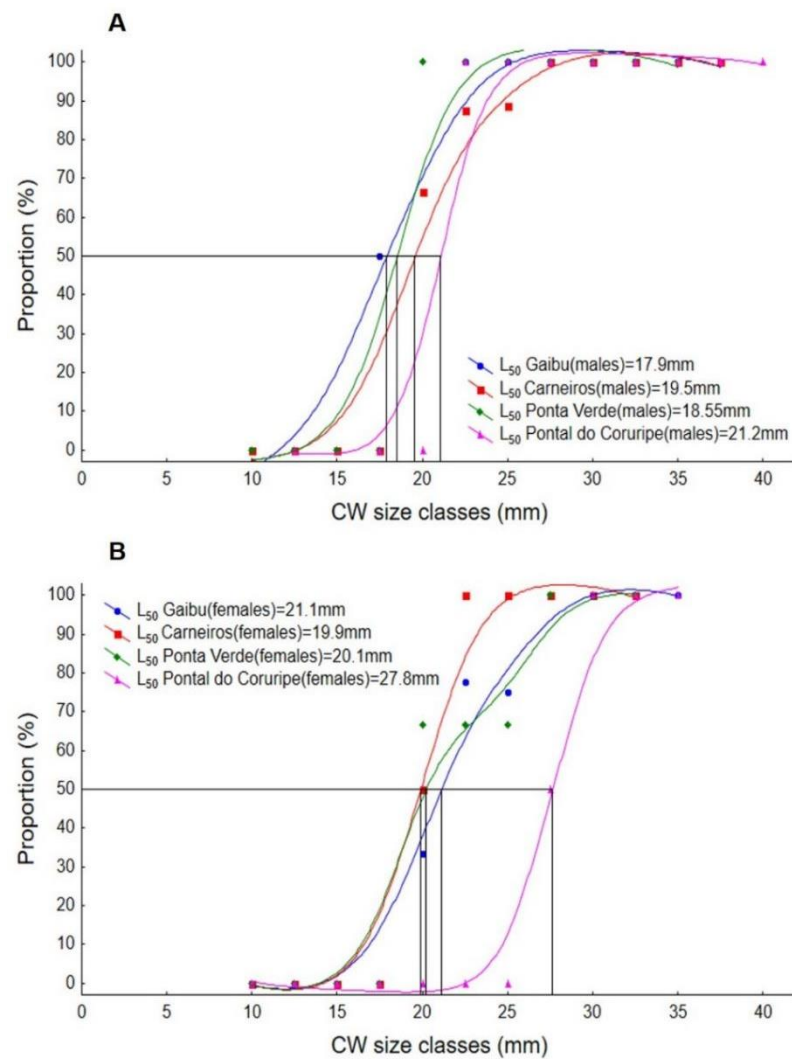


Figure 4. Physiological maturity (L_{50}) curve for males (A) and females (B) of *Eriphia gonagra* from Gaibu, Carneiros, Ponta Verde and Pontal do Coruripe, northeastern Brazil.

IMPACT OF OIL SPILL ON ABUNDANCE AND SEX RATIO

Sex ratio of *E. gonagra* differed significantly ($p < 0.05$) from the proposed 1:1 (M:F) proportion only at Pontal do Coruripe that exhibited a 1:0.42 ratio (male-biased) (Table 3). Despite oil spill hit Gaibu, Carneiros and Pontal do Coruripe between August and September 2019, there was no significant changes in the distribution of genders of *E. gonagra* before (BOS) and after (AOS) oil spill (χ^2 test < 3.84) (Table 4). During sampling in November 2019, it was observed many reef crevices obstructed with oil (Figure 5A), and even some oiled animals (Figures 5B and 5C). However, no dead individuals were found after the oil spill, that was supposed to be expected since sampling was carried out several weeks after the oil spill.

Table 3. Sex ratio of *Eriphia gonagra* from Gaibu, Carneiros, Ponta Verde and Pontal do Coruripe, northeastern Brazil. Ratios are calculated for several carapace width (CW, in mm) size classes.

CW size classes (mm)	Gaibu (M:F)	Carneiros (M:F)	Ponta Verde (M:F)	Pontal do Coruripe (M:F)
10.0 — 15.0	0:1	1:0	0:0	1:0
15.0 — 20.0	1:0*	1:1	1:0.5	1:0.5
20.0 — 25.0	1:1.71	1:1	1:1	1:0.2
25.0 — 30.0	1:1.05	1:0.58	1:1.33	1:0.6
30.0 — 35.0	1:1.83	1:1.83	1:0.8	1:2
35.0 — 40.0	1:0.33	1:0	1:0	1:0
40.0 — 45.0	0:0	0:0	0:0	1:0
Total	1:1.1	1:0.87	1:0.88	1:0.42*

*proportion differing significantly between the sexes ($p < 0.05$)

Table 4. Sex ratio (M:F) of *Eriphia gonagra* at the four study sites before (BOS) and after (AOS) the oil spill that hit the Brazilian coast between August and September, 2019. Ratios are calculated for several carapace width (CW, in mm) size classes.

Size class CW (mm)	Gaibu		Carneiros		Ponta Verde		Pontal do Coruripe	
	BOS	AOS	BOS	AOS	BOS	AOS	BOS	AOS
10.0 — 15.0	0:0	0:0	1:0	0:0	0:0	0:0	1:0	1:0
15.0 — 20.0	1:0	1:0*	1:1	1:1	1:1	1:0	1:0	1:0.5
20.0 — 25.0	1:2	1:1.33	1:0.57	1:1.75	1:0.75	1:1.5	1:0*	1:0.2
25.0 — 30.0	1:1	1:1.14	1:0.66	1:0.54	0:1	1:1	1:1	1:0.6

30.0 — 35.0	1:1.75	1:2	1:2.5	1:1.5	1:0.5	1:2	1:1.5	1:2
35.0 — 40.0	1:0.25	1:0.5	1:0	1:0	1:0	0:0	1:0	1:0
40.0 — 45.0	0:0	0:0	0:0	0:0	0:0	0:0	1:0	0:0
Total	1:1.12	1:1.06	1:0.75	1:1	1:0.7	1:1.14	1:0.35*	1:0.75

*proportion differing significantly between the sexes ($p < 0.05$)

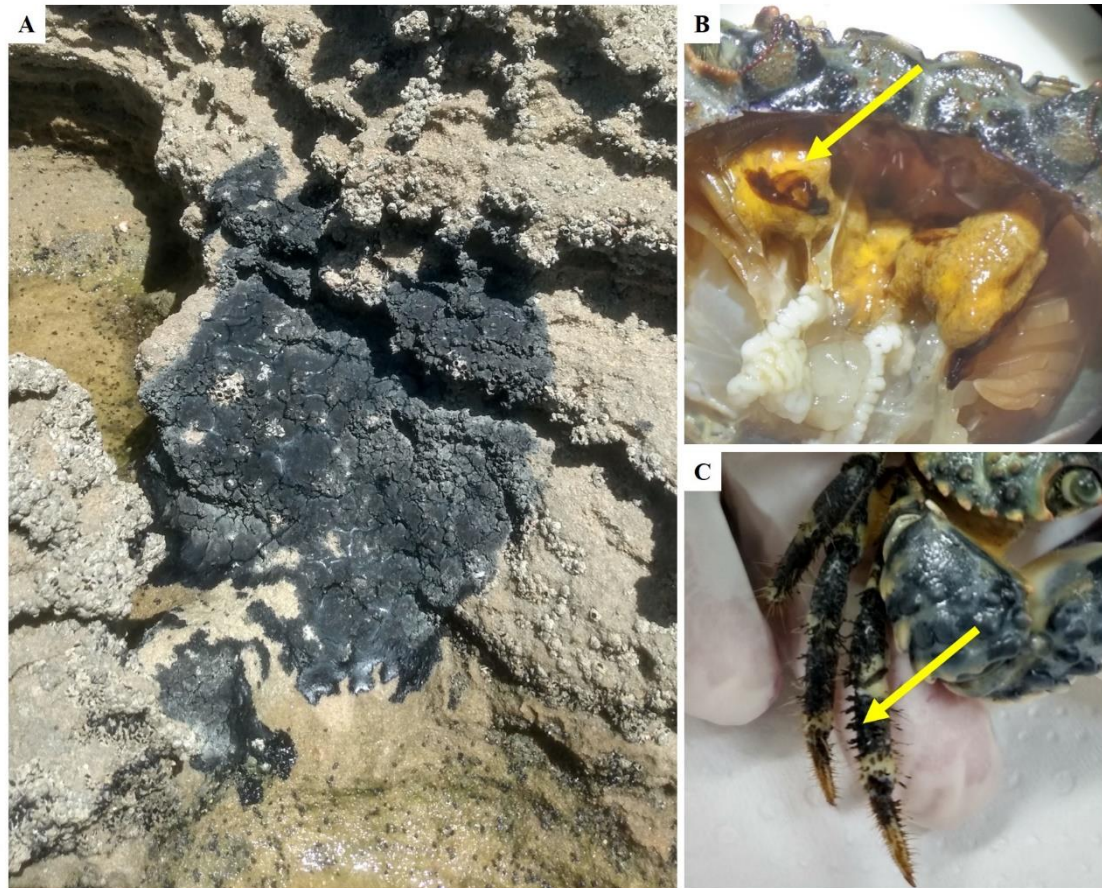


Figure 5. (A) Oil stuck to a reef surface at Gaibu. (B) Oil over hepatopancreas of *Eriphia gonagra*. (C) Oil stuck to bristles of pereopods of *Eriphia gonagra*.

DISCUSSION

In this study, it was observed a decrease in the number of large individuals of *E. gonagra* at Ponta Verde and Gaibu followed by Carneiros. The opposite trend was observed in the least touristically visited site (Pontal do Coruripe) across all *E. gonagra* size classes. The beaches of Ponta Verde and Gaibu are more urbanized among all study sites (Pádua et al., 2016; Vasconcelos et al., 2019). However, reef trampling is more problematic at Ponta Verde

since visitors walk over the reef for accessing the lighthouse that is a well-known tourist spot. Intense visitation on reefs can cause changes in community structure, including reduction of algal and coral cover. This changes micro-habitats, decreases shelters for some species, and causes a decrease on abundance of top predators, modifying composition of local biodiversity (Brossnan and Crumrine, 1994; Stevčić et al., 2018). A decrease in size of *Eriphia verrucosa* (Forskål, 1775) crabs as well as population decrease has been also observed by Stevčić et al. (2018) on Spanish rocky shores exposed to intense anthropogenic interference.

On average, females of *E. gonagra* were larger than the males in two out of four study sites (Carneiros and Pontal do Coruripe). This is in agreement with findings of Góes and Fransozo (1998) for the same species at Ubatuba (São Paulo, Brazil). Similar findings have been observed for other species of the superfamily Eriphioidea such as *Eriphia sebana* (Shaw and Nodder, 1803) in Hong Kong, China (Coombes and Seed, 1992) and *Menippe nodifrons* Stimpson, 1859 in Ubatuba, Brazil (Rodrigues-Alves et al., 2013). Conversely, males larger than females were found at Gaibu and Ponta Verde in this study. Such findings have been also found for the same species in Recife (Pernambuco, Brazil) (Araújo et al., 2016) and for *P. transversus* in Ceará and São Paulo also in Brazil, and Portugal and Lebanon (Furtado-Ogawa, 1977; Flores and Negreiros-Fransozo, 1999a; Flores and Paula, 2002; Arab et al., 2015, respectively). Sexual dimorphism with males larger than females is common in grapsoid crabs (Flores and Paula 2002; Castiglioni et al., 2011; Santana et al., 2018). Although less frequent, it can also occur in eriphioid crabs as it has been observed in Spain for *E. verrucosa* (Forskål, 1775) (Pérez-Miguel et al., 2017). In this study, *E. gonagra* males larger than females were collected in sites with greater tourist visitation (Gaibu and Ponta Verde). This stressor can influence the capture effort of the species in sites with high trampling. In such conditions, females and juveniles tend to hide in reef crevices while males remain over the reef (Stevčić et al., 2018), facilitating their visibility and capture by researchers. In other locations, there

would be a greater effort to capture individuals in reef crevices which may increase the capture rate of large females.

Eriphia gonagra males were more abundant in the larger size classes (> 35 mm) in the four sites although differences were not statistically. Prevalence of males in the larger size classes was also recorded for the same species in Ubatuba (> 38 mm) by Andrade et al. (2014) and in Recife (> 17.5 mm) by Araújo et al. (2016). Males are generally more numerous in the larger size classes because females drive their energy for egg production and nutrition after puberty molting, whereas males need to grow to become stronger for territorial disputes and fights for females (Araújo et al., 2012; Santana et al., 2018). Larger females that are physiologically mature for reproduction tend to spend more time hidden when they are ovigerous. In addition, they are stronger than smaller females and consequently more difficult to be captured which might contribute for the observed deviation in sex ratio (Abele et al., 1986; Campos and Oshiro, 2001).

In this study, for males, the variables GL vs. CW represented the best fit for estimating relative growth. The same variables were pointed out as the most suitable for performing morphometric analysis in *E. gonagra* at Ubatuba (Góes and Fransozo, 1997; Bertini et al., 2007). Those variables were also used for modeling growth of *M. nodifrons* in Ubatuba and the grapsoid crab *Plagusia depressa* (Fabricius, 1775) in Pontal do Coruripe (Alagoas, Brazil) (Rocha et al., 2019). For analyzing allometric growth in crabs, AW (for females), GL and CP (for males) are useful variables because growth rates usually change after individuals reach the puberty moulting (Castiglioni and Coelho, 2011; Araújo et al., 2012). In males, the growth of gonopods, increase the size variation in carapace width of females able to copulate with them, and chelipeds are developed for aiding in courtship and territorial disputes (Warner, 1970; Pescinelli et al., 2015).

The positive allometric growth of AW in juvenile and adult females and GL in juvenile

males of *E. gonagra* as well as the negative allometry in adult males have been previously described for the superfamily Eriphioidea (Góes and Fransozo, 1997; Bertini et al., 2007; Araújo et al., 2016). In this study, findings of Carneiros and Ponta Verde are in agreement with data previously published in the literature. Conversely, the negative allometric growth of juvenile males at Gaibu and juvenile males and females at Pontal do Coruripe are novel for *E. gonagra*. At Gaibu, parameter b in the growth curve of juveniles was close to isometry although still negative ($b = 0.971$). Anyways, it was also larger than for adults ($b = 0.938$), similarly to growth rates found in the other sites. A higher rate of gonopod growth in juvenile stage was recorded for *P. transversus* by Flores and Negreiros-Fransozo (1999b). These authors associated such findings with differentiation of the gonopods that occurs in this ontogenetic stage, marking the major sexual dimorphism among brachyuran species. At Pontal do Coruripe, the change in the pattern of allometry, different from that observed in the other areas, coinciding with the highest value of physiological maturity of the males. Thus, in the area where the animals matured later, juveniles showed a lower rate of growth of the dependent variable than in other areas, with an increase in gonopod development after the molt puberty (Santos et al., 2018). The limited number of juvenile females ($n = 2$) sampled at Pontal do Coruripe hinders any comparison with data from other sites.

The slight female-biased sex ratio (1:1.1; M:F) of *E. gonagra* observed at Gaibu was also observed by Góes and Fransozo (2000) (0.93:1; M:F). Similarly to this study, the authors did not find statistically significant differences. Conversely, females of *E. gonagra* were significantly more abundant than males at Boa Viagem Beach, Brazil (0.82:1; M:F) (Araújo et al., 2016) and in sandstone reefs built by *Phragmatopoma lapidosa* Kinberg, 1866 in Praia Grande, Brazil (1:1.4; M:F) (Andrade et al., 2014).

Environmental pressure, food availability, migration, differential behavior between sexes, and abiotic factors are among elements that can modify the predicted sex ratio of 1:1

(Wenner, 1972; Góes and Fransozo, 2000). These variables can provide clues for understanding the significant bias toward males of *E. gonagra* at Pontal do Coruripe (1:0.42; M:F). They may also explain why large males are more frequently observed in this site than in other reefs (Table 3). Another study at Pontal do Coruripe found that males of the grapsoid *Plagusia depressa* occur at higher proportion than females (1.1:1; M:F) (Rocha et al., 2019). Pontal do Coruripe is exposed to lesser tourist activity than the other sites and consequently has less trampling. As a result, males that prefer to get out of shelter are less susceptible to being trampled (Furtado-Ogawa, 1977; Abele et al., 1986; Arab et al., 2015) and can be more easily captured. In the other sites (Gaibu, Carneiros and Ponta Verde), there are fewer apparent animals and the effort for capturing them in crevices is greater, boosting the probability of catching females that spend more time hidden than males. Other eriphioid crabs such as *Menippe nodifrons* that are caught in rocks and have similar hiding habits also exhibit sex ratios biased toward males. (Rodrigues-Alves et al., 2013).

Oil spills represent occasional environmental impact, usually as a consequence tanker accident or platform leaks (Krebs and Burns, 1977; Yim et al., 2020). In the 2019 oil spill that hit coastal environments of northeastern Brazil, many marine species were affected because large patches of oil impacted beaches and reefs inhabited by intertidal organisms (Araújo et al., 2020; Lourenço et al., 2020; Soares et al., 2020). Oily material stucked to substrates can cause risks in the short, mid and long term since toxic chemicals may bioaccumulate in organisms and potentially cause physiological, morphological and behavioral changes (Burger et al., 1991; Soares-Gomes et al., 2010). *E. gonagra* individuals did not show significant immediate differences in capture before and after oil spill.

CONCLUSION

Although the species did not show immediate visible responses to the oil spill, about

the sex ratio, or complete disappearance of the species, it is important to continue monitoring this and other species of marine animals, since various sublethal damages can be observed, but which can still affect behavior, reproduction, feeding and survival of the species in that environment. The present study, therefore, provides important information on how this species responds to different impacts, both acute and chronic, on the environment, serving as a basis for future studies with this and other crustacean species.

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6 ARTIGO 3 - GENOTOXIC AND MORPHOLOGICAL DAMAGES IN TWO SPECIES OF REEF CRABS EXPOSED TO SEDIMENTS CONTAMINATED BY PAHS, ORGANOCHLORINES AND TRACE METALS

Abstract: The present study aimed to investigate the influence of Polycyclic Aromatic Hydrocarbons (PAHs), Organochlorine compounds (OCPs) and Trace Metals (TMs) in sediments upon the health of brachyurans crabs in four tropical reef environments. Crabs of the species *Pachygrapsus transversus* and *Eriphia gonagra* were manually captured in four reef areas of northeastern Brazil. Hemolymph cells were analyzed for the presence of micronucleated cells, which are indicators of potential environmental stress. Sediment samples from the reefs were collected to investigate organics (PAHs and OCPs) and trace metals, respectively. During the studied period, at the end of August 2019, slick spots with tons of crude oil reached the beaches and estuaries in the northeast and part of southeastern coast of Brazil. After the oil spill, were captured some individuals with morphological changes and oiled specimens of *Eriphia gonagra* and with morphological changes. The frequencies of micronucleated cells of both species increased significantly in all affected areas. PAHs and OCs concentrations are related to genotoxic and morphological damages. Besides generating subsidies for environmental education actions and data on how human actions affect marine organisms, it is expected that these results will help understand how species react to these and other environmental stresses, not only on the Brazilian coast, but all over the planet.

Keywords: micronucleus test, oil spill, anthropogenic impact, malformation

INTRODUCTION

With the growing urbanization of coastal areas over the centuries, aquatic environments have been impacted by human actions and anthropogenic stressors such as sewage, heavy metals, pesticides (including organochlorines – OCPs), polycyclic aromatic hydrocarbons (PAHs), among others. Some contaminants such as PAHs and OCPs are chemically stable,

hydrophobic and are associated with both consolidated and unconsolidated substrates. They can remain unaltered in an environment for a long time, becoming bioavailable for organisms that interact with the benthic ecosystem (Griscom and Fisher, 2004; Caruso and Alaburda, 2008; Ferreira et al., 2010). These contaminants can affect species, causing morphological, genotoxic and behavioral changes or even death (Torreiro-Melo et al., 2015; Mendes, 2017; Hong et al., 2018; Huang et al., 2019; Jesus et al., 2020; Souza et al., 2021). Anthropogenic impacts of metals and organic pollutants have been investigated through assessment of sublethal effects in tissues, urine or hemolymph (Fillmann et al., 2004; Pinheiro et al., 2021).

Benthic crustaceans and mollusks are the most abundant animals in tropical reefs (Garcia-Hernandez et al., 2014; Silva et al., 2020). Two of the most abundant crab species in reefs of the southwestern Atlantic are *Eriphia gonagra* (Fabricius, 1781) and *Pachygrapsus transversus* (Gibbes, 1850) (Araújo et al., 2016). These crabs inhabit intertidal zones, living in crevices of rocky shores, sandstone and coral reefs. *P. transversus* also lives over pier pillars and roots of the red mangrove *Rhizophora mangle* L. (Abele, 1976; Melo, 1996). Both species are sympatric and their interactions have already been studied (see e.g. Abele et al., 1986; Araújo et al., 2016).

Several studies on the effects of anthropogenic activities and environmental contamination on crustaceans have been carried out, mainly on species of commercial value (Turoczy et al., 2001; Souza et al., 2008; Nudi et al., 2010; Magalhães et al., 2012; Rodrigues et al., 2013; Pinheiro et al., 2013; Hong et al., 2018). *P. transversus* and *E. gonagra* are abundant and relatively resilient and have been often used as indicators of reef environmental health (Rodrigues et al., 2020; Barros et al., 2020; Azevedo-Farias et al., 2021; Santana et al., 2022).

This study investigated the concentration of PAHs, OCPs and trace metals in sediments of reef environments that are exposed to different anthropogenic impacts in northeastern Brazil. Correlation between contamination and morphological and genotoxic damages in crabs *E. gonagra* and *P. transversus* were investigated in four study sites. Over the course of this research, a major oil spill event hit more than 3000 km across the coast of Brazil and impacted sensitive ecosystems including sites investigated here (Lourenço et al., 2020). This was an unexpected opportunity for observing changes in the health of crabs, comparing samplings carried out before and after the oil spill.

MATERIAL AND METHODS

Study area

The reefs studied are located in four sites across the northeastern coast of Brazil: Gaibu (8° 20" S, 34° 56' W), Carneiros (8° 42' S, 35° 4' W), Ponta Verde (9° 39' S, 35° 41' W) and Pontal do Coruripe (10° 9' S, 36° 7' W) (Figure 1). All sites have sandstone reefs covered by algae and corals that house several other species. Each site was chosen based on human activities developed in its surroundings and have different sources of contamination for the local marine environment (see Santana et al., 2022b). In addition to chronic pollution, three out of four reefs (except Ponta Verde) were affected by oil spill in late 2019. This event left oil residues stuck to sandreef surfaces despite all cleaning efforts (Figure 2).

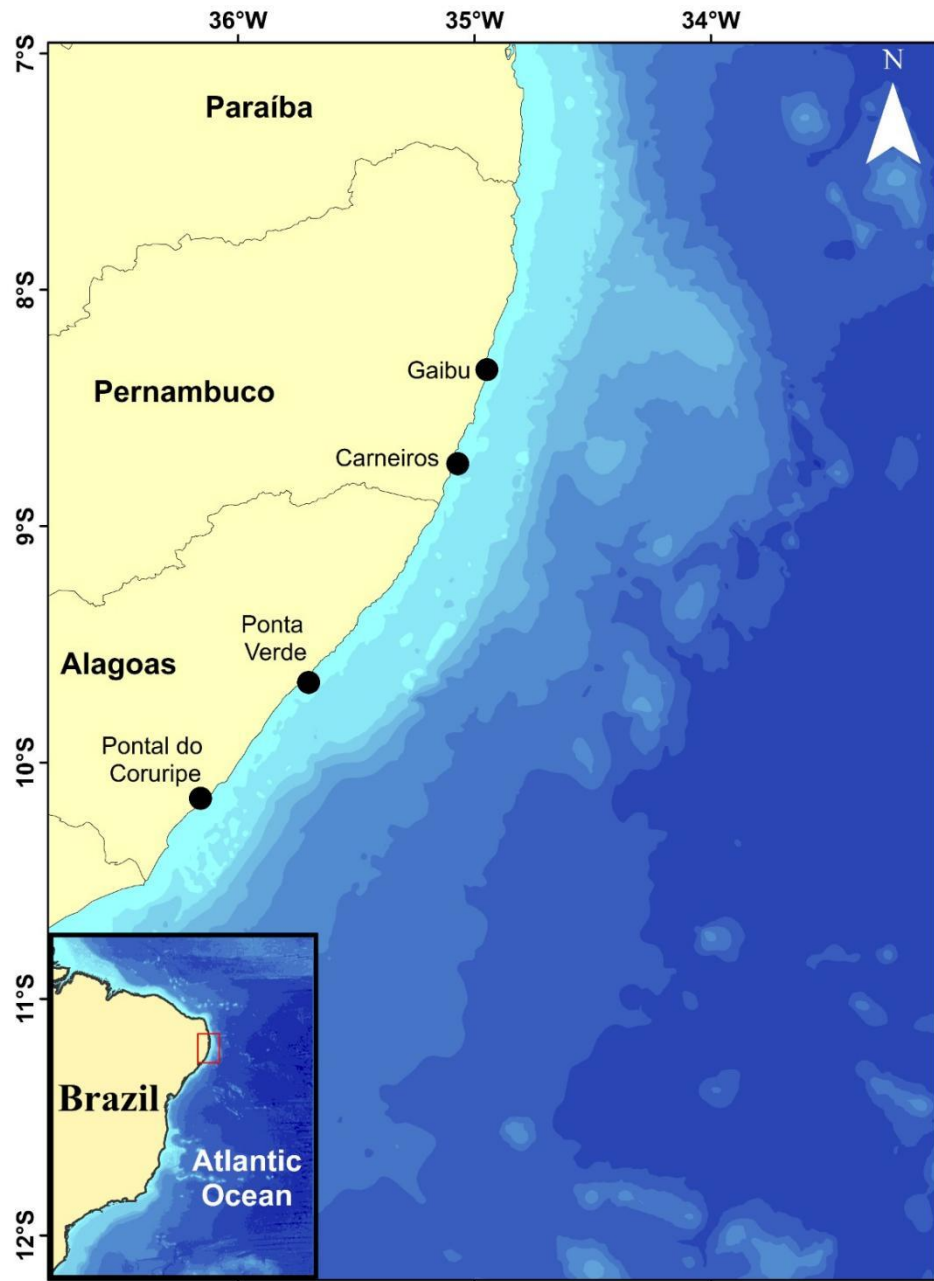


Figure 1. Study sites in northeastern Brazil: Gaibu and Carneiros located in Pernambuco State, and Ponta Verde and Pontal do Coruripe located in Alagoas State.

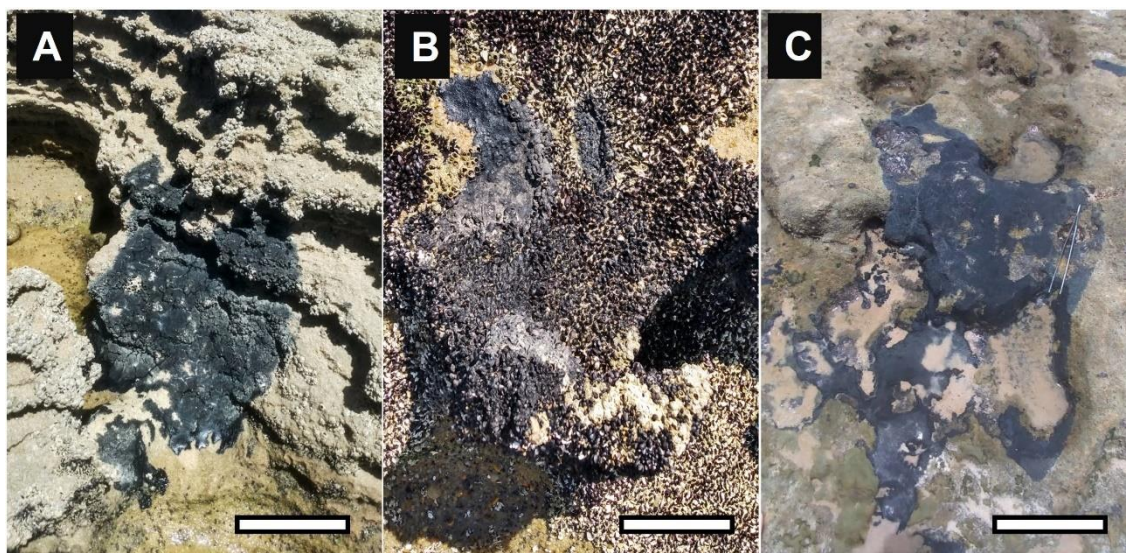


Figure 2. Oil patches stuck to sandstone reefs at (A) Gaibu, (B) Carneiros and (C) Pontal do Coruripe in November, 2019 (i.e. after the oil spill event). Scale bar: 15 cm.

Sampling procedures

Sampling was carried out in February, May, August and November 2019, and February 2020 during low tide. Four quadrats (30x30m) were sampled at each reef site (Supplementary S1). Air and water temperature and salinity were measured using a thermometer and a refractometer, respectively (Supplementary S2). For the analysis of PAHs and OCPs, surface sediment was collected from pools on the top of reefs with a stainless-steel spoon and samples were placed in aluminum containers previously combusted at 450 °C for 4 hours. For trace metal analysis, surface sediment was collected with a plastic spoon and stored in plastic bags previously cleaned with 10% hydrochloric acid (HCl). Both samples were temporarily stored in a cooler containing ice for transportation to the laboratory where they were kept in a freezer at -20 °C until analysis.

The largest possible number of the reef crabs *E. gonagra* and *P. transversus* were collected by two researchers over a two-hour period (30 minutes in each quadrat). The caught

specimens were transported alive to the laboratory in plastic containers with pebbles, sediment, and water for replicating the natural environment and avoiding stress to the animals.

Laboratory procedures

Chemical analysis and sediment properties

Organic matter and sediment grain size

The analysis of organic matter was performed according to Davies (1974). Briefly, an aliquot of 4 g of sediment from each sample was weighed and combusted in a furnace at 450 °C for 6 hours. Organic matter content was estimated gravimetrically by difference between initial and final sample weight.

Sediment grain size was determined by dry sieving method (Suguio, 1973). A 30 g aliquot of sediment was weighed and shaken through sieves with different meshes for 12 minutes. The material retained on each sieve was weighed for calculation of percentages of the following fractions: gravel, coarse sand, medium sand, fine sand, and mud (silt + clay).

Polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs)

Samples were freeze-dried, ground and passed through a 500- μ m mesh sieve. Aliquots of 15 g of sediment were used for PAHs and OCPs analysis. Exactly 100 μ L of PAH (acenaphthene-d10, phenanthrene-d10, and chrysene-d12 at 1000 ng mL⁻¹) and OCP (DBOFB and TCMX at 1000 ng mL⁻¹) internal standards were added to the samples. Sediments were Soxhlet-extracted with 80 mL of a mixture containing n-hexane:dichloromethane (1:1; v/v) for 8 hours. The extracts were concentrated down to 1 mL and split into two equal parts. The first one was cleaned up and fractionated in a column filled with silica/alumina (5% deactivated) for PAH determination. The column was sequentially eluted with 10 mL of n-hexane and 15 mL

of a mixture of n-hexane:dichloromethane (7:3; v/v). Just the last 15 mL were collected for PAH analysis (Arruda-Santos et al., 2018). The second part of the extract was cleaned up in a column filled with alumina (5% deactivated) (7:3; v/v) for OCP analysis (Zanardi-Lamardo et al., 2019). Both fractions were injected in a gas chromatograph (Agilent Technologies 7820A GC System) equipped with a fused silica capillary column (HP-5MS, 30m x 25 mm x 0.25 μ m) and coupled to a mass spectrometer (Agilent Technologies 5975C MS) (GC-MS). Full details on these chromatographic procedures are described elsewhere (e.g. Zanardi-Lamardo et al., 2019).

This study investigated the 16 priority PAHs listed by USEPA (United States Environmental Protection Agency) based on their toxic potential. For OCPs, α -HCH, β -HCH, γ -HCH, δ -HCH, aldrin, dieldrin, endrin, endrin aldehyde, endrin ketone, α -chlordane, γ -chlordane, heptachlor, heptachlor epoxide, endosulfan I, endosulfan II, endosulfan sulfate, o,p'-DDT, p,p'-DDT, o,p'-DDD, p,p'-DDD, o,p'-DDE, p,p'-DDE, metoxychlor, and mirex. Quality control was based on laboratory blanks, internal standards recoveries, and duplicate analysis of certified reference material (SRM 1944 –New York/New Jersey Waterway Sediment acquired from the United States National Institute of Standards and Technology, NIST).

Recovery of the internal standards ranged from 45 to 115%. Average recovery of analytes in the SRM 1944 was $81 \pm 19\%$ of the certified concentrations. The limit of quantification (LOQ) was calculated as the ratio of the lowest concentration in the analytical curve to the mass of sediment extracted, resulting in 0.06 ng g^{-1} for PAHs and OCPs (Wade and Cantillo, 1994; Lauenstein and Cantillo, 1998). Concentrations are reported on a dry weight basis.

Trace metals (TM)

Samples were manually pulverized, using pestle and mortar, to particle sizes of a limit of 500 µm. An aliquot of 100 g was sent to SGS GEOSOL Laboratory (<https://www.sgsgeosol.com.br>) for metal analysis. The extraction procedure included multiacid digestion with HNO₃, HF, HClO₄ and HCl. A total of 9 elements were analyzed: Ag, As, Cd, Cr, Cu, Hg, Ni, Pb and Zn. These metals were identified and quantified by inductively coupled plasma mass spectrometry (ICP-MS). The standard reference material (Canadian Certified Reference Material TILL-3) fell within the standard deviation of the certified concentrations for all elements analyzed. The coefficient of variation (CV) between duplicates ranged from 4 to 12%.

Micronucleus test

Crabs sampled in the field received an identification code. Hemolymph was punctured in the pereopod joint using a hypodermic syringe with 21-gauge needle containing an anticoagulant solution (Scarpato et al., 1990; Pinheiro et al., 2013; Shields, 2017). The micronucleus test followed the protocol described by Scarpato et al. (1990). The prepared slides were analyzed under an optical microscope using a 100x lens with immersion oil (Schmid, 1995; Cabral, 2017). In each slide, 1000 cells were counted for estimating micronucleus frequency according to the formula $MNF(\%) = (\text{micronucleated cells} / \text{total of counted cells}) \times 100$ (Duarte et al., 2012). Classification of suitable hemocytes followed descriptions done by Matozzo and Marin (2010).

After extracting the hemolymph, specimens were weighed and measured in carapace width (CW), using a precision balance (0.001 g) and a precision caliper (0.01 mm). External morphology was carefully analyzed, searching for alterations that were photographed when found. Crabs were finally dissected by a dorsal incision bordering the carapace in order to check integrity of internal structures.

GB1	0.7 ± 0.8	19.1 ± 12.1	49.9 ± 12.8	29.5 ± 12.8	1.0 ± 1.0	0.3 ± 0.2
GB2	1.4 ± 1.2	16.7 ± 5.5	40.9 ± 13.6	38.5 ± 16.8	2.4 ± 1.2	0.7 ± 0.3
GB3	0.5 ± 0.4	19.2 ± 8.8	52.3 ± 15.5	27.3 ± 18.8	0.7 ± 0.5	0.1 ± 0.1
GB4	1.9 ± 3.4	21.7 ± 10.3	62.0 ± 16.4	13.3 ± 9.7	1.1 ± 1.9	0.5 ± 0.7
Carneiros						
CA1	5.4 ± 9.9	18.0 ± 19.8	17.2 ± 15.5	59.0 ± 27.9	0.4 ± 0.3	0.5 ± 0.3
CA2	7.6 ± 8.2	47.4 ± 15.6	17.5 ± 8.0	24.8 ± 14.3	2.7 ± 2.2	0.5 ± 0.3
CA3	1.9 ± 1.0	51.1 ± 14.3	29.1 ± 5.8	16.0 ± 12.9	1.9 ± 1.4	0.5 ± 0.4
CA4	2.2 ± 1.6	54.0 ± 28.5	31.5 ± 19.5	10.9 ± 14.2	1.4 ± 2.6	0.3 ± 0.2
Ponta Verde						
PV1	5.3 ± 4.0	31.9 ± 11.3	21.6 ± 12.5	39.7 ± 22.5	1.6 ± 2.6	0.4 ± 0.2
PV2	4.2 ± 3.1	41.2 ± 14.7	29.1 ± 13.5	24.4 ± 28.0	1.1 ± 1.4	0.4 ± 0.2
PV3	2.5 ± 3.0	42.3 ± 25.6	25.0 ± 11.9	28.1 ± 30.0	2.2 ± 4.3	0.5 ± 0.2
PV4	2.1 ± 2.9	41.6 ± 26.6	23.7 ± 13.9	31.2 ± 34.3	1.5 ± 2.1	0.3 ± 0.1
Pontal do Coruripe						
PC1	1.6 ± 1.1	19.4 ± 7.2	14.4 ± 5.5	62.9 ± 13.9	1.7 ± 2.6	0.8 ± 1.0
PC2	1.2 ± 0.8	18.0 ± 18.1	18.0 ± 12.4	59.5 ± 28.8	3.3 ± 2.4	0.2 ± 0.1
PC3	1.9 ± 2.2	20.7 ± 23.0	15.6 ± 5.3	60.5 ± 29.5	1.4 ± 2.1	0.2 ± 0.1
PC4	1.6 ± 2.1	23.0 ± 20.7	29.8 ± 21.8	44.6 ± 33.9	1.2 ± 1.6	0.2 ± 0.1

PAHs

Total PAHs, defined as the sum of 17 congeners (naphthalene (Nap), 2-methylnaphthalene (M-Nap), acenaphtylene (Acy), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IP), dibenzo[a,h]anthracene (DahA), benzo[ghi]perylene (BghiP)), ranged from 0.2 ng g⁻¹ at Pontal do Coruripe in Aug/19 to 633 ng g⁻¹ at Gaibu in Nov/19 (Table 2 and Supplementary S3, S4, S5, S6, and S7). Concentrations reported for each sample within a site three months before the oil spill were similar and exhibited no statistical difference ($p > 0.05$). Therefore, they were grouped as before oil spill (BOS) (Figures 3, 4, 5 and 6). This similarity did not occur for sampling campaigns in November/19 and February/20, so they were labeled as right after oil spill (RAOS) and recovery

(REC) post oil spill, respectively. Average sediment contamination before and after oil spill showed an increase in sites impacted by large oil patches (Figures 3 and 6). Interestingly, concentrations are in the same order of magnitude at Carneiros and Ponta Verde, with a slight increasing trend not statistically significant ($p > 0.05$) (Table 2).

Table 2. Average concentration and standard deviation of $\Sigma_{17}\text{PAHs}$ detected in sediment samples from the four reef sites in northeastern Brazil.

Month/Area	Gaibu	Carneiros	Ponta Verde	Pontal de Coruripe
	$\Sigma_{17}\text{PAHs}$ (ng g ⁻¹ dry weight)			
Feb/19	1.2 ± 0.6	1.4 ± 1.7	2.1 ± 2.2	1.6 ± 1.2
May/19	2.2 ± 2.0	2.3 ± 1.7	1.3 ± 0.3	4.3 ± 2.1
Aug/19	10.2 ± 8.3	1.7 ± 1.3	7.7 ± 6.9	0.6 ± 0.3
Nov/19	164 ± 313	1.8 ± 0.8	2.8 ± 1.7	266 ± 179
Feb/20	3.6 ± 5.1	6.1 ± 1.7	3.5 ± 3.5	13.4 ± 7.4

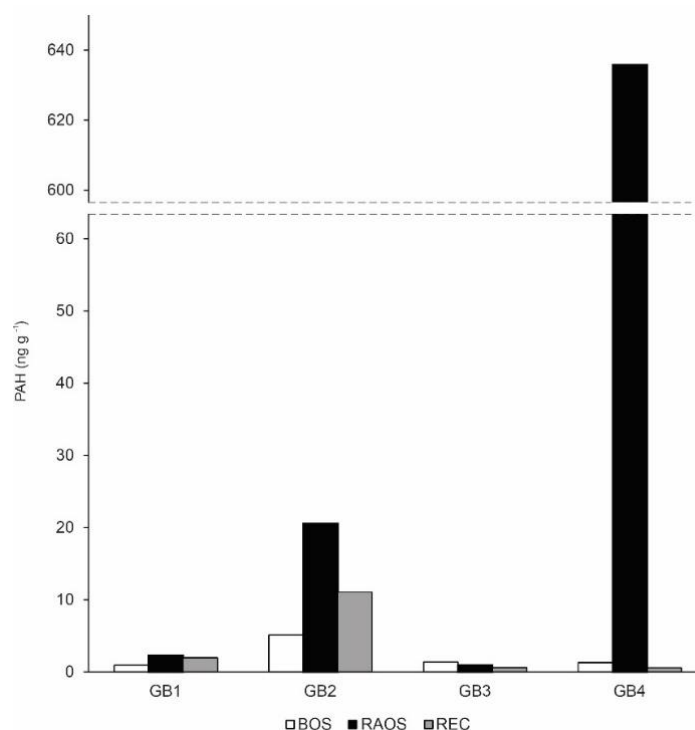


Figure 3. Total PAH ($\Sigma_{17}\text{PAH}$) concentrations in surface sediments collected before oil spill (BOS), right after oil spill (RAOS), and during recovery (REC), post oil spill at Gaibu, Pernambuco, northeastern Brazil.

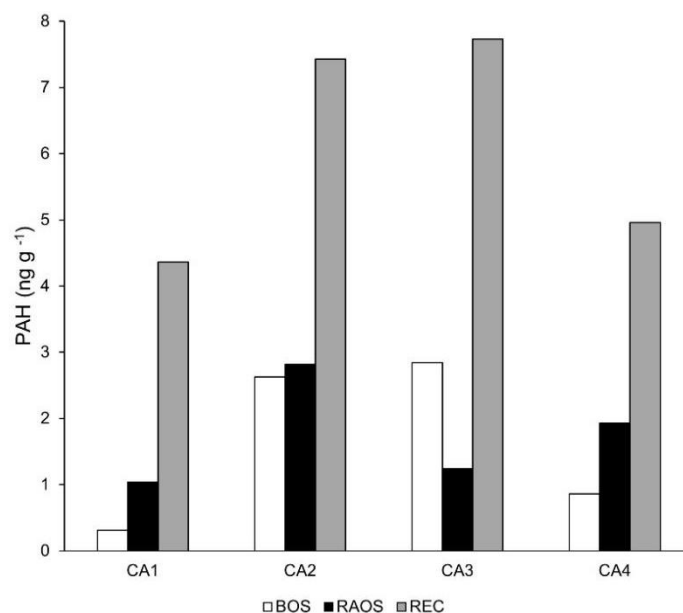


Figure 4. Total PAH ($\Sigma_{17}\text{PAH}$) concentrations in surface sediments collected before oil spill (BOS), right after oil spill (RAOS), and during recovery (REC) post oil spill at Carneiros, Pernambuco, northeastern Brazil.

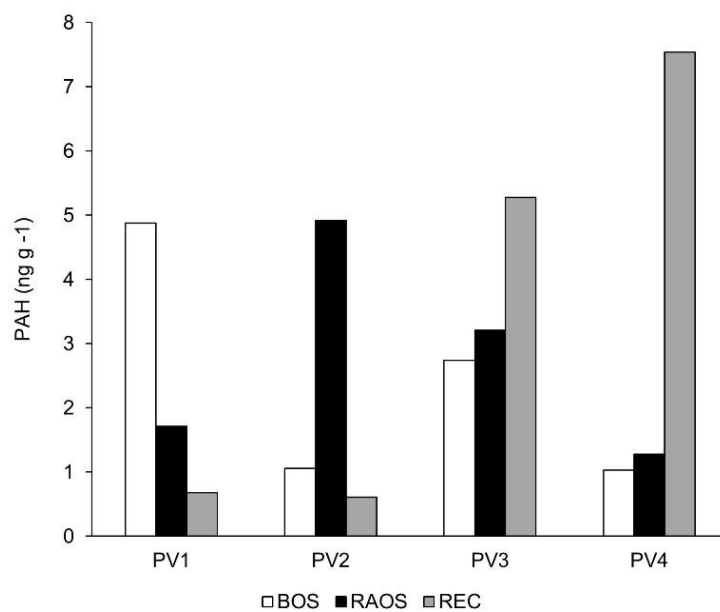


Figure 5. Total PAH ($\Sigma_{17}\text{PAH}$) concentrations in surface sediments collected before oil spill (BOS), right after oil spill (RAOS), and during recovery (REC) post oil spill at Ponta Verde, Alagoas, northeastern Brazil.

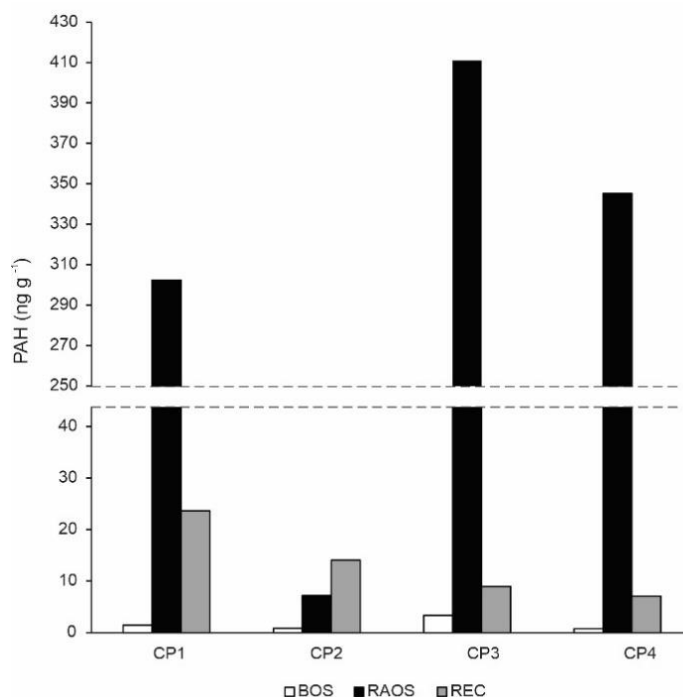


Figure 6. Total PAH ($\Sigma_{17}\text{PAH}$) concentrations in surface sediments collected before oil spill (BOS), right after oil spill (RAOS), and during recovery (REC) post oil spill at Pontal do Coruripe, Alagoas, northeastern Brazil.

The most representative PAHs in sediments before oil spill were phenanthrene, pyrene, naphthalene and fluoranthene. Phenanthrene remained as the most abundant compound post-spill followed by chrysene, pyrene, fluorene, and benzo[a]pyrene (Supplementary S3, S4, S5, S6 and S7). Individual PAH concentrations were compared with sediment quality guidelines proposed by NOAA (Long et al., 1995 and MacDonald et al., 1996). Such guidelines have two benchmarks: TEL (threshold effect level), below which harmful effects on organisms are not expected, and PEL (probable effect level), above which adverse effects on biota are likely (Macdonald et al., 1996). A few compounds (acenaphthene, fluorene and phenanthrene) were above the TEL in sediments sampled in November/19 (RAOS) at samples GB4, PC1, PC3 and PC4, with the highest incidence at GB4 (Supplement S6).

OCPs

Based on PAH and trace metal results, analysis of OCPs was performed only in one monthly sample from each reef site. Among all OCPs analyzed, just seven were detected above the limit of quantification (γ -HCH, aldrin, endrin, p,p'-DDT o,p'-DDD, p,p'-DDE and methoxychlor (Table 3). Concentrations of OCPs ranged from n.d. to 2.0 ng g⁻¹ in November/19 at Pontal do Coruripe. None of these OCPs showed concentrations higher than the proposed effects range low (ERL) and effects range median (ERM) (values from Long et al.,1995 and MacDonald et al., 1996 and in Supplementary S8).

Table 3. Organochlorine pesticides (OCPs) (ng g⁻¹ dry weight) observed at sediment samples from Gaibu, Carneiros, Ponta Verde and Pontal do Coruripe.

Month	ST	β -HCH	γ -HCH	Aldrin	Eldrin	p,p'-DDT	o,p'-DDD	o,p'-DDE	p,p'-DDE	Methoxychlor
Feb/19	GB2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	CA4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	PV4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	CP3	n.d.	0.09	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
May/19	GB1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	CA2	<0.067	n.d.	n.d.	0.20	n.d.	n.d.	n.d.	n.d.	0.13
	PV4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<0.067
	PC4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Aug/19	GB2	n.d.	1.19	n.d.	n.d.	0.11	n.d.	n.d.	n.d.	0,12
	CA1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	PV4	n.d.	n.d.	0.12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	CP3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nov/19	GB2	n.d.	n.d.	0.37	n.d.	0.19	n.d.	n.d.	0.09	0.18
	CA4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	PV1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	PC1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.00
Feb/20	GB4	n.d.	n.d.	n.d.	n.d.	n.d.	<0.067	n.d.	n.d.	n.d.
	CA1	n.d.	n.d.	n.d.	0.52	n.d.	0.09	n.d.	n.d.	n.d.
	PV1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<0.067	n.d.	n.d.

PC1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<0.067	0.28	0,51
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TMs

A total of 9 elements were analyzed, but only 6 were quantified: As, Cr, Cu, Ni, Pb and Zn (Supplementary 9). The trace metals ranged from 493 to 3601 ng g⁻¹ in Nov/19 at Gaibu (GB4 and GB2, respectively). Concentrations did not vary significantly between sites (Supplementary S10).

Biological responses

Pachygrapsus transversus

A total of 385 specimens of *P. transversus* were captured, 217 males and 168 females (including 66 ovigerous females). Captured males had CW ranging from 16.65 mm to 39.36 mm (mean $\pm$ SD = 29.46 $\pm$ 4.59 mm). Females ranged from 14.65 mm to 35.84 mm (mean $\pm$ SD = 25.37 $\pm$ 4.24 mm). The frequency of micronucleated cells (MNF) ranged between 0.25 and 1.50% (0.50 $\pm$ 0.28%). In order to observe effects of oil spill on *P. transversus*, MNF was compared between sampling periods: BOS (Feb/19, May/19 and Aug/19), RAOS (Nov/19) e REC (Feb/20). MNF of *P. transversus* presented an increasing from BOS to RAOS in all affected areas (Figure 7). In fact, they were statistically different at Gaibu. MNF in sampling periods BOS and REC did not differ significantly in any area ($p > 0.05$), suggesting that the frequency of micronucleated cells has returned to the original pattern. At Gaibu, values significantly different between BOS and RAOS ($p = 0.0003$), and RAOS and REC ($p = 0.002$). At Carneiros, the MNF values were significantly different between the RAOS and REC, suggesting a reduction in the rate of micronucleated cells over time.

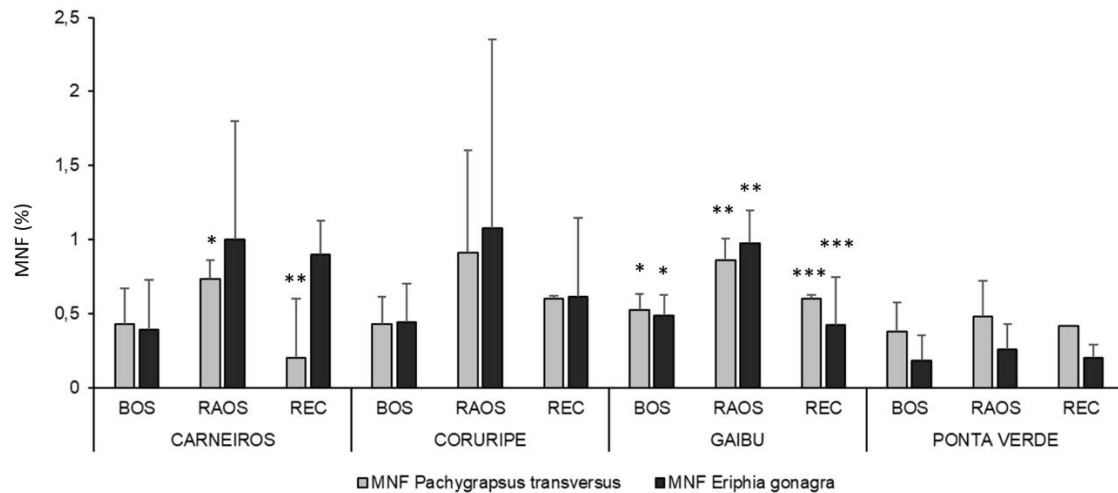


Figure 7. Mean value (with standard deviation bar) of micronucleated cell frequency (MNF) in individuals of *Eriphia gonagra* and *Pachygrapsus transversus* collected before oil spill (BOS), right after oil spill (RAOS) and during recovery (REC) post oil spill at Gaibu, Carneiros, Ponta Verde and Pontal do Coruripe, northeastern Brazil. Legend: * differs significantly from BOS; ** differs significantly from RAOS; and *** differs significantly from REC.

Eriphia gonagra

A total of 221 specimens of *E. gonagra* were captured, 118 males and 103 females (including 31 ovigerous females). Carapace width (CW) in males ranged from 19.78 to 34.79 mm (mean $\pm$ SD = 26.07 ± 5.10 mm) while in females they ranged from 14.65 to 35.84 mm (mean $\pm$ SD = 25.37 ± 4.24 mm). MNF ranged between 0.1 and 2.5% (mean $\pm$ SD = $0.43 \pm 0.46\%$), with the highest value at Pontal do Coruripe in Nov/19 (Figure 7). As observed for *P. transversus*, MNF in *E. gonagra* showed an increasing trend immediately after the oil spill followed by a decrease during the recovery period (February/20) in all sites. This was also observed at Ponta Verde samples where the lowest variation was observed. Likewise observed for *P. transversus*, MNF of *E. gonagra* at Gaibu differed significantly between BOS and RAOS ($p = 0.003$), and RAOS and REC ($p = 0.001$).

Morphological changes in crabs

None of *P. transversus* presented unusual characteristics such as scars, injuries, and spots on carapace, chelipeds and/or abdomen. After oil spill, individuals of *E. gonagra* were identified with morphological damages in reefs from Pernambuco: 7 specimens at Gaibu and 10 at Carneiros during sampling campaigns of Nov/2019 and Feb/2020 (Table 4). Ten oiled individuals were captured with the oil residues mainly attached to the setae of pereopods (Figure 8), but only one presented morphological alterations. All of them presented high MNF ranging from 1 to 2.5% (25 micronucleated cells per mille), with an average of $1.2 \pm 0.5\%$ for Gaibu individuals and $1.8 \pm 0.5 \%$ for Carneiros.

Table 4. Number of individuals recorded with injuries, and description of morphological damages found in *Eriphia gonagra* from Gaibu and Carneiros, northeastern Brazil.

Gaibu (n=7)					
Station	F	OvF	M	Total	Morphological damages
Nov/19					
#GB1	1	-	-	1	Circular spots with a purple center, white mid-region and a purple edge on two abdominal somites (n=1) (Fig 9B)
#GB2	1	1	1	3	Circular spots with a white inner part with a purple edge on the ventral surface of the cheliped propodus (n=2); White spots on the dorsal region of the carapace (n=1F) (Fig 8B)
#GB3	-	-	1	1	Circular spots with a purple center, white mid-region and a purple edge on the dorsal surface of the carapace (n=1) (Fig 9A)
Feb/20					
#GB2	-	1	1	2	Circular spots with a white inner part with a purple edge on the ventral surface of the cheliped propodus (n=1OvF; 1M)
Carneiros (n=10)					
Station	F	OvF	M	Total	Morphological damages
Nov/19					
#CA2	-	-	2	2	Injury to the right side of the 1 st sternite, with structural deformity and purple edges (Fig 9C) (n=1); Circular spots with a white inner part with a purple edge on the ventral surface of the cheliped propodus (n=1)
Feb/20					
#CA3	1	2	1	4	Circular spots with a white inner part with a purple edge on the ventral surface of the cheliped propodus (Fig 9D) (n=4)
#CA4	2	1	1	4	Circular spots with a white inner part with a purple edge on the ventral surface of the cheliped propodus (n=4)

F: non-ovigerous females; OvF: ovigerous females; M: males. See the station's list in Material and Methods.



Figure 8. **A:** Individual of *Eriphia gonagra* with the carapace under normal conditions and cheliped with mechanical trauma; **B:** individual of *Eriphia gonagra* with white spots on carapace (red arrows) and oil residues in the setae of the pereopods (black arrows)

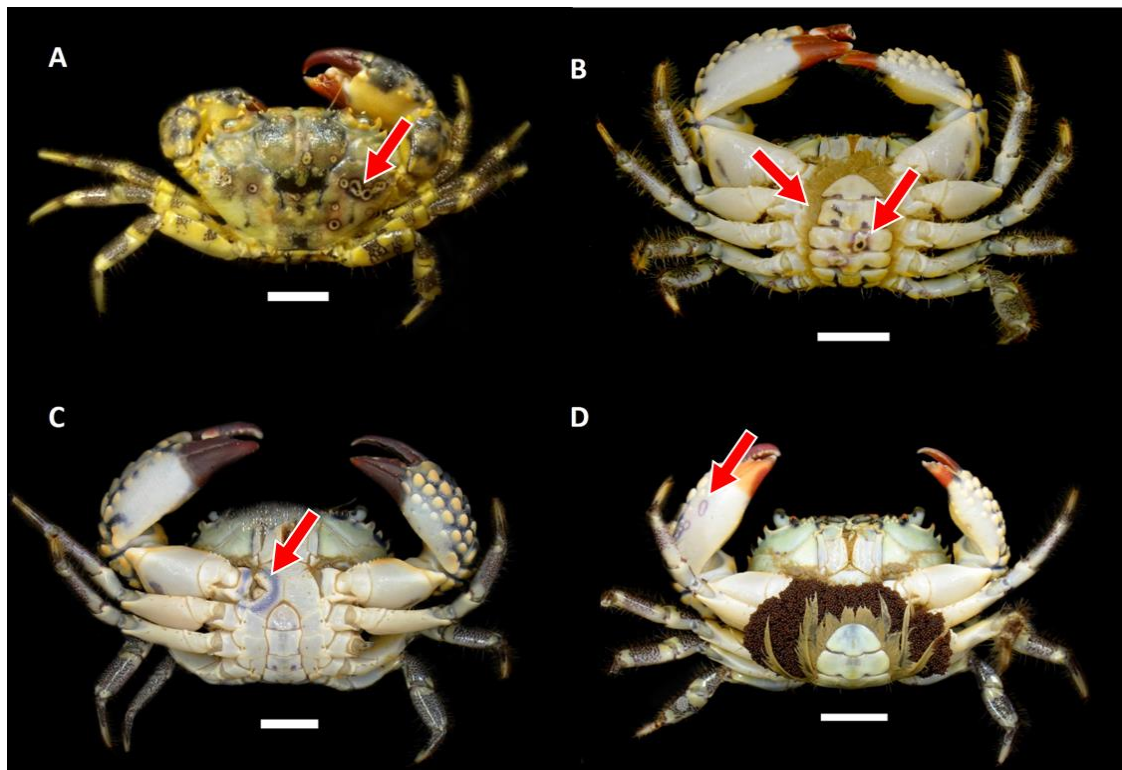


Figure 9. Individuals of *Eriphia gonagra* with morphological damages at Gaibu (**A:** male; **B:** female) and Carneiros (**C:** male; **D:** ovigerous female) (scale bar: 1mm). Red arrows are highlighting damages.

Integrated Analysis

According to the Spearman's rank-order correlation, positive and significant associations were observed between Σ OCPs and MNF in *P. transversus* ($r_s = 0.71$; $p = 0.018$) at Ponta Verde, MNF and Σ TM in *P. transversus* at Carneiros ($r_s = 0.55$; $p = 0.01$), and Σ_{17} PAHs and MNF in *P. transversus* at Gaibu ($r_s = 0.50$; $p = 0.02$) and Pontal do Coruripe ($r_s = 0.451$; $p = 0.04$). Pearson's correlations were positive and significant between frequency of spots (%) and MNF in *E. gonagra* at Gaibu ($r = 0.71$; $p = 0.0004$) and Carneiros ($r = 0.66$; $p = 0.01$), and between Σ OCPs and MNF in *P. transversus* ($r = 0.91$; $p = 0.03$) and *E. gonagra* ($r = 0.99$; $p = 0.001$) at Pontal do Coruripe.

PCA applied to sediment data, contamination, and morphological and genotoxic damage evidenced a direct influence of higher values of organic contaminants on axis 1 and secondarily grain size and Σ TM (Figure 10). The first two axes of the PCA together explained 51.8% of the total data variation (Table 6). Axis 1 (30.5%) showed the formation of two groups. The first one is positioned in the positive quadrant and formed by samples PC1 (REC and RAOS), GB2 (BOS) and CA1 (REC), associated with vectors MNF of *E. gonagra*, Σ OCPs, and Σ HPA. The second, inversely positioned group to the anterior group, is formed by samples GB1, PC3, PC4 and PV4 (BOS), and GB4 and PV1 (RAOS). Axis 2 (21.2%) grouped in the positive coordinates samples CA2 (BOS), and CA4, GB2 e PV1 (RAOS) associated with the vectors gravel, coarse sand, medium sand, Σ TM, MNF of *P. transversus*, and frequency of spots (FS%) of *E. gonagra*. At negative coordinates, samples CA1, CA4, PV4, GB2 and PC3 (BOS) were associated with vectors fine sand and silt/clay.

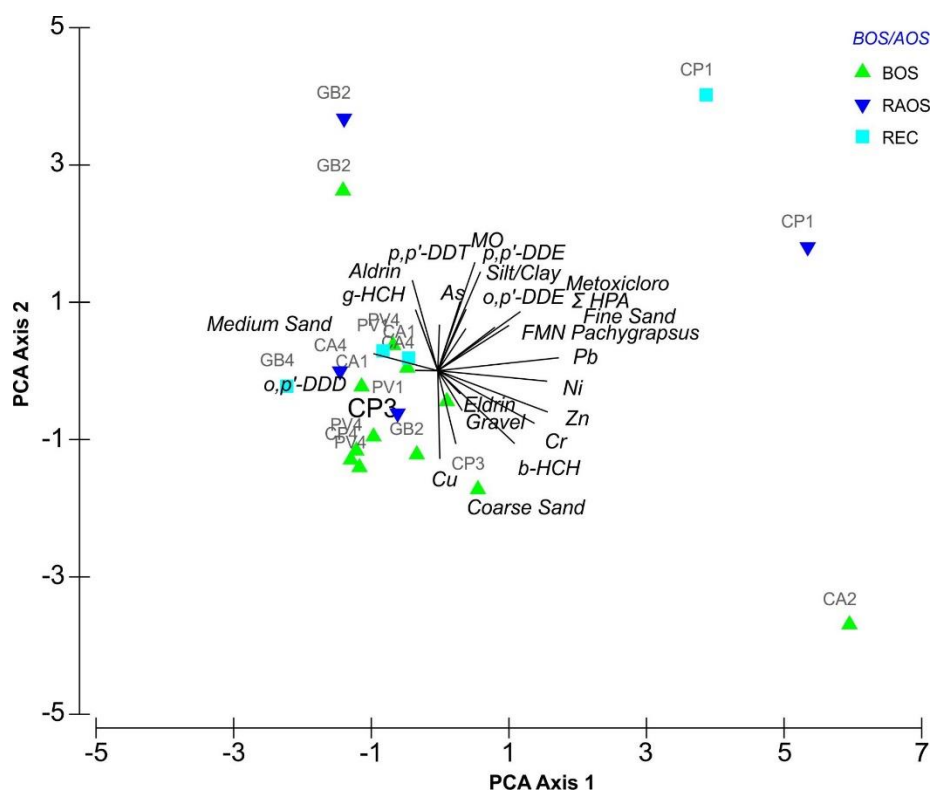


Figure 10. Ordination results of the principal component analysis (PCA) based on micronucleus frequency (MNF%) of *Pachygrapsus transversus*, $\sum_{17}\text{PAHs}$, $\sum\text{OCPs}$ and $\sum\text{TM}$ concentrations, organic matter (OM%) and grain size (%). Samples were categorized as before oil spill - BOS (February, May and August 2019), right after oil spill - RAOS (November 2019), and recovery post oil spill - REC (February 2020).

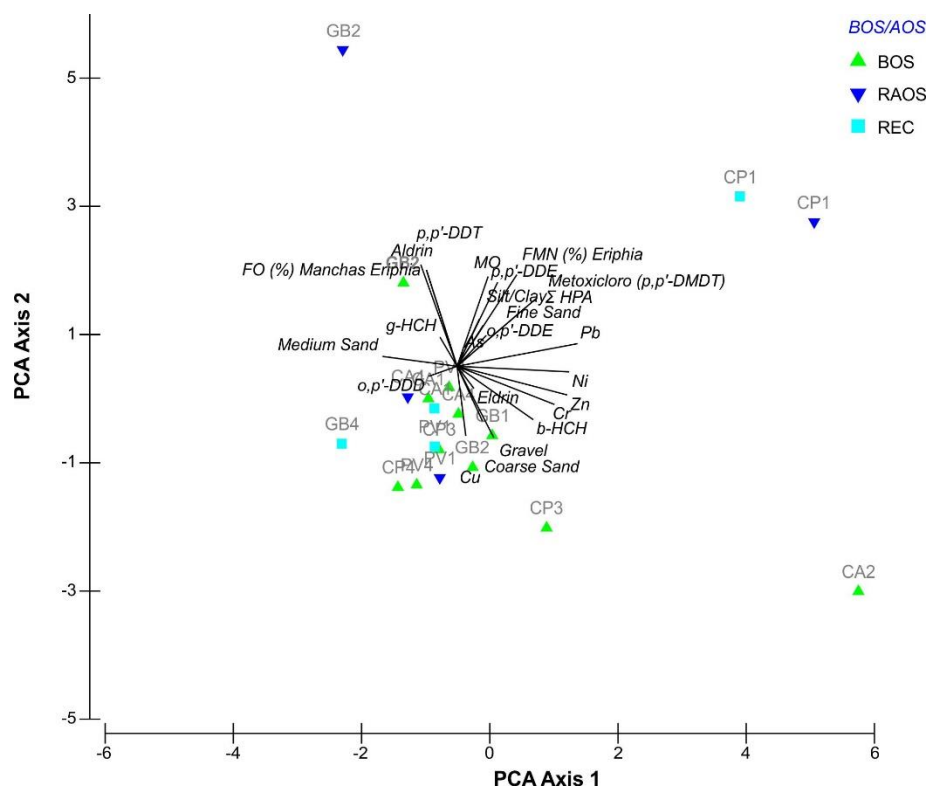


Figure 11. Ordination results of the principal component analysis (PCA) based on micronucleus frequency (MNF%) of *Eriphia gonagra*, Σ_{17} PAHs, Σ OCPs and Σ TM concentrations, organic matter (OM%) and grain size (%). Samples were categorized as before oil spill - BOS (February, May and August 2019), right after oil spill - RAOS (November 2019), and recovery post oil spill - REC (February 2020).

DISCUSSION

After the 2019 oil spill, many studies have been carried out in a variety of coastal environments, assessing the impacts of PAHs on marine and estuarine ecosystems of northeastern Brazil (Magalhães et al., 2022. Fernandes et al., 2022). In the case of sandreefs, oily material tend to stick and cover substrates, suffocating sessile species such as bivalves and barnacles or blocking crevices where crustaceans inhabit (Silva et al., 1997; Santana et al., 2022). Oil spill in coastal areas presents a high risk to the environment and impacts include

diverse damages and socioeconomic losses (Araújo et al., 2020). Oil can impact fishing, activities on industries that use raw marine resources, and tourism (Estevo et al., 2021).

The finest sediment grains have greater capacity to retain organic matter as well as contaminants of various types, including PAHs, OCPs e TMs (Bayen, 2012; Zahra et al., 2014; Maciel et al., 2015, 2016; Zanardi-Lamardo et al., 2019). For this reason, studies analyzing contaminants in sediments from coastal environments have been mainly performed in areas with lower hydrodynamics such as harbors and estuaries (Silva et al., 1997; Souza et al., 2008; Yogui et al., 2018). Even though this study analyzed unconsolidated substrate with larger grain size (lower OM retention capacity), it was possible to investigate PAHs in sediments probably due to the chronic input of these compounds to the environment, particularly after an oil spill event.

During this study, relatively low concentrations of PAHs were detected and quantified before the oil spill event, indicating chronic although subtle contamination. In this study, sites were selected because they exhibit different types of impact that can be potential sources of PAHs such as river runoff, recreational and fishing boats (Pontal do Coruripe and Carneiros), sewage (Ponta Verde and Gaibu), proximity to ports and large urban areas (Gaibu and Ponta Verde, respectively). Three out of four sites were impacted by oil spill. This was reflected on a significant increase on PAH concentrations in sediment sampled in November 2019 (RAOS). Before oil spill, Gaibu and Ponta Verde had the highest concentrations of PAHs. However, Ponta Verde was not affected by oil spill, and became the site with the lowest levels of PAHs. In fact, PAHs average concentration in samples from Ponta Verde remained relatively constant over the study period (Table 2).

Gaibu and Pontal do Coruripe showed a fast recovery since PAHs decreased in Feb/20 after a sharp increase in Nov/19. Recovery time may vary due to various environmental

parameters such as temperature and hydrodynamics (Yim et al., 2017) but mainly due to the magnitude of chronic inputs from various PAH sources.

Rivers may carry all types of contaminants to marine environments (Falcão et al. (2020). All sites (except Ponta Verde) are under influence of streams which probably explains their relatively higher amount of OCPs compared to Ponta Verde that it is influenced just by rainwater outfalls (Pádua et al., 2016; Santana et al., 2022). Pontal do Coruripe is the site with the lowest diversity of OCPs in sediments (γ -HCH, p,p'-DDE and methoxychlor) but at higher concentrations. Prior to reaching Pontal do Coruripe, the Coruripe Creek flows through sugarcane mills, crops and rural areas that certainly are contaminated by pesticide residues.

The use of OCPs such as DDT to control pests in agriculture and disease vectors was common in the past but they were banned in Brazil in the 1980s (agriculture) and 1990s (public health campaigns) (Magalhães et al., 2012). DDTs and their metabolites are lipid-soluble and can cause damage to organisms once they come in contact dermally, orally or by inhalation (D'Amato et al., 2002). The higher concentration of DDT metabolites (DDD and DDE) points out to an ancient environmental contamination since they are formed mainly during biological or chemical degradation of DDT (Fernícola et al., 1985).

Yogui et al. (2018) reported o,p'-DDD at sites located near the mouth of Tatuoca Creek in Suape estuary that is about 10 km south of Gaibu reefs. The authors suggested that this compound came from river or estuarine areas with low oxygen. The OCP o,p'-DDD was also found at Carneiros that receives input from estuarine waters of the Formoso Creek. In this study, DDT was detected at Gaibu during (August) and after (November) the wet season when freshwater flow increases and can carry contaminants to the coastal reef sites.

Some OCPs were present in three out of four study sites (Pontal do Coruripe, Carneiros and Gaibu). Methoxychlor, for instance, can cause changes in crustaceans, and delay ecdysis in larval and juvenile stages of *Metacarcinus magister* (Dana, 1852) (Armstrong et al., 1976).

Other studies highlight the influence and effects of OCPs on crustaceans (Souza et al., 2008; Magalhães et al., 2012 Mothershead II et al., 1991).

TM concentrations varied greatly between months, especially over the wet season. The highest and lowest values were reported for samples collected at Gaibu in November. GB2 exhibited the highest concentrations of metals and the second highest content of OM among all sites investigated. The largest variations were in samples GB2, GB3, GB4, and PC2 - all of them contained finer sediments.

PAH and MNF in *Pachygrapsus* were positively and strongly correlated at Gaibu and Pontal do Coruripe. Similar associations have been also observed in other crustaceans such as *Carcinus aestuarii* (Fossi et al., 2000) *U. cordatus* (Nudi et al., 2010; Cabral, 2017), and *Litopenaeus vannamei* (Reyes, 2020). Micronucleus rates showed a potencial of recovery in February 2020 for both species in all areas, including Ponta Verde and Carneiros that exhibited no PAH variation. This could seem unusual, but MNF reflects the presence of other stressors, including human activities. The environment can recover after oil spills due to degradation and proper cleaning (Soares et al., 2020). This phenomenon has been reported for animals living in association with algae that were collected oiled the week after the oil spill (Craveiro et al., 2021). This paper recorded alterations in the community associated to the algae, with recovery observed a few months after the oil spill in northeastern Brazil. It is interesting to note that Pontal do Coruripe and Gaibu exhibited the highest concentrations, and levels of chrysene and benzo[a]pyrene in sediment were above ERL and TEL in November. Such a finding can be pointed out as a potential stressor to the animals.

Certain morphological, physiological, and behavioral changes can occur in crabs after exposure to environmental stressors such as metals (Qin et al., 2012) and PAHs (Chase *et al.*, 2013). This has been observed in *Callinectes sapidus* (Mothershead II et al., 1991), *Scylla* sp. (Uno et al., 2010), and *Portunus trituberculatus* (Wen and Pan, 2016). Some morphological

problems can be triggered from a significant increase in micronucleated cells. FS in *Eriphia* exhibited a positive correlation with the frequency of micronucleated cells. Pinheiro and Toledo (2010) also noted higher MNF in individuals of *Ucides cordatus* with malformations in southeastern Brazil.

Prevalence of malformations and diseases in the carapace of crustaceans have been associated with environmental stress, intensive aquaculture (in species of commercial interest) and contamination as well as alterations in ecdysis events (Weis and Kim, 1988; Gregatti and Negreiros-Fransozo, 2009). In these cases, deformation abnormalities have been attributed to genetic factors linked to accidents during moulting (Mantelatto et al., 2000), action of chemicals from industrial sewage spills, commercial or leisure boats, and even to the action of parasites and ectobionts.

CONCLUSION

Oil spill increased PAH concentrations in sediments right after the accident. However, all sites were previously impacted by lower, chronic contamination from various sources. Besides, agriculture activities contributed with organochlorines compounds to sediments of the study areas, except Ponta Verde where metals prevailed.

The two crab species investigated in this study are sensitive to sediment contamination but responded in different ways. Although genotoxic damages of both species correlated with OCPs, *Pachygrapsus transversus* showed more sensibility to oil since there was a positive correlation between PAHs and the frequency of micronucleated cells. On the other hand, individuals of *Eriphia gonagra* were captured with morphological changes which are positively related to an increase of genotoxic alterations after oil spill. Environments that have been

impacted showed a rapid recovery since it was seen a decrease in genetic effects and contaminant concentration in sediments.

Future research is needed to further understand how both species of crab respond to each contaminant group separately. Considering that DNA macro lesions were related to an increase of PAHs in all areas, both crab species can be used as bioindicators for oil spill impact using micronucleus test as a parameter of response to PAHs exposure. Additionally, *Eriphia gonagra* is sensitive to such a contamination that causes morphological damage.

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MATERIAL SUPLEMENTAR

Supplementary S1. Location and characteristics of the sampling stations.

ST	Coordinates	Description of the area and potential impacts
Gaibu		
GB1	8°19'55.36"S 34°57'0.52"W	Protected margin of the sandstone strand
GB2	8°19'47.14"S 34°56'59.81"W	Protected margin of the sandstone strand, in a region with mangrove tree formation on the reefs
GB3	8°19'39.52"S 34°56'58.95"W	Near the exposed margin of the sandstone strand, in front of the outlet of a stream with sewage input
GB4	8°19'31.07"S 34°56'58.04"W	Near the exposed margin of the sandstone strand;
Carneiros		
CA1	8°42'18.78"S 35° 4'45.11"W	Protected margin of the sandstone strand, closest to the beach and with a high concentration of tourists (trampling)
CA2	8°42'15.84"S 35° 4'43.87"W	Region at the mouth of Formoso river. Protected margin of the sandstone strand, with mangrove tree formation on the reefs
CA3	8°42'12.65"S 35° 4'42.48"W	Region at the mouth of the Formoso river. Near the inner margin of the sandy strand. Intense presence of leisure boats and swimmers in natural pools.
CA4	8°42'9.60"S 35° 4'40.86"W	Near the outer margin of the sandstone strand. Region at the mouth of the Formoso river, with intense presence of leisure boats and tourists in natural pools.
Ponta Verde		
PV1	9°39'57.51"S 35°41'43.72"W	Region near the beach, in front of a stormwater gallery outlet, with sewage connections, and with a high concentration of bathers (trampling)
PV2	9°39'59.92"S 35°41'34.63"W	Near the lighthouse installed on the reef structure, with intense tourist visitation (trampling)
PV3	9°39'53.94"S 35°41'33.12"W	Near the installation of fishing corrals on the reef structure
PV4	9°39'50.47"S 35°41'39.84"W	Region closer to the beach, with intense tourist visitation (trampling)
Pontal do Coruripe		
PC1	10° 9'48.26"S 36° 8'9.46"W	Inner margin of the sandstone strand. Region at the mouth of the Coruripe River, with the presence of small fishing boats.
PC2	10° 9'45.52"S 36° 8'7.95"W	Inner margin of the sandstone strand. Region in the mouth of the Coruripe River.
PC3	10° 9'43.31"S 36° 8'6.39"W	Area of interruption of the sandstone strand, in an area exposed to wave action.
PC4	10° 9'39.91"S 36° 8'4.52"W	Near the outer margin of the sandstone strand, with tourist circulation (trampling)

Supplementary S2. Variations in the air and water temperatures, and salinity in Gaibu, Carneiros, Ponta Verde and Pontal do Coruripe, between February 2019 and February 2020

	Air (°C)				
	Feb/ 19	May/ 19	Aug/ 19	Nov/ 19	Feb/ 20
Gaibu	28.9	26.9	27.6	28.3	28.5
Carneiros	28.5	27	27.8	29.9	28.2
Ponta Verde	27.8	27	26.3	29.5	28.2
Pontal de Coruripe	28.2	26.3	27.8	28	28
	Water(°C)				
	Feb/ 19	May/ 19	Aug/ 19	Nov/ 19	Feb/ 20
Gaibu	28.3	26.2	28.3	30	29.4
Carneiros	28.7	27.3	28.1	32.8	29.3
Ponta Verde	28.1	27.8	26.5	31.5	28.9
Pontal de Coruripe	28.5	26.5	28.2	30.4	29
	Salinity				
	Feb/ 19	May/ 19	Aug/ 19	Nov/ 19	Feb/ 20
Gaibu	35.7	34.7	35.6	35.5	35.1
Carneiros	35	34	35.5	35.1	35.5
Ponta Verde	35.2	35.7	36.6	36.5	35.9
Pontal de Coruripe	34.7	33	34.6	35.5	35
	Precipitation (mm) ¹				
	Feb/ 19	May/ 19	Aug/ 19	Nov/ 19	Feb/ 20
Gaibu (Cabo de Santo Agostinho)	179.5	175.8	210.9	2.9	70.9
Carneiros (Tamandaré)	130.9	270.8	226.8	0,7	94.6
Ponta Verde (Maceió)	39.31*	163.4*	128.9*	6.3*	97.6**
Pontal do Coruripe (Coruripe)	27.5*	53.7*	90.6*	13.8*	72.6**

¹Source: Instituto Agronômico de Pernambuco (Cabo de Santo Agostinho and Tamandaré) and Secretaria de Meio Ambiente e Recursos Hídricos (Maceió and Coruripe)

* Stations: *Maceió (Cruz das Almas)* and *Usina Coruripe - Fz.Praia*

* Due to lack of data, in February 2020, the stations evaluated were *Maceió (IMA)* and *Coruripe*.

Supplementary S3. Concentration of individual and total polycyclic aromatic hydrocarbons (PAHs) in the superficial sediment in February/19 in reef áreas (Gaibu, Carneiros, Ponta Verde and Pontal do Coruripe) in Northeastern Brazil. Sediment quality guidelines available in Long et al. (1995) and MacDonald et al. (1996) were used for comparison. All concentrations are expressed in ng g^{-1} dry weight. l.o.q.: limite of quatification

Compounds/Stations	GB1	GB2	GB3	GB4	CA1	CA2	CA3	CA4	PV1	PV2	PV3	PV4	CP1	CP2	CP3	CP4
Naphthalene (Nap)	0.4	0.3	0.3	0.2	0.2	0.5	0.2	0.4	0.3	0.2	0.2	0.2	0.2	0.1	0.4	0.2
2-Methyl-naphthalene (M-Nap)	0.1	<l.o.q	ND	0.1	<l.o.q	0.1	ND	0.1	<l.o.q	ND	<l.o.q	<l.o.q	ND	ND	0.4	0.1
Acenaphtylene (Acy)	<l.o.q	<l.o.q	<l.o.q	<l.o.q	<l.o.q	<l.o.q	<l.o.q	<l.o.q	<l.o.q	ND	ND	ND	ND	ND	<l.o.q	ND
Acenaphthene (Ace)	ND	ND	ND	ND	ND	ND	ND	ND	<l.o.q	<l.o.q	<l.o.q	0.1	ND	ND	ND	ND
Fluorene (Fl)	ND	ND	ND	ND	ND	<l.o.q	ND	ND	0.1	ND	<l.o.q	0.1	ND	ND	<l.o.q	ND
Phenanthrene (Phe)	0.2	0.5	0.3	0.4	0.2	0.6	0.2	0.2	0.5	0.2	0.2	ND	0.4	0.2	0.6	0.3
Anthracene (Ant)	<l.o.q	0.1	<l.o.q	0.1	ND	0.2	ND	<l.o.q	0.1	ND	0.1	ND	<l.o.q	ND	0.1	<l.o.q
Fluoranthene (Flu)	<l.o.q	0.2	<l.o.q	<l.o.q	ND	0.5	ND	0.1	0.4	<l.o.q	0.2	ND	0.3	0.1	0.3	<l.o.q
Pyrene (Pyr)	0.1	0.5	0.2	0.3	<l.o.q	0.5	0.1	0.1	0.8	0.1	0.3	ND	0.3	0.2	0.4	0.2
Benzo[a]anthracene (BaA)	ND	<l.o.q	ND	<l.o.q	ND	0.1	ND	ND	0.2	ND	0.1	ND	0.1	<l.o.q	0.1	ND
Chrysene (Chr)	ND	0.2	<l.o.q	ND	ND	0.4	ND	<l.o.q	0.5	<l.o.q	0.2	ND	0.2	0.1	0.3	<l.o.q
Benzo[b]fluoranthene (BbF)	ND	0.1	ND	ND	ND	0.2	ND	<l.o.q	0.2	ND	0.1	ND	0.1	ND	0.2	ND
Benzo[k]fluoranthene (BkF)	ND	0.1	ND	ND	ND	0.4	ND	<l.o.q	0.2	ND	0.3	ND	0.1	ND	0.1	ND
Benzo[a]pyrene (BaP)	ND	ND	ND	ND	ND	0.2	ND	ND	0.6	ND	0.6	ND	ND	ND	0.1	ND
Indeno[1,2,3-cd]pyrene (IP)	ND	ND	ND	ND	ND	<l.o.q	ND	ND	0.2	ND	0.2	ND	ND	ND	ND	ND
Dibenzo[a,h]anthracene (DahA)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzo[ghi]perylene (BgHiP)	ND	0.1	ND	ND	ND	0.4	ND	ND	0.6	ND	0.2	ND	0.1	ND	0.2	ND

Supplementary S4. Concentration of individual and total polycyclic aromatic hydrocarbons (PAHs) in the superficial sediment in May/19 in reef áreas (Gaibu, Carneiros, Ponta Verde and Pontal do Coruripe) in Northeastern Brazil. Sediment quality guidelines available in Long et al. (1995) and MacDonald et al. (1996) were used for comparison. All concentrations are expressed in ng g⁻¹ dry weight. l.o.q.: limite of quatification

Compounds/Stations	GB1	GB2	GB3	GB4	CA1	CA2	CA3	CA4	PV1	PV2	PV3	PV4	CP1	CP2	CP3	CP4
Naphthalene (Nap)	0.4	0.5	0.4	0.5	0.1	0.3	1.0	0.8	0.3	0.2	0.2	0.2	0.2	0.7	0.6	0.4
2-Methyl-naphthalene (M-Nap)	0.1	0.2	0.1	<l.o.q	<l.o.q	0.1	0.5	0.3	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1
Acenaphtylene (Acy)	<l.o.q	0.1	<l.o.q	<l.o.q	<l.o.q	<l.o.q	0.1	<l.o.q	ND	ND	ND	ND	<l.o.q	0.1	0.1	ND
Acenaphthene (Ace)	<l.o.q	ND	ND	ND	ND	<l.o.q	0.1	<l.o.q	ND	ND	ND	ND	ND	ND	ND	ND
Fluorene (Fl)	ND	ND	ND	ND	ND	0.1	0.1	<l.o.q	ND	ND	ND	ND	ND	ND	ND	ND
Phenanthrene (Phe)	0.2	0.4	0.1	0.1	0.1	0.6	0.9	0.5	0.4	0.5	0.6	0.3	0.4	0.5	0.5	0.7
Anthracene (Ant)	0.1	0.2	<l.o.q	<l.o.q	ND	<l.o.q	0.1	<l.o.q	<l.o.q	<l.o.q	<l.o.q	<l.o.q	ND	0.1	<l.o.q	0.1
Fluoranthene (Flu)	<l.o.q	0.5	0.1	0.1	<l.o.q	0.2	0.4	0.1	0.1	0.1	0.1	0.1	0.1	1.0	0.6	0.7
Pyrene (Pyr)	0.1	0.4	0.1	0.1	0.1	0.4	0.3	0.2	0.3	0.3	0.5	0.3	0.5	1.2	0.9	2.3
Benzo[a]anthracene (BaA)	<l.o.q	0.2	0.1	0.1	ND	0.1	0.1	<l.o.q	<l.o.q	ND	ND	<l.o.q	ND	0.3	0.2	ND
Chrysene (Chr)	<l.o.q	0.3	0.1	0.1	<l.o.q	0.1	0.1	<l.o.q	0.1	0.1	<l.o.q	<l.o.q	<l.o.q	0.6	0.3	ND
Benzo[b]fluoranthene (BbF)	ND	0.9	0.1	0.3	ND	0.1	0.2	0.1	ND	ND	ND	ND	ND	0.5	0.3	ND
Benzo[k]fluoranthene (BkF)	ND	0.2	0.1	<l.o.q	ND	0.1	0.1	<l.o.q	ND	ND	ND	ND	ND	0.4	0.2	ND
Benzo[a]pyrene (BaP)	ND	0.3	ND	ND	ND	0.1	0.2	ND	ND	ND	ND	ND	ND	0.3	0.2	ND
Indeno[1,2,3-cd]pyrene (IP)	ND	0.4	<l.o.q	ND	ND	0.1	0.2	ND	ND	ND	ND	ND	ND	0.3	0.2	ND
Dibenzo[a,h]anthracene (DahA)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzo[ghi]perylene (BgHiP)	ND	0.6	0.1	ND	ND	0.2	0.2	<l.o.q	ND	ND	ND	ND	ND	0.4	0.3	ND

Supplementary S5. Concentration of individual and total polycyclic aromatic hydrocarbons (PAHs) in the superficial sediment in August/19 in reef áreas (Gaibu, Carneiros, Ponta Verde and Pontal do Coruripe) in Northeastern Brazil. Sediment quality guidelines available in Long et al. (1995) and MacDonald et al. (1996) were used for comparison. All concentrations are expressed in ng g^{-1} dry weight. l.o.q.: limite of quatification

Compounds/Stations	GB1	GB2	GB3	GB4	CA1	CA2	CA3	CA4	PV1	PV2	PV3	PV4	CP1	CP2	CP3	CP4
Naphthalene (Nap)	1.1	0.1	0.7	0.6	0.1	0.3	0.7	0.2	0.2	0.2	0.5	0.2	0.5	0.6	<l.o.q	0.5
2-Methyl-naphthalene (M-Nap)	0.4	<l.o.q	0.3	0.1	<l.o.q	0.1	0.2	0.2	0.9	<l.o.q	0.1	3.7	0.1	0.1	<l.o.q	0.1
Acenaphthylene (Acy)	0.2	<l.o.q	0.1	<l.o.q	<l.o.q	<l.o.q	0.1	ND	ND	ND	<l.o.q	ND	<l.o.q	<l.o.q	<l.o.q	<l.o.q
Acenaphthene (Ace)	ND	ND	ND	ND	ND	<l.o.q	ND	ND	1.0	ND	ND	2.9	<l.o.q	ND	ND	ND
Fluorene (Fl)	0.2	ND	0.3	ND	ND	0.1	<l.o.q	ND	2.4	ND	ND	3.8	<l.o.q	<l.o.q	<l.o.q	<l.o.q
Phenanthrene (Phe)	1.8	0.9	1.1	0.4	0.1	0.6	0.2	0.2	5.3	0.3	0.5	4.5	0.1	0.1	0.1	0.1
Anthracene (Ant)	0.7	0.3	0.3	0.5	ND	<l.o.q	<l.o.q	<l.o.q	0.8	ND	ND	0.6	<l.o.q	ND	ND	<l.o.q
Fluoranthene (Flu)	3.3	1.2	1.2	0.2	<l.o.q	0.2	0.3	0.1	0.1	0.1	0.3	0.1	<l.o.q	<l.o.q	<l.o.q	<l.o.q
Pyrene (Pyr)	2.7	1.5	1.4	0.3	0.1	0.4	0.3	0.1	0.2	0.5	1.6	0.2	<l.o.q	<l.o.q	0.1	<l.o.q
Benzo[a]anthracene (BaA)	1.1	0.4	0.3	0.1	ND	0.1	0.1	<l.o.q	<l.o.q	ND	ND	<l.o.q	<l.o.q	<l.o.q	ND	ND
Chrysene (Chr)	1.5	0.4	0.5	0.1	<l.o.q	0.1	0.1	0.1	<l.o.q	<l.o.q	<l.o.q	<l.o.q	<l.o.q	<l.o.q	<l.o.q	<l.o.q
Benzo[b]fluoranthene (BbF)	1.9	0.7	0.5	0.2	ND	0.1	0.2	ND	ND	ND	ND	ND	0.1	<l.o.q	ND	ND
Benzo[k]fluoranthene (BkF)	0.8	0.3	0.2	<l.o.q	ND	0.1	0.2	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzo[a]pyrene (BaP)	1.3	0.5	0.3	ND	ND	0.1	0.2	ND	ND	ND	ND	ND	<l.o.q	<l.o.q	ND	ND
Indeno[1,2,3-cd]pyrene (IP)	2.1	0.7	0.4	ND	ND	0.1	0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dibenzo[a,h]anthracene (DahA)	0.3	<l.o.q	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzo[ghi]perylene (BghiP)	2.7	0.9	0.6	ND	ND	0.2	0.1	ND	<l.o.q	ND	ND	<l.o.q	<l.o.q	ND	ND	ND

Supplementary S6. Concentration of individual and total polycyclic aromatic hydrocarbons (PAHs) in the superficial sediment in November/19 in reef áreas (Gaibu, Carneiros, Ponta Verde and Pontal do Coruripe) in Northeastern Brazil. Sediment quality guidelines available in Long et al. (1995) and MacDonald et al. (1996) were used for comparison. All concentrations are expressed in ng g⁻¹ dry weight. l.o.q.: limite of quatification

Compounds/Stations	GB1	GB2	GB3	GB4	CA1	CA2	CA3	CA4	PV1	PV2	PV3	PV4	CP1	CP2	CP3	CP4
Naphthalene (Nap)	0.2	0.9	0.5	0.2	0.2	0.2	0.1	0.6	0.7	4.0	0.9	0.3	0.5	0.2	0.6	0.5
2-Methyl-naphthalene (M-Nap)	0.1	0.5	<l.o.q	2.6	0.1	0.1	0.1	0.2	0.3	ND	0.5	0.3	6.8	<l.o.q	10.7	7.8
Acenaphtylene (Acy)	<l.o.q	0.2	<l.o.q	ND	<l.o.q	ND	<l.o.q	<l.o.q	<l.o.q	ND	0.1	<l.o.q	ND	<l.o.q	0.2	0.2
Acenaphthene (Ace)	ND	ND	ND	5.9	<l.o.q	ND	ND	ND	ND	ND	0.1	<l.o.q	6.5	ND	7.9	7.0
Fluorene (Fl)	ND	0.6	ND	18.0	ND	ND	<l.o.q	ND	ND	ND	0.1	ND	22.7*	ND	29.6*	26.5*
Phenanthrene (Phe)	0.3	1.8	0.2	224.8	0.3	0.5	0.3	0.3	0.3	0.3	0.7	0.4	121.8	0.2	159.6	139.0
Anthracene (Ant)	<l.o.q	0.3	<l.o.q	10.4	<l.o.q	<l.o.q	<l.o.q	ND	<l.o.q	ND	<l.o.q	<l.o.q	9.7	0.1	12.8	11.7
Fluoranthene (Flu)	0.3	2.7	ND	24.0	0.1	0.3	0.1	0.2	0.1	0.2	0.2	0.1	9.3	0.5	11.6	9.9
Pyrene (Pyr)	0.3	2.9	0.2	80.0	0.2	1.0	0.3	0.7	0.2	0.3	0.5	0.1	45.0	1.4	60.8	43.9
Benzo[a]anthracene (BaA)	0.2	1.0	ND	64.2	<l.o.q	<l.o.q	<l.o.q	ND	ND	<l.o.q	<l.o.q	<l.o.q	17.5	1.0	24.9	19.4
Chrysene (Chr)	0.2	1.1	0.1	92.5	0.1	0.2	0.1	<l.o.q	<l.o.q	0.1	<l.o.q	0.1	41.4	2.4	57.6	48.4
Benzo[b]fluoranthene (BbF)	ND	1.6	ND	28.0	ND	0.1	0.1	ND	ND	ND	<l.o.q	<l.o.q	4.8	0.3	7.7	7.8
Benzo[k]fluoranthene (BkF)	ND	0.9	ND	10.3	ND	<l.o.q	<l.o.q	ND	ND	ND	<l.o.q	<l.o.q	2.1	0.3	2.5	2.3
Benzo[a]pyrene (BaP)	ND	1.2	ND	46.6	ND	0.1	<l.o.q	ND	ND	ND	<l.o.q	ND	8.9	ND	14.0	10.1
Indeno[1,2,3-cd]pyrene (IP)	0.4	2.0	ND	2.5	ND	0.1	ND	ND	ND	ND	ND	ND	0.5	ND	1.2	1.2
Dibenzo[a,h]anthracene (DahA)	ND	0.3	ND	9.2	ND	ND	ND	ND	ND	ND	ND	ND	1.4	0.3	3.0	3.0
Benzo[ghi]perylene (BghiP)	0.3	2.4	ND	13.9	ND	0.1	ND	ND	ND	ND	ND	ND	3.3	0.5	6.4	6.3

Bold: acima do TEL; * acima do ERL, according to supplementary material X.

Supplementary S7. Concentration of individual and total polycyclic aromatic hydrocarbons (PAHs) in the superficial sediment in February/20 in reef areas (Gaibu, Carneiros, Ponta Verde and Pontal do Coruripe) in Northeastern Brazil. Sediment quality guidelines available in Long et al. (1995) and MacDonald et al. (1996) were used for comparison. All concentrations are expressed in ng g^{-1} dry weight. l.o.q.: limite of quatification

Compounds/Stations	GB1	GB2	GB3	GB4	CA1	CA2	CA3	CA4	PV1	PV2	PV3	PV4	CP1	CP2	CP3	CP4
Naphthalene (Nap)	0.2	0.5	0.4	0.5	0.3	0.4	0.5	0.1	0.4	0.2	0.1	0.1	0.7	0.3	0.2	0.2
2-Methyl-naphthalene (M-Nap)	0.1	<l.o.q	<l.o.q	<l.o.q	0.3	0.2	0.3	<l.o.q	0.1	0.1	0.1	0.2	0.2	0.2	<l.o.q	0.1
Acenaphtylene (Acy)	<l.o.q	<l.o.q	<l.o.q	<l.o.q	0.1	<l.o.q	0.1	ND	<l.o.q	<l.o.q	ND	ND	0.1	<l.o.q	<l.o.q	<l.o.q
Acenaphthene (Ace)	0.1	ND	ND	ND	ND	ND	0.1	ND	ND	ND	<l.o.q	0.2	ND	ND	<l.o.q	ND
Fluorene (Fl)	ND	ND	ND	ND	ND	ND	0.3	ND	ND	<l.o.q	0.3	1.3	ND	ND	<l.o.q	<l.o.q
Phenanthrene (Phe)	0.3	0.2	0.1	0.1	0.3	0.4	1.8	0.9	0.1	0.2	4.2	5.3	1.2	0.5	0.4	0.3
Anthracene (Ant)	0.1	<l.o.q	0.1	<l.o.q	<l.o.q	0.1	0.2	<l.o.q	ND	<l.o.q	ND	ND	0.2	<l.o.q	0.1	<l.o.q
Fluoranthene (Flu)	0.4	0.2	<l.o.q	<l.o.q	0.2	0.5	0.5	0.6	<l.o.q	<l.o.q	0.1	0.1	1.5	0.4	0.3	0.2
Pyrene (Pyr)	1.8	0.2	<l.o.q	<l.o.q	0.2	0.6	0.8	3.3	<l.o.q	0.1	0.4	0.3	2.7	1.0	1.2	0.9
Benzo[a]anthracene (BaA)	2.1	0.1	<l.o.q	<l.o.q	0.1	0.2	0.4	<l.o.q	ND	<l.o.q	<l.o.q	<l.o.q	2.1	1.1	1.1	1.0
Chrysene (Chr)	4.4	0.1	<l.o.q	<l.o.q	0.2	1.2	0.7	<l.o.q	<l.o.q	0.1	0.1	0.1	5.9	3.2	2.8	2.3
Benzo[b]fluoranthene (BbF)	1.4	0.2	ND	ND	0.5	0.8	0.4	0.1	ND	ND	<l.o.q	ND	1.6	1.0	0.7	0.4
Benzo[k]fluoranthene (BkF)	0.3	<l.o.q	ND	ND	0.1	0.3	0.3	<l.o.q	ND	ND	<l.o.q	ND	1.2	0.4	ND	0.1
Benzo[a]pyrene (BaP)	1.4	ND	ND	ND	0.1	0.6	0.4	<l.o.q	ND	ND	ND	ND	2.7	1.2	0.8	0.8
Indeno[1,2,3-cd]pyrene (IP)	0.2	0.2	ND	ND	0.9	0.7	0.4	ND	ND	ND	ND	ND	1.0	0.5	0.3	0.2
Dibenzo[a,h]anthracene (DahA)	0.6	ND	ND	ND	0.2	0.2	<l.o.q	ND	ND	ND	ND	ND	0.5	0.2	0.3	0.2
Benzo[ghi]perylene (BghiP)	1.4	0.3	ND	ND	0.9	1.2	0.5	<l.o.q	ND	ND	ND	ND	2.1	1.0	0.8	0.6

Supplementary S8. Sediment guidelines quality (Long et al., 1995 and MacDonald et al., 1996)

ERL=effects range-low; ERM=effects range-median; TEL=threshold effects level; PEL=probable effects level (in ng.g⁻¹ dry weight)

Compounds	TEL	ERL	PEL	ERM
Naphthalene (Nap)	34.60	160	391	2100
2-Methyl-naphthalene (M-Nap)	-	70	-	670
Acenaphthylene (Acy)	5.87	44	128	640
Acenaphthene (Ace)	6.71	16	88.9	500
Fluorene (Fl)	21.2	19	144	540
Phenanthrene (Phe)	86.7	240	544	1500
Anthracene (Ant)	46.9	85.3	245	1100
Fluoranthene (Flu)	113	600	1494	5100
Pyrene (Pyr)	153	665	1398	2600
Benzo[a]anthracene (BaA)	74.8	261	693	1600
Chrysene (Chr)	108	384	846	2800
Benzo[b]fluoranthene (BbF)	-	320	-	1880
Benzo[k]fluoranthene(BkF)	-	280	-	1620
Benzo[a]pyrene (BaP)	88.8	430	763	1600
Indeno[1,2,3-cd]pyrene (IP)	-	-	-	-
Dibenzo[a,h]anthracene (DahA)	6.22	63.4	135	260
Benzo[ghi]perylene (BghiP)	-	430	-	1600
ΣPAHs	1.684	4.022	16.770	44.792
p,p'-DDT	1.19	1	4.77	7
p,p'-DDE	2.07	2.2	374	27
ΣDDTs	3.89	1.58	51.7	46.1

Supplementary S9. Metals (ng g⁻¹ dry weight) observed at sediment samples from Gaibu, Carneiros, Ponta Verde and Pontal do Coruripe, Northeastern Brazil.

	Gaibu					Carneiros					Ponta Verde					Pontal do Coruripe				
MT	feb/19	may/19	aug/19	nov/19	feb/20	feb/19	may/19	aug/19	nov/19	feb/20	feb/19	may/19	aug/19	nov/19	feb/20	feb/19	may/19	aug/19	nov/19	feb/20
As	-	-	11	10	11	-	-	11	11	-	11	-	-	11	11	13	11	-	-	13
Cr	2	3	1	0	0	0	3	1	0	1	0	0	0	0	0	3	1	0	3	1
Cu	4	3	-	-	-	5	5	-	-	-	4	4	4	-	-	2	6	6	-	-
Ni	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	4	-	-	3	4
Pb	-	-	-	-	-	-	12	-	-	-	-	20	-	-	-	-	-	-	11	9
Zn	3	4	2	1	2	1	7	2	0	3	1	2	0	-	0	3	1	0	6	5

7 CONSIDERAÇÕES FINAIS

Foi constatada a contaminação do sedimento, mesmo que em concentrações relativamente baixas para alguns compostos, como os OCPs. As concentrações de HPAs foram baixas nas primeiras três campanhas (fevereiro, maio e agosto de 2019), mas apresentaram um aumento significativo a partir do mês de novembro de 2019 em todas as áreas analisadas. Este aumento foi maior nas áreas atingidas por grandes quantidades de óleo durante o desastre que atingiu o Nordeste do Brasil. Entretanto, na coleta subsequente (fevereiro de 2020) as concentrações já eram significativamente menores, sugerindo uma recuperação do ambiente em termos de contaminação aguda.

Durante o período logo após o acidente (novembro de 2019), aumentou a frequência de micronúcleos na hemolinfa de *P. transversus* e *E. gonagra*. Ela foi positivamente correlacionada ao aumento da contaminação do sedimento nas áreas estudadas. Para *E. gonagra*, houve correlação positiva com as maiores concentrações de HPAs e OCPs, enquanto para *P. transversus* a correlação foi com metais. Quanto aos danos morfológicos causados pelo óleo, as espécies responderam de maneira diferente. Foram encontrados indivíduos de *E. gonagra* com manchas e má-formações na carapaça e apêndices no período após o acidente (novembro de 2019 e fevereiro de 2020). Porém, *P. transversus* não apresentou má formações.

Para *E. gonagra* não foram observadas alterações populacionais significativas. Para *P. transversus* houve uma alteração na razão sexual, com a redução do número de fêmeas em todos os locais atingidos pelo derramamento de óleo. Quanto ao tamanho médio da largura da carapaça e maturidade sexual, em Ponta Verde foram detectados os menores valores para *P. transversus* - um local com impactos crônicos tais como área altamente urbanizada, turismo e pisoteio.

Levando todas estas conclusões em consideração, pode-se confirmar parcialmente a hipótese inicial deste trabalho, de que as populações dos caranguejos braquiúros analisados sofreram alterações morfológicas e lesões no DNA relacionadas com a contaminação do sedimento. No entanto, das duas espécies, apenas *P. transversus* apresentou alterações populacionais significativas. Para análises futuras, o estudo completo de como esses contaminantes afetam as espécies podem ser realizados utilizando outros marcadores, como a presença de metabólitos de HPAs em urina, por exemplo.

Estudos abordando como os impactos antrópicos e contaminantes orgânicos podem afetar as espécies marinhas são muito importantes e fornecem subsídios para a elaboração de

legislação, criação de áreas de unidades de conservação e ações de educação ambiental para população. Deve-se conhecer para preservar! Apesar das espécies serem resilientes e ainda habitarem áreas contaminadas, os animais estão sofrendo alterações morfológicas, genéticas, fisiológicas e comportamentais. Em longo prazo, isso pode se traduzir em mudanças estruturais significativas nas comunidades de caranguejos, eventualmente afetando toda a teia trófica do ecossistema recifal.

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