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NYKON JEFFERSON DE ALBUQUERQUE CRAVEIRO

MACROFAUNA DO FITAL: QUAIS CARACTERÍSTICAS DAS MACROALGAS CONTROLAM A COMUNIDADE MACROBÊNTICA ASSOCIADA?

Recife
2023

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Área de concentração: Oceanografia Biológica.

Orientador: Prof. Dr. José Souto Rosa Filho.

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“*No fundo no fundo, tudo é Bentos*”

RESUMO

As macroalgas desempenham um papel fundamental no aumento da complexidade e da biodiversidade dos recifes tropicais, sendo colonizadas por muitas espécies de animais e plantas epibênticas associadas. As macroalgas são muito diversas e apresentam diferentes características físicas e químicas, o que pode influenciar na ocorrência e distribuição de sua epifauna nas macroalgas. No entanto, a maioria dos estudos focam em apenas uma dessas características. Diante disso, este estudo teve como objetivo responder se a estrutura das comunidades epifaunísticas associadas às macroalgas dos recifes tropicais refletem a complexidade estrutural e a composição química das macroalgas. Para isso, amostras das macroalgas *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* e *Gelidiella acerosa* foram coletadas nos recifes de Enseada dos Corais, Nordeste do Brasil, e analisadas quanto à complexidade estrutural (altura, biomassa, diâmetro da área e do perímetro, e índice de intertícios), compostos químicos (minerais, metabólitos primários e metabólitos secundários majoritários) e identificação taxonômica da epifauna. Em síntese, as Rhodophyta foram as macroalgas mais complexas, com maior conteúdo químico, e também apresentaram a maior abundância e riqueza da epifauna, sendo a herbivoria o habito alimentar mais comum. Variações significativas na riqueza, abundância e composição da epifauna diferiram entre as espécies de macroalgas, especialmente devido ao conteúdo de carboidratos, neofitadieno, potássio e o índice de intertícios. Esses fatores das macroalgas estão relacionados à origem taxonômica e à adaptação às condições ambientais e biológicas desses organismos. Os resultados indicam fortemente que a abundância, a riqueza e a distribuição da epifauna nas macroalgas dos recifes de arenito da costa tropical são determinados pela complexidade e pela composição química das macroalgas. Os espaços intersticiais e o componente químico são usados pelas espécies epifaunais como refúgio/proteção e como fonte de alimento, respectivamente, mostrando que as macroalgas Rhodophyta, especialmente a *P. perforata*, é o melhor substrato para o estabelecimento de uma comunidade epibêntica mais rica e diversa.

Palavras-chave: macroalgas; complexidade; compostos químicos; epifauna.

ABSTRACT

Macroalgae are crucial in enhancing the intricacy and diversity of tropical reefs, providing a habitat for numerous animal species and associated epibenthic plants. Macroalgae exhibit varied physical and chemical traits, which may affect the presence and dispersion of epifauna in macroalgae. Nonetheless, the majority of studies tend to emphasize only one of these traits. The objective of this study is to investigate whether the structural complexity and chemical composition of tropical reef macroalgae is reflected in the epifaunal community structure associated with it. To achieve this, we obtained samples of the macroalgae *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata*, and *Gelidiella acerosa* from Enseada dos Corais reefs in Northeast Brazil. We then conducted analyses to determine the structural complexity (height, biomass, area and perimeter diameter, and intertissue index), chemical compounds (minerals, primary metabolites, and major secondary metabolites), and taxonomic identification of the epifauna. We took care to use precise technical terminology, ensure objectivity, and maintain a formal register throughout the text. Consistent citation and footnote styles were also followed, and any technical abbreviations were adequately explained. Rhodophyte were found to be the most complex macroalgae, exhibiting the highest chemical content. They also displayed the greatest abundance and richness of epifauna, with herbivory being the most common feeding behavior. Significant variations in the richness, abundance, and composition of epifauna differed among macroalgae species, particularly due to the content of carbohydrates, neophytadiene, potassium, and the intertissue index. The taxonomic origin and environmental and biological conditions of macroalgae are related factors. The abundance, richness, and distribution of epifauna on sandstone reefs of the tropical coast are influenced by the chemical composition and complexity of macroalgae, as the results indicate. The interstices and chemical composition serve as a protective refuge and food source, respectively, for epifaunal species. These findings indicate that Rhodophyte macroalgae, particularly P. perforata, provide the optimal substrate for creating a more diverse and robust epibenthic community.

Keywords: macroalgae; complexity; chemical compounds; epifauna.

SUMÁRIO

1 INTRODUÇÃO

As macroalgas são um grupo diversificado de organismos fotossintéticos, classificados como algas vermelhas (Rhodophyta), verdes (Chlorophyta) ou marrons (Ochrophyta, Phaeophyceae), de acordo com sua pigmentação, composição das paredes celulares e reservas de polissacarídeos (Lüning, 1990; Lewis e McCourt, 2004; Hanelt, 2008). As macroalgas são organismos excepcionalmente resistentes, que surgiram entre 1 e 1,6 bilhões de anos AP, e sobreviveram a vários eventos de extinção em massa (Butterfield, 2000; Bengtson, 2017). Em todo o mundo, sabe-se da existência de quase 12.000 espécies de algas marinhas (Guiry e Guiry, 2023), das quais 1.707 foram registradas nas águas tropicais e subtropicais do Atlântico Ocidental (Wynne, 2022).

No ambiente marinho as macroalgas são predominantes e são um componente crucial dos ecossistemas costeiros bentônicos, que dominam essas comunidades nos ambientes rasos em grande parte do mundo (Horta et al., 2001; Tano et al., 2016; Aued et al., 2018). Nos recifes tropicais de arenito espalhados ao longo da costa brasileira, as macroalgas desempenham a função de produtores primários (Horta et al., 2001; Fulton et al., 2019; Pessarrodona et al. 2022), além de aumentar a complexidade ambiental e contribuir para a purificação da água ao absorver o excesso de nutrientes e poluentes (Dijkstra et al. 2017; Cotas et al., 2023; Farghali et al., 2023).

As macroalgas como parte do ambiente fital, geram ecossistemas complexos que também fornecem habitats e alimentos para uma ampla gama de organismos marinhos, principalmente, por meio da atenuação das condições ambientais e disponibilidade de alimento (Christie et al. 2009; Kraan, 2013; Cotas et al., 2023). As macroalgas são usadas como habitat tanto pelas algas epífitas quanto pela fauna (invertebrados e vertebrados) (Christie et al. 2009, Edgar, 1983). A enorme diversidade estrutural das macroalgas aumenta a complexidade do substrato, a disponibilidade de alimentos (propriamente as macroalgas ou pelos detritos acumulados) e abrigo, o que pode favorecer o desenvolvimento de comunidades de animais e plantas muito diversas e abundantes (Bates, 2009, Christie et al. 2009, Hayward, 1980).

Dentre os animais, o grupo dominante nas macroalgas dos recifes são os invertebrados marinhos, principalmente compostos por anfípodes, poliquetas e moluscos (Corte et al., 2012; Jacobucci e Leite, 2014; Hamdy et al., 2018; Barbosa et al., 2019; Longo et al., 2021, Leite et al., 2021). A ocorrência e estabelecimento da fauna associada às macroalgas é moldada por fatores ambientais, como a hidrodinâmica, a frequência de imersão/emersão e exposição ao ar, bem como as características das macroalgas, como morfologia, complexidade e composição química (metabólitos secundários e conteúdo nutricional), que podem variar de acordo com os táxons de macroalgas e as condições ambientais (Cacabelos et al. 2010, Carvalho e Roque, 2000, Gan et al. 2019, Steneck e Dethier, 1994).

A variação na morfologia e complexidade das macroalgas tem se mostrado um importante preditor da diversidade e abundância das comunidades epifaunais associadas a essas macroalgas (Taylor e Cole, 1994; Chemello e Milazzo, 2002; Jacobucci e Leite, 2014; Vieira et al. 2018; Gan et al., 2019; Duarte et al. 2020; Leite et al., 2021). Essa complexidade está relacionada a uma série de variáveis, como o grau de ramificação, o comprimento, o volume e a biomassa das macroalgas, suas dimensões fractais e sua cobertura de macroalgas epífitas e hidroides (McAbendroth et al., 2005; Dibble e Thomaz, 2006; Leite et al., 2007; Carvalho et al., 2018; Duarte et al., 2020; Leite et al., 2021). As macroalgas mais comuns encontradas em águas tropicais rasas também variam consideravelmente em sua morfologia, incluindo formas filamentosas, folhosas, achatadas e ramificadas (Steneck e Dethier, 1994).

Em termos de composição química, as macroalgas também influenciam a distribuição da fauna associada. O teor de nutrientes pode atuar como um atrativo (Jormalainen et al., 2001; Barile et al., 2004; Duarte et al., 2010; Machado et al. 2018; Fernandes et al., 2021) e alguns metabólitos secundários podem repelir e/ou impedir o consumo pela epifauna (Hay et al., 1987; Hay e Fenical, 1988; Sudatti et al, 2018; Pereira et al. 2020); porém fatores físicos como luz, salinidade e temperatura (Sudatti et al., 2011; Biancacci et al., 2022) e biológicos como a herbivoria (Peckol et al., 1996; Pereira et al., 2017; Sudatti et al., 2018) controlam a variação das concentrações dos compostos químicos. A pressão dos herbívoros também modula e induz a produção desses compostos químicos defensivos pelas macroalgas (Peckol et al., 1996; Pereira et al., 2017). Os compostos produzidos pelas macroalgas dependendo de seu filo, estágio de crescimento e condições ambientais, e podem conter quantidades e tipos variados desses compostos (Ibanez et al., 2012; Belghit et al., 2017; Vinuganesh et al., 2022). Entre os compostos bioativos das macroalgas, destacam-se os metabólitos secundários, como alcaloides, fenóis, terpenos, esteróis e ácidos graxos (Ahmad et al., 1993; Mtolera e Semesi, 1996; Ibanez et al. 2012). Esses compostos podem ter atividade ecológica (anti-herbivoria) e biológica (antimicrobianas) (Hay e Duffy, 1987; Anjali et al., 2019; Biancacci et al. 2022).

Essa variedade de compostos químicos também apresenta uma ampla e reconhecida atividade biológica, com enorme potencial de aplicação nos setores alimentício, cosmético e farmacológico (Gamero-Veja et al., 2020; Vasconcelos et al., 2021; Lomartire et al., 2022; Farghali et al., 2023). Os compostos derivados das macroalgas com atividades antimicrobianas, por exemplo, podem inibir ou retardar o crescimento de bactérias (atividade antibacteriana) e fungos (atividade antifúngica). Pesquisas demonstraram a eficácia antimicrobiana dos extratos de macroalgas (Fables et al., 1995; Sukatar et al., 2006; Rushdi et al., 2021), o que desperta o interesse da indústria farmacêutica em utilizar esses compostos bioativos (Anjali et al., 2019; Lomartire e Gonçalves, 2022; Aravinth et al., 2023), com o objetivo de produzir medicamentos mais eficientes e com menos efeitos colaterais para o tratamento e a prevenção de doenças causadas por microrganismos em humanos ou animais (Shanmughapriya et al. 2008; Pérez et al. 2016).

2 HIPÓTESES

H1: Macroalgas das espécies *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* e *Gelidiella acerosa* apresentam complexidades estruturais e composições químicas diferentes.

H2: Os extratos brutos das macroalgas apresentam atividade biológica frente a bactérias e fungos.

H3: Macroalgas estruturalmente mais complexas abrigam uma comunidade epifaunal mais rica, abundante e diversa.

H4: Macroalgas com maior valor nutricional (micro e macro nutrientes) e menor teor de metabólitos secundários majoritários apresentam uma comunidade epifaunal mais rica, abundante e diversa.

3 OBJETIVOS

3.1. Objetivo Geral

Descrever as variações na estrutura das comunidades epifaunal das macroalgas *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* e *Gelidiella acerosa* em recifes tropicais considerando a complexidade e composição química das macroalgas.

3.2. *Objetivos específicos*

Descrever as comunidades epifaunal das macroalgas *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* e *Gelidiella acerosa* de recifes tropicais, utilizando como descritores a composição taxonômica, a riqueza, a densidade, a diversidade e a equitatividade;

Caracterizar a complexidade estrutural das macroalgas *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* e *Gelidiella acerosa*;

Quantificar os metabólitos secundários majoritários das macroalgas *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* e *Gelidiella acerosa*;

Avaliar a atividade antibacteriana e antifúngica dos extratos das macroalgas *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* e *Gelidiella acerosa*;

Quantificar os macro nutrientes (lipídios totais, proteínas totais, carboidratos totais e cinzas) e micro nutrientes (Na, K, Ca, Mg, Fe, Cu, Zn, Mn e Cr) das macroalgas *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* e *Gelidiella acerosa*;

Identificar a contribuição da complexidade estrutural e da química das macroalgas na estruturação das comunidades macrobentônicas associadas as macroalgas *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* e *Gelidiella acerosa*.

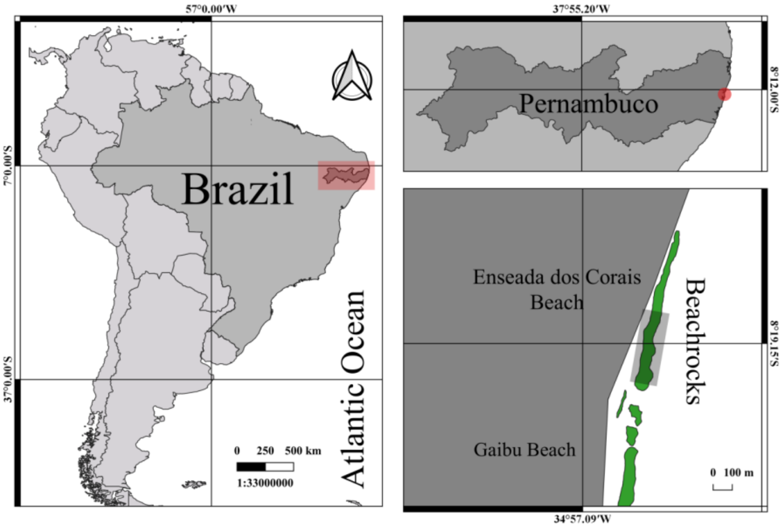
4 METODOLOGIA

4.1 Área de estudo

As amostras foram coletadas na praia da Enseada dos Corais (08°19'09.6" S, 034°56'53.7" W) no nordeste do Brasil - (Figura 1), que tem 3 km de comprimento e recifes de arenito (beachrock) que correm paralelamente à costa (Vasconcelos et al. 2013), com fortes correntes de maré no canal que separa a beachrock da praia e tem uma alta dinâmica de sedimentos siliciclásticos (Bérgamo et al., 2022). O regime de maré é do tipo mesotidal semidiurno, com alturas de maré variando de 0,7 m a 2,5 m (Pereira et al., 2016; Schettini et al. 2016). A temperatura média da água é de 27°C, com salinidade de aproximadamente 36, bons níveis de oxigenação e baixa turbidez (Bezerra et al., 2021; Domingues et al., 2017).

O clima é típico da zona tropical, onde as incidências solares são intensas e a temperatura média anual é próxima a 28ºC (Macedo et al. 2004, Vasconcelos et al. 2013, Domingues et al., 2017). A região tem duas estações bem definidas: uma estação seca, de setembro a fevereiro, e uma estação chuvosa, de março a agosto, onde no verão austral (estação seca), predominam ventos sudeste relativamente intensos e frequentes, criando correntes sul-norte. No inverno austral (estação chuvosa), os ventos predominantes são os nordeste mais fracos, que invertem as correntes (Lira et al. 2010).

Figura 1 – Localização da área de estudo (retângulo cinza) nos recifes de arenitos (beachrocks) da praia da Enseada dos Corais.



Fonte: O autor, 2023.

4.2 Escolha das espécies de macroalgas

Os recifes são colonizados por uma rica ficoflora, sendo as espécies *Ulva lactuca* Linnaeus, 1753, *Padina gymnospora* (Kützing) Sonder 1871, *Palisada perforata* (Bory) K.W.Nam, 2007 e *Gelidiella acerosa* (Forsskål) Feldmann & Hamel, 1934 perenes e abundantes (Vasconcelos et al. 2013; Vasconcelos et al., 2019) e devido a isso foram selecionadas para os estudos. A espécie *U. lactuca* é uma macroalga verde com um talo semelhante a uma folha, composto por lâminas lisas. A macroalga marrom *P. gymnospora* tem um talo em forma de leque coberto por pequenos "pelos" que permitem a fixação da fauna. A *P. perforata* e a *G. acerosa* são macroalgas vermelhas com talos corticados e diferentes tipos de ramificação. Enquanto a *P. perforata* é completamente cilíndrica e forma ramificações densas ao redor do ápice, lembrando um pinheiro, a *G. acerosa* tem uma base cilíndrica que se comprime em direção ao ápice e ramificação pinada. Essas macroalgas estão entre as espécies de algas marinhas mais abundantes e dominantes no recife do nordeste do Brasil (Steneck and Gethier, 1994; Vasconcelos et al., 2019).

4.3 Coleta das macroalgas

Para o estudo da epifauna, dez frondes de cada espécie de macroalga foram coletadas durante a maré baixa em dezembro (2018), fevereiro, abril e junho de 2019, e fixadas em formalina salina a 4% tamponada com tetraborato de sódio. Antes de separar as frondes do substrato, elas foram cobertas com um saco plástico, evitando que a epifauna escapasse.

Para os estudos da complexidade e da química, amostras de cada espécie de macroalga (aproximadamente 1 kg de alga fresca por espécie) foram coletadas aleatoriamente à mão na zona entremarés dos recifes de arenito durante a maré baixa em dezembro de 2018, fevereiro, abril e junho de 2019. Após a coleta, as amostras foram lavadas cuidadosamente em água do mar para remover a fauna aderida, as epífitas e as partículas de areia e, em seguida, armazenadas em caixas térmicas contendo gelo para o transporte até o laboratório.

4.4 Triagem da fauna

Cada amostra foi peneirada em uma malha de 0,3 mm com água corrente para remover a macrofauna. Todos os indivíduos da macrofauna destacados das macroalgas foram contados por dez gramas de macroalgas seca (ind.$10g^{-1}$ de macroalgas secas) e foram identificados no menor nível taxonômico possível. A abundância da epifaunal (N: o número de indivíduos por grama de alga), a riqueza (S: o número total de táxons), a diversidade (λ: índice de Simpson) e a uniformidade (J: índice de Pielou) foram calculadas para cada amostra.

4.5 Análises de complexidade das macroalgas

Para cada espécie de macroalga, cinco frondes foram então colocadas em uma folha de papel branco, estendida o máximo possível e prensada, antes de ser seca por 72 horas em uma estuda a 60°C. Em seguida, cada fronde foi removida da prensa botânica e fotografada com uma câmera digital Nikon Coolpix AW100. Essas fotografias foram usadas para medir a altura (cm), as dimensões fractais da área (Da) e do perímetro (Dp) da fronde e seu índice espacial intersticial (IS), usando o programa ImageJ. As dimensões fractais foram calculadas de acordo com o método proposto por McAbendroth et al. (2005) e Kovalenko et al. (2009), e o índice espacial intersticial (ISI) foi calculado de acordo com Dibble e Thomaz (2006). Após o processamento, as frondes foram pesadas em uma balança analítica (± 0,0001 g).

4.6 Preparação das amostras para as análises químicas das macroalgas

Para os compostos químicos, as amostras foram lavadas em água corrente para remover o sal, secas à temperatura ambiente e moídas até obter um pó fino, para determinar os metabólitos primários (carboidratos totais, proteínas solúveis, lipídios totais, cinzas, minerais como Ca, Mg, Fe, Cu, Zn, Mn, Cr, Na e K) e os metabólitos secundários principais.

Para determinar os metabólitos primários, as amostras em pó foram analisadas conforme descrito na seção 4.6.1, abaixo.

Para os metabólitos secundários, as amostras do pó de algas marinhas foram extraídas com uma solução 2:1 de diclorometano (Neon - Pureza: 99,5%) e metanol (Neon - Pureza: 99,8%). Após 72 horas, os extratos foram filtrados e o solvente foi removido por evaporação sob pressão reduzida, a uma temperatura máxima de 40°C em um evaporador rotativo. Os extratos brutos secos foram posteriormente analisados conforme descrito na seção 4.6.2, abaixo. Os rendimentos dos extratos brutos secos de cada espécie de macroalga marinha foram calculados com base no peso seco das amostras.

4.6.1 Determinação dos metabólitos primários

Teor de cinzas

O teor de cinzas da biomassa seca das macroalgas marinhas foi determinado pelo método descrito por Robledo e Freile-Pelegrin (1997), com modificações. Amostras de 2 g foram calcinadas a 300°C por aproximadamente 1 h e depois a 800°C por 2 h. No final do processo, os cadinhos contendo as cinzas foram resfriados em um dessecador e a massa de cinzas foi determinada pela equação: Cinza total (g)=acm-crm, em que acm = massa de cinzas (g) + massa do cadinho (g) e crm = massa do cadinho (g).

Teor de proteínas

O teor de proteína da biomassa seca das macroalgas marinhas foi determinado usando o método descrito por Kjedahl, com um fator de 6,25 sendo adotado para a conversão dos valores do teor de nitrogênio total (AOAC, 2005).

Teor de carboidratos

Os carboidratos solúveis foram extraídos da biomassa seca das macroalgas marinhas usando ácido tricloroacético a 5% (Merck - Pureza: 98%) e as concentrações foram determinadas pelo método colorimétrico de ácido sulfúrico fenólico descrito por Dubois et al. (1956). A porcentagem de carboidratos solúveis foi calculada com base na absorção a 490 nm em um espectrofotômetro (Hewlett-Packard, modelo 8453), que foi comparado a um padrão de glicogênio.

Teor de lipídios

O teor de lipídios da biomassa seca das macroalgas marinhas foi determinado pela extração em um aparelho Soxhlet por 8 h, usando éter de petróleo (Sigma-Aldrich - Pureza: 100%) como solvente. O material extraído foi seco em uma estufa a 105±2ºC até atingir um peso constante.

Teor de minerais

A quantidade de Ca, Mg, Fe, Cu, Zn, Mn e Cr foi determinada pela dissolução de 2,0 g da biomassa seca de algas marinhas em 10 ml de ácido nítrico a 2% (Alphatec - Pureza: 65%), que foi então quantificada em um espectrofotômetro de absorção atômica Shimadzu AA-6300. O conteúdo de Na e K foi determinado em um Fotômetro de Chama Digimed DM-61.

4.6.2 Determinação dos metabólitos secundários majoritários

As amostras foram analisadas em um cromatógrafo a gás acoplado a um espectrômetro de massa Shimadzu QP2010 Ultra, equipado com uma coluna capilar RTX-5MS de 30 m de comprimento (5% difenil / 95% dimetil polissiloxano), com um diâmetro interno de 0,25 mm e espessura de filme de 0,25 µm. O gás de arraste foi o hélio 5.0 (Pureza: 99,9990%) com uma taxa de fluxo de 1 mL/min. A temperatura inicial do forno foi de 40°C, com uma rampa de aquecimento inicial de 5°C/min até 220°C e 20°C/min até 280°C. O modo de injeção foi splitless, com 1 microlitro sendo injetado. A execução durou 25 minutos. Os espectros de massa foram obtidos por ionização de impacto de elétrons (EI) de 70eV, com a fonte de íons sendo mantida a uma temperatura de 250°C. Os bancos de dados NIST08, NIST08+S e FFNSC 1.3 foram usados para comparação, obedecendo a uma similaridade mínima de 90%. As substâncias com uma concentração acima de 5% foram consideradas compostos majoritários.

4.7 Preparação das amostras para os ensaios biológicos

A partir dos extratos brutos secos obtidos de cada espécie de macroalga, estes foram submetidos aos ensaios biológicos para avaliação da atividade antimicrobiana sobre bactérias, fungos leveduriformes e fungos filamentosos. Os produtos foram pesados e devidamente solubilizados em 150 µL (5%) de dimetilsulfóxido (DMSO) e adicionados100 µL (2%) de Tween 80, completando-se o volume final com água destilada esterilizada q.s.p. 3 mL. Dessa forma, foi obtida a concentração inicial dos produtos de 1024 µg/mL e diluído em série até 32 µg/mL (Cleeland; Squires, 1991; Nascimento et al., 2007; Pereira et al., 2014).

Meios de cultura

Os meios de cultura utilizados nos ensaios para avaliação da atividade biológica foram Brain Heart Infusion (BHI), Agar Sabouraud Dextrose (ASD)- adquiridos da Difco Laboratories Ltd, USA, France, para manutenção, respectivamente, das cepas de bactérias e fungos. Enquanto que para os ensaios de atividade biológica, foram usados caldo Brain Heart Infusion (BHI) para as bactérias e meio RPMI 1640 com L-glutamina e sem bicarbonato para os fungos (Difco Laboratories Ltd, USA, France e INLAB, São Paulo, Brasil). Todos os meios foram preparados conforme as descrições dos fabricantes.

Micro-organismos

Para os ensaios de atividade biológica dos produtos testes, foram utilizadas as seguintes cepas:

- Bactérias: *Staphylococcus aureus* ATCC-25923, S. aureus LM 01 (MRSA), *Escherichia coli* ATCC-25922, E. coli LM 02 (ESBL).

- Fungos: *Candida albicans* ATCC-76485, *C. albicans* LM-85, *C. tropicalis* ATCC-13803, *C. glabrata* ATCC-90030, *C. neoformans* FCF-119, *Aspergillus flavus* ATCC-4603, *Penicillium citrium* ATCC-40011, *Sporothrix brasiliensis* IPEC 100 e *S. brasiliensis* LM 492/20.

Os micro-organismos pertencem a MICOTECA do Laboratório de Micologia, Departamento de Ciências Farmacêuticas (DCF), Centro de Ciências da Saúde (CCS) da Universidade Federal da Paraíba (UFPB). As cepas foram mantidas em BHI/bactérias e em ASD/fungos à temperatura de 4°C. Foram utilizados para os ensaios, repiques de 24 - 48 horas em BHI/ASD, respectivamente, para bactérias e leveduras, incubados a 35 ± 2°C.

Inóculo

Para preparação do inóculo, as colônias obtidas de culturas das cepas de fungos em meio ASD, foram suspensas em solução fisiológica a 0,9% estéril e ajustadas de acordo com o tubo 0,5 da escala padrão de Mcfarland para obtenção de aproximadamente 1-5 x 106 UFC/mL para as cepas fúngicas e 1-5 x 108 UFC/mL para as cepas bacterianas (CLSI, 2015; CLSI, 2008; Hadaceck; Greeger, 2000; Cleeland; Squires, 1991; Antunes et al., 2006; Freire et al., 2014).

Determinação da concentração inibitória mínima (CIM)

Os ensaios de atividade antimicrobiana foram realizados conforme os protocolos de Cleeland e Squires (1991), Eloff (1998) e CLSI (2008). A determinação da CIM das amostras sobre cepas bacterianas e fúngicas foram realizadas através da técnica de microdiluição em caldo, com placa para cultura de células (TPP/ SWITZERLAND/EUROPA) contendo 96 poços com fundo em “U”. Inicialmente, foram distribuídos 100 µL de caldo RPMI/BHI duplamente concentrado nos poços das placas de microdiluição. Em seguida, 100 µL dos produtos solubilizados foram dispensados nas cavidades da primeira linha da placa. E por meio de uma diluição seriada a uma razão de dois, foram obtidas concentrações de 1024 até 16 µg/mL.

Por fim, foi adicionado 10 µL das suspensões das cepas bacterianas e fúngicas nas cavidades, onde cada coluna da placa refere-se, especificamente, a uma espécie. Paralelamente, foram realizados os controles: micro-organismos (CBHI + bactérias e RPMI + leveduras), para comprovação da viabilidade das cepas, meio de cultura (RPMI/CBHI), para comprovação da esterilidade e controle com antimicrobianos: gentamicina (64 µg/mL) para inibição das bactérias e fluconazol (256 µg/mL) para inibição dos fungos. As placas preparadas foram assepticamente fechadas e submetidas à incubação numa temperatura de 35 ± 2°C por 24 - 48 horas para os ensaios com bactérias e leveduras.

No caso do ensaio biológico com as bactérias, após 24 h de incubação, foi adicionado 20 µL de solução do corante resazurina a 0,01% (INLAB), reconhecido como um indicador colorimétrico de óxido-redução (Mann and Markan, 1998). O ensaio foi incubado a 35 ± 2°C por 24 - 48 h. Após a mudança de coloração do corante (azul para vermelho), considerou-se como indicador de crescimento microbiano; e se a cor permanece azul, significa a ausência de crescimento microbiano. A CIM para cada produto foi definida como a menor concentração capaz de inibir visualmente o crescimento microbiano e/ou verificado pela permanência da coloração do corante indicador.

O produto foi considerado ativo quando inibiu, pelo menos, 50% dos micro-organismos utilizados nos ensaios de atividade biológica (Cleeland and Squires, 1991; Hafidh et al., 2011). E a Concentração Inibitória Minìma /CIM, foi considerada e interpretada como ativa ou inativa, conforme os seguintes critérios: até 600 µg/mL= forte atividade; 600-1500 µg/mL= moderada atividade; > acima de 1500 µg/mL=fraca atividade ou produto inativo (Holetz et al., 2002; Sartoratto et al., 2004; Houghton et al., 2007).

4.8 Análises estatísticas

Para comparar a complexidade estrutural (altura, biomassa, Da, Dp, ISI) e o composto químico (metabólitos primários e secundários) das macroalgas, e os descritores da comunidade epifaunal (riqueza de espécies, abundância, equitabilidade e diversidade) entre as espécies de macroalgas, foi realizada uma ANOVA de uma via [dados log(x+1)] transformada, seguida pelo teste de Tukey. A PERMANOVA (unidirecional) e o CAP foram baseados em matrizes de semelhança calculadas usando a distância euclidiana [dados transformados em raiz quarta - complexidade estrutural e composto químico das macroalgas] e a semelhança de Bray Curtis (dados transformados em raiz quarta - comunidade epifaunal). A relação entre complexidade estrutural, composto químico e comunidade epifaunal foi avaliada por meio de modelos lineares baseados em distância (DistLM) e análise de redundância baseada em distância (dbRDA) usando o procedimento "Step-wise" e AIC como critério de seleção para os melhores modelos. As análises estatísticas foram realizadas com o Primer 7.0 +PERMANOVA e o STATISTICA® 12 (Clark e Warwick 2001). Em todas as análises, foi considerado um nível de significância de 5%.

5 RESULTADOS

Os resultados obtidos estão apresentados na forma de artigos científicos, onde cada artigo corresponde a uma seção da tese. Estes apresentam uma sequência dos seguintes temas: Composição química, Complexidade estrutural, Atividade biológicas dos extratos brutos, Interação entre assembleia de poliquetas e macroalgas, e comunidade epifaunal e macroalgas.

A seção 6 trata da caracterização química das macroalgas *Ulva lactuca, Padina gymnospora, Palisada perforata* e *Gelidiella acerosa*.

A seção 7 analisa a atividade antifúngica e antibacteriana dos extratos brutos das macroalgas *Ulva lactuca, Padina gymnospora, Palisada perforata* e *Gelidiella acerosa*.

A seção 8 caracteriza as assembléias de poliquetas nas macroalgas *Ulva lactuca, Padina gymnospora, Palisada perforata* e *Gelidiella acerosa*, identificando quais características das macroalgas podem ser os determinantes mais importantes da variação dessas assembléias.

A seção 9 caracteriza a epifauna das macroalgas *Ulva lactuca, Padina gymnospora, Palisada perforata* e *Gelidiella acerosa*, identificando quais características físicas e químicas das macroalgas controlam a comunidade.

6 ARTIGO 1 - CHEMICAL COMPOUNDS OF FOUR SEAWEEDS, *ULVA LACTUCA, PADINA GYMNOSPORA, PALISADA PERFORATA AND, GELIDIELLA ACEROSA* FROM TROPICAL COASTAL REEFS OF NORTHEASTERN BRAZIL

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Abstract

Seaweed extracts have an enormous potential for the prospection of chemical compounds with industrial applications. The present study describes the chemical composition (total carbohydrates, proteins, lipids, ash, minerals, and major secondary metabolites) found in the dichloromethane/methanol extracts of four species of seaweed (*Ulva lactuca*, *Padina gymnospora*, *Palisada perforata*, and *Gelidiella acerosa*) collected of Pernambuco, on the tropical coast of northeastern Brazil. Carbohydrate content ranged from 14.35% to 48.52% of Dry Weight (DW), soluble proteins from 7.49% to 14.98% DW, total lipids 0.40–8.92% DW, and ash 18.51–37.02% DW. The concentrations of macro elements (Ca, Mg, K, and Na) ranged from 900 to 4902.12 mg.Kg algae dry weight^{-1} and those of trace elements (Fe, Mn, Cu, Zn, and Cr), from 0.08 to 4462.8 mg.Kg algae dry weight^{-1}. The lowest concentrations of Ca, Mg, Na, K, Mn, and Cu were recorded in *P. gymnospora*, and the highest in *P. perforata*. High concentrations of terpenes and fatty acids were recorded in all the extracts, and neophytadiene was the major compound in all species. Phytol and palmitic acid were also found in all the seaweed species, albeit in smaller quantities than neophytadiene. Phytone was only recorded in *U. lactuca*. The red algae had the highest concentrations of most chemical compounds. Overall, these findings indicate that the four seaweed species have considerable potential as a source of chemical compounds for human use.

Key words: primary metabolites; secondary metabolites; benthic seaweeds, sandstone reefs.

Resumo

Os extratos de algas marinhas têm um enorme potencial para a prospecção de compostos químicos com aplicações industriais. O presente estudo descreve a composição bioquímica (carboidratos totais, proteínas, lipídios, cinzas, minerais e principais metabólitos secundários) encontrada nos extratos de diclorometano/metanol de quatro espécies de algas marinhas (*Ulva lactuca, Padina gymnospora, Palisada perforata* e *Gelidiella acerosa*) coletadas em Pernambuco, na costa tropical do nordeste do Brasil. O conteúdo de carboidratos variou de 14,35% a 48,52% do peso seco (PSA), proteínas solúveis de 7,49% a 14,98% PSA, lipídios totais de 0,40 a 8,92% PSA e cinzas de 18,51 a 37,02% PSA. As concentrações de macroelementos (Ca, Mg, K e Na) variaram de 900 a 4902,12 mg.Kg de peso seco de algas^{-1} e as de elementos-traço (Fe, Mn, Cu, Zn e Cr), de 0,08 a 4462,8 mg.Kg de peso seco de algas^{-1}. As concentrações mais baixas de Ca, Mg, Na, K, Mn e Cu foram registradas em *P. gymnospora*, e as mais altas em *P. perforata*. Altas concentrações de terpenos e ácidos graxos foram registradas em todos os extratos, e o neofitadieno foi o principal composto em todas as espécies. O fitol e o ácido palmítico também foram encontrados em todas as espécies de algas marinhas, embora em quantidades menores do que o neofitadieno. A fitona só foi registrada na *U. lactuca*. As algas vermelhas apresentaram as maiores concentrações da maioria dos compostos bioquímicos. Em geral, essas descobertas indicam que as quatro espécies de algas marinhas têm um potencial considerável como fonte de compostos químicos para uso humano.

Palavras-chave: metabólitos primários; metabólitos secundários; macroalgas marinhas bentônicas, recifes de arenito.

1. Introduction

Seaweeds are a diverse group of photosynthetic organisms, which are classified as red (Rhodophyta), green (Chlorophyta) or brown algae (Ochrophyta, Phaeophyceae), according to their pigmentation, the composition of their cell walls, and their polysaccharide reserves (Lüning, 1990; Hanelt, 2008; Lewis and McCourt, 2004). Seaweeds are exceptionally resilient organisms, which arose between one and 1.6 billion years ago, and have survived numerous mass extinction events (Butterfield, 2000; Bengtson, 2017). Worldwide, nearly 12,000 seaweed species are known to exist (Guiry and Guiry, 2023), of which, 1,707 have been recorded in the tropical and subtropical waters of the western Atlantic (Wynne, 2022).

Seaweeds play important roles as primary producers in aquatic ecosystems, as well as enhancing environmental complexity, and contributing to the purification of the water by absorbing excess nutrients and pollutants (Dijkstra et al. 2017; Cotas et al., 2023; Farghali et al. 2023). Macroalgae also provide habitats and food for a wide range of marine organisms, primarily through the attenuation of environmental conditions and the accumulation of periphyton (Christie et al. 2009; Kraan, 2013; Cotas et al., 2023). As seaweeds are exposed constantly to environmental stressors in their natural environments, they produce a variety of unique metabolites that have a wide range of commercial and industrial applications in many different sectors (Leandro et al., 2019; Biris-Dorhoi et al., 2020; Echave et al., 2022; Farghali et al., 2023).

Seaweed can be a rich source of nutrients and bioactive compounds such as minerals (calcium, magnesium, potassium, and iron), trace elements (zinc, manganese and selenium), fiber, proteins, carbohydrates, lipids, and vitamins, including vitamins A, B, C, E, and K (Marinho-Soriano et al., 2006; Gamero-Vega et al., 2020; Echave et al., 2022). Seaweed extracts also tend to have high concentrations of secondary metabolites (phenolic and halogenated compounds, sterols, and terpenes) that have antioxidant, anti-inflammatory, anti-cancer, antiviral, bactericide, anti-fungal, and immune-modulating properties (Anjali et al., 2019; Biancacci et al. 2022). The specific chemical composition of a seaweed will depend on a ranged of factors, including genetic variation, geographic distribution, and environmental conditions (Marinho-Soriano et al. 2006; Belghit et al., 2017; Vinuganesh et al. 2022).

Almost 300 species of seaweed are exploited in some way by humans, and Yaris et al. (2016) have predicted that more than 500 million tons (dry weight) of seaweed will be consumed annually by 2050. The global seaweed market was worth US$9.9 billion in 2021, and is predicted to grow at an annual rate of 2.3% from 2022 to 2030 (Skyquest, 2023). A large number of studies have demonstrated the potential of chemical compounds extracted from marine seaweed for the production of human foodstuffs (Rameshkumar et al., 2013; Ramero-Vega et al., 2020), cosmetics (Suganthy et al. 2013; Vasconcelos et al. 2021), fertilizers (Pereira and Cotas, 2019; Mukrjee et al., 2020) and medicinal drugs (Bhardwaj et al., 2020; Santos et al., 2020; Lomartire and Gonçalves, 2022). These compounds may also have a range of other industrial (Kılınç et al. 2013; Polat et al., 2021) and biotechnological uses (Desai et al., 2021; Zhang et al., 2022).

Given this potential, understanding the specific makeup of the nutrients and bioactive compounds found in marine seaweed species can be extremely valuable, especially in a world with a growing population and ever-increasing demand for food and medication. In particular, seaweed could supply the protein needed by many populations while conserving natural resources (Koyande et al. 2021). In this context, the present study investigated the chemical constituents, including minerals, and primary and major secondary metabolites, of the red algae *Gelidiella acerosa* (Forsskål) Feldmann & Hamel 1934 and *Palisada perforata* (Bory) K.W.Nam 2007, the brown alga *Padina gymnospora* (Kützing) Sonder 1871, and the green alga *Ulva lactuca* Linnaeus 1753 from tropical sandstone reefs of the coast of northeastern Brazil.

2. Materials and Methods

2.1 Study area

The study was conducted of the beach of Enseada dos Corais in Pernambuco, on the tropical Brazilian coast. This beach is approximately three kilometers long, and has offshore sandstone reefs running parallel to the coastline (Vasconcelos et al., 2013). These intertidal reefs are colonized densely by macroalgal species that are typical of the tropical phytogeographic region, such as *Palisada perforata* and *Gelidiella acerosa*, and species of the genera *Centrocera*s, *Ulva*, and *Padina* (Vasconcelos et al., 2019). *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata*, and *Gelidiella acerosa* are extremely abundant at Enseada dos Corais, where they occur throughout the year (Vasconcelos et al 2019, Bergamo et al 2022). The local climate is tropical humid, with a mean temperature of 28ºC and two well-defined seasons. The dry season lasts from September to February, and the rainy, from March through August (Bezerra et al., 2021). The tidal regime is of the mesotidal semi-diurnal type, with tide heights ranging from 0.7 m (neap tide) to 2.5 m, on the spring tide (Pereira et al., 2017).

2.2 Sample collection

Samples of *Gelidiela acerosa*, *Palisada perforata*, *Padina gymnospora* and *Ulva lactuca* (approximately 1 kg of fresh alga per species) were collected randomly by hand from the intertidal zone of the sandstone reefs during the low spring tide in December 2018. After collection, the samples were washed thoroughly in seawater to remove the attached fauna, epiphytes, and sand particles, and then stored on ice in a cooler for transportation to the laboratory.

2.3 Preparation of the seaweed for chemical analysis

In the laboratory, the samples were washed under running water to remove the salt, dried at room temperature, and ground to a fine powder. To determine the primary metabolites (chemical composition), the powdered samples were analyzed as described in section 2.4, below. For secondary metabolites, samples of the seaweed powder were extracted using a 2:1 solution of dichloromethane (Neon - Purity: 99.5%) and methanol (Neon - Purity: 99.8%). After 72 hours, the extracts were filtered, and the solvent was removed by evaporation under reduced pressure and a maximum temperature of 40°C in a rotary evaporator. The dried extracts were subsequently analyzed as described in section 2.5, below. The yields of the extracts of each seaweed were calculated based on the dry weight of the samples.

2.4 Determination of the primary metabolites

2.4.1 Ash

The ash content of the dry seaweed biomass was determined by the method described by Robledo and Freile-Pelegrin (1997), with modifications. Samples of 2 g were calcined at 300°C for approximately 1 h and then at 800°C for 2 h. At the end of the process, the crucibles containing the ash were cooled in a desiccator and the mass of ash was determined by the equation:

$$Totalash(g) = acm - crm$$

where, acm = ash mass (g) + crucible mass (g), and crm = crucible mass (g).

2.4.2 Protein

The protein content of the dry seaweed biomass was determined using the method described by Kjedahl, with a factor of 6.25 being adopted for the conversion of the values from the total nitrogen content (AOAC, 2005).

2.4.3 Carbohydrates

Soluble carbohydrates were extracted from the dry seaweed biomass using 5% trichloroacetic acid (Merck - Purity: 98%) and the concentrations were determined by the phenolic sulphuric acid colorimetric method described by D Dubois et al. (1956). The percentage of soluble carbohydrates was calculated based on the absorption at 490 nm in a spectrophotometer (Hewlett-Packard, model 8453), which was compared to a glycogen standard.

2.4.4 Lipids

The lipid content of the dry seaweed biomass was determined by extraction in a Soxhlet apparatus for 8 h, using petroleum ether (Sigma-Aldrich - Purity: 100%) as the solvent. The extracted material was dried in an oven at 105±2ºC until reaching a constant weight.

2.4.5 Minerals

The amount of Ca, Mg, Fe, Cu, Zn, Mn, and Cr was determined by dissolving 2.0 g of the dried seaweed biomass in 10mL of 2% nitric acid (Alphatec - Purity: 65%), which was then quantified in a Shimadzu AA-6300 Atomic Absorption Spectrophotometer. The Na and K content was determined in a DM-61 Digimed Flame Photometer.

2.5 Determination of the secondary metabolites

The samples were analyzed in a gas chromatograph coupled to a Shimadzu QP2010 Ultra mass spectrometer equipped with a 30-m long RTX-5MS capillary column (5% Diphenyl / 95% dimethyl polysiloxane), with an internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium 5.0 (Purity: 99.9990%) with a flow rate of 1 mL/min. The starting oven temperature was 40°C, with an initial heating ramp of 5°C/min to 220°C and 20°C/min to 280°C. The injection mode was splitless, with 1 microliter being injected. The run lasted 25 min. The mass spectra were obtained by 70eV electron impact ionization (EI), with the ion source being maintained at a temperature of 250°C. The NIST08, NIST08+S, and FFNSC 1.3 databases were used for comparison, complying with a minimum similarity of 90%. Substances with a concentration over 5% were considered to be majority compounds.

2.6 Statistical analysis

A one-way Analysis of Variance (ANOVA) was applied to the comparison of the amounts of primary and secondary metabolites recorded among the different seaweed species (fixed factor), using the log (x+1) transformed data. When the results of the ANOVA were significant, Tukey's *post hoc* test was applied for pairwise comparisons. These analyses were run in STATISTICA® 12, and a significance level of 95% was considered in all cases.

3. Results

The yields of the crude extracts from the four algal species are shown in Table 1. *Palisada perforata* had the highest yield (1.9%), followed by *Gelidiella acerosa* (1.2%), *Padina gymnospora* (1.1%), and *Ulva lactuca* (0.7%).

Table 1. Yield of the crude extracts obtained from the the four seaweed species (*Ulva lactuca*, *Padina gymnospora*, *Palisada perforata*, and *Gelidiella acerosa*) sampled from the coastal sandstone reefs of the tropical coast of northeastern Brazil.

Phylum	Species	Amount of dried seaweed collected (g)	Dried extract (g)	Yield (%)
Chlorophyta	*Ulva lactuca*	140.5	1.1	0.7
Phaeophyta	*Padina gymnospora*	115.4	1.2	1.1
Rhodophyta	*Gelidiella acerosa*	90.6	1.1	1.2
	Palisada perforata	262.8	5.0	1.9

Primary metabolites

The content of carbohydrates (F = 1202.7, $p < 0.01$), proteins (F = 88.2, $p < 0.01$), lipids (F = 2408.4, $p < 0.01$), and ash (F = 385.5, $p < 0.01$) varied significantly among the four seaweed species (Figure 1). Significantly lower amounts of all these metabolites were found in the brown alga *P. gymnospora*, while the red algae *P. perforata* had significantly more carbohydrates and lipids than the other species. The highest protein content was recorded in *P. perforata*, and *U. lactuca*, and *P. perforata* also contained significantly more ash than the other species (Figure 1).

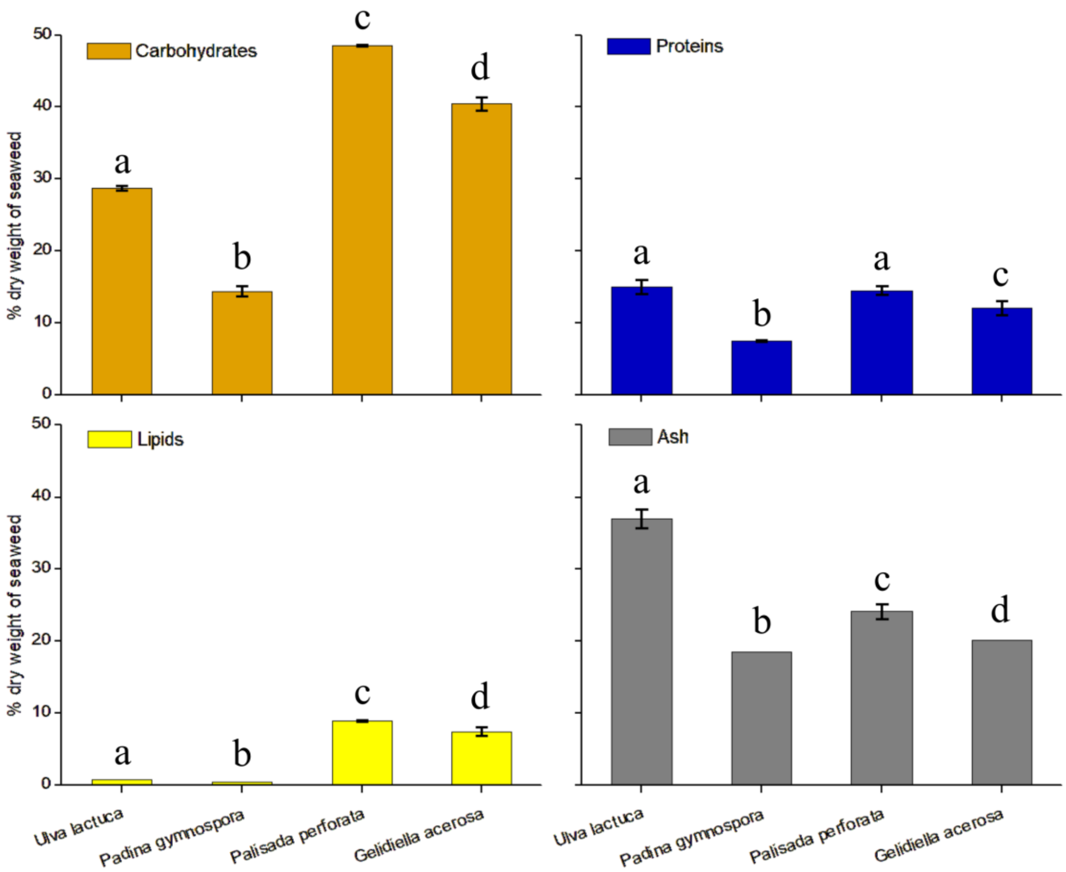

Figure 1. Mean ± SE concentrations (n = 3) of the primary metabolites found in samples of the seaweeds *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata*, and *Gelidiella acerosa* collected from sandstone reefs of the tropical coast of northeastern Brazil. Different letters above the columns in the same graph indicate a significant difference between the pair of species, based on the results of Tukey's *post hoc* test.

Minerals

With the exception of Cr (F = 1.34, p = 0.33), the concentrations of all the minerals varied significantly among the study species (Figure 2). The greatest variation was recorded in Ca (F = 2135, $p < 0.01$), Mg (F = 2512, $p < 0.01$), Fe (F = 60087, $p < 0.01$), Na (F = 3721, $p < 0.01$), and Mn (F = 290549, $p < 0.01$). Less marked, but still significant variation was recorded in K (F = 1983, $p < 0.01$), Cu (F = 124.8, $p < 0.01$), and Zn (F = 37.33, $p < 0.01$). In general, higher concentrations of most minerals (Ca, Mg, Na, K, Mn, Cu) were recorded in *P. gimnospora*. However, *U. lactuca* returned exceptionally high concentration of Fe and Zn (Figure 2).

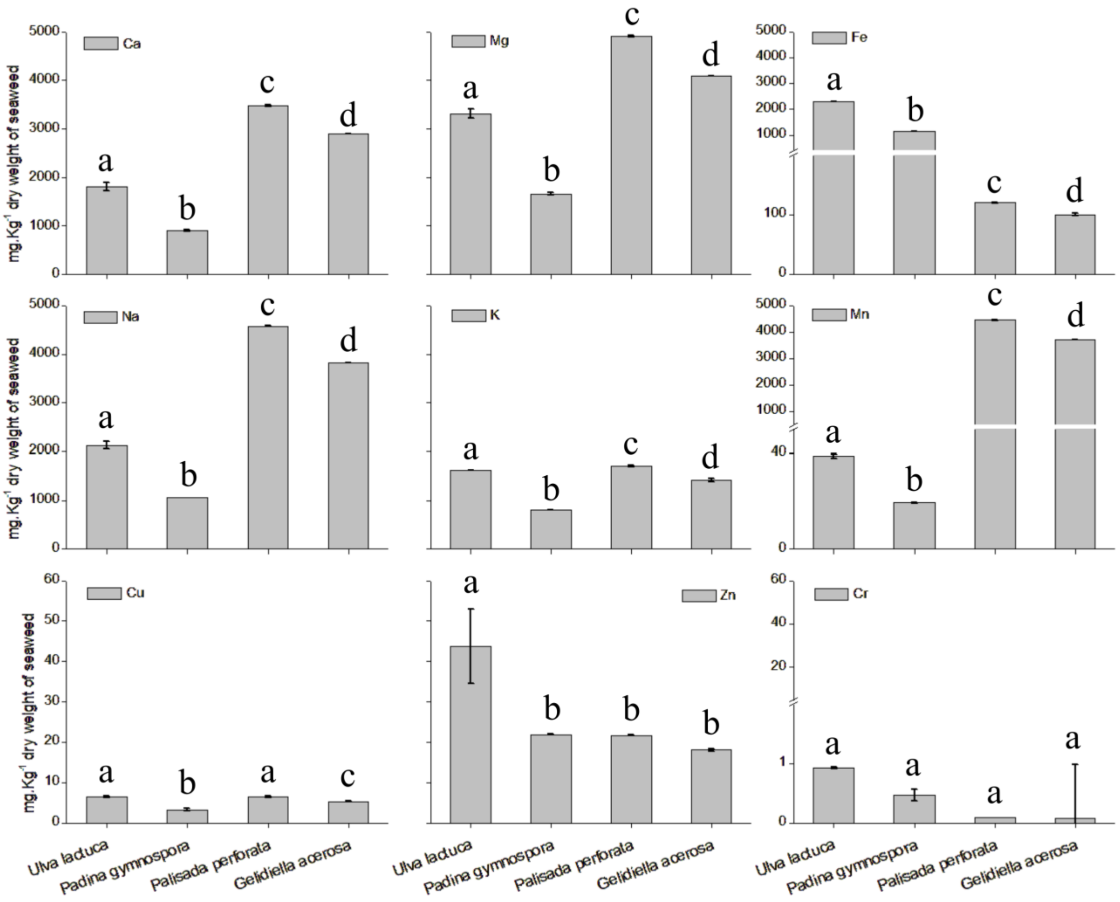

Figure 2. Mean ± SE concentrations (n = 3) of the minerals recorded in the seaweed species (*Ulva lactuca*, *Padina gymnospora*, *Palisada perforata*, and *Gelidiella acerosa*) collected from sandstone reefs of the tropical coast of northeastern Brazil. Different letters above the columns in the same graph indicate a significant difference between the pair of species, based on the results of Tukey's *post hoc* test.

Major secondary metabolites

The major chemical groups were terpenes and fatty acids. Neophytadiene, phytol, and palmitic acid were recorded in all four species, and neophytadiene was the major compound. *Palisada perforata* was the species with the highest concentrations of secondary metabolites (neophitadine: 23.89% DW, phytol: 8.29% DW; palmitic acid: 8.32% DW), while *U. lactuca* had the lowest concentrations, except for phytone, which was present only in this species.

Figure 3. Chemical structure of the major secondary metabolites found in *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* and *Gelidiella acerosa* from the sandstone reefs of the tropical coast of northeastern Brazil.

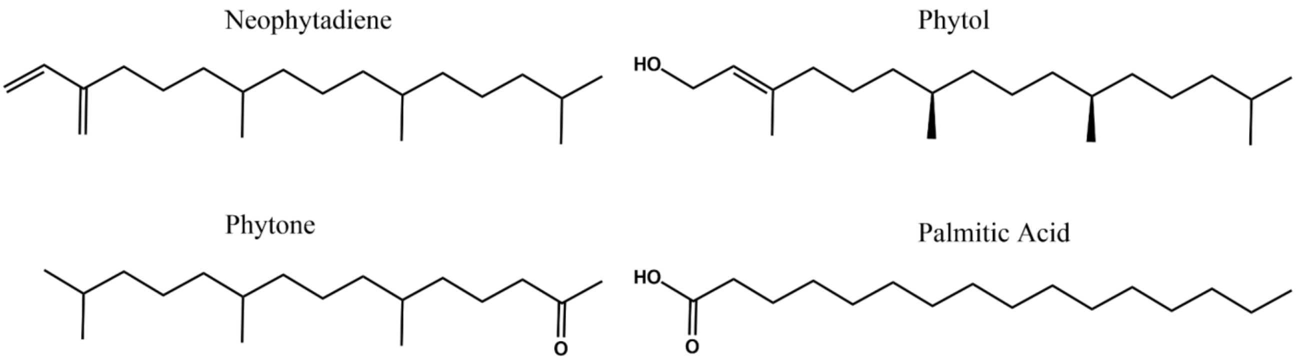

Table 2. Major compounds, Retention Time (RT), and the relative abundance (%) of the secondary metabolites recorded in *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata*, and *Gelidiella acerosa* from the sandstone reefs of the tropical coast of northeastern Brazil.

Compounds	Chemical group	*U. lactuca*		*P. gymnospora*		*P. perforata*		*G. acerosa*	
		RT	%	RT	%	RT	%	RT	%
Neophytadiene	Terpenes	18.69	17.60	18.712	23.89	18.68	29.04	18.73	24.13
Phytol		19.14	8.47	19.164	8.29	19.13	11.29	19.18	09.69
Palmitic acid	Fatty acids	20.01	6.20	20.035	8.32	19.99	08.10	20.05	07.75
Phytone	Ketones	18.77	6.05	-	-	-	-	-	-

4. Discussion

The four seaweed species – *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* and *Gelidiella acerosa* – sampled from the sandstone reefs of Enseada dos Corais, on the tropical coast of Brazil have very distinct chemical compositions. Seaweeds produce primary and secondary metabolites in response to both abiotic and biotic factors (Belghit et al., 2017; Biris-Dorhoi et al., 2020), and the chemical composition of these organisms is known to vary according to genetic variation, geographic distribution, and environmental conditions, such as salinity, temperature, luminosity, and growth habitats (Marinho-Soriano et al., 2006; Vasconcelos et al., 2019; Vinuganesh et al., 2022). While they occupy the same reefs, the study species belong to different phyla, which is probably the principal determinant of the differences observed in their chemical composition, given the known variation among brown, red, and green algae (Kumar et al, 2011; Sahul Hamid et al., 2019; Al Sharie et al., 2020).

Carbohydrates were the most abundant compound found in all the species, which is typical of the known chemical composition of seaweeds (Marinho-Soriano et al. 2006; Rohani-Ghadikolaei et al. 2012; Biancacci et al. 2022). Carbohydrates derived from seaweeds are classified as fucoidan, alginate, carrageenan, ulvan, laminarin, and cellulose and hemicellulose, depending on their chemical composition (Goñi et al., 2020). Marine algae contain relatively large amounts of polysaccharides including mucopolysaccharides, and cell-wall and storage polysaccharides, which account for 4–76% of their total dry weight (Usman et al., 2017). Many of these carbohydrates function as either a structural component of the cell wall or as storage molecules in the plastids, which provide the energy required for various metabolic processes (Khairy and El-Shafay, 2013).

The red algae *P. perforata* had the highest carbohydrate content (49.7% DW). The taxonomic group is the principal determinant of the occurrence, composition, and structure of the carbohydrates found in marine seaweeds (Usman et al., 2017) and each class of macroalga produces its own unique compounds (Rioux and Turgeon, 2015). Red algae have κ-carrageenan polysaccharides and agar composed of a variety of monomers (Cian et al., 2015; Gamero-Vega et al., 2020). Although few studies have compared the carbohydrate content of the different types of seaweed, that is, green, red, and brown algae, Mohammadi et al. (2013) recorded the highest carbohydrate content in the red alga *Gracillaria corticata* (41.72%) and the lowest in the brown alga *Colpomenia sinuous* (11.3%). Ilhami et al. (2021) obtained similar findings, and concluded that red macroalgae typically have a higher carbohydrate content than either brown or green macroalgae.

The maximum protein content was recorded in the green alga *U. lactuca* (14.98% DW) and the minimum value, in the brown alga *P. gimnospora* (1.49% DW). In algae, protein plays a crucial role in processes such as enzymatic catalysis, transport and storage, and mechanical sustentative control (Rameshkumar et al., 2013). Protein content may vary considerably among species, seasons, and environmental conditions (Mishra et al. 1993; Biancacci et al. 2022), although marine macoralgae tend to contain high concentrations of essential amino acids, lectins, glycoproteins, and phycobiliproteins (Echave et al., 2022). As observed in the present study, red (12.5–35.2% DW) and green algae (9.6–23.3% DW) tend to have a higher protein content than brown algae – 4.5–16.8% DW (Ibañez and Cifuentes, 2013; Biancacci et al., 2022; Echave et al., 2022).All the study species had a relatively low lipid content, with the highest concentrations being recorded in the red algae (8.9% DW in *P. perforata* and 7.43% DW in *G. acerosa*) and the lowest in the brown alga *P. gymnospora* (0.4% DW). In general, seaweeds are not considered to be a rich source of lipids (Ratana-Arporn & Chirapart, 2006), and tend to contain approximately 4% DW (Herbreteau et al. 1997; Polat and Ozogul, 2008). The results of the present study are in partial disagreement with previous studies, such as those of Rohani-Ghadikolae et al. (2012) and Barot et al. (2019), which recorded a higher lipid content in green algae in comparison with red and brown species. It is important to note here, however, that other studies have focused on different species and have used alternative analytical procedures, which means that any such comparisons should be treated with caution.

The ash content varied widely among the study species (18.51–37.02% DW). A high ash content is typical of seaweed (Fuentes et al., 2000), and is generally around 20–25 g.100 g^{-1} (Di-Filippo-Herrera et al., 2018; Tapia-Martínez et al., 2019), although higher values have been recorded in some studies (Rupérez, 2002: 20.6–39.3% DW; Mohammadi et al., 2013: 15.84–33.68% DW; Jeliane et al., 2022: 31–36% DW). Most algae have a greater ash content than terrestrial plants, and some of the trace elements found in seaweeds are rare or absent in terrestrial plants (Rameshkumar et al., 2013). The ash content of a plant tends to correlate with its mineral content (Kumar et al. 2011; Siddique et al. 2013; Praiboon et al. 2018), as observed in the present study. The ash of edible seaweed is known to contain larger amounts of macrominerals (8.083–17.875 mg.100 g^{-1}; Na, K, Ca, Mg) and trace elements (5.1–15.2 mg.100 g^{-1}; Fe, Zn, Mn, Cu) than edible terrestrial plants (Rupérez, 2015; Leandro et al., 2020).

With the exception of Cr, the mineral content of the different elements (Ca, Mg, Fe, Na, K, Mn, Cu, and Zn) varied significantly among the study species. These minerals play a vital role in the growth, development, and protein synthesis of seaweed (Huerta-Diaz et al. 2007; Ismail et al. 2017) and the availability of these nutrients can affect the production of metabolites by marine alga e (Gaubert et al. 2019). Seaweeds can absorb minerals selectively from the seawater and accumulate them in their thallus (Rafia Azmat et al. 2006). In general, the composition and concentrations of minerals found in seaweeds are species- and location-specific (Rohani-Ghadikolaei et al. 2012). Minerals such as Ca, Mg, K, and Na are important for the development of the plant, and are generally present in larger quantities in marine algae than in freshwater species (Nisizaka, 2006).

The principal groups of secondary metabolites recorded in the present study were terpenes (neophytadiene and phytol) and fatty acids (palmitic acid), although both groups varied considerably in their abundance among the different seaweed species. The red algae *P. perforata* had a higher content of these metabolites in comparison with the green alga *U. lactuca*. Both chemical groups are typical of seaweeds (Kim et al. 2011; Andrade et al 2013; Teixeira et al. 2019) and neophytadiene, phytol, and palmitic acid often comprise the major compounds of terpenes and fatty acids in these organisms (Abdel-Aal et al., 2015; Santos et al., 2020). In general, secondary metabolites are excretory produced under stressful conditions, such as exposure to ultraviolet radiation, shifts in temperature and salinity, and pressures from competitors and herbivores (Vergés et al. 2008; Uhrich et al. 2016; Gaubert et al. 2019), and are predominantly phenolic and halogenated compounds, sterols, terpenes, and small peptides (Stengel et al., 2011; Rosa et al., 2019).

Neophytadiene and phytol are the predominant terpenes in many types of seaweed, and their potential industrial applications have been the focus of several studies (Aziz et al., 2019; Santos et al., 2020). Phytol is an isoprenoid compound derived primarily from chlorophyll (Moraes et al., 2014), and is known to have antinociceptive, antioxidant (Santos et al., 2013; Santos et al., 2015), antimicrobial (Anjali et al., 2019), and immunostimulatory activity in humans (Venkata et al., 2012). Like phytol, neophytadiene is a diterpene with known antibacterial (Anjali et al., 2019) and antioxidant activity (Santos et al., 2017). Bhardwaj et al., (2020) also found that neophytadiene extracted from the brown macroalga *Turbinaria ornata* inhibited significantly the production of nitric oxide and inflammatory cytokines in *in vivo* and *in vitro* experiments.

Palmitic acid is the most abundant saturated fatty acid found in green, brown, and red algae (Rohani-Ghadikolaei et al., 2012). This compound has known antioxidant, antifungal, and antibacterial activity (Anjali et al., 2019; Aravinth et al., 2023), and may protect the seaweed against physical, chemical, and biological stressors (Vasconcelos et al., 2021). While phytone was found only in *U. lactuca* in the present study, it has been observed in other species of green, red, and brown macroalgae (Kajiwara et al. 1990; Kajiwara et al. 1991). The presence of this compound may be the result of the hydrolysis of chlorophyll or bacteriochlorophyll-a photoproducts (Marchand and Rontani 2003; Rontani et al 2003) or the biodegradation of phytol (Rontani and Acquaviva 1993; Cikos et al. 2022).

5. Conclusions

The chemical compounds, including minerals and primary and secondary metabolites, of the macroalgal species Ulva lactuca, Padina gymnospora, Palisada perforata, and Gelidiella acerosa, sampled from sandstone reefs of the tropical coast of northeastern Brazil, are described in this study. In general, these species are rich in proteins (all four), carbohydrates (primarily the red and green algae), and lipids (the red algae in particular). The red and green algae also have high concentrations of essential minerals, while the red and brown algae have the highest concentrations of the major secondary metabolites identified in this study. These findings indicate that the four study species have considerable potential as a source of chemical compounds for human use in a world with a continuously growing population, and ever-increasing demand for foodstuffs and medication. The study also emphasizes the need for further studies to better evaluate the potential of marine seaweeds for commercial, industrial, and pharmaceutical uses, and contribute to the development of industrial products derived from natural marine resources.

Author contribuitions

NC: research conceptualization, data collection, picture editing, data analysis and interpretation, writing - original draft, review and editing.

FFS: chemical data analysis and interpretation, writing - original draft, review and editing.

MSN: chemical data analysis and interpretation, writing - original draft

JF: chemical data analysis and interpretation, writing - original draft.

YM: picture editing, chemical data analysis and interpretation, writing - original draft.

JSRF: research conceptualization, data analysis and interpretation, writing - original draft, review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Ethics and Permits

None of the research reported in this article required research permits.

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7 ARTIGO 2 - ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACT OF THE MACROALGAE *ULVA LACTUCA, PADINA GYMNOSPORA, PALISADA PERFORATA* AND *GELIDIELLA ACEROSA* FROM TROPICAL COASTAL SANDSTONE REEFS

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Abstract

Macroalgae produce a wide variety of chemical compounds with diverse biological activities. The present study evaluated the antimicrobial activity of crude extracts of the macroalgae *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* and *Gelidiella acerosa* collected from tropical coastal sandstone reefs in Enseada dos Corais beach, were dried and powder samples were extracted with a solution of dichloromethane and methanol (2:1). The results obtained in this study showed that of all the macroalgae from tropical coastal sandstone reefs exhibit antifungal activity. *Ulva lactuca* have strong activity against *C. albicans* and *C. neoformans*. On the other hand, the crude extract of the macroalgae showed that none could inhibit the growth of the bacterial strains and filamentous fungi. The species used have great potential for antifungal properties, but further studies with different extraction methods are needed. This work may provide a basis for future studies of bioactive products derived from macroalgae, which may serve as natural sources of products for future medical applications.

Key words: Macroalgae, chemical compounds, biological activities, antimicrobial properties.

Resumo

As macroalgas produzem uma ampla variedade de compostos químicos com diversas atividades biológicas. O presente estudo avaliou a atividade antimicrobiana de extratos brutos das macroalgas Ulva lactuca, Padina gymnospora, Palisada perforata e Gelidiella acerosa coletadas de recifes de arenito da costa tropical da praia da Enseada dos Corais, foram secas e as amostras em pó foram extraídas com uma solução de diclorometano e metanol (2:1). Os resultados obtidos nesse estudo mostraram que todas as macroalgas dos recifes de arenito da costa tropical apresentam atividade antifúngica. A Ulva lactuca tem forte atividade contra C. albicans e C. neoformans. Por outro lado, o extrato bruto das macroalgas mostrou que nenhuma delas foi capaz de inibir o crescimento das cepas bacterianas e dos fungos filamentosos. As espécies usadas têm grande potencial para propriedades antifúngicas, mas são necessários mais estudos com diferentes métodos de extração. Este trabalho pode fornecer uma base para estudos futuros de produtos bioativos derivados de macroalgas, que podem servir como fontes naturais de produtos para futuras aplicações médicas.

Palavras-chave: Macroalgas, compostos químicos, atividades biológicas, propriedades antimicrobianas.

1. Introduction

Macroalgae are photosynthetic organisms that are classified into three groups based on their pigmentation: red algae (Rhodophyta), green algae (Chlorophyta), or brown algae (Ochrophyta, Phaeophyceae) (Horta et al., 2001). Macroalgae produce a wide variety of chemical compounds and are recognized for their diverse biological activity and enormous potential for application in the food, cosmetic, and pharmacological sectors (Gamaro-Veja et al., 2020; Vasconcelos et al., 2021; Lomartire et al., 2022; Farghali et al., 2023).

Depending on their phylum, growth stage, and environmental conditions, macroalgae may contain varying amounts and types of bioactive compounds (Ibanez et al., 2012; Belghit et al., 2017; Vinuganesh et al., 2022). Among the bioactive compounds in macroalgae, secondary metabolites such as alkaloids, phenols, terpenes, sterols and fatty acids are notable (Ahmad et al., 1993; Mtolera and Semesi, 1996; Ibanez et al. 2012). These compounds can possess ecological activity (e.g. anti-herbivory) and biological activity (e.g. antimicrobial) (Hay and Duffy, 1987; Anjali et al., 2019; Biancacci et al. 2022).

Antimicrobial compounds can inhibit or slow the growth of bacteria (antibacterial activity) and fungi (antifungal activity). Research has demonstrated the antimicrobial efficacy of macroalgae extracts (Fables et al., 1995; Sukatar et al., 2006; Rushdi et al. 2021), which has piqued the interest of the pharmaceutical industry in utilizing these bioactive compounds (Anjali et al. 2019; Lomartire and Goncalves, 2022; Aravinth et al., 2023), aimed to produce more efficient drugs with fewer side effects for the treatment and prevention of microorganism-caused diseases in humans or animals (Shanmughapriya et al. 2008; Perez et al. 2016).

Knowled of the biological activity of crude macroalgae extracts is important for initial studies in the search for bioactive compounds that could be used in treatments against pathogens. The aim of this study was to evaluate the antimicrobial activity of crude extracts of the macroalgae *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* and *Gelidiella acerosa* from sandstone reefs of the tropical coast.

2. Materials and methods

2.1 Collection of macroalgae and obtaining extracts

The macroalgal samples Ulva lactuca, Padina gymnospora, Palisada perforata and Gelidiella acerosa were collected from tropical coastal sandstone reefs in the Enseada dos Corais beach. The macroalgaes were initially washed in seawater to remove fauna, epiphytes and sand particles, placed in polythene bags, labelled and kept in a cool box with ice until transported to the laboratory. At the laboratory, the samples were washed in running water to remove salt, any remaining organisms and sediment. Subsequently, the macroalgae were laid out and dried at room temperature for five days and after this period, ground to powder.

For the determination of secondary metabolites, the macroalgae powder samples were extracted with a solution of dichloromethane and methanol (2:1). After 72 hours, the extracts were filtered and the solvent removed by evaporation under reduced pressure and maximum temperature of 40 °C in a rotary evaporator.

2.2 *Identification of chemical constituents (major secondary metabolites) - Gas chromatography coupled to mass spectrometry (GC-MS).*

The analyses of the major secondary metabolites in the crude extract of the macroalgae *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* and *Gelidiella acerosa* were performed in a Gas Chromatograph coupled to a Mass Spectrometer model GCMS-QP2010 Ultra - Shimadzu, equipped with a capillary RTX-5MS column (5% Diphenyl / 95% dimethyl polysiloxane), 30 m long, 0.25 mm internal diameter and 0.25 µm film thickness. The carrier gas was helium 5.0 with a flow rate of 1 mL/min. The initial oven temperature was 40 °C, with an initial heating ramp of 5 °C/min to 220 °C and 20 °C/min to 280 °C. The injection mode was splitless, with 1 microliter being injected. The run lasted 25 min. Mass spectra were obtained by 70eV electron impact ionization (EI); the ion source was maintained at a temperature of 250 °C. The NIST08, NIST08+S and FFNSC 1.3 databases were used for comparison, complying with minimum 90% similarity. It was considered a majority compound with concentration greater than 5%.

2.3 Microbial assay

2.3.1. Preparation of samples and culture media

The algae extracts were subjected to biological assays to evaluate their antimicrobial activity against bacteria, yeast-like fungi, and filamentous fungi. The products were weighed and dissolved in 150 µL (5%) dimethyl sulfoxide (DMSO) and added 100 µL (2%) Tween 80, with the final volume completed with sterile distilled water to a total of 3 mL. This resulted in an initial concentration of the products at 1024 µg/mL, which was then serially diluted to 32 µg/mL (Cleeland and Squires, 1991; Nascimento et al., 2007; Pereira et al., 2014). The culture media used in the biological activity assays were Brain Heart Infusion (BHI) and Sabouraud Dextrose Agar (ASD) acquired from Difco Laboratories Ltd, USA, France, for the maintenance of bacterial and fungal strains, respectively. For the biological activity assays, Brain Heart Infusion broth (BHI) was used for bacteria, while RPMI 1640 medium with L-glutamine and without bicarbonate (Difco Laboratories Ltd, USA, France, and INLAB, São Paulo, Brazil) was used for fungi. All media were prepared according to the manufacturer's instructions.

2.3.2. Microorganisms and inoculum

The following strains were used for the microbiological activity assays of the test products: *Staphylococcus aureus* ATCC-25923, *S. aureus* LM 01 (MRSA), *Escherichia coli* ATCC-25922, *E. coli* LM 02 (ESBL), *Candida albicans* ATCC-76485, *C. albicans* LM-85, *C. tropicalis* ATCC-13803, *C. glabrata* ATCC-90030, *C. neoformans* FCF-119, *Aspergillus flavus* ATCC-4603, *Penicillium citrium* ATCC-40011, *Sporothrix brasiliensis* IPEC 100, and *S. brasiliensis* LM 492/20. The strains were maintained in BHI (bacteria) and ASD (fungi) at 4°C. For the assays, subcultures of 24-48 hours in BHI/ASD were used for bacteria and yeast, respectively, and incubated at 35 ± 2°C. To prepare the inoculum, colonies obtained from cultures of fungal strains on ASD medium were suspended in sterile 0.9% saline solution and adjusted to approximately 1-5 x 106 CFU/mL for fungal strains and 1-5 x 108 CFU/mL for bacterial strains, according to the 0.5 tube of the McFarland standard scale (Cleland; Squires, 1991; Antunes et al., 2006; CLSI, 2008; Freire et al., 2014; CLSI, 2015).

2.3.3. Minimum inhibitory concentration (MIC) determination

The antimicrobial activity assays were performed following the protocols of Cleeland and Squires (1991), Eloff (1998), and CLSI (2008). The determination of MIC for bacterial and fungal strains was carried out using the broth microdilution technique in U-bottom 96-well cell culture plates (TPP/Switzerland/Europe). Initially, 100 µL of double-concentrated RPMI/BHI broth was distributed into the wells of the microdilution plates. Subsequently, 100 µL of the dissolved products were added to the wells of the first row of the plate. Serial two-fold dilutions were performed to obtain concentrations ranging from 1024 to 16 µg/mL. Finally, 10 µL of bacterial and fungal suspensions were added to the wells, with each column of the plate specifically referring to a species. Parallel controls were performed, including microorganisms (CBHI + bacteria and RPMI + yeast) to verify strain viability, culture medium (RPMI/CBHI) to confirm sterility, and antimicrobial controls: gentamicin (64 µg/mL) for bacterial inhibition and fluconazole (256 µg/mL) for fungal inhibition. The prepared plates were aseptically sealed and incubated at a temperature of 35 ± 2°C for 24-48 hours for bacterial and yeast assays.

In the case of the biological assay with bacteria, after 24 hours of incubation, 20 µL of a 0.01% resazurin dye solution (INLAB) was added, which is recognized as a colorimetric indicator of redox reactions (Mann and Markan, 1998). The assay was then incubated at 35 ± 2°C for 24-48 hours. A change in color of the dye from blue to red was considered an indicator of microbial growth, while a blue color indicated the absence of microbial growth. The MIC for each product was defined as the lowest concentration that visually inhibited microbial growth and/or was confirmed by the persistence of the color of the indicator dye. The product was considered active if it inhibited at least 50% of the microorganisms used in the biological activity assays (Cleeland and Squires, 1991; Hafidh et al., 2011). The Minimum Inhibitory Concentration (MIC) was classified and interpreted as active or inactive based on the following criteria: up to 600 µg/mL = strong activity; 600-1500 µg/mL = moderate activity; >1500 µg/mL = weak activity or inactive product (Holetz et al., 2002; Sartoratto et al., 2004; Houghton et al., 2007).

3. Results

3.1 Major compound in the crude extract of macroalgae

In the analysis of GC/MS spectra of the crude extracts from the four studied macroalgae species, the compound Neofitadiene was identified as the major component in all macroalgae species (Table 1).

Table 1. Majority compound, Retention Time (RT) and Abundance (Area in %) of the crude extracts of the seaweed *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* and *Gelidiella acerosa* colected in tropical coastal sandstone reefs.

Macroalgae		Compounds			
		Neophytadiene	Phytol	Palmitic acid	Phytone
U. lactuca	RT	18.686	19.140	20.007	18.767
	%	17.60	08.47	06.20	06.05
P. gymnospora	RT	18.712	19.164	20.035	-
	%	23.89	08.29	08.32	-
P. perforata	RT	18.680	19.132	19.998	-
	%	29.04	11.29	08.10	-
G. acerosa	RT	18.725	19.179	20.054	-
	%	24.13	09.69	07.75	-

3.2 Antibacterial and antifungal activity

The biological activity assays' results evaluating the antimicrobial activity of the crude extracts from *U. lactuca*, *P. gymnospora*, *P. perforata*, and *G. acerosa* are presented in Table 2. The *U. lactuca* and *P. gymnospora* crude extracts exhibited activity against all fungal species, excluding filamentous fungi, in concentrations ranging from 256 to 1024 µg/mL. The crude extracts from *P. perforata* and *G. acerosa* did not display any activity against the growth of fungal species *C. glabrata* and *C. neoformans*, respectively. Additionally, *P. perforata* and *G. acerosa* showed no activity against filamentous fungi growth, similar to *U. lactuca* and *P. gymnospora*. Moreover, the antibacterial activity results showed that none of the tested extracts could inhibit the growth of the bacterial strains utilized.

Table 2. Evaluation of the Minimum Inhibitory Concentration (MIC - µg/mL) crude extracts of macroalgae *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* and *Gelidiella acerosa* colected in the tropical coastal sandstone reefs.

Microorganisms tested	Extract (µg/mL)				Control			
	Ulva lactuca	*Padina gymnospora*	*Palisada perforata*	*Gelidiella acerosa*	CM	Microorganisms	Fluc	Gent
S. aureus ATCC-25923	+	+	+	+	-	+	NA	32
S. aureus LM 01 (MRSA)	+	+	+	+	-	+	NA	32
E. coli ATCC_25922	+	+	+	+	-	+	NA	32
E. coli ATCC_25922	+	+	+	+	-	+	NA	32
C. albicans ATCC - 90028	1024	1024	512	1024	-	+	128	NA
C. albicans LM-37	256	512	512	1024	-	+	256	NA
C. tropicalis ATCC-13803	512	512	512	512	-	+	256	NA
C. glabrata ATCC-90030	512	1024	512	+	-	+	256	NA
C. neoformans FCF-119	256	512	+	512	-	+	128	NA
A. flavus ATCC-4603	+	+	+	+	-	+	512	NA
P. citrinum INCQS-4001	+	+	+	+	-	+	512	NA
S. brasiliensis IPEC 100	+	+	+	+	-	+	+	NA
S. brasiliensis LM 492/20	+	+	+	+	-	+	+	NA

Footnotes: (+) *Microorganism growth*; (-) *No microbial growth observed; (NA) Not applicable; (CM) Culture medium; (Fluc) Fluconazole; (Gent) Gentamicin.*

4. Discussion

Studies have confirmed the potential of marine macroalgae as a source of bioactive compounds. Among the major phytochemicals of the crude extracts, the concentrations of neophytadiene and phytol were higher in the red algae, *Palisada perforata* and *Gelidiella acerosa*, and in palmitic acid in *Padina gymnospora*. These phytocompounds include the major terpene and fatty acid compounds found in macroalgae, (Abdel-Aal et al., 2015; Pérez et al. 2016; Santos et al., 2020), with recognized antimicrobial activity (Chandrasekaran et al. 2011; Swamy et al. 2015; Kumara Swamy et al. 2017). Additionally, other compounds such as polysaccharides, sterols, phenols, and flavonoids also exhibiting antimicrobial activit, but not focused in this study. These findings highlight the potential of marine macroalgae as a source of compounds and indicate the need for further research to explore their use (Shah et al., 2022; Rengasamy et al., 2020; Michalak et al., 2022).

In the present study, no antibacterial activity was observed in the crude extracts of macroalgae, nor was there any activity against filamentous fungi. Factors such as seasonality, the solvent used or the state of the macroalgae (fresh or dried) are aspects that interfere with the process of obtaining bioactive compounds and their biological activity (Paul and Puglisi, 2004; Kandhasamy and Arunachalam, 2008; Shanmughapriya et al., 2008; Banu and Mishra, 2018). In our study, crude extracts of macroalgae were obtained from dried biomass using a mixture of dichloromethane and methanol solvents (2:1). Tuney et al. (2006) and Shanmughapriya et al. (2008) show that the organic extract of fresh macroalgae has the highest antibacterial activity compared to the dried extracts. In addition, studies show that extracts using chloroform as a solvent are more effective in obtaining antibacterial compounds (Pandithurai et al., 2015; Banu and Mishra, 2018). However, the dried methanolic extracts of the macroalgae *Gelidiela acerosa* showed strong antimicrobial activity (Elsie et al., 2011), and *Ulva lactuca* (Saritha et al., 2013), but not observed for *Palisada perforata* (Moheimanian et al. 2023). Acetone-dried extracts of the macroalgae *Padina gymnospora* showed good antimicrobial activity (Rosaline et al., 2012). In addition, the antimicrobial activity also varies between macroalgal species due to different chemical components present in the extract such as phenols, flavonoids, triterpenoid (Rojas et al. (1992); Cordeiro et al. 1995; Tuney et al. 2006).

All extracts of macroalgae showed antifungal activity in *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. neoformans*, with *Padina gymnospora, Palisada perforata* and *Gelidiella acerosa* have low or moderate activity, while *U. lactuca* showed strong activity in *C. albicans* and *C. neoformans.* This lower activity for a given organism may be related to seasonality, where studies have shown differences in antimicrobial activities from different seasons for *Ulva lactuca* (Deveau et al. 2016). Methanol and ethyl acetate dried extract of the macroalgae *Ulva flexuosa* showed moderate antifungal activity in fungal strains *C. albicans* (ATCC 10231), with a minimum inhibitory concentration (MIC) value of 15 mg.mL^{-1} for both extracts (Mashjoor et al. 2015), whereas for dichloromethane:metanol (2:1) dried extract of the *Ulva lactuca* in the present study, the MIC was 0.256 mg.mL^{-1} for *C. albicans* (LM-37) and *C. neoformans* (FCF-119), with antifungal effects also follow a similar trend to the standard (Fluconazole). In the study developed by Ibraheem et al. 2012, the fungus *Candida albicans* (IMRU 3669), was strongly inhibited by acetone extract of *U. lactuca*. For this fungus, the macroalgae of the *Ulva* genus, in different extraction solvents, show excellent inhibition efficiency.

In conclusion, the results obtained in this study showed that dichloromethane:metanol (2:1) dried extract of the all macroalgae from tropical coastal sandstone reefs exhibit antifungal activity. *Ulva lactuca* have strong activity in *C. albicans* and *C. neoformans*. On the other hand, the crude extract of the macroalgae showed that none could inhibit the growth of the bacterial strains and filamentous fungi. The disparity between our findings and those from other studies on antibacterial activity may stem from various factors. One likely cause could be the seasonal variation of the macroalgae compounds or the could be differences in the extraction method of the active metabolites. This work may provide a foundation for future studies involving bioactive products derived from macroalgae, which may serve as natural sources of products for upcoming medical applications.

Author contribuitions

NC: research conceptualization, data collection, picture editing, data analysis and interpretation, writing - original draft, review and editing.
GFS: data analysis, writing - original draft, review and editing.
EOL: antimicrobial activity, data analysis and interpretation, writing - original draft
CM: data analysis, writing - original draft.
YM: picture editing, chemical data analysis and interpretation, writing - original draft.
JSRF: research conceptualization, data analysis and interpretation, writing - original draft, review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Ethics and Permits

None of the research reported in this article required research permits.

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8 ARTIGO 3 - MACROALGAE TRAITS AND SEASONALITY AS DRIVERS OF POLYCHAETE ASSEMBLAGES ON MACROALGAE OF TROPICAL SANDSTONE REEFS

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Abstract

Benthic macroalgae are very important in structuring the biota of coastal reefs and are colonized by several species of animals and plants. The present study describes the polychaete assemblages associated with the macroalgae *Gelidiella acerosa, Palisada perforata*, *Padina gymnospora*, and *Ulva lactuca* on tropical sandstone reefs on the Brazilian coast and correlates the complexity of the macroalgae with the polychaete assemblages. Samples (10 fronds of each macroalgal species) were collected randomly during the dry (December 2018 and February 2019) and rainy (April and June 2019) seasons. The height, fractal dimension of the area and perimeter, and the Index of interstices were determined from macroalga photographs using the ImageJ software. Significant seasonal differences were found in the architecture of the macroalgae, and *G. acerosa* and *P. perforata* had the most complex structure. The macroalgae were colonized by 25 taxa of mobile polychaetes from seven families (Dorvilleidae, Eunicidae, Heisionidae, Polynoidae, Phyllodocidae, Syllidae and Nereididae), with dominance of syllids and nereidids in both seasons. Polychaetes were mostly carnivores and omnivores in the dry and rainy seasons, respectively. *Platynereis dumerillii* was the most abundant and frequent polychaete species. The structure of the assemblages varied significantly among macroalgae. *G. acerosa* and *P. perforata* had the most species-rich assemblages. The most important traits of macroalgae that structure polychaete assemblages are height, diameter of the area (Da), and diameter of the perimeter (Dp), with slight variation these traits between macroalgal species. Seasonal changes and macroalgal complexity (traits) were important drivers for polychaete assemblages on macroalgae in tropical coastal sandstone reefs.

Key words: Animal-plant interactions, tropical reefs, seaweed beds, macrobenthos, phytal environment, Polychaeta, epifaunal

Resumo

As macroalgas bentônicas são muito importantes na estruturação da biota dos recifes costeiros e são colonizadas por várias espécies de animais e plantas. O presente estudo descreve as assembleias de poliquetas associadas às macroalgas *Gelidiella acerosa*, *Palisada perforata*, *Padina gymnospora* e *Ulva lactuca* em recifes tropicais de arenito na costa brasileira e correlaciona a complexidade das macroalgas com as assembleias de poliquetas. As amostras (10 frondes de cada espécie de macroalga) foram coletadas aleatoriamente durante as estações seca (dezembro de 2018 e fevereiro de 2019) e chuvosa (abril e junho de 2019). A altura, a dimensão fractal da área e do perímetro e o índice de interstícios foram determinados a partir de fotografias de macroalgas usando o software ImageJ. Foram encontradas diferenças sazonais significativas na arquitetura das macroalgas, e a G. acerosa e a P. perforata apresentaram a estrutura mais complexa. As macroalgas foram colonizadas por 25 táxons de poliquetas móveis de sete famílias (Dorvilleidae, Eunicidae, Heisionidae, Polynoidae, Phyllodocidae, Syllidae e Nereididae), com predominância de syllids e nereidids em ambas as estações. Os poliquetas eram em sua maioria carnívoros e onívoros nas estações seca e chuvosa, respectivamente. Platynereis dumerillii foi a espécie de poliqueta mais abundante e frequente. A estrutura das assembléias variou significativamente entre as macroalgas. *G. acerosa* e *P. perforata* apresentaram os conjuntos mais ricos em espécies. As características mais importantes das macroalgas que estruturam as assembleias de poliquetas são a altura, o diâmetro da área (Da) e o diâmetro do perímetro (Dp), com uma pequena variação dessas características entre as espécies de macroalgas. As mudanças sazonais e a complexidade das macroalgas (características) foram fatores importantes para as assembleias de poliquetas em macroalgas nos recifes de arenito da costa tropical.

Palavras-chave: Interações entre animais e plantas, recifes tropicais, bancos de macroalgas, macrobentos, ambiente fital, Polychaeta, epifauna

1. Introduction

Macroalgae are used as a habitat by several epiphytic algae and animals (invertebrates and vertebrates) in coastal marine environments (Edgar, 1983; Christie et al., 2009). The enormous structural diversity of the macroalgae can increase the complexity of the substrate and the availability of food (either the macroalgae themselves or the detritus accumulated in their structures) and shelter, which may support very diverse and abundant animal and plant communities (Hayward, 1980; Bates, 2009; Christie et al., 2009). The presence of macroalgae generate complex ecosystems (the phytal environment), in which many organisms depend on these plants for feeding, reproduction and protection, with surprisingly high productivity (Masunari and Forneris, 1981; Gan et al., 2019). The fauna associated with macroalgae is driven by environmental factors, such as hydrodynamics, the frequency of immersion/emersion, exposure to the air, and the characteristics of the macroalgae, such as morphology, complexity, and chemical composition (secondary metabolites and nutritional content), which may all vary according to the macroalgal taxa and environmental conditions (Steneck and Dethier, 1994; Carvalho and Roque, 2000; Cacabelos et al., 2010; Gan et al., 2019). The most diverse and abundant animal groups in the phytal environment include the benthos, represented primarily by crustaceans, mollusks, polychaetes, and echinoderms (Azevedo, 1992; Corte et al., 2012; Jacobucci and Leite, 2014). Polychaetes are often dominant, in terms of both abundance and species richness, in the phytal of marine macroalgae. Polychaetes exploit seaweed beds primarily as a refuge from predation (Bailey-Brock et al., 1980; Serrano et al., 2006) and as a place to feed (Hamdy et al., 2018).

The variation in macroalgal morphology and complexity have been shown to be an important predictor of the diversity and abundance of the epifaunal communities associated with these plants (Taylor and Cole, 1994; Chemello and Milazzo, 2002; Jacobucci and Leite, 2014; Vieira et al., 2018; Gan et al., 2019). This complexity is related to a number of variables, such as the degree of branching, the length, volume, and biomass of the plants, their fractal dimensions, and their coverage of epiphytic macroalgae and hydroids (McAbendroth et al., 2005; Leite et al., 2007; Carvalho et al., 2018; Duarte et al., 2020a). The most common macroalgae found in shallow tropical waters also vary considerably in their morphology, including filamentous, leafy, flattened, and branched forms (Steneck and Dethier, 1994). Sandstone reefs are abundant bordering the Brazilian tropical coast and are the primary consolidated substrate for macroalgae fixation (Maida and Ferreira, 1997).

The present study describes the relationship between the polychaete assemblages of the study reef in Pernambuco and four species of macroalga – *Palisada perforata* (Rhodophyta, Rhodomelaceae), *G. acerosa* (Rhodophyta, Gelidiellaceae), *Padina gymnospora* (Ochrophyta, Dictyotaceae), and *Ulva lactuca* (Chlorophyta, Ulvaceae). In particular, the study aimed to understand the role of macroalgae complexity and seasonality in structuring the polychaete assemblages of tropical coastal sandstone reefs. We tested the hypothesis that in tropical coastal sandstone reefs the more structurally complex macroalgae are colonized by more abundant and species-rich polychaete assemblages, and more diverse and rich assemblages occur during the dry season. We also attempted to identify which macroalgal traits are the most important drivers of the polychaete assemblages associated with the different macroalgal species.

2. Materials and Methods

Study area

The samples were collected in Enseada dos Corais (08◦19′ 09.6″ S, 034◦56′ 53.7″ W) (Northeastern Brazil) (Fig. 1), which is 3 km long and has sandstone (beachrock) reefs that run parallel to the coast (Vasconcelos et al., 2013). Some 36 macroalgal species occur on these reefs, with dominance of *Palisada perforata* (Bory) K.W. Nam, *Ulva* Linnaeus, *Gelidiella acerosa* (Forssk.) Feldmann, Hamel, *Centroceras* Kütz. *Padina* Adanson, Sphacelariaceae J. Decaisne, and *Sargassum* C. Agardh (Vasconcelos et al., 2013, 2019). The region’s climate is typical of the tropical zone, where the solar incidences intense and the mean annual temperature is close to 28 ◦C (Macˆedo et al., 2004; Vasconcelos et al., 2013; Domingues et al., 2017). There are two well-defined seasons, a dry season, from September to February, and a rainy season, from March to August. The mean temperature of the water is 27 ◦C, with salinity of approximately 36, high level of dissolved oxygen and low turbidity (Domingues et al., 2017). The local tides are semidiurnal mesotides (Schettini et al., 2016). In the austral summer (dry season), relatively intense and frequent southeasterly winds predominate, creating south-north currents. In the austral winter (rainy season), the predominant winds are weaker northeasterlies, which invert the currents (Lira et al., 2010).

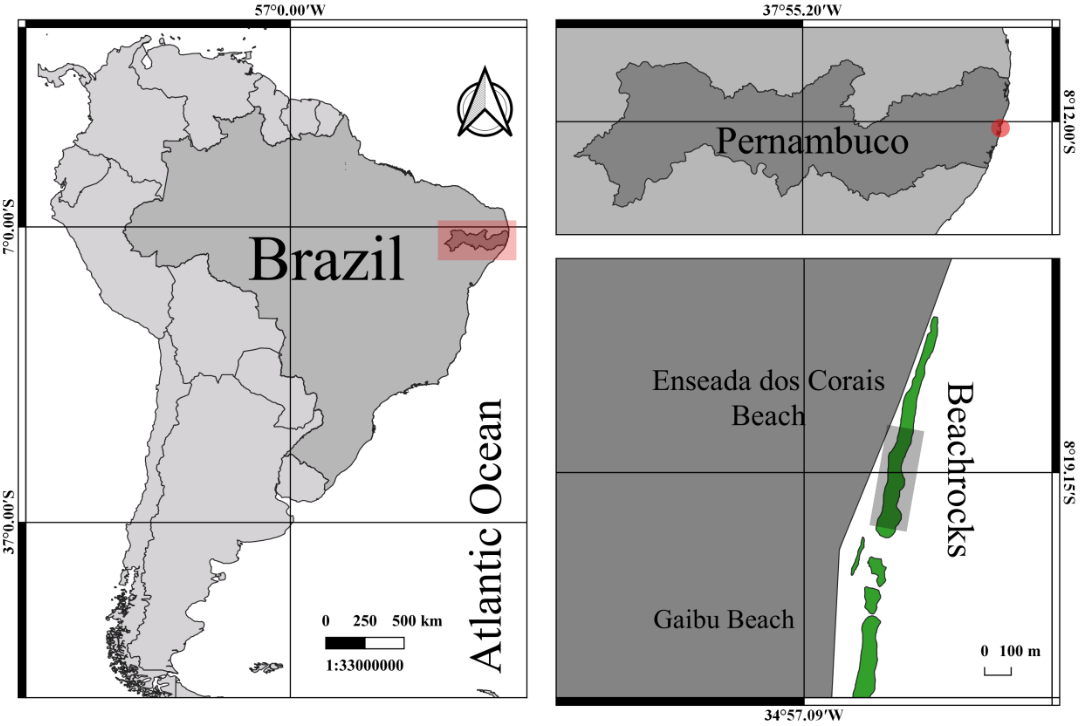

Fig. 1 – Location of the study area (gray rectangle) on the sandstones beachrocks reefs of Enseada dos Corais beach.

Sampling

Samples (10 fronds) of *Gelidiela acerosa* (corticated), *Palisada perforata* (corticated), *Padina gymnospora* (foliose), and *Ulva lactuca* (foliose) were collected randomly from the study reefs in December (2018) and February (2019) (the dry season), and Abril and June 2019 (the rainy season). These macroalgae represent different morphofunctional groups and are very abundant, and they are found throughout the year on the coastal reefs of tropical Brazil (Seteneck & Dethier, 1994; Soares and Fujii, 2012; Vasconcelos et al., 2019). Prior to collection, each specimen was covered with a plastic bag, to prevent the escape of its motile fauna, before being detached from the substrate with a spatula and then fixed in 4% formalin saline buffered with sodium tetraborate.

Laboratory analysis

In the laboratory, the samples were immersed in fresh water and shaken vigorously several times. The water was then filtered through a 0.3 mm mesh to collect the polychaetes. The polychaetes were identified to the lowest possible taxonomic level and counted (Le´on-Gonz´ales et al., 2009, 2021; Steiner et al., 2021; Amaral et al., 2021). The feeding habit and mobility of polychaetes associated with macroalgae are important to understand the functional aspects of these communities, like the polychaetes preferences for macroalgae as a food source (i.e., the macroalgae itself or the organisms that reside within it) and/or for shelter and protection. The feeding habit and mobility of the polychaete species were classified according to Fauchald and Jumars (1979) and Jumars et al. (2015). The polychaete feeding guilds used were herbivores, carnivores, and omnivores.

Each macroalgal frond was then placed on a sheet of white paper, spread out as far as possible, and pressed, before being dried for 72 h in an oven at 60 ◦C. After drying ach frond was removed from the botanical press and photographed using a Nikon Coolpix AW100 digital camera. The fronds were then weighed on an analytical balance (±0.0001 g).

Macroalgae complexity can be evaluated based on several traits (McAbendroth et al., 2005; Dibble and Thomaz, 2006; Thomaz and Cunha, 2010; Carvalho et al., 2018; Duarte et al., 2020a,b). So, we used the photographs to measure the height (cm), the fractal dimensions of the area (Da) and perimeter (Dp) and the interstitial spatial index (ISI), using the ImageJ program. The fractal dimensions were calculated following the method proposed by McAbendroth et al. (2005) and Kovalenko et al. (2009), and ISI was calculated according to Dibble and Thomaz (2006).

The macroalgae height (H) was measured in the central line (base to apex) of each image (orange dotted line) (Fig. 2). To calculate Da and Dp, the *box count* algorithm was utilized in the ImageJ software. A grid comprising squares varying from 2 to 64 pixels was superimposed on the picture until a sufficient number of squares encompassed all the coverage. This quantity is denoted by N(s), where "s" indicates the scale examined (Thomaz and Cunha, 2010). Da represents the measure of the area covered by the macroalgae (Fig. 2b), while Dp indicates the perimeter area of the macroalgae (Fig. 2c).

To calculate the Interstitial Space Index (ISI), two vertical black-dashed lines and one orange dotted line, along with three black-dashed horizontal lines, were superimposed on each image to delineate the frond’s top, middle, and bottom (Fig. 2a). Subsequently, the spaces between the macroalgae’s structural elements, i.e., the intercepted interstices, were quantified and measured for each line. The formula to calculate the index is ISI = $fh/lh + fv/lv$. Here, fh represents the average frequency of interstices intercepted per centimeter on the horizontal axis, lh refers to the average length of the interstices along the horizontal axis, fv refers to the average frequency of interstices intercepted per centimeter on the vertical axis, and lv represents the average length of the interstices along the vertical axis (Dibble and Thomaz, 2006).

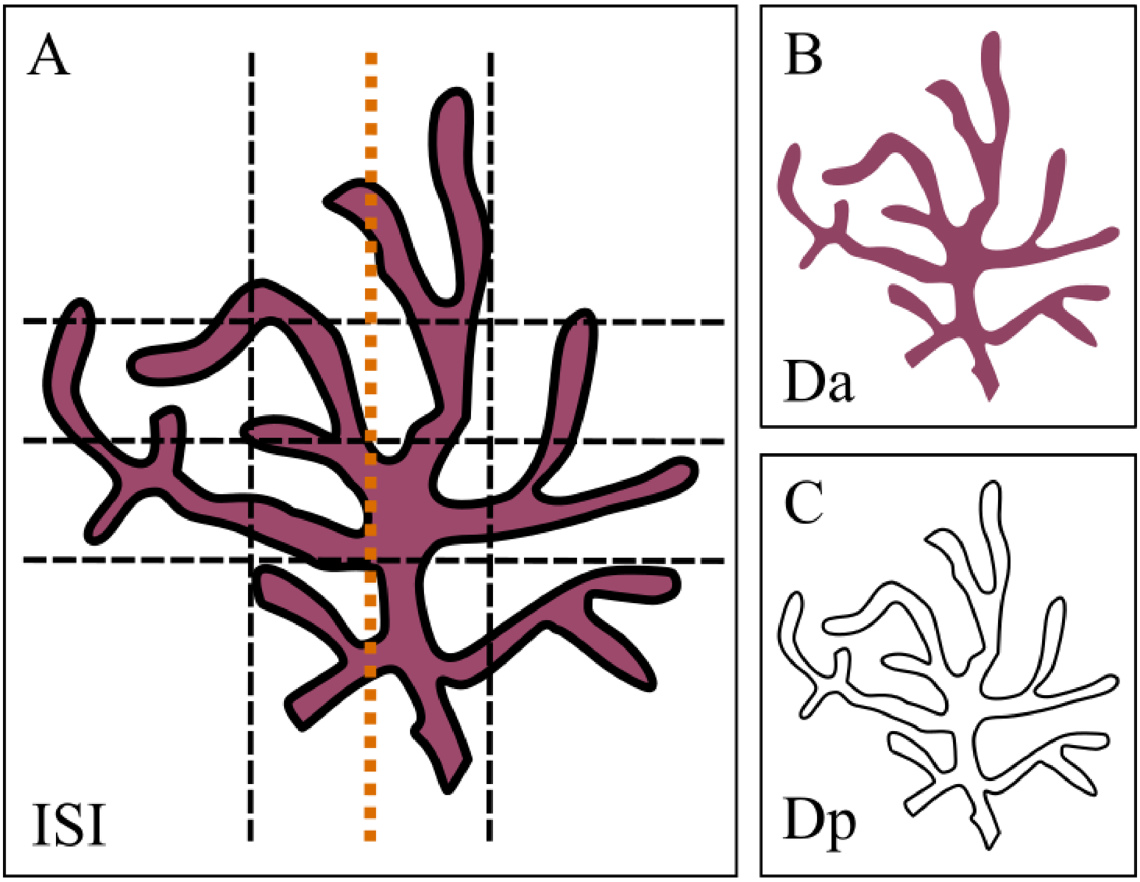

Fig. 2 – Schematic model to measure the interstitial spatial index (A), the fractal diameter of the area (Da) (B) and the fractal diameter of the perimeter (Dp) (C) of each macroalgae frond. Height (H) measurement (orange dotted line).

Statistical analysis

A two-way ANOVA (with data log (x+1) transformed) was used to compare macroalgal traits (height, Da, Dp and ISI) and polychaete assemblage descriptors (abundance - N = number of individuals per gram of macroalga, richness - S = total number of taxa, diversity - 1-λ = Simpson's index, and evenness - J = Pielou's index), among the macroalgal species and between seasons. Tukey's test was applied *post hoc* for pairwise comparisons. Multi-Dimensional Scaling (MDS) ordinations, with bootstrap-means, were used to graphically represent the samples (macroalgae and polychaetes) from the dry and rainy seasons. The complexity of the macroalgae and the structure of the polychaete assemblages were compared among macroalgal species and seasons using a two-way PERMANOVA. The relationship between the morphological attributes of the macroalgae and the structure of the polychaete assemblages was assessed using Distance-based Linear Models (DistLMs), with the "best" procedure and the AIC as the criteria for the selection of the best models. The PERMANOVA, MDS, and DistLM were based on resemblance matrices calculated using Euclidian distance (data log (x+1) transformed) for the macroalgae, and Bray Curtis similarity (data fourth-root transformed data) for the polychaetes. The statistical analyses were run in Primer 6.0 +PERMANOVA and STATISTICA® 12. A significance level of 5% was considered in all analyses.

3. Results

Macroalgal traits

The highest and heaviest fronds were recorded in *Gelidiella acerosa* in both seasons. On the other hand, *Ulva lactuca* was the shortest alga and had the lowest biomass (Fig. 3). *Padina gymnospora* and *U. lactuca* had higher fractal dimension of the area (Da) than *G. acerosa* and *Palisada perforata*, whereas the fractal dimensions of the perimeter (Dp) was higher in *G. acerosa* and *P. perforata* than in *Padina gymnospora* and *U. lactuca*. The Da and Dp were generally greater in the rainy season (Fig. 3, Table 1). The interstitial spatial index (ISI) varied significantly among the different macroalgae, but not between seasons, although the ISI values were invariably higher in the rainy season (Fig. 3, Table 1). *Gelidiella acerosa* had significantly higher ISI than all the other macroalgae in both seasons (Fig. 3) (dry season - *G. acerosa* (14.94) > *P. perforata* (5.74) > *P. gymnospora* (1.20) > *U. lactuca* (1.10); rainy season - *G. acerosa* (28.37) > *P. perforata* (7.18) > *U. lactuc*a (2.12) > *P. gymnospora* (1.75).

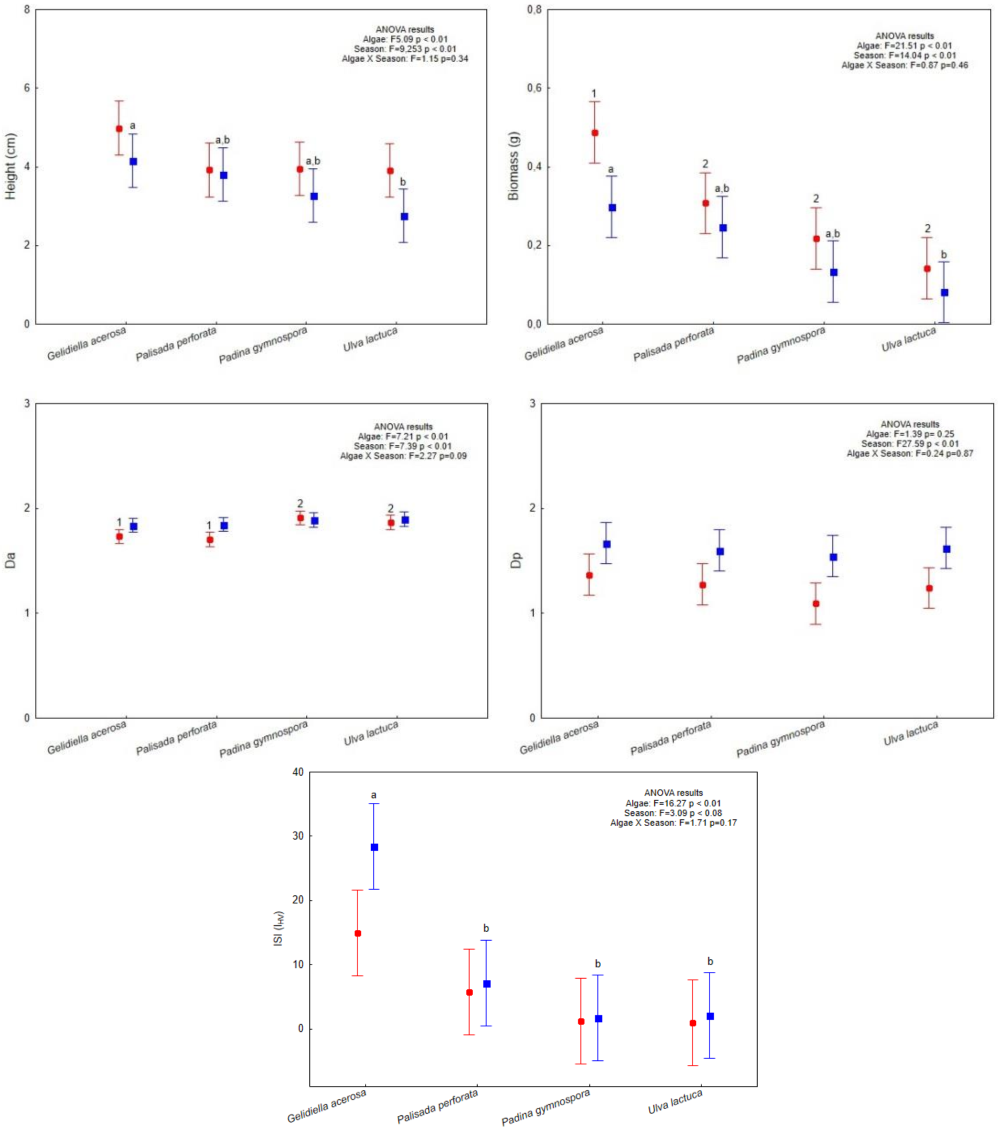

Fig. 2 – Traits (mean ± standard deviation) of the macroalgae sampled on the sandstone reefs of the tropical coast of northeastern Brazil. Red circles = dry season, blue squares = rainy season. Different numbers (dry season) or letters (rainy season) over pairs of values in the same graph indicate a significant difference between the species, based on Tukey's test.

Table 1. ANOVA results for the traits of the macroalgae from sandstone reefs of the tropical Brazilian coast.

Height					
	df	SS	MS	F	p
Macroalgae	3	0.82	0.27	5.10	**< 0.01**
Season	1	0.49	0.49	9.25	**< 0.01**

Macroalgae x Season	3	0.18	0.06	1.15	0.34
Error	72	3.85	0.05		
Fractal dimension of the area (Da)					
	df	SS	MS	F	p
Macroalgae	3	0.25	0.08	7.21	< **0.01**
Season	1	0.08	0.09	7.39	< **0.01**
Macroalgae x Season	3	0.08	0.03	2.27	0.09
Error	72	0.83	0.01		
Fractal dimension of the perimeter (Dp)					
	df	SS	MS	F	p
Macroalgae	3	0.40	0.13	1.39	0.25
Season	1	2.65	2.65	27.59	< **0.01**
Macroalgae x Season	3	0.07	0.02	0.23	0.87
Error	72	6.93	0.10		
Interstitial space index (ISI)					
	df	SS	MS	F	p
Macroalgae	3	5461.80	1820.60	16.27	< **0.01**
Season	1	345.26	345.26	3.09	0.08
Macroalgae x Season	3	575.31	191.77	1.71	0.17
Error	72	8054.46	111.87		

df: degrees of freedom, SS: sum of squares, MS: mean square

In the MDS plot of the macroalgal traits two groups of samples can be observed in both seasons (Fig. 4). One group is formed by the more complex macroalgae (*G. acerosa* and *P. perforata*) and the other group includes the less complex species (*P. gymnospora* and *U. lactuca*). Most traits varied significantly among species and between seasons, albeit with no significant interaction among the factors. *Gelidiella* was significantly more complex than most other macroalgae in both seasons. The macroalgae traits was more variable among the species in the dry season (Table 2).

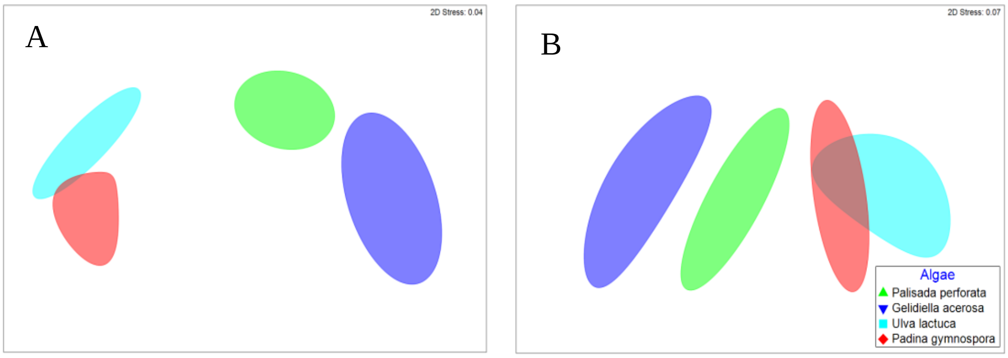

Fig. 3 – The permutational MDS plots (95% bootstrap) of the traits of the macroalgae collected from the sandstone reefs of the tropical Brazilian coast. A = dry season, B = rainy season.

Table 2 – Results of the PERMANOVA of the traits of the macroalgae collected from the sandstone reefs of the tropical Brazilian coast, at Enseada dos Corais in Pernambuco.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Macroalgae	3	119.8	39.93	12.91	< 0.01	998
Season	1	42.02	42.01	13.58	< 0.01	998
Macroalgae x Season	3	10.42	3.47	1.12	0.32	996
Residual	72	222.77	3.1			
Total	79	395				

Pairwise

	Dry season			Rainy season		
Group	t	P(perm)	Unique Perms	t	P(perm)	Unique perms
Palisada perforata, G. acerosa	2.16	< 0.01	993	1.22	0.21	989
Palisada perforata, U. lactuca	3.35	< 0.01	991	2.00	0.02	996
Palisada perforata, Padina gymnospora	3.85	< 0.01	995	1.66	0.06	995
G. acerosa, U. lactuca	4.18	< 0.01	988	3.15	< 0.01	996
G. acerosa, Padina gymnospora	4.30	< 0.01	992	2.94	< 0.01	990
U. lactuca, Padina gymnospora	1.21	0.20	993	0.98	0.38	993

Polychaete assemblages

The polychaetes belonged to 25 taxa distributed in the families Dorvilleidae, Eunicidae, Heisionidae, Polynoidae, Phyllodocidae, Syllidae, and the Nereididae (Table 3). The Syllidae was the most speciose and abundant family in both seasons (Table 3), while *Platynereis dumerillii* was the most frequent and abundant species. All the polychaetes were vagile. Three feeding gruilds were recorded: herbivores, carnivores and omnivores. In the dry season carnivores (18 species) and omnivores (12 species) were the most abundant, with dominance of *Syllis* sp. and *Haplosyllis spongicola* (carnivores) and Syllinae spp. (omnivore). In the rainy season the assemblages were dominated by omnivores (13 species) and herbivores (12 species), with the herbivore *P. dumerillii* and the omnivores of the subfamily Syllinae being the most abundant taxa (Table 3).

Table 3 – Seasonal variation in the abundance (ind.g^{-1} of dried macroalgae) of polychaetes recorded on four macroalga species growing on sandstone reefs of the tropical Brazilian coast. Ul = *Ulva lactuca*; Pg = *Padina gymnospora*; Pp = *Palisada perforata*; Ga = *Gelidiella acerosa*.

Taxon	Mean±standard deviation number of individuals per gram of dried alga recorded in the:								Feeding guild
	Dry season				Rainy season				
	Ul	Pg	Pp	Ga	Ul	Pg	Pp	Ga	
POLYNOIDAE									
Lepidonotus variabilis	0	0	0	0.7±1.4	0	0	0	0	Omnivore
DORVILEIDAE	0	0	0	0.2±0.7	0	0	0	0	Carnivore
EUNICIDAE									
Eunice sp.	0	0	0	0.2±0.7	0	0	0	3±9.1	Omnivore
Eunice rubra	0	0	0	0.7±0.9	0.8±2.4	0	0	2.4±2.8	Herbivore
Marphysa sp.	0.7±2.1	2.1±3.3	2.2±4.4	0.2±0.7	1.6±4.9	0	2.7±8	0.6±1.2	Herbivore
Lysidice collaris	0	0	0	0.2±0.7	0	2±6	1.7±3.2	1.7±2.9	Herbivore
HEISIONIDAE									
Oxydromus sp.	0	2.7±4.4	0	0	0	0	0	0.8±2.3	Carnivore
NEREIDIDAE									
Platynereis dumerillii	3.7±7.4	3.7±5.9	11.9±10.4	1.2±2.2	64.1±152.7	5±7.8	8.9±10	2.5±4.2	Herbivore
Pseudonereis gallapaguensis	0	0	3.1±7.1	1±2.9	0	0	3.1±6.2	0.9±1.4	Carnivore
PHYLLODOCIDAE									
Pterocirrus sp.	0	0	0.4±1.2	0	0	0	0	0	Carnivore
Eumida sp.	0	0.8±2.3	0	0	0	0	0	0	Carnivore
Eteone sp.	0	0	0	0	0.7±2.2	0	0	0	Carnivore
SYLLIDAE									
Syllinae spp.	5.6±7.1	1±3.1	2.6±3.1	0.2±0.6	8±11.6	5.6±16.9	6.4±8.1	1.5±2.9	Omnivore
Syllis sp.	0	2.6±4.5	0.3±1	2.3±4.3	0	0	0	0	Carnivore
Haplosyllis spongicola	0	1.4±2.8	1.1±3.3	0.2±0.7	0	0	0	3±9.1	Carnivore

Opisthodonta sp.	0	0	4.3±9.9	1.2±3.5	0	0	2.7±8.2	0	Carnivore
Opstosyllis sp.	0	0	1.6±2.6	0.5±1.4	0	0	0	0	Omnivore
Dentatysyllis sp.	5.5±16.4	0	0	0.2±0.7	0	0	0	0	Carnivore
Trypanosyllis sp.	0	0.5±1.6	0.4±1.2	0	0	0	0.4±1.1	1.2±2.7	Carnivore
Eurysyllis sp.	0	0	0	0	0	0	0	0.6±1.2	Omnivore
Inermosyllis sp.	0	0	1.9±3.4	0.4±1.3	6.6±10.9	0	3.2±5.7	3.2±5.8	Omnivore
Exogone sp.	0	0	0.4±1.3	0	1.6±4.9	0	0	0	Omnivore
Dentatysyllis coralinae	0	0	2±5.9	0	0	0	3.6±10.7	7.5±15.5	Omnivore
Amblyosyllis spectabilis	0	0	0.4±1.2	0	0	0	0	0	Omnivore
Megasyllis sp.	0	0	0	0	0	0.8±2.3	0	0	Omnivore
All polychaetes	15.4±22.9	14.8±17.1	32.7±33.7	9.5±18.5	83.4±156.1	13.4±19.7	32.6±29.1	28.9±45.7	
Richness	4	8	14	15	7	4	9	13	

In the dry season *P. perforata* had the highest abundance of polychaetes (327 ind.g□ 1 dry alga), whereas in the rainy season the higher abundance was recorded in *U. lactuca* (834 ind.g□ 1 dry alga). On the other hand, the lowest abundances were recorded in *G. acerosa* (95 ind.g^{-1} dry alga) in the dry season and in *P. gymnospora* (134 ind.g□ 1 dry alga) in the rainy season. The richness was highest in *G. acerosa* in both season (dry:15 taxa, rainy season: 13 taxa). The minimum richness was recorded in *U. lactuca* in the dry season and in *P. gymnospora* in the rainy season (Table 3). Richness, abundance, diversity and evenness varied significantly among macroalgae species, but not between seasons, and there was no significant interaction among factors. Richness and abundance were significantly higher in *P. perforata* and *P. gymnospora* (Fig. 5, Table 4).

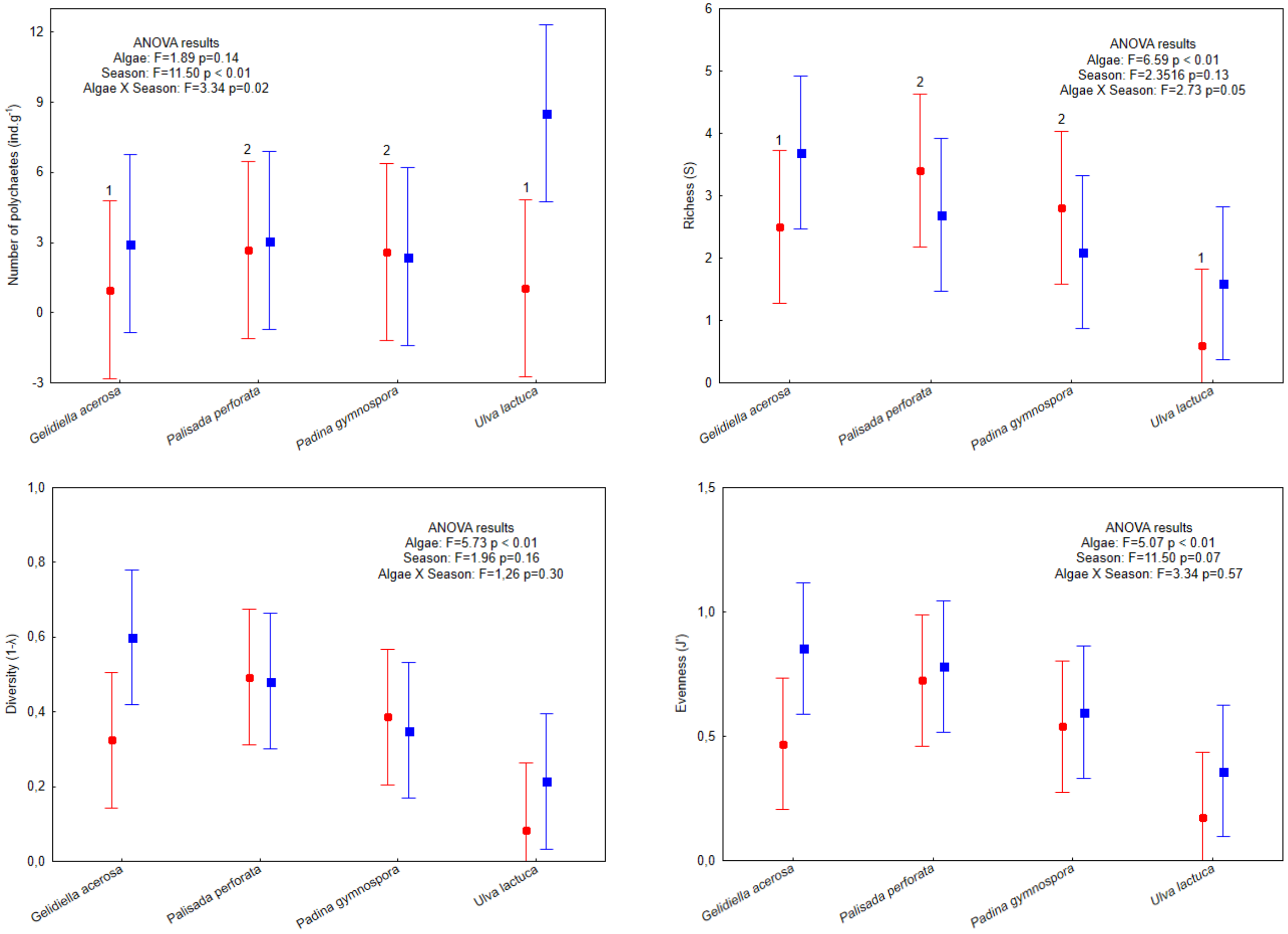

Fig. 5 – Descriptors (mean ± standard deviation) of the polychaete assemblages retrieved from four macroalgal species growing on sandstone reefs of the tropical Brazilian coast. Red circles = dry season, blue squares = rainy season. Different numbers (dry season) or letters (rainy season) over pairs of values in the same graph indicate a significant difference between the species, based on Tukey's test.

Table 4. ANOVA results for the descriptors of the polychaete assemblages on macroalgae from sandstone reefs of the tropical Brazilian coast.

Abundance					
	df	SS	MS	F	p
Macroalgae	3	8.89	2.96	1.89	0.14
Season	1	18.01	18.01	11.50	< **0.01**
Macroalgae x Season	3	15.67	5.22	3.33	**0.02**
Error	72	112.80	1.57		
Richness					
	df	SS	MS	F	p
Macroalgae	3	5.54	1.84	6.59	< **0.01**
Season	1	0.66	0.66	2.35	0.13
Macroalgae x Season	3	2.29	0.76	2.73	0.05
Error	72	20.16	0.28		
Diversity					
	df	SS	MS	F	p
Macroalgae	3	1.42	0.47	5.73	< **0.01**
Season	1	0.16	0.16	1.96	0.16
Macroalgae x Season	3	0.31	0.10	1.26	0.29
Error	72	5.96	0.08		
Evenness					
	df	SS	MS	F	p
Macroalgae	3	2.68	0.89	5.06	< **0.01**
Season	1	0.59	0.59	3.36	0.07
Macroalgae x Season	3	0.36	0.12	0.68	0.58
Error	72	12.68	0.18		

df: degrees of freedom, SS: sum of squares, MS: mean square

In the MDS plots it is possible to identify, particularly in the dry season, four groups, each one representing a macroalgae species (Fig. 6A). The internal dissimilarity (spread) of the groups was higher in the rainy season, when the samples groups were closer, especially among *P. perforata*, *P. gymnospora* and *U. lactuca* (Fig. 6B). The structure of the polychaete assemblages varied significantly among macroalgae and between seasons, with a significant interaction among factors (Table 5). Most results of the pairwise tests between macroalgal species were significant in the dry season. In the rainy season the differences between macroalgal species were less common. The most distinct polychaete assemblages were recorded on *G. acerosa* in both seasons (Table 5).

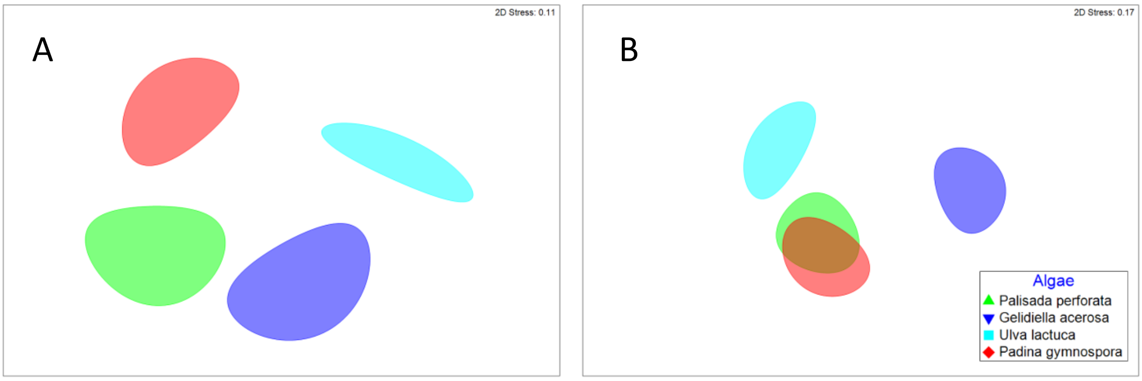

Fig. 6 – The permutational MDS plots (95% bootstrap) of the data on the polychaetes found on the macroalgae collected from the sandstone reefs of the tropical Brazilian coast. A = dry season, B = rainy season.

Table 5 – Results of the PERMANOVA of the polychaete assemblages found on the macroalgae collected from the sandstone reefs of the tropical Brazilian coast, at Enseada dos Corais in Pernambuco.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique perms**
Macroalgae	3	27706	9234.4	3.11	< 0.01	998
Season	1	14238	14238	4.79	< 0.01	999
Macroalgae x Season	3	14317	4772.5	1.60	0.04	998
Residual	72	2.1419E+05	2974.8			
Total	79	2.7045E+05				

Pair-Wise						
	Dry season			Rainy season		
Groups	t	P(perm)	Unique perms	t	P(perm)	Unique perms
Palisada perforata, G. acerosa	16.62	0.02	982	14.42	0.04	992
Palisada perforata, U. lactuca	22.58	< 0.01	917	10.17	0.4	990
Palisada perforata, Padina gymnospora	13.84	0.07	990	0.58	0.94	992

G. acerosa, U. lactuca	16.92	0.02	587	15.40	0.02	989
G. acerosa, Padina gymnospora	18.25	< 0.01	982	13.63	0.05	994
U. lactuca, Padina gymnospora	20.44	< 0.01	920	0.97	0.47	995

df: degrees of freedom, SS: sum of squares, MS: mean square, Unique perms: unique permutiations

There were significant relationships between polychate assemblages and macroalgae in the dry and rainy seasons (Table 6). In *G. acerosa* Da and Dp were significantly related with the whole community in the rainy season. The whole community was significantly related with Da in both seasons in *P. perforata*. In this macroalgae, during the dry season there were significant correlations between abundance and height, and richness with ISI. In *P. gymnospora* the abundance and richness were significantly related with height in the dry season, and in the rainy season Dp significantly explained the variations in richness. The whole community was significantly related with *U. lactuca* Dp in the rainy season."

Table 6. Best distance-based linear model (DistLM) fitted for the relationship between polychate assemblage descriptors and macroalgae. Samples from sandstone reefs of the tropical Brazilian coast.

Gelidiella acerosa					
Varia	AI	SS(tra	Pseud	P	Pro
Whole community					
Dry					
H	75.	3156.7	2.02	0.	20.
Rainy					
Da	77.	6911.9	3.32	**0.**	29.
Dp	50	7740.8	3.92	<	32.
Abundance					
Dry					
H	-	2.49	3.30	0.	30.
Rainy					
Da	-	0.65	1.67	0.	17.
Dp	12.67	1.13	3.43	0.	30.
ISI		0.11	0.24	0.	2.9
Richness					
Dry					
H	4.9	5.94	4.30	0.	34.
Rainy					
Da		0.34	0.61	0.	1.5

Dp	-	0.31	0.55	0.	6.4

Palisada perforata

Varia	AI	SS(tra	Pseud	P	Pro
Whole community					
<u>Dry</u>					
H	77.		2.04	0.	20.
Da			2.99	**0.**	27.
Dp	08		1.36	0.	14.
ISI			2.14	0.	21.
<u>Rainy</u>					
Da	75.	4099.3	2.65	**0.**	24.
Abundance					
<u>Dry</u>					
H	-	2.89	10.37	**0.**	56.
ISI	13.22	0.18	0.28	0.	3.4
<u>Rainy</u>					
H	-	1.87	2.77	0.	25.
Richness					
<u>Dry</u>					
H	-	2.21	3.96	0.	33.
ISI	11.98	2.80	11.85	**0.**	42.
<u>Rainy</u>					
ISI	-	0.32	0.45	0.	05.

Padina gymnospora

Varia	AI	SS(tra	Pseud	P	Pro
Whole community					
<u>Dry</u>					
H	77.	4814.2	2.36	0.	22.
ISI	01	2024.1	0.85	0.	09.
<u>Rainy</u>					
Dp	78.	3560.6	1.71	0.	17.
Abundance					
<u>Dry</u>					
H	-	2.50	8.27	**0.**	50.
Dp	10.31	0.33	0.58	0.	06.
<u>Rainy</u>					
H	-	0.23	0.79	0.	8.9
Richness					
<u>Dry</u>					
H	-	3.27	8.61	**0.**	51.
<u>Rainy</u>					
Dp	-	2.13	9.29	**0.**	53.

Ulva lactuca

Varia	AIC	SS(tra	Pseu	P	Pro

Whole community					
Dry					
H	71.08	2645.5	2.30	0.	22.
Da		823.99	0.60	0.	06.
ISI		2923.4	2.62	0.	24.
Rainy					
Dp	76.4	6303.4	3.62	**0.**	31.
Abundance					
Dry					
H	-1.16	2.88	2.66	0.	24.
ISI		4.68	5.45	0.	40.
Rainy					
H	3.80	3.81	3.11	0.	27.
Richness					
Dry					
H	-8.97	1.84	4.19	0.	23.
ISI		2.18	4.88	0.	37.
Rainy					
ISI	-	0.60	1.08	0.	11.

H = Height, Da = fractal dimensions of the area, Dp = fractal dimensions of the perimeter, ISI = interstitial spatial index, and Prop. (%) = proportion of variability explained."

4. Discussion

The traits of the macroalgae varied significantly between species and seasons. *Gelidiella acerosa* was the most complex species, with highest values of height, Dp, and ISI. The maximum height was recorded in the dry season, whereas Da, Dp and ISI were highest in the rainy season. Variations in structure are adaptive responses to the environment, which may occur over time or in space (Stewart and Carpenter, 2003; Stelling-Wood et al., 2020). These adaptations may range from changes in growth and morphology to shifts in the chemical composition of the plant, and are caused by factors such as precipitation, nutrient input, hydrodynamics, photoperiod, and biological interactions (Diaz-Pulido et al., 2007; Jorge et al., 2012; Sirison and Burnett, 2020).

Although seasonal changes in environmental characteristics in the tropics are not as intense as those observed in temperate regions, the tropics are marked by major shifts in rainfall rates throughout the year (Bontempo Filho et al., 2022). These processes may, in turn, lead to major alterations in the characteristics of marine waters, particularly in coastal areas (Seibold and Berger, 2017; Mclachlan and Defeo, 2018). On the coast of

Pernambuco the dry season (September through February) is very dry (mean monthly rainfall = 40–51 mm), while the rainy season (March through August) have mean monthly rainfall of 180–212 mm (Vasconcelos et al., 2013). The high rainfall in the rainy season decreases the salinity of the water through increased fluvial input, which also produced an increased input of organic matter, suspended particulate matter, and nutrients to the coast (Kolm et al., 2016; Melo et al., 2020). There is also an increase in wind intensity during this period, which involves the generation of larger and more frequent waves (Lira et al., 2010) and more intense return currents (MacMahan et al., 2006; Maia et al., 2014), which all create maximum hydrodynamic intensity on the reefs (Bérgamo et al., 2022; Bontempo Filho et al., 2022).

The size and biomass of the macroalgae decreased during the rainy season, while there was an increase in their fractal dimensions and the intersticial index in the dry season. The macroalgal morphology reflects both their taxonomic identity and response to environmental stimuli (Steneck and Watling, 1982; Vadas and Steneck, 1988; Steneck and Dethier, 1994). Macroalgae respond to hydrodynamic pressures by changing the shape of their fronds, reducing the area exposed in the water (Wolcott, 2007; Martone et al., 2012), while strengthening their support structures (Stewart and Carpenter, 2003). The reduction in the height and biomass of the macroalgae observed in the rainy season results from the accidental breakage of their fronds, and this survival strategy during periods when wave action and coastal currents are more intense. In the rainy season, when hydrodynamics is more intense, the macroalgae tend to be more branched, thereby increasing the area of contact with the water for the assimilation of nutrients and photosynthesis (Rice and Schuepp, 1995; Stewart and Carpenter, 2003). Increased branching also enhances fractal complexity (Gee and Warwick, 1994; McAbendroth et al., 2005) and interstitial spaces (Dibble and Thomaz, 2006; Thomaz and Cunha, 2010), as observed in the rainy season on the sandstone reefs of Enseada dos Corais.

The polychaete assemblage was dominated by mobile syllids and nereidids in both seasons. Syllids and nereidids are typical dominant polychaete groups found in association with the macroalgae (Fraschetti et al., 2002; Magalh˜aes and Bailey-Brock, 2014; Hamdy et al., 2018). The Syllidae and Nereididae are abundant and diverse families (Giangrande et al., 2000; Rouse and Pleijel, 2001), and have a diversity of feeding habits (Giangrande et al., 2000; Rouse and Pleijel, 2001; Jumars et al., 2015) and reproductive strategies (Rouse and Pleijel, 2001; Ribeiro et al., 2018), being capable of colonizing different substrates, such as macroalgae, seagrasses, and even other animals (Bone and

San Martín, 2003; Cunha et al., 2013). Most syllids are carnivores or omnivores, and thus may benefit from the relative abundance of prey found in vegetated habitats (Vizzini et al., 2002; Antoniadou and Chintiroglou, 2006) or from deposits accumulated in the abundant and diverse spaces among the branches, blades, and thalii of the macroalgae (Carvalho et al., 2018). In turn, the herbivore feeding habits of some nereidids, which may ingest the macroalgal thalli or the associated epiphytes (Fauchald and Jumars, 1979), favor their occurrence on the macroalgae (Menge, 1978; Raffaelli, 2000; Hamdy et al., 2018).

The herbivore *Platynereis dumerilii* was dominant on most macroalgae, in particular *Palisada perforata,* and it is not uncommon (Cunha et al., 2013; Quir´os-Rodríguez et al., 2013; Ba-Akdah et al., 2016). This species occurs frequently in shallow vegetated habitats, on hard bottoms covered with both macroalgal beds and seagrass meadows (¨Ozpolat et al., 2021). Due to their herbivore feeding habits (San Martín and Bone, 2001; Cunha et al., 2013), this species is likely using the macroalgae or their epyphites as a food source (Ba-Akdah et al., 2016). The preference of *P. dumerilii* for *P. perforata* coincides with the fact that this alga is colonized extensively by epiphytic algae, diatoms, and dinoflagellates (Soares and Fujii, 2012; Ferreira and Bertotti, 2021; Fawzy, 2022). There was a major shift in the abundance of this species between seasons, with more individuals in the rainy season. Changes in environmental conditions are known to have a strong impact on the speed and synchronization of the reproductive cycle of polychaetes (Giangrande, 1997; Fischer et al., 2010). In a study of *Laeonereis culveri* in the same area as the present study, Santos et al. (2003) observed polychaetes reproducing year-round and found a strong correlation between oocyte growth and organic matter content of the sediment, which indicates that the predominant role of temperature, as observed in temperate species, is probably not valid for tropical nereidids. The greater abundance of *P. dumerilii* recorded during the rainy season in the present study is consistent with this hypothesis, given that the higher rainfall rates on the Pernambuco coast during this period lead to a more intense flow and discharge of the local rivers, leading to an increase in the input of organic matter to coastal areas.

The polychaete assemblages varied considerably among the macroalgal species, with *G. acerosa* and *P. perforata* having the most abundant and speciose communities. The complexity of macroalgae can be evaluated based on attributes such as their length (Carvalho et al., 2018), biomass (Duarte et al., 2020a), fractal dimensions (McAbendroth et al., 2005), interstitial indices (Dibble and Thomaz, 2006; Thomaz and Cunha, 2010), and coverage of epibionts (Carvalho et al., 2018). Based on these traits, the red corticated macroalgae *G. acerosa* and *P. perforata*, with their greater biomass, height, Dp, and ISI, are more complex than the foliose species *Padina gymnospora* and *Ulva lactuca*. The macroalgae complexity has a strong effect on the structure and function of the invertebrate communities of the marine macroalgae of the phytal zone (Chemello and Milazzo, 2002; Duarte et al. 2020a, 2020b, Carvalho et al., 2018). The more complex algae support a greater abundance, richness, and diversity of invertebrates, given that they have more interstitial spaces, which provide more areas for attachment (Duarte et al., 2020b; Gan et al., 2019), more food (Chemello and Milazzo, 2002; Jacobucci and Leite, 2014), more spaces for shelter (Chemello and Milazzo, 2002, Quir´os-Rodríguez et al., 2013), and support a greater variety of niches (Barbosa et al., 2019). On the other hand, *U. lactuca* and *P. gymnospora,* the least complex macroalgae, had the lowest abundance and richness of polychaetes. These macroalgae are leafy (Steneck and Dethier, 1994), which makes it difficult for other terstices (Hacker and Steneck, 1990) and refuges (Zamzow et al., 2010).

In addition to architectural complexity, the chemical composition (primary and secondary metabolites and nutrients) of macroalgae may also influence their selection and colonization by organisms in the phytal zone (Cruz-Rivera and Hay, 2000; Hurd et al., 2014; Gaubert et al., 2019). Although the chemical characteristics of macroalgae were not assessed in this study, they are important for the associated fauna. The effects of these chemical characteristics on the on the macrobenthos will depend primarily on whether the organisms use the macroalgae as a substrate, shelter, refuge or a source of food (Hay et al., 1987; Hay and Steinberg, 1992; Machado et al., 2019; Fernandes et al., 2021). Macroalgae with a higher lipid, protein, and vitamin content favor macroinvertebrates, given that they are used as a source of food, and the availability of these nutrients increases the efficiency with which a consumer converts food into biomass (Mattson, 1980; Sterner and Hesson, 1994). Secondary metabolites, such as phenolic and halogenated compounds, sterols, terpenes, and small peptides (Schmitt et al., 1995; Bedoux and Bourgougnon, 2015; Pereira et al., 2023), are produced by the macroalgae in response to biotic and abiotic stressors such as desiccation, shifts in temperature, herbivory, and colonization by epibionts (Cardozo et al., 2007; Stengel et al., 2011; Kooke and Keurentjes, 2012). In this case, macroalgae with a high secondary metabolite content tend to be poor in macrofaunal species (Baumgartner et al., 2009; Amsler et al., 2014; Fernandes et al., 2021).

In conclusion, this study helps to further understanding the responses of macrofauna to macroalgal characteristics (traits) on the phytal in tropical coastal reefs. Macroalgae morphological complexity exerts a strong influence on the polychaetes, and the most complex macroalgae have more species-rich assemblages. Most polychaetes are mobile herbivores, omnivores or carnivores, mainly Nereididae and Syllidae, which benefit from the increased number of preys in vegetated habitats, deposits accumulated on the macroalgae, or are using macroalgae and their epyphites as a food source. The most important macroalgae trait in structuring polychaeta assemblages were height, Da and Dp, with slightly variation these traits between macroalgal species. Seasonal changes and macroalgal complexity (traits) were important drivers for polychaete assemblages on macroalgae in tropical coastal sandstone reefs. This study represents a baseline for the relationships between polychaetes and macroalgae in tropical coastal reefs.

Author contribuitions

NC: research conceptualization, data collection, picture editing, data analysis and interpretation, writing - original draft, review and editing.

JSRF: research conceptualization, data analysis and interpretation, writing - original draft, review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Ethics and Permits

None of the research reported in this article required research permits.

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9 ARTIGO 4 - TROPICAL EPIFAUNAL COMMUNITIES DRIVEN BY THE STRUCTURAL COMPLEXITY AND CHEMICAL COMPOUNDS OF MACROALGAE

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Abstract

Macroalgae play a critical role in increasing the complexity and biodiversity of tropical reefs, highlighting its actuation as habitat and vectors of drive of epibenthic communities associated. This study aimed to answer whether the structure of the epifauna communities associated with macroalgae from tropical reefs of Brazil reflects the structural complexity and chemical composition of macroalgae. A total of forty samples of *Ulva*, *Padina*, *Palisada* and *Gelidiella* were collected from the reefs of Enseada dos Corais, Northeast Brazil, and analyzed for structural complexity (e.g., height, biomass, Da, Dp, ISI), chemical compounds (e.g., mineral, primary and secondary metabolites) and for taxonomic identification of the epifauna. In the synthesis, the Rhodophyta were the most complex and with the higher chemical content. As for the macrofauna, a total of 46,499 individuals belonging to 47 taxa were collected, composed of Crustacea (59%), Mollusca (28.9%), Annelida (8.5%), and Platyhelminthes (3.6%). Herbivory was the most common feeding habit (90%). Significant variations in richness, abundance and composition of epifauna differed among the macroalgal species, especially due to the content of carbohydrates, neophytadiene, potassium, and the ISI. These macroalgal factors are related to taxonomic origin and adaptation to environmental and biological conditions. The results of this study strongly indicate that the abundance, richness, and distribution of epifauna in macroalgae from the sandstone reefs of the tropical coast of Brazil are determined by the macroalgae's complexity and chemical composition. The interstitial spaces and the chemical component are used by epifaunal species as refuge and protection and as a source of food, respectively, showing that Rhodophyta macroalgae, especially *P.*

perforata, are the best substrate for the establishment of a more complex epibenthic community.

Keywords: Phytal; Macroalgae; Habitat complexity; Primary and secondary metabolites; Benthos.

Resumo

As macroalgas desempenham um papel fundamental no aumento da complexidade e da biodiversidade dos recifes tropicais, destacando-se sua atuação como habitat e vetor de movimentação das comunidades epibênticas associadas. Este estudo teve como objetivo responder se a estrutura das comunidades epifaunísticas associadas às macroalgas dos recifes tropicais do Brasil reflete a complexidade estrutural e a composição química das macroalgas. Um total de quarenta amostras de *Ulva, Padina, Palisada* e *Gelidiella* foram coletadas nos recifes da Enseada dos Corais, Nordeste do Brasil, e analisadas quanto à complexidade estrutural (por exemplo, altura, biomassa, Da, Dp, ISIIhv), compostos químicos (por exemplo, metabólitos minerais, primários e secundários) e identificação taxonômica da epifauna. Na síntese, as Rhodophytas foram as mais complexas e com maior conteúdo químico. Quanto à macrofauna, foi coletado um total de 46.499 indivíduos pertencentes a 47 táxons, compostos por Crustacea (59%), Mollusca (28,9%), Annelida (8,5%) e Platyhelminthes (3,6%). A herbivoria foi o habito alimentar mais comum (90%). Variações significativas na riqueza, abundância e composição da epifauna diferiram entre as espécies de macroalgas, especialmente devido ao conteúdo de carboidratos, neofitadieno, potássio e ISI. Esses fatores de macroalgas estão relacionados à origem taxonômica e à adaptação às condições ambientais e biológicas. Os resultados deste estudo indicam fortemente que a abundância, a riqueza e a distribuição da epifauna nas macroalgas dos recifes de arenito da costa tropical do Brasil são determinadas pela complexidade e pela composição química das macroalgas. Os espaços intersticiais e o componente químico são usados pelas espécies epifaunais como refúgio e proteção e como fonte de alimento, respectivamente, mostrando que as macroalgas Rhodophyta, especialmente a *P. perforata*, são o melhor substrato para o estabelecimento de uma comunidade epibêntica mais complexa.

Palavras chave: Fital; macroalgas; complexidade do habitat; metabólitos primários e secundários; bentos.

1. Introduction

Macroalgae are a crucial component of benthic coastal ecosystems and dominate this communities in shallow marine environments worldwide (Horta et al., 2001; Tano et al. 2016; Aued et al., 2018). On the tropical sandstone reefs scattered along the Brazilian coastline, macroalgae perform a crucial function as primary producers (Horta et al. 2001; Fulton et al. 2019; Pessarrodona et al. 2022) being a complex environments that host abundant and diverse biological communities (Fulton et al., 2019; Tano et al., 2016). Furthermore, they offer a variety of benefits such as refuge, nursery, and food sources as well as a habitat for diverse species (Duffy and Hay, 1991; Bates, 2009; Borst et al., 2019; Chen et al., 2021).

The macroalgae are very diverse (Hanelt, 2008) and show different physical (Steneck and Dethier, 1994; Cotas et al., 2023) and chemical (Belght et al., 2017; Gamero-Vega et al., 2020; Echave et al., 2022) characteristics. The macroalgae increase the complexity and biodiversity of the coastal zone for offering microhabitats and food inside their structures and on the consume by macrofauna (Christie et al. 2009; Kovalenko et al. 2012; Carvalho et al., 2017; Leite et al. 2021; Cotas et al., 2023).

The dominant group present in reef macroalgae are marine invertebrates, composed of amphipods, polychaetes, molluscs (Corte et al. 2012; Jacobucci and Leite, 2014; Hamdy et al. 2018; Barbosa et al. 2019; Longo et al. 2021, Leite et al., 2021). There are different feeding habits among the epiphytic macrofauna organisms, such as carnivores, herbivores and omnivores that are part of trophic arrangement of community. The epifauna associated with macroalgae respond to the physical characteristics (Cacabelos et al. 2010; Gan et al. 2019; Leite et al. 2021) and chemical composition (Hay and Fenical, 1988; Duffy & Hay, 1994; Sterner and Hesson, 1994, Wang et al. 2018) of the macroalgae.

Several studies have demonstrated a positive correlation between the morphological complexity of macroalgae and the abundance and diversity of associated epifauna in terms of physical characteristics (Chemello and Milazzo, 2002; Gan et al. 2019; Duarte et al. 2020; Leite et al., 2021). The physical characteristics of macroalgae are influenced by several factors, such as the presence of epiphytes and hydroids, branching, biomass, length, fractal dimensions, and interstitial index (McAbendroth et al., 2005; Dibble and Thomaz, 2006; Carvalho et al., 2018; Duarte et al., 2020; Leite et al., 2021). Generally, in the marine environment, habitat complexity determines the composition and structure of biotic communities. Greater complexity results in increased species richness and higher organism abundance (Kovalenko et al. 2012, Carvalho et al. 2017, Leite et al. 2021).

In terms of chemical composition, macroalgae also influences the distribution of associated fauna. Nutrient content can act as an attractant (Jormalainen et al., 2001; Barile et al., 2004; Duarte et al., 2010; Machado et al. 2018; Fernandes et al., 2021) and secondary metabolites can control epifauna. (Hay et al., 1987; Hay and Fenical, 1988; Sudatti et al., 2018; Pereira et al. 2020). Physical factors such as light, salinity and temperature (Sudatti et al., 2011; Biancacci et al., 2022) and biological factors such as herbivory (Peckol et al., 1996; Pereira et al., 2017; Sudatti et al., 2018) control the variation in chemical compound concentrations. Herbivore pressure also modulates and induces the production of defensive chemical compounds by macroalgae (Peckol et al., 1996; Pereira et al., 2017). However, studies suggest that the nutritional value of the macroalgae may be more relevant than the effects of defensive metabolites (Cruz-Rivera and Hay, 2003; Duate et al. 2011).

Our hypothesis was that the structure of the epifauna communities associated with macroalgae from tropical reefs of Brazil reflects the structural complexity and chemical composition of macroalgae. Thus, our main objective was to answer whether the structure of the epifauna communities of the macroalgae from tropical reefs of Brazil responds primarily to the structural complexity of the macroalgae or to their chemical composition.

2. Materials and methods

Study area

The collection areas were the sandstone reefs located of the beach of Enseada dos Corais (08° 19' 24.74" S - 034° 56' 56.85 W), which are on average 50 m offshore, 42.5 m in width, 0.69 m high, and extends for 2.93 km (Vasconcelos, 2016), along the tropical coast of Northeast Brazil. Enseada dos Corais Beach has strong tidal currents in the channel that separates the beachrock from the beach and have a high sediment dynamic of siliciclastic sediment (Bérgamo et al., 2022). The region has two distinct seasons: a dry season between September and February, and a rainy season from March to August, with an average water temperature around 27°C salinity of approximately 36 (Bezerra et al., 2021; Domingues et al., 2017).

Field procedures

The studied macroalga were *Ulva lactuca* Linnaeus, 1753, *Padina gymnospora* (Kützing) Sonder 1871, *Palisada perforata* (Bory) K.W.Nam, 2007 and *Gelidiella acerosa* (Forsskål) Feldmann & Hamel, 1934. *Ulva lactuca* is a green macroalga with a leaf-like thallus consisting of smooth blades. The brown macroalga *Padina gymnospora* has a fan-like thallus covered with small "hairs" that allow fauna to attach. *Palisada perforata* and *Gelidiella acerosa* are both red macroalgae with corticated thalli and different types of branching. While *P. perforata* is completely cylindrical and forms dense ramifications around the apex, resembling a pine tree, *G. acerosa* has a cylindrical base that compresses toward the apex and pinnate branching. These macroalgae are among the most abundant and dominant seaweed species on the reef in northeastern Brazil (Vasconcelos et al., 2019).

The samples were taken in the intertidal zone in the summer of 2018. For the epifaunal study ten fronds of each macroalgae species were collected and fixed with fixing it in 4% formalin saline buffered with sodium tetraborate. Before detaching the fronds from the substrate they were covered with a plastic, preventing epifauna from escaping. For the study of structural complexity and chemical compounds approximately 1 kg of fresh macroalgae per species was randomly collected by hand, thoroughly washed in seawater to remove attached fauna, epiphytes, and sand particles, and then kept refrigerated in a thermal container for transport to the laboratory.

Laboratory procedures

Epifauna

Each sample was sieved on a 0.3 mm mesh with running water to remove the macrofauna. All individuals of macrofauna detached were counted per ten grams of macroalgae (ind.10g^{-1} of dry macroalgae) and were identified in the lowest possible taxonomic level.

Structural complexity and chemical compounds of the macroalgae

For structural complexity, three fronds of each macroalgae species were photographed using a Nikon Coolpix AW100 digital camera. These photographs were used to measure the height (cm), the fractal dimensions of the area (Da) and perimeter (Dp) of the frond, and its interstitial spatial index (ISI_{Ihv}) as described in Craveiro et al. (Section eight of the thesis).

For chemical compounds, the samples were washed under running water to remove the salt, dried at room temperature, and ground to a fine powder. To determine the primary metabolites (total carbohydrates, soluble proteins, total lipid, ash, minerals as Ca, Mg, Fe, Cu, Zn, Mn, Cr, Na, and K) and major secondary metabolites was determined as described in Craveiro et al. (Section six of the thesis).

Statistical analysis

To compare structural complexity (height, biomass, Da, Dp, ISI_{Ihv}) and chemical compound (primary and secondary metabolites) of macroalgae, and epifaunal community descriptors (richness species and abundance) among macroalgae species a one-way ANOVA [data log(x+1)] transformed, followed by Tukey's test, was performed. PERMANOVA (one-way) and CAP were based on resemblance matrices calculated using Euclidean distance [data fourth root transformed – structural complexity and chemical compound of macroalgae] and Bray Curtis similarity (data fourth root transformed – epifaunal community). The relationship between structural complexity, chemical compound and epifaunal community was assessed using distance-based linear models (DistLM) and distance-based redundancy analysis (dbRDA) using the "Step-wise" procedure and AIC as selection criterion for the best models. Statistical analyses were performed using Primer 7.0 +PERMANOVA and STATISTICA® 12 (Clark and Warwick 2001). In all analyses a significance level of 5% was considered.

3. Results

Structural complexity and chemical compounds of the macroalgae

All macroalgae were short with height, not significantly different among species, ranging from 3.31 ± 0.52 cm in *Palisada perforata* to 5.72 ± 0.11 cm in *Gelidiella acerosa* (Tab. 1). The Dp also did not vary significantly among species. On the other hand, the Da values significantly varied among the majority of species (*Padina gymnospora* > *Ulva lactuca* > *P. perforata* = *G. acerosa*), and the ISI_{Ihv} was significantly higher in *P. perforata* and *G. acerosa* than in other macroalgae.

Most mineral contents significantly varied among macroalgae species. The contents of Ca, Mg, Na, K, Mn, Cu and carbohydrates were minimum in *P. gymnospora* and maximum in *P. perforata*. Maximum and minimum amounts of Fe, Zn and Cr were recorded respectively in *Ulva lactuca* and *G. acerosa*. *P. perforata* had the highest lipid content. The highest and lowest concentration of proteins and ashes were recorded in *U. lactuca*, while *P. gymnospora* had the lowest. The secondary metabolites neophytadieno, phytol and palmitic acid were present in all macroalgae. *Ulva lactuca* was the only one to contain phytone. *P. perforata* and *G. acerosa* had the highest contents of neophytadiene and phytol, while *P. gymnospora* had the highest amount of palmitic acid. *U. lactuca* had generally significantly lower values of all secondary metabolites.

Table 1 - Structural complexity and chemical compounds of the macroalgae from sandstone reefs of the Brazilian tropical coast. Results are presented as mean ± standard deviation (n = 3).

Descriptors	Macroalgae species				F^1 (ANOVA)	Tukey's test results^3
	Ul	Pg	Pp	Ga		
Height (cm)	4.75±1.15	4.84±1.45	3.31±0.52	5.72±0.11	3.36^{ns}	
Fractal area dimension (Da)	1.82±0.02	1.89±0.05	1.8±0.02	1.79±0.05	5.24*	Pg>Ul>Pp=Ga
Fractal perimeter dimension (Dp)	1.56±0.38	1.19±0.23	1.29±0.03	1.38±0.04	1.59^{ns}	
ISI_{Ihv}^2	1.21±0.65	1.25±1.14	6.17±0.89	5.84±3.29	8.20*	Pp=Ga>Ul=Pg
Ca ($mg.Kg^{-1}$)	1800±78	900±10	3468.12±20	2890.1±0.1	2135.00*	Pp>Ga>Ul>Pg
Mg ($mg.Kg^{-1}$)	3310±97.3	1655±23	4902.12±22	4085.1±0.58	2511.91*	Pp>Ga>Ul>Pg
Fe ($mg.Kg^{-1}$)	2312.7±0.87	1156.35±10	120±0.7	100±2	60087.30*	Ul>Pg>Pp>Ga
Na ($mg.Kg^{-1}$)	2125±79.3	1062.5±0	4580.64±13	3817.2±2	3720.63*	Pp>Ga>Ul>Pg
K ($mg.Kg^{-1}$)	1621±0.7	810.5±0.1	1707.6±20	1423±34	1982.65*	Pp>Ul>Ga.Pg
Mn ($mg.Kg^{-1}$)	38.77±0.7	19.39±0.1	4462.8±13	3719±1	290549.43*	Pp>Ga>Ul>Pp
Cu ($mg.Kg^{-1}$)	6.5±0.2	3.25±0.3	6.41±0.2	5.34±0.12	124.80*	Pp=Ul>Ga>Pg
Zn ($mg.Kg^{-1}$)	43.8±9.2	21.9±0.1	21.72±0.1	18.1±0.34	37.33*	Ul>Ga=Pp=Pg
Cr ($mg.Kg^{-1}$)	0.93±0.01	0.47±0.1	0.1±0	0.35±0.54	1.34^{ns}	
Total carbohydrates (%)	28.7±0.3	14.35±0.7	48.52±0.1	40.43±0.9	1202.75*	Pp>Ga>Ul>Pa
Total protein (%)	14.98±1	7.49±0.1	14.46±0.6	12.05±1	88.20*	Ul=Pp>Ga<Pg
Total lipids (%)	0.79±0	0.4±0	8.92±0.1	7.43±0.6	2408.40*	Pp>Ga>Ul>Pg
Total ash (%)	37.02±1.3	18.51±0	24.13±1	20.11±0	385.48*	Ul>Pp>Ga>Pg
Neophytadiene (%)	17.6±0.5	23.89±0.5	29.04±0.5	24.13±0.5	262.70*	Pp>Ga=Pg>Ul
Phytol (%)	8.47±0.5	8.29±0.5	11.29±0.5	9.69±0.5	21.06*	Pp>Ga=Pg=Ul
Palmitic acid (%)	6.2±0.5	8.32±0.5	8.1±0.5	7.75±0.5	11.87*	Pg=Pp=Ga>Ul
Phytone (%)	6.05±0.5	0	0	0	2262.49*	Ul>Pg=Pp=G

[1]*: $p<0.05$; ^{ns}: not-significant. [2]ISI_{Ihv}: interstitial spatial index. [3]Ul: *Ulva lactuca*; Pg: *Padina gymnospora*; Pp: *Palisada perforata*; Ga: *Gelidiella acerosa*.

The set macroalgal characteristics (structural complexity and chemical compounds) varied significantly among species. The resemblance was maximum between *P. perforata* and *G. acerosa* (Table 2). In the CAP plot it is possible to identify four groups, composed by the samples from each macroalgae species. This result is confirmed by the high values of success in the sample classification (100% samples correctly classified) and canonical correlations (δ^2 CAP1 = 0.99; δ^2 CAP2 = 0.98). Amounts of neophytadide, phytone, ash and Cu were the variables most important for separating the groups (Fig. 1).

Table 2 - PERMANOVA results for structural complexity and chemical compounds of the macroalgae (n = 3 per macroalgae) from sandstone reefs of the Brazilian tropical coast.

Source of variation	DF[1]	SS[2]	MS[3]	Pseudo-F	Up[4]	P(MC)[5]
Macroalgae	3	212.96	70.99	19.56	7313	< 0.01
Error	8	29.036	3.6295			

	Ul	Pg	Pp	Ga
Ulva lactuca	2.8678			
Padina gymnospora	7.5449*	2.7343		
Palisada perforata	7.5806*	8.5056*	1.0003	
Gelidiella acerosa	7.3805*	7.4809*	3.8748*	3.0418

Footnote: [1]Degree of Freedom; [2]Sum of Squares; [3]Mean Square; [4]Unique permutations; [5]P(MC): Monte Carlo permutation. *: $p < 0.05$. Ul: *Ulva lactuca*; Pg: *Padina gymnospora*; Pp: *Palisada perforata*; Ga: *Gelidiella acerosa*.

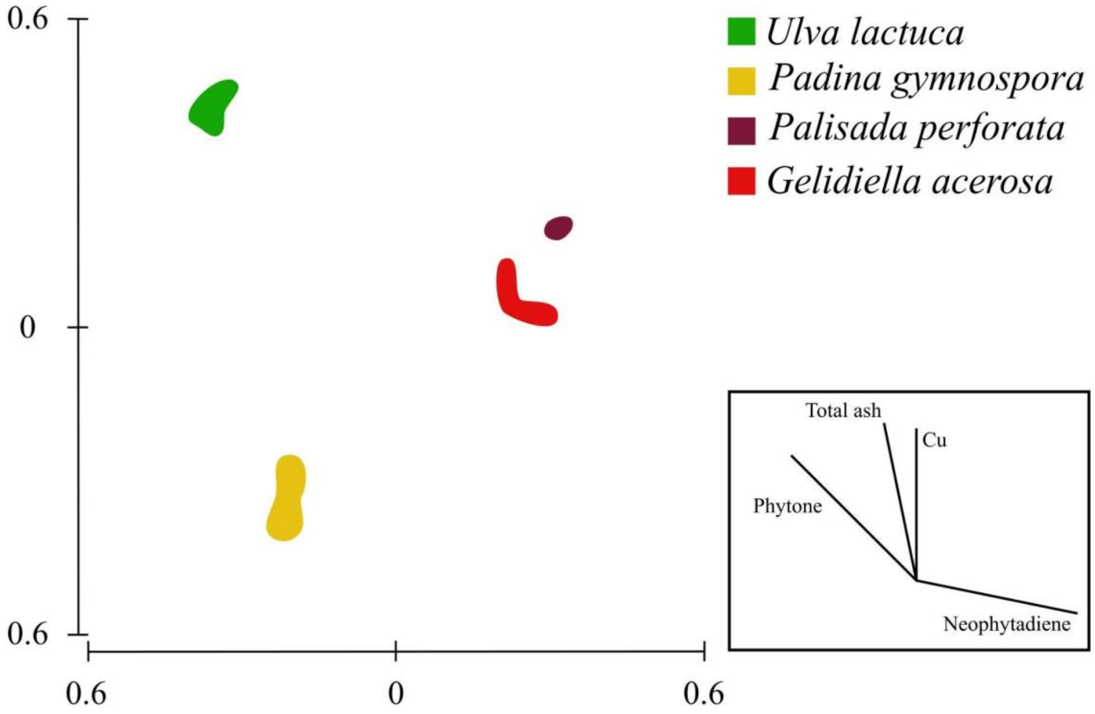

Figure 1 – CAP plot based on structural complexity and chemical composition of the marine macroalgae from sandstone reefs of the Brazilian tropical coast.

Epifaunal community

There were 46,499 individuals belonging to 47 taxa distributes in Platyhelminthes (=Turbellaria) (3.6%), Annelida (=Polychaeta) (8.5%), Mollusca (mainly Gastropoda) (28.9%) and Crustacea (mainly Amphipoda) (59%). Crustaceans, especially amphipods, were dominant in all macroalgae, except in *G. acerosa*, where the most abundance were molluscs (especially the gastropod *Eulithidium affine*) (Figure 2). Annelids were more abundant in red algae, and least abundant in *P. gymnospora*. The highest number of amphipods (e.g., *Apohyale media*, *Hyale nigra*, *Ampithoe suapensis*, and *A. raimondi),* grastropod molluscs (e.g., *Eullithium affine, Diodora* sp., and *Fissurela* sp.), and anellids (e.g., *Platyenereis dumerillii*, *Pseudonereis gallapaguensis,* and *Opisthodonta* sp.) occured in *P. perforata*. Herbivore was the feeding habit most common (90 %), followed by carnivore (7%), omnivore (2%) and depositivore (1%). The maximum and minimum richness and abundance were recorded in *P. perfotara* (13 ± 6 taxa, 1112 ± 451 ind.10g^{-1}) and *P. gymnospora* (6 ± 3 taxa, 24 ± 101 ind.10g^{-1}) respectively (Table 3).

Table 3 – Epifaunal abundance (ind.10g^{-1} of dry macroalgae) recorded on macroalgae from sandstone reefs of the Brazilian tropical coast. Results are presented as mean ± standard deviation (n = 5).

	Macroalgae				
Taxa	*Ulva lactuca*	*Padina gymnospora*	*Palisada perforata*	*Gelidiella acerosa*	**Feeding Guild**
Platyhelminthes					
Turbellaria sp.	202±386	20±29	52±38	59±79	Carnivore
Mollusca					
Diodora sp.	0	6±14	337±365	0	Herbivore
Eullithidium affine	282±259	270±200	861±561	476±387	Herbivore
Eullithidium bellum	6±14	0	0	0	Herbivore
Mitrela dichroa	0	7±15	0	0	Carnivore
Fissurela sp.	6±14	0	272±411	106±121	Herbivore
Fissurela rosea	20±44	0	0	5±11	Herbivore
Nudibranchia sp.	20±44	0	0	0	Omnivorous
Ischnochiton sp.	0	0	7±15	10±22	Omnivorous
Annelida					
Lepidonotus variabilis	0	0	0	5±11	Omnivorous
Eunice rubra	0	0	0	14±9	Carnivorous
Marphysa sp.	14±32	0	15±20	5±11	Omnivorous

Oxydromus sp.	0	16±36	0	5±11	Herbivorous
Platynereis dumerillii	74±101	0	163±131	14±32	Herbivorous
Pseudonereis gallapaguensis	0	0	62±102	19±43	Herbivorous
Pterocirrus sp.	0	0	8±18	0	Carnivorous
Syllinae spp	75±72	21±46	8±18	0	Omnivorous
Syllis sp.	0	0	7±15	42±60	Carnivorous
Haplosyllis spongicola	0	0	22±49	5±11	Carnivorous
Opisthodonta sp.	0	0	86±141	23±52	Carnivorous
Opstosyllis sp.	0	0	23±35	10±22	Carnivorous
Dentatysyllis sp.	0	0	0	5±11	Omnivorous
Trypanosyllis sp.	0	0	8±18	0	Carnivorous
Inermosyllis sp.	0	0	34±48	0	Carnivorous
Amblyosyllis spectabilis	0	0	8±18	0	Carnivorous
Arthropoda (Crustacea)					

Pycnogonida sp.	0	0	0	7±11	Omnivorous
Acanthonyx petiverii	0	0	21±20	0	Omnivorous
Ampithoe marcuzzi	0	1024±280	0	5±11	Herbivore
Ampithoe ramondi	0	0	217±344	0	Herbivore
Ampithoe sp.	0	70±156	0	0	Herbivore
Ampithoe suapensis	0	41±92	118±149	23±33	Herbivore
Apohyale media	327±269	21±46	2060±1425	114±182	Herbivore
Caprella penantis	0	0	0	12±17	Detritivore
Chondrochelia dubia	20±44	7±15	24±54	0	Herbivore
Cymadusa sp.	19±28	21±46	0	0	Herbivore
Cymodoce brasiliensis	0	39±70	8±18	0	Detritivore
Elasmopus longipropodus	0	76±105	0	0	Herbivore
Elasmopus souzafilhoi	0	0	16±36	0	Herbivore
Elasmopus sp.	0	0	0	7±11	Herbivore
Epialtus brasiliensis	6±14	26±41	7±15	0	Omnivorous
Hyale nigra	0	29±33	685±1531	14±32	Herbivore
Microphrys bicornutus	20±44	14±19	0	0	Omnivorous
Sinelobus stanfordi	0	6±14	0	0	Herbivore

Stenothoe sp.	0	0	0	12±17	Herbivore	
Spheromopis mourei	20±44	29±33	0	0	Detritivore	
Tethygeneia sp.	11±25	261±355	37±29	14±21	Herbivore	
Descriptors					**ANOVA**	**Tukey's result**
Richness	6 ± 3	8 ± 3	13 ± 6	10 ± 8	$F = 2.44^{ns}$	-
Abundance (ind.$10g^{-1}$)	24 ± 101	44 ± 173	112 ± 451	22 ± 95	$F = 4.93^{*}$	Pp=Pg>Ul>Ga

The structure of communities varied significantly among all macroalgae species. *P. perforata* and *G. acerosa* had the most similar communities (Tab. 4). In the CAP plot it is possible to identify four groups, composed by the samples from each macroalgae species (Fig. 2). This result is confirmed by the high values of success in the sample classification (95% samples correctly classified) and canonical correlations (δ^2 CAP1 = 0.97; δ^2 CAP2 = 0.78). The specimens *Ampithoe marcuzzi*, *Apohyale media*, Fissurella sp, Syllis sp. and Syllinae spp had the highest correlation with the clusters.

Table 4 - PERMANOVA results of the epifaunal community on macroalgae (n = 5 per macroalgae) from sandstone reefs of the Brazilian tropical coast.

Source of variation	df[1]	SS[2]	MS[3]	Pseudo-F	Up [4]	P(MC)[5]
Macroalgae	3	23300	7766.7	5.64	9911	< 0.01
Error	16	22016	1376			
		Similarity/dissimilarity within/between groups				
		Ul	Pg	Pp	Ga	
Ulva lactuca		42.247				
Padina gymnospora		22.249*	48.581			
Palisada perforata		33.377*	22.180*	57.784		
Gelidiella acerosa		29.767*	24.068*	37.960*	46.355	

Footnote: [1]Degree of Freedom; [2]Sum of Squares; [3]Mean Square; [4]Unique permutations; [5]P(MC): Monte Carlo permutation. *: $p < 0.05$. Ul: *Ulva lactuca*; Pg: *Padina gymnospora*; Pp: *Palisada perforata*; Ga: *Gelidiella acerosa*.

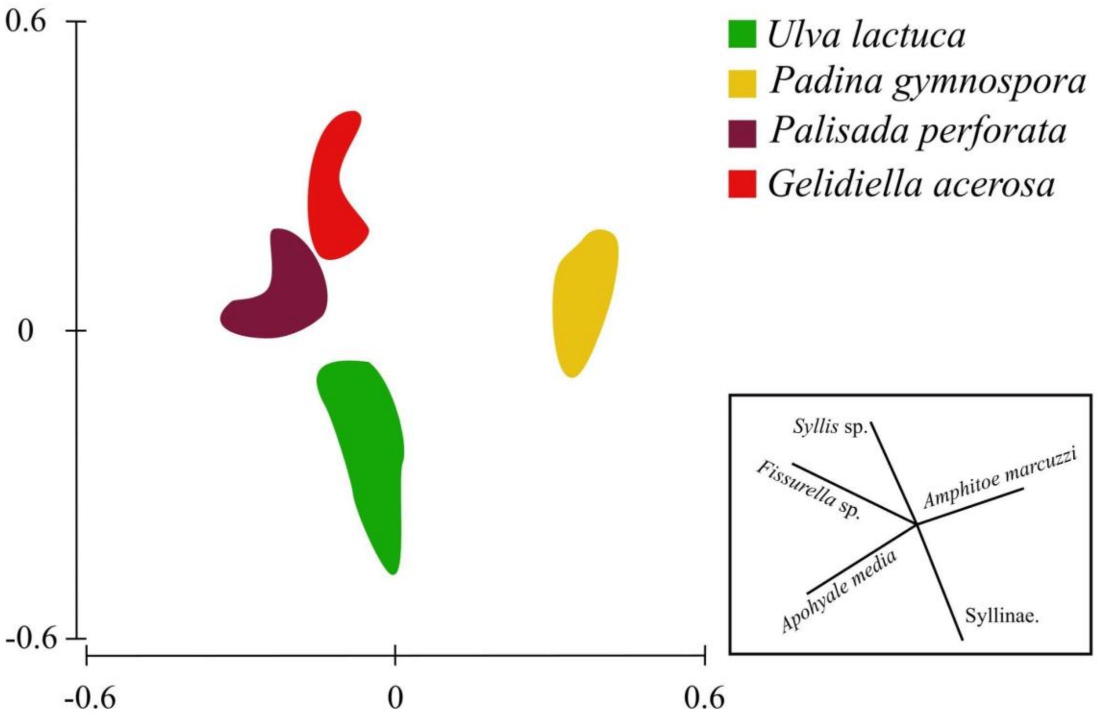

Figure 2. CAP plot based on the epifaunal communities in marine macroalgae from sandstone reefs of the Brazilian tropical coast.

Relationship between epifaunal and macroalgal characteristics

The best linear model fitted explained 99% of the variation of the whole communities, 36% of the richness and 63% of the abundance. The content of carbohydrates, neophytadiene, and potassium, and the Index of interstitial spaces (ISI$_{(\mathrm{Ihv})}$) were the factors that best explained the variation of the epifauna (Figure 3; Table 5).

Table 5 – Fitted model (DistLM) for the relationship between structural complexity and chemical compounds, and epifaunal communities in marine macroalgae from sandstone reefs of the Brazilian tropical coast.

Whole communities			
AIC: 60.12	$R^{\wedge 2}$: 0.99		RSS: 287.4
Variable	**Pseudo-F**	**P**	**Prop. (%)**
Total carbohydrates	3.937	< 0.01	0.28
Potassium	3.221	< 0.01	0.24
ISI$_{(\mathrm{Ihv})}$	2.987	< 0.01	0.23
Neophytadiene	2.367	0.02	0.19
Richness			
AIC: -5.78	$R^{\wedge 2}$: 0.36		RSS: 0.44
Variable	**Pseudo-F**	**P**	**Prop. (%)**
ISI$_{(\mathrm{Ihv})}$	5.69	0.04	0.36
Abundance			
AIC: -35.55	$R^{\wedge 2}$: 0.63		RSS: 4.50
Variable	**Pseudo-F**	**P**	**Prop. (%)**
Neophytadiene	4.221	0.06	0.30
Dry weight	8.000	0.02	0.33

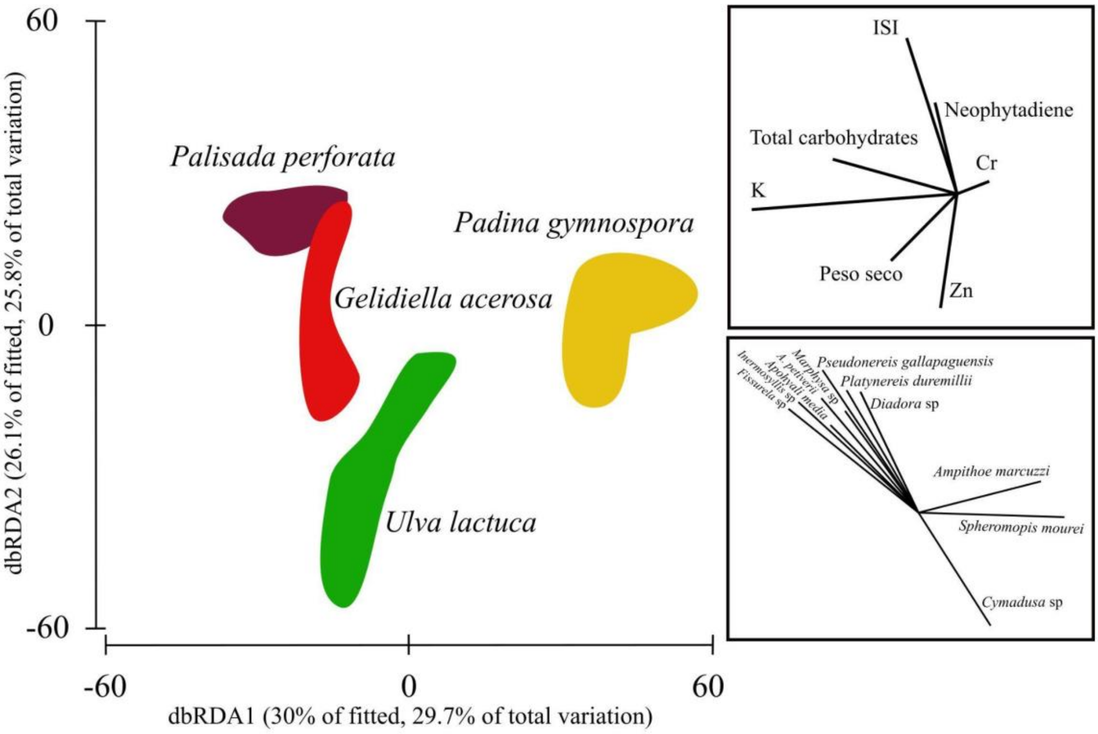

Figure 3 – Adjusted models (dbRDA) for the relationship between structural complexity and chemical compounds with epifaunal community, richness and abundance in macroalgae from sandstone reefs of the Brazilian tropical coast.

4. Discussion

The species of macroalgae studied significantly differ in architectural complexity, chemical composition and the structure of associated macrofaunal communities. The red macroalgae *Palisada perforata* and *Gelidiella acerosa* had generally greater complexity (higher height, Da, and $\mathrm{ISI}_{\mathrm{IHV}}$), nutritional values (highest contents of minerals, lipids, carbohydrates, and protein), and higher richness and abundant communities than *Padina gymnospora* and *Ulva lactuca*. The benthic fauna was composed predominantly by herbivores of 47 taxa belonging to Platyhelminthes (=Turbellaria), Annelida (=Polychaeta), Mollusca (mainly Gastropoda), and Crustacea (mainly Amphipoda). Amphipods were dominant in all macroalgae, except *G. acerosa*, and Annelids were more abundant in red algae.

The structural complex of substratum (e.g., $\mathrm{ISI}_{\mathrm{IHV}}$ and macroalgae weight), chemical defenses (e.g., metabólitos secundários), and nutritional content of plants (e.g., carbohydrates, neophytadiene, and potassium), has been shown to play a critical role in the structure of the whole communities, as well as in the richness and abundance of the phytal macrobenthic communities, specially in food preferences of herbivores (Hay and Fenical, 1988, 1992; Jormalainen et al., 2001; Cruz-Rivera and Hay, 2003; Barile et al., 2004). These characteristics highlight the Rhodophyta, in special *P. perforata*, as the most complex macroalgae with the higher height, fractal dimension of area, and interstitial space, describing *Palisada* and *Gelidiella* as good substrates of life for epiphytic macrofauna.

The Rhodophyta species studied are corticated and have a long, fine, and cylindrical stem, and are very branched, specially in the apex part. On the other hand, the species studied of Ochrophyta and Chlorophyta are featured by a foliose stem, less branched, and arrangement in fan-shaped (Steneck and Dethier, 1994). These differents structural complexities of macroalgae present a relationship with taxonomic origin and adaptation to the environmental and biological conditions (Steneck, 1982; Steneck and Dethier, 1994), such as seasonal (Sirison and Burnett, 2020), hydrodynamic (Martone et al. 2012), and herbivory (Leite et al., 2021; Longo et al., 2021). The increase of complexity occur especially due the higher ramification of stem, as more observed in the Rhodophytes (*P. perforata* and *G. acerosa*) than in *P. gymnospora* and *U. lactuca*, resultant of a consecutive higher amount of interstitial spaces (McAbendroth et al., 2005; Dibble and Thomaz, 2006; Thomaz and Cunha, 2010). These interstitial spaces can be used as habitats of refuge and protection by the epifauna against environmental and biological conditions, such as desiccation and predation (Holbrook & Schmitt 1988; Hixon & Menge 1991; Hooper & Davenport, 2006).

Among the phylum of macrofauna and macroalgal species, the Annelids [=Polychaete] presented a preference and also exclusivity in macroalgae more complex, favoring the occurrence of a higher among of species, such as observed for Crustacea [=Amphipoda] (Jacobucci and Leite, 2014; Carvalho et al., 2018) and Molluscs (Chemello and Millazo, 2002; Barbosa et al. 2019; Duarte et al., 2020a, b).

The chemical compounds with the highest mineral, carbohydrate, lipid, and major secondary metabolite contents were found in the red macroalgae *Palisada perforata* and *Gelidiella acerosa*. In addition to this, the nutritional value, greater exemplified by the high protein and carbohydrates contents (Jormalainen et al. 2001; Amsler et al. 2005; Carvalho et al., 2018; Fernandes et al., 2021), result in a better palatability of *P. perforata* and *G. acerosa* (Duffy and Hay, 1991; Duffy and Paul, 1992; Bolser and Hay, 1996). The palatability is an important factor by the fauna, especially to herbivores (the most abundant feeding habit of epifauna), favoring the establishment of more complex epiphytic communities in the Rhodophyta species (Hacker e Steneck, 1990).

The chemical composition of macroalgae varies based on genetic diversity, geographic distribution, and environmental factors, such as salinity, temperature, and light exposure (Marinho-Soriano et al., 2006; Vasconcelos et al., 2019; Vinuganesh et al., 2022). Although the studied species inhabit the same reefs, these macroalgae belong to different phylum and naturally have distinct chemical compositions (Kumar et al., 2011; Sahul Hamid et al., 2019; Al Sharie et al., 2020). Based on Belghit et al. (2017), these characteristics are particularly associated with the different variations in the metabolic profiling and composition of these marine algae, illustrating their taxonomic classification.

About other chemical components, trace elements such as copper, zinc, and iron are also considered essential for organism development (Kress et al., 1998; Circumcision et al., 2018), performing multiple biological functions including oxygen transport, neurotransmitter synthesis, and energy metabolism. In this study, concentrations of Cu and Zn were found to be low in all macroalgae. However, the concentration of Fe was high in *Ulva lactuca* and *Padina gymnopora*. Additionally, there was low organism richness, particularly in polychaetes, with only 3 and 2 taxa identified, respectively.

The presence of certain compounds in macroalgae can impact epifauna, particularly gastropod molluscs, which are sensitive to metal contamination. High concentrations of these compounds can lead to a decrease in richness and abundance (Longo et al., 2021, 2023). Our hypothesis is that the high concentration of Fe, combined with the lower structural complexity of *Padina* and *Ulva*, may play a key role in regulating the epifauna, including polychaetes. In addition, *P. gymnospora* contains compounds that can deter consumption by organisms. The study by Pereira et al. (2023) demonstrated the significance of the new hydrocarbon derived from the ethyl acetate extract (See Pereira et al. 2023) in effectively reducing consumption by the herbivorous echinoderm *L. variegatus*.

With regards to mollusks, Baumgartner et al. (2009) and Barbosa et al. (2019) investigated the behavior and structure of the mollusk community in various species of macroalgae, including *Padina gymnospora, Dictyopteris justii*, and *Sargassum polyceratium*. The researchers found that the chemical defenses and complexity of macroalgae could affect the diet selection of marine mollusks, leading to quantitative variations in their food intake. In more complex macroalgae and sacoglossan mollusks, an increase in richness, abundance, diversity, and functional characteristics is observed. In experiments involving the macroalga *Fucus distichus*, the herbivorous gastropod *Littorina sitkana* exhibited a preference for those with lower amounts of polyphenolic compounds.

For herbivorous amphipods, an increase in abundance and diversity was observed by Leite et al. (2021) as biomass of the macroalgae increased. This increase in biomass may favor these amphipods that feed on the macroalgae they inhabit and/or the epiphytes that occur on them, as noted by Jacobucci & Leite (2008, 2014). Amphipods may use chemical signals to identify habitats suitable as a refuge while also offering greater nutritional value for food (Fernandes et al., 2021). However, the amphipod *Orchestoidea tuberculata* was not affected by the structure or type of macroalgae or secondary metabolites, but instead influenced by nutritional content such as protein and carbohydrate content when selecting food resources (Duarte et al., 2010). For herbivores, nutrient content of the diet may be especially crucial for their growth, fecundity and fitness (Cruz-Rivera and Hay, 2000a, b). Herbivorous species can optimize their diet by preferential consumption of macroalgae with a higher nutritional value (Barile et al., 2004), suggesting that carbohydrate content could also play a significant role in the food preference patterns of herbivorous invertebrates (Jormalainen et al., 2001).

Another essential aspect controlling the appearance of herbivores in macroalgae is the existence of predators or rivals, which impact food consumption and habitat selection (Reynolds and Bruno, 2013; Beermann and Boos, 2015; Lurig et al., 2016; Beermann et al., 2018). Beermann et al. (2018) examined the habitat preferences and dietary habits of the marine mesograzer, *Echinogammarus marinus*, and found that the availability of food is limited by the presence of predators and competitors, leading the organisms to choose more protected habitats.

However, herbivory can trigger chemical defenses leading to increased production of anti-herbivory compounds, thereby limiting consumption by herbivores (Pereira et al. 2017; Sudatti et al. 2018; Pereira et al. 2020).

Our findings suggest that the distribution of epifauna associated with macroalgae is not controlled by a single factor. The results of this study strongly indicate that the abundance, richness, and distribution of epifauna in macroalgae from the sandstone reefs of the tropical coast of Brazil are determined by the macroalgae's complexity and chemical composition. For instance, *Palisada perforata* has a higher epifauna diversity and abundance due to its higher complexity and chemical content.

Author statement

NC: research conceptualization, data collection, picture editing, data analysis and interpretation, writing - original draft, review and editing.

JSRF: research conceptualization, data analysis and interpretation, writing - original draft, review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Ethics and Permits

None of the research reported in this article required research permits.

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10 CONCLUSÃO

Avaliando a composição química das macroalgas na seção 6 observamos que as macroalgas vermelhas, *Palisada perforata* e *Gelidiela acerosa*, apresentam a maiores concentrações de nutrientes, metabólitos primários e secundários, indicando que as macroalgas têm um potencial considerável como fonte de compostos bioquímicos para uso humano.

Na seção 7 a fim de testar o efeito dos extratos das macroalgas na atividade antimicrobiana, os extratos obtidos a apartir da solução de diclorometano e metanol foram testados frente a diferentes cepas de bactétias e fungos (filamentosos e não fialmentosos). Dentre as espécies estudadas as macroalgas dos recifes de arenito da costa tropical apresentam atividade antifúngica, com a *Ulva lactuca* apresentando forte atividade contra *C. albicans e C. neoformans*, podendo essa macroalga servir como fontes naturais de produtos para futuras aplicações médicas.

Para verificar a influência da complexidade das macroalgas de diferentes perídos na assembleia de poliquetas, na seção 8 realizamos coletas das quatro espécies de macroalgas em dois momentos no período seco e dois no período chuvoso, onde as características físicas (altura, dimensões fractais da área e do perímetro e seu índice espacial intersticial) foi aferida. Foi observado que as características das assembléias de poliquetas variaram entre as diferentes macroalgas, e *G. acerosa* e *Palisada perforata* apresentaram as assembléias mais ricas em espécies e também como as mais complexas. As mudanças sazonais nas características ambientais e a complexidade das macroalgas foram os principais fatores da estrutura das assembleias de poliquetas.

Na seção 9, para verificar se a estrutura das comunidades de epifauna associadas às macroalgas dos recifes tropicais reflete as caraterísticas físicas (complexidade estrutural) e/ou químicas (composição química) das macroalgas. As quatro espécies de macroalgas foram analisadas quanto à complexidade estrutural (altura, biomassa, dimensões fractais da área e do perímetro e seu índice espacial intersticial) e compostas químicas (minerais, metabólitos primários e metabólitos secundários majoritários). Os resultados deste estudo sugerem fortemente que a abundância, a riqueza e a distribuição da epifauna nas macroalgas dos recifes de arenito da costa tropical são determinadas pela complexidade e pela composição química das macroalgas. Os espaços intersticiais e o componente químico são usados pelas espécies epifaunais como refúgio/proteção e como fonte de alimento, respectivamente, mostrando que as macroalgas Rhodophytes, especialmente a *P. perforata*, são o melhor substrato para o estabelecimento de uma comunidade epibêntica mais complexa.

Concluímos então que nossos estudos mostram que a distribuição da epifauna associada às macroalgas não é controlada por um único fator, onde a abundância, a riqueza e a distribuição da epifauna nas macroalgas dos recifes de arenito da costa tropical são determinadas pela complexidade e pela composição química das macroalgas, sendo o índice de interstícios, teor de carboidratos, potássio e neofitadieno os fatores que melhor ajudam a entender a distribuição da fauna nas macroalgas.

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APÊNDICE A - IMMEDIATE EFFECTS OF THE 2019 OIL SPILL ON THE MACROBENTHIC FAUNA ASSOCIATED WHITH MACROALGAE ON THE TROPICAL COAST OF BRAZIL

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Immediate effects of the 2019 oil spill on the macrobenthic fauna associated with macroalgae on the tropical coast of Brazil

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ABSTRACT

The 2019–2020 Brazil oil spill disaster affected several ecosystems and species-rich areas. The shallow-water reefs of Paiva beach are among the best-preserved coastal environments in the State of Pernambuco (NE Brazil), but were severely affected by oil arrival at the end of 2019. The reefs are densely covered by macroalgae as important biogenic substrates for benthic fauna. Based on that, herein, we provide a baseline assessment of the immediate impacts of the oil spill on the structure of epifaunal communities associated with the algae *Jania capillacea* and *Penicillus capitatus*. The benthic communities in both algae simplified (reduction of species richness and abundance) soon after oil arrival, while opportunistic taxa increased. After two months, the macrofaunal communities restructured to almost pre-disaster levels. However, polychaeta diversity remained low. Despite the apparently fast recovery of reef macrobenthos, a more detailed, long-term monitoring is necessary to evaluate the chronic effects.

1. Introduction

Oil spills are one of the most destructive environmental impacts in marine ecosystems, particularly coastal zones, and can affect human populations, the physical environment and biota at all trophic levels (Weiss, 2015; McLachlan and Defeo, 2018). Biota are impacted by high toxicity of oil components, mainly hydrocarbons, which can represent 90% of the total chemical compounds (Tissot and Welte, 1984; UNEP, 1992; NRC, 2003). Oil can severely impact faunal and floral populations by physical (smothering, reduced light), habitat (altered pH, decreased dissolved oxygen, decreased food availability), and ecotoxicological effects (Kennish, 1997; Weiss, 2014, 2015; Yim et al., 2020). Several studies have demonstrated the effects of petroleum derived compounds on organisms, which include changes in their swimming capacities, prey capture, predation and reproduction, thus demonstrating sublethal and lethal effects on abundance and diversity (Maciel et al., 2015; Torreiro-Melo et al., 2015; Weiss, 2015). In this sense, assessing the acute (short-term) and chronic (long-term) impacts of oil spills on marine biodiversity is of utmost importance (Weiss, 2014; Yuewen and Adzigbli, 2018).

Traces of crude oil from a "mysterious" source were recorded for the first time in late August of 2019 in a few localities on the Northeast Coast of Brazil (Escobar, 2019; Araujo et al., 2020). The oil spread rapidly during the following months, reaching more than 3000 km along the Brazilian coast by January of 2020 (Lourenço et al., 2020; Magris and Giarrizzo, 2020). More than 5000 tons of crude oil had already been removed within approximately five months (Brum et al., 2020). However, stranded oil fragments in some of the affected areas have been reported by the media since then and are expected to keep appearing sporadically for years to come due resuspension from the continental shelf and/or mangroves (Lourenço et al., 2020). In the meantime, geochemical analyses have found that the crude oil that reached the Brazilian Coast was compatible with Venezuelan oil (Oliveira et al., 2020). The oil reached 1000 localities and 55 marine protected areas (Soares et al., 2020b), affecting several coastal ecosystems such as mangroves (489.83 km^2), beaches (185.3 km^2), intertidal coral reefs (45.95 km^2) (Magris and Giarrizzo, 2020) and seagrass meadows (Magalhães et al., 2020). This disaster represents the most extensive and most severe environmental impact for the Brazilian coast, the South Atlantic Ocean, and tropical oceans so far (Soares et al., 2020a, 2020b).

Along the 194 km long Pernambuco coast, coral reefs are very

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abundant on the inner shelf, occurring parallel to the coastline as patches or elongated bank reefs attached to the coast or at depths of 5–10 m (Laborel, 1970; Laborel-Deguen et al., 2019). These coastal reefs, first described by Darwin (1841), are basically rocky bodies composed of mostly quartz sand grains, cemented by calcium carbonate. The intertidal reefs are densely colonized by macroalgal assemblages that are typically found in the tropical phytogeographic region (Horta et al., 2001). Most of the 1676 tons of oil that arrived on the coast of Pernambuco was deposited between October 19 and 28 of 2019 (Brazilian Navy, 2019; SPG, 2019; Câmara et al., 2020). Upon reaching the coast, oil stains quickly covered extensive areas of sandy beaches and coral reefs with a thick layer of oil. Immediately after the wide publication of images and alerts broadcast on television, non-governmental organizations and civilians started collecting the oil, mainly by hand (frequently without adequate protection), and much oil was rapidly collected at sea or along the beaches (Soares et al., 2020b).

The impacts of the oil arrival were acute and immediate, killing turtles, fish, dolphins and birds, in addition to decimating millions of small animals as invertebrates and plants, whose deaths were not easily perceived by the naked eye. Although less "popular" to the general public, these small organisms are key components of complex food webs and may be good indicators of hidden environmental impacts (Roberts et al., 2008). Despite this, no detailed studies about the consequences of the Brazilian oil spill on macrobenthic fauna have been published until now. Besides impacting several coastal ecosystems, the oil spill severely affected traditional fishing communities (artisanal fishing), since seafood items, such as mollusks, crustaceans and fishes, that constitute the basis of their diets and income were no longer allowed to be sold or consumed (Araujo et al., 2020; Ramalho and Santos, 2020).

Although several studies about the marine life of Pernambuco have been conducted since 1950, surprisingly little information exists concerning reef intertidal communities. In July 2019, a research project was started to understand the role of macroalgae morphology and chemical composition in structuring macrobenthos at Paiva beach (one of the best-preserved Brazilian coral reefs), and on the 21st of October 2019 LaBen's researchers were collecting samples on this beach when oil stains started arriving at the reefs. During the 2019 oil spill these reefs were severely impacted, since more than 1000 tons of oil were collected in just one week in the Cabo de Santo Agostinho municipality, where the Paiva's reefs are located (week between 19 and 28 October 2019) (SPG, 2019). This presented a unique opportunity to study the acute (short-term) effects of crude oil on the benthic macrofauna associated with seaweed in tropical coastal reefs. This paper describes the immediate impacts of the 2019 oil spill on the structure of macrobenthic communities associated with *Jania capillacea* and *Penicillus capitatus* on coastal coral reefs of Paiva beach (Northeastern Brazil).

2. Material and methods

2.1. Study area

Paiva beach (8°16′46.4″S and 34°56′47.1″W) is an open ocean sandy beach approximately 7.8 km long (Holanda et al., 2020) (Fig. 1). Paiva beach, located on the southern Pernambuco coast, has also some of the best-preserved coral reef areas on the tropical coast of Brazil (Vasconcelos et al., 2019) and is surrounded by two Protected Areas (MPA Estuários dos rios Jaboatão e Pirapama and PA Mata de Camaçari) (Barbosa, 2016). The climate is tropical hot/humid, with average annual temperatures above 25 °C throughout the year (Domingues et al., 2017). The tidal regime is of the mesotidal semi-diurnal type, with tide height varying between 0.7 m (neap tide) and 2.5 m (spring tide) (Domingues et al., 2017). Wave climate in the region presents minimum and maximum significant heights of 0.97 m and 3.37 m, with mean of 1.5 m (Pereira and Nogueira Neto, 2010). Parallel to the coastline there are several lines of discontinuous and elongated beach rocks (Laborel, 1970). The reef line closest to the beach is up to 2.5 km long and 1 km wide and is colonized by dense algal beds (Vasconcelos et al., 2019).

2.2. Sampling and statistical analysis

Ten samples of *Jania capillacea* and *Penicillus capitatus* were collected manually (OMB - One Month Before Oil Arrival - 24-IX-2019) and two (TMB - Two Months Before Oil Arrival - 26-VIII-2019) months before the oil spill, on the week of oil arrival (WOA - Week of Oil Arrival - 21-X-2019), and one (OWA - One Week After Oil Arrival - 28-X-2019) and two (TWA - Two Weeks After Oil Arrival - 04-XI-2019) weeks and one

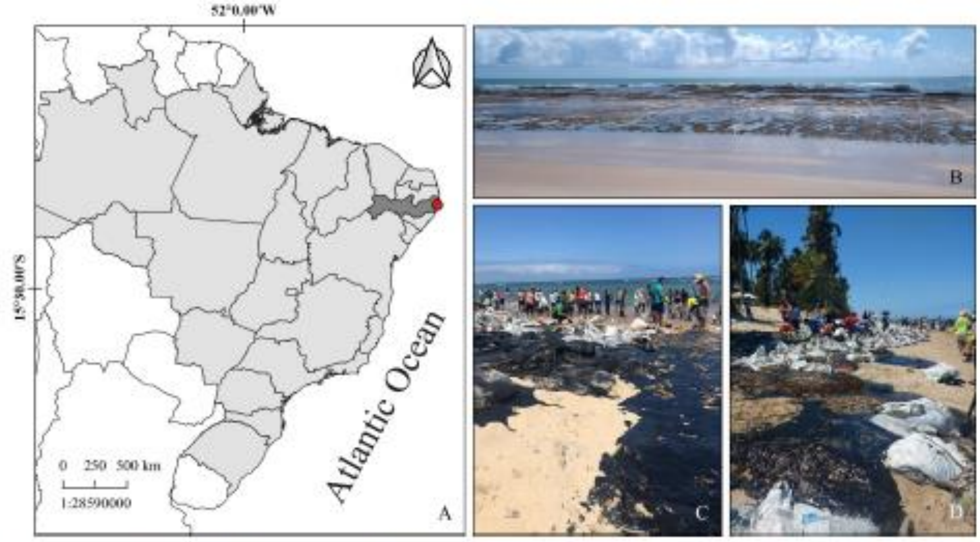

Fig. 1. Location of the study area, Paiva beach (red circle) (A). (B) Overview of the coral reefs before the oil spill. (C) and (D) Volunteers collecting the oil. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(OMA - One Month After Oil Arrival - 27-XI-2019) and two months (TMA - Two Months After Oil Arrival - 26-XII-2019) after the disaster. Collections were made by hand at random points on the reef within the affected area. Algae fronds were wrapped with plastic bags to prevent motile animals from escaping and then removed from the substrate. Samples were fixed with 4% saline formalin. In the laboratory, the samples were sieved (0.3 mm mesh size) and the retained epifaunal organisms were identified to the lowest taxonomic level possible and counted.

For each sample, richness (total taxa present), density (ind.100 ml^{-1} of algae), diversity (Simpson Index - 1-λ) and evenness (Pielou J index) were calculated. These descriptors were compared among algae and sampling occasions using a two-way Analysis of Variance (ANOVA) with data log $(x + 1)$ transformed. When ANOVA results were significant, Tukey test was used as a post hoc test. For each algae Metric Multidimensional Scaling analyses (MDS) using bootstrap-average were performed to represent samples from distinct sampling occasions (before, during and after the oil arrival) and to produce 95% bootstrap regions (Clarke et al., 2014). A two-way Permutational Analysis of Variance (Permanova) (Anderson et al., 2008), was used to compare fauna among sampling occasions per algal species. These analyses were based on resemblance matrices calculated with Bray Curtis similarity (data square root transformed). Analyses were performed using Statistica 12 and PRIMER 7 + PERMANOVA considering a 5% significance level.

3. Results

A total of 19,446 specimens belonging to 40 taxa were collected (*Jania capillacea* - 16,702 individuals, 35 taxa; *Penicillus capitatus* - 2744 individuals, 31 taxa). The main phyla were Cnidaria, Platyhelminthes, Annelida, Mollusca, Arthropoda and Echinodermata. Crustaceans were dominant in both algae, particularly amphipods and the isopod *Janaira gracilis*. In addition to these taxa, ostracods and tanaids were the most abundant crustaceans in *J. capillacea* algae. Among polychaetes, the second most abundant group, syllids were dominant in *P. capitatus* and the sabellid *Branchiomma luctuosum* dominated in *J. capillacea*. Gastropods were the dominant mollusks (third most abundant phylum in the samples) in *P. capitatus*, whereas bivalves and chitons dominated in *J. capillacea*. In *J. capillacea*, only four families (Sabellidae, Nereididae, Syllidae and Capitellidae) of the 11 polychaetes families registered until the week of oil arrival were observed in the samples collected from the second week after oil arrival (Supplementary material).

In the week of the disaster several algae and organisms had oil adhered to them (Fig. 2). In *J. capillacea* richness, diversity and evenness were significantly lower during the week of oil arrival, whereas density was significantly higher this week and significantly dropped the following week (Fig. 3 and Table 1). Richness in *P. capitatus* was minimal one week after the oil spill and significantly increased posteriorly, and the other descriptors did not vary significantly over time (Fig. 3 and Table 1). Much of the increase in total abundance after the oil arrival in both algae was due to the high number of the polychaete *Branchiomma*

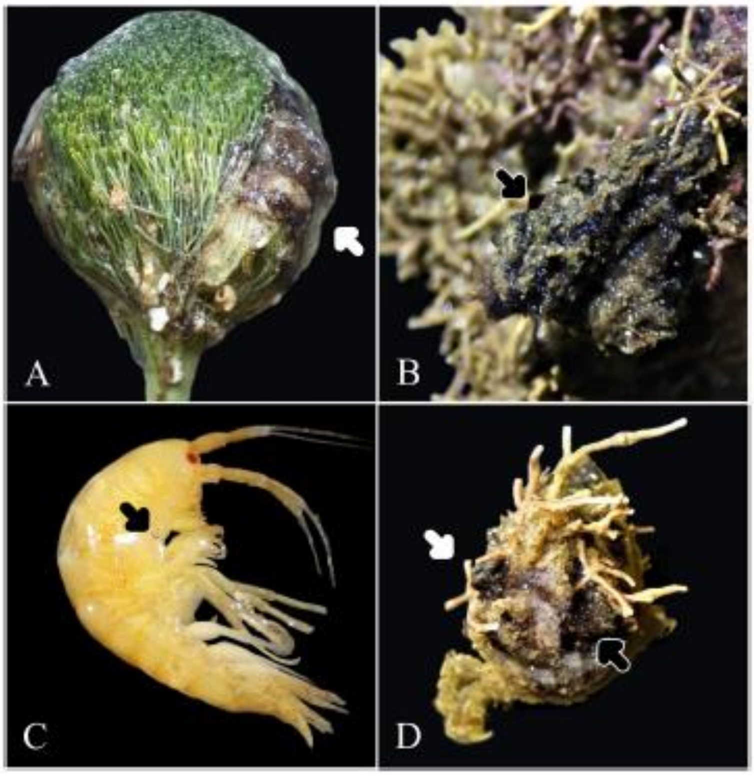

Fig. 2. Oil adhered on *Jania capillacea* and *Penicillus capitatus* and on some associated invertebrates in Paiva beach. (A) *P. capitatus* - white arrows indicate the oil attached to the seaweed; (B) *J. capillacea* - black arrows indicate the oil attached to the seaweed; (C) Amphipod collected in *P. capitatus* - black arrows indicate the oil attached to the carapace; and (D) *Acanthonyx* sp. collected in *J. capillacea* - black and white arrows indicate the oil attached to carapace.

luctuosum and the isopod *Janaira gracilis*, which increased tenfold after the disaster (Supplementary material).

In the MDS plots it is possible to observe that most samples collected before the spill are on the right side of the graph and those collected before the disaster are plotted on the left side (Fig. 4). In *P. capitatus* the samples from the week of the oil arrival were still similar to the ones collected before the disaster (right side of the graph). In *J. capillacea* the samples from the week of oil arrival were the most different from the others (Fig. 4). In both algae, the epifaunal communities significantly changed after the oil spill (Table 2). Significant changes in *J. capillacea* communities occurred in the week of oil arrival, whereas changes were only observed one week later in *P. capitatus*. In both algae after two months the communities were similar to those collected before the disaster (Table 2).

4. Discussion

Significant differences were observed in the structure of epibenthic fauna associated with macroalgae after the oil spill. The immediate effects of oil on epifauna were distinct among algae, being stronger in *J. capillacea* than in *P. capitatus*, as indicated by the significant changes on richness and abundance values of the communities associated with *J. capillacea* on the week of the arrival, and its slower recovery posteriorly. Since *Jania* generally occurs around tide pool borders, they may be susceptible to oil immediately upon arrival (O'Brien and Dixon, 1976). The effects of petroleum hydrocarbons in the marine environment can be either acute or chronic (Weiss, 2015) and the changes in the structure of benthic communities due to polluting oil may be immediate and persist from days to years (NRC, 2003).

The number of individuals and taxa, and the structure of the communities varied significantly between the species of *Jania capillacea* and *Penicillus capitatus*. The macroalgal traits such as size and morphological complexity can greatly affect local populations and communities (Munari et al., 2015; Carvalho et al., 2018). The considerably lower number of macrobenthic organisms found in *Penicillus* may be related to its compact shape with tiny interstices (Hacker and Steneck, 1990). On the other hand, *Jania*, characterized by cylindrical intergenicula and dichotomous branches throughout with marginal and axial conceptacles (Johansen and Silva, 1978), attracts more diverse fauna by providing habitat, refuge and grazing areas for numerous fish and invertebrate species (Steller et al., 2003; Chenelot et al., 2011; Bhaduri and Wolf, 2017).

In both algae the macrofauna was dominated by arthropods (more than 70% of total individuals), mainly amphipods and isopods. Typically, gammarid amphipods are the most abundant taxa in macroalgae associated fauna (Taylor and Cole, 1994; Lippert et al., 2001; Jacobucci and Leite, 2002; Carvalho et al., 2018). Amphipods have morphological and behavioral adaptations that enable them to be very successful in macroalgae phytal, such as chelated and hooked appendages that enhance their ability to hold onto algae firmly (Hagerman, 1966; Fenwick, 1976; Tararam et al., 1985), capacity to colonize distinct habitats and high diversity of feeding habits and reproduction modes (Cruz-Rivera and Hay, 2001; Carvalho et al., 2018). In the tropics, some isopods such as *Janaira gracilis*, an abundant species in Paiva, have high fertility and continuous reproduction throughout the year, which may lead to high abundances and recovery capacity (Tararam and Wakabara, 1981).

Amphipods are considered good indicators of contamination caused

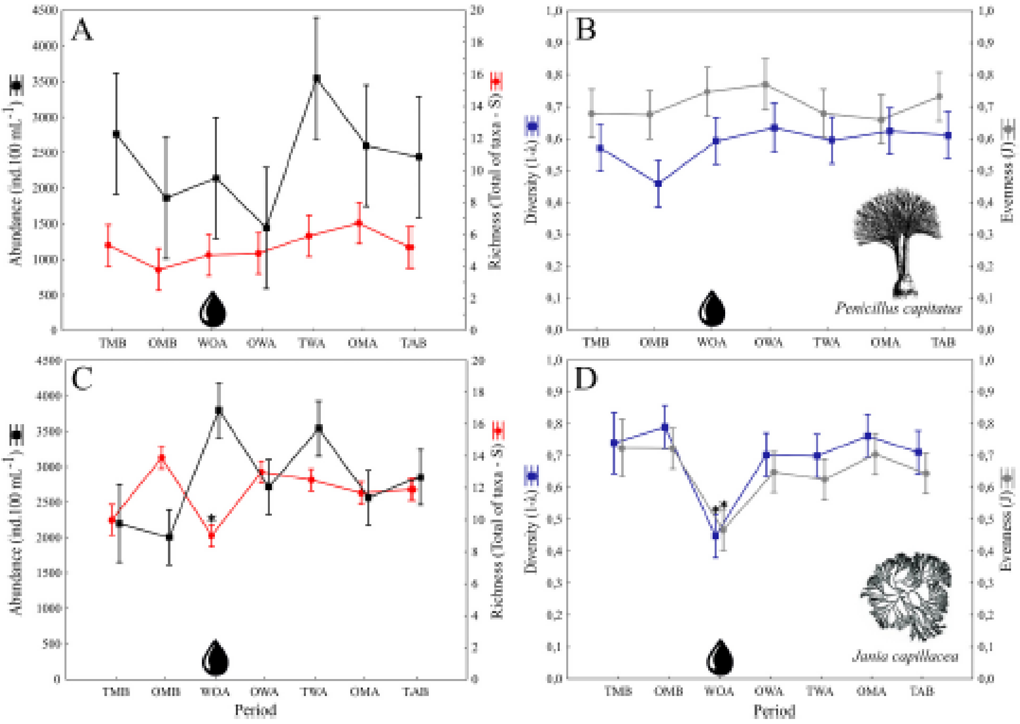

Fig. 3. Mean values (± standard deviation) of density, richness, diversity and evenness of epifauna associated with algae *Jania capillacea* (A and B) and *Penicillus capitatus* (C and D). TMB: two months before oil arrival, OMB: one month before oil arrival, WOA: week of oil arrival, OWA: one week after oil arrival, TWA: two weeks after oil arrival, OMA: one month after oil arrival, TAB: two months after oil arrival. The drop indicates the week of oil arrival.

Table 1
Summary of ANOVA results.

Source of variation	Abundance		Richness		Diversity		Evenness	
	F	p	F	p	F	p	F	p
Jania capillacea	3.15	<0.01	6.03	<0.01	10.84	<0.01	7.35	<0.01
Penicillus capitatus	2.16	0.06	1.74	0.13	2.56	0.03	1.20	0.32

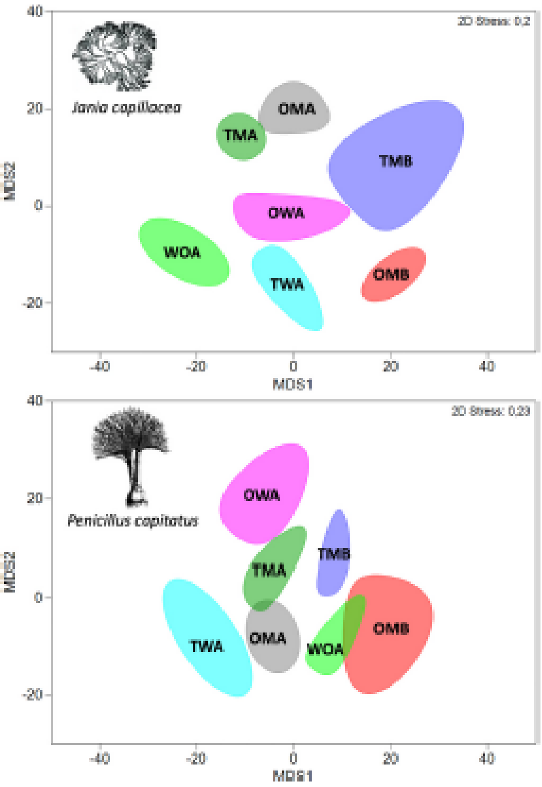

Fig. 4. Plots of MDS with 95% bootstrap regions for each macroalgae species. TMB: two months before oil arrival, OMB: one month before oil arrival, WOA: week of oil arrival, OWA: one week after oil arrival, TWA: two weeks after oil arrival, OMA: one month after oil arrival, TMA: two months after oil arrival.

Table 2
Summary of Permanova results. Asterisks indicate significant differences.

Jania capillacea

Source of variation	df	SS	MS	Pseudo-F	P (Perm)	Unique permutations
Sampling occasion	6	19,776	3296.1	5.99	<0.01	999
Residuals	58	31,913	550.23			

Average similarity between/within sampling occasions[a]

	TMB	OMB	WOA	OWA	TWA	OMA	TMA
TMB	59,61						
OMB	61.35	72.29					
WOA	56.18*	53.66*	71.06				
OWA	58.28*	60.26*	61.1*	64.73			
TWA	57.47*	60.29*	60.39*	63.57*	69.01		
OMA	57.62*	56.59*	55.01*	60.86*	59.61*	65.12	
TMA	56.58	54.94	60.28*	63.85*	62.16*	66.95	69.25

Penicillus capitatus

Source	df	SS	MS	Pseudo-F	P (Perm)	Unique permutations
Sampling occasion	6	12,083	2313.8	1.93	<0.01	998
Residuals	63	65,653	1042.1			

Average similarity between/within sampling occasions

	TMB	OMB	WOA	OWA	TWA	OMA	TMA
TMB	62.68						
OMB	54.79	48.73					
WOA	62.32*	57.39	66.51				
OWA	52.43*	44.26*	53.03*	49.32			
TWA	53.80*	46.76*	55.16*	46.47*	52.76		
OMA	63.44	56.86	64.87*	53.43*	57.53	67.25	
TMA	61.19	53.98	62.07*	55.35*	54.34	64.55	61.26

[a] TMB: two months before oil arrival. OMB: one month before oil arrival. WOA: week of oil arrival, OWA: one week after oil arrival, TWA: two weeks after oil arrival, OMA: one month after oil arrival, TAB: two months after oil arrival.

by oil due to their high sensitivity, widespread distribution in the environment, and low mobility (Lourenço et al., 2019). It is known that oil components can have negative effects on their reproduction (Yuewen and Adzigbli, 2018) and ability to recolonize algae (Grande et al., 2012). However, since amphipods have different feeding habits, hydrocarbon concentrations may vary in these organisms (Lourenço et al., 2019). Omnivores and generalist feeders might be more exposed to oil since they ingest a greater diversity of contaminant sources compared to those with more restricted diets (Lourenço et al., 2019).

Acute effects of oil spills may have short durations and limited impact, or they may have long-term population- or community-level impacts depending on the timing and duration of the oil spill and the number and types of benthic organisms affected (Newey and Seed, 1995). In general, soon after oil spill events there is a significant reduction in diversity, whereas some tolerant and opportunistic species increase their populations (e.g., tanaids and sabellid worms in this study) (Suchaneck, 1993; Yu et al., 2013). This pattern is consistent with the changes observed in the fauna associated with *J. capillacea*, which reacted soon after the oil arrived. The macrofauna in *P. capitatus* had similar changes, although less clearly, one week after the disaster.

The changes in polychaeta assemblages (diversity reduction and arrival of opportunistic species) were probably the main immediate impacts of oil arrival, particularly in *J. capillacea*. Polychaetes, which rank among the benthic invertebrates as the most tolerant to several types of environmental impacts (Kennish, 1997), may have different responses to oil contamination (Yuewen and Adzigbli, 2018). In some cases, multivariate approaches based on polychaeta species composition are much more sensitive than univariate analyses for discriminating impacts (Guerra-García and García-Gómez, 2004). A reduction of polychaeta diversity is usually related to community simplification (e.g., reduction in richness and high dominance of certain few taxa) after oil spills (NRC, 2003; Saleh, 2012).

After one or two weeks of oil arrival, the abundance of the stress-tolerant sabellid *Branchiomma luctuosum* increased strongly in both algae. The recolonization of oil-disturbed habitats is usually initiated by opportunistic polychaeta species (Kennish, 1997). *B. luctuosum* is an early colonizer of stressed habitats, and quickly becomes widespread and abundant especially in areas degraded by anthropogenic impacts (Licciano et al., 2007; Arias et al., 2013; Giangrande et al., 2017). The high tolerance to pollution and reproductive strategies of this benthic species lead to its success in colonizing and spreading over wide areas in a relatively short time, giving this species high invasive capacity (Lezzi et al., 2016).

After two months, there were no more significant differences in the structure of epifaunal communities of both algae before and after the disaster. The vulnerability of intertidal reefs to oiling is dependent on its position, topography and composition. Impacts in exposed, steep and plain rocky shorelines are generally less severe than those on protected, plain and more irregular (Moore and Guzmán, 2005). In turn, the impacts of oil spills on the biota of reefs are known to differ according to several factors, such as type and amount of spilled oil, rock shape, weathering of oil, methods and intensity of cleanup, and weather conditions (Gelin et al., 2003; Seo et al., 2014; Jung et al., 2017; Albert et al., 2018; Yim et al., 2020).

In Paiva beach, it is possible to observe an open ocean tropical sandy beach with high wave action, a semi-diurnal tidal regime and high-water temperature, which can help explain the rapid recovery of epifauna. Wave action tends to detach the oil from the surface of rocks and macroalgae and transport it to higher shore levels (Grande et al., 2012). Waves with significant height of up to 1.8 m in Paiva beach in October (Pereira and Nogueira Neto, 2010) might have been the reason that after some days of the oil spill most of the oil was concentrated along the high tide mark while the lower parts were already clear (Castège et al., 2014; Jung et al., 2017). In addition, the semi-diurnal tidal regime in Paiva (Holanda et al., 2020) favors, twice a day, the gradual removal and transport of oil from the lower shore to the upper shore. Finally, water temperatures above 26 °C in October (Domingues et al., 2017) may enhance microbial breakdown of the oil (Zekri and Chaalal, 2005; Ribicic et al., 2018).

Clean-up processes can also have strong impacts on the recovery of the intertidal macrofauna and macroalgae after oil spills (Serrano et al., 2005; Dave and Ghaly, 2011; Castège et al., 2014; Shigenaka, 2014). The use of chemicals dispersants, mechanical scraping and high-pressure hot water flushing in some oil spills caused more impacts on the biota than the oil itself (Southward and Southward, 1978; Houghton et al., 1993; Moore et al., 1995; Dave and Ghaly, 2011; Stankovich and Simeonova, 2018). None of these procedures were performed in Paiva beach. Instead, the rapid manual removal of oil, still at sea or along the beach (Soares et al., 2020b), may have been crucial to reduce the contact time of the organisms with the oil and thus decreasing the negative impacts on the biota. In addition, since most oil arrived during high tides, it is probable that much oil passed over the reefs, causing a lesser suffocating effect on the organisms. It is probable that intertidal rocky bottoms, as in Paiva's reefs, recover more quickly than other affected environments, e.g., sandy beaches, seagrass meadows and mangroves, where oiled material accumulates and is more difficult to remove (Bejarano and Michel, 2010; Magalhães et al., 2020).

Relatively few studies have focused on the effects of oil contamination in coral reefs epifauna (Roberts et al., 2008), especially in the tropics, since most large oil spills (>700 tons) in the last 50 years have occurred in temperate and polar areas, mainly in the northern hemisphere (ITOPF, 2020). This study, as the first to present quantitative data on the effects of the 2019 Brazilian oil spill in the benthic fauna associated with macroalgae in the Paiva beach, one of the most preserved reefs in Northeastern Brazil, will contribute to a better understanding of the effects of oil contamination on the benthic fauna of tropical coastal reefs. A detailed assessment of the environmental impacts of the 2019 oil spill in the coastal reefs was hindered by the lack of appropriate baseline data, since little information exists concerning intertidal communities in the tropical coast of Brazil.

In conclusion, although the changes in epifauna at the community level were relatively small in our study, a species-level analysis of the most representative taxa could reveal a more detailed scenario, since different taxa may have distinct responses to oil contamination (Roberts et al., 2008). Besides that, oil spills on seaweeds can have cumulative and cascading effects in the environment (O'Brien and Dixon, 1976). Considering the role of macroalgae as important habitat-forming species in the study area, long-term, species-level monitoring of the phytal communities affected by this extensive oil spill in Tropical South Atlantic is pivotal.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that there is no competing interest.

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