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INSTITUTO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
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COMUNIDADE BACTERIANA ASSOCIADA À ÁGUA DE RIOS E CORAIS SAUDÁVEIS E BRANQUEADOS DA ESPÉCIE *Siderastrea stellata* NA ÁREA DE PROTEÇÃO AMBIENTAL COSTA DOS CORAIS - ALAGOAS

MACEIÓ - ALAGOAS

2021

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Diversidade Biológica e Conservação nos Trópicos, Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas, como requisito para obtenção do título de Doutor em CIÊNCIAS BIOLÓGICAS, área de concentração em Conservação da Biodiversidade Tropical.

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RESUMO

O branqueamento de corais é visto como o resultado da atuação sinérgica de diversos estressores globais e locais, como a presença de rios, que dentre outros fatores alteram a composição da microbiota associada aos corais. Com o objetivo de verificar a composição da comunidade bacteriana associada à água dos rios Santo Antônio e Manguaba e a comunidade bacteriana associada a corais saudáveis e branqueados em diferentes pontos na Área de Proteção Ambiental Costa dos Corais (APACC) - AL, foram aplicadas as técnicas de microarranjo de DNA e de metataxonomia, além de extensivas análises estatísticas e de bioinformática, como predição de grupos funcionais e do conteúdo metagenômico. Com relação aos rios, os resultados indicam que uma parte significativa das Unidades Taxonômicas Operacionais (OTUs) identificadas foram capazes de sobreviver à transição de água doce para água do mar, sendo várias delas pertencentes a gêneros envolvidos na patogênese humana. A proporção BBC:A e a predição funcional sugerem que ambos os rios do estudo estão sujeitos à contaminação fecal e entrada de xenobióticos, e que as comunidades bacterianas foram mais homogêneas nesses ambientes. Com relação aos corais da espécie *Siderastrea stellata*, grupos comumente associados ao processo de branqueamento, como Vibrionaceae e Burkholderiaceae, apresentaram valores de abundância pouco expressivos, e as principais diferenças entre as duas condições (saudável x branqueado) se deram principalmente no enriquecimento de grupos mais “raros” da microbiota, mas com funções já relatadas como importantes para o holobionte, em indivíduos saudáveis. Os índices de α-diversidade indicaram uma média semelhante de riqueza e diversidade de acordo com os tratamentos e recifes, mas foram significativamente diferente apenas temporalmente. O afluxo de água do Rio Santo Antônio parece aumentar a homogeneidade entre a microbiota de corais da região, principalmente durante a estação chuvosa, sustentado principalmente pela análise da β-diversidade e pelo grande número de *Amplicon Sequence Variants* (ASV’s) pertencentes à microbiota *core*. Sugerimos que as ações de proteção adotadas para os recifes sejam amplamente estendidas ao ambiente ao redor, já que podem estar sendo afetados pelo aporte de componentes bióticos e abióticos oriundos das atividades que ocorrem ao longo do curso dos rios, e que táxons mais raros associados aos corais sejam levados em consideração nesses estudos.

Palavras-chave: *Siderastrea stellata*, bactérias, branqueamento, predição funcional, PhyloChip™.

ABSTRACT

Coral bleaching is seen as the result of the synergistic action of several global and local stressors, such as the presence of rivers, which, among other factors, alter the composition of the microbiota associated with corals. In order to verify the composition of the bacterial community associated with the water of the Santo Antônio and Manguaba rivers and the bacterial community associated with healthy and bleached corals at different points in the Costa dos Corais Environmental Protection Area (APACC), microarray techniques were applied DNA and metataxonomy, as well as extensive statistical and bioinformatics analysis, such as prediction of functional groups and metagenomic content. Regarding rivers, the results indicate that a significant part of the Operational Taxonomic Units (OTUs) identified were able to survive the transition from freshwater to seawater, several of them belonging to genera involved in human pathogenesis. The BBC:A ratio and the functional prediction suggest that both rivers in the study are subject to fecal contamination and entry of xenobiotics, and that bacterial communities were more homogeneous in these environments. Regarding the corals of the species *Siderastrea stellata*, groups commonly associated with the bleaching process, such as Vibrionaceae and Burkholderiaceae, presented low abundance values, and the main differences between the two conditions (healthy vs. bleached) were mainly due to the enrichment of more groups. “rare” of the microbiota, but with functions already reported as important for the holobiont, in healthy individuals. The α-diversity indices indicated a similar mean of richness and diversity according to treatments and reefs, but were significantly different only temporally. The inflow of water from the Santo Antônio River seems to increase the homogeneity among the coral microbiota of the region, mainly during the rainy season, supported mainly by the β-diversity analysis and by the large number of Amplicon Sequence Variants (ASV's) belonging to the core microbiota. We suggest that the protection actions adopted for the reefs be broadly extended to the surrounding environment, as they may be affected by the input of biotic and abiotic components from activities that occur along the course of rivers, and that rarer taxa associated with corals are taken into account in these studies.

Keywords: *Siderastrea stellata,* bacteria, bleaching, functional prediction, PhyloChip.

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1 APRESENTAÇÂO

Os recifes de coral constituem as maiores estruturas marinhas construídas por organismos vivos, fornecendo diversos serviços ecossistêmicos e também econômicos importantes principalmente para regiões costeiras (FISHER *et al*., 2015). Apesar disso, diversos impactos, muitos desses potencializados ou gerados por ação antrópica, ameaçam a sobrevivência dos corais ao redor do mundo (HUGHES *et al*., 2017). A ocupação costeira de ambientes aquáticos, aliada ao crescimento urbano, constituem problemas atuais a conservação dos recifes de coral, e junto com fatores decorrentes das mudanças climáticas, estão levando a uma crise global dos recifes de coral (VANWONTERGHEM E WEBSTER, 2020).

Como consequência, é cada vez mais frequente o surgimento de surtos de doenças e fenômenos/síndromes afetando os corais, sendo o mais importante o branqueamento, que vem acontecendo de forma cada vez mais intensa e frequente, com eventos globais mais prolongados e acontecendo em intervalos de tempo cada vez mais curtos, dificultando a recuperação dos indivíduos afetados (EAKIN; SWEATMAN; BRAINARD, 2019; SKIRVING *et al*., 2019). O branqueamento é classicamente visto como uma disbiose entre o coral e as zooxantelas fotossintetizantes presentes em seus tecidos, que são expulsas após certas condições fisiológicas serem afetadas, dando assim um aspecto pálido à colônia e consequentemente levando a uma perda de grande parte da sua fonte de nutrientes (HAWKINS, 2014).

De um ponto de vista microbiológico, os corais passaram a ser vistos como seres holobiontes que abrigam em seus compartimentos anatômicos uma ampla gama de micro-organismos responsáveis por realizar as mais diversas funções, incluindo ciclagem de nutrientes e proteção contra patógenos oportunistas (BOURNE E MUNN, 2005; MERA; BOURNE, 2018; ROHWER *et al*., 2002) . Essa microbiota extremamente rica associada aos corais vem sendo cada vez mais estudada na tentativa de entender os mecanismos por trás de sua persistência e na busca de identificar membros-chave para a manutenção da saúde do hospedeiro. Essa comunidade parece ser vital para a sobrevivência dos corais, principalmente em momentos de estresse, onde mudanças rápidas

em sua composição fornecem um mecanismo adaptativo eficiente capaz de amortecer os impactos ambientais (RESHEF *et al*., 2006; ROSENBERG *et al*., 2007). Entretanto, alguns micro-organismos, incluindo bactérias, vírus e fungos, parecem estar associados ao surgimento de algumas doenças oportunistas, como é o caso de alguns membros do gênero *Vibrio,* como *V. shiloi*, *V. coralliilyticus,* por exemplo, são frequentemente associados ao surgimento do branqueamento, sendo que para alguns deles o mecanismo de virulência já foi elucidado (DENNIS, 2018; KUSHMARO *et al*., 1997, 2001). Além disso, mudanças maiores na composição da comunidade microbiana parecem estar relacionadas ao processo de adoecimento, geralmente partindo de um estado mutualista ou comensal para um estado patogênico, com maior dominância de alguns grupos oportunistas (LIU *et al*., 2016; MAO-JONES *et al.*, 2010).

A Área de Proteção Ambiental Costa dos Corais (APACC) representa hoje uma das maiores unidades federais de conservação marinha no Brasil, estendendo-se por praticamente toda a costa norte do estado de Alagoas e servindo de fonte de renda para milhares de pessoas da região. Infelizmente, com a ocupação costeira não só da região marítima, mas também da margem dos diversos rios que deságuam na região, o processo de branqueamento parece ter se tornando mais frequente, mesmo em períodos onde não há estresse termal (MAGEL *et al*., 2019). Até então, nenhum estudo propôs analisar a questão microbiológica associada ao branqueamento dos corais da região, como, por exemplo, de que forma a comunidade bacteriana associada aos corais varia de acordo com o estado de saúde do hospedeiro e estação do ano.

Nessa tese, foram utilizadas técnicas de biologia molecular que permitiram avaliar a comunidade bacteriana ao longo do curso de dois rios (Santo Antônio e Manguaba) que deságuam em recifes de coral da região da APACC e associada a corais da espécie *Siderastrea stellata* em condições saudáveis ou afetados pelo branqueamento. Com isso, foi possível trazer conhecimento sobre as condições das adjascências dos recifes de coral, contribuindo para o desenvolvimento de novas medidas de proteção que incluam também essas áreas e não só as zonas recifais, e dos aspectos microbiológicos relacionados ao branqueamento da espécie de coral *Siderastrea stellata*, bastante comum e importante aos recifes de coral brasileiros. De forma mais

específica, o documento foi divido em dois capítulos, ambos apresentados na forma de artigo científico:

1) No primeiro, foi utilizado o PhyloChip™ (método derivado da técnica de microarranjo de DNA) como ferramenta molecular para avaliar a comunidade bacteriana ao longo do curso de dois grandes rios, Santo Antônio e Manguaba, ambos dentro da porção alagoana da APACC, que deságuam sobre recifes de coral das respectivas regiões. Além disso, dados físico-químicos e biológicos dos pontos de coleta foram aferidos mensalmente durante todo o ano de 2018. Dessa forma, foi possível estimar a qualidade da água coletada quanto à possíveis contaminantes de origem antrópica, através da presença e abundância de grupos bacterianos já relacionados à doenças humanas e da predição de categorias funcionais putativas associadas à degradação de diversos compostos xenobióticos, além de fornecer um panorama sobre as condições físico-químicas de ambos os rios;
2) No segundo, as diferenças entre a comunidade bacteriana associada a corais saudáveis e branqueados coletados em duas regiões da porção alagoana da APACC, Barra de Santo Antônio e Maragogi, durante os períodos seco e chuvoso de 2019, foram analisadas utilizando o sequenciamento massivo através da plataforma Illumina. Os dados gerados foram analisados taxonomicamente e funcionalmente (de forma putativa, através de softwares específicos) a fim de determinar grupos possivelmente associados à manutenção da saúde dos corais ou ao desenvolvimento do branqueamento nos indíviduos afetados. Assim, foi mostrado que a condição do branqueamento nos corais coletados não parece estar associada à presença de patógenos já relacionados ao fenômeno, mas sim associada à variações sazonais que afetam a fisiologia do hospedeiro e a capacidade deste em abrigar micro-organismos capazes de suprir suas carências metabólicas durante esses períodos de transição.

1.1 Referências Bibliográficas

BOURNE, D. G.; MUNN, C. B. Diversity of bacteria associated with the coral *Pocillopora damicornis* from the Great Barrier Reef. **Environmental Microbiology**, v. 7, n. 8, p. 1162–1174, 2005.

DENNIS, M. M. **Diseases of Coral**. v. 54. 2018.

EAKIN, C. M.; SWEATMAN, H. P. A.; BRAINARD, R. E. The 2014–2017 global-scale coral bleaching event: insights and impacts. **Coral Reefs**, v. 38, n. 4, p. 539–545, 2019.

HAWKINS, C. Coral Bleaching - A review of the causes and consequences. **A reef manager's guide to coral bleaching**. 2014.

HUGHES, T. P. *et al*. Coral reefs in the Anthropocene. **Nature**, v. 546, n. 7656, p. 82–90, 2017.

KUSHMARO, A. *et al*. Bleaching of the coral *Oculina patagonica* by Vibrio AK-1. **Marine Ecology Progress Series**, v. 147, n. 1–3, p. 159–165, 1997.

KUSHMARO, A. *et al*. *Vibrio shiloi* sp. nov., the causative agent of bleaching of the coral Oculina patagonica. **International Journal of Systematic and Evolutionary Microbiology**, 2001.

LIU, Z. *et al*. Differences in microbial communities between healthy and bleached coral *Acropora solitaryensis* from Xisha Islands, South China Sea. **Marine Biology Research**, v. 12, n. 10, p. 1101–1108, 2016.

MAO-JONES, J. *et al*. How microbial community composition regulates coral disease development. **PLoS Biology**, 2010.

MERA, H.; BOURNE, D. G. Disentangling causation: complex roles of coral-associated microorganisms in disease. **Environmental Microbiology**, v. 20, n. 2, p. 431–449, 2018.

RESHEF, L. *et al*. The Coral Probiotic Hypothesis. **Environmental Microbiology**, v. 8, n. 12, p. 2068–2073, 2006.

ROHWER, F. *et al*. Diversity and distribution of coral-associated bacteria. **Marine Ecology Progress Series**, v. 243, p. 1–10, 2002.

ROSENBERG, E. *et al*. The role of microorganisms in coral health, disease and evolution. **Nature Reviews Microbiology**, v. 5, n. 5, p. 355–362, 2007.

SKIRVING, W. J. *et al*. The relentless march of mass coral bleaching: a global perspective of changing heat stress. **Coral Reefs**, v. 38, n. 4, p. 547–557, 2019.

2 REVISÃO DE LITERATURA

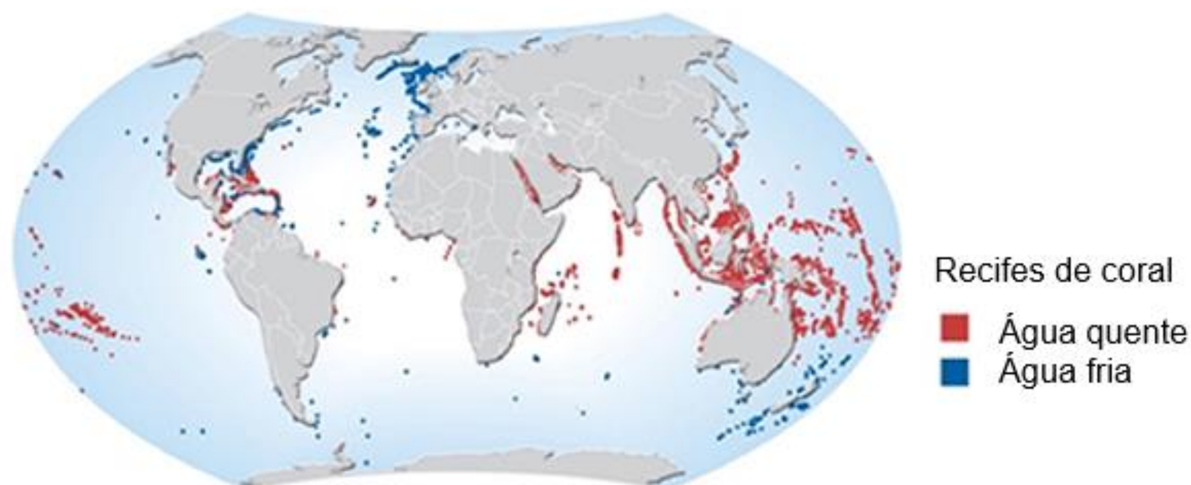
2.1 Os recifes de coral

Os corais escleractíneos ou hermatípicos (massivos) e algas coralíneas crustosas são os principais responsáveis, através do depósito de carbonato de cálcio, por consolidarem os recifes de coral, que representam as maiores estruturas construídas por organismos marinhos. Atualmente, as algas coralíneas são consideradas responsáveis ainda por aumentar a diversidade e fortalecer a estrutura dos recifes de coral antigos (WEISS E MARTINDALE, 2017). Estima-se que, apesar de ocuparem apenas 0,2% dos oceanos em todo mundo (Figura 1) (HOEGH-GULDBERG *et al.*, 2017), os recifes coralíneos tornam-se críticos para a sobrevivência dos ecossistemas marinhos tropicais graças a sua elevada produtividade, abrigando algo entre 550.000 e 1.330.000 espécies de organismos multicelulares, dos quais uma grande parcela ainda permanece desconhecida (FISHER *et al.*, 2015).

Os recifes de coral fornecem bens ecossistêmicos sobre os quais dependem mais de 500 milhões de pessoas que habitam as regiões costeiras dos 109 países onde ocorrem, agindo como barreiras naturais contra danos causados por ação das ondas e tempestades (FERRARIO *et al.*, 2014) ou constituindo uma fonte fundamental de alimento para muitas populações costeiras através da pesca, turismo e recreação (WILKINSON, 2004). Levando em consideração características como a região recifal e suas adjascências, cerca de 30% dos recifes do mundo são economicamente importantes para esse setor, com um valor total anual calculado em quase US$ 36 bilhões (SPALDING *et al.*, 2017).

Além de toda a rica biodiversidade, que abrange desde pequenos invertebrados até grandes mamíferos, os recifes ainda abrigam uma grande gama de micronichos onde há dominância de diversos micro-organismos (BOURNE; MORROW; WEBSTER, 2016; HERNANDEZ-AGREDA *et al.*, 2018; HERNANDEZ-AGREDA; GATES; AINSWORTH, 2017; PEIXOTO *et al.*, 2017).

Figura 1: Distribuição mundial dos recifes de coral.



Adaptado de: HOEGH-GULDBERG *et al.*, 2017.

Apesar de toda sua importância, os recifes de coral sofreram um declínio significativo ao longo das últimas quatro décadas, apresentando perda global estimada em 19% de cobertura total (WEAR E THURBER, 2015). No Caribe Mexicano, uma região que conta com 16 Áreas Protegidas Naturais, por exemplo, estima-se que os corais representem apenas 15 a 20% da cobertura total, devido às doenças causadas por micro-organismos e eventos catastróficos em resposta a variações climáticas e ambientais (RIOJA-NIETO E ÁLVAREZ-FILIP, 2019).

Monitoramentos de longo prazo sugerem que o declínio global da cobertura dos corais é causado principalmente pela pesca excessiva, mudanças climáticas e distúrbios devido ao escoamento de rios enriquecidos com nutrientes e sedimentos (Figura 2) (PANDOLFI *et al.*, 2003; VANWONTERGHEM E WEBSTER, 2020). Eventos climáticos extremos até então resultantes dessas mudanças climáticas podem ser classificados principalmente como: sistemas de tempestade ciclônicos (como tornados, furacões e ciclones), capazes de causar danos estruturais aos recifes de coral e estão associados à mudança de dominância de corais para macroalgas através da interação com outros fatores locais (MADIN E CONNOLLY, 2006); ondas de calor (associadas principalmente à fenômenos globais de branqueamento), com possíveis consequências como o achatamento da estrutura do recife de coral e redução na produção de carbonato de cálcio; formação de novas comunidades de peixes persistentes (redução no

número de espécies herbívoras, por exemplo); estado composicional alternativo com dominância de macroalgas; e períodos prolongados de secas (COUCH *et al.*, 2017; GRAHAM *et al.*, 2015; LANGE; PERRY, 2019; ROBINSON *et al.*, 2019).

Figura 2: Principais serviços ecossistêmicos fornecidos pelos recifes de coral e os principais impactos antropogênicos que os afetam.

Adaptado de: VANWONTERGHEM; WEBSTER, 2020.

Em águas costeiras, a descarga direta de esgoto é uma importante fonte de poluição, que pode elevar as concentrações de nutrientes do ambiente recifal, favorecendo principalmente o crescimento de algas, que liberam compostos alelopáticos nocivos aos corais e depletam os níveis de oxigênio no local, causando morte por hipóxia (ALTIERI *et al.*, 2017; REOPANICHKUL *et al.*, 2009). O problema é recorrente em praticamente todos as regiões que abrigam recifes de coral em sua costa, e dentre as centenas de compostos diferentes que podem ser encontrados nessas águas, os mais comuns são água doce,

nutrientes inorgânicos, agentes patogênicos, sólidos em suspensão, sedimentos, metais pesados e outras toxinas (WEAR E THURBER, 2015).

Mais recentemente, outro fator vem sendo apontado como importante ameaça emergente aos recifes de coral. O plástico, tanto na forma de macro (fragmentos maiores que 50 mm) quanto micro (tradicionalmente definido como resíduos de produtos feitos de plástico liberados no ambiente e decompostos em fragmentos menores que 5 mm) (ARTHUR; BAKER; BAMFORD, 2009). Os danos causados pelo microplástico aumentam à medida que o coral passa a ingeri-lo, já que sua forma e tamanho são bastante similares ao do zooplâncton presente na coluna de água. As consequências incluem uma falsa sensação de saciedade que não vem acompanhada dos nutrientes necessários para a sobrevivência do coral, o bloqueio de cavidades internas, maior gasto de energia com sua remoção e intoxicação por possíveis poluentes adicionados ao material plástico durante sua fabricação (BEDNARZ, *et al.*, 2021; HALL *et al.*, 2015). Esses fatores são potencializados durante períodos de estresse termal, onde o coral passa a depender ainda mais da heterotrofia para se alimentar devido a ausência das zooxantelas (AXWORTHY; PADILLA-GAMIÑO, 2019).

Outra consequência é a colonização por potenciais patógenos associados ao plástico, capazes de perturbar as relações normais entre hospedeiro-simbionte, como os grupos bacterianos Rhodobacterales, Vibrionaceae, Rhodobacteraceae e Flavobacteraceae, e o protozoário *Halofolliculina* spp., que apresentam membros oportunistas conhecidamente associados a doenças de coral, incluindo o branqueamento (FENG *et al.*, 2020).

Os macroplásticos bentônicos (aqueles que ficam retidos na estrutura coralínea) são suspeitos de aumentar a suscetibilidade a doenças de corais de 4% para 89% através de abrasões físicas e lesões nos tecidos do coral, e afetar mais facilmente os corais estruturalmente complexos (LAMB *et al.*, 2016; 2018). Juntos, estressores climáticos e antrópicos levam à mudanças na composição das comunidades bacterianas dos recifes de coral, comprometendo o funcionamento normal dos hospedeiros e predispondo-os à infecções oportunistas (HARVELL *et al.*, 1999; LESSER *et al.*, 2007).

2.2 Os recifes de coral brasileiros

Os recifes de coral brasileiros estão distribuídos principalmente ao longo da costa da região Nordeste, se estendendo por cerca de 3.000 km, onde são registradas grandes comunidades coralíneas desde o Parcel de Manuel Luís - Maranhão (0°53' S, 44°16' W) até os recifes de Viçosa, na área de Abrolhos (18°01' S, 39°17' W). Algumas ilhas oceânicas mais afastadas da costa, como o Atol das Rocas e Fernando de Noronha, também abrigam estruturas recifais (FERREIRA E MAIDA, 2006).

Estes são principalmente remendos ou recifes de bancos alongados; alguns estão ligados à costa (seu arranjo espacial e alongamento sugerem que a maioria deles pode ter crescido sobre linhas de rocha de praia) (CORREIA, 2011) ou afastados a vários quilômetros, geralmente alinhados paralelamente à costa em profundidades de aproximadamente 5–10 m mas também podem ser encontrados a 20–25 m de profundidade (SANTOS *et al.*, 2007).

Apesar de descoberto há cerca de 40 anos, apenas recentemente foi reconhecido o Grande Recife Amazônico, uma enorme estrutura formada principalmente por algas calcáreas, localizado entre o Brasil e o Caribe (considerado um possível corredor ecológico profundo) e sob influência direta das águas do Rio Amazonas, responsável por introduzir uma grande quantidade de sedimento na região, o que dificulta o crescimento de organismos fotossintetizantes (DE MAHIQUES *et al.*, 2019; MOURA *et al.*, 2016).

As áreas recifais são consideradas prioritárias para a conservação da biodiversidade no Oceano Atlântico, pois embora não sejam tão extensas (representando aproximadamente 5% dos recifes do Atlântico), possuem uma alta taxa de endemismo entre as espécies de corais (MOURA, 2000).

O pesquisador Jacques Laborel é o responsável por um dos trabalhos mais abrangentes sobre os recifes de coral brasileiros, realizado na década de 60 durante sua tese de doutorado pela Universidade de Marseille (LABOREL, 1970). Apesar de ter enfrentado sérios problemas logísticos, o estudo forneceu uma descrição qualitativa e semi-quantitativa dos recifes brasileiros ao longo de quase toda a costa Nordeste. Posteriormente, o importante projeto Coral Vivo atualizou essas informações, mostrando que a fauna de corais escleractíneos do Brasil apresenta quatro características distintas: i) baixa diversidade (cerca 16

corais-pétreos zooxantelados, três corais-negros, 17 octocorais e cinco hidrocorais) quando comparada com a encontrada nos recifes do Atlântico Norte, por exemplo, dos quais os principais construtores de recifes são formas arcaicas, remanescentes de uma antiga fauna de corais que remonta ao Terciário; ii) os principais construtores de recifes são espécies endêmicas das águas brasileiras, bem adaptadas a altas condições hidrodinâmicas e com ocorrência sujeita à altos graus de turbidez; iii) grandes descontinuidades em sua distribuição e iv) são predominantemente compostos por formas massivas (LEÃO *et al.*, 2016; ZILBERBERG *et al.*, 2016).

Estima-se que mais de 70 milhões de pessoas (cerca de 40% da população) vivam na zona costeira brasileira, uma das áreas mais densamente povoadas do país, mas bastante irregularmente distribuida (MARRONI; ASMUS, 2013). Sob diversas perspectivas, a pesca (em suas mais diversas modalidades), junto com o turismo, ainda é uma das atividades mais importantes dessas regiões, mas é apontada, ao lado do estresse termal, como um dos principais estressores para os recifes de coral brasileiros (MAGRIS; GRECH E PRESSEY, 2018). Sinais típicos atribuídos à sobrepesca são relatados pelos pescadores e administradores locais: queda na produção, menor tamanho médio dos peixes e viagens mais longas para pegar menos peixes (LEÃO E OLIVEIRA, 2019).

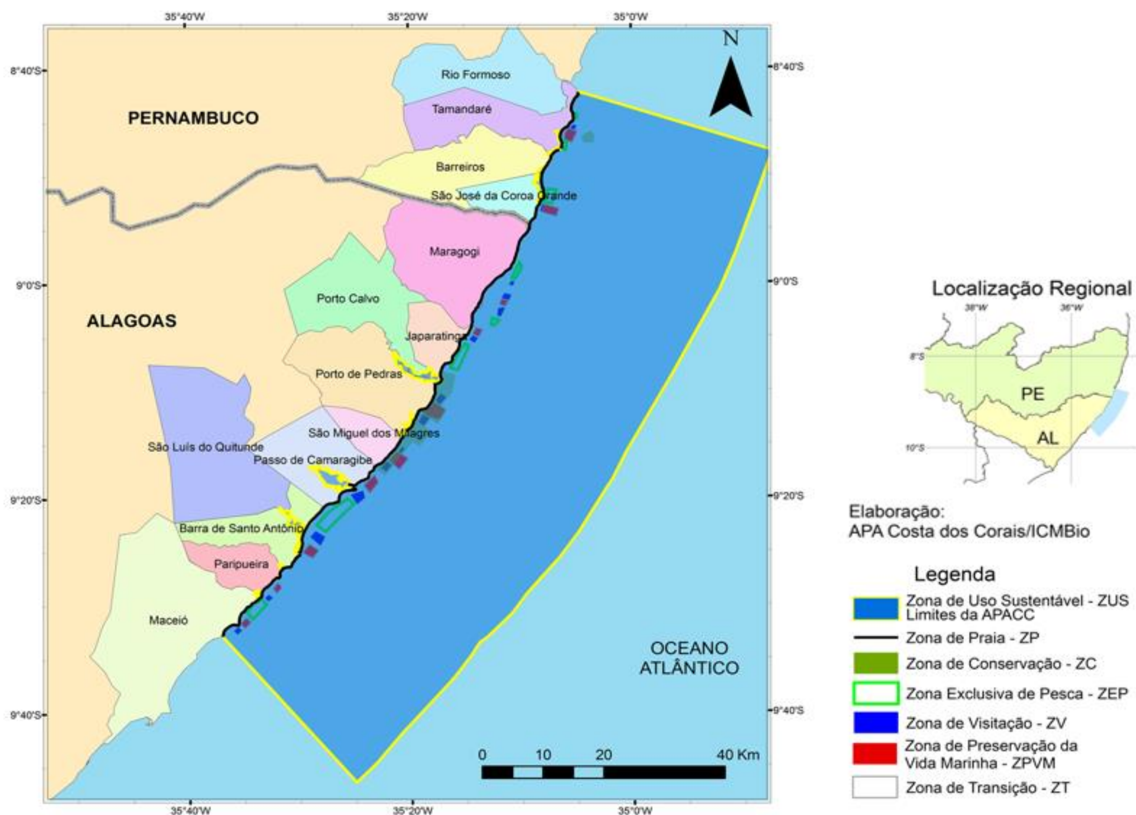
Enquanto isso, o turismo crescente, que vem passando por um cenário com vários projetos de desenvolvimento em andamento, apresenta-se tanto como oportunidade quanto ameaça. Danos causados por âncoras, o encalhe de embarcações, a introdução de lixo, a atividade de mergulhadores que se apoiam ou esbarram em corais e as caminhadas no topo do recife representam danos potenciais aos recifes de coral (SPANÓ, LEÃO E KIKUCHI, 2008).

Além disso, o desenvolvimento urbano desenfreado nas áreas costeiras, relacionado principalmente ao despejo de esgoto não tratado e lixo urbano estão causando, em certos locais, um aumento anormal de nutrientes que trarão consequências dramáticas para o equilíbrio ecológico do meio ambiente (WEAR *et al.*, 2021). Ainda, os recifes próximos à costa estão cada vez mais expostos a um influxo de sedimentos de origem terrestre, principalmente graças ao aumento do escoamento costeiro, que pode ser atribuído à expansão da agricultura e da indústria e o consequente desmatamento da Mata Atlântica, inicialmente para

plantações de cana-de-açúcar e coco, depois para permitir a exploração madeireira, e, nas últimas décadas, para o cultivo de eucalipto para uso industrial (LEÃO E OLIVEIRA, 2019).

Na costa do estado de Alagoas é possível encontrar numerosas formações tanto de recifes coralíneos quanto de arenito que se estendem desde o litoral até poucos metros de profundidade (CEDRO *et al.*, 2007). Com o intuito de preservar a biodiversidade marinha associada a essas formações de uma parcela importante do litoral nordestino, foi implementada em 1997, entre os estados de Pernambuco e Alagoas, a Área de Preservação Ambiental Costa dos Corais (APACC), considerada uma das maiores unidades de conservação federal marinha do Brasil, abrangendo uma área total de aproximadamente 4135 km² e se estendendo por cerca de 135 km de praias e mangues (Figura 3) (FERREIRA *et al.*, 2003; ICMBIO, 2013).

Figura 3: Mapa da APA Costa dos Corais, mostrando sua extensão desde Alagoas a Pernambuco e as zonas propostas no plano de manejo.



Fonte: ICMBio, 2013.

A área é considerada um imenso berçário da vida marinha, contando com a presença de diversas espécies ameaçadas de extinção, como tartarugas marinhas e o peixe-boi, e abrigando cerca 185 espécies de peixes e dez de corais, sendo sete dessas endêmicas, como *Mussismilia hispida* e *Millepora braziliensis* (FERREIRA E MAIDA, 2006; ICMBIO, 2013). Um levantamento da diversidade em alguns recifes da APACC indicou uma grande predominância geral de algas, enquanto que entre os corais dominaram os zoantídeos como *Palythoa caribaeorum e Zoanthus* sp., mas com ocorrências significativas de corais escleractíneos como *Siderastrea stellata* e *Favia gravida* em alguns pontos de amostragem (STEINER *et al*., 2015).

2.3 Os cnidários e a espécie *Siderastrea stellata*

Os cnidários são membros fundamentais de comunidades epibentônicas, especialmente em águas tropicais rasas, onde atuam, principalmente junto com as algas calcáreas, como importantes formadores dos recifes de corais (DALY *et al*., 2007; SANTHANAM, 2020). O termo Cnidaria tem origem do grego *knidos* (urticante), por todos os integrantes do grupo apresentarem organelas chamadas cnidas, dispostas de um longo filamento com espinhos retráteis e substâncias tóxicas, utilizadas para sua defesa e captura de presas (THORP E ROGERS, 2014). O filo Cnidaria apresenta aproximadamente 13000 espécies, quase todas consideradas de alguma maneira peçonhentas, enquanto cerca de 200 (menos que 1%) são potencialmente perigosas para humanos (SANTHANAM, 2020), e compreende dois clados bem definidos por aspectos morfológicos e características relacionadas ao ciclo de vida: Anthozoae e Medusozoa. O clado Anthozoa é representado por duas subclasses: Octocorallia e Hexacorallia. Esta última compreende os corais escleractínios (principais formadores de recifes dentre os cnidários), os corais negros, as anêmonas-do-mar e os zoantídeos (revisitado por MCFADDEN *et al*., 2021).

A espécie *Siderastrea stellata* (Verril, 1868) é caracterizada como um organismo colonial, zooxantelado e massivo, reconhecida por sua notável plasticidade ecológica e resistência à diversas variáveis ambientais, como sedimentação, ação de ondas e variações de temperatura e salinidade (Figura 4) (LEÃO *et al*. 2003). A espécie pertence ao pequeno gênero *Siderastrea* (de

Blainville, 1830), composto por apenas cinco espécies, duas (*S. savignyana* e *S. glynni*) das quais restritas aos oceanos Pacífico e Índico (BUDD E GUZMAN, 1994). As demais espécies, *S. siderea, S. radians* (ampla ocorrrência principalmente no Caribe, mas com registros também no Brasil) (NEVES *et al*. 2008) e *S. stellata* compõem o chamado "complexo *Siderastrea* do Atlântico" (definido por VERON, 1995 e revisado morfologicamente e molecularmente por GARCÍA *et al*., 2017).

Figura 4: Colônia saudável de *Siderastrea stellata* no recife de coral da Barra de Santo Antônio - 2019.

Autor: José Gilmar.

A taxonomia deste grupo é considerada complicada, principalmente pela grande variação e sobreposição de caracteres diagnósticos. Algumas características, como o número de septos, por exemplo, parecem ser mais eficientes em distinguir algumas espécies, como *S. stellata* e *S. radians* (MENEZES *et al*., 2014). A filogenia foi resolvida usando a região ITS (*internal transcribed spacer* – espaçador transcrito interno) como um marcador molecular, que ainda mostrou uma divergência profunda entre as espécies do Pacífico

Ocidental e do Atlântico e que *S. glynni* não é derivado de *S. savignyana*, mas sim que compartilha similaridades genéticas com *S. siderea*, levantando questionamentos sobre sua dispersão (FORSMAN *et al*., 2005). Acreditava-se que a distribuição de *S. stellata* era limitada ao território costeiro brasileiro, apresentando ocorrência em praticamente todos os recifes brasileiros, abragendo toda a extensão costeira entre o Maranhão e Rio de Janeiro (CASTRO E PIRES, 2001), com ocorrências nos recifes adjacentes à foz do rio Amazonas (CORDEIRO *et al*., 2015). Entretanto, um estudo recente usando dados morfológicos e moleculares determinou a primeira ocorrência da espécie no Golfo do México (GARCÍA *et al.,* 2017).

Reprodutivamente, *S. stellata* é classificada como uma espécie gonocórica, com uma alta razão sexual feminina/masculina e ciclo reprodutivo anual. As larvas, que aparentemente adquirem as zooxantelas do pólipo que as liberam, apresentam tamanho variável entre 500 µm a 1,4 mm de diâmetro e são lançadas pela boca, ao contrário do que ocorre com *S. radians* (hermafrodita), que as libera através das pontas dos tentáculos (NEVES E DA SILVEIRA, 2003).

A espécie é descrita como sendo marrom-avermelhada com paredes pálidas, ou rosada com tonalidades azuladas (SANTOS *et al*., 2004), mas nenhum deles associou esses diferentes padrões de cor a eventos sazonais de branqueamento. Tais alterações de pigmentação podem estar associadas às condições locais de estresse gerado por fatores biológicos e/ou ambientais, incluindo impactos antropogênicos sobre o uso da terra (SASSI *et al*., 2014).

Dados sugerem que as condições ambientais influenciam a densidade de zooxantelas e outros microssimbiontes, que também parecem estar intimamente relacionadas às condições de saúde do hospedeiro. Por outro lado, há uma importante variação sazonal, com alguns fatores, como a maior densidade de zooxantelas e outros microssimbiontes, estando mais relacionados ao período seco, enquanto que a chuva atua na desestabilização dos simbiontes dentro das colônias, resultando inclusive em alterações na pigmentação do coral (SASSI *et al.,* 2015).

As alterações de pigmentação incluem formas de pigmentos de baixa e alta fluorescência que parecem fornecer um sistema de proteção fotobiológica capaz de dissipar o excesso de energia em comprimentos de onda de baixa

atividade fotossintética, aumentando assim a resistência ao branqueamento durante períodos de estresse termal (ROTH *et al*., 2010; SALIH *et al*., 2000).

A composição da comunidade microbiana associada à espécie já foi explorada de forma descritiva usando bibliotecas do gene 16S rDNA. Os principais filos observados foram Proteobacteria, Bacteroidetes, Actinobacteria, Cyanobacteria e Planctomycetes, enquanto que os OTU's (*Operational Taxonomic Unit* – Unidade Taxonômica Operacional) mais abundantes foram identificados como *Acidovorax* sp. (Betaproteobacteria) e *Acinetobacter* sp. (Gammaproteobacteria) (LINS-DE-BARROS *et al*., 2010). Em estudo posterior, a microbiota foi comparada entre um grupo saudável e um grupo branqueado, sendo demostrada uma diversidade procariótica (bactérias e arquéias) ligeiramente maior em indivíduos saudáveis, cuja microbiota foi dominada principalmente por Betaproteobacteria, do que branqueados, dominada por Alphaproteobacteria e Gammaproteobacteria, enquanto que o oposto ocorreu em relação aos plastídios de algas eucarióticas associados (LINS-DE-BARROS *et al*., 2013). Além disso, membros da sua microbiota pertencentes ao gênero *Bacillus* sp. já foram avaliados quanto a atividade antimicrobiana contra diferentes linhagens de *Salmonella* sp. e *Escherichia coli,* e oito linhagens se mostraram promissoras contra ao menos duas das dez bactérias alvo (MOURA *et al.,* 2021).

2.4 Os corais como seres holobiontes

Até pouco tempo atrás, os corais eram considerados o produto de uma interação mutualista entre o coral e eucariontes dinoflagelados do gênero *Symbiodinium*, comumente chamadas de zooxantelas, reportadas pela primeira vez em 1883 por Karl Brandt e isoladas em meio de cultura nos anos 1950 (KLEBS, 1984). Estima-se que esses micro-organismos tenham evoluído há aproximadamente 160 milhões de anos, na época da segunda radiação adaptativa de corais escleractínicos durante o período jurássico médio (LAJEUNESSE *et al*., 2018).

Essa simbiose estendeu a capacidade metabólica do coral por meio de transferências metabólicas e da transferência horizontal de genes, o que contribuiu para o sucesso ecológico dos hospedeiros. As microalgas fornecem

grande parte das necessidades energéticas dos seus hospedeiros ao transferir carbono fixado fotossinteticamente para o coral, além de produzirem grandes quantidades de oxigênio molecular que permite a respiração eficiente pelo coral (WANGPRASEURT *et al.*, 2017) e que quando saturado leva a formação de radicais livres que ajudam o hospedeiro na prevenção de infecções por patógenos (BANIN *et al.*, 2003). Também já foi demonstrado que os corais podem abrigar diferentes linhagens de *Symbiodinium,* indicando um papel importante da dinâmica dessas comunidades sobre a resiliência do hospedeiro (SILVA-LIMA *et al.*, 2015).

A família Symbiodiniaceae foi recentemente revisada e reorganizada em sete gêneros, entre os quais, quatro são conhecidos por se associarem comumente a corais escleractíneos: *Symbiodinium*, *Breviolum*, *Cladocopium* e *Durusdinium*, enquanto os gêneros *Fugacium* e *Gerakladium* são apenas associados ocasionais (LAJEUNESSE *et al.*, 2018).

Os primeiros estudos de microbiologia de corais utilizaram métodos dependentes de cultivo e se concentraram na microbiota associada ao muco, encontrando uma grande diversidade bacteriana (DUCKLOW E MITCHELL, 1979). Entretanto, estima-se que cerca de 99% dos micro-organismos marinhos não sejam cultiváveis, principalmente por requerem condições nutricionais e/ou físicas complexas difíceis de serem replicadas em laboratório (FUHRMAN E CAMPBELL, 1998).

Diversos fatores contribuem para esse viés metodológico, como o fato desses micro-organismos frequentemente existirem em conjuntos interdependentes que funcionam como unidades metabólicas, já que esses cenários até então não podem ser replicados com a precisão necessária em condições laboratoriais (AINSWORTH *et al.*, 2010). Além disso, os micro-organismos frequentemente existem em relações altamente específicas com seus hospedeiros, e a ausência de fatores-chave, como metabólitos produzidos por outros membros da comunidade, também dificultam o cultivo (HANDELSMAN, 2004).

O surgimento das técnicas independentes de cultivo e das tecnologias de sequenciamento de nova geração, capazes de identificar até mesmo as espécies raras e menos abundantes no ambiente (GILLES *et al.*, 2011; PETROSINO *et al.*, 2009; QUAIL *et al.*, 2012; ROGERS E VENTER, 2005; SUNAGAWA *et al.*,

2010), ajudaram a revelar uma comunidade microbiana rica e diversa associada aos corais, incluindo uma maioria de novas espécies. Assim, esses organismos passaram a ser vistos como seres holobiontes, abrigando diversos micro-organismos em seus tecidos, como fungos, arquéias, protozoários, vírus e bactérias, que formam uma comunidade complexa que aparentemente coevoluiu com os corais (HARRIS *et al*., 2001; ROHWER *et al*., 2002). Posteriormente, Bourne e Munn utilizaram ambas as abordagens, dependentes e independentes de cultivo, para investigar a diversidade de bactérias associadas ao coral *Pocillopora damicornis* e encontraram diferentes grupos dominando os resultados observados de acordo com a técnica utilizada (BOURNE E MUNN, 2005).

Diversos trabalhos ao redor do mundo passaram a analisar a microbiota de várias espécies de coral buscando entender quais os padrões e processos ecológicos que norteiam essa associação (BOURNE *et al*., 2005; RITCHIE, 2006; RODER *et al.,* 2014; SUN *et al*., 2014; SWEET *et al*., 2011), e esse microbioma passou a ser visto como uma das mais complexas biosferas estudadas (HERNANDEZ-AGREDA *et al*., 2017).

No Brasil, a primeira caracterização da microbiota de uma espécie de coral foi realizada utilizando bibliotecas do gene 16S rDNA de bactérias do muco da espécie endêmica *Mussismilia brazilienses* e da água circundante (REIS, 2009), e após isso vários, trabalhos se dedicaram a explorar a diversidade bacteriana associada aos corais pertencentes à costa brasileira e sua relação com fatores locais (CARLOS; TORRES; OTTOBONI, 2013; LEITE *et al*., 2018; PAULINO *et al*., 2016; PEIXOTO *et al.,* 2018).

Taxonomicamente, os principais grupos de bactérias marinhas associadas ao muco e ao tecido de corais identificados através dessas técnicas são Proteobacteria (principalmente das classes Alpha- e Gammaproteobacteria), Firmicutes, Bacteroidetes, Planctomycetes, Actinomycetes e Cyanobacteria (BOURNE *et al*., 2016). Sabe-se ainda que os micro-organismos associados aos corais podem ser comensais, mutualistas ou patogênicos, com densidades estimadas de 1×10^2 a $>1\times10^6$ células por centímetro quadrado de tecido hospedeiro (BLACKALL *et al*., 2015).

A diversidade dessa microbiota geralmente excede um número de milhares de unidades taxonômicas operacionais (OTU's) em algumas espécies

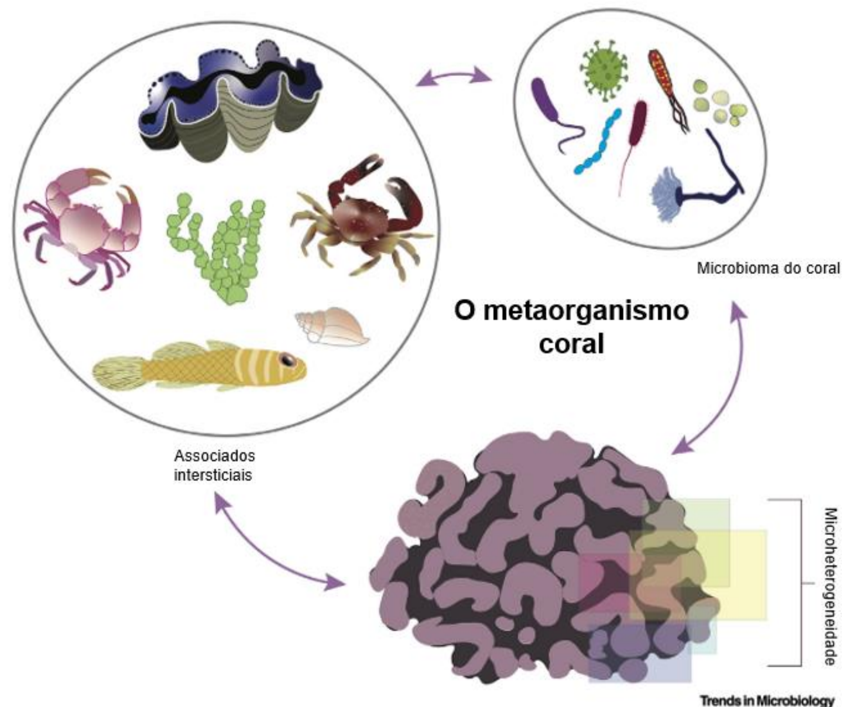
hospedeiras, sendo influenciada por uma série de fatores, incluindo características fisiológicas do hospedeiro, localização dentro da subestrutura de coral (tecido, cavidade gástrica, muco, esqueleto) e estágio da história de vida (AINSWORTH *et al*., 2010; MOUCHKA *et al*., 2010; SWEET *et al*., 2011). Além disso, eventos biológicos, como competição de algas, reprodução e doenças, bem como mudanças nas variáveis ambientais, incluindo temperatura, pH, nutrientes e carbono orgânico dissolvido, geram mudanças na composição, riqueza e abundância dessas bactérias associadas aos corais (AINSWORTH *et al*., 2017).

Essas interações mutualistas entre coral-microbiota, entretanto, não ocorrem isoladamente; outras espécies também interagem com as comunidades microbianas associadas ao coral, e como consequência acabar por desempenhar um papel importante na condução da dinâmica do recife: mais de 800 espécies de invertebrados associados já foram identificadas em associação aos corais, sendo que mais da metade destas dependem dos corais para sobreviver (STELLA *et al*., 2011).

Embora a inclusão de comunidades microbianas seja crítica para a compreensão da ecologia do coral, esta versão do metaorganismo negligencia o "macrobioma" dos associados intersticiais do coral, que molda as interações dos micro-organismos do coral. Um dos benefícios claros de integrar o microbioma e o macrobioma são suas implicações potenciais para a restauração de corais. Por exemplo, já foi demonstrado que a introdução de espécies que conferem resiliência aos corais pode aumentar o sucesso inicial, bem como a longevidade dos recifes restaurados (SHAVER E SILLIMAN, 2017).

Pensando nisso, trabalhos mais recentes sugerem pensar de forma mais crítica sobre o holobionte, levando em consideração também como os associados intersticiais afetam as interações coral-micro-organismo, e então foi sugerido o conceito do coral como um metaorganismo, enfatizando as relações entre o próprio hospedeiro, seu microbioma (zooxantelas e micro-organismos que habitam todos os seus compartimentos estruturais) e demais associados intersticiais (por exemplo, invertebrados residentes, macroalgas e peixes criptobentônicos) (Figura 5) (AINSWORTH; RENZI; SILLIMAN, 2020).

Figura 5: O coral como um metaorganismo, formando associações com macro (associados intersticiais) e micro-organismos (microbioma).

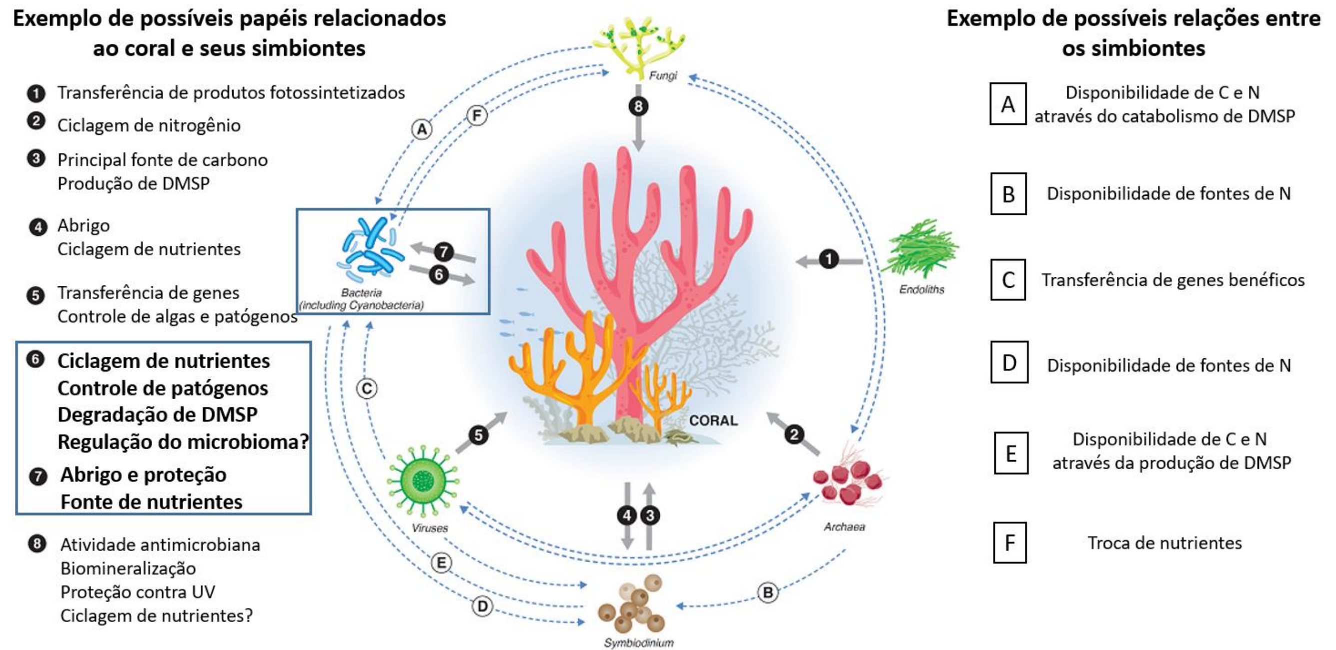


Adaptado de: AINSWORTH; RENZI; SILLIMAN, 2020.

2.5 O papel dos micro-organismos na saúde dos corais

Diversos estudos mostram que os micro-organismos associados aos corais podem desempenhar um importante papel na saúde do hospedeiro, provendo uma fonte de alimento e ocupando nichos específicos, incluindo a participação em diversos ciclos biogeoquímicos (RITCHIE, 2006), além de fornecerem nutrientes vitais como fósforo, metais e vitaminas (Figura 6) (BOURNE, 2016).

Figura 6: Exemplos de interações entre hospedeiro e microbiota e papéis desempenhados por micro-organismos nesse ambiente.



Adaptado de PEIXOTO *et al.*, 2017.

Os corais satisfazem suas necessidades nutricionais explorando dois sistemas separados: compartilhando produtos fotossintéticos produzidos por suas algas simbiontes e capturando material orgânico particulado com a ajuda de seus tentáculos (RESHEF *et al.*, 2006). Como a fixação de nitrogênio é um processo realizado principalmente por procariotos, é razoável supor que alguns representantes desses grupos beneficiarão o coral holobionte através desse mecanismo (RÄDECKER *et al.*, 2015), além de promoverem a ciclagem de enxofre (RAINA *et al.*, 2009), um componente chave nas proteínas, coenzimas e metaloproteínas e assimilado principalmente através do sulfato dissolvido (SIEVERT, 2007).

Quando a disponibilidade de nitrogênio diminui no ambiente, os micro-organismos associados podem fornecer nitrogênio orgânico ao hospedeiro, que geralmente abriga espécies bacterianas nitrificantes (transforma amônio em nitratos e eventualmente nitritos) e desnitrificantes (reduz nitritos e nitratos em dinitrogênio) capazes de transformar resíduos relacionados ao nitrogênio produzidos pelo hospedeiro em compostos não tóxicos. Isso ajuda a prevenir o acúmulo de resíduos nitrogenados que podem alterar a homeostase dos corais.

Além disso, comparações com a abundância e taxa de expressão do gene *nifH* demonstraram que a fixação de nitrogênio compensa a baixa absorção heterotrófica de nitrogênio em corais autotróficos (POGOREUTZ *et al.*, 2017; RÄDECKER *et al.*, 2015).

A competição por espaço e nutrientes entre os micro-organismos é ainda uma força seletora que estimula a produção de metabólitos secundários, consequentemente auxiliando no combate de possíveis patógenos para o hospedeiro e mantendo a comunidade microbiana estável (ROSENBERG *et al.*, 2007). Entre estes antagonistas de corais, já foram identificadas algumas espécies dos gêneros *Vibrio*, *Photobacterium*, *Bacillus* e *Halomonas* (ROSENBERG E FALKOVITZ, 2004; RITCHIE, 2006).

As bactérias comensais, que utilizam as glicoproteínas poliméricas e lipídios que compõem o muco como fonte de carbono e nitrogênio, podem interromper o estabelecimento de patógenos invasores dentro do muco do coral através de métodos amensalistas, como a interferência em suas atividades metabólicas e enxameação, que podem reduzir a ocorrência de doenças de coral (Tabela 1) (KREDIET *et al.*, 2013a). De outra maneira, a alta concentração de antibióticos dentro da camada mucosa impede que novos patógenos se instalem na superfície do coral, embora os patógenos também possam produzir antibióticos para favorecer seu estabelecimento (KREDIET *et al.*, 2013b).

Além da produção desses metabólitos secundários, a proteção contra esses patógenos ocorre por meio de diversos mecanismos, incluindo exclusão competitiva e inibição do *quorum sensing,* um mecanismo molecular envolvido na proteção contra patógenos, onde a produção de autoindutores usados para sinalização química permite que as bactérias modulem sua expressão gênica de acordo com a densidade da população (GOLBERG *et al.*, 2011). Esses sinais são usados para regular atividades fisiológicas, como simbiose, virulência, motilidade e produção de antibióticos, e já foi demonstrado que sua inibição está relacionada com a prevenção de infecções (CERTNER; VOLLMER, 2018; KALIA *et al.*, 2019).

Tabela 1: Exemplos de táxons já relacionados na literatura a funções importantes na associação com o coral holobionte.

Papel	Descrição	Exemplo	Referências
Metabolismo	Fornece nitrogênio para *Symbiodinium* e para o coral	*Cyanobacteria*, *Synenochocous*, *Prochlorococcous*, *Vibrio* sp. *Rhizobiales*	Lesser *et al.*, 2004; Olson *et al.*, 2009
	Fornece nitrogênio para *Symbiodinium* durante o estágio larval dos corais	*Altermonas* sp., *Vibrio alginolyticus, Rhizobiales*	Lesser *et al.*, 2007, Lema *et al.*, 2012
	Ciclagem do enxofre	*Roseobacter, Spongiobacter, Vibrio, Altermonas*	Raina *et al.*, 2009
Proteção	Atividade antimicrobiana contra os patógenos *Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium* e *Serratia marsescens*	*Photobacterium, Halomonas, Exiguobacterium, Bacillus, Altermonas*	Ritchie, 2006
	Atividade antibiofilme contra *Pseudomonas aeruginosa* através da produção de biosurfactante	*Providencia rettgeri, Bacillus flexus, Psychrobacter* sp.	Padmavathi *et al.*, 2014
	Interações antagonistas em ensaios de difusão em ágar, incluindo inibição de *Vibrio shiloi*	Vibrionales, Alteromonadales (e.g., *Pseudoaltermonas*), Bacteroidetes	Rypien *et al.*, 2010
	Inibição de *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Vibrio fluvialis* e os fungos *Penicillium* sp., *Aspergillus niger, Candida albicans*	*Bacillus, Pseudomonas*	ElAhwany *et al.*, 2013
	Sua baixa abundância facilita a proliferação de grupos oportunistas	Actinobacteria	Zaneveld *et al.*, 2016
	Atividade antimicrobiana contra *Serratia marcescens,* um patógeno associado à doença de coral "*White Pox*"	*Exiguobacterium, Bacillus* sp.	Krediet *et al.*, 2012; Pereira *et al.*, 2017
	Bactérias predatórias que consomem os patógenos de coral *Vibrio corallyticus* e *Vibrio harveyii*	*Halobacteriovorax* sp.	Welsh *et al.*, 2016
	Atividade antimicrobiana exibida contra outros isolados obtidos do muco de corais	*Pseudoalteromonas*	Kvennefors *et al.*, 2011
Recrutamento	Aumento significativo no assentamento larval, sugerindo um fator de sinalização extracelular	*Roseivivax* sp. 46E8 (Alphaproteobacterium)	Sharp *et al.*, 2015

Alguns trabalhos demonstram a influência de micro-organismos no desenvolvimento e resiliência de corais hospedeiros, onde, por exemplo, a combinação de biofilmes e compostos produzidos por populações específicas de bactérias funcionariam emitindo múltiplos sinais que coordenam o estabelecimento e metamorfose de estágios larvais (HADFIELD, 2011). Em outro caso, o tratamento de culturas de larvas de corais com antibióticos foi suficiente para impedir seu estabelecimento e metamorfose, sugerindo que a ação das bactérias associadas às larvas é fundamental para que o ciclo de vida desses corais continue (VERMEIJ *et al.*, 2009).

Embora os micro-organismos possam ser transmitidos vertical e horizontalmente nos corais, também existem estratégias mistas. A transmissão vertical garante a herdabilidade de membros das comunidades microbianas, permitindo que os hospedeiros transfiram espécies microbianas específicas e bem-sucedidas para sua progênie (BERNASCONI *et al.*, 2019). Por outro lado, a aquisição da microbiota também pode ocorrer por meio de sua incorporação ao muco que envolve os feixes óvulo-esperma (LEITE *et al.*, 2017). No entanto, a aquisição contínua de membros microbianos do ambiente circundante enfraquece a associação hereditária ao longo do tempo de vida do hospedeiro (WILLIAMS *et al.*, 2015). Por fim, estipula-se que inicialmente a composição da microbiota tenha um estágio inicial relacionada a herança materna, e que ao longo do tempo de vida atinge a maturidade por meio de estágios sucessionais regidos principalmente pela aquisição horizontal de novos membros, com potencial para gerar redundância funcional dentro do microbioma (HERNANDEZ-AGREDA; LEGGAT; AINSWORTH, 2019).

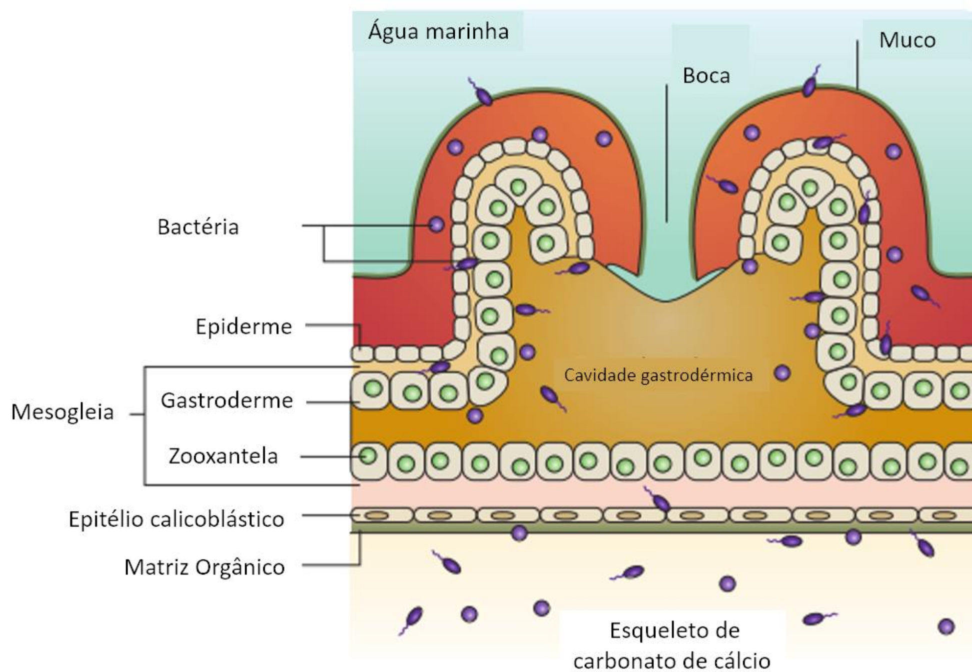
A microbiota associada aos corais não é apenas crucial para o *fitness*, mas também mostra-se sensível à perturbações ambientais e ao estado fisiológico do seu hospedeiro (MAO-JONES *et al.*, 2010; ZHANG *et al.*, 2021). Diversos fatores ambientais, como temperatura, intensidade da luz e fluxo da água, conhecidos por variar diariamente e sazonalmente, influenciam tanto o organismo hospedeiro quanto a microbiota associada (ULSTRUP *et al.*, 2006).

Os efeitos desses estressores ambientais sobre o hospedeiro e sua microbiota incluem: perda de muco da superfície do coral, redução da oferta de muco na camada superficial mucosa do coral, mudanças na disponibilidade de nutrientes, deterioração da função dinoflagelada endossimbionte e também

ruptura do tecido do hospedeiro. Consequentemente, as mudanças em termos da composição taxonômica microbiana e funcional (metagenoma) representam um processo evolutivo em ação que poderia potencialmente produzir traços fenotípicos adaptativos hereditários (ROBBINS *et al.*, 2019; ZILBER-ROSENBERG; ROSENBERG, 2008).

Um dos pontos determinantes da composição da microbiota é a própria estrutura corporal dos corais, que apresenta três possíveis diferentes habitats para micro-organismos, cada um apresentando variações na disponibilidade de nutrientes, oxigênio e na forma como interagem com o meio circundante, por exemplo, refletindo em comunidades associadas distintas (Figura 7) (BOURNE *et al.*, 2016; LI *et al.*, 2014; TOUT *et al.*, 2014).

Figura 7: Esquema com os compartimentos que compõem a estrutura básica dos corais.



Adaptado de: ROSENBERG *et al.*, 2007.

O primeiro é representado pela camada mucosa superficial, composta principalmente por proteínas, lipídios, polissacarídeos e glicoproteínas (DUCKLOW E MITCHELL, 1979), o que difere em composição e abundância de recursos da água do mar ou do sedimento marinho (TREMBLAY *et al.*, 2011). O muco, que corresponde a cerca de 30% da massa de tecido seco das colônias

de coral (YAMASHIRO *et al.*, 1999), fornece proteção contra UV, dessecação e aumento da carga de sedimentos (BOURNE *et al.*, 2016), além de aumentar a resistência do organismo através de vários mecanismos, que incluem: a formação de uma barreira física entre o coral e o ambiente; o transporte mucociliar de micro-organismos para remoção via ingestão pelo coral hospedeiro, evitando assim a colonização por micro-organismos invasores; e atuando como um meio para difusão de compostos com propriedades antimicrobianas (BROWN E BYTHELI, 2005).

Diversos estudos demonstram que esta camada suporta uma comunidade bacteriana benéfica diversa e abundante (CARLOS *et al.*, 2013; MCKEW *et al.*, 2012) incluindo fixadores de nitrogênio e decompositores de quitina (DUCKLOW, 1979; GLASL *et al.*, 2016). A composição da comunidade microbiana residente nesse compartimento é determinada principalmente por dois mecanismos: (i) a composição química (por exemplo, nutrientes e sinalizadores), viscosidade e espessura do muco, e (ii) os receptores específicos associados à superfície do coral e camada mucosa (JATKAR *et al.*, 2010; TEPLITSKI E RITCHIE, 2009).

Após, temos os tecidos, que incluem a cavidade gastrodérmica e epiderme, e o esqueleto de carbonato de cálcio. Em ambas as camadas de tecido (ectoderme e gastroderme) do pólipo, bactérias associadas às células são frequentemente encontradas em estruturas chamadas agregados microbianos associados a células (*Cell Associated Microbial Agregates* - CAMAs), que provavelmente consistem em uma única espécie bacteriana (WORK; AEBY, 2014).

O esqueleto é um substrato poroso com características físico-químicas únicas, afetado principalmente pela luz, oxigênio, pH e acesso a componentes químicos, que abriga a maior diversidade de bactérias em relação a outros microhabitats, além de fungos e algas verdes (revisado por PERNICE *et al.*, 2020 e TRIBOLLET, 2008). Estima-se que a comunidade endolítica satisfaça cerca de 50% das necessidades totais de nitrogênio do coral (FERRER E SZMANT, 1988), além de fornecer carbono graças a grupos fotossintetizantes, como bactérias do filo Cyanobacteria, que podem manter a nutrição do coral em momentos de estresse, como o branqueamento (FINE E LOYA, 2002). Espécies de cianobactérias marinhas filamentosas, como *Plectonema terebrans*, *Mastigocoleus testarum* e *Halomicronema excentricum*, são relatadas como os

primeiros procariotos descritos formando bandas verdes em esqueletos de coral (LE CAMPION ALSUMARD; GOLUBIC; HUTCHINGS, 1995). Estudos sugerem que os complexos conjuntos de micro-organismos que habitam esse micronicho podem influenciar a saúde dos corais principalmente por meio de seu importante papel na bioerosão, produção primária e ciclo de nutrientes (principalmente nitrogênio) (PERNICE *et al.*, 2020).

Cada um desses compartimentos do coral atua como uma espécie de micronicho, apresentando características bioquímicas diferentes, e são afetados de forma diferente por fatores abióticos, como o grau de penetração da luz solar e acidificação dos oceanos, capazes de determinar a presença de distintos tipos de *Symbiodinum* e bactérias endolíticas presentes no esqueleto, por exemplo (BLACKALL *et al.*, 2015). Além disso, acredita-se que certas substâncias inibidoras de crescimento e/ou adesão bacteriana produzidas tanto pelo coral quanto pela sua microbiota podem contribuir com a seleção de bactérias marinhas capazes de colonizar de forma mais eficiente os tecidos dos corais (SWEET *et al.*, 2011).

Baseado nessas observações, foi proposta a hipótese probiótica, sugerindo que mudanças nas comunidades microbianas sob diferentes condições ambientais permitiam uma rápida e versátil adaptação do coral holobionte, que contaria com o enorme arsenal genético dos micro-organismos associados (RESHEF *et al.*, 2006). Juntos com o tempo significativamente mais curto no surgimento de novas gerações, a enorme diversidade taxonômica e metabólica do microbioma fornecem um potencial considerável para os micro-organismos contribuam de forma rápida para uma eficiente resposta adaptativa do holobionte (TORDA *et al.*, 2017). Um dos fatos apresentados para sustentar a hipótese consiste que apesar de não apresentarem um sistema imune adaptativo, os corais podem desenvolver resistência a diversos patógenos (NAIR *et al.*, 2005), e mudanças na microbiota normal, geralmente observadas antes de sinais de estresses visíveis nas colônias, podem ser usadas como bioindicadores de doenças ambientais (PANTOS *et al.*, 2003).

Além disso, foi sugerido que existe um microbioma central estável e persistente associado aos corais (em alguns casos até espécie-específicos), composto principalmente por membros benéficos para a saúde do hospedeiro, que interage com uma porção maior e altamente variável, influenciada e

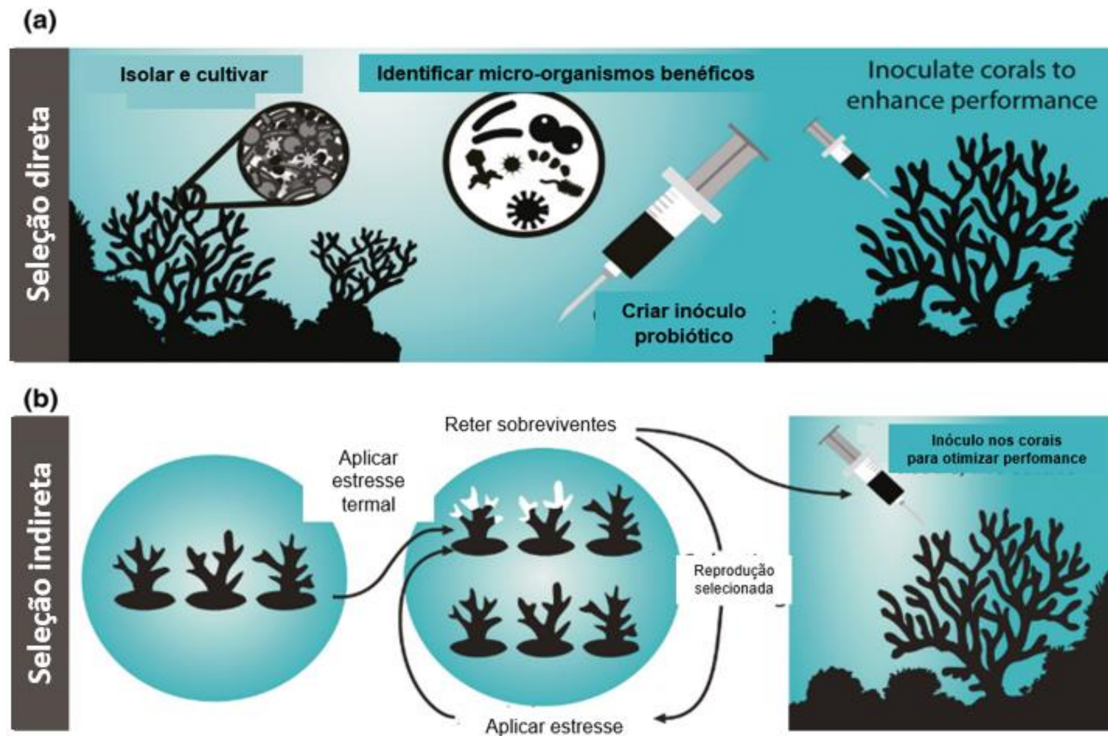
responsiva a fatores bióticos (associados ao hospedeiro) e abióticos (associados ao ambiente recifal e também às atividades antrópicas que são exercidas nele) (AINSWORTH *et al.*, 2015; HERNANDEZ-AGREDA *et al.*, 2017). Esse nível de especificidade com seus simbiontes bacterianos associados sugerem um processo de coevolução adaptativa entre o hospedeiro coral e sua microbiota, já sendo demonstrado que uma microbiota específica a determinadas espécie de coral pode estar correlacionada com a filogenia de seu hospedeiro (ou seja, a filossimbiose) (O'BRIEN *et al.*, 2020; POLLOCK *et al.*, 2018).

Após, foi proposto o termo "Micro-organismos benéficos para os corais" (*Benefical Microorganisms for Corals* – BMC), que refere-se diretamente aos micro-organismos simbiontes que atuam na manutenção e proteção do equilíbrio fisiológico dos corais, de forma semelhante as rizobactérias promotoras de crescimento nas plantas (PEIXOTO *et al.*, 2017).

Com base nesses conceitos, entrou em discussão a possibilidade de pôr em prática métodos de engenharia microbiana (definida como a manipulação experimental de micro-organismos individuais, comunidades microbianas ou do próprio hospedeiro, no caso nos mecanismos envolvidos na interação com sua microbiota associada) voltados para o aumento da performance e fitness dos corais frente às mudanças climáticas e ambientais, principalmente como uma possível ferramenta para a conservação (DAMJANOVIC *et al.*, 2017; VAN OPPEN *et al.*, 2015; 2017).

Entretanto, essa possibilidade continua em desenvolvimento inicial, com um dos primeiros estudos empíricos bem-sucedidos de manipulação do microbioma dos corais explorando a possibilidade de usar inóculos microbianos para aumentar a resistência dos corais à poluição por óleo (FRAGOSO *et al.*, 2015). Métodos tradicionalmente usados no melhoramento de plantas usando engenharia microbiana são propostos para fundamentar a técnica voltada para os corais: a seleção de um grupo benéfico específico ou de uma comunidade microbiana para melhorar o desempenho ou fitness do hospedeiro ou a seleção de uma característica específica do hospedeiro (fenótipo), permitindo uma seleção indireta das funções do microbioma (Figura 8) (EPSTEIN *et al.*, 2019).

Figura 8: Esquema mostrando maneiras como a engenharia microbiana pode ser aplicada em benefício da saúde dos corais: a) seleção direta, com micro-organismos benéficos selecionados em laboratório e inoculados em colônias de coral a fim de melhorar sua resistência frente à variações ambientais e b) seleção indireta, onde colônias que demostrarem maior resistência à um estresse termal aplicado intencionalmente servem como base para criar um inóculo composto pela microbiota associada a seu tecido e muco.



Adaptado de: EPSTEIN *et al.*, 2019.

Diferentes métodos de entrega de consórcios microbianos benéficos vêm sendo testados, incluindo o uso de rotíferos que previamente acumulavam os micro-organismos em seu trato digestivo e depois eram ativamente ingeridos por corais da espécie *Pocillopora damicornis* (ASSIS *et al.*, 2020) e através de inóculo direto de quatro culturas bacterianas puras em experimento controlado usando aquários, também com a espécie *P. damicornis* (ZHANG *et al.*, 2021). Uma outra possibilidade em pauta é o uso de probióticos baseados em Symbiodiniaceae para melhorar a recuperação durante ou pós-branqueamento. Curiosamente, os micro-organismos inoculados não foram detectados nos tecidos do coral após o fim do experimento, sugerindo que tenham sido usados

como suplemento nutricional ao invés de formarem novas associações simbióticas (MORGANS *et al.*, 2020).

2.6 O branqueamento dos corais e suas consequências

Apesar da extrema relevância econômica e ecológica, os recifes de coral são hábitats criticamente ameaçados que experimentaram nos últimos 30 anos declínios globais de aproximadamente 30% sobre sua população mundial (HUGHES *et al.*, 2003). Globalmente, o fenômeno mais preocupante é o branqueamento, caracterizado pela interrupção da interação simbiótica entre os corais hospedeiros e as zooxantelas endossimbiontes (OPPEN E LOUGH; 2018), um processo geralmente causado pelo aumento da temperatura e conhecido como branqueamento termal, ou através da infecção por um patógeno (geralmente pertencente ao gênero bacteriano *Vibrio*) que atinge as zooxantelas ou o coral hospedeiro (KUSHMARO *et al.*, 1996; 1997; OPPEN E LOUGH; 2018; ROSENBERG E FALKOVITZ, 2004).

Eventos de branqueamento foram amplamente registrados pela primeira vez em 1983 e sua causa foi relacionada à ocorrência de um forte El Niño, que também é responsável pelos principais grandes eventos globais de branqueamento que vieram à seguir (EAKIN; SWEATMAN; BRAINARD, 2019; HUGHES *et al.*, 2018). O aumento da temperatura e da intensidade de radiação solar causa o branqueamento reduzindo a capacidade do sistema fotossintético nas zooxantelas para processar a luz (WARNER *et al.*, 1999). Quando as temperaturas excedem determinados limiares, a luz que entra chega a sobrecarregar o aparelho fotossintético, resultando na produção de espécies reativas de oxigênio que danificam as estruturas celulares, e como os corais não toleram altos níveis dessas moléculas tóxicas eles devem expulsar as zooxantelas para evitar danos nos tecidos (DIAZ *et al.*, 2016).

Pouco depois de relatado o branqueamento de *Oculina patagonica* pela primeira vez em 1995, e através da aplicação do postulado de Koch, foi demonstrado que, nesse caso, o agente causal era a bactéria da espécie *Vibrio shiloi* (KUSHMARO *et al.*, 2001). Esta espécie em particular produz uma superóxido dismutase extracelular (SOD) que protege o patógeno dentro do coral à 30 ºC, mas não a 16 ºC, permitindo a infecção e persistência no tecido

hospedeiro, além da produção de uma toxina peptídica extracelular que inibe a fotossíntese das algas, ocasionando o branqueamento do coral (BANIN *et al*. 2003).

Neste caso, o fenômeno é o resultado da expressão de genes de virulência pertencentes a membros da microbiota e regulados pela temperatura, que produzem um fator de adesão que permite a ligação das bactérias ao coral, além da produção de toxinas extracelulares que inibem a fotossíntese, afetando assim as zooxantelas (ROSENBERG E FALKOVITZ, 2004). Se as populações de simbiontes não forem restauradas dentro de semanas ou meses após um evento de branqueamento, é provável que haja mortalidade total ou parcial do coral afetado (HOEGH-GULDBERG *et al*., 2007).

Nas últimas duas décadas, os eventos de branqueamento foram relatados com maior frequência em grandes escalas geográficas, incluindo três grandes eventos pan-tropicais em 1998, 2010 e 2014/17 (considerado até então o mais longo, extenso, intenso e “mortal”), e o número de recifes atingidos simultaneamente aumenta a cada evento (DONNER; RICKBEIL; HERON, 2017; HUGHES *et al*., 2017; SKIRVING *et al*., 2019). Uma série de evidências científicas indica que tais eventos de branqueamento em massa estão intimamente associados com temperaturas de superfície do mar anormalmente elevadas em grande escala (AINSWORTH *et al*., 2016), já que aumentos de temperatura de apenas 1~2ºC são suficientes para desencadear eventos de branqueamento em massa, principalmente em regiões onde os corais já vivem perto de seus limites térmicos máximos (COLES E BROWN, 2003; FITT *et al*., 2001). As piores predições feitas por modelos climáticos globais apontam para um cenário onde cerca de metade dos recifes irão experimentar eventos anuais de branqueamento até 2050 (VAN HOOIDONK *et al*., 2016). Com isso, a capacidade de aclimatação dos corais pode não ser rápida o suficiente e estima-se que mais de 90% dos recifes de todo o mundo estarão em risco de degradação (GROTTOLI *et al*., 2014).

No Brasil, um dos registros mais antigos de branqueamento extenso já publicados remonta a 1998, no Parque Estadual Marinho do Parcel do Manuel Luiz - Maranhão, região com uma grande diversidade de diferentes grupos de corais, dos quais poucos membros não foram afetados, e foi associado a um aumento de ao menos 1º C em relação à média máxima mensal esperada.

(AMARAL; HUDSON; STEINER, 1998). Entretanto, dados gerados entre 1993 e 1996 e até então não publicados foram compilados e publicados, indicando um significativo evento de branqueamento infelizmente não devidamente quantificado entre 1993-1994 (CASTRO E PIRES, 1999).

Em 2003, um evento menor, mas que ocorreu simultaneamente em algumas regiões (incluindo pontos da APACC, como Maragogi e Tamandaré), foi registrado por alguns autores (FERREIRA *et al.*, 2006). A região de Abrolhos teve seus dados refentes à eventos de branqueamento ocorridos entre 1993 até 2005 revisados pela primeira em 2008, e mostrou que recifes com distâncias diferentes em relação à costa estão sujeitos à valores médios de aumento na temperatura superficial da água (>0.25° C próximo a costa e >0.5° C para distâncias maiores) e tempo de persistência diferentes (LEÃO; KIKUCHI; OLIVEIRA, 2008). A situação dos recifes da região foi novamente avaliada em 2010, e além dos registros de branqueamento, foi possível notar também um aumento tanto no número de doenças observadas quanto de espécies de coral afetadas (KRUG *et al.*, 2012; LEÃO *et al.*, 2010; MIRANDA; CRUZ; LEÃO, 2013).

Dados sobre o evento de 2010, mas dessa vez na região Nordeste, foram publicados em 2016, e relatou a sobrevivência da maior parte das espécies atingidas após quatro anos (DIAS E GONDIM, 2016). Em todos os casos, o El Niño parece ser o principal gatilho, mas geralmente outros fatores locais atuam de forma sinérgica no surgimento do branqueamento (LEÃO *et al.*, 2016). O grande evento global de branqueamento de 2014-2017 serviu de gatilho para que diversos autores reforçassem ou propusessem novos pontos de vista em relação aos recifes de coral brasileiros, que passaram a ser caracterizados como refúgio natural: a mortalidade entre os corais durante esses eventos era muito baixa (cerca de 3%, muito menor do que a média global); a turbidez característica ao qual os corais brasileiros estão submetidos parece fornecer uma proteção principalmente nos recifes costeiros; uma maior tolerância ao aporte de nutrientes e associações simbióticas mais flexíveis entre zooxantelas e hospedeiros (MIES *et al.*, 2020; TEIXEIRA *et al.*, 2019).

Casos de branqueamento pontuais geralmente surgem da ação de diversos estressores locais geralmente decorrentes de ações antrópicas, como a poluição, a pesca, o turismo e a descarga de esgoto em ambientes costeiros, ou eventos climáticos que afetam a estabilidade do ambiente, como furacões e

enchentes. A presença de rios que deságuam nos recifes de coral constitui outra importante fonte de estresse, pois além de representarem uma das principais fontes de sedimento, nutrientes, e água doce, geralmente transportam uma série de componentes de origem antrópica, incluindo xenobióticos, como herbicidas e pesticidas oriundos de práticas agrícolas que ocorrem próximo a margem, e esgoto não-tratado despejado como consequência da urbanização sem planejamento (FABRICIUS, 2005; PIRES *et al.*, 2015). Na região Nordeste do Brasil, por exemplo, o cultivo de cana-de-açucar, o aumento na sedimentação e o desenvolvimento urbano costeiro estão entre os principais problemas relacionados ao adoecimento dos recifes de coral costeiros (LEÃO *et al.*, 2016).

A susceptibilidade ao branqueamento varia entre as espécies, profundidade e região, e é influenciada pela morfologia do coral, pela resposta fisiológica tanto do animal quanto dos endossimbiontes e pelo tipo de endossimbiontes abrigados pelo coral (SUGGETT E SMITH, 2020). Já a resiliência ao branqueamento é determinada pelo resultado de três aspectos fundamentais: resistência ao branqueamento, capacidade de sobreviver ao estado branqueado (tolerância) e taxa de recuperação do recife após mortalidade de corais (OPPEN E LOUGH; 2018). Fatores intrínsecos à resistência incluem: tipos de zooxantelas associadas, composição da comunidade microbiana, níveis de aminoácidos semelhantes a micosporina e características genéticas do hospedeiro. Mesmo que sobrevivam, as colônias branqueadas geralmente sofrem com severas consequências, como: uma taxa de reprodução muito menor do que a dos corais que não têm branqueamento; crescimento reduzido, provavelmente devido aos efeitos combinados do estresse e à redução da oferta de energia após a diminuição das densidades de zooxantelas; imunidade reduzida a patógenos, tornando-os mais suscetíveis à doença (DUNN *et al.*, 2012; GROTTOLI *et al.*, 2004).

Durante o processo de branqueamento, também ocorrem mudanças significativas na fisiologia do coral, o que acaba afetando também seu perfil lipídico. O conjunto de moléculas lipídicas em um sistema biológico é definido como lipidoma, e o campo de estudo do espectro lipídico e a determinação de seu papel biológico é denominado lipidômica (WENK, 2005). Por muito tempo, a medição integral dessas moléculas (o nível de lipídios comuns, o conteúdo das principais classes de lipídios e a composição dos ácidos graxos) foi uma das

principais abordagens metodológicas para estudar a eficácia de diversos parâmetros associados aos corais, incluindo aspectos reprodutivos, aquisição de nutrientes de sua relação simbiótica e recuperação frente à danos estruturais e eventos de branqueamento (SIKORSKAYA E IMBS, 2020).

Com o avanço nas tecnologias de análise e com a preocupação crescente com os corais, hoje sabe-se que as alterações causadas pelo processo de branqueamento são refletidas também no perfil lipídico das zooxantelas. Por exemplo, estudos mostraram que diferentes tipos de simbiontes reagem de forma diferente ao estresse termal, o que afeta o perfil de sua composição lipídica (RODRIGUES; GROTTOLI; PEASE, 2008; YAMASHIRO; OKU; ONAGA, 2005). A composição lipídica e de ácidos graxos de culturas de zooxantelas de *Symbiodinium goreauii* do clado C1 e *Symbiodinium* sp. do clado D1 foi estudada comparativamente em diferentes temperaturas, e os resultados indicam que a exposição de longo prazo a uma temperatura elevada levou a uma diminuição na quantidade relativa de C18 PUFA em zooxantelas lipídicas de ambos os tipos, mas o limiar térmico visível de mudanças em lipídios foi menor para o clado C1 (KNEELAND *et al.*, 2013).

Nos recifes severamente danificados, a recuperação é dependente da quantidade da reserva energética do coral (LEVAS *et al.*, 2013) e sua capacidade de se alimentar heterotroficamente (GROTTOLI *et al.*, 2006; HOULBRÈQUE E FERRIER-PAGÈS, 2009), além da chegada de larvas de coral adequadas que sobreviveram ao evento de branqueamento em outros lugares, bem como a sua bem sucedida colonização, sobrevivência e crescimento (CONNELL *et al.*, 1997). Mesmo supondo que as condições favoreçam o recrutamento, o processo de recuperação está sujeito a oferta de larvas e aos muitos riscos que enfrentam os corais jovens, como predação, sufocamento por sedimentos ou algas, crescimento excessivo por outros corais, entre outros (GILMOUR, 1999).

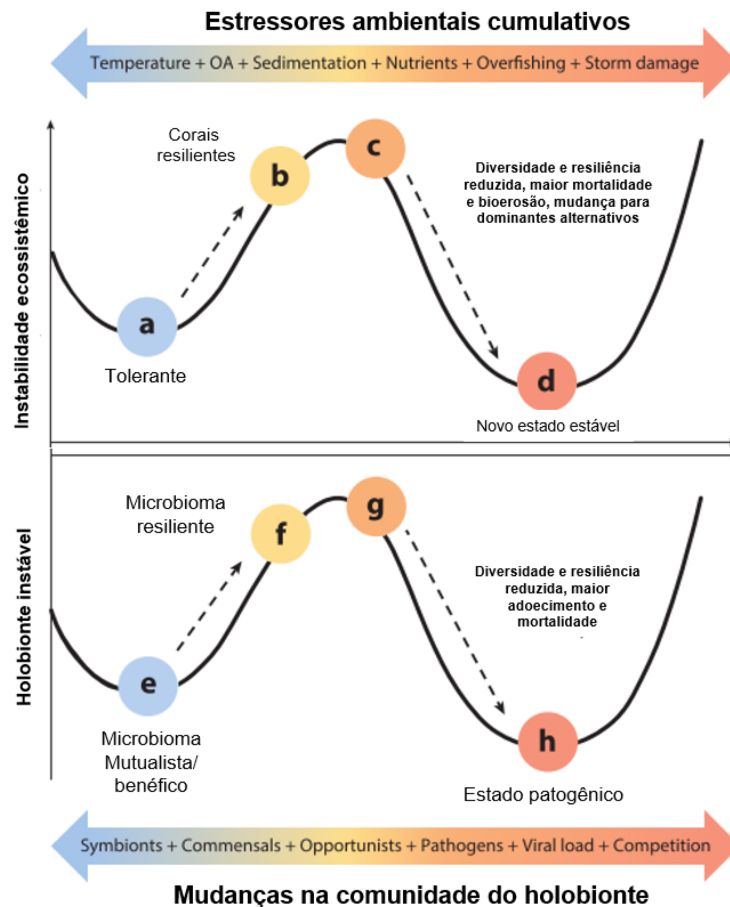
A qualidade da água é outro fator que pode afetar, entre outras coisas, o sucesso do processo de fertilização dos corais, potencialmente colocando severas limitações na capacidade de recuperação das comunidades de coral após a mortalidade induzida pelo branqueamento (NEGRI E HEYWARD, 2000). Isso se dá, por exemplo, através do estimulo no crescimento de algas, o que por sua vez pode reduzir o recrutamento de larvas e aumentar a competição por espaço no recife (MCCLANAHAN *et al.*, 2002).

2.7 A microbiota associada aos corais e sua relação com o branqueamento

Tradicionalmente, uma doença é definida como qualquer deficiência que interfira ou modifique o desempenho das funções normais de um organismo, incluindo respostas a fatores ambientais, agentes infecciosos, defeitos inerentes ou congênitos ou diferentes combinações desses fatores (FORRESTER, 2007). Infelizmente, devido os poucos sinais macroscópicos produzidos pelos corais para indicar um funcionamento fisiológico deficiente, é surpreendentemente difícil identificar doenças afetando corais (WOODLEY *et al.*, 2015). O futuro estado dos recifes de corais será determinado pela capacidade do ecossistema de responder as crescentes perturbações ambientais, permanecendo um sistema dominado por corais (HUGHES *et al.*, 2003; NYSTROM E FOLKE, 2001).

Nesses ecossistemas, a comunidade microbiana é extremamente sensível às mudanças ambientais, e após a perturbação, permanecem alteradas e funcionalmente diferentes por longos períodos de tempo. De forma um tanto genérica, um microbioma considerado saudável (eubiose) representa uma comunidade diversificada e altamente estruturada, fundamental para a capacidade de um hospedeiro de sobreviver a mudanças nas condições ambientais (MUELLER E SACHS, 2015). Quando exposta a perturbações ambientais persistentes, a simbiose hospedeiro-microbioma pode ficar comprometida, levando a uma mudança imprevisível na estrutura da comunidade microbiana, denominada disbiose (RODER *et al.*, 2014). As atividades desempenhadas pela microbiota associada pode mitigar ou mesmo prevenir as respostas de branqueamento, suportando o equilíbrio interno entre o hospedeiro e Symbiodiniaceae e mantendo taxas estáveis de fotossíntese (PEIXOTO *et al.*, 2017). Por outro lado, uma disbiose microbiana pode interromper a estabilidade da atividade fotossintética e a troca de nutrientes perfeitamente ajustada entre corais e seus parceiros dinoflagelados (RÄDECKER *et al.*, 2021). Curiosamente, esses estados disbióticos e as mudanças resultantes na rede metabólica do holobionte podem ocorrer muito rapidamente, até mesmo antes dos primeiros sinais visuais de branqueamento (Figura 9) (BOURNE *et al.*, 2008).

Figura 9: a) Mudanças macroscópicas relacionadas aos corais de acordo com impactos ambientais e b) alterações na microbiota como resposta a estresses, com mudanças na natureza das interações ecológicas no holobionte.



Adaptado de: BOURNE *et al.*, 2016.

Apesar de toda a problemática associada, o processo de disbiose entre os corais e sua microbiota abre a possibilidade de aquisição de novas capacidades adaptativas às mudanças ambientais. Tal processo adaptativo ocorre desde que o hospedeiro coral possa rapidamente voltar a um estado saudável, recuperando uma assembléia de Symbiodiniaceae mais adequada, seja através do recrutamento de espécies exógenas do ambiente circundante (*shifting*) ou ajustando a abundância relativa de espécies nativas encontradas em baixa abundância (*shuffling*) (BAKER, 2003; HUME *et al.*, 2016). Ambos os processos podem refletir em estados transitórios de branqueamento, esperando que o holobionte se adapte e se recupere para um estado saudável. Nesse contexto, os demais membros da microbiota atuariam como um amortecedor

temporário das consequências desse estado disbiótico, mantendo funções essenciais ao enfrentar perturbações de curta duração (MATTHEWS *et al.*, 2020).

De maneira geral, hoje o branqueamento é visto microbiologicamente como uma alteração na composição da microbiota normal do hospedeiro, que passa para um estado alternativo onde grupos funcionalmente importantes tem suas populações reduzidas (BOURNE *et al.*, 2008; 2016; LITTMAN *et al.*, 2011). Essas mudanças na comunidade microbiana em resposta aos distúrbios são propostas como interessantes para monitoramento, uma vez que podem preceder os sinais visuais de branqueamento e necrose do tecido (GLASL; WEBSTER; BOURNE, 2017). Além disso, vários estudos relataram mudanças relacionadas ao metabolismo do microbioma, como uma transição de comportamentos predominantemente autotróficos para heterotróficos, juntamente com mudanças nos perfis de metabólitos secundários, metabolismo de enxofre e nitrogênio, motilidade e quimiotaxia, e utilização de ácidos graxos e lipídios (THURBER *et al.*, 2009).

Comparações entre metagenomas de corais saudáveis e branqueados submetidos a estresses ambientais, como aumento da temperatura superficial e acidez da água, demonstraram alterações na abundância de membros relacionados a ciclagem de enxofre, fosfato, carboidratos e ácidos graxos dentro do coral, por exemplo (LITTMAN *et al.*, 2011; WEBSTER *et al.*, 2016).

As características da comunidade microbiana associada aos corais branqueados frequentemente incluem um aumento na diversidade geral (incremento na riqueza), na variabilidade (menos uniformidade) e menor estabilidade (BOILARD et al., 2020; POOTAKHAM et al., 2018). É comum observar uma redução em simbiontes bacterianos mutualísticos importantes, como as espécies de *Endozoicomonas*, frequentemente dominantes e supostamente capazes de prevenir a disfunção mitocondrial e promover a gliconeogênese, auxiliar no ciclo de enxofre e proteger o coral de patógenos de branqueamento, potencialmente através da produção de metabólitos relacionados ao *quorum sensing* ou compostos antimicrobianos (DING *et al.*, 2016).

Há, ainda, um aumento de bactérias oportunistas e patógenos potenciais, como espécies do gênero *Vibrio*, associado também a um aumento em genes

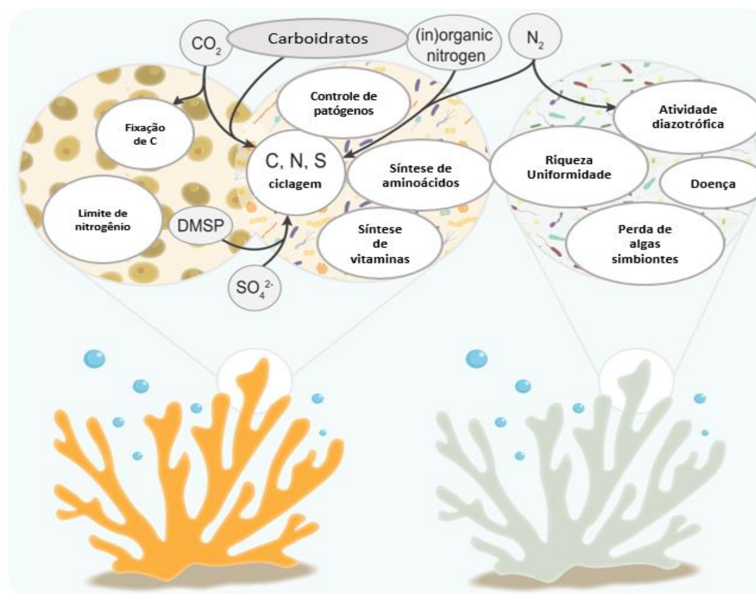
relacionados a fatores de virulência (MORROW; MULLER; LESSER, 2018), embora já tenha sido demonstrado que alguns corais, como *Porites lobata* (HADAIDI *et al.*, 2017) e *Orbicella faveolata* (TRACY *et al.*, 2015), foram capazes de manter microbiomas estáveis durante eventos de branqueamento. Sob temperaturas elevadas, alguns membros desse gênero foram mais eficazes ao usar substratos-chave encontrados no muco do coral (por exemplo, α-D-glucopiranosidase), permitindo-lhes superar as bactérias comensais do coral (FRYDENBORG *et al.*, 2014).

De forma geral, foram propostos três estágios de sucessão de disbiose holobionte para definir o processo de branqueamento dos corais: 1) Disbiose adaptativa sem reestruturação da comunidade de *Symbiodiniaceae*, onde um aumento na proporção de cianobactérias e plastídios nas comunidades microbianas sob estresse atua como um mecanismo potencial para compensar a redução da atividade fotossintética relacionadas a uma menor abundância de Symbiodiniaceae, geralmente associada também a uma proliferação de bactérias fixadoras de nitrogênio (diazotróficas) (ROSENBERG; ZILBER-ROSENBERG, 2011); 2) Disbiose adaptativa com reestruturação da comunidade de Symbiodiniaceae com ou sem branqueamento temporário, envolvendo a reestruturação simbionte bacteriana e dinoflagelada, como por exemplo um aumento nas cianobactérias durante o branqueamento do coral, capaz de contribuir na manutenção da estabilidade da atividade fotossintética a fim de atender aos requisitos nutricionais do coral durante o branqueamento transicional (ROSENBERG *et al.*, 2007); 3) Disbiose adaptiva/traumática com perda de Symbiodiniaceae e invasão de grupos oportunistas, levando à morte do holobionte, nos casos onde a perda do simbionte dinoflagelado é permanente e o microbioma não pode mais manter a resistência contra invasões de patógenos oportunistas (por exemplo, devido a uma diminuição na produção de substâncias antimicrobianas), com consequente quebra irreversível no equilíbrio do coral hospedeiro (BOILARD, *et al.*, 2020; MAO-JONES *et al.*, 2010).

O processo de diazotrofia (ou seja, bactérias e arquéias que fixam o gás nitrogênio atmosférico em uma forma mais utilizável, como amônio) suporta evidências que sugerem que existe uma ligação crítica entre o branqueamento do coral e a disponibilidade de nitrogênio ambiental (Figura 10). De fato, um aumento na aquisição de nitrogênio por meio da heterotrofia contribuiu para

reduzir a fotoinibição e o tempo de recuperação pós-branqueamento (FERRIER-PAGÈS *et al.*, 2010; GROTTOLI *et al.*, 2006; HOOGENBOOM *et al.*, 2012). Entretanto, níveis mais altos de nitrogênio fornecidos por bactérias diazotróficas provavelmente liberariam as zooxantelas de *Symbiodinium* completamente do crescimento N-limitado e causariam altas taxas de divisão celular e translocação reduzida de fotossintatos para o coral, contribuindo potencialmente para a resposta ao branqueamento (SUESCÚN-BOLÍVAR; TRAVERSE; THOMÉ, 2016).

Figura 10: Variações ocorridas na microbiota e nos ciclos biogeoquímicos entre corais saudáveis e branqueados.



Adaptado de: BAKER e GLYNN, 2008.

Um dos efeitos do estresse térmico sobre os corais é a mudança na composição, volume e viscosidade da camada mucosa: em alguns casos há o aumento de matéria orgânica e quantidade de muco secretada pelo coral (NIGGL *et al.*, 2009), associada ou não a uma redução em sua viscosidade, que também pode estar correlacionado com uma mudança na comunidade microbiana associada. Por exemplo, uma queda na abundância relativa de Gammaproteobacteria foi associada a uma mudança no conteúdo de açúcares de fucose e manose, enquanto um aumento em cianobactérias foi correlacionado com mudanças nos conteúdos de arabinose e xilose na espécie de coral *Acropora muricata* (LEE *et al.*, 2016). De maneira inesperada, essas

mudanças estruturais na comunidade tiveram início antes de que sinais visíveis de branqueamento fossem observados nas colônias, sugerindo que a composição do muco pode ser usada como um possível bioindicador de condições de estresse do coral.

O branqueamento pode interferir ainda na remoção de sedimentos, através da redução do número de mucócitos epiteliais e na quantidade de muco nas camadas gastrodérmicas mais profundas (FREEMAN *et al.*, 2001). O aumento do acúmulo de sedimentos em corais branqueados pode levar à formação de placas de muco, necrose e, em última instância, mortalidade. Todas essas mudanças relacionadas aos efeitos do estresse termal sobre a camada mucosa podem levar a um ambiente que é menos estável e mais atraente para micro-organismos oportunistas e patógenos do que simbiontes benéficos (MAO-JONES *et al.*, 2010).

2.8 Referências Bibliográficas

AINSWORTH, T. D.; FORDYCE, A. J.; CAMP, E. F. The other microeukaryotes of the coral reef microbiome. **Trends in Microbiology**, v. 25, n. 12, p. 980–991, 2017.

AINSWORTH, T. D.; RENZI, J. J.; SILLIMAN, B. R. Positive Interactions in the coral macro and microbiome. **Trends in Microbiology**, v. 28, n. 8, p. 602–604, 2020.

AINSWORTH, T. D.; THURBER, R. V.; GATES, R. D. The future of coral reefs: a microbial perspective. **Trends in Ecology and Evolution**, v. 25, n. 4, p. 233–240, 2010.

ALTIERI, A. H. *et al*. Tropical dead zones and mass mortalities on coral reefs. **Proceedings of the National Academy of Sciences of the United States of America**, v. 114, n. 14, p. 3660–3665, 2017.

ARTHUR, C.; BAKER, J. E.; BAMFORD, H. A. Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008, University of Washington Tacoma, Tacoma, WA, USA. Group, 2009.

ASSIS, J. M. *et al*. Delivering beneficial microorganisms for corals: rotifers as carriers of probiotic bacteria. **Frontiers in Microbiology**, v. 11, n. December, p. 1–9, 2020.

AXWORTHY, J. B.; PADILLA-GAMIÑO, J. L. Microplastics ingestion and heterotrophy in thermally stressed corals. **Scientific Reports**, v. 9, n. 1, p. 1–8, 2019.

BAKER, A. C. Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of Symbiodinium. **Annual Review of Ecology, Evolution, and Systematics**, v. 34, p. 661–689, 2003.

BANIN, E. *et al*. Superoxide dismutase is a virulence factor produced by the coral bleaching pathogen *Vibrio shiloi*. *Current Microbiology*, v. 46, n. 6, p. 418–422, 2003.

Bednarz, V. *et al.* The Invisible Threat: How nicroplastics endanger corals. **Frontiers Young Minds**. 9:574637, 2021.

BERNASCONI, R. *et al*. Establishment of coral-bacteria symbioses reveal changes in the core bacterial community with host ontogeny. **Frontiers in Microbiology**, v. 10, n. July, p. 1–16, 2019.

BLACKALL, L. L.; WILSON, B.; VAN OPPEN, M. J. H. Coral-the world’s most diverse symbiotic ecosystem. **Molecular Ecology**, v. 24, n. 21, p. 5330–5347, 2015.

BOILARD, A. *et al*. Defining coral bleaching as a microbial dysbiosis within the coral holobiont. **Microorganisms**, v. 8, n. 11, p. 1–26, 2020.

BOURNE, D. *et al*. Changes in coral-associated microbial communities during a bleaching event. **The ISME journal**, v. 2, n. 4, p. 350–363, 2008.

BOURNE, D. G.; MORROW, K. M.; WEBSTER, N. S. Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. **Annual Review of Microbiology**, v. 70, n. 1, p. annurev-micro-102215-095440, 2016.

BOURNE, D. G.; MUNN, C. B. Diversity of bacteria associated with the coral *Pocillopora damicornis* from the Great Barrier Reef. **Environmental Microbiology**, v. 7, n. 8, p. 1162–1174, 2005.

BROWN, B. E.; BYTHELL, J. C. Perspectives on mucus secretion in reef corals. **Marine Ecology Progress Series**, v. 296, p. 291–309, 2005.

BUDD, A.F.; GUZMÁN, H.M. *Siderastrea glynni*, a new species of scleractinian coral (Cnidaria: Anthozoa) from the eastern Pacific. **Proceedings of the Biological Society of Washington.** 107:591–599. 1994.

CARLOS, C.; TORRES, T. T.; OTTOBONI, L. M. M. Bacterial communities and species-specific associations with the mucus of Brazilian coral species. **Scientific Reports**, v. 3, n. 1964, p. 1–7, 2013.

CASTRO, C. B.; PIRES, D. O. A bleaching event on a Brazilian coral reef. **Revista Brasileira de Oceanografia**, v. 47, n. 1, p. 87–90, 1999.

CASTRO, C. B.; PIRES, D. O. Brazilian coral reefs: What we already know and what is still missing. **Bulletin of Marine Science**, v. 69, n. 2, p. 357–371, 2001.

CERTNER, R. H.; VOLLMER, S. V. Inhibiting bacterial quorum sensing arrests coral disease development and disease-associated microbes. **Environmental Microbiology**, v. 20, n. 2, p. 645–657, 2018.

CORDEIRO, R.T.S;NEVES, B.M; ROSA-FILHO, J.S; PÉREZ, C.D. Mesophotic coral ecosystems occur offshore and north of the Amazon River. **Bulletin Marine Science**, 91(4): 491-510. 2015.

COLES, S. L.; BROWN, B. E. Coral bleaching - Capacity for acclimatization and adaptation. **Advances in Marine Biology**, v. 46, p. 183–223, 2003.

COLÍN GARCÍA, N. A. *et al*. Comparative molecular and morphological variation analysis of *Siderastrea* (Anthozoa, Scleractinia) reveals the presence of *Siderastrea stellata* in the Gulf of Mexico. **Biological Bulletin**, v. 232, n. 1, p. 58–70, 2017.

CORREIA, M.D. Scleractinian corals (Cnidaria: Anthozoa) from reef ecosystems on the Alagoas coast, Brazil. **Journal of the Marine Biological Association of the United Kingdom**, v.91, p. 659-668, 2011.

COSTA, R. *et al*. Diversity and antagonistic potential of *Pseudomonas* spp. associated to the rhizosphere of maize grown in a subtropical organic farm. **Soil Biology and Biochemistry**, v. 38, n. 8, p. 2434–2447, 2006.

COUCH, C. S. *et al*. Mass coral bleaching due to unprecedented marine heatwave in Papahānaumokuākea Marine National Monument (Northwestern Hawaiian Islands). **PLoS ONE**, v. 12, n. 9, 2017.

DALY, M. *et al*. The phylum Cnidaria: A review of phylogenetic patterns and diversity 300 years after Linnaeus. **Zootaxa**, n. 1668, p. 127–182, 2007.

DAMJANOVIC, K. *et al*. The contribution of microbial biotechnology to mitigating coral reef degradation. **Microbial Biotechnology**, v. 10, n. 5, p. 1236–1243, 2017.

DE MAHIQUES, M. M. *et al*. Insights on the evolution of the living Great Amazon Reef System, equatorial West Atlantic. **Scientific Reports**, v. 9, n. 1, p. 1–8, 2019.

DENNIS, M. M. Diseases of Coral. **Journal of Wildlife Diseases**. v. 54, 2018.

DIAS, T. L. P.; GONDIM, A. I. Bleaching in scleractinians, hydrocorals, and octocorals during thermal stress in a northeastern Brazilian reef. **Marine Biodiversity**, v. 46, n. 1, p. 303–307, 2016.

DIAZ, J. M. *et al*. Species-specific control of external superoxide levels by the coral holobiont during a natural bleaching event. **Nature Communications**, v. 7, n. May, p. 1–10, 2016.

DING, J. Y. *et al*. Genomic insight into the host-endosymbiont relationship of *Endozoicomonas montiporae* CL-33T with its coral host. **Frontiers in Microbiology**, v. 7, p. 1-15, 2016.

DONNER, S. D.; RICKBEIL, G. J. M.; HERON, S. F. A new, high-resolution global mass coral bleaching database. **PLoS ONE**, v. 12, n. 4, p. 1–17, 2017.

DUCKLOW, H. W.; MITCHELL, R. Composition of mucus released by coral reef coelenterates. **Limnology and Oceanography**, v. 24, n. 4, p. 706–714, 1979.

DUNN, S. R. *et al*. Thermal stress promotes host mitochondrial degradation in symbiotic cnidarians: Are the batteries of the reef going to run out? **PLoS ONE**, v. 7, n. 7, p. 1-14, 2012.

EAKIN, C. M.; SWEATMAN, H. P. A.; BRAINARD, R. E. The 2014–2017 global-scale coral bleaching event: insights and impacts. **Coral Reefs**, v. 38, n. 4, p. 539–545, 2019.

ELAHWANY, A. M. D. et al. Phylogenetic diversity and antimicrobial activity of marine bacteria associated with the soft coral *Sarcophyton glaucum*. **Journal of Basic Microbiology**, v. 55, n. 1, p. 2–10, 2015.

ENRÍQUEZ, S.; MÉNDEZ, E. R.; IGLESIAS-PRIETO, R. Multiple scattering on coral skeletons enhances light absorption by symbiotic algae. **Limnology and Oceanography**, v. 50, n. 4, p. 1025–1032, 2005.

EPSTEIN, H. E. *et al*. Microbiome engineering: enhancing climate resilience in corals. **Frontiers in Ecology and the Environment**, v. 17, n. 2, p. 100–108, 2019.

FABRICIUS, K. E. Effects of terrestrial runoff on the ecology of corals and coral reefs: Review and synthesis. **Marine Pollution Bulletin**, v. 50, n. 2, p. 125–146, 2005.

FENG, L. *et al*. Investigating the composition and distribution of microplastics surface biofilms in coral areas. **Chemosphere**, v. 252, p. 1-14, 2020.

FERRARIO, F. *et al*. The effectiveness of coral reefs for coastal hazard risk reduction and adaptation. **Nature Communications**, v. 5, n. May, p. 1–9, 2014.

FERREIRA, B. P. *et al*. The status of Coral Reefs in Brazil. **Proceedings of the 10th International Coral Reef Symposium**, v. 1015, n. November 2016, p. 1011–1015, 2006.

FERREIRA, B. P.; MAIDA, M. Monitoramento dos Recifes de Coral do Brasil: situação e perspectivas. **Ministério do Meio Ambiente**, 2006.

FERREIRA, B. P. *et al*. **Interações entre a pesca artesanal e o turismo em Tamandaré, Costa dos Corais**. II Congresso sobre Planejamento e Gestão das Zonas Costeiras dos Países de Expressão Portuguesa, 2003.

FERRER, L. M.; SZMANT, A. M. Nutrient regeneration by the endolithic community in coral skeletons. **Proceedings of the 6th International Coral Reef Symposium**, p. 1-4, 1988.

FERRIER-PAGÈS, C. *et al.* Experimental assessment of the feeding effort of three scleractinian coral species during a thermal stress: Effect on the rates of photosynthesis. **Journal of Experimental Marine Biology and Ecology**, v. 390, n. 2, p. 118–124, 2010.

FITT, W. K. *et al*. Coral bleaching: Interpretation of thermal tolerance limits and thermal thresholds in tropical corals. **Coral Reefs**, v. 20, n. 1, p. 51–65, 2001.

FISHER, R. *et al*. Species richness on coral reefs and the pursuit of convergent global estimates. **Current Biology**, v. 25, n. 4, p. 500–505, 2015.

FORRESTER, D. J. Essentials of Disease in Wild Animals. **Journal of Wildlife Diseases**, 2007.

FORSMAN, Z. H. *et al*. An ITS region phylogeny of *Siderastrea* (Cnidaria: Anthozoa): Is *S. glynni* endangered or introduced? **Coral Reefs**, v. 24, n. 2, p. 343–347, 2005.

FRAGOSO ADOS SANTOS, H. *et al*. Impact of oil spills on coral reefs can be reduced by bioremediation using probiotic microbiota. **Scientific Reports**, v. 5, p. 1–11, 2015.

FREEMAN, R. K. *et al*. Coral bleaching: Interpretation of thermal tolerance limits and thermal thresholds in tropical corals. **Coral Reefs**, v. 20, n.1, p. 51-65, 2001.

FRYDENBORG, B. R. *et al*. Temperature-dependent inhibition of opportunistic *Vibrio* pathogens by native coral commensal bacteria. **Microbial Ecology**, v. 67, n. 2, p. 392–401, 2014.

FUHRMAN, J. A.; CAMPBELL, U. Microbial microdiversity. **Nature**, p. 410-411, 1998.

GILLES, A. *et al*. Accuracy and quality assessment of 454 GS-FLX Titanium pyrosequencing. **BMC Genomics**, v. 12, p. 2-11, 2011.

GILMOUR, J. Experimental investigation into the effects of suspended sediment on fertilisation, larval survival and settlement in a scleractinian coral. **Marine Biology**, v. 135, n. 3, p. 451–462, 1999.

GLASL, B.; HERNDL, G. J.; FRADE, P. R. The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. **ISME Journal**, v. 10, n. 9, p. 2280–2292, 2016.

GLASL, B.; WEBSTER, N. S.; BOURNE, D. G. Microbial indicators as a diagnostic tool for assessing water quality and climate stress in coral reef ecosystems. **Marine Biology**, v. 164, n. 4, p. 91, 2017.

GRAHAM, N. A. J. *et al*. Predicting climate-driven regime shifts versus rebound potential in coral reefs. Nature, v. 518, n. 7537, p. 94–97, 2015.

GOLBERG, K. *et al*. Characterization of Quorum Sensing Signals in Coral-Associated Bacteria. **Microbial Ecology**, 2011.

GROTTOLI, A. G.; RODRIGUES, L. J.; PALARDY, J. E. Heterotrophic plasticity and resilience in bleached corals. **Nature**, v. 440, n. 7088, p. 1186–1189, 2006.

HADAIDI, G. *et al*. Stable mucus-associated bacterial communities in bleached and healthy corals of *Porites lobata* from the Arabian Seas. **Scientific Reports**, v. 7, n. November 2016, p. 1–11, 2017.

HADFIELD, M. G. Biofilms and marine invertebrate larvae: what bacteria produce that larvae use to choose settlement sites. **Annual review of marine science**, v. 3, p. 453–470, 2011.

HALL, N. M. *et al*. Microplastic ingestion by scleractinian corals. **Marine Biology**, v. 162, n. 3, p. 725–732, 2015.

HANDELSMAN, J. Metagenomics: Application of Genomics to Uncultured Microorganisms. **Microbiology and Molecular Biology Reviews**, v. 68, n. 4, p. 669–685, 2004.

HARRIS, J. B. *et al*. Diversity of bacteria associated with the Caribbean coral Montastraea franksi. **Coral Reefs**, v. 20, n. 1, p. 85–91, 2001.

HARVELL, C. D. *et al*. Emerging marine diseases - Climate links and anthropogenic factors. **Science**, v. 285, n. 5433, p. 1505–1510, 1999.

HERNANDEZ-AGREDA, A.; GATES, R. D.; AINSWORTH, T. D. Defining the Core Microbiome in Corals’ Microbial Soup. **Trends in Microbiology**, v. 25, n. 2, p. 125–140, 2017.

HERNANDEZ-AGREDA, A. *et al*. Rethinking the coral microbiome: Simplicity exists within a diverse microbial biosphere. **mBio**, v. 9, n. 5, p. e00812-18, 2018.

HERNANDEZ-AGREDA, A.; LEGGAT, W.; AINSWORTH, T. D. A place for taxonomic profiling in the study of the coral prokaryotic microbiome. **FEMS Microbiology Letters**, v. 366, n. 6, 2019.

HOEGH-GULDBERG, O. *et al*. Coral reefs under rapid climate change and ocean acidification. **Science (New York, N.Y.)**, v. 318, n. 5857, p. 1737–1742, 2007.

HOOGENBOOM, M. O. *et al*. Effects of light, food availability and temperature stress on the function of photosystem II and photosystem I of coral symbionts. **PLoS ONE**, v. 7, n. 1, 2012.

HOULBRÈQUE, F.; FERRIER-PAGÈS, C. Heterotrophy in tropical scleractinian corals. **Biological Reviews**, v. 84, n. 1, p. 1–17, 2009.

HUGHES, T. P. *et al*. Climate Change , Human Impacts , and the Resilience of Coral Reefs. **Science**, v. 301, n. August, p. 929–934, 2003.

HUGHES, T. P. *et al*. Global warming and recurrent mass bleaching of corals. **Nature**, v. 543, n. 7645, p. 373–377, 2017.

HUGHES, T. P. *et al*. Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. **Science**, v. 359, n. 6371, p. 80–83, 2018.

HUME, B. C. C. *et al*. Ancestral genetic diversity associated with the rapid spread of stress-tolerant coral symbionts in response to holocene climate change. **Proceedings of the National Academy of Sciences of the United States of America**, 2016.

ICMBIO. Plano de manejo da APA Costa dos Corais. p. 74, 2013.

JATKAR, A. A. *et al*. Measuring mucus thickness in reef corals using a technique devised for vertebrate applications. **Marine Biology**, v. 157, n. 2, p. 261–267, 2010.

KALIA, V. C. et al. Quorum sensing inhibitors as antipathogens: biotechnological applications. **Biotechnology Advances**, v. 37, n. 1, p. 68–90, 2019.

Klebbs, G. Ein kleiner Beitrag zur Kenntnis der Peridineen. **Botanische Zeitung Jahrg**. 10, 46–47 (1884).

KNEELAND, J. *et al*. Lipid biomarkers in *Symbiodinium* dinoflagellates: New indicators of thermal stress. **Coral Reefs**, v. 32, n. 4, p. 923–934, 2013.

KNOWLTON, N.; JACKSON, J. **Corals and Coral Reefs**. [s.l: s.n.] 2018.

KREDIET, C. J. *et al*. Members of native coral microbiota inhibit glycosidases and thwart colonization of coral mucus by an opportunistic pathogen. **ISME Journal**, v. 7, n. 5, p. 980–990, 2013.

KREDIET, C. J. *et al*. Coral-associated micro-organisms and their roles in promoting coral health and thwarting diseases. **Proceedings of the Royal Society, Biological Sciences**, 2013b.

KRUG, L. A. *et al*. Characterization of coral bleaching environments and their variation along the Bahia state coast, Brazil. **International Journal of Remote Sensing**, v. 33, n. 13, p. 4059–4074, 2012.

KUSHMARO, A. *et al*. Bacterial infection and coral bleaching. **Nature**, v. 380, n. 6573, p. 396, 1996.

KUSHMARO, A. *et al*. Effect of temperature on bleaching of the coral *Oculina patagonic*a by *Vibrio* AK-1. **Marine Ecology Progress Series**, v. 171, p. 131–137, 1998.

KUSHMARO, A. *et al*. *Vibrio shiloi* sp. nov., the causative agent of bleaching of the coral *Oculina patagonica*. **International Journal of Systematic and Evolutionary Microbiology**, 2001.

KVENNEFORS, E. C. E. *et al*. Regulation of bacterial communities through antimicrobial activity by the coral holobiont. **Microbial Ecology**, v. 63, n. 3, p. 605–618, 2012.

LAJEUNESSE, T. C. *et al*. Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. **Current Biology**, v. 28, n. 16, p. 2570- 2580.e6, 2018.

LAMB, J. B. *et al*. Reserves as tools for alleviating impacts of marine disease. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 371, n. 1689, 2016.

LAMB, J. B. *et al*. Plastic waste associated with disease on coral reefs. **Science**, v. 359, n. 6374, p. 26–29, 2018.

LANGE, I. D.; PERRY, C. T. Bleaching impacts on carbonate production in the Chagos Archipelago: influence of functional coral groups on carbonate budget trajectories. **Coral Reefs**, v. 38, n. 4, p. 619–624, 2019.

LAJEUNESSE, T.C. *et al*. Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. **Curr. Biol**. v. 28, p. 2570–2580, 2018.

LAZCANO-PÉREZ, F. *et al*. A purified *Palythoa* venom fraction delays sodium current inactivation in sympathetic neurons. **Toxicon**, v. 82, p. 112-116, 2014.

LE CAMPION ALSUMARD, T.; GOLUBIC, S.; HUTCHINGS, P. Microbial endoliths in skeletons of live and dead corals: *Porites lobata* (Moorea, French Polynesia). **Marine Ecology Progress Series**, v. 117, n. 1–3, p. 149–158, 1995.

LEÃO, Z. M. A. N., *et al*. Corals and Coral Reefs of Brazil. In: Jorge Cortés (**Ed.). (Org.). Latin America Coral Reefs. 1aed.Amsterdam: Elsevier Science**, 2003.

LEÃO, Z. M. A. N. *et al*. Status of eastern Brazilian coral reefs in time of climate changes. **Pan-American Journal of Aquatic Sciences**, v. 5, n. 2, p. 52–63, 2010.

LEÃO, Z. M. A. N. *et al*. Brazilian coral reefs in a period of global change: A synthesis. **Brazilian Journal of Oceanography**, v. 64, n. Special Issue 2, p. 97–116, 2016.

LEÃO, Z. M. A. N.; KIKUCHI, R. K. P. DE; OLIVEIRA, M. DE D. M. DE. Branqueamento de corais nos recifes da Bahia e sua relação com eventos de anomalias térmicas nas águas superficiais do oceano. **Biota Neotropica**, v. 8, n. 3, p. 69–82, 2008.

LEÃO, Z. M. A. N.; KIKUCHI, R. K. P.; OLIVEIRA, M. D. M. **The coral reef province of Brazil**. n. 2, v. 1, 2019.

LEITE, D. C. A. *et al*. Coral bacterial-core abundance and network complexity as proxies for anthropogenic pollution. **Frontiers in Microbiology**, v. 9, n. APR, p. 1–11, 2018.

LEE, S. T. M. *et al*. Mucus sugar content shapes the bacterial community structure in thermally stressed *Acropora muricata*. **Frontiers in Microbiology**, v. 7, n. MAR, p. 1–11, 2016.

LEITE, D. C. A. *et al*. Broadcast spawning coral *Mussismilia hispida* can vertically transfer its associated bacterial core. **Frontiers in Microbiology**, v. 8, n. February, p. 1–12, 2017.

LEMA, K. A.; WILLIS, B. L.; BOURNEB, D. G. Corals form characteristic associations with symbiotic nitrogen-fixing bacteria. **Applied and Environmental Microbiology**, v. 78, n. 9, p. 3136–3144, 2012.

LESSER, M. P. *et al*. Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. **Science**, v. 305, n. 5686, p. 997–1000, 2004.

LESSER, M. P. Coral reef bleaching and global climate change: Can corals survive the next century? **Proceedings of the National Academy of Sciences**, v. 104, n. 13, p. 5259–5260, 2007.

LI, J. *et al*. Bacterial dynamics within the mucus, tissue and skeleton of the coral Porites lutea during different seasons. **Scientific Reports**, v. 4, p. 7320, 2014.

LINS-DE-BARROS, M. M. *et al*. Archaea, bacteria, and algal plastids associated with the reef-building corals siderastrea stellata and mussismilia hispida from Búzios, South Atlantic Ocean, Brazil. **Microbial Ecology**, v. 59, n. 3, p. 523–532, 2010.

LINS-DE-BARROS, M.; PIRES, D. O. Comparison of the reproductive status of the scleractinian coral *Siderastrea stellata* throughout a gradient of 20o of latitude. **Brazilian Journal of Oceanography**, v. 55, n. 1, p. 67–69, 2007.

LITTMAN, R.; WILLIS, B. L.; BOURNE, D. G. Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. **Environmental Microbiology Reports**, v. 3, n. 6, p. 651–660, 2011.

MAGRIS, R. A.; GRECH, A.; PRESSEY, R. L. Cumulative human impacts on coral reefs: Assessing risk and management implications for brazilian coral reefs. **Diversity**, v. 10, n. 2, p. 1–14, 2018.

MAIDA, M. AND; FERREIRA, B. P. Coral Reefs of Brazil : Overview and Field Guide. **Proceedings of the 8th International Coral Reef Symposium**, vol. 1, 1997.

MAO-JONES, J. *et al*. How microbial community composition regulates coral disease development. **PLoS Biology**, v. 8, n. 3, 2010.

MARRONI, E. V.; ASMUS, M. L. Historical antecedents and local governance in the process of public policies building for coastal zone of Brazil. **Ocean and Coastal Management**, v. 76, p. 30–37, 2013.

MATTHEWS, J. L. *et al*. *Symbiodiniaceae*-bacteria interactions: rethinking metabolite exchange in reef-building corals as multi-partner metabolic networks. **Environmental Microbiology**, v. 22, n. 5, p. 1675–1687, 2020.

MCCLANAHAN, T.; POLUNIN, N.; DONE, T. Ecological states and the resilience of coral reefs. **Ecology and Society**, v. 6, n. 2, 2002.

MCFADDEN, C. S. *et al*. Phylogenomics, Origin, and Diversification of Anthozoans (Phylum Cnidaria). **Systematic biology**, v. 70, n. 4, p. 635–647, 2021.

MCKEW, B. A. *et al*. Characterization of geographically distinct bacterial communities associated with coral mucus produced by *Acropora* spp. and *Porites* spp. **Applied and Environmental Microbiology**, v. 78, n. 15, p. 5229–5237, 2012.

MENEZES, N. *et al*. Morphological variation in the atlantic genus *Siderastrea* (Anthozoa, Scleractinia). **Papeis Avulsos de Zoologia**, v. 54, n. 16, p. 199–208, 2014.

MIES, M. *et al*. South Atlantic Coral Reefs Are Major Global Warming Refugia and Less Susceptible to Bleaching. **Frontiers in Marine Science**, v. 7, n. June, p. 1–13, 2020.

MIRANDA, R. J.; CRUZ, I. C. S.; LEÃO, Z. M. A. N. Blanqueamiento de corales en el arrecife de Caramuanas (Bahía de Todos los Santos, Brasil) durante el evento El Niño 2010. **Latin American Journal of Aquatic Research**, v. 41, n. 2, p. 351–360, 2013.

MORGANS, C. A. *et al*. Symbiodiniaceae probiotics for use in bleaching recovery. **Restoration Ecology**, v. 28, n. 2, p. 282–288, 2020.

MORRI, C.; BIANCHI, C. N. Cnidarian zonation at Ilha do Sal (Arquipelago de Cabo Verde). **Beitrage zur Palaontologie**, v.20, n. 41-49, 1995.

MORROW K.M., MULLER E., LESSER M.P. How does the coral microbiome cause, respond to, or modulate the bleaching process?. In: van Oppen M., Lough J. (eds) **Coral Bleaching. Ecological Studies (Analysis and Synthesis)**, V 233, 2018.

MOUCHKA, M. E.; HEWSON, I.; HARVELL, C. D. Coral-associated bacterial assemblages: Current knowledge and the potential for climate-driven impacts. **Integrative and Comparative Biology**, v. 50, n. 4, p. 662–674, 2010.

MOURA, S. R. DE *et al*. Screening of *Bacillus* sp. isolated from coral *Siderastrea stellata* for antimicrobial activity against enteropathogenic strains of *Salmonella* and *Escherichia coli*. **Brazilian Journal of Development**, v. 7, n. 1, p. 3385–3397, 2021.

MOURA, R. L. *et al*. An extensive reef system at the Amazon River mouth. **Science Advances**, v. 2, n. 4, p. 1–12, 2016.

MUELLER, U. G.; SACHS, J. L. Engineering Microbiomes to Improve Plant and Animal Health. **Trends in Microbiology**, v. 23, n. 10, p. 606–617, 2015.

NAIR, S. V. *et al*. Macroarray analysis of coelomocyte gene expression in response to LPS in the sea urchin. Identification of unexpected immune diversity in an invertebrate. **Physiological Genomics**, v. 22, p. 33-47, 2005.

NEGRI, A. P.; HEYWARD, A. J. Inhibition of fertilization and larval metamorphosis of the coral *Acropora millepora* (Ehrenberg, 1834) by petroleum products. **Marine Pollution Bulletin**, v. 41, n. 7–12, p. 420–427, 2000.

NEVES, E. G. *et al*. Genetic variation and population structuring in two brooding coral species (*Siderastrea stellata* and *Siderastrea radians*) from Brazil. **Genetica**, v. 132, n. 3, p. 243–254, 2008.

NEVES, E. G.; DA SILVEIRA, F. L. Release of planula larvae, settlement and development of *Siderastrea stellata* Verrill, 1868 (Anthozoa, Scleractinia). **Hydrobiologia**, v. 501, p. 139-147, 2003.

NIGGL, W. *et al*. First evidence of coral bleaching stimulating organic matter release by reef corals. **Proceedings of the 11th International Coral Reef Symposium**, Ft. Lauderdale, Florida, 7-11 July 2008, Session 19. 2009.

NYSTRÖM, M.; FOLKE, C. Spatial resilience of coral reefs. **Ecosystems**, v. 4, n. 5, p. 406–417, 2001.

O'BRIEN, P. A. *et al*. Diverse coral reef invertebrates exhibit patterns of phylosymbiosis. **ISME Journal**, v. 14, n. 9, p. 2211–2222, 2020.

OPPEN, M. J. H. VAN; LOUGH, J. M. **Coral Bleaching: Patterns, Processes, Causes and Consequences**. Second Edition. Springer, 2018.

PANDOLFI, J. M. *et al*. Global trajectories of the long-term decline of coral reef ecosystems. **Science**, v. 301, n. 5635, p. 955 LP – 958, 15 ago. 2003.

PAULINO, G. V. B. *et al*. Compositional shifts in bacterial communities associated with the coral *Palythoa caribaeorum* due to anthropogenic effects. **Marine Pollution Bulletin**, v. 114, n. 2, p. 1024–1030, 2017.

PEIXOTO, R. *et al*. Beneficial Microorganisms for Corals (BMC): proposed mechanisms for coral health and resilience. **Frontiers in Microbiology**, v. 8, n. March, p. 1–16, 2017.

PEREIRA, L. B. *et al*. Diversity and antimicrobial activity of bacteria isolated from different Brazilian coral species. **FEMS Microbiology Letters,** v. 364, n. 16, p. 1–8, 2017.

PERNICE, M. *et al*. Down to the bone: the role of overlooked endolithic microbiomes in reef coral health. **ISME Journal**, v. 14, n. 2, p. 325–334, 2020.

PETROSINO, J. F. *et al*. Metagenomic pyrosequencing and microbial identification. **Clinical Chemistry**, v. 55, n. 5, p. 856–866, 2009.

PIRES, N. L. *et al*. Impacts of the urbanization process on water quality of Brazilian Savanna rivers: The case of Preto River in Formosa, Goiás state, Brazil. **International Journal of Environmental Research and Public Health**, v. 12, n. 9, p. 10671–10686, 2015.

POGOREUTZ, C. *et al*. Nitrogen fixation aligns with *nifH* abundance and expression in two coral trophic functional groups. **Frontiers in Microbiology**, v. 8, n. JUN, p. 1–7, 2017.

POLLOCK, F. J. *et al*. The urgent need for robust coral disease diagnostics. **PLoS Pathogens**, v. 7, n. 10, 2011.

POLLOCK, F. J. *et al*. Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. **Nature Communications**, v. 9, n. 1, p. 4921, 2018.

POOTAKHAM, W. *et al*. Dynamics of coral-associated microbiomes during a thermal bleaching event. **MicrobiologyOpen**, v. 7, n. 5, p. 1–16, 2018.

QUAIL, M. *et al*. A tale of three next generation sequencing platforms: comparison of Ion torrent, pacific biosciences and illumina MiSeq sequencers. **BMC Genomics**, v. 13, n. 1, p. 1, 2012.

RÄDECKER, N. *et al*. Nitrogen cycling in corals: the key to understanding holobiont functioning? **Trends in Microbiology**, v. 23, n. 8, p. 1–8, 2015.

RÄDECKER, N. *et al*. Heat stress destabilizes symbiotic nutrient cycling in corals. **Proceedings of the National Academy of Sciences of the United States of America**, v. 118, n. 5, 2021.

REIS, A. M. M. *et al*. Bacterial diversity associated with the Brazilian endemic reef coral *Mussismilia braziliensis*. **Journal of Applied Microbiology**, v. 106, n. 4, p. 1378–1387, 2009.

REOPANICHKUL, P. *et al*. Sewage impacts coral reefs at multiple levels of ecological organization. **Marine Pollution Bulletin**, v. 58, n. 9, p. 1356–1362, 2009.

RESHEF, L. *et al*. The Coral Probiotic Hypothesis. **Environmental Microbiology**, v. 8, n. 12, p. 2068–2073, 2006.

RIOJA-NIETO, R.; ÁLVAREZ-FILIP, L. Coral reef systems of the Mexican Caribbean: Status, recent trends and conservation. **Marine Pollution Bulletin**, v. 140, n. July 2018, p. 616–625, 2019.

RITCHIE, K. B. Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. **Marine Ecology Progress Series**, v. 322, p. 1–14, 2006.

ROBBINS, S. J. *et al*. A genomic view of the reef-building coral *Porites lutea* and its microbial symbionts. **Nature Microbiology**, v. 4, n. 12, p. 2090–2100, 2019.

ROBINSON, J. P. W. *et al*. Thermal stress induces persistently altered coral reef fish assemblages. **Global Change Biology**, v. 25, n. 8, p. 2739–2750, 2019.

RODER, C. *et al*. Bacterial profiling of White Plague Disease in a comparative coral species framework. **The ISME Journal**, v. 8, n. 1, p. 31–39, 2014.

RODRIGUES, L. J.; GROTTOLI, A. G.; PEASE, T. K. Lipid class composition of bleached and recovering *Porites compressa* Dana, 1846 and *Montipora capitata* Dana, 1846 corals from Hawaii. **Journal of Experimental Marine Biology and Ecology**, v. 358, n. 2, p. 136–143, 2008.

ROGERS, Y. H.; VENTER, J. C. Genomics: Massively parallel sequencing. **Nature**, v. 437, n. 7057, p. 326–327, 2005.

ROHWER, F. *et al*. Diversity and distribution of coral-associated bacteria. **Marine Ecology Progress Series**, v. 243, p. 1–10, 2002.

ROSENBERG, E. *et al*. The role of microorganisms in coral health, disease and evolution. **Nature reviews. Microbiology**, v. 5, n. 5, p. 355–362, 2007.

ROSENBERG, E.; FALKOVITZ, L. The *Vibrio shiloi*/*Oculina patagonica* model system of coral bleaching. **Annual Review of Microbiology**, v. 58, p. 143–159, 2004.

ROSENBERG, E.; ZILBER-ROSENBERG, I. Symbiosis and development: The hologenome concept. **Birth Defects Research Part C - Embryo Today: Reviews**, 2011.

ROTH, M. S. *et al*. Green fluorescent protein regulation in the coral *Acropora yongei* during photoacclimation. **Journal of Experimental Biology**, v. 213, n. 21, p. 3644–3655, 2010.

RYPIEN, K. L.; WARD, J. R.; AZAM, F. Antagonistic interactions among coral-associated bacteria. **Environmental Microbiology**, v. 12, n. 1, p. 28–39, 2010.

SALIH, A. *et al*. Fluorescent pigments in corals are photoprotective. **Nature**, v. 408, n. 6814, p. 850–853, 2000.

SANTHANAM, R. Biology of Marine Cnidarians [Phylum Cnidaria (= Coelenterata)]. **Biology and ecology of venomous marine snails**.p. 7-27, 2016.

SANTOS, M. G. *et al*. Morphological variation of *Montastrea cavernosa* and *Siderastrea stellata* (Cnidaria: Scleractinia) from the states of Maranhão, Paraíba and Pernambuco, Brasil. **Biologia Geral e Experimental**, v. 5, n. 1, p. 5–11, 2004.

SASSI, C. F. C. *et al*. Pigmentation patterns of *Siderastrea stellata* Verrill, 1868 (Cnidaria, Scleractinia) from coastal reefs in Northeastern Brazil and its relation with zooxanthellae and other microsymbionts. **Pan-American Journal of Aquatic Sciences**, v. 9, n. 3, p. 207–222, 2014.

SASSI, R. *et al*. Pigmentation changes in *Siderastrea* spp. during bleaching events in the costal reefs of northeastern Brazil. **Latin American Journal of Aquatic Research**, v. 43, n. 1, p. 176–185, 2015.

SHAVER, E. C.; SILLIMAN, B. R. Time to cash in on positive interactions for coral restoration. **PeerJ**, v. 2017, n. 6, 2017.

SIEVERT, S. M.; KIENE, R. P.; SCHULZ-VOGT, H. N. Microbes and Major Elemental Cycles. In: **Oceanography**. [s.l: s.n.]. v. 20p. 117–123.

SKIRVING, W. J. *et al*. The relentless march of mass coral bleaching: a global perspective of changing heat stress. **Coral Reefs**, v. 38, n. 4, p. 547–557, 2019.

SIKORSKAYA, T. V.; IMBS, A. B. Coral lipidomes and their changes during coral bleaching. **Russian Journal of Bioorganic Chemistry**, v. 46, n. 5, p. 643–656, 2020.

SILVA-LIMA, A. W. *et al*. Multiple symbiodinium strains are hosted by the brazilian endemic corals *Mussismilia* spp. **Microbial Ecology**, v. 70, n. 2, p. 301–310, 2015.

SPALDING, M. *et al*. Mapping the global value and distribution of coral reef tourism. **Marine Policy**, v. 82, n. January, p. 104–113, 2017.

SPANÓ, S.; LEAO, Z.; KIKUCHI, R. Diagnóstico do estado de conservação dos recifes em franja do Parque Nacional Marinho dos Abrolhos. **OLAM - Ciência & Tecnologia**, v. 8, p. 245–277, 1 jan. 2008.

Santhanam, R. **Biology and Ecology of Venomous Marine Cnidarians. Springer**.2020.

SANTOS, C. *et al.* Mapemanento de Recifes Submersos na Costa do Rio Grande do Norte, NE Brasil: Macau a Maracajaú, **Revista Brasileira de Geofísica,** 25 (Suple. 1): 27-36, 2007.

STEINER, A. Q. *et al.* Zonação de recifes emersos da Área de Proteção Ambiental Costa dos Corais, Nordeste do Brasil. **Iheringia. Série Zoologia**, v. 105, n. 2, p. 184–192, 2015.

STELLA, J. S. *et al.* Coral-associated invertebrates: Diversity, ecological importance and vulnerability to disturbance. In: **Oceanography and Marine Biology: An Annual Review**. p. 43-104, 2011.

SUESCÚN-BOLÍVAR, L. P.; TRAVERSE, G. M. I.; THOMÉ, P. E. Glycerol outflow in *Symbiodinium* under osmotic and nitrogen stress. **Marine Biology**, v. 163, n. 5, 2016.

SUGGETT, D. J.; SMITH, D. J. Coral bleaching patterns are the outcome of complex biological and environmental networking. **Global Change Biology**, v. 26, n. 1, p. 68–79, 2020.

SUN, K. *et al.* Indole diterpenoids and isocoumarin from the fungus, *Aspergillus flavus*, isolated from the prawn, Penaeus vannamei. **Marine Drugs**, v. 12, n. 7, p. 3970–3981, 2014.

SUNAGAWA, S.; WOODLEY, C. M.; MEDINA, M. Threatened corals provide underexplored microbial habitats. **PLoS ONE**, v. 5, n. 3, p. 1–7, 2010.

SWEET, M. J.; CROQUER, A.; BYTHELL, J. C. Bacterial assemblages differ between compartments within the coral holobiont. **Coral Reefs**, v. 30, n. 1, p. 39–52, 2011.

TEPLITSKI, M.; RITCHIE, K. How feasible is the biological control of coral diseases? **Trends in Ecology and Evolution**, v. 24, n. 7, p. 378–385, 2009.

THORP, J. H.; ROGERS, D. C. **Ecology and General Biology - Freshwater Invertebrates**. 2014.

THURBER, R. V. *et al.* Metagenomic analysis of stressed coral holobionts. **Environmental Microbiology**, v. 11, n. 8, p. 2148–2163, 2009.

TORDA, G. et al. Rapid adaptive responses to climate change in corals. **Nature Climate Change**, v. 7, n. 9, p. 627–636, 2017.

TOUT, J. *et al.* Variability in microbial community composition and function between different niches within a coral reef. **Microbial Ecology**, p. 1–13, 2014.

TRACY, A. M. *et al.* Persistent shifts in Caribbean coral microbiota are linked to the 2010 warm thermal anomaly. **Environmental Microbiology Reports**, v. 7, n. 3, p. 471–479, 2015.

TRIBOLLET, A. The boring microflora in modern coral reef ecosystems: A review of its roles. **Current Developments in Bioerosion**. **Anais**.2008.

ULSTRUP, K. E. *et al*. Intra-colonial variability in light acclimation of zooxanthellae in coral tissues of *Pocillopora damicornis*. **Marine Biology**, v. 149, n. 6, p. 1325–1335, 2006.

VAN HOOIDONK, R. *et al*. Local-scale projections of coral reef futures and implications of the Paris Agreement. **Scientific Reports**, v. 6, n. December, p. 1–8, 2016.

VAN OPPEN, M. J. H. *et a*l. Building coral reef resilience through assisted evolution. **Proceedings of the National Academy of Sciences of the United States of America**, v. 112, n. 8, p. 2307–2313, 2015.

VAN OPPEN, M. J. H. *et al*. Shifting paradigms in restoration of the world's coral reefs. **Global Change Biology**, v. 23, n. 9, p. 3437–3448, 2017.

VANWONTERGHEM, I.; WEBSTER, N. S. Coral reef microorganisms in a changing climate. **iScience**, v. 23, n. 4, p. 100972, 2020.

VAUGHAN, T.W.; WELLS, J.W. Revision of the suborders, families, and genera of the Scleractinia. **Geological Society of Ameria Special Papers**, 44: 343. 1943.

VERMEIJ, M. J. A. *et al*. Survival and settlement success of coral planulae: Independent and synergistic effects of macroalgae and microbes. **Oecologia**, v. 159, n. 2, p. 325–336, 2009.

VERON, J.E.N. Corals in space and time: The biogeography and evolution of the Scleractinia. **UNSW Press**, Sydney, 336p. 1995.

WANGPRASEURT, D. *et al*. In vivo microscale measurements of light and photosynthesis during coral bleaching: Evidence for the optical feedback loop? **Frontiers in Microbiology**, v. 8, 2017.

WARNER, M. E.; FITT, W. K.; SCHMIDT, G. W. Damage to photosystem II in symbiotic dinoflagellates: A determinant of coral bleaching. **Proceedings of the National Academy of Sciences**, v. 96, n. 14, p. 8007–8012, 2002.

WEAR, S. L. *et al*. Sewage pollution, declining ecosystem health, and cross-sector collaboration. **Biological Conservation**, v. 255, 2021.

WEAR, S. L.; THURBER, R. V. Sewage pollution: Mitigation is key for coral reef stewardship. **Annals of the New York Academy of Sciences**, v. 1355, n. 1, p. 15–30, 2015.

WEBSTER, N. S. *et al*. Host-associated coral reef microbes respond to the cumulative pressures of ocean warming and ocean acidification. **Nature Publishing Group**, n. JANUARY, p. 1–9, 2016.

WEISS, A.; MARTINDALE, R. C. Crustose coralline algae increased framework and diversity on ancient coral reefs. **PLoS ONE**, v. 12, n. 8, p. 1–12, 2017.

WELLS ,J.W. Scleractinia. In: Moore RC, editor. Treatise on invertebrate paleontology: coelenterata. Lawrence: **Geological Society of America and University of Kansas Press**. p. F328–443. 1956

WELSH, R. M. et al. Bacterial predation in a marine host-associated microbiome. **ISME Journal**, v. 10, n. 6, p. 1540–1544, 2016.

WENK, M. R. The emerging field of lipidomics. **Nature Reviews Drug Discovery**, v. 4, n. 7, p. 594–610, 2005.

WILKINSON, C. Status of Coral Reefs of the World : **Coral Reefs**, 2002.

WILLIAMS, A. D. *et al*. Age-related shifts in bacterial diversity in a reef coral. **PLoS ONE**, v. 10, n. 12, 2015.

WORK, T. M.; AEBY, G. S. Microbial aggregates within tissues infect a diversity of corals throughout the Indo-Pacific. **Marine Ecology Progress Series**, v. 500, p. 1–9, 2014.

YAMASHIRO, H. *et al*. Composition of lipids, fatty acids and sterols in Okinawan corals. **Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology**, v. 122, n. 4, p. 397–407, 1999.

YAMASHIRO, H.; OKU, H.; ONAGA, K. Effect of bleaching on lipid content and composition of Okinawan corals. **Fisheries Science**, v. 71, n. 2, p. 448–453, 2005.

ZHANG, Y. *et al*. Shifting the microbiome of a coral holobiont and improving host physiology by inoculation with a potentially beneficial bacterial consortium. **BMC Microbiology**, v. 21, n. 1, p. 1–14, 2021.

ZILBERBERG, C. *et al*. **Conhecendo os Recifes Brasileiros: Rede de Pesquisas Coral Vivo**. [s.l: s.n.] 2016.

ZILBER-ROSENBERG, I.; ROSENBERG, E. Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. **FEMS Microbiology Reviews**, v. 32, n. 5, p. 723–735, 2008.

3 OBJETIVOS

O objetivo geral desta tese foi investigar como a microbiota associada aos rios Santo Antônio e Manguaba varia ao longo de seus cursos até desaguarem em regiões recifais localizadas logo após suas fozes, como também avaliar, a partir de uma perspectiva microbiológica, o branqueamento dos corais em duas regiões da APA Costa dos Corais (APACC), Maragogi e Barra de Santo Antônio.

3.2 OBJETIVOS ESPECÍFICOS

3.2.1 – Analisar variações nas comunidades bacterianas ao longo do curso dos rios Santo Antônio e Manguaba, ambos pertencentes a APACC, e identificar quais grupos bacterianos estão sendo introduzidos nos recifes de corais adjacentes à cada respectiva foz;

3.2.2 – Analisar variações no perfil funcional predito das comunidades bacterianas ao longo do curso de dois rios pertencentes a APACC e inferir possíveis xenobióticos que possam estar sendo despejados no ambiente através das rotas metabólicas encontradas;

3.2.3 – Avaliar a composição bacteriana de corais saudáveis e branqueados dos recifes de coral da Barra de Santo Antônio, que sofre influência do rio Santo Antônio, e de Maragogi;

3.2.3 – Avaliar o perfil funcional putativo e o conteúdo metagenômico predito das bactérias associadas a corais saudáveis e branqueados em dois recifes de coral pertencentes a APACC.

4 CAPÍTULO 1

Bacterial community and environmental factors associated to rivers runoff and their possible impacts on coral reef conservation

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Abstract

Rivers potentially carry out important components as result of anthropogenic stressors for coral reefs. Molecular techniques are increasingly being used for monitoring biological and chemical monitoring of rivers and reefs. Here, we use PhyloChips™ to process surface water samples collected along two rivers and associated reefs in an environmental protection area in northeastern Brazil. Our results indicate that a significant part of Operational Taxonomic Units (OTUs) identified were able to survive the transition from freshwater to seawater, several of them belonging to genera implicated in human pathogenesis. The BBC:A ratio and functional prediction suggests that both study rivers are subject to fecal contamination and xenobiotics input, and that the bacterial communities were more homogeneous in these environments. We suggest that protection actions adopted for reefs should be broadly extended to the surrounding environment, and that other bacterial group (besides culturable coliforms) should be included in routine water quality monitoring.

Introduction

The relative importance of different anthropogenic pressures (e.g. overfishing, coastal development and agriculture in catchment areas) in the degradation of coral reef ecosystems has changed over time (Bellwood et al., 2019). The effects of these changes can be seen in an increase in generalized stress responses such as coral bleaching events (Sully et al., 2019). Reducing anthropogenic pressures on coral reefs is typically addressed through restricting use through zoning, a common territorial planning instrument that seeks to reconcile environmental preservation with sustainable use of shared of natural resources (Steiner et al., 2015). However, this type of action is very focused on the marine region and often does not take into account the surrounding environment.

While protecting coral reefs from over-exploitation of natural resources is important, it does not address other stressors such as climate change and pollution. Reefs close to populated coastlines are particularly vulnerable to exposure to contaminants from rivers that flow through urban areas on their way to the sea. Such rivers can have a pronounced biophysical effect on the surrounding coastline, introducing sediments, nutrients and freshwater, which contribute to changes in salinity and pH, for example (Fabricius, 2005). These rivers may also act as a source of contamination due to discharge of sewage and xenobiotics (Pires et al., 2015). Increasing urbanization in many tropical coastal areas means that pollution entering coastal ecosystems is increasing, with potentially deleterious effects on coral reefs (Wear and Thurber, 2015).

Some consequences related to pollution impact on coral reefs have already been reported, including strong links between sewage-associated human pathogens and the development of coral diseases in Caribbean and Guam, for example (Sutherland et al., 2010; Redding et al., 2013). Other studies have indicated a reduction in the native microbial community associated with corals subjected to pollution from river discharge (Leite et al., 2018). The rivers themselves also host a diverse microbial community whose structure could be influenced by spatial variability in physicochemical and biotic parameters, and which may be used as a general indicator of environmental quality (Tiquia, 2010).

One of the oldest and most commonly used proxies to asses pathogen risk in water bodies is the quantification of fecal indicator bacteria (FIB), mainly *Escherichia coli* and/or enterococci (Gerba, 2014). However, when released into the sea, *E. coli* cells are negatively exposed to a complex array of biotic and abiotic factors which trigger a viable but nonculturable state which can seriously interfere with standard culturing approaches (Neill, 2004). Though difficult to monitor, while in this “hibernation” state, these bacterial cells still maintain some metabolic activity, infective capacity and pathogenic potential. (Grimes et al., 1986; Hassard et al., 2016). Such limitations of standard monitoring technologies has lead to the development of other, more sensitive, assays such as those based on DNA microarray analysis (Lemarchand et al., 2004).

PhyloChip™ is a widely used and commercially available DNA microarray analyzer, that has been shown to be a highly effective for studying microbial communities in water samples, with the potential to specify sources of fecal contamination (Dubinsky et al., 2016). One advantage of this method compared to next generation sequencing is the replicable detection of rare taxa (as infrequent as 0.01% of microbial community). This is important as sources are diluted and degraded within microbial communities from diverse environmental backgrounds (Zhou et al., 2015). This technology already has been used for monitoring several types of environments, including airborne bacteria for bioterrorism surveillance (Brodie et al., 2007), wastewater treatment systems (Xia et al., 2010) and microbial source tracking of pathogens in coastal urban watersheds (Wu et al., 2010). In addition, with the emergence of new bioinformatics tools, data obtained from PhyloChip™ can provide even more information, including functional predictions, allowing a deeper view of the environment (Langille et al., 2013).

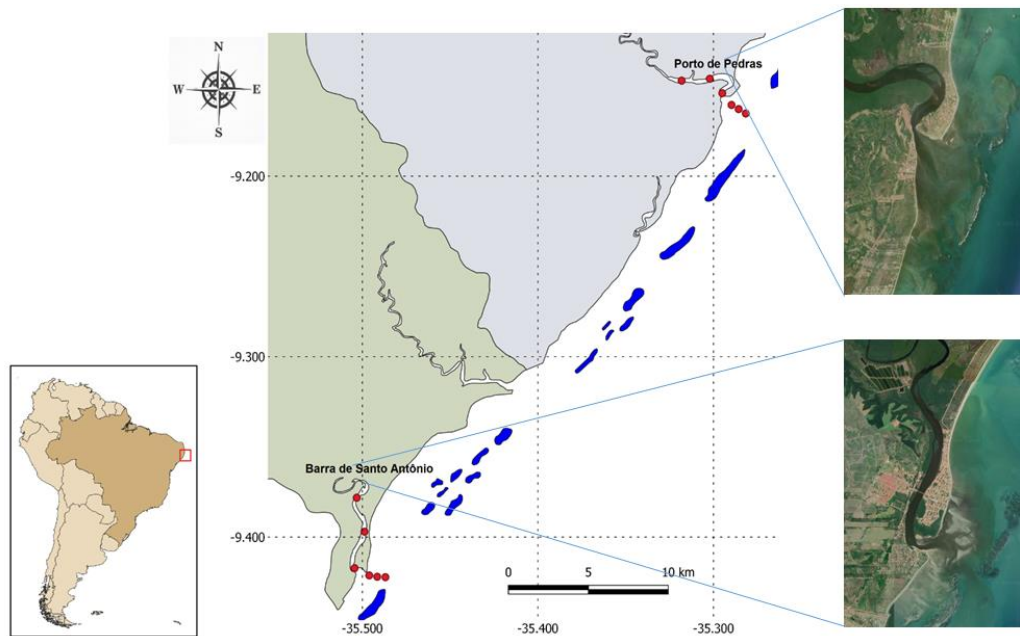
Our main objective in this study was to use the PhyloChip™ system to quantify variations in microbial communities along the course of two tropical rivers and to identify which bacterial groups are being introduced into nearby coral reefs located in *Costa dos Corais* marine protected area. We also performed monthly monitoring of several physicochemical variables known to influence coral health, as salinity, turbidity and pH, for example.

Material and methods

Study area

Our study was conducted in the *Área de Proteção Ambiental Costa dos Corais* (APACC), located in the eastern region of the State of Alagoas – Brazil. APACC is one of the largest federal marine conservation units in Brazil, covering approximately 4,135 km² and extending for about 135 km of beaches and mangroves (Ferreira and Maida, 2006). This region is heavily used for fishing and tourism and has low rates of basic sanitation, with discharge of untreated sewage directly into rivers a common occurence. Samples were collected in two sites included in the management plan of APACC: a) the Santo Antonio river, with extension of approximately 18.5 km and an estuary that divides a small town with approximately 16,000 inhabitants; b) the Manguaba river, with extension of approximately 42 km, located in the municipality of Porto de Pedras with population of approximately 8,000 inhabitants (Figure 1). According to the latest census conducted by the Instituto Brasileiro de Geografia e Estatística (IBGE, 2011), the percentage of adequate sewage is low at both points, with values of 23.4% in Barra de Santo Antônio and 4% in Porto de Pedras. Throughout its course, both rivers support several economic activities, especially agricultural, until finally flowing into a coastal region with abundant in shore reefs.

Figure 1: Map showing the collection points at each site (red) and the coral reefs (blue). The samples were collected along the river about 2 ~ 2.5 km apart, while at sea the samples were collected right after the mouth, separated from each other by 500m, until reaching the coral reefs.



Water sampling and DNA extraction to microbial community analysis

Surface water collection was carried out at morning on April 17 and 18, 2018, during low tide (when the freshwater moves further towards the ocean). At each site, surface water samples (200 mL) were collected at three points along the rivers (about 2.5 km away from each other) and three more points after the respective estuary mouth, 500 m apart, until they reached the coral reefs, totaling 12 samples (Figure 1). In this way we were able to track the variation in the community along an environmental gradient, identifying which bacterial groups are able to resist the transition between freshwater and marine environments. The sample points of Barra de Santo Antônio are denoted as *BST* and the points for Porto de Pedras as *PP*.

To isolate the total DNA of seawater and river samples we used 0.22 µm polycarbonate filter membranes with adsorbed microbial cells resulting from the filtration of 200 mL of water. These were cut into pieces before being added to PowerBead Tubes. DNA was extracted using a PowerSoil DNA Isolation Kit

(MoBio) following the manufacturer`s instructions. Finally, the DNA was resuspended in 50 mL sterile Milli-Q water and stored at -20 °C.

1.1.1 16S rDNA amplification, purification and product quantification

Following DNA extraction and quantification with Qubit (Invitrogen™), replicate PCR was performed to amplify genes encoding 16S rDNA using primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) for bacteria (Lane, 1991). Each PCR reaction contained 1×Ex Taq buffer (Takara Bio Inc., Japan), 0.025 units/μl Ex Taq polymerase (Takara Bio Inc, Otsu, Japan), 200uM dNTP mixture, 1.0μg/μl BSA, 300 nM each primer and 0.5 ng genomic DNA (gDNA) as template. Each sample was amplified in four 25μl reactions that spanned a 50–56 °C gradient in annealing temperatures to minimize PCR bias due to variable template annealing efficiencies and random priming effects (DeSantis et al., 2007). PCR conditions were: 95 °C (3 min), followed by 25 cycles 95 °C (30s), 50–56 °C (30s), 72 °C (2 min), followed by a final extension 72 °C (10 min). Amplicons from each reaction were pooled for each sample, purified with the QIAquick PCR purification kit (Qiagen, Valencia, CA), and eluted in 50μL elution buffer. Concentrated PCR product (1 μl) was quantified on a 2% agarose E-gel using the Low Range Quantitative DNA Ladder (Invitrogen, Carlsbad, CA) and the software QuantityOne™.

PhyloChip™ analysis and data obtaining

Up to 500 ng of purified PCR product from water samples was applied to each PhyloChip™ following procedures previously described (Hazen et al., 2010). Briefly, 16S rDNA amplicons were combined in a mixture of amplicons at known concentrations (spike-mix), fragmented using DNAse1 (Invitrogen™, Carlsbad, CA) and labeled with biotin according to the protocol recommended by Affymetrix Prokaryotic Arrays. The labeled products were hybridized overnight at 48° C and 60 RPM. The matrices were then washed, stained and scanned using a GeneArray Scanner (Affymetrix™, Santa Clara, CA, USA) to provide raw PhyloChip™ data in the form of fluorescent image files. Details on probe selection,

probe scoring, data acquisition, and preliminary data analysis are provided elsewhere. The data obtained from the CEL files (produced through GeneChip Microarray Analysis Suite, version 5.1) were sized by adjusting the average intensity of the thickness mix to 10,000 to compensate for small differences in the scanning intensities in different chips.

Operational taxonomic unit (OTU) selection for data analysis differed slightly from Hazen et al. (2010): all OTU's passing PhyCA analysis Stage 1 criteria in this dataset were considered for further analyses, allowing the inclusion of Unclassified OTUs, which would normally be excluded by Stage 2 analysis. In this approach, the presence of different bacterial OTU's was determined by positive hybridization of multiple probes that correspond to distinguishing 16S rDNA gene polymorphisms (average of 37 probes/OTU). The hybridization score for an OTU was calculated as the mean intensity of the perfectly matching probes exclusive of the maximum and minimum. The PhyloChip™ probes for almost 60,000 different microbial taxonomic units that represent 147 phyla, 1,123 classes, 1,219 orders, and 1,464 families according to the placement of its member organisms in the taxonomic outline as maintained. The microarray includes 1,016,064 probe features, the majority of which target 16S rDNA gene sequences that are useful for differentiating taxa. Additional probes are used for quality management, processing controls, image orientation, and normalization controls.

Statistical and data analysis

Intensity values, which are correlative with the relative abundance of taxa, were log_2 transformed before analysis. Statistical analyses were performed in the R software environment version 3.4.2 (R CoreTeam, 2017), using the packages Phyloseq (McMurdie and Holmes, 2013) and Ampvis2 (Andersen et al., 2018). Due to the nature of data obtained using PhyloChip™, we chose to analyze the results based on Jaccard distance matrix to presence/absence of operational taxonomic units (OTU's) and Bray-Curtis distance matrix to relative abundance (normalized fluorescence intensity) of each observed OTU. Non-parametric multidimensional scaling (nMDS) analyses were performed to graphically

represent relationships between the microbial abundance and composition variability between environments in multidimensional space, and a Canonical Analysis of Principal Coordinates (CAP) was performed to identify major environmental factors influencing the microbial community composition at each sample site. We performed a PERMANOVA to determine differences between the environmental factors along the sampling points and to check its influence to dissimilarities in observed microbial communities. Differences in community composition between groups were investigated using the analysis of similarity (ANOSIM), where R values range from 0 to 1, with values close to 1 indicating strong separation between groups, moderately overlapping for R statistics near 0.5 and values close to 0 indicating no significant separation. OTU's were clustered according genus level and the Similarity Percentage (SIMPER) method was used with vegan package in software R to determine the cumulative contribution of each taxon to dissimilarities between river and seawater microbial communities at each site based on average Bray–Curtis dissimilarities.

Potential pathogens were identified at the genus level using as reference a list of human pathogenic bacteria proposed by Fang et al. (2018), which incorporates data from the German Culture Collection (DSMZ, Braunschweig, Germany) and previous studies. The ratio BBC:A was calculated as proposed by Wu et al. (2010). The number of OTU's in the classes of Bacilli, Bacteroidetes, Clostridia, and α-proteobacteria were tallied when the positive fraction was equal to 1. The BBC:A ratio incorporates the relative richness of OTU's prevalent in these 4 bacterial classes associated with fecal and non-fecal samples to reflect possible fecal inputs, rather than the use of single organism presence or absence. The count for unique OTU's in each of the class was normalized by dividing by the total number of OTU's in each class detectable by dividing by the total number of OTU's in each class detectable by the G3 PhyloChip™. The denominators were predetermined based on the number of OTU's assigned for each bacterial class on by the G3 PhyloChip™.

We used Phylogenetic Investigation of Communities by construction of Unobserved States (PICRUSt) version 1.0.0 (Langille et al., 2013) with the default parameters on the Microbiome Analyst web platform (Dhariwal et al., 2017) for functional gene prediction with reference to the KEGG (Kyoto Encyclopedia of

Genes and Genomes), based on the 16S rDNA-based taxonomy and presence x absence data. This method predicts the functional composition of each sample and was used mainly to check for genes associated with xenobiotic degradation in order to verify if it can also provide us with some information, even if presumptive, about the presence of chemical components in the region.

Physical-chemical and biological parameters

Monthly monitoring was carried out between Oct/2017 and Sept/2018, and several parameters (pH, temperature, salinity, turbidity, dissolved oxygen and chlorophyll) were assessed using a model Ysi 6600V2 sonde. Also, 100ml of water was collected and sent on ice to the laboratory to analyze bathing data through the Most Probable Number (MPN) method of *Escherichia coli* and total coliforms, according to Environmental Protection Agency (USEPA, 2006). One day was randomly chosen each month in order to generate data during both high and low tide. Monthly precipitation data was obtained through Inmet (National Institute of Meteorology).

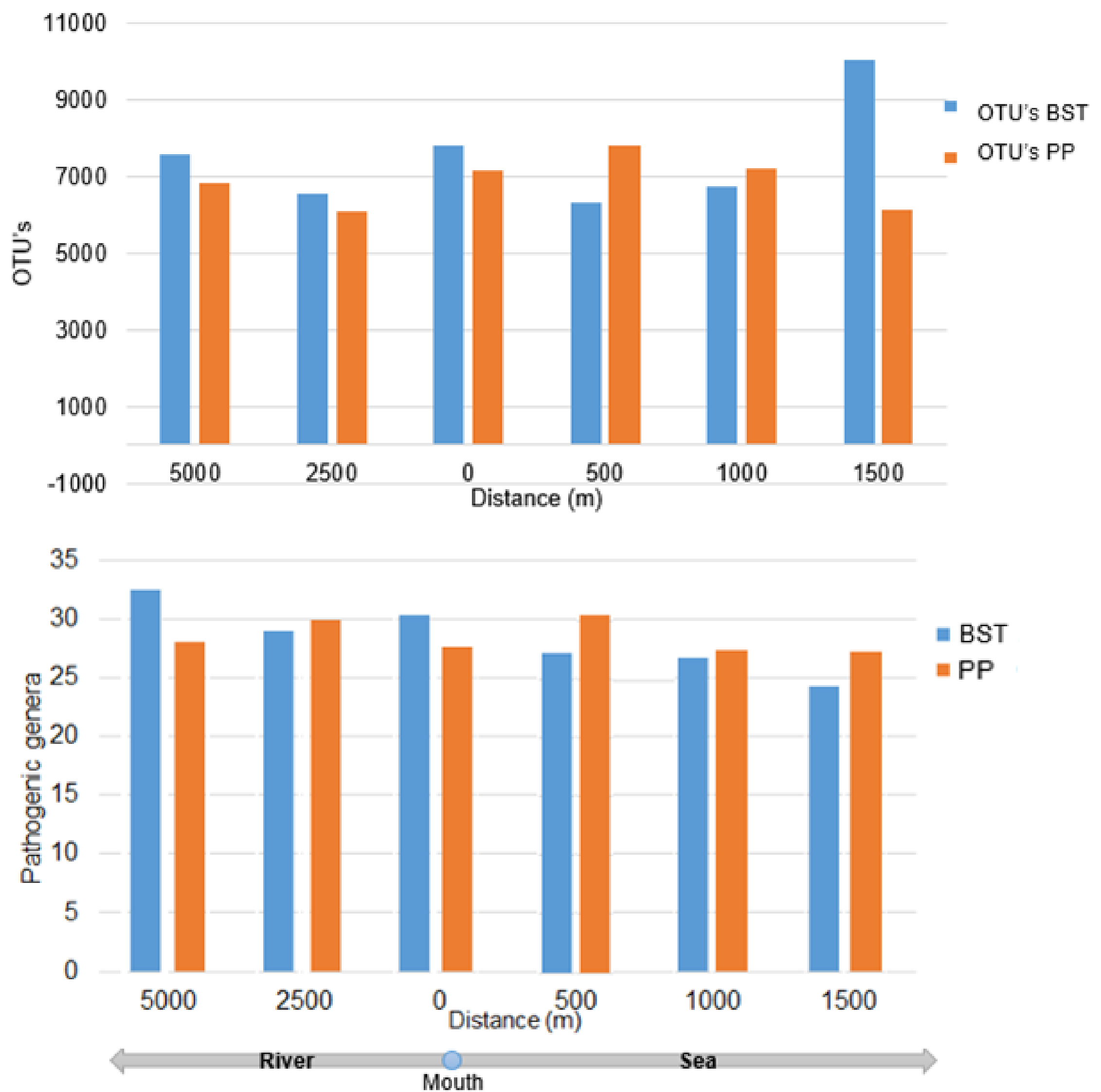
Results

Richness, diversity of microbial communities and associated physicochemical data

The G3 PhyloChip™ method was used to gain insights into the structural diversity of bacteria in rivers and seawater samples from two different sites. A total of 17,898 OTU's were detected, with a mean of 7,192 OTU's per sample, with a minimum of 6,116 OTU's and a maximum of 10,046 OTU's (Figure 2). These figures correspond to 39 phyla, 107 classes, 162 orders, 288 families and 589 genera. Overall, the highest number of OTU's belonged to Proteobacteria (46%), followed by Firmicutes (18%), Actinobacteria (11%), Bacteroidetes (7%) and Acidobacteria (3%), accounting to approximately 85% of all observed taxa. This taxonomic distribution was maintained even when comparing intensity values, with changes only in phylum Verrucomicrobia (Supplementary figure 1). Among Proteobacteria, the highest numbers of OTU's were affiliated to

Gammaproteobacteria, followed by Beta-, Gamma-, Alpha-, Delta- and Epsilonproteobacteria, respectively.

Figure 2: A) Number of OTU's observed per sample; B) Number of genera associated to human pathogens per sample.



On the day of collection of samples for analysis with PhyloChip™, some environmental parameters in PP (temperature, dissolved oxygen and salinity) showed a gradual increase along the sampling points from river to reef. Other parameters (pH, turbidity, chlorophyll and *E. coli*) declined (Supplementary Table 1). In BST, we observed a similar pattern but with less accentuated variations. Turbidity gradually increased from river to reef, while salinity increased sharply

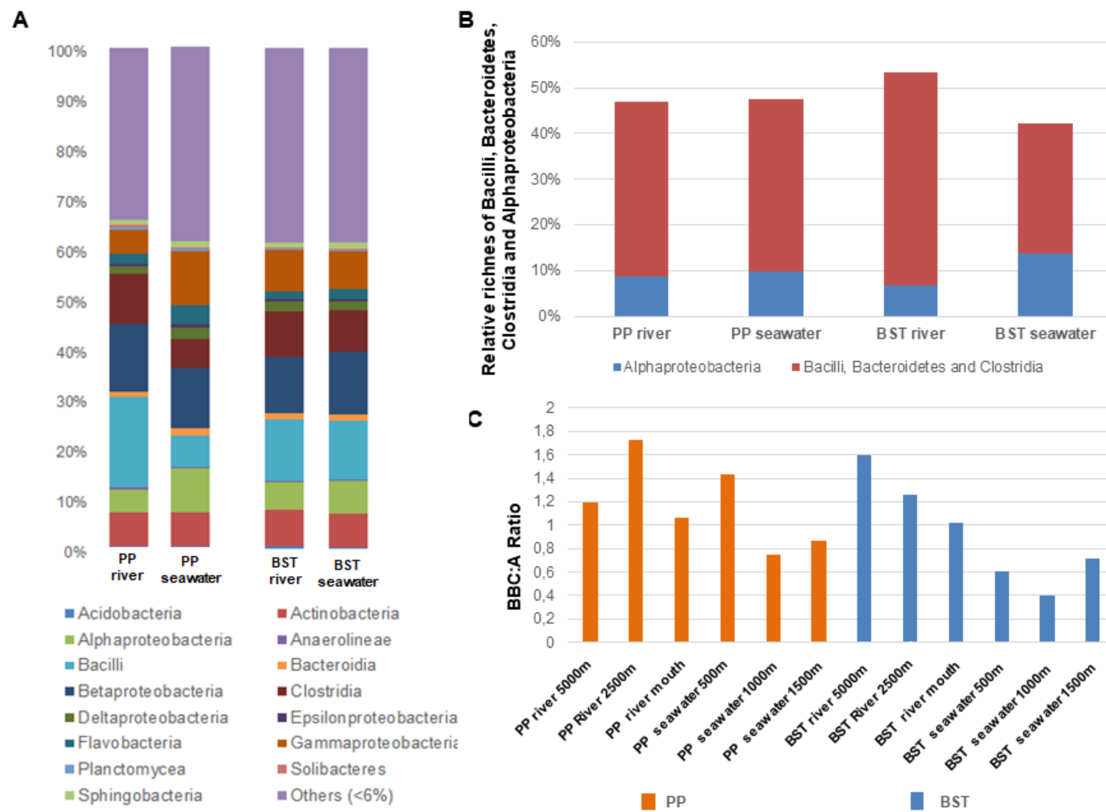
after reaching the farthest point in the sea just above the reefs. The *E. coli* count was lower at this site. The total coliforms count was very high (MPN = 16,000) at all points of both sites (Supplementary Table 1).

Taxonomic composition along the collection points

The proportion of Proteobacteria per sample substantially increased along the course of river BST (41.8% at first point) until the last sampling point (54%), but remained more or less constant in PP (47.6% at the first sampling point and 49.8% at the last point) (Supplementary figure 1). The relative abundance of Actinobacteria was constant at all points and sites, while Firmicutes decreased from 29.4% at first point to 17.9% at last in BST and from 23.5% to 20.9% to PP, after a sudden increase at the first seawater sampling point (26.7%). The relative abundance of Bacteroidetes increased along the points of BST (from 6.8% to 8.4%), but remained more or less constant at PP (7% at the beginning and 6.8% at the end). Phyla with relative abundance (intensity values) less than 1%, such as Verrucomicrobia and Acidobacteria, were relatively constant throughout all the samples.

The percentage of OTU's per class varied between sites and points on the environmental gradient (Figure 3A). Most of classes showed a greater variation in relative abundance (intensity values) in, while in PP values were similar. At BST, classes as Bacilli and Clostridia, for example, were more representative at the first point of the river (17.9% and 11.6%, respectively) than at the last point at sea (8.5% and 9.3%, respectively). Groups such as the Gammaproteobacteria and Alphaproteobacteria increased their abundance gradually along the sampling points from river to reef.

Figure 3: A) Relative richness (%) of main classes, with samples clustered by site and environment; B) Relative richness of Bacilli, Bacteroidetes, Clostridia and Alphaproteobacteria, with samples clustered by site and environment; C) BBC:A ratio calculated to each sample.



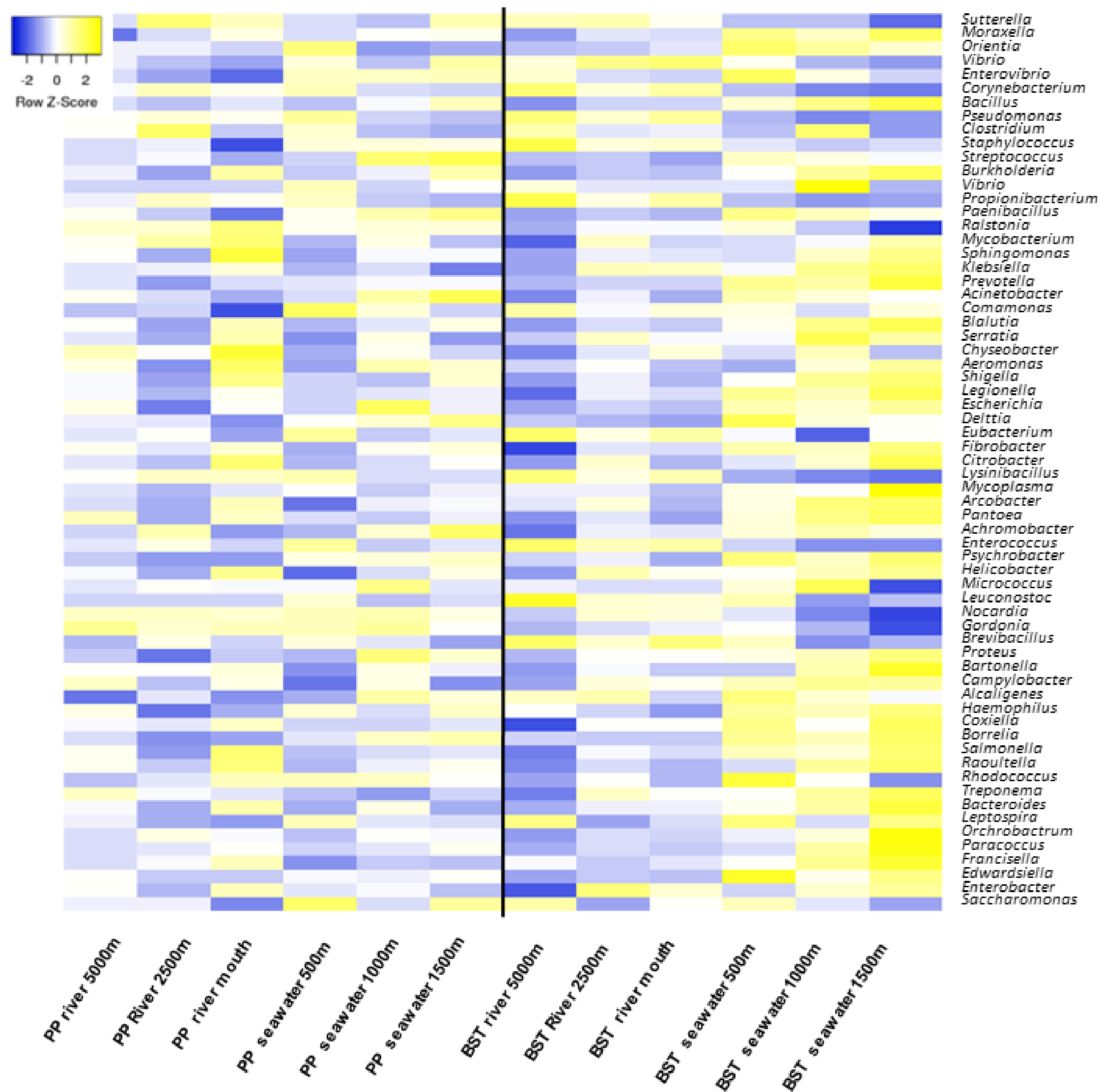
Ratio of Bacilli, Bacteroidetes and Clostridia to Alphaproteobacteria (BBC:A)

The BBC:A ratio uses the relative richness of 4 bacterial classes representative of the fecal (BBC: Bacilli, Bacteroidetes and Clostridia) and the non-fecal (A: Alphaproteobacteria) bacterial communities (Wu et al., 2010). The combined relative richness of Bacilli, Bacteroidetes and Clostridia was 46.6% of detected bacteria in the BST river and 38.3% in PP river. In seawater it was 28.5% in BST and 37.7% in PP (Figure 3B). The relative richness of Alphaproteobacteria in the BST and PP rivers corresponded to 6.7% and 8.6%, respectively. In seawater, this value rises to 13.5% in BST and 9.8% in PP. The relative richness ratio BBC:A was higher than 1 at all sampling points of both rivers, which according to established criteria is indicative of a possible fecal or sewage influence. However, these values started to decrease after the first point in seawater in BST, while it only happened after 1000m in PP (Figure 3C).

Presence of genera associated with human pathogens

As expected, several human opportunistic pathogens were identified throughout the stream, with few site-to-site variations in relative abundances. Among the 589 genera detected, 65 present species already related to human pathogenesis. Of these, *Bacillus*, *Corynebacterium*, *Staphylococcus*, *Pseudomonas*, *Burkholderia* and *Vibrio* showed moderate to high relative abundance (> 0.4%). The relative abundance (intensity values) per sample of most of these groups increases in BST from river towards the sea, being most evident from the first sampling point after the city and until reaching the reefs (Figure 4). On the other hand, the abundance of most this potential pathogenic groups decreases from the seawater sampling points in PP. Among the 20 most abundant genera, 10 are related to pathogens and showed fluctuations in their abundance according to environmental parameters and collection site. These variations occurred principally with the genera *Corynebacterium* and *Staphylococcus*, while *Pseudomonas*, *Clostridium*, *Streptococcus* and *Faecalibacterium* had stable relative abundance values throughout all sampling points. *Bacillus* showed a clear decrease along the river Santo Antonio toward the coral reefs (starting with 9.4% and reaching 3%). These same genera had more consistent abundance values in the sampling points of PP. The relative abundance of *Vibrio* was low (less than 1%) in all environments.

Figure 4: Heatmap of the 65 genera associated to human pathogens found, organized in order to show the variation along the rivers and marine region of each collection point.

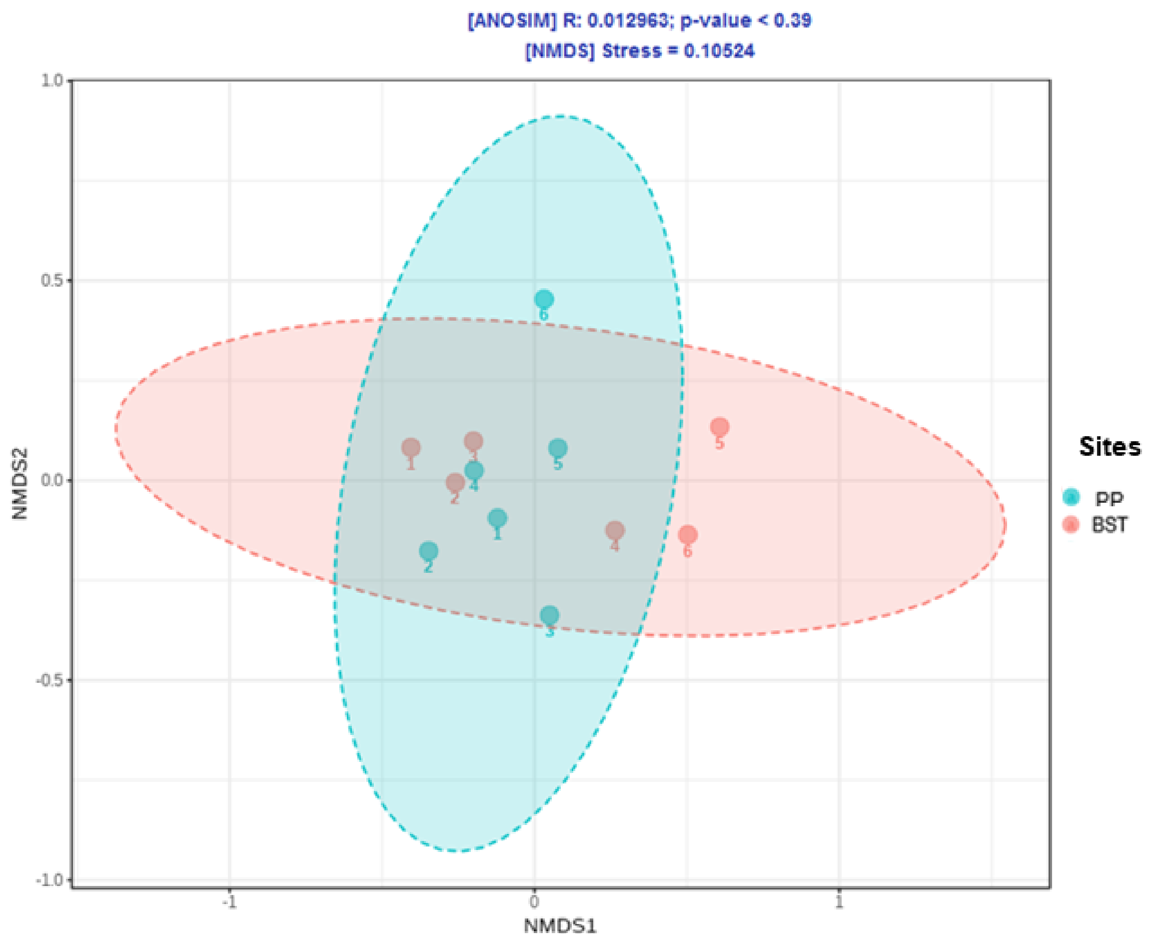


Statistical analysis

The nMDS using presence/absence (Jaccard matrix) (stress = 0.1) and relative abundance (Bray-Curtis matrix) (stress = 0.04) data showed similar results, with marked differences among bacterial communities related to seawater samples at both sites, while samples from same river were more similar (Figure 5). Comparisons between the seawater and river environments using presence/absence data and Jaccard distance showed higher taxonomical variability at BST (ANOSIM R=0.74) than PP (R=0.18), and moderate overall compositional variability based on relative abundance (intensity) data and the Bray-Curtis matrix (R=0.55 and R=0.33, respectively), suggesting that

dissimilarities between environments were higher at BST due principally to differences at OTU composition and to a lesser extent due to relative abundance.

Figure 5: Non-Metrical Dimensional Scale (NMDS) at OTU level based on Bray-Curtis distance matrix, where: 1, 2 and 3) Points at rivers; 4, 5 and 6) Points at seawater.



A canonical analysis of principal coordinates (CAP) was performed using abundance data to determine the correlation between physicochemical properties and bacterial community composition (Supplementary figure 2). The graph represented 76.4% of the total variation in bacterial community composition and indicates that communities between samples from rivers associated similarly to chlorophyll, turbidity, pH and *E. coli* abundance. Meanwhile, communities in seawater samples were more strongly linked to variations in temperature, dissolved oxygen and salinity - factors that, as expected, slightly varied in this environment and which seem to be responsible

for different OTU composition. The results of PERMANOVA analysis indicate that only salinity varied significantly, suggesting that it better explains differences in abundance between samples from rivers and seawater at BST (R^2 = 0.72) than in PP (R^2 = 0.35). Using presence/absence data, these variables explain only 30% of community richness and taxonomic composition, and according to the PERMANOVA none of the variables was statistically relevant (R^2 <0.3) to justify this fact.

In order to identify the bacteria most sensitive to changes from freshwater to seawater, intensity values were compared between groups of samples through a SIMPER analysis with OTU's clustered at genus level. Dissimilarities between river and seawater samples at BST were, according cumulative importance values, mainly due to abundance values of OTU's belonging to an unclassified Proteobacteria (0.29), *Diaphorobacter* (0.39), *Corynebacterium* (0.44), *Aquabacterium* (0.49), *Candidatus Pelagibacter* (0.53), *Bacillus* (0.57), *sfA* (0.60), *Pseudomonas* (0.63), *Staphylococcus* (0.65) and *Burkholderia* (0.66). In PP the dissimilarity between environments was due to abundance values of OTU's belonging to an unclassified Proteobacteria (0.21), followed by genus *Bacillus* (0.36), *Staphylococcus* (0.42), *Corynebacterium* (0.47), *Clostridium* (0.56), *sfA* (0.58), *Aquabacterium* (0.61), *Paenibacillus* (0.62), *Flavobacterium* (0.64), *Streptococcus* (0.66) and *Pseudomonas* (0.67).

Core microbiome at each environment and site

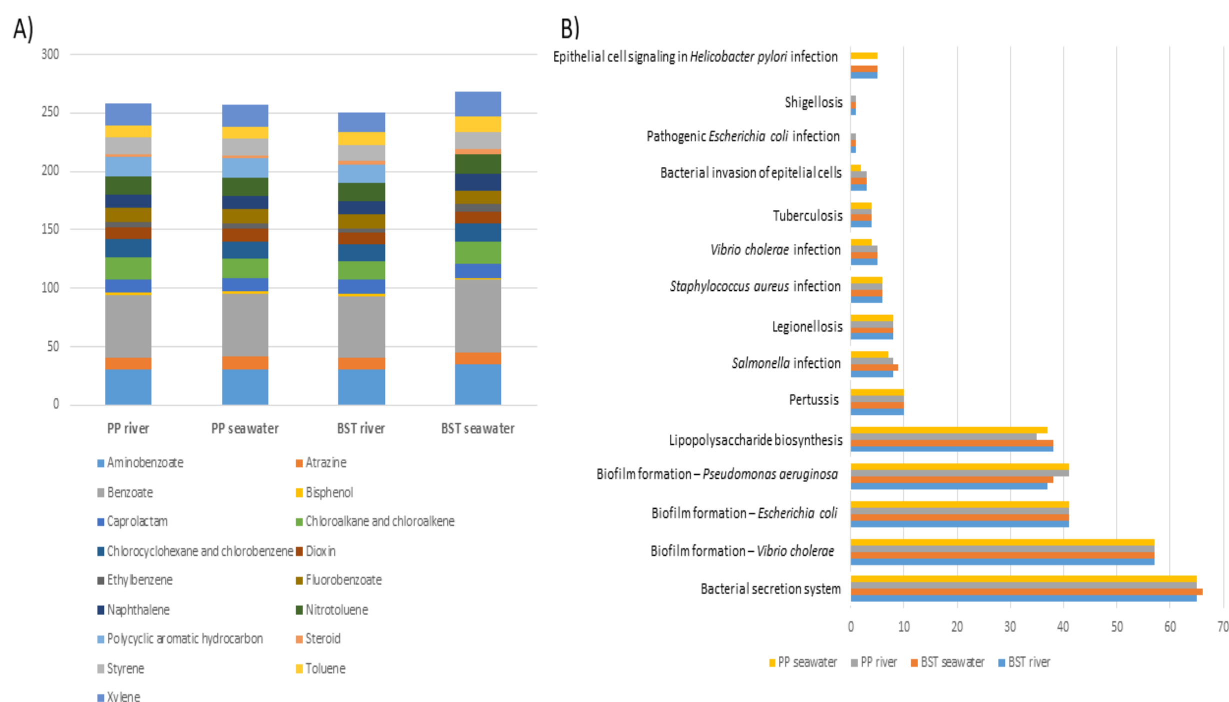
There was a high degree of overlap of OTUs between samples from rivers and seawater collected at same site (Supplementary figure 3). Core analysis indicates that 42.85% (n=4,765) of OTU's found at BST river were common to all three samples from this environment, while the PP river shared 34.22% (n=3,815) of OTU's between its samples, indicating a moderately conservative composition along the course of these rivers. Seawater samples showed similar pattern, with core values ranging from 28.05% (n=3,607) at BST to 30.99% (n=3,815) at PP. However, these values increase when comparing samples grouped according river and seawater from the same sites, resulting in a core composed of 46.9% OTUs (n=2,467, corresponding to 110 genera) in BST and 61.49% OTU's (n=2,579, corresponding to 124 genera) in PP. This indicates that in addition to

each environment hosting unique groups, possibly more adapted to its unique conditions, they still share a high diversity of OTUs. Moreover, 31 genera related to human pathogens were found among the all samples from PP, while in BST this value drops to 26.

Prediction of functional content

The functional prediction using PICRUSt analysis returned 6,885 genes, classified into 371 functional KEGG or orthology pathways at level 3, which included any biological reaction and regulation of gene expression. We identified 16 pathways associated with xenobiotic compound degradation in both environments, and according to literature, at least 10 of them are related to the use of pesticides. The pathways with the highest numbers of identified genes were benzoate (62), followed by aminobenzoate (35), xylene (21) and polycyclic aromatic hydrocarbon (17) – though these values varied according to sampling location. In PP, river and seawater samples has a similar profile, but in BST samples from seawater points showed an increase in the number of genes associated with benzoate, aminobenzoate and xylene degradation pathways (Figure 6A).

Figure 6: A) Richness of genes related to xenobiotic degradation pathways predicted through PICRUSt with samples clustered according site and environment; Richness of genes related to bacterial pathogenesis predicted through PICRUSt with samples clustered according site and environment.



We also explored the predicted genes related to bacterial pathogens and antibiotic resistance, and found 15 pathways that play some role in the infectious process. The pathways with the highest numbers of identified genes were: Bacterial secretion system (66), Biofilm formation - *Vibrio cholera* (57), Biofilm formation - *Escherichia coli* (41), Biofilm formation - *Pseudomonas aeruginosa* (41) and Lipopolysaccharide biosynthesis (38) (Figure 6B). Few genes related to antibiotic resistance routes were identified, the most expressive being Cationic antimicrobial peptide resistance (31), beta-Lactam resistance (16) and Vancomycin resistance (7), at both collection sites (Supplementary figure 04).

Monitoring of physico-chemical and biological variables

Physico-chemical data (from monthly monitoring performed between Ago/2017 and Sep/2018) were analyzed to quantify their dynamics and to compare these with the literature to detect possible risk factors (Supplementary Table 2). The annual average temperature within the PP river (28.64 ºC) did not

significantly differ from that of the seawater immediately after the river mouth (28.54 ºC). This pattern was repeated in BST where the average water temperature was 29.12 ºC in the river and 28.91 ºC in the seawater. Both sites had higher temperatures in Jan/2018, ranging from 31 to 29.25 ºC, followed by a decrease that reached a minimum of 26.5 ºC in June/2018 - after which temperatures started to rise again. As expected, salinity values were lower at rivers (means of PP = 2.14 ppt and BST = 6.38 ppt) but increased along the course until the farthest seawater point (means of PP = 20.38 and BST = 24.69). This result follows a pattern of increasing rainfall, which began in Nov/2017, reached a peak in March/2018 and continued until May/2018, when it started to decrease. As expected, we could observe a decreasing gradient of turbidity along the rivers (mean of 21 NTU) until the farthest point at seawater (mean of 8.63 NTU), but PP showed higher means before the rainy season and the opposite occurred at BST. Levels of Chlorophyll-α also followed this pattern, but the mean production was higher in the BST river (α = 2.22) than in PP river (α = 1.1), while in the seawater points the values were more similar (approximately α = 0.4). pH values were significantly higher at first sampling point (mean of 8.48) in the PP river than the last sampling point in the seawater (7.69) ($p < 0.05$). However, minor differences were observed along the BST sampling points (mean of 7.79 at first point and 7.64 at the last point). Dissolved oxygen at both sites was much lower (less than 80% concentration) in the rivers than the seawater points (averages greater than 102%), especially in the points before the estuary.

Total coliforms generally were higher than MPN = 16,000 all points along the course of both rivers, but they usually started to decrease after 500 meters of seawater. In a few months we observed significant values as much as 1,500 meters after the river mouth (right above the reefs) (Supplementary Table 2). A similar situation was observed for *E. coli*, but a more homogeneous pattern was observed along Barra de Santo Antonio, with a high account of these bacteria (MPN = 16,000) only reaching 500 meters into the seawater, at which point values suddenly decreased to close to zero. Only a few months showed significant abundance of *E. coli* on top of reefs at both sites (mean of MPN = 5,091 at PP and MPN = 0 at BST).

Discussion

Microbial communities in surface waters are highly responsive to perturbation, shifting with tidal cycles, salinity gradients, dissolved organic matter concentration, and chemical stress (Crump et al., 2004). According to our statistical analysis, bacterial communities from freshwater and seawater in BST showed more dissimilarity between in composition and relative abundance, indicating less influence of the freshwater community on the reefs at this site. The small variation in salinity was probably sufficient to affect the composition of bacterial communities at both sites and in the different environments. Sensitivity of microbial communities to variations in salinity is well documented, including the response time of community composition shift within a 24-hour period (Wu et al., 2010), and the development of lineage-specific adaptations in estuaries due to tidal fluctuations (Lozupone and Knight, 2007).

Our results suggest that both rivers are contaminated with human waste. Previous studies indicates that *E. coli* is a more reliable indicator of fecal pollution and occurrence of pathogens in water than total coliforms (Edberg et al., 2000; Leclerc et al., 2002), and is currently used by the government agency responsible for monitoring water quality off the APACC coast. We recorded a high density of total coliforms at all points, but a low density of *E. coli*. Coliform bacteria other than *E. coli* also occur in water as a result of run-off from soil or from growth on decaying vegetation, but it counts gives an indication of the general level of the microbiological quality of water (Neill, 2004).

The most abundant phyla in our samples were Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes, three of which are associated to sewage/fecal pollution. Similar abundance profile has already been observed in previous works involving samples of wastewater and urban rivers (Xia et al., 2010, Jin et al., 2018). Proteobacterial sequences dominated our samples, with an average of 45% OTU's per sample (Supplementary figure 1). This taxon is the largest and most phenotypically diverse lineage, and is known to contain a high proportion of bacteria associated with fecal sources and human diseases. Firmicutes is one of the markers for identifying presence of human feces (Zheng et al., 2009), while numerous species of Bacteroidetes are associated with the gut microbiota of many mammals (Shanks et al., 2011), including humans (Mariat et al., 2009).

Indeed, some species within this phyla have been proposed as effective alternative fecal indicators (García-Aljaro et al., 2019).

Molecular-based methods indicate that most pathogenic genera are commonly found in urban-domestic sewage waters, but their relative abundance in bacterial community is usually low (<1%). Our results revealed a high diversity and relative abundance of potential pathogens, many of which are associated with human stool, animal faeces (particularly swine and cattle) and domestic WWTP's, as already observed in works with surface waters of urban rivers (e.g., *Bacillus*, *Corynebacterium*, *Clostridium, Faecalibacterum, Pseudomonas* and *Vibrio*) (Jin et al., 2018; Jordaan et al., 2019; Osunmakinde et al., 2019). Furthermore, these groups are among the OTU's core that persisted along the length of freshwater-marine gradient. In estuarine areas, these pathogens are often associated with sediments and flocs (suspended sediments) and typically arise from contamination of fresh and marine waters with human sewage and can be transported from upstream sources to estuarine and coastal waters, especially during heavy rain or flood events that may then impact on recreational and shellfish growing waters (Shannon et al., 2007; Wang et al., 2014).

This is supported by the BBC: A ratio, which provided evidence of possible fecal or sewage contamination in both rivers. Our data suggests that more detailed microbial community characterization may allow researchers to move away from generic, single indicators to a community-indicator approach for assessing fecal contamination or environments conducive to pathogen growth. This strengthens the justification for using PhyloChip™ as a powerful alternative for accurately and reproducibly detecting and monitoring the microbial population at different environments. Furthermore, these data highlight the need to for a more integrative approach to reef monitoring, including coastal environments in coral reef monitoring and conservation actions. This will be especially important where there is terrestrial runoff due from urban centers or agricultural lands.

Although more regularly applied in combination with next generation sequencing data, some articles have already used PICRUST to predict functional content of microbial communities analyzed with PhyloChip (Huang et al., 2015; Iwai et al., 2014). Through this workflow, we predicted the microbial functional features for our samples and were able to focus our analysis on xenobiotic

degradation, bacterial pathogenesis and antibiotic resistance. Notably, were identified several xenobiotic degradation functions in both marine and freshwater environments, many of which are related to pesticide use and fuel production. This makes sense, since agricultural activity is common along the water courses in both regions, especially the cultivation of sugarcane which has vinasse as a by-product. The consequences of vinasse discharge in effluents includes the proliferation of microorganisms that deplete the oxygen dissolved in the water, death of aquatic animals and plants, and making contaminated water bodies more difficult to be used as sources of potable water (Laime et al., 2011). The runoff of pesticides from agricultural lands already is a key concern for the health of the Great Barrier Reef, since relatively low levels of these residues can reduce the productivity of corals damaging the partnership between host and symbiont, resulting in the expulsion of zooxanthellae from the coral host (Lewis et al., 2009; Markey et al., 2007). Also, benzene, ethylbenzene toluene and xylene are part of the aromatic hydrocarbons that are present in gasoline, which may be being released into the environment by local fisherman's vessels (Filho et al., 2016).

Among the mechanisms of pathogenicity, the most predicted genes belonged to the “Bacterial Secretion System” category, related to the delivery of polypeptides in the host environment, many of them being effectors or toxins (Green and Mecsas, 2016). The other pathways richest in predicted genes belonged to categories related to the production of biofilms in *Vibrio cholerae*, *E. coli* and *P. aeruginosa*. Interestingly, we observed a low number of genes related to “Pathogenic *E. coli* infection” pathway at both sampling points, which may be an indication that these strains are not so virulent, more related to commensal strains. Is already known that *E. coli* strains vary in several phenotypic characteristics, such as ability to form biofilms and utilization of carbon sources, and this diversity has been explained by the impact of the genomic makeup of the organism adapting to the host intestinal or the extraintestinal natural environments (Van Elsas et al., 2011). However, since it represents a prediction and other results support the presence of other pathogens, we suggest that virulence analyzes be carried out concurrently with the bathing samples in order to have a better understanding of the problem in these two locations.

Fortunately, few resistance genes have been predicted, the most expressive category being related to resistance to cationic antimicrobial peptides, whose members are widely distributed in nature, existing in organisms from insects to plants to mammals and non-mammalian vertebrates as a immunological mechanism against several pathogens (Brown and Hancock, 2006). Antibiotic resistance has been shown to be able to spread throughout the environment through mechanisms such as urban and agricultural runoff, wind, and biological forces such as animals and humans (Allen et al., 2010). It is also possible that genes that confer resistance to antibiotics in pathogens may have an alternative purpose in their original host, which may explain their presence in different compartments of the environment without typical selective pressures (Martinez, 2009). Apparently, these genes are not being carried in significant numbers to the region's coral reefs. This predictive method thus appears to provide a robust measure of functional differences, and together with microbial community data, reinforces the use of PhyloChip™ as a powerful alternative for accurately and reproducibly detecting and monitoring different environments.

Physicochemical and biological variables

Water temperature is an important indicator of the health of aquatic systems because of its direct relationship with how much oxygen can be dissolved in the water (Caroline Joyce and Todd Viola, 2016). The results of our monthly monitoring indicate that even during the summer (corresponding to the period between December and March in Brazil) the temperature measurements obtained just above the coral reefs were not high enough to trigger bleaching (generally 1 °C increase in daily temperature) at either site (Safaie et al., 2018).

On the other hand, several other environmental factors may be synergistically affecting the coral reefs of these regions. The turbidity values that we observed immediately above both coral reefs were high (means between 5.81 to 8.63 NTU) - values greater than 5.5 NTU in seawater are capable of decreasing coral cover to less than 30% (Coles and Ruddy, 1995; Otero and Carbery, 2005). Most sediments are imported into coastal marine systems through rivers, and their reach depends on the size of the grains transported (Fabricius, 2005). The turbidity over coral reef systems may act as a modulator of local biodiversity

favoring the growth of more resistant groups, such as sponges and gorgonians (Carricart-Ganivet and Merino, 2001).

In the open ocean, salinity varies little, but in an estuary it varies daily, seasonally, by location and with tidal cycles. It is affected by high temperatures and by large amounts of rain (Caroline Joyce and Todd Viola, 2016). As these corals are subjected to successive cycles of saline stress due to variation in the tide, the data collected needs to be carefully interpreted. During high tides, values found at seawater are between those reported in the literature (approximately 33~36 ppt), but the decrease observed during low tide (values lower than 10 ppt) indicate that, as expected, these corals are subject to very abrupt seasonal variations in salinity. It has previously been shown that the duration of exposure at moderately low salinities (22-25 ppt) is a critical transition factor for survivorship of sensitive species, and this variable is considered as a structuring force for near-shore coral communities (True, 2012).

Bacterial counts for total coliform and *E. coli* are normally orders of magnitude higher at the freshwater end than at the seaward end of an estuary, notwithstanding the daily fluctuations due to tidal fluctuations (Pachepsky and Shelton, 2011). The persistence of *Salmonella* and *E.coli* was largely unaffected in brackish/saline water until concentrations reached 25 ppt (parts per thousand). The salinity value of seawater is approximately 35 ppt, suggesting that these organisms would not survive for very long in seawater but could survive in brackish waters (Chandran et al., 2013). We found consistently high FIB values during our monthly monitoring, principally related to thermotolerant coliforms. The values related to *E. coli* found in seawater were particularly high, especially in Porto de Pedras, where the gradient of degradation along the collection points was not as consistent as in Barra de Santo Antônio. At both sites the variation between the first point in seawater (500 m after the mouth of the river) and the last one (just above the reefs) was almost significant ($p = 0.06$). These differences are probably related to the clearly higher average salinity observed in seawater sampling points at Barra de Santo Antônio.

Conclusion

Early prediction of ecosystem stress is critical for an effective implementation of local management and restoration strategies on threatened reef sites. The high OTU overlap between freshwater and marine environments, the indicators of fecal contamination, the number of pathogen-related genera and the predicted richness and abundance of genes related to xenobiotic degradation, combined with the observations from monthly monitoring, strongly suggest that studied rivers are suffering significant anthropogenic impacts. More robust molecular tools applied to the study of microbial communities are an alternative to be considered, since they allow greater exploratory prognosis and can identify situations that would go unnoticed when using the traditional methods based on culture of enteric groups. In general, our results indicate that coral reefs are being impacted by several factors resulting from different activities occurring along the riverbanks. The incorporation of microbial community data into environmental monitoring programs could thus improve the prediction and management of environmental pressures. Factors such as the presence of agribusiness and the discharge of sewage along the course of the rivers should be regularly evaluated, since both are possible sources of environmental contamination. There are another three more rivers with similar size and characteristics within this environmental protected area, and these are probably suffering from the same problems reported in this paper. Our study clearly indicates that conservation actions must extend beyond the reef environment and include interventions that minimize the impacts caused by the low water quality of the rivers present in the region.

Declaration of competing interest

None.

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Author contributions

Gustavo Paulino: Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization; Ciro Ramon: Formal analysis, Writing - Review & Editing, Visualization; Cinta Gomez: Validation, Resources; Gary Andersen: Conceptualization, Resources, Supervision; Melissa Landell: Conceptualization, Supervision, Project administration, Funding acquisition

References

Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K.A., Davies, J., Handelsman, J., 2010. Call of the wild: Antibiotic resistance genes in natural environments. Nat. Rev. Microbiol. https://doi.org/10.1038/nrmicro2312

Andersen, K.S., Kirkegaard, R.H., Karst, S.M., Albertsen, M., 2018. ampvis2: an R package to analyse and visualise 16S rDNA amplicon data. bioRxiv 299537. https://doi.org/10.1101/299537

Bellwood, D.R., Pratchett, M.S., Morrison, T.H., Gurney, G.G., Hughes, T.P., Álvarez-Romero, J.G., Day, J.C., Grantham, R., Grech, A., Hoey, A.S., Jones, G.P., Pandolfi, J.M., Tebbett, S.B., Techera, E., Weeks, R., Cumming, G.S., 2019. Coral reef conservation in the Anthropocene: Confronting spatial mismatches and prioritizing functions. Biological Conservation 236, 604–615. https://doi.org/10.1016/j.biocon.2019.05.056

Brodie, E.L., DeSantis, T.Z., Moberg Parker, J.P., Zubietta, I.X., Piceno, Y.M., Andersen, G.L., 2007. Urban aerosols harbor diverse and dynamic bacterial populations. Proceedings of the National Academy of Sciences of the United States of America. https://doi.org/10.1073/pnas.0608255104

Brown, K.L., Hancock, R.E.W., 2006. Cationic host defense (antimicrobial)

peptides. Curr. Opin. Immunol. https://doi.org/10.1016/j.coi.2005.11.004

Caroline Joyce, Todd Viola, 2016. Monitoring estuarine water quality. NOAA Ocean Data Education.

Carricart-Ganivet, J.P., Merino, M., 2001. Growth responses of the reef-building coral Montastraea annularis along a gradient of continental influence in the southern Gulf of Mexico. Bulletin of Marine Science.

Chandran, A., Suson, P.S., Thomas, A.P., Hatha, M., Mazumder, A., 2013. Survival of multi-drug resistant enteropathogenic *Escherichia coli* and *Salmonella paratyphi* in Vembanadu lake as a function of saltwater barrier along southwest coast of India. Journal of Water and Health. https://doi.org/10.2166/wh.2013.221

Coles, S.L., Ruddy, L., 1995. Comparison of water quality and reef coral mortality and growth in southeastern Kane'ohe Bay, O'ahu, Hawai'i, 1990 to 1992, with conditions before sewager diversion. Pacific Science 49, 247–265.

Crump, B.C., Hopkinson, C.S., Sogin, M.L., Hobbie, J.E., 2004. Microbial biogeography along an estuarine salinity gradient: Combined influences of bacterial growth and residence time. Applied and Environmental Microbiology 70, 1494–1505. https://doi.org/10.1128/AEM.70.3.1494-1505.2004

DeSantis, T.Z., Brodie, E.L., Moberg, J.P., Zubieta, I.X., Piceno, Y.M., Andersen, G.L., 2007. High-density universal 16S rDNA microarray analysis reveals broader diversity than typical clone library when sampling the environment, in: Microbial Ecology. https://doi.org/10.1007/s00248-006-9134-9

Dhariwal, A., Chong, J., Habib, S., King, I.L., Agellon, L.B., Xia, J., 2017. MicrobiomeAnalyst: A web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Research 45, W180–W188. https://doi.org/10.1093/nar/gkx295

Dubinsky, E.A., Butkus, S.R., Andersen, G.L., 2016. Microbial source tracking in impaired watersheds using PhyloChip and machine-learning classification. Water Research 105, 56–64. https://doi.org/10.1016/j.watres.2016.08.035

Edberg, S.C., Rice, E.W., Karlin, R.J., Allen, M.J., 2000. *Escherichia coli*: the best biological drinking water indicator for public health protection. Symposium

series (Society for Applied Microbiology) 106S-116S. https://doi.org/10.1111/j.1365-2672.2000.tb05338.x

van Elsas, J.D., Semenov, A. V., Costa, R., Trevors, J.T., 2011. Survival of Escherichia coli in the environment: Fundamental and public health aspects. ISME J. https://doi.org/10.1038/ismej.2010.80

Fabricius, K.E., 2005. Effects of terrestrial runoff on the ecology of corals and coral reefs: Review and synthesis. Marine Pollution Bulletin 50, 125–146. https://doi.org/10.1016/j.marpolbul.2004.11.028

Fang, T., Cui, Q., Huang, Y., Dong, P., Wang, H., Liu, W.T., Ye, Q., 2018. Distribution comparison and risk assessment of free-floating and particle-attached bacterial pathogens in urban recreational water: Implications for water quality management. Science of the Total Environment 613–614, 428–438. https://doi.org/10.1016/j.scitotenv.2017.09.008

Ferreira, B.P., Maida, M., 2006. Monitoramento dos recifes de corais do Brasil, Ministério do Meio Ambiente. https://doi.org/10.1007/s10531-013-0611-4

Filho, C.M.C., Neto, M.N.L., Teixeira, R.S., Pais, A.A.C.C., Valente, A.J.M., 2016. Development and optimization of an HPLC–DAD method for quantification of six petroleum hydrocarbon compounds in aqueous samples. Journal of Liquid Chromatography and Related Technologies. https://doi.org/10.1080/10826076.2016.1274998

García-Aljaro, C., Blanch, A.R., Campos, C., Jofre, J., Lucena, F., 2019. Pathogens, faecal indicators and human-specific microbial source-tracking markers in sewage. Journal of Applied Microbiology 126, 701–717. https://doi.org/10.1111/jam.14112

Gerba, C.P., 2014. Indicator Microorganisms, in: Environmental Microbiology: Third Edition. https://doi.org/10.1016/B978-0-12-394626-3.00023-5

Green, E.R., Mecsas, J., 2016. Bacterial Secretion Systems: An Overview. Microbiol. Spectr. https://doi.org/10.1128/microbiolspec.vmbf-0012-2015

Grimes, D.J., Atwell, R.W., Brayton, P.R., Palmer, L.M., Rollins, D.M., Roszak, D.B., Singleton, F.L., Tamplin, M.L., Colwell, R.R., 1986. The fate of enteric pathogenic bacteria in estuarine and marine environments. Microbiological sciences 3, 324–329.

Hassard, F., Gwyther, C.L., Farkas, K., Andrews, A., Jones, V., Cox, B., Brett, H., Jones, D.L., McDonald, J.E., Malham, S.K., 2016. Abundance and distribution of enteric bacteria and viruses in coastal and estuarine sediments-A review. Frontiers in Microbiology. https://doi.org/10.3389/fmicb.2016.01692

Hazen, T.C., Dubinsky, E.A., DeSantis, T.Z., Andersen, G.L., Piceno, Y.M., Singh, N., Jansson, J.K., Probst, A., Borglin, S.E., Fortney, J.L., Stringfellow, W.T., Bill, M., Conrad, M.E., Tom, L.M., Chavarria, K.L., Alusi, T.R., Lamendella, R., Joyner, D.C., Spier, C., Baelum, J., Auer, M., Zemla, M.L., Chakraborty, R., Sonnenthal, E.L., D'Haeseleer, P., Holman, H.Y.N., Osman, S., Lu, Z., Van Nostrand, J.D., Deng, Y., Zhou, J., Mason, O.U., 2010. Deep-sea oil plume enriches indigenous oil-degrading bacteria. Science. https://doi.org/10.1126/science.1195979

Huang, Y.J., Nariya, S., Harris, J.M., Lynch, S. V., Choy, D.F., Arron, J.R., Boushey, H., 2015. The airway microbiome in patients with severe asthma: Associations with disease features and severity. Journal of Allergy and Clinical Immunology 136, 874–884. https://doi.org/10.1016/j.jaci.2015.05.044

IBGE, 2011. IBGE.Instituto Brasileiro de Geografi a e Estatística. Censo demográfico 2010 [WWW Document]. IBGE. Instituto Brasileiro de Geografi a e Estatística. Censo demográfico 2010.

Iwai, S., Huang, D., Fong, S., Jarlsberg, L.G., Worodria, W., Yoo, S., Cattamanchi, A., Davis, J.L., Kaswabuli, S., Segal, M., Huang, L., Lynch, S. V., 2014. The lung microbiome of Ugandan HIV-infected pneumonia patients is compositionally and functionally distinct from that of San Franciscan patients. PLoS ONE 9. https://doi.org/10.1371/journal.pone.0095726

Jin, D., Kong, X., Cui, B., Jin, S., Xie, Y., Wang, X., Deng, Y., 2018. Bacterial communities and potential waterborne pathogens within the typical urban surface waters. Sci. Rep. https://doi.org/10.1038/s41598-018-31706-w

Jordaan, K., Comeau, A.M., Khasa, D.P., Bezuidenhout, C.C., 2019. An integrated insight into the response of bacterial communities to anthropogenic contaminants in a river: A case study of the Wonderfonteinspruit catchment area, South Africa. PLoS One.

https://doi.org/10.1371/journal.pone.0216758

Laime, E.M.O., Fernandes, P.D., Oliveira, D.C.D.S., Freire, E.D.A., 2011. Possibilidades tecnológicas para a destinação da vinhaça : uma revisão. Revista Trópica - Ciências Agrárias e Biológicas. https://doi.org/10.5935/0100-4042.20140138

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G., Huttenhower, C., 2013. Predictive functional profiling of microbial communities using 16S rDNA marker gene sequences. Nature Biotechnology 31, 814–821. https://doi.org/10.1038/nbt.2676

Leclerc, H., Mossel, D.A.A., Edberg, S.C., Struijk, C.B., 2002. Advances in the Bacteriology of the Coliform Group: Their Suitability as Markers of Microbial Water Safety. Annual Review of Microbiology 55, 201–234. https://doi.org/10.1146/annurev.micro.55.1.201

Leite, D.C.A., Salles, J.F., Calderon, E.N., Castro, C.B., Bianchini, A., Marques, J.A., van Elsas, J.D., Peixoto, R.S., 2018. Coral bacterial-core abundance and network complexity as proxies for anthropogenic pollution. Frontiers in Microbiology 9, 1–11. https://doi.org/10.3389/fmicb.2018.00833

Lemarchand, K., Masson, L., Brousseau, R., 2004. Molecular biology and DNA microarray technology for microbial quality monitoring of water. Critical Reviews in Microbiology. https://doi.org/10.1080/10408410490435142

Lewis, S.E., Brodie, J.E., Bainbridge, Z.T., Rohde, K.W., Davis, A.M., Masters, B.L., Maughan, M., Devlin, M.J., Mueller, J.F., Schaffelke, B., 2009. Herbicides: A new threat to the Great Barrier Reef. Environmental Pollution 157, 2470–2484. https://doi.org/10.1016/j.envpol.2009.03.006

Lozupone, C.A., Knight, R., 2007. Global patterns in bacterial diversity. Proceedings of the National Academy of Sciences of the United States of America 104, 11436–11440. https://doi.org/10.1073/pnas.0611525104

Mariat, D., Firmesse, O., Levenez, F., Guimarăes, V.D., Sokol, H., Doré, J., Corthier, G., Furet, J.P., 2009. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. BMC Microbiology 9, 1–6. https://doi.org/10.1186/1471-2180-9-123

Markey, K.L., Baird, A.H., Humphrey, C., Negri, A.P., 2007. Insecticides and a fungicide affect multiple coral life stages. Marine Ecology Progress Series 330, 127–137. https://doi.org/10.3354/meps330127

Martinez, J.L., 2009. Environmental pollution by antibiotics and by antibiotic resistance determinants. Environ. Pollut. https://doi.org/10.1016/j.envpol.2009.05.051

McMurdie, P.J., Holmes, S., 2013. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE. https://doi.org/10.1371/journal.pone.0061217

Neill, M., 2004. Microbiological indices for total coliform and *E. coli* bacteria in estuarine waters. Marine Pollution Bulletin 49, 752–760. https://doi.org/10.1016/j.marpolbul.2004.05.016

Osunmakinde, C.O., Selvarajan, R., Mamba, B.B., Msagati, T.A.M., 2019. Profiling bacterial diversity and potential pathogens in wastewater treatment plants using high-throughput sequencing analysis. Microorganisms. https://doi.org/10.3390/microorganisms7110506

Otero, E., Carbery, K.K., 2005. Chlorophyll a and turbidity patterns over coral reefs systems of La Parguera Natural Reserve, Puerto Rico. Revista de Biologia Tropical 53, 25–32.

Pachepsky, Y.A., Shelton, D.R., 2011. *Escherichia coli* and fecal coliforms in freshwater and estuarine sediments. Critical Reviews in Environmental Science and Technology 41, 1067–1110. https://doi.org/10.1080/10643380903392718

Pires, N.L., Muniz, D.H. de F., Kisaka, T.B., Simplicio, N. de C.S., Bortoluzzi, L., Lima, J.E.F.W., Oliveira-Filho, E.C., 2015. Impacts of the urbanization process on water quality of Brazilian Savanna rivers: The case of preto river in formosa, Goiás state, Brazil. International Journal of Environmental Research and Public Health 12, 10671–10686. https://doi.org/10.3390/ijerph120910671

R CoreTeam, D.C., 2017. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. https://doi.org/10.1007/978-3-540-74686-7

Redding, J.E., Myers-Miller, R.L., Baker, D.M., Fogel, M., Raymundo, L.J., Kim, K., 2013. Link between sewage-derived nitrogen pollution and coral disease severity in Guam. Mar. Pollut. Bull. https://doi.org/10.1016/j.marpolbul.2013.06.002Safaie, A., Silbiger, N.J., McClanahan, T.R., Pawlak, G., Barshis, D.J., Hench, J.L., Rogers, J.S., Williams, G.J., Davis, K.A., 2018. High frequency temperature variability reduces the risk of coral bleaching. Nature Communications 9, 1–12. https://doi.org/10.1038/s41467-018-04074-2

Shanks, O.C., Kelty, C.A., Archibeque, S., Jenkins, M., Newton, R.J., McLellan, S.L., Huse, S.M., Sogin, M.L., 2011. Community structures of fecal bacteria in cattle from different animal feeding operations. Applied and Environmental Microbiology 77, 2992–3001. https://doi.org/10.1128/AEM.02988-10

Shannon, K.E., Lee, D.Y., Trevors, J.T., Beaudette, L.A., 2007. Application of real-time quantitative PCR for the detection of selected bacterial pathogens during municipal wastewater treatment. Science of the Total Environment 382, 121–129. https://doi.org/10.1016/j.scitotenv.2007.02.039

Steiner, A.Q., Amaral, F.M.D., Amaral, J.R. de B.C. do, Sassi, R., Barradas, J.I., 2015. Zonação de recifes emersos da Área de Proteção Ambiental Costa dos Corais, Nordeste do Brasil. Iheringia. Série Zoologia 105, 184–192. https://doi.org/10.1590/1678-476620151052184192

Sully, S., Burkepile, D.E., Donovan, M.K., Hodgson, G., van Woesik, R., 2019. A global analysis of coral bleaching over the past two decades. Nature Communications 10, 1–5. https://doi.org/10.1038/s41467-019-09238-2

Sutherland, K.P., Porter, J.W., Turner, J.W., Thomas, B.J., Looney, E.E., Luna, T.P., Meyers, M.K., Futch, J.C., Lipp, E.K., 2010. Human sewage identified as likely source of white pox disease of the threatened Caribbean elkhorn coral, Acropora palmata. Environmental Microbiology 12, 1122–1131. https://doi.org/10.1111/j.1462-2920.2010.02152.x

Tiquia, S.M., 2010. Metabolic diversity of the heterotrophic microorganisms and potential link to pollution of the Rouge River. Environmental Pollution 158, 1435–1443. https://doi.org/10.1016/j.envpol.2009.12.035

True, J.D., 2012. Salinity as a structuring force for near shore coral communities. Proceedings of the 12th International Coral Reef Symposium, Cairns,

Australia, 9-13 July 2012 9–13.

USEPA, 2006. Volunteer Estuary Monitoring: A Methods Manual. EPA-842-B-06-003 Washington, D.C. : United States Environmental Protection Agency (USEPA).

Wang, Z.H., Yang, J.Q., Zhang, D.J., Zhou, J., Zhang, C.D., Su, X.R., Li, T.W., 2014. Composition and structure of microbial communities associated with different domestic sewage outfalls. Genetics and Molecular Research 13, 7542–7552. https://doi.org/10.4238/2014.September.12.21

Wear, S.L., Thurber, R.V., 2015. Sewage pollution: Mitigation is key for coral reef stewardship. Annals of the New York Academy of Sciences 1355, 15–30. https://doi.org/10.1111/nyas.12785

Wu, C.H., Sercu, B., van de Werfhorst, L.C., Wong, J., deSantis, T.Z., Brodie, E.L., Hazen, T.C., Holden, P.A., Andersen, G.L., 2010. Characterization of coastal urban watershed bacterial communities leads to alternative community-based indicators. PLoS ONE 5, e11285. https://doi.org/10.1371/journal.pone.0011285

Xia, S., Duan, L., Song, Y., Li, J., Piceno, Y.M., Andersen, G.L., Alvarez-Cohen, L., Moreno-Andrade, I., Huang, C.L., Hermanowicz, S.W., 2010. Bacterial community structure in geographically distributed biological wastewater treatment reactors. Environmental Science and Technology 44, 7391–7396. https://doi.org/10.1021/es101554m

Zheng, G., Yampara-Iquise, H., Jones, J.E., Andrew Carson, C., 2009. Development of Faecalibacterium 16S rDNA gene marker for identification of human faeces. Journal of Applied Microbiology 106, 634–641. https://doi.org/10.1111/j.1365-2672.2008.04037.x

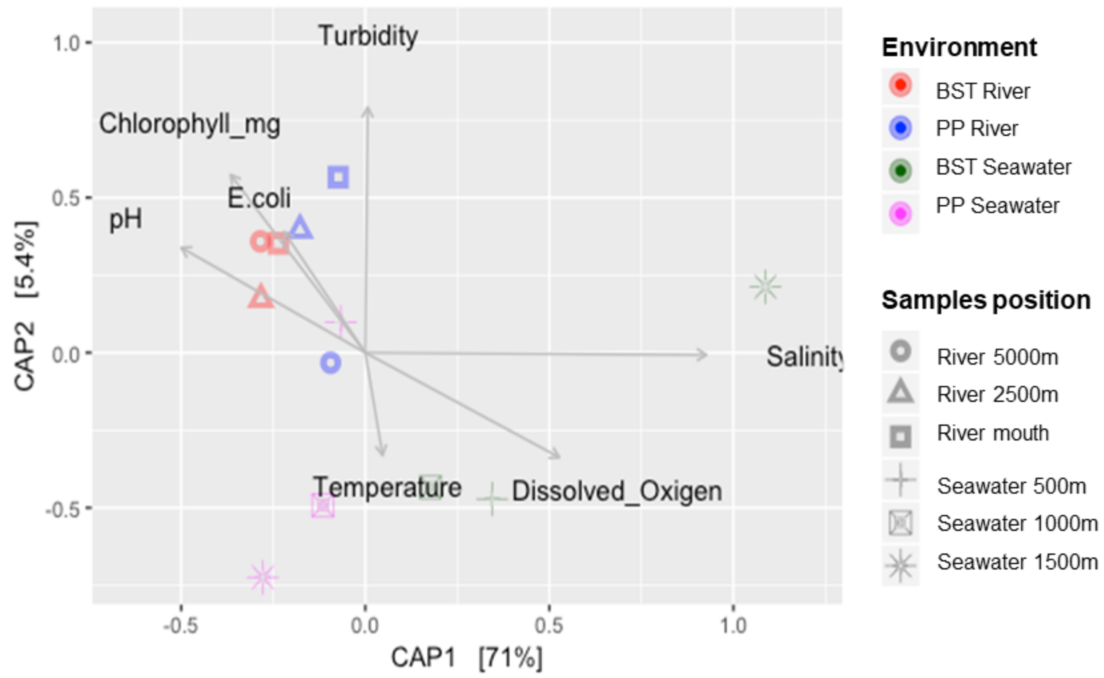
Zhou, J., He, Z., Yang, Y., Deng, Y., Tringe, S.G., Alvarez-Cohen, L., 2015. High-throughput metagenomic technologies for complex microbial community analysis: Open and closed formats. mBio 6. https://doi.org/10.1128/mBio.02288-14

Supplementary Material

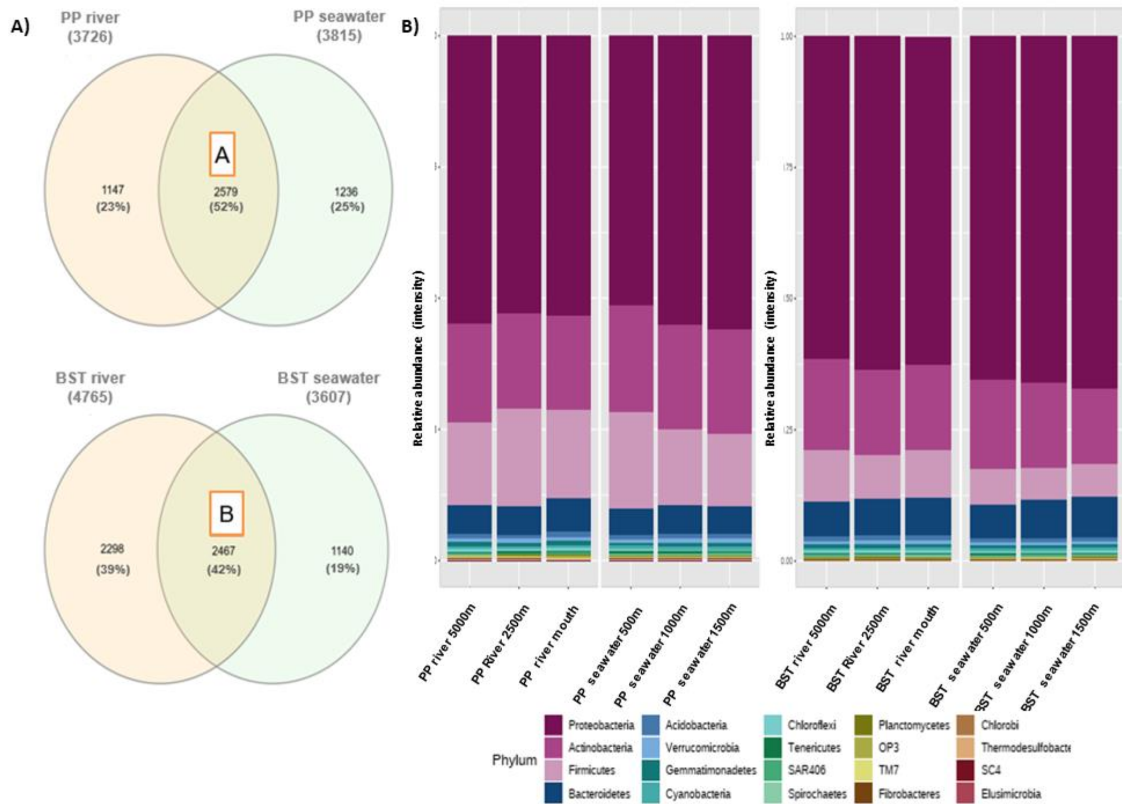
Supplementary figure 1: Relative abundance (intensity values) (%) of most prevalent phylum at each sample and environment.

	PP river 5000m	PP River 2500m	PP river mouth	PP seawater 500m	PP seawater 1000m	PP seawater 1500m	BST river 5000m	BST River 2500m	BST river mouth	BST seawater 500m	BST seawater 1000m	BST seawater 1500m
Proteobacteria	47.6	45.5	47.3	44.7	48.6	49.8	41.8	45.8	44.1	49.2	50.9	54
Firmicutes	23.5	26.7	23.3	26.7	21.8	20.9	29.4	24.8	27.1	20.7	19.6	17.9
Actinobacteria	14	13.7	13.5	14.7	14.7	14.6	14.6	14.2	13.9	15.1	14.1	12
Bacteroidetes	7	6.5	7.6	6.2	7	6.8	6.8	7.3	7.2	7.3	8	8.4
Verrucomicrobia	1.3	1.2	1.4	1.2	1.4	1.3	1	1.2	1.1	1.1	1.1	1.1
Acidobacteria	1.2	1.2	1.3	1.2	1.2	1.2	1.1	1.2	1.2	1.2	1.1	1.1
Cyanobacteria	1	0.9	1	0.9	0.9	0.9	0.9	1	1	1	1	1.2
Chloroflexi	0.8	0.8	0.8	0.8	0.8	0.7	0.8	0.8	0.8	0.8	0.7	0.7
Planctomycetes	0.6	0.6	0.7	0.6	0.7	0.7	0.6	0.7	0.6	0.6	0.6	0.7
Tenericutes	0.6	0.5	0.5	0.6	0.5	0.5	0.6	0.6	0.6	0.6	0.6	0.6
Gemmatimonadetes	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Spirochaetes	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
TM7	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1
Chlorobi	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Nitrospirae	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Fusobacteria	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
WS3	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Fibrobacteres	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Thermi	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
SAR406	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

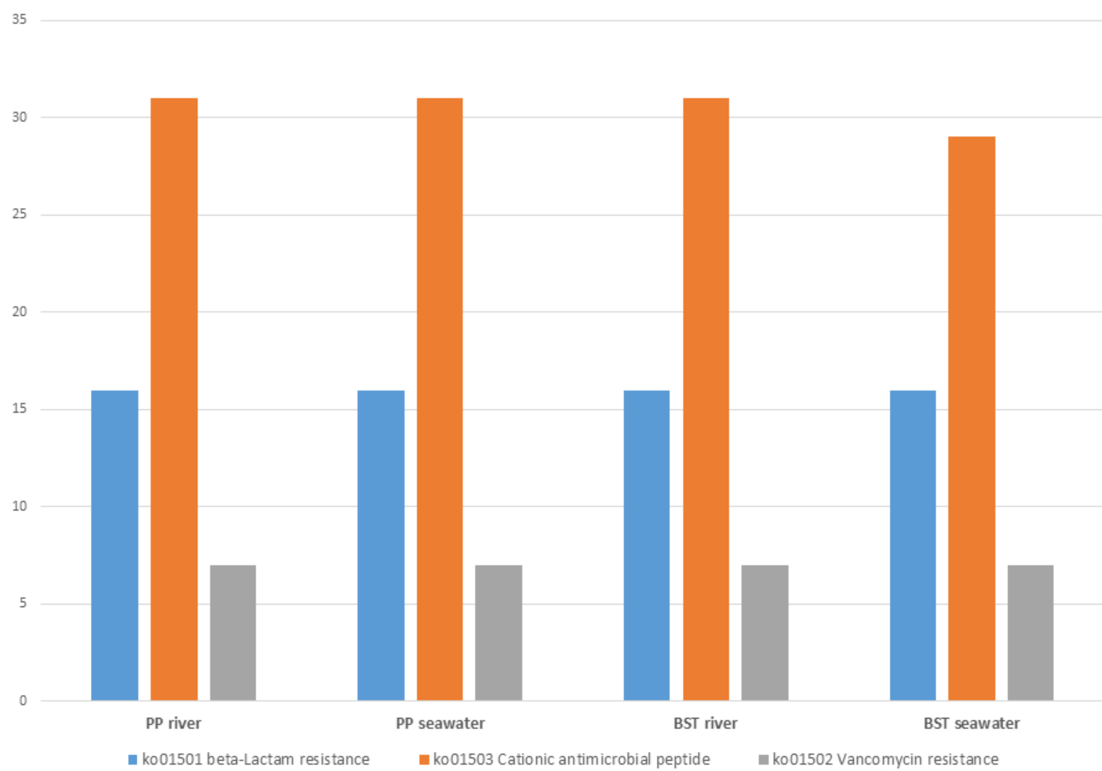
Supplementary figure 2: Canonical analysis of principal coordinates (CAP) performed to visualize the correlation between physicochemical properties and the bacterial community composition.



Supplementary figure 3: A) Venn diagrams with core microbiome (N) between environments at each site; B) Relative abundance (intensity values) of main phyla related to core microbiome.



Supplementary figure 4: A) Richness of genes related to antibiotic resistance pathways predicted through PICRUSt with samples clustered according site and environment.



Supplementary Table 1: Values of the environmental variables measured on the day of water collection for analyzes with PhyloChip™.

Sites	Temperature (°C)	Salinity (ppt)	pH	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	Turbidity (NTU)	Chlorophyll (RFU)	Chlorophyll (mg/L)	Thermotolerant Coliforms (NMP/100 mL)	*E. coli* (NMP/100 mL)
PP river 5000m	27,59	0,03	9,3	52,8	4,15	12	1	4,3	>16.000	>16.000
PP river 2500m	27,7	0,02	8,5	49,3	3,86	38	1,1	4,8	>16.000	>16.000
PP river mouth	28,47	0,27	7,5	61,1	4,72	30,5	0,8	3,8	>16.000	5.100
PP seawater 500m	28,19	0,17	7,0	60,8	4,71	19,5	0,1	0,2	>16.000	2.200
PP seawater 1000m	28,21	0,19	7,4	61,6	4,79	5	0,3	1,8	>16.000	0
PP seawater 1500m	29,27	2,13	,87	70,4	5,3	3,4	0,6	2,1	>16.000	0
BST river 5000m	26,35	0,04	7,7	45,4	3,65	22	0,2	3,65	>16.000	2.200
BST river 2500m	26,36	0,16	7,5	51	4,09	10	0,8	3,5	>16.000	0
BST river mouth	26,46	0,35	6,8	51,1	4,09	10,8	0,2	1	>16.000	0
BST seawater 500m	26,64	0,61	6,8	47,6	3,8	13,7	0,1	0,3	>16.000	0
BST seawater 1000m	27,15	2,6	6,3	60,2	4,7	15,4	0,1	0,2	>16.000	0
BST seawater 1500m	27,85	12,3	6,3	74,6	5,46	16	0,4	1,8	>16.000	0

Supplementary Table 2: Values of the environmental variables measured along the monthly monitoring using a multiparameter sonde.

	Temperature											
Points	**oct/17**	**nov/17**	**dec/2017**	**jan/18**	**feb/2018**	**mar/18**	**apr/2018**	**may/18**	**june/18**	**july/18**	**ago/18**	**sep/18**
PP (Beggining)	29,52	30,3	26,87	29,77	31,48	27,89	27,59	27,31	25,87	25,74	26,55	27,89
PP (Middle)	31,31	30,26	29,53	30,32	31,77	27,86	27,7	27,01	26,43	25,48	26,74	27,86
PP (Mouth)	30,51	30,92	28,75	29,14	31,42	27,7	28,47	26,92	26,25	26,86	27,13	27,7
PP (Sea - 500m)	28,98	29,72	28,58	29,19	32	27,66	28,19	27,21	26,25	26,18	27,55	27,66
PP (Sea - 1000m)	29,44	29,65	29,05	29,7	32,87	27,85	28,21	27,81	26,97	26,57	26,88	27,85
PP (Sea - 1500m)	29,58	28,28	28,6	29,86	29,3	28,28	29,27	27,73	26,88	26,55	26,74	28,28
BST (Beggining)	28,64	28,95	31,18	30,74	31,14	29,32	26,35	27,24	26,57	25,15	27,19	29,32
BST (Middle)	28,25	29,22	32	29,88	31,01	29,39	26,36	27,53	27,78	25,63	28,04	29,39
BST (Mouth)	28,62	29,35	31,29	30,46	31,33	30,3	26,46	28,31	27,64	26,12	27,86	30,3
BST (Sea - 500m)	29,09	29,68	31,74	29,98	31,2	30,03	26,64	28,38	28,07	26,23	28,03	30,03
BST (Sea - 1000m)	28,1	28,88	28,41	29,62	30,7	28,39	27,15	28,46	27,13	26,8	27,59	28,39
BST (Sea - 1500m)	27,3	27,96	28,29	29,56	29,87	28,78	27,85	28,43	27,15	27,07	27,34	28,78

	pH											
Points	**oct/17**	**nov/17**	**dec/2017**	**jan/18**	**feb/2018**	**mar/18**	**apr/2018**	**may/18**	**june/18**	**july/18**	**ago/18**	**sep/18**
PP (Beggining)	6,76	6,83	8,04	8,82	8,1	8,79	9,35	8,04	8,89	8,83	9,75	8,79
PP (Middle)	6,97	7,23	7,95	8,23	7,94	8,52	8,58	7,97	8,37	7,7	9,09	8,52
PP (Mouth)	7,27	8,13	7,6	7,64	7,96	8,36	7,51	7,5	7,8	7,5	8,37	8,36
PP (Sea - 500m)	8,46	8,56	7,8	7,37	7,37	8,24	7,09	7,31	7,1	7,8	8,18	8,24
PP (Sea - 1000m)	8,32	8,59	7,77	7,28	7,33	8,07	7,45	7,62	6,92	8,1	8,25	8,07
PP (Sea - 1500m)	7,97	8,55	8	7,33	8,2	8,23	6,87	8	7,61	7,93	8,17	8,23
BST (Beggining)	8,31	8,46	8,38	8,04	8,04	8,86	7,72	8,38	7,59	8,83	8,17	8,86
BST (Middle)	8,31	8,7	7,8	8,02	8,02	8,86	7,5	7,8	7,65	8,8	8,17	8,86
BST (Mouth)	7,41	8,65	7,83	7,88	7,84	8,3	6,88	7,9	7,68	8,1	8,08	8,3
BST (Sea - 500m)	8,84	8,62	7,76	8,1	7,8	8,08	6,83	7,73	6,95	8,41	8,23	8,08
BST (Sea - 1000m)	8,48	8,59	8	7,94	8,2	8,25	6,37	7,7	7,4	7,9	8,58	8,25
BST (Sea - 1500m)	8,15	8,51	7,9	7,96	8,01	8,15	6,3	7,9	7,43	8,05	8,57	8,15

	Salinity (ppt)											
Points	**oct/17**	**nov/17**	**dec/2017**	**jan/18**	**Feb/2018**	**mar/18**	**Apr/2018**	**May/18**	**June/18**	**July/18**	**ago/18**	**sep/18**
PP (Beggining)	-	-	1,94	0,38	6,7	3,51	0,03	4,56	0,01	0,37	0,37	3,51
PP (Middle)	-	-	3,37	0,89	6,7	5,28	0,02	1,23	0,02	1,46	1,46	5,28
PP (Mouth)	-	-	29,44	2,69	11,31	9,46	0,27	0,28	0,42	6,56	6,56	9,46
PP (Sea - 500m)	-	-	30,7	10,41	19,01	23,52	0,17	9,24	0,47	7,66	7,66	23,52
PP (Sea - 1000m)	-	-	35,1	14,89	24,88	25,63	0,19	40,89	1,27	6,82	6,82	25,63
PP (Sea - 1500m)	-	-	35,2	15,66	34,6	27,39	2,13	39,1	2,94	9,67	9,67	27,39
BST (Beggining)	-	-	1,61	23,41	15,45	9,57	0,04	1,7	0,5	0,98	0,98	9,57
BST (Middle)	-	-	6,3	34,29	27,7	9,57	0,16	25,85	0,8	0,15	0,15	9,57
BST (Mouth)	-	-	26,38	34,41	35,92	28,58	0,35	44,3	5,54	0,58	0,58	28,58
BST (Sea - 500m)	-	-	18,2	34,97	37,29	18,58	0,61	44,63	8,832	0,69	0,69	18,58
BST (Sea - 1000m)	-	-	34,6	34,97	38,55	35,34	2,6	45,91	10,14	5,94	5,94	35,34
BST (Sea - 1500m)	-	-	34,8	35,21	38,84	36,58	12,3	24,47	10,21	8,97	8,97	36,58

	Dissolved Oxygen (%)											
Points	**oct/17**	**nov/17**	**dec/2017**	**jan/18**	**feb/2018**	**mar/18**	**apr/2018**	**may/18**	**june/18**	**july/18**	**ago/18**	**sep/18**
PP (Beggining)	62,9	94,7	71,8	51	63,8	104,2	52,8	97,3	72,4	77,1	77,5	104,2
PP (Middle)	98,1	113,2	75	65,2	65	105,1	49,3	70	74,6	79,2	86,4	105,1
PP (Mouth)	98,1	111,2	90,7	90,34	86,4	104,2	61,1	64,5	79,7	99,2	104	104,2
PP (Sea - 500m)	108,5	163,2	93,8	83,4	123	105,1	60,8	93,5	83,2	98,4	116	105,1
PP (Sea - 1000m)	117,4	111,3	108,1	97,1	106	102,3	61,6	111,9	99,3	103,7	106,7	102,3
PP (Sea - 1500m)	109,5	114,5	113,2	98,4	107	96,2	70,4	112,8	97,1	108,5	108,5	96,2
BST (Beggining)	36,2	43,2	47,7	107,5	82	110,8	45,4	77,6	95	71,5	37,4	110,8
BST (Middle)	36,2	86,1	81	107,7	109,6	110,8	51	106,6	126,9	80	131	110,8
BST (Mouth)	91,1	105	121,2	106,4	115,5	122	51,1	116	128,1	93,3	130	122
BST (Sea - 500m)	127,3	102	135,8	103,2	115,8	124	47,6	115	132,1	96	123	124
BST (Sea - 1000m)	110,5	101,8	115,1	104	113	115	60,2	105	104,9	116,1	118	115
BST (Sea - 1500m)	104,13	102,9	114	103	103	114	74,6	100,6	106,6	115,9	106	114

	Turbidity (NTU)											
Points	**oct/17**	**nov/17**	**dec/2017**	**jan/18**	**feb/2018**	**mar/18**	**apr/2018**	**may/18**	**june/18**	**july/18**	**ago/18**	**sep/18**
PP (Beggining)	14,1	23	29,5	60	39	23	12	12	20,5	16,4	17,2	23
PP (Middle)	14,7	7,1	20	58	31,7	15,4	38,6	38,6	17,5	17	20,2	15,4
PP (Mouth)	14,7	7,4	6,9	36,2	11,2	7	30,5	30,5	19	14,6	9,9	7
PP (Sea - 500m)	7,6	7,8	5,9	27,7	17,6	7,2	19,5	19,5	15,5	7,9	6,2	7,2
PP (Sea - 1000m)	3,2	6,6	4,6	12	34,7	8,3	5	5	9,4	6	8,6	8,3
PP (Sea - 1500m)	3,1	4,9	4,1	14,4	7,2	5,5	3,7	3,7	7,4	7,4	2,8	5,5
BST (Beggining)	3,1	22,1	9,5	12,8	19,9	9,2	22	22	21,8	18,8	8	9,2
BST (Middle)	3,1	15,7	15,2	9	13,4	9,2	10	10,8	13,91	21	5,6	9,2
BST (Mouth)	9,5	7,7	11,2	10,3	9,8	9	10,8	10,8	16,2	23,6	9,8	9
BST (Sea - 500m)	6,3	8,4	10,2	8,2	4,8	8,8	13,7	13,5	14,4	19,17	4	8,8
BST (Sea - 1000m)	5,2	9,3	6,9	7,2	3,9	4,3	15,4	15,6	15,4	19,2	5,3	4,3
BST (Sea - 1500m)	3,7	14,3	-2,5	8,1	6,1	6	16	17,8	10,7	12,5	4,9	6

	Chllorophyll (RFU)											
Points	**oct/17**	**nov/17**	**dec/2017**	**jan/18**	**feb/2018**	**mar/18**	**apr/2018**	**may/18**	**june/18**	**july/18**	**ago/18**	**sep/18**
PP (Beggining)	-0,1	0,2	3,707	0,1	0,1	2,4	1	1,3	0,4	0,6	1,1	2,4
PP (Middle)	0,2	1,4	2,2	0,2	0,2	2,2	1,1	0,2	0,2	0,6	2,3	2,2
PP (Mouth)	0,2	-0,8	4,1	0,1	0,1	0,7	0,8	0,1	0,3	1	2	0,7
PP (Sea - 500m)	3,5	-1	3	0,2	0,1	0,7	0,1	0,4	0,6	0,2	1,4	0,7
PP (Sea - 1000m)	2,1	-0,18	1,8	0,6	0,1	0,9	0,3	0,3	0,1	0,1	1,6	0,9
PP (Sea - 1500m)	1	0	0,4	0,6	0	0,4	0,6	0,2	0,5	0	0,7	0,4
BST (Beggining)	-0,5	4,9	-0,2	1,9	2,5	4	0,2	1,3	2,1	1,1	1,4	4
BST (Middle)	-0,5	7,7	0,2	0,5	2,3	4	0,8	2,2	2	1,6	1,8	4
BST (Mouth)	8,5	3,8	2,6	0,1	1	0,7	0,2	1,6	1,2	2,8	2,1	0,7
BST (Sea - 500m)	6,8	2	2,2	0,1	0,1	1,4	0,1	1,4	1,4	3	0,4	1,4
BST (Sea - 1000m)	2	1,5	0,2	0,2	0,1	0,2	0,1	0,4	0,4	5,2	0,2	0,2
BST (Sea - 1500m)	0,8	1,6	0,8	0	0,1	0,2	0,4	0,2	0,1	0,3	0,2	0,2

	Total Coliforms (MPN/100 mL)											
Points	**oct/17**	**nov/17**	**dec/2017**	**jan/18**	**feb/2018**	**mar/18**	**apr/2018**	**may/18**	**june/18**	**july/18**	**ago/18**	**sep/18**
PP (Beggining)	16.000	9.200	16000	16000	16000	16000	16000	16000	16000	16.000	16000	16000
PP (Middle)	16.000	9.200	16000	16000	16000	16000	16000	16000	16000	16.000	16000	16000
PP (Mouth)	16.000	0	16000	16000	16.000	16000	16000	16000	16000	5.100	16000	16000
PP (Sea - 500m)	16.000	0	9.200	16000	9.200	9.200	16000	16000	16000	5.100	16000	9.200
PP (Sea - 1000m)	9.200	0	9.200	16.000	5.100	9.200	16000	9.200	16000	2.200	16000	9.200
PP (Sea - 1500m)	9.200	0	5.100	16.000	0	9.200	16000	2.200	16000	2.200	0	9.200
BST (Beggining)	16.000	16.000	16000	9.200	16000	16000	16000	16000	16000	16000	16000	16000
BST (Middle)	16.000	16000	16000	5.100	16000	16000	16000	16000	16000	16000	16000	16000
BST (Mouth)	16000	16000	16000	5.100	5.100	9.200	16000	16.000	16.000	16000	16000	9.200
BST (Sea - 500m)	9.200	16.000	16000	2.200	0	5.100	16000	2.200	2.200	16000	16000	5.100
BST (Sea - 1000m)	0	9.200	5.100	0	0	5.100	16000	2.200	0	16.000	16000	5.100
BST (Sea - 1500m)	0	0	0	0	0	0	16000	0	0	2.200	0	0

	E. coli (MPN/100 mL)											
Points	**oct/17**	**nov/17**	**dec/2017**	**jan/18**	**feb/2018**	**mar/18**	**apr/2018**	**may/18**	**june/18**	**july/18**	**ago/18**	**sep/18**
PP (Beggining)	16.000	16.000	16000	16000	16000	16000	16000	9.200	16000	16.000	16000	16000
PP (Middle)	16.000	16.000	16000	16000	16000	16000	16000	2.200	16000	16.000	16000	16000
PP (Mouth)	16.000	0	16000	16000	5.100	16000	5.100	9.200	16000	5.100	16000	16000
PP (Sea - 500m)	16.000	0	9.200	16000	2.200	9.200	2.200	16000	16.000	5.100	16000	9.200
PP (Sea - 1000m)	9.200	0	9.200	16.000	0	9.200	0	5.100	16.000	2.200	16000	9.200
PP (Sea - 1500m)	9.200	0	5.100	16.000	0	9.200	0	5.100	5.100	2.200	0	9.200
BST (Beggining)	16.000	16.000	16.000	9.200	2.200	16000	2.200	16000	16000	16.000	16000	16000
BST (Middle)	16.000	5.100	16.000	5.100	0	16000	0	16000	16000	5.100	16000	16000
BST (Mouth)	16000	16000	16.000	5.100	0	9.200	0	16.000	5.100	5.100	16000	9.200
BST (Sea - 500m)	9.200	16.000	9.200	2.200	0	0	0	2.200	2.200	5.100	16000	0
BST (Sea - 1000m)	0	5.100	9.200	0	0	0	0	2.200	0	5.100	16000	0
BST (Sea - 1500m)	0	0	0	0	0	0	0	0	0	0	0	0

	Acummulated rain (Inmet)											
	out/17	**nov/17**	**dez/17**	**jan/18**	**fev/18**	**mar/18**	**abr/18**	**mai/18**	**jun/18**	**jul/18**	**ago/18**	**set/18**
BST	58,2	11,9	45,7	144,4	125,6	128,2	424,8	241,4	149	232,4	67	69,4
PP	20,0	20,5	50,5	109,3	125,3	219,1	285,1	119,9	113,5	66	26,2	32,1

5 CAPÍTULO 2

Dynamics between rainy and dry season in the microbiota of healthy and bleached individuals of coral *Siderastrea stellata* in response to environmental changes and local stressors[1]

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Abstract

Despite coral bleaching being mainly associated with global warming, the importance of local stressors is growing. Here, we investigated the microbiota associated to mucus and tissue of endemic coral *Siderastrea stellata*, collected from coral reefs of Barra de Santo Antônio (subject to river runoff) and Maragogi, both within a Brazilian federal marine conservation unit, during rainy and dry season. We used Illumina MiSeq to sequencing the V4 region of 16S rDNA and several packages to process data, perform statistical analysis and functional predictions. Differences between treatments were mainly associated with the enrichment of several less abundant taxa involved in important functions, essential putative prokaryotic functional groups and predicted metabolic pathways in healthy than bleached individuals. Was not possibly to observe a dominance of groups already associated to bleaching as Vibrionaceae or Burkholderiaceae. The influx of freshwater seems to increase the homogeneity between individuals in Barra de Santo Antonio, especially during the rainy season. In the other hand, in Maragogi it happened mainly during dry season. Understanding how the coral microbiota behaves and how bleaching appears in response to different situations, in addition to determining persistent groups over environmental changes, is essential to improve approaches related to increase coral resistance.

Introduction

Coral reefs are essential to a healthy marine ecosystem and are important, directly or indirectly, for at least 500 million people living in coastal regions around the world (Burke et al., 2011). Unfortunately, corals around the world are facing a crisis mainly related to increasingly frequent and intense bleaching events as a consequence of prolonged periods of increases in sea surface temperature. The most recent (2014-2017) is considered the most severe, widespread and longest-lasting ever recorded (Baker et al., 2008; Hughes et al., 2017; Macneil et al., 2019, Stuart-Smith et al., 2018, Sully et al., 2019). Coral bleaching is a stress response that results in a breakdown of the relationship between a coral host and its symbiotic algae (genus: *Symbiodinium*), responsible for transferring photosynthetically fixed carbon to its host (Glynn, 1993). Failure to maintain this symbiotic relationship leads to a reduction of *Symbiodinium* abundance and photosynthetic pigment loss, giving the coral a pale appearance called bleaching (Jokiel and Coles, 1990).

However, this “crisis” is also a result of direct local and regional-scale human impacts on their environment through accelerated industrialization, urbanization, and agriculture (Lapointe et al., 2019) which includes actions such as pollution, overfishing, tourism and sewage discharge in coastal environments (Zaneveld et al., 2016; Ziegler et al., 2016). From a local perspective, the presence of Rivers that flow into coral reefs can act as a natural stress source due to sediment, nutrients, freshwater (which contributes to saline stress) and potential pathogens input. It may also transport xenobiotics, such as herbicides and pesticides from agricultural practices occurring near the shore, and sewage discharged as a result of urbanization (Fabricius, 2005; Pires et al., 2015).

After the landmark work of Rohwer and colleagues (2002), corals are now considered holobiont organisms formed by complex interactions between the host animal, *Symbiodinium* and an array of other microorganisms known to perform several essential functions to coral host (reviewed by Bourne et al., 2016; Morrow et al., 2018; Raina et al., 2009). In addition, it has been suggested recently that there is a ubiquitous core microbiome associated with corals that are likely beneficial to host health, together with functional niche fillers and other highly variable microbial community members (Ainsworth et al., 2015;

Hernandez-Agreda et al., 2017). The coral microbiome can be modulated by the host (Reshef et al., 2006; Rosenberg et al., 2009), and at the same time, it is highly dynamic and especially affected by changes in environmental conditions (Hernandez-Agreda et al., 2018; Lin et al., 2016; Yang et al., 2017). Further observations led to the Probiotic Hypothesis, a model that suggests that changes in the microbial communities under different environmental conditions would allow a quick and versatile adaptation of coral holobiont, primarily through the enormous genetic arsenal of associated microorganisms (RESHEF et al., 2006). More recently, the term Benefical Microorganisms for Corals (BMC) has been proposed referring directly to the symbiotic microorganisms that can act in the maintenance and protection of the physiological balance of corals (PEIXOTO et al., 2017).

Due to the emergence of this approach related to coral studies, bleaching is also been seen as a change in the composition of the host's microbiota, which turns from a healthy state to a dysfunctional state where functionally important groups have their populations reduced (BOURNE et al., 2008; LITTMAN et al., 2011 BOURNE et al., 2016). Usually, alterations in microbial diversity and the occurrence of opportunistic microbial taxa such as Vibrionaceae and Pseudoaltermonadaceae are closely associated with the development of diseases as bleaching (Bourne et al., 2009; Rosenberg and Falkovitz, 2004). Comparisons of healthy and bleached coral metagenomes subjected to environmental stresses, such as increased surface temperature and water acidity, for example, have shown changes in the abundance of groups related to the cycling of sulfur, nitrogen, carbohydrates and fatty acids within the coral, for example (Littman et al., 2011; Webster and Reusch, 2017).

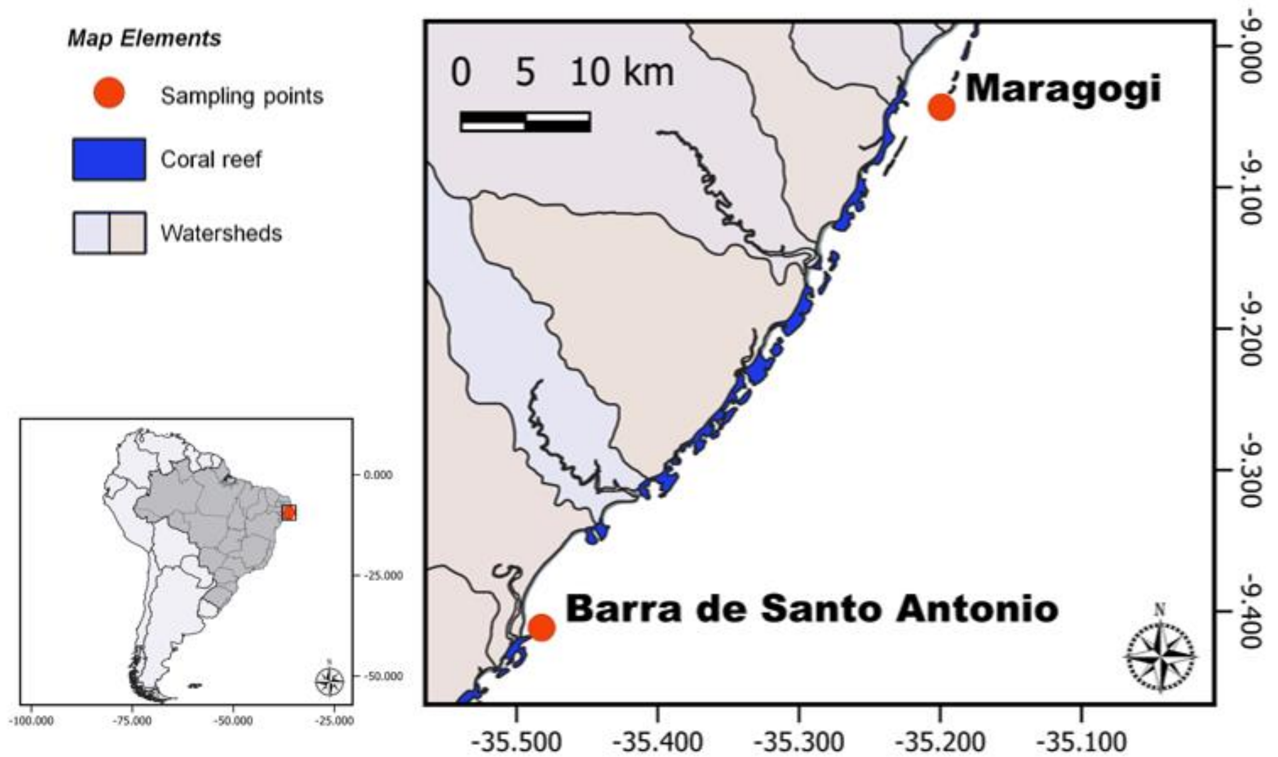
In this work, we explored the bacterial diversity and its predicted functional groups and metagenomic content associated to healthy and bleached colonies of coral *Siderastrea stellata*, a zooxanthellated stony coral endemic to Brazil (Knowlton and Jackson, 2013), collected in two different reefs, one of them influenced by runoff from a local river, to determine dynamics of coral-associated microbiota along space and time in order to explore possible local adaptations that might be helping to keep some individuals healthy even though stress related to local environmental condition.

Material and methods

Study area

The study was conducted in the *Área de Proteção Ambiental Costa dos Corais* (APACC), located in the eastern region of the Alagoas State – Brazil. APACC is one of the largest federal marine conservation units in Brazil, covering approximately 4,135 km² and extending for about 135 km of beaches and mangroves (Walker and Schlacher, 2014). Samples were collected in two sites included in the management plan of APACC: a) Barra de Santo Antonio (Barra) (S09.41159 W35.48230), where coral reefs are approximately 2,4 km away from the coast and receive the influx of freshwater from Santo Antonio River, with an extension of approximately 18.5 km. This area was chosen because it presents known seasonal variations in environmental factors (mainly temperature, salinity, turbidity and pH) and a microbial community associated with freshwater and reef surrounding seawater that indicated a possible slight of anthropic contamination, as demonstrated in a recent study (Paulino et al., 2020); b) Maragogi (S09.04336 W35.19904), where coral reefs are located approximately 3.5 km off the coast, acting as a control point, as it is not influenced by the discharge of any local river, and comprise a sandstone bank with a thin framework formed by corals, calcareous algae and vermetid mollusks, and human visits are concentrated to specific locations at low-tide (Figure 1). Permission for this study was obtained from the regulatory institution Instituto Brasileiro do Meio Ambiente (license no. L. 32723-1).

Figure 1: Map showing the collection points at each site (red) and the coral reefs (blue). The samples were collected during low tide, and randomly on each reef, to avoid bias.



Physicochemical properties of the seawater and fecal indicator analysis

At the sampling moment, the physical-chemical parameters (pH, temperature, salinity, turbidity, dissolved oxygen and chlorophyll) of the reef's surrounding seawater were measured using Ysi 6600V2. Also, 100 mL of water were collected at each point and sent on ice to the laboratory, where the f data were analyzed through the Most Probable Number (MPN) counting of *Escherichia coli* and *Enterococcus* sp. (total coliforms), where the samples are initially inoculated into Lauril Tryptose broth, and after that, aliquots of the tubes that showed positive results are transfered to Brilliant Green and EC broths, according to Environmental Protection Agency (USEPA, 2006). Precipitation data were obtained from the website of the state agency Secretariat for the Environment and Water Resources of Alagoas State (Secretaria de Estado do Meio Ambiente e dos Recursos Hídricos – SEMARH).

Coral sampling and tissue and mucus extraction

The collections were carried out in April and September of 2019, corresponding to the rainy and dry season, respectively. In each reef, we used a punch core and hammer to collect 3 healthy and 3 bleached colonies of *S. stellata* at 1.5 up to 2 meters depth. We considered as bleached the individuals that had lost at 100 % of colony surface coloration and healthy colonies completely brown, according to the work of Sassi et al. (2014), who described the pigmentation patterns of this species under different local environmental conditions. Samples were stored in sterile plastic bags and kept on ice until the laboratory.

Samples were washed with sterile seawater for removing debris and then sprayed with TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 8.0) using an airbrush (80 PSI) (Yang et al., 2017). This method physically separated the coral mucus and tissue from the skeleton and suspended the cellular material in a slurry. The slurry was homogenized and centrifuged at 6.000 rpm for 10 min to form pellets comprised of coral tissue and mucus. The supernatant was removed and the slurry resuspended in 1 mL of TE (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 8.0) then kept at -20 °C until performing DNA extractions.

Coral DNA extraction

The DNA extraction protocol was followed as proposed by Sun et al (2014), with some modifications. Initially, samples were frozen in liquid nitrogen and then ground using pestle and mortar. Next, 1 mL of CTAB extraction buffer (4 g CTAB, 16.4 g NaCl, 20 mL 1 M Tris-HCl, 8 mL 0.5 M EDTA, and 200 mL distilled water) was added to the pellets followed by vortex during 5 min. After incubation at 65°C for 1 h, the tubes were centrifuged at 14.000 rpm for 10 min. The supernatants were then transferred to tubes on ice and extracted with an equal volume of phenol/chloroform until the interface was clean. Genomic DNA was precipitated overnight with 0.3 M sodium acetate (pH 5.2) and isopropanol at -20°C and collected by centrifugation at 14.000 rpm for 20 min. After removal of the supernatant, the pellets were washed with 1 mL of ethanol 70 % and left to dry at environmental temperature. The DNA was resuspended in 50 mL sterile Milli-Q water A and stored at −20°C.

16S Amplification and sequencing

For amplicon sequencing on the Illumina MiSeq platform, the variable V4 region of the 16S rDNA gene was amplified using the primer pair 515F-Y (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACNVGGGTWTCTAAT-3′) that targets bacterial and archaeal communities (Walters et al., 2016). Primers contained Illumina adapter overhangs. Polymerase Chain Reactions were performed in triplicate (10–20 ng of DNA) with a 2x Kapa HiFi HotStart Ready PCR kit and a final primer concentration of 0.25 µM in a final reaction volume of 25 µL. The following PCR conditions were used: initial denaturing at 95 °C for 3 min, 35 cycles each at 98 °C for 20 s, 55°C for 30 s, and 72 °C for 30 s, followed by a final extension step at 72 °C for 5 min. The PCR products were visually assessed via 1% agarose gel electrophoreses (5 µL) and subsequently cleaned, indexed (Nextera XT indexing adapters), and purified with AMPure XP beads. Amplicon PCRs for each sample were quantified on the BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA) and by QuBit (Quant-IT dsDNA Broad Range Assay Kit, Invitrogen, Carlsbad, CA, USA) and pooled in equimolar ratios. Amplicon pools were paired-end sequenced (2x150 bp) on the Illumina MiSeq sequencing platform using v2 chemistry according to the manufacturer's specifications. Sequencing was performed at the Genomics and Bioinformatics Center of Drug Research and Development Center of Federal University of Ceará, Brazil.

Quality filtering and sequencing analysis

We choose the QIIME 2 package (version 2020.11) to process the raw sequences and perform most of the further analysis (Bolyen et al., 2019). In brief, samples were imported and demultiplexed with q2-DEMUX plugin and paired-ended merged with PEAR (Paired-End reAd merger) v0.98. Reads were filtered (total expected error or total sum of the error probabilities >1), dereplicated, chimera removed and the remaining high-quality reads were binned into Amplicon Sequence Variants (ASVs) 250bp longer with q2-DADA2 plugin (Callahan et al., 2016). The representative sequence for each feature was aligned against a pre-trained SILVA v138 database and taxonomically annotated using VSEARCH-based consensus taxonomy classifier at default settings (Rognes et

al., 2016). To further analysis, singletons, non-bacteria, mitochondria, chloroplasts or features that remained unidentified (i.e, “NA”) at the kingdom level were removed from the dataset. The raw dataset has been deposited under accession number PRJNA747031 in the NCBI SRA.

Data visualization and statistical analysis

Qiime2 artifacts were imported to R software through qiime2R (v0.99.5) package and statistical analysis was conducted using Phyloseq version 1.34.0 (MCMURDIE, HOLMES, 2013). The dataset was rarefied according to the minimum sample, which showed to be sufficient according to the rarefaction curve. We calculated three alpha-diversity metrics that put more or less weight on common species: (1) Observed richness, (2) Shannon exponential, which weighs ASVs by their frequency, and (3) Simpson multiplicative inverse, which overweighs abundant ASVs. After, we performed a Mann-Whitney pairwise test to check if those metrics differ significantly between coral condition and seasonality. A Permutational Multivariate Analysis of Variance (PERMANOVA) using distance matrices (PERMANOVA/Adonis) (Oksanen et al. 2018) was performed based on Bray–Curtis dissimilarity index using 999 permutations to determine the degree to which the physicochemical factors explained the microbial community composition of the samples ($p < 0.05$).

We generated graph information about relative abundance at each taxonomic level and a Non-parametric multidimensional scaling (nMDS) based on Bray-Curtis distance metric to graphically represent relationships between the microbial abundance and composition variability between sites and health condition in multidimensional space. Differences between bacterial communities associated to both treatments at each site and season were investigated using the Analysis of Similarity (ANOSIM). Differential-Abundance analysis at each taxonomic level were performed using DESeq2 package with adjusted p-value cutoff = 0.05 to verify which groups showed variations between healthy and bleached coral-associated communities.

The PIME (Prevalence Interval for Microbiome Evaluation) package (ROESCH et al., 2020) was used to determine the core microbiome among the best prevalence range predicted by its supervised machine learning Random

Forest algorithm. The importance of each ASV in finding core microbiome differences between treatments at the best prevalence interval was also determined by PIME. In the end, a validation step was applied to check for possible type I errors (finding false-positive results).

The functional profile of bacterial communities was predicted using FAPROTAX (Functional Annotation of Prokaryotic Taxa) (Louca et al., 2016), through python script collapse_table.py using the ASV table generated by QIIME2 as input. For predicting the metagenomics content through 16S rDNA sequences of two different treatments, PIPHILLIN pipeline (IWAI et al., 2016) was applied with the support of Kyoto Encyclopedia of Genes and Genomes (KEGG - May, 2017 release) and a threshold of 0.97. The DESeq2 package was used to detect differentially abundant KEGG orthologues (KO) between treatments, and a PCA (Principal Coordinate Analysis) was performed to graphically represent relationships between functional content.

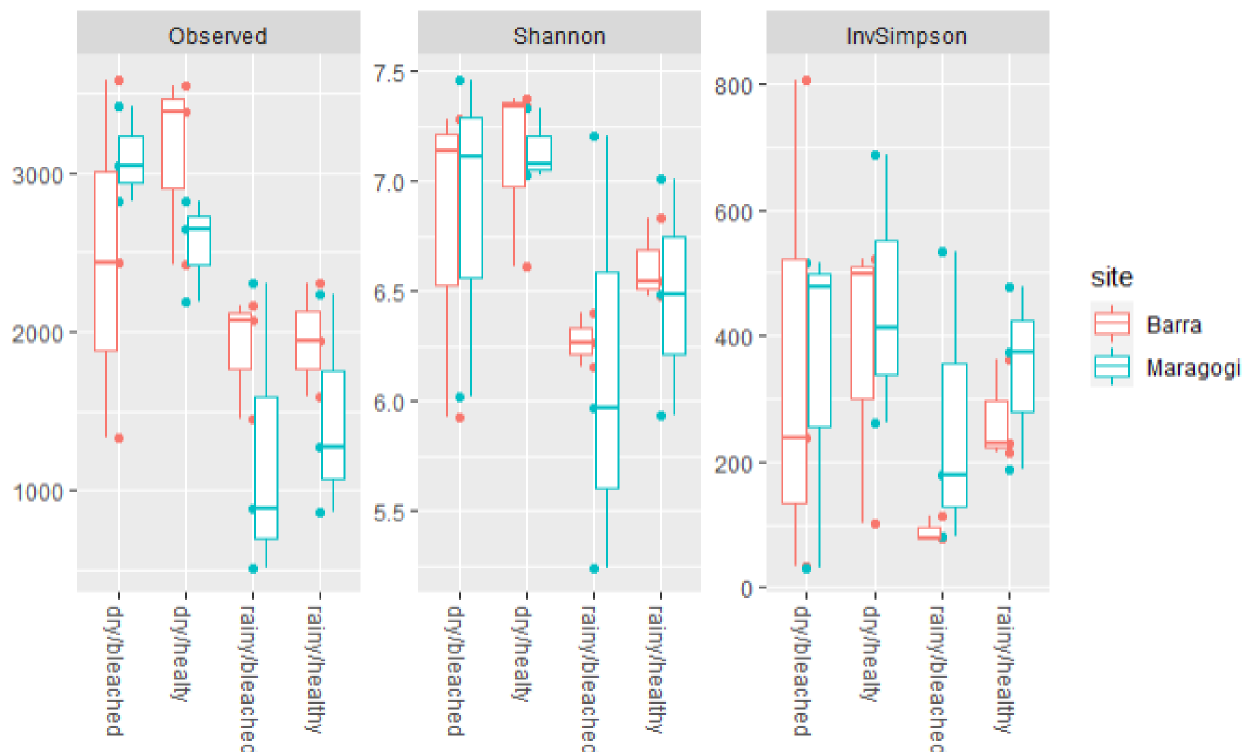
Results

From 3.4 million raw reads generated by sequencing, 2.14 million reads remained after quality control. Initially were formed 31.645 ASVs, but 25.146 ASVs were maintained after the exclusion of sequences which not belonged to bacteria or were not taxonomically assigned. In the end, the total sequences per sample ranged from 54.671 to 123.191.

The physicochemical parameters were characteristic of each region and season, with temperatures below the threshold usually associated with bleaching in tropical environments (> 30°C). Based on total coliforms and *E. coli* count, none of the points showed signs of fecal pollution (MPN = 0) at the sampling moment. Precipitation data showed significant differences between seasons ($p < 0,05$), with rainy season starting in April (accumulated average of 172.8 mm in Barra and 175.8 mm in Maragogi) and ending in August, while in September Barra accumulated 39.9 mm of rain and Maragogi 56.6 mm (Supplementary Table 1). The results of PERMANOVA analysis indicate that only precipitation significantly influenced differences between samples from the same reef at different seasons ($F = 1.8135$, $R^2 = 0.21385$ and p-value = $1e^{04}$).

According to Shannon, Inverse Simpson and Observed ASV's indexes, bleached corals in Barra harbored a higher richness and diversity average than healthy corals during the rainy season, while in the dry season this characteristic was found among healthy samples. In Maragogi, it was possible to observe the inverse scenario, where bleached corals were richer and more diverse during the dry period, while in other moment healthy corals showed this characteristic. However, this difference was not statistically significant under any of these conditions (Mann-Whitney pairwise test p-values > 0.1). When comparing these indexes according to seasonality, the average richness and diversity were higher in both treatments and coral reefs during the dry period, with significant differences in relation to the rainy period in all data sets (Mann-Whitney pairwise test p-values < 0.05).

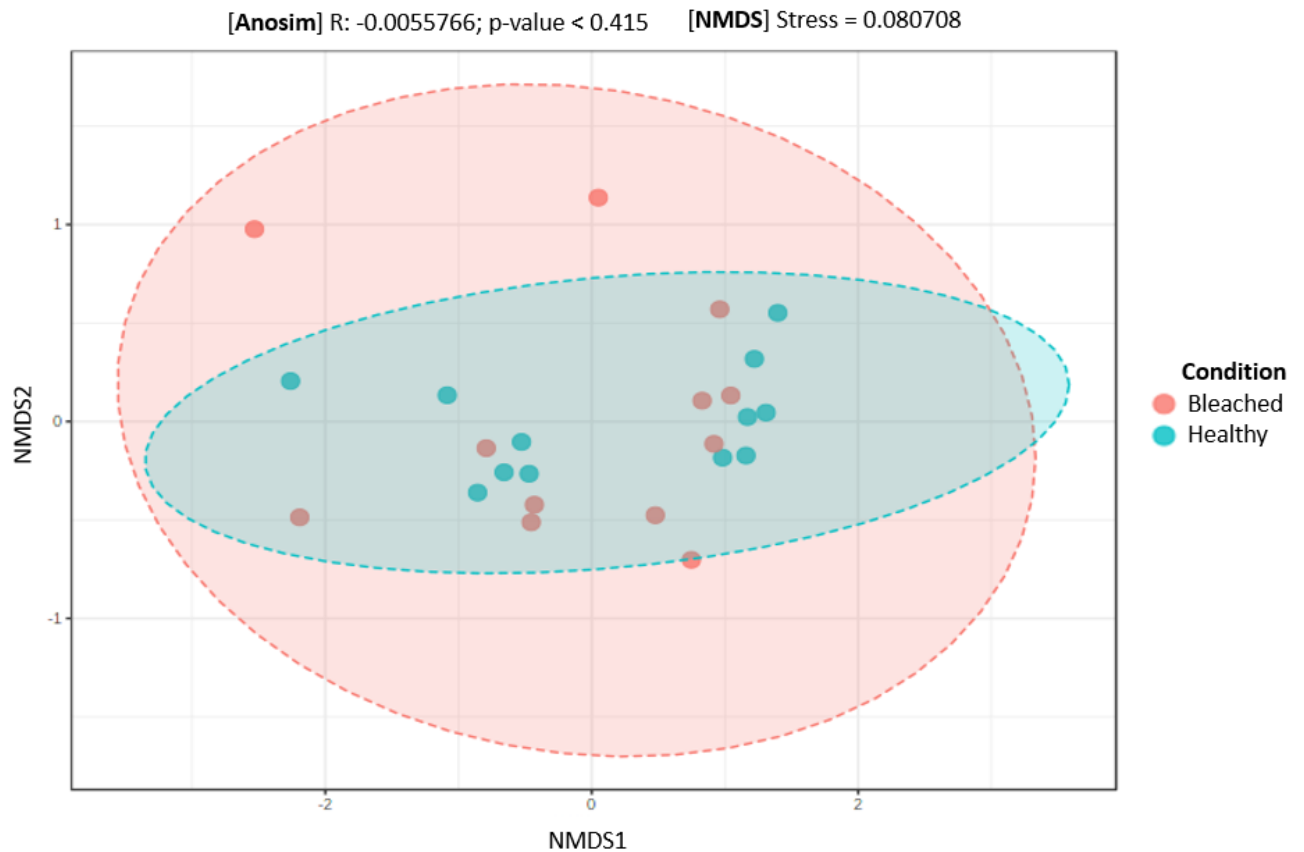
Figure 2: Main alpha-diversity indexes with samples grouped according to: treatment (healthy or bleached), reef (Barra de Santo Antônio or Maragogi) and season (Rainy = April and Dry = September).



A suite of multivariate analyses was performed to test for differences between the bacterial communities across health condition (by comparing healthy

and bleached samples) at each reef and season. According to Non-Metric Multidimensional Scaling analysis based on Bray-Curtis distance, *S. stellata* bacterial communities were highly dynamic, responding mainly to seasonality than health condition or site (ANOSIM = R: 0.78, p-value < 0.001; ANOSIM = R: -0.005, p-value < 0.41 and ANOSIM = R: 0.07, p-value < 0.09, respectively), with minimum overlap between the 95% confidence ellipses representing each sampling month (Figure 3). As for the coral condition, healthy and bleached corals from Barra showed bacterial communities with moderate dissimilarity during the rainy season (R: 0.44 and significance = 0.2), while it was greater in the dry season (R: -0.14 and significance = 0.9). In Maragogi the results were reversed, with the microbiota in the rainy season showing higher dissimilarity between healthy and bleached hosts (R: -014 and significance = 0.8) than in the dry period, where this overlap was moderate (R: 0.44 and significance = 0.1).

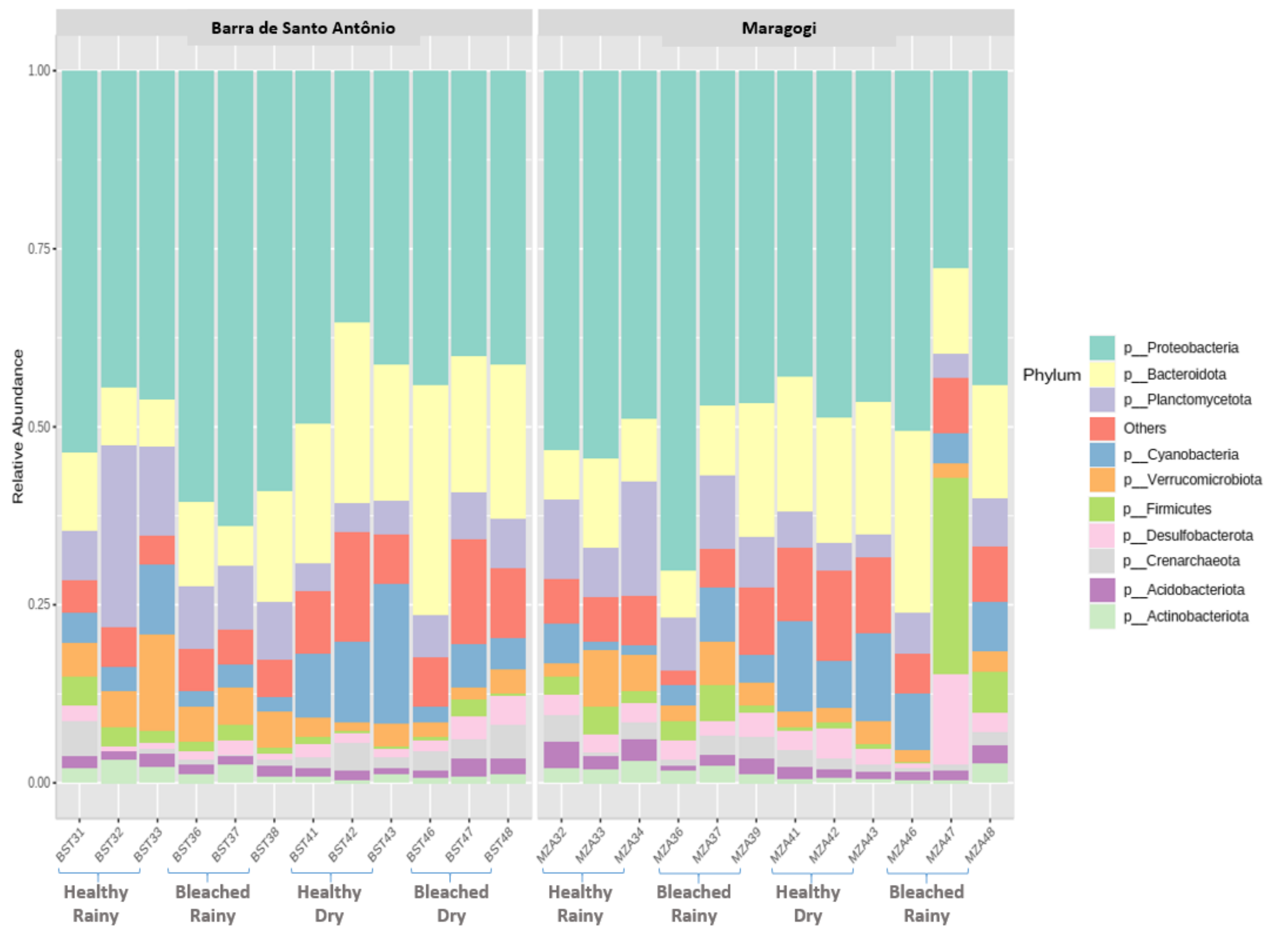
Figure 3: Non-Metrical Dimensional Scale (nMDS) plot of the Bray–Curtis based dissimilarity matrix of bacterial communities colonizing the mucus and tissue of healthy and bleached samples of coral *Siderastrea stellata* collected during the dry and rainy seasons of two distinct coral reefs.



We analyzed the top proportionally bacterial taxa in the whole data and tried to identify groups whose abundance significantly varied between both treatments using the algorithm DESeq2. At phylum category, Proteobacteria dominated the bacterial community of all samples, except for MZA47 (bleached sample from Maragogi; relative abundance: 27.34 ± 57.58%), followed by Bacteroidota (5.7~30.22%), Planctomycetota (5.02~24.96%), Verrucomicrobiota (2.43~14.32%), Desulfobacterota (12.86%) and Cyanobacteria (0.46~7.9%) (Figure 4). Overall, Alpha- and Gammaproteobacteria (15.38~32.97% and 14.28~36.25%, respectively), dominated the bacterial community at class level, followed by Bacteroidia (6.89~29.97%) and Planctomycetes (1.57~23.83%) (Supplementary figure 1). At the family level, minor representative taxa comprised ±50% of microbiota. Rhodobacteraceae was the most representative, especially in samples collected during the dry season, where it divided dominance with Flavobacteriaceae (Supplementary figure 2). Meanwhile, Pirellullaceae was one of the most abundant families in all samples during rainy season. Among bleached samples, all collected during rainy season in Barra showed a greater

abundance of Burkholderiaceae, and this fact was not repeated in any other site or season.

Figure 4: Relative abundance of top-10 phyla. Samples grouped according to season and coral condition.



As seasonality was the main discriminating factor among microbial communities, further analyzes were performed with the data set divided according to each coral reef and season. Differential abundance analyzes showed that the differences between healthy and bleached corals could be attributed mainly to “rare” members of the microbiota, which varied in response to seasonality. The results at family level are available in table 1, while the results at upper taxonomic levels are available in Supplementary Table 2. In short, during the month of April in Barra, the phyla WS2, Deinococcota and Sva0485 (log 2 Fold-Change (log2FC): -81 ~ -94), the WS2, Sva0485 and Deinococci classes (log2FC: -81 ~ -93), 10 families and seven genera showed enrichment in bleached corals. In September, a much smaller group of taxa showed this characteristic, formed only by the phylum NKB15 and one of its classes (log2FC: ~ 21), in addition to 3 families and 6 genera. Concerning to healthy corals in both seasons, taxa already related to important functions in nutrient cycling, such as the classes Nitrospiria, Cyanobacteriia and Chlorobia, showed significant

enrichment in this treatment. In Maragogi, no phylum showed significant differential abundance between treatments in April, while the Thermodesulfovibrionia class, seven families and nine genera showed enrichment in bleached corals. More groups showed enrichment in healthy corals, composed by one class, 14 families and 16 genera. In September, bleached corals showed a larger group of enriched taxa in this condition, composed by phyla Firmicutes and Sumerlaeota (log2FC: -21 ~ -46), three classes and six families, while in healthy corals this group was formed only by the classes Syntrophobacteria and Deinococci (log2FC: 21 ~ 69).

Table 1: Family level significant changes in abundance using DESEq2 algorithm associated with enrichment in healthy (+) or bleached (-) samples at both sites during different seasons, ranked accord to significance.

Barra - April			
Family	**log2FC**	**Pvalues**	**FDR**
Burkholderiaceae	-23.169	0.00066179	0.012574
Caulobacteraceae	-3.164	7,23E-03	2,34E-01
Competibacteraceae	20.315	2,02E-03	7,25E-02
Defluviicoccaceae	55.126	0.00093015	0.015813
Izemoplasmatales	69.536	0.00046637	0.0094149
Marinimicrobia	20.905	8,87E-04	4,78E-02
Methylophagaceae	20.527	1,52E-03	6,14E-02
Nitrospiraceae	21.239	5,51E-04	3,57E-03
Rhodocyclaceae	20.641	1,29E-03	5,97E-02
Rikenellaceae	21.62	1,39E-04	1,49E-02
SAR116_clade	-14.027	1,44E-07	4,64E-05
Solimonadaceae	-52.072	4,86E-01	0.0013084
Spirosomaceae	-77.963	0.00070447	0.012641
Sva0485	-86.139	7,72E-01	0.001782
Sva1033	21.815	5,00E-05	8,07E-03
Tenderiaceae	-78.199	0.00036851	0.0079353
Trueperaceae	-82.917	6,88E-01	0.0017088

Barra - September			
Family	**log2FC**	**Pvalues**	**FDR**
Chlorobiaceae	22.846	8,48E-08	3,16E-05
Fodinicurvataceae	-22.495	1,92E-06	3.58e-08
NKB15	-21.978	2,53E-05	3,14E-03
Cardiobacteriaceae	20.696	1,20E-03	1,12E-01
Marinomonadaceae	75.604	5,54E-02	0.0004134
Endozoicomonadaceae	78.416	0.0004797	0.024962
Leptospiraceae	51.724	0.00049109	0.024962
BIrii41	75.698	0.00054079	0.024962
SG8_4	-5.79	0.00065669	0.024962
Candidatus_Campbellbacteria	93.544	0.00066922	0.024962

Maragogi - April			
Family	log2FC	Pvalues	FDR
Milano_WF1B_44	-23.188	3,21E-06	5,96E-04
Streptococcaceae	-23.086	3,79E-06	5,96E-04
Competibacteraceae	22.88	6,34E-06	6,64E-04
Nitriliruptoraceae	22.55	1.04e-09	8,16E-04
Eubacterium_coprostanoligenes	-22.143	2,07E-05	1,30E-03
Candidatus_Peribacteria	21.628	4,78E-05	2,50E-03
Desulfobacteraceae	-21.376	9,39E-05	3,44E-03
Pla4_lineage	21.194	9,73E-05	3,44E-03
Terasakiellaceae	21.143	1,08E-04	3,44E-03
WD2101_soil_group	21.187	1,10E-04	3,44E-03
Cycloclasticaceae	21.129	1,23E-04	3,51E-03
Gaiellaceae	21.067	1,39E-04	3,64E-03
Synechococcales	-10.09	0.00030295	0.0073173
Solirubrobacteraceae	10.469	0.001438	0.032194
Hydrogenophilaceae	10.374	0.0015799	0.032194
Alteromonadaceae	-10.353	0.0016404	0.032194
NS9_marine_group	10.138	0.0017687	0.032668
Rhodocyclaceae	9.663	0.0027283	0.047594
Tenderiaceae	-96.345	0.0031989	0.049521
Alcaligenaceae	9.458	0.0032631	0.049521
Fusibacteraceae	95.443	0.0033119	0.049521

Maragogi - September			
Family	log2FC	Pvalues	FDR
Sulfurimonadaceae	-25.525	2,05E-08	3,63E-05
Chthoniobacteraceae	-24.074	9,79E-08	1,16E-04
Caminicellaceae	-15.483	9,80E-06	8,70E-04
Sumerlaeaceae	-21.745	2,50E-04	1,78E-02
Sedimenticolaceae	-82.237	0.00030333	0.017947

In a preliminary step, we assessed whether the Out Of Bag (OOB) error rate for our unfiltered data was >0.1, which indicated that PIME filtering would effectively remove noise. The best prevalence interval calculated for all our data sets was 70% (OOB=0). The importance of ASVs to differentiate between healthy and bleached corals were measured by Mean Decrease Accuracy. The validation step showed randomized errors corresponded with the predicted prevalence cutoff value of 0.70 indicating an absence of false positives (Type I error). Table 2 indicates the top 10 ASVs according to their Mean Decrease Accuracy for each data set. In Barra, the filtered dataset after selecting members of microbiota that were present in at least 70% of all samples comprised 386 ASVs in April, of which 46 ASVs (encompassing 36 families) were primarily responsible for the differences between treatments, while in September this value dropped to 320 ASVs, but more ASVs were associated with differences between treatments (63 ASVs, corresponding to 40 families). In Maragogi, we identified a core composed of 123 ASVs in April, of which 33 ASVs, classified as 30 families, were the main responsible for dissimilarities between treatments. In September, we observed an increase in the number of ASVs (358) that met the established prevalence threshold, also reflected in a greater number of ASVs responsible for differences

between healthy and bleached corals (58 ASVs, classified in 41 families). In general, the classes that showed more variation were Alphaproteobacteria, Gammaproteobacteria and Bacteroidia. Curiously, only in the rainy season members of the Vibrionaceae family figured in this category (Barra = Mean Decrease Accuracy = 0.002; Maragogi = Mean Decrease Accuracy = 0.0046).

Table 2: Top-10 ASVs according to importance measured by Mean Decrease Accuracy using PIME package to differentiate treatments (healthy and bleached) between coral samples at each site and season, with closest microbial relative taxonomic level assigned.

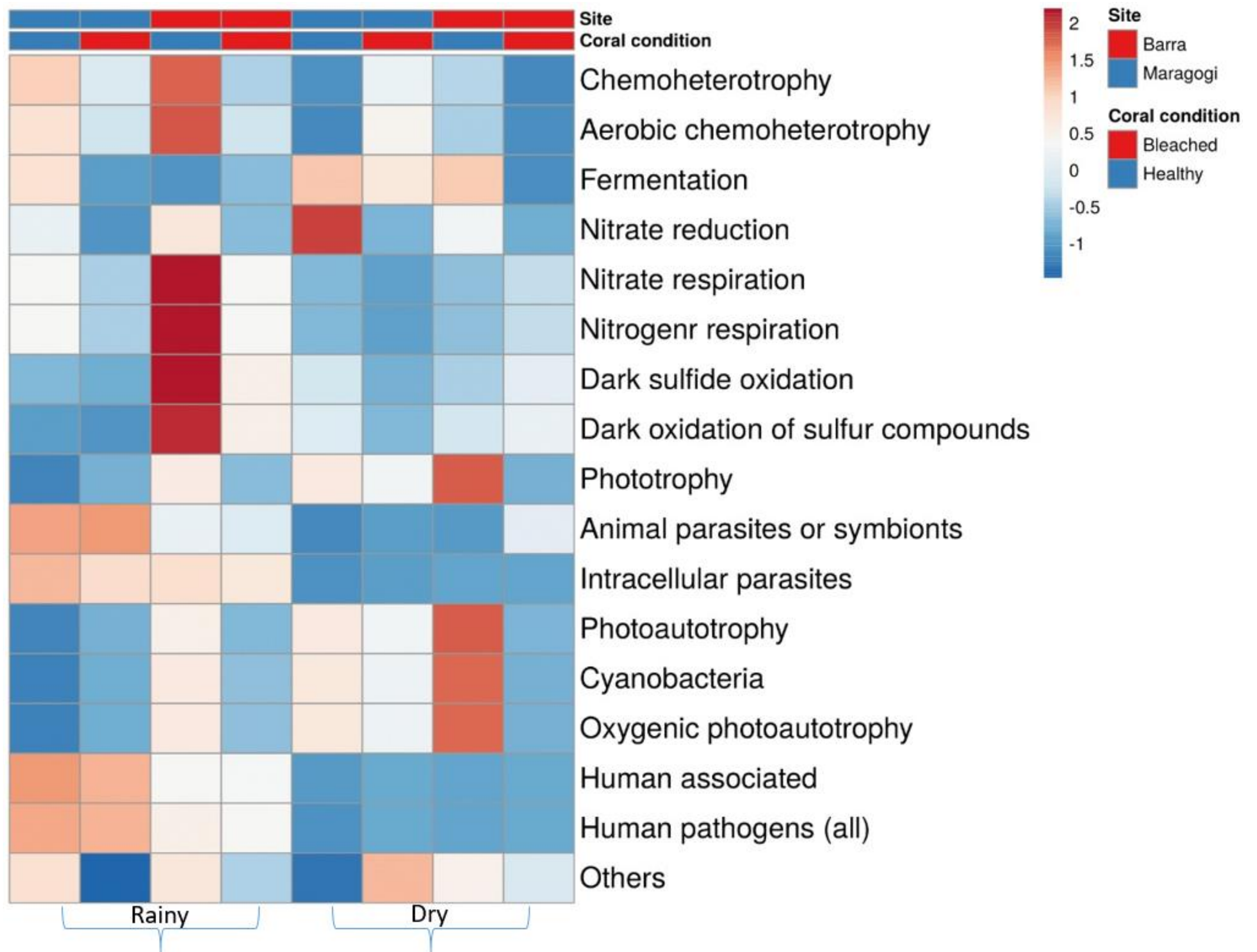
Barra de Santo Antônio - April				
Bleached	**Healthy**	**MeanDecrease Accuracy**	**MeanDecrease Gini**	**Closest microbial relative taxonomic level**
0.006	0.004	0.004666667	0.032000000	UBA10353_marine_group
0.006	0.004	0.004666667	0.020000000	Verrucomicrobiales
0.004	0.004	0.004000000	0.018000000	*Rhodopirellula*
0.004	0.004	0.004000000	0.016000000	Clostridiaceae
0.004	0.004	0.004000000	0.017333333	Dadabacteriales
0.004	0.004	0.004000000	0.012000000	Robiginitalea
0.004	0.003	0.003333333	0.039333333	Halieaceae
0.003	0.004	0.003333333	0.014666667	*Woeseia*
0.004	0.003	0.003333333	0.020666667	Flavobacteriaceae
0.003	0.004	0.003333333	0.011333333	Propionibacteriaceae
Barra de Santo Antônio - September				
Bleached	**Healthy**	**MeanDecrease Accuracy**	**MeanDecrease Gini**	**Closest microbial relative taxonomic level**
0.006	0.006	0.006000000	0.031333333	Thermoanaerobaculaceae
0.006	0.006	0.006000000	0.017333333	*Fluviicola*
0.006	0.005	0.005333333	0.021333333	Saprospiraceae
0.005	0.006	0.005333333	0.027333333	Desulfocapsaceae

0.007	0.004	0.005000000	0.028666667	Rhodobacteraceae
0.005	0.005	0.004666667	0.020000000	*Fluviicola*
0.004	0.006	0.004666667	0.029333333	Flavobacteriaceae
0.004	0.005	0.004333333	0.014000000	Cryomorphaceae
0.004	0.005	0.004000000	0.023333333	Alteromonadales
0.004	0.004	0.004000000	0.022000000	Sva0485
Maragogi - April				
Bleached	**Healthy**	**MeanDecrease Accuracy**	**MeanDecrease Gini**	**Closest microbial relative taxonomic level**
0.011	0.008	0.008666667	0.03000000	Burkholderiales
0.010	0.007	0.008000000	0.04933333	*Actibacter*
0.007	0.008	0.007333333	0.03733333	Kiloniellaceae
0.008	0.007	0.007333333	0.02933333	Gammaproteobacteria
0.005	0.006	0.005333333	0.02866667	Kiloniellaceae
0.006	0.005	0.005333333	0.03533333	Gammaproteobacteria
0.006	0.005	0.005333333	0.04533333	*Blastopirellula*
0.006	0.005	0.005333333	0.04600000	Sandaracinaceae
0.006	0.005	0.005333333	0.02333333	Caulobacter
0.005	0.005	0.005000000	0.05000000	Acidobacteriota
Maragogi - September				
Bleached	**Healthy**	**MeanDecrease Accuracy**	**MeanDecrease Gini**	**Closest microbial relative taxonomic level**
0.004	0.006	0.004666667	0.017333333	Alloprevotella
0.004	0.004	0.004000000	0.016000000	Woeseia
0.004	0.004	0.004000000	0.020000000	Sphingobacteriales
0.004	0.004	0.004000000	0.015333333	Planctomycetota
0.004	0.004	0.004000000	0.011333333	Calorithrix
0.004	0.004	0.004000000	0.030666667	*Fluviicola*
0.004	0.004	0.004000000	0.016000000	Phormidesmiales
0.004	0.003	0.003333333	0.016000000	Gammaproteobacteria

0.004	0.003	0.003333333	0.017333333	Calorithrix
0.004	0.003	0.003333333	0.012000000	*Woeseia*

The analysis with FAPROTAX database assigned 6109 ASVs to 63 microbial functional groups, which were then ranked according to relative abundance. There was a general decrease in some important functions between healthy and bleached condition, as Chemoheterotrophy, Aerobic Chemoheterotrophy, categories related to photosynthetic activity (Phototrophy, Photoautotrophy, Cyanobacteria and Oxygenic Photoautotrophy), Ureolysis and Nitrate Reduction (Figure 5). These putative functions also followed a seasonal shift in both reefs, with a higher predominance of photosynthetic activity during the dry season. In general, categories somehow related to the nitrogen cycle had their relative abundance reduced in bleached corals, except for nitrification (Supplementary Figure 3). On the other hand, categories related to association with animals (including humans) were more abundant in the rainy season at both sites and treatments than the dry season.

Figure 5: Top most abundant prokaryotic functions predicted observed in each reef and season (April = dry and September = rainy) using FAPROTAX.



The prediction of metagenomic content using the PIPHILLIN platform returned 9320 KO and 402 KEGG pathways from 747 inferred genomes. The visualization of the PCA indicated a complete overlap between, indicating that there are not so many overall functional dissimilarities between treatments (Supplementary figure 4). Differential abundance analysis using DESeq2 indicated that 58 KO showed significant variation between treatments. From these, 46 KO showed enrichment in healthy corals, corresponding to 30 pathways mainly associated with Metabolic pathways (10), Biosynthesis of secondary metabolites (9), Porphyrin and chlorophyll metabolism (7) and Microbial metabolism in diverse environments (5) (Supplementary Table 3). On the other hand, the 12 KO enriched in bleached corals were associated with 10 pathways,

and only one of them (Epithelial cell signaling in *Helicobacter pylori* infection) can be directly associated to pathogenic processes.

Discussion

The species *S. stellata* is known to harbor a lower density of zooxanthellae than other species of Brazilian corals, which ends up reducing the environmental requirements and ensuring better adaptability conditions in face of environmental variations, such as sedimentation, wave action, temperature and salinity variations, characteristics that make the species suitable for the project proposal (Costa et al., 2001; Leão et al., 2003). The greater dominance of Proteobacteria (mainly Alpha- and Gammaproteobacteria), in addition to other co-dominant groups, such as Bacteroidota (mainly Flavobacteria) and Cyanobacteria, seems to be common in corals, being considered ubiquitous in mucus and tissue samples besides strongly responsive to the reef environment, yet retain host species-specific associations (Blackall et al., 2015, Hernandez-Agreda et al., 2017; Huggett & Apprill, 2019; Hussien et al., 2019). Members of Rhodobacteraceae are known to contribute to nitrogen availability through the degradation of chitin produced by symbiotic zooxanthellae (Bourne et al., 2016), and were found in varying relative abundances more according to the season than coral health status. Cyanobacteria became more abundant mainly during the dry season at both reefs and the treatments, which may be associated with greater photosynthetic activity and could be crucial for the survival of the coral when it loses its endosymbiotic algae (Schlichter et al., 1995). Pirellulaceae (phylum Planctomycetes) was present in all samples, in variable abundance, and it is classified as anaerobic ammonia-oxidizing bacteria thought to contribute through the removal of metabolic waste within the host microbiome (Mohamed et al., 2010).

Furthermore, it was possible to observe a differential abundance of some ASVs, mainly associated with minor groups, between treatments at each site. In addition, the taxa involved in this differentiation varied according to seasonality. In Barra, most groups enriched in healthy corals are somewhat associated with the cycling of essential nutrients, mainly nitrogen, such as the phylum Nitrospirota, the classes Nitrospiria, Marinimicrobia, Chlorobia and Cyanobacteriia and the

families Rikenellaceae, Nitrospiraceae and Rhodocyclaceae. Of families that showed enrichment in bleached corals, only Burkholderiaceae was previously reported as important taxa in diseased scleractinians. In Maragogi, this enrichment was even more noticeable among rarer taxa, and in none of the stations there were taxa already associated with bleaching. In addition, fewer groups showed significant differential abundance between the two treatments during the dry period, indicating greater stability in the community during this period. In general, healthy individuals of *S. stellata* presented associated bacterial communities that on average were slightly more diverse, rich and homogeneous than bleached corals. In addition, we observed a great seasonal heterogeneity, with each reef presenting distinct bacterial communities between samples collected during the rainy and the dry season, independent of health condition. Maragogi exhibited greater alpha-diversity and less dissimilarity between bacterial communities during the dry season. This seasonal dynamic in the bacterial community, mainly correlated to dissolved oxygen and rainfall, was already observed in other species, as *Porites lutea* (Li et al., 2014), *Astrangia poculata*, which exhibited the highest alpha diversity and the lowest inter-individual beta diversity in spring (Sharp et al., 2017) and other corals (Welsh et al., 2015; Zaneveld et al., 2016). These compositional shifts may occur in response to the transition from quiescence to an increase in holobiont metabolic rates in spring (Glasl et al., 2016) and may help enable the holobiont to tolerate, and adapt to, environmental stress conditions (Ziegler et al., 2019).

More important, it was not possible to identify a bacterial community as clearly pathogenic as observed when bleaching results in thermal stress, usually as a response to increases in water temperature to 30 ~ 31º C for long term stress (Morrow et al., 2018), and commonly associated to high abundances of Vibrionaceae, a family that constitutes an opportunistic and potentially pathogenic bacterial group commonly linked to bleaching, for example (Garren et al., 2016), and besides being common to find a greater abundance diseased corals subjected to stress (mainly thermal) (Hussien et al., 2019), we did not see this in our study. Furthermore, it may be associated with the Coral Probiotic Hypothesis, where rapid changes in the bacterial community in response to environmental variations would be a quick way of adapting the holobiont (Reshef et al., 2006). In short, the maintenance of this rare microbiota, but which has

members known to play important roles related to nutrient cycling, seems to guarantee resistance to seasonal variations in the environment that may cause a temporary bleaching state, which is usually linked to a dysbiotic state in bacterial communities.

One of our hypotheses was that the Santo Antônio River, through the input of its microorganisms and anthropic influence, would make more homogeneous bacterial communities in Barra. By contrast, in Maragogi this happened during the rainy season, when healthy and bleached corals showed relative similarity between their microbiotas, whereas after that only healthy individuals exhibited this characteristic. A recent study at the same location analyzed the microbiota of both River and the reef region using the PhyloChip microarray technique showed that a considerable amount of human pathogens are carried along its course, with signs of fecal contamination, but that it decreases when it reaches the point in the sea where the reef on which we collect is located (Paulino et al., 2020). In general, this region was the one with the greatest bacterial richness and diversity, which is supported by previous work showing corals on impacted reefs harbor higher bacterial diversity and richness than those further from the disturbance (Claar et al., 2020; Morrow et al., 2012). In addition, Barra was the reef with the largest members of the core community, with the highest number also being observed during the rainy season. One of the few recent studies that also has a river in its study area showed higher relative abundances of the variable bacterial populations and more site-stable ASVs related to greater proximity to the coast, also during the rainy season (Leite et al., 2018). This suggests that rivers play a structuring role for bacterial communities associated with corals, making them richer, more diverse and homogeneous especially during the rainy season, when their influence is greatest.

Due to the important functions performed, the core microbiota present in reef environment usually acts by protecting corals against climatic and anthropogenic stresses (Grottoli et al., 2018). After we identified the core microbiota between treatments, it was possible to observe that its size and composition varied according to season. Nevertheless, those members responsible for differentiating the core between treatments also varied, composed by a large number of less abundant families, but in a way, members of the

Burkholderiaceae, Flavobacteriaceae and Rhodobacteraceae contributed with most ASVs. This seasonal variability in the microbiome has been suggested to be an indication that these associations are responsive to environmental variables, biological events, and also to factors that result in stress response in the host (Hernandez-Agreda et al., 2017). It also reinforces recent data that says that more than 90% of this bacterial community associated with corals are formed by transient phylotypes (environmentally responsive community members) (Hernandez-Agreda et al., 2018). Our study reinforces this observation through our finding of the instability caused by the presence of Santo Antônio River in Barra and possibly tourism in Maragogi, which may contribute to the dynamics of microbial communities through the input of new microorganisms in these environments. Taxa identified as core member of healthy corals microbiota can be considered in the category of beneficial microorganisms for corals proposed by Peixoto et al. (2017), which also proposed, for example, that specific nitrogen-fixing bacteria could be inoculated during events of thermal stress, allowing the coral to stay alive until environmental conditions return to a state closer to optimal. Burkholderiaceae and Rhodobacteraceae have already been found to belong to the core microbiota of other coral species, as *Acropora* sp., *Pocillopora* sp. and *Porites lutea* (Gardner et al., 2019; Gonzalez-Zapata et al., 2018; Pootakham et al., 2018), suggesting an important role for coral health. For example, members of Rhodobacteraceae are known to show a high metabolic versatility which includes the utilization of various organic and inorganic compounds (Pohlner et al., 2019).

Besides heterotrophic feeding, corals largely depend on their microbiome for proficient nutrient acquisition and recycling in oligotrophic seawaters (Bourne et al., 2016). The results of the prediction of functional groups and of the metagenomic content indicated in healthy individuals a greater abundance and enrichment of groups and routes related to the acquisition of nutrients, mainly to photosynthetic and nitrogen cycling, which might be keeping homeostasis. At both sites, these functional groups were more abundant during the dry season and in healthy individuals, likely to be related to the higher incidence of sunlight and less sediment suspended in water. Proportional shifts in categories related to photosynthesis and respiration may indicate that the coral microbiome changed from a system driven by autotrophy to one comprised predominately of

heterotrophic modes of metabolism, but the reduction in important functional groups related to host's health has already been demonstrated in bleached corals and may lead to a lower recovery rate of the coral, that without them resistance period to disease may be shorter (Littman et al., 2011). For example, nitrogen-cycling microbes appear to be ubiquitous and consistent members of the coral microbiome, being crucial for the acquisition and retention of nitrogen to sustain primary productivity (i.e., photosynthesis) (Rädecker et al., 2015), and it's acquisition through heterotrophy reduce post-bleaching photoinhibition and recovery times (Grottoli et al., 2006).

Conclusions

Bleaching is one of the main global phenomena related to the coral crisis and has been studied at all scales of the holobiont, including the microbiota. Differentiating the situation of illness resulting from local and seasonal stressors can contribute to understanding how changes in the microbiota contribute to the homeostasis of the host coral. We did not observe a clearly pathogenic state as previously reported in other studies related to bleached coral microbiota. We suggest that bleaching has arisen as a result of environmental changes mainly associated with seasonality, causing a misbalance mainly in rarer groups of the bacterial community that also reflected in the putative functional profile, which showed fewer prokaryotic groups and metabolic pathways related to the nutrient acquisition. Some of the most abundant families in all reefs and stations do not have previous data on association with corals, which raises more questions about the importance of these bacteria in the holobiont. The presence of a river close to one of the points apparently led to fewer dissimilarities between bacterial communities, mainly during the rainy season, highlighting the relevance of environments surrounding coral reefs. Although the difference in higher taxonomic levels is more related to variations in relative abundance, there was also a change related to treatments and the seasonality in the predicted functions, which may be related to the process of acclimatization and adaptation of the holobiont in face of local environmental changes. Determining persistent groups over space and time is essential to improve approaches related to increasing coral resistance. In this context, some bacterial "rare" families appeared to be

more prevalent in the community associated with *S. stellata*, and with more studies, they may be able to contribute to these approaches.

Acknowledgements

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Bibliography

Ainsworth, T.D., et al., 2015. The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. ISME J. 9, 2261–2274. https://doi.org/10.1038/ismej.2015.39

Bolyen, E., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. https://doi.org/10.1038/s41587-019-0209-9

Bourne, D.G., et al., 2009. Microbial disease and the coral holobiont. Trends Microbiol. 17, 554–562. https://doi.org/10.1016/j.tim.2009.09.004

Bourne, D.G., Morrow, K.M., Webster, N.S., 2016. Insights into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems. Annu. Rev. Microbiol. 70, 317–340. https://doi.org/10.1146/annurev-micro-102215-095440

Burke, L., Reytar, K., Spalding, M., Perry, A., 2011. Reefs at Risk Revisited Executive Summary, Reefs at Risk Revisited.

Callahan, B.J., et al., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869

Claar, D.C., McDevitt-Irwin, J.M., Garren, M., Vega Thurber, R., Gates, R.D., Baum, J.K., 2020. Increased diversity and concordant shifts in community structure of coral-associated Symbiodiniaceae and bacteria subjected to chronic human disturbance. Mol. Ecol. 29, 2477–2491.

https://doi.org/10.1111/mec.15494

Costa, C.F. da, Amaral, F.D., Sassi, R., 2001. Branqueamento Em *Siderastrea stellata* (Cnidaria, Scleractinia) Da Praia De Gaibu, Pernambuco, Brasil. Rev. Nord. Biol.

Fabricius, K.E., 2005. Effects of terrestrial runoff on the ecology of corals and coral reefs: Review and synthesis. Mar. Pollut. Bull. 50, 125–146. https://doi.org/10.1016/j.marpolbul.2004.11.028

Ferreira, B.P., Maida, M., 2006. Monitoramento dos Recifes de Coral do Brasil: situação e perspectivas, Monitoramento dos recifes de coral do Brasil. https://doi.org/M744

Gardner, S.G., et al., 2019. Coral microbiome diversity reflects mass coral bleaching susceptibility during the 2016 El Niño heat wave. Ecol. Evol. 9, 938–956. https://doi.org/10.1002/ece3.4662

Garren, M., Son, K., Tout, J., Seymour, J.R., Stocker, R., 2016. Temperature-induced behavioral switches in a bacterial coral pathogen. ISME J. 10, 1363–1372. https://doi.org/10.1038/ismej.2015.216

Glasl, B., Herndl, G.J., Frade, P.R., 2016. The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. ISME J. 10, 2280–2292. https://doi.org/10.1038/ismej.2016.9

Gleason, D.F., Wellington, G.M., 1993. Ultraviolet radiation and coral bleaching. Nature 365, 836–838. https://doi.org/10.1038/365836a0

Glynn, P.W., 1993. Coral reef bleaching: ecological perspectives. Coral Reefs 12, 1–17. https://doi.org/10.1007/BF00303779

Gonzalez-Zapata, F.L., et al., 2018. Holobiont diversity in a reef-building coral over its entire depth range in the mesophotic zone. Front. Mar. Sci. 5, 1–13. https://doi.org/10.3389/fmars.2018.00029

Grottoli, A.G., et al., 2018. Coral physiology and microbiome dynamics under combined warming and ocean acidification. PLoS One 13, 1–22. https://doi.org/10.1371/journal.pone.0191156

Grottoli, A.G., Rodrigues, L.J., Palardy, J.E., 2006. Heterotrophic plasticity and resilience in bleached corals. Nature 440, 1186–1189. https://doi.org/10.1038/nature04565

Hernandez-Agreda, A., Gates, R.D., Ainsworth, T.D., 2017. Defining the Core Microbiome in Corals' Microbial Soup. Trends Microbiol. 25, 125–140. https://doi.org/10.1016/j.tim.2016.11.003

Hernandez-Agreda, A., et al., 2018. Rethinking the coral microbiome: Simplicity exists within a diverse microbial biosphere. MBio 9, e00812-18. https://doi.org/10.1128/mBio.00812-18

Huggett, M.J., Apprill, A., 2019. Coral microbiome database: Integration of sequences reveals high diversity and relatedness of coral-associated microbes. Environ. Microbiol. Rep. 11, 372–385. https://doi.org/10.1111/1758-2229.12686

Hussien, E., Juhmani, A.S., AlMasri, R., Al-Horani, F., Al-Saghir, M., 2019. Metagenomic analysis of microbial community associated with coral mucus from the Gulf of Aqaba. Heliyon 5, e02876. https://doi.org/10.1016/j.heliyon.2019.e02876

Jokiel, P.L., Coles, S.L., 1990. Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. Coral Reefs. https://doi.org/10.1007/BF00265006

Knowlton, N., Jackson, J., 2013. Corals and Coral Reefs, Encyclopedia of Biodiversity: Second Edition. https://doi.org/10.1016/B978-0-12-384719-5.00237-9

Lapointe, B.E., Brewton, R.A., Herren, L.W., Porter, J.W., Hu, C., 2019. Nitrogen enrichment, altered stoichiometry, and coral reef decline at Looe Key, Florida Keys, USA: a 3-decade study. Mar. Biol. https://doi.org/10.1007/s00227-019-3538-9

Leão, Z.M.A.N., Kikuchi, R.K.P., Testa, V., 2003. Corals and coral reefs of Brazil, in: Latin American Coral Reefs. pp. 9–52. https://doi.org/10.1016/B978-044451388-5/50003-5

Leite, D.C.A., et al., 2018. Coral bacterial-core abundance and network complexity as proxies for anthropogenic pollution. Front. Microbiol. 9, 1–11. https://doi.org/10.3389/fmicb.2018.00833

Li, J., et al., 2014. Bacterial dynamics within the mucus, tissue and skeleton of the coral *Porites lutea* during different seasons. Sci. Rep. 4, 7320. https://doi.org/10.1038/srep07320

Lin, C.H., et al., 2016. Seasonal changes in bacterial communities associated with healthy and diseased *Porites coral* in southern Taiwan. Can. J. Microbiol. 62, 1021–1033. https://doi.org/10.1139/cjm-2016-0100

Littman, R., Willis, B.L., Bourne, D.G., 2011. Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. Environ. Microbiol. Rep. 3, 651–660. https://doi.org/10.1111/j.1758-2229.2010.00234.x

Louca, S., Parfrey, L.W., Doebeli, M., 2016. Decoupling function and taxonomy in the global ocean microbiome. Science (80). 353, 1272–1277. https://doi.org/10.1126/science.aaf4507

Mohamed, N.M., Saito, K., Tal, Y., Hill, R.T., 2010. Diversity of aerobic and anaerobic ammonia-oxidizing bacteria in marine sponges. ISME J. 4, 38–48. https://doi.org/10.1038/ismej.2009.84

Morrow, K.M., Moss, A.G., Chadwick, N.E., Liles, M.R., 2012. Bacterial associates of two caribbean coral species reveal species-specific distribution and geographic variability. Appl. Environ. Microbiol. 78, 6438–6449. https://doi.org/10.1128/AEM.01162-12

Morrow, K.M., Muller, E., Lesser, M.P., 2018. How Does the Coral Microbiome Cause, Respond to, or Modulate the Bleaching Process? Coral Bleaching: Patterns, Processes, Causes and Consequences. https://doi.org/10.1007/978-3-319-75393-5_7

Paulino, G.V.B., et al., 2020. Bacterial community and environmental factors associated to rivers runoff and their possible impacts on coral reef conservation. Mar. Pollut. Bull. 156, 111233. https://doi.org/10.1016/j.marpolbul.2020.111233

Peixoto, R.S., et al., 2017. Beneficial microorganisms for corals (BMC): Proposed mechanisms for coral health and resilience. Front. Microbiol. 8, 1–16. https://doi.org/10.3389/fmicb.2017.00341

Pires, N.L., et al., 2015. Impacts of the urbanization process on water quality of Brazilian Savanna rivers: The case of Preto River in formosa, Goiás state, Brazil. Int. J. Environ. Res. Public Health 12, 10671–10686. https://doi.org/10.3390/ijerph120910671

Pohlner, M., Dlugosch, L., Wemheuer, B., Mills, H., Engelen, B., Reese, B.K., 2019. The majority of active Rhodobacteraceae in marine sediments belong to uncultured genera: A molecular approach to link their distribution to environmental conditions. Front. Microbiol. 10. https://doi.org/10.3389/fmicb.2019.00659

Pootakham, W., et al., 2018. Dynamics of coral-associated microbiomes during a thermal bleaching event. Microbiologyopen 7, 1–16. https://doi.org/10.1002/mbo3.604

Rädecker, N., et al., 2015. Nitrogen cycling in corals: The key to understanding holobiont functioning? Trends Microbiol. 23, 490–497. https://doi.org/10.1016/j.tim.2015.03.008

Raina, J.B., Tapiolas, D., Willis, B.L., Bourne, D.G., 2009. Coral-associated bacteria and their role in the biogeochemical cycling of sulfur. Appl. Environ.

Microbiol. 75, 3492–3501. https://doi.org/10.1128/AEM.02567-08

Reshef, L., et al., 2006. The Coral Probiotic Hypothesis. Environ. Microbiol. 8, 2068–2073. https://doi.org/10.1111/j.1462-2920.2006.01148.x

Rognes, T., et al., 2016. VSEARCH: A versatile open source tool for metagenomics. PeerJ 2016. https://doi.org/10.7717/peerj.2584

Rohwer, F., Seguritan, V., Azam, F., Knowlton, N., 2002. Diversity and distribution of coral-associated bacteria. Mar. Ecol. Prog. Ser. 243, 1–10. https://doi.org/10.3354/meps243001

Rosenberg, E., Falkovitz, L. 2004. The *Vibrio shiloi/Oculina patagonica* model system of coral bleaching. Annu. Rev. Microbiol. 58, 143–159. https://doi.org/10.1146/annurev.micro.58.030603.123610

Rosenberg, E., et al. 2007. The role of microorganisms in coral health, disease and evolution. Nat. Rev. Microbiol. 5, 355–362. https://doi.org/10.1038/nrmicro1635

Rosenberg, E., Kushmaro, A., Kramarsky-Winter, E., Banin, E., Yossi, L., 2009. The role of microorganisms in coral bleaching. ISME J. 3, 139–146. https://doi.org/10.1038/ismej.2008.104

Sassi, C.F.C., Sassi, R., Gorlach-Lira, K., De Lima, R.D.C.P. 2014. Pigmentation patterns of *Siderastrea stellata* Verrill, 1868 (Cnidaria, Scleractinia) from coastal reefs in Northeastern Brazil and its relation with zooxanthellae and other microsymbionts. Panam. J. Aquat. Sci. 9, 207–222.

Schlichter, D., Zscharnack, B., Krisch, H. 1995. Transfer of Photoassimilates from Endolithic Algae to Coral Tissue. Naturwissenschaften 82, 561–564. https://doi.org/10.1007/s001140050234

Sharp, K.H., et al. 2017. Season, but not symbiont state, drives microbiome structure in the temperate coral *Astrangia poculata*. Microbiome 5, 120. https://doi.org/10.1186/s40168-017-0329-8

Stuart-Smith, R.D., et al. 2018. Ecosystem restructuring along the Great Barrier Reef following mass coral bleaching. Nature 560, 92–96. https://doi.org/10.1038/s41586-018-0359-9

Sully, S., et al. 2019. A global analysis of coral bleaching over the past two decades. Nat. Commun. 10, 1–5. https://doi.org/10.1038/s41467-019-09238-2

Sun, W., Zhang, F., He, L., Li, Z., 2014. Pyrosequencing reveals diverse microbial community associated with the zoanthid *Palythoa australiae* from the South China Sea. Microb. Ecol. 67, 942–950. https://doi.org/10.1007/s00248-014-0395-4

Walker, S.J., Schlacher, T.A., 2014. Limited habitat and conservation value of a young artificial reef, Biodiversity and Conservation. https://doi.org/10.1007/s10531-013-0611-4

Walters, W., et al. 2016. Improved Bacterial 16S rDNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys. mSystems 1, e0009-15. https://doi.org/10.1128/msystems.00009-15

Webster, N.S., Reusch, T.B.H., 2017. Microbial contributions to the persistence of coral reefs. ISME J. 11, 2167–2174. https://doi.org/10.1038/ismej.2017.66

Welsh, R., et al. 2015. Alien vs. Predator: Pathogens open niche space for opportunists, unless controlled by predators. https://doi.org/10.7287/peerj.preprints.1537

Yang, S.H., et al. 2017. Long-term survey is necessary to reveal various shifts of microbial composition in corals. Front. Microbiol. 8, 1–11. https://doi.org/10.3389/fmicb.2017.01094

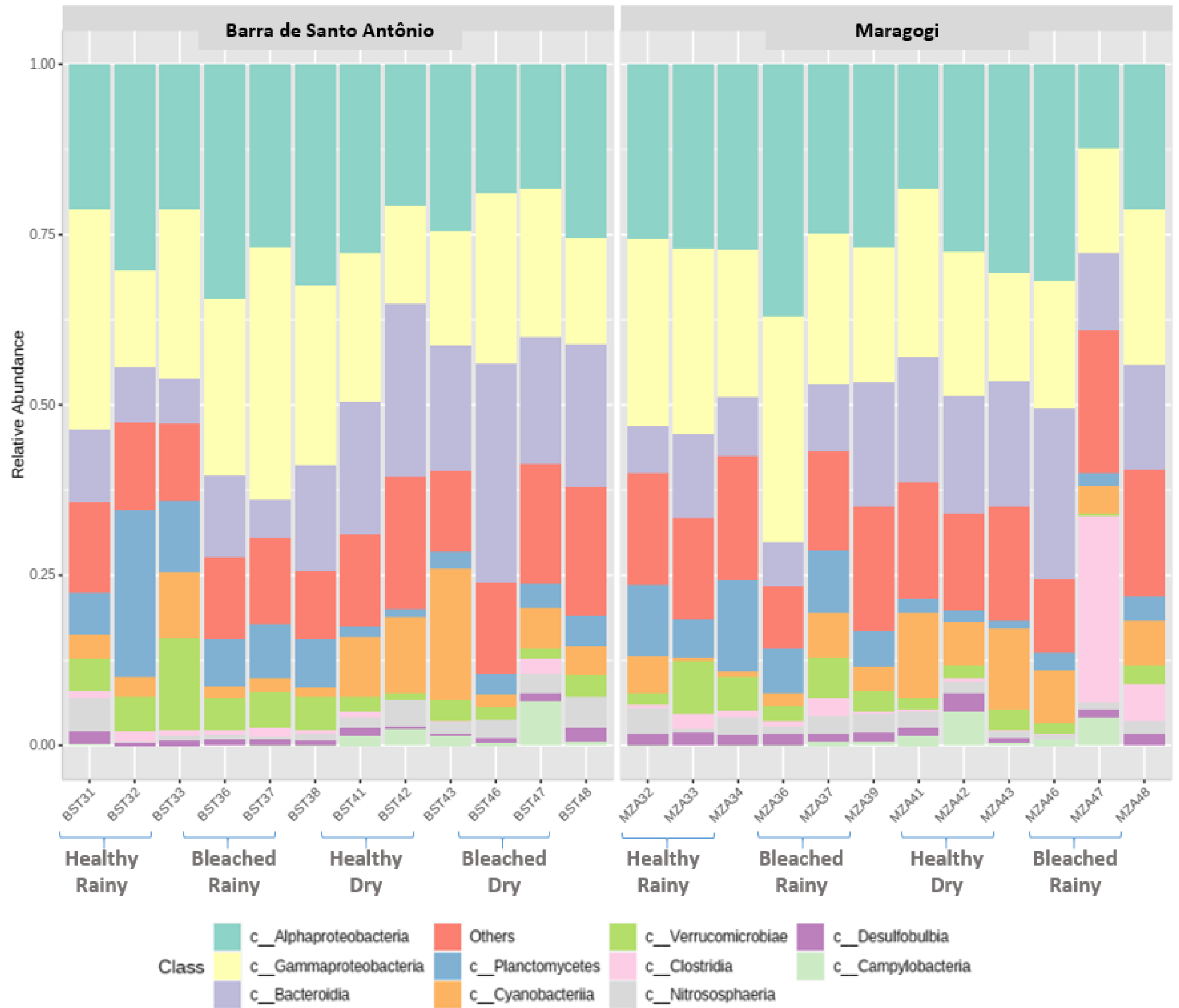
Zaneveld, J.R., Burkepile, D.E., Shantz, A.A., Pritchard, C.E., McMinds, R., Payet, J.P., Welsh, R., Correa, A.M.S., Lemoine, N.P., Rosales, S., Fuchs, C., Maynard, J.A., Thurber, R.V., 2016. Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. Nat. Commun. 7, 1–12. https://doi.org/10.1038/ncomms11833

Ziegler, M., et al. 2019. Coral bacterial community structure responds to environmental change in a host-specific manner. Nat. Commun. 10. https://doi.org/10.1038/s41467-019-10969-5

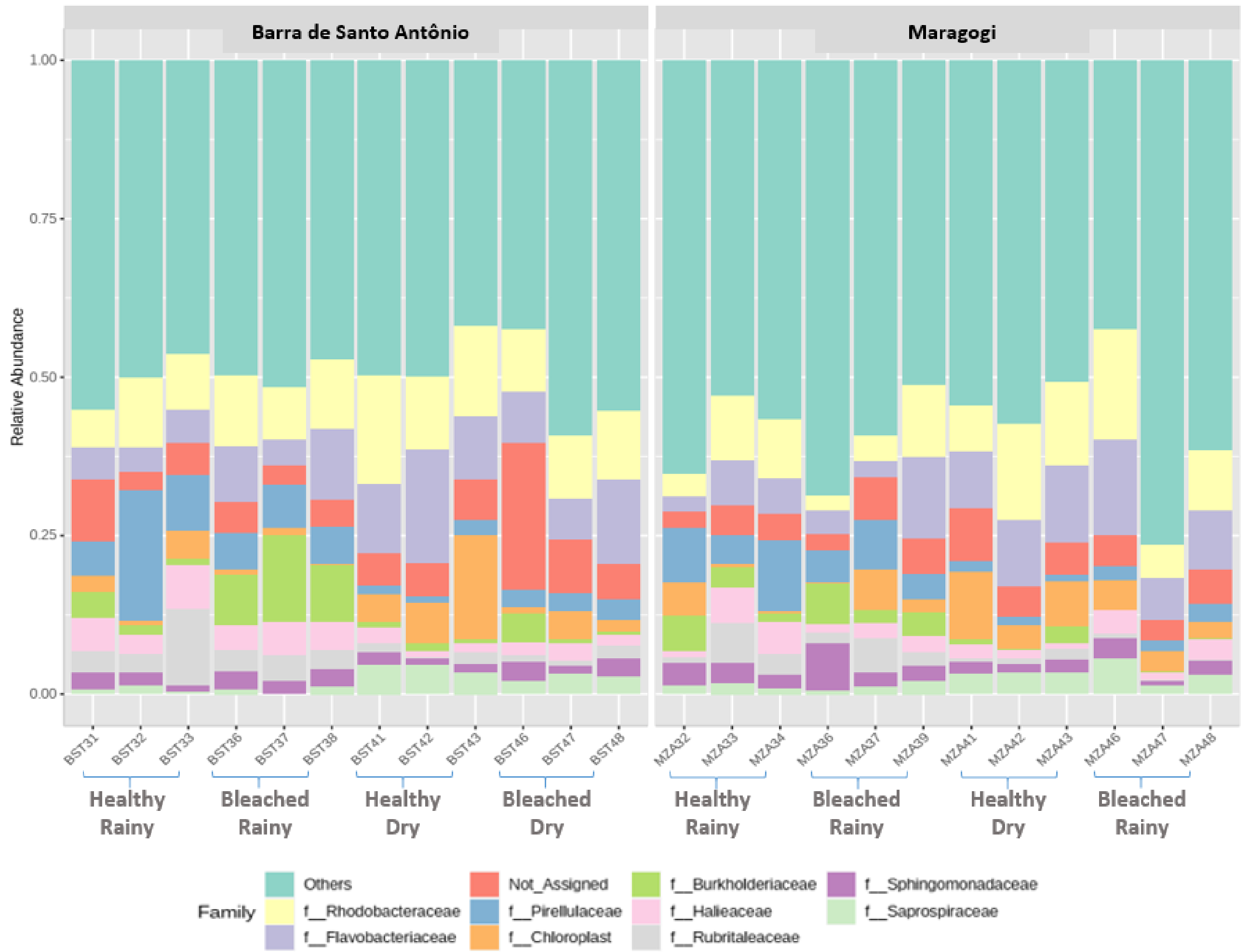
Ziegler, M., et al. 2016. Coral microbial community dynamics in response to anthropogenic impacts near a major city in the central Red Sea. Mar. Pollut. Bull. 105, 629–640. https://doi.org/10.1016/j.marpolbul.2015.12.045

Supplementary Figures

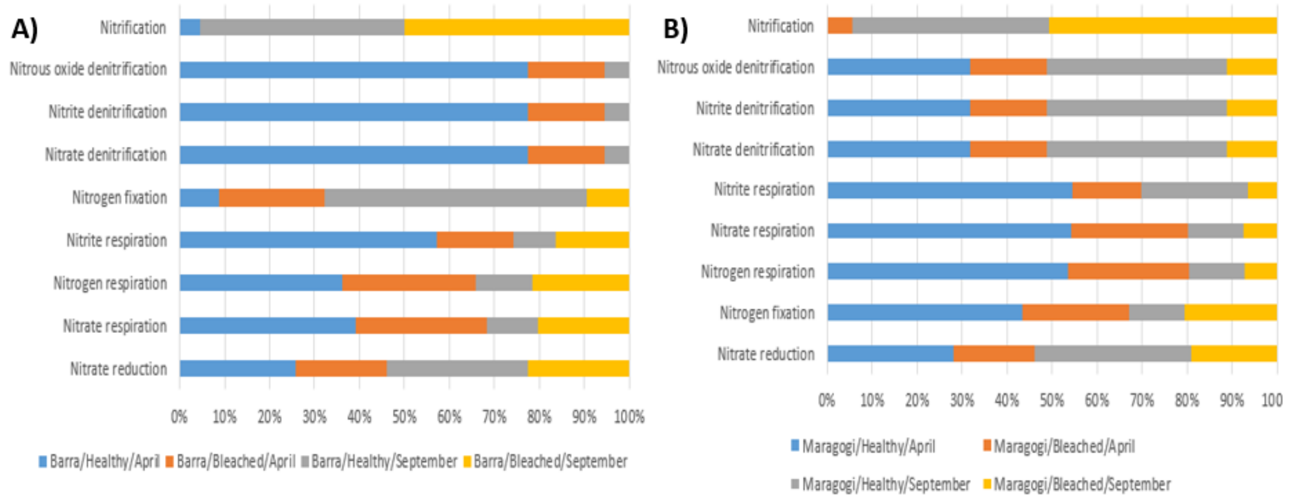
Supplementary figure 1: Relative abundance of top-10 classes. Samples grouped according to season and coral condition.



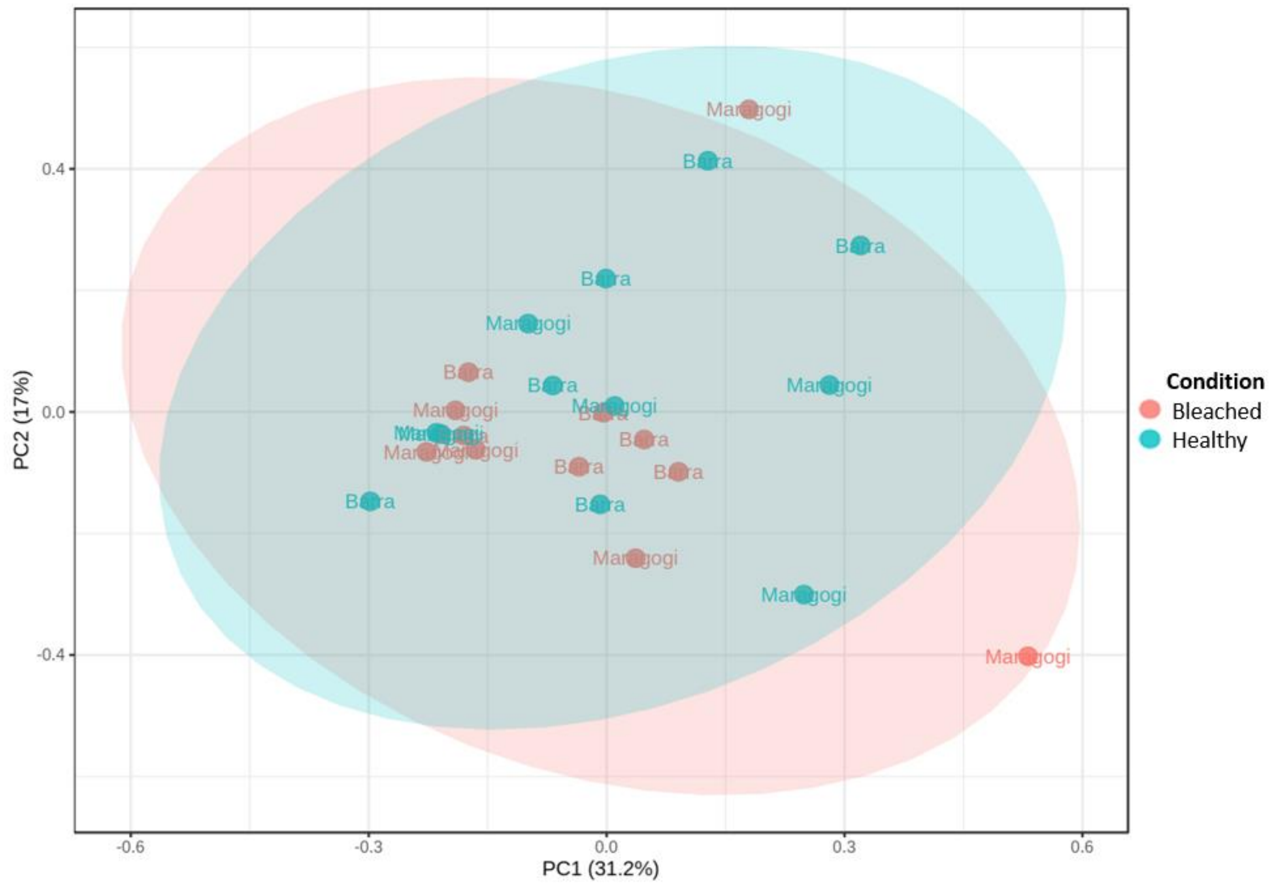
Supplementary figure 2: Relative abundance of top-10 families. Samples grouped according to season and coral condition.



Supplementary figure 3: Relative abundance of functional groups related to nitrogen utilization predicted using FAPROTAX in A) Barra de Santo Antônio and B) Maragogi.



Supplementary figure 4: Principal Coordinates Analysis (PCA) plot based on predicted metagenomic content of healthy and bleached samples of coral *Siderastrea stellata* collected during the dry and rainy seasons of two distinct coral reefs.



Supplementary Tables

Supplementary table 1: Values of physicochemical parameters measured during each collection.

Site	Month	Temperature (°C)	pH	Salinity	Chlorophyll (mg/l)	Turbidity (NTU)	Dissolved oxygen (%)	Dissolved oxygen (mg/l)	Total coliforms	*E. coli*
Barra	April	29.52	8.4	34.2	0	0.1	123.8	8.63	0	0
Maragogi	April	29.55	8.6	33.25	0.4	0.1	104.6	6.63	0	0
Barra	September	29.99	9.0	32.94	0	-2	162	10.22	0	0
Maragogi	September	28.76	9.1	32.98	0.2	0	147.8	9.51	0	0

Supplementary table 2: Taxa that according to the DESeq2 algorithm showed statistically significant differential abundance between the two treatments, where (+) indicates enrichment in healthy corals and (-) in bleached corals.

Barra de Santo Antônio - April

Taxa	log2FC	lfcSE	Pvalues	FDR
p__WS2	-94.053	16.077	4,91E-05	1,86E-03
p__Nitrospirota	21.469	39.083	3,95E-04	5,81E-03
p__Marinimicrobia_(SAR406_clade)	21.367	39.084	4,58E-04	5,81E-03
p__Deinococcota	-81.715	19.854	3,86E-01	0.00036672
p__Sva0485	-85.481	21.311	6,04E-01	0.00045938
c__Nitrospiria	21.529	34.401	3,89E-06	3,58E-04
c__Marinimicrobia_(SAR406_clade)	21.309	34.957	1,09E-05	5,01E-04
c__WS2	-93.713	17.383	7,00E-04	2,15E-02
c__Sva0485	-85.731	21.639	7,44E-01	0.0017107
c__Deinococci	-81.649	21.628	0.00015991	0.0029423
c__Cyanobacteriia	17.683	0.59509	0.0029634	0.045439
f__SAR116_clade	-14.027	20.767	1,44E-07	4,64E-05
f__Sva1033	21.815	37.307	5,00E-05	8,07E-03
f__Rikenellaceae	21.62	38.096	1,39E-04	1,49E-02
f__Nitrospiraceae	21.239	39.086	5,51E-04	3,57E-03
f__WS2	-94.942	17.474	5,53E-04	3,57E-03
f__Marinimicrobia_(SAR406_clade)	20.905	39.087	8,87E-04	4,78E-02
f__Rhodocyclaceae	20.641	39.095	1,29E-03	5,97E-02
f__Methylophagaceae	20.527	39.098	1,52E-03	6,14E-02
f__Competibacteraceae	20.315	39.087	2,02E-03	7,25E-02
f__Caulobacteraceae	-3.164	0.63854	7,23E-03	2,34E-01
f__Solimonadaceae	-52.072	12.819	4,86E-01	0.0013084
f__Trueperaceae	-82.917	20.831	6,88E-01	0.0017088
f__Sva0485	-86.139	21.792	7,72E-01	0.001782
f__Tenderiaceae	-78.199	21.956	0.00036851	0.0079353
f__Izemoplasmatales	69.536	19.871	0.00046637	0.0094149
f__Burkholderiaceae	-23.169	0.68045	0.00066179	0.012574
f__Spirosomaceae	-77.963	23.013	0.00070447	0.012641
f__Defluviicoccaceae	55.126	1.665	0.00093015	0.015813

Barra de Santo Antônio - September

Taxa	log2FC	lfcSE	Pvalues	FDR
p__NKB15	-21.935	38.507	1,22E-04	6,37E-03
p__Cyanobacteria	21.252	0.51985	4,35E-01	0.0011304
p__Deinococcota	22.495	0.59044	0.00013909	0.0024109
p__Patescibacteria	3.173	0.85104	0.00019268	0.0025048
p__Latescibacterota	-0.76694	0.39198	0.050398	0.49758
p__Spirochaetota	23.074	12.143	0.057413	0.49758
c__NKB15	-21.865	31.364	3,14E-08	3,67E-06
c__MB_A2_108	20.827	34.812	2,19E-05	1,28E-03
c__Chlorobia	23.12	39.074	3,28E-05	1,28E-03
c__Leptospirae	52.339	11.353	4,03E-02	0.00011778
c__Cyanobacteriia	23.095	0.59107	9,34E-01	0.0021846
f__Chlorobiaceae	22.846	33.448	8,48E-08	3,16E-05
f__Fodinicurvataceae	-22.495	35.327	1,92E-06	3.58e-08
f__NKB15	-21.978	36.878	2,53E-05	3,14E-03
f__Cardiobacteriaceae	20.696	3.91	1,20E-03	1,12E-01
f__Marinomonadaceae	75.604	16.641	5,54E-02	0.0004134
f__Endozoicomonadaceae	78.416	22.457	0.0004797	0.024962
f__Leptospiraceae	51.724	14.839	0.00049109	0.024962
f__BIrii41	75.698	2.188	0.00054079	0.024962
f__SG8_4	-5.79	16.994	0.00065669	0.024962
f__Candidatus_Campbellbacteria	93.544	27.498	0.00066922	0.024962

Maragogi - April

Taxa	log2FC	lfcSE	Pvalues	FDR
c__Thermodesulfovibrionia	-22.155	3.91	1,46E-04	1,46E-02
c__Pla4_lineage	20.915	3.909	8,77E-04	4,39E-02
f__Milano_WF1B_44	-23.188	36.874	3,21E-06	5,96E-04
f__Streptococcaceae	-23.086	36.865	3,79E-06	5,96E-04
f__Competibacteraceae	22.88	37.013	6,34E-06	6,64E-04
f__Nitriliruptoraceae	22.55	36.948	1.04e-09	8,16E-04
f__Eubacterium_coprostanoligenes_group	-22.143	36.952	2,07E-05	1,30E-03
f__Candidatus_Peribacteria	21.628	36.942	4,78E-05	2,50E-03
f__Desulfobacteraceae	-21.376	37.231	9,39E-05	3,44E-03
f__Pla4_lineage	21.194	36.953	9,73E-05	3,44E-03
f__Terasakiellaceae	21.143	36.975	1,08E-04	3,44E-03
f__WD2101_soil_group	21.187	37.072	1,10E-04	3,44E-03
f__Cycloclasticaceae	21.129	37.097	1,23E-04	3,51E-03
f__Gaiellaceae	21.067	37.127	1,39E-04	3,64E-03
f__Synechococcales_Incertae_Sedis	-10.09	27.928	0.00030295	0.0073173
f__Solirubrobacteraceae	10.469	32.849	0.001438	0.032194
f__Hydrogenophilaceae	10.374	32.834	0.0015799	0.032194
f__Alteromonadaceae	-10.353	32.881	0.0016404	0.032194
f__NS9_marine_group	10.138	32.425	0.0017687	0.032668

f__Rhodocyclaceae	9.663	32.244	0.0027283	0.047594
f__Tenderiaceae	-96.345	32.682	0.0031989	0.049521
f__Alcaligenaceae	9.458	3.215	0.0032631	0.049521
f__Fusibacteraceae	95.443	32.495	0.0033119	0.049521

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Taxa	log2FC	lfcSE	Pvalues	FDR
p__Sumerlaeota	-21.738	31.319	3,90E-08	1,80E-06
p__Firmicutes	-46.177	10.853	2,09E-01	0.00048171
c__Sumerlaeia	-21.687	39.093	2,90E-04	3,07E-02
c__Syntrophobacteria	72.904	19.145	0.00014006	0.0074231
c__Clostridia	-51.083	14.024	0.00027003	0.0095412
c__Deinococci	20.124	0.62004	0.0011723	0.031067
c__Methylomirabilia	-69.713	22.213	0.0016989	0.036018
f__P_palmC41	-25.348	31.365	6,40E-12	2,27E-09
f__Sulfurimonadaceae	-25.525	38.082	2,05E-08	3,63E-05
f__Chthoniobacteraceae	-24.074	37.208	9,79E-08	1,16E-04
f__Caminicellaceae	-15.483	2.533	9,80E-06	8,70E-04
f__Sumerlaeaceae	-21.745	3.902	2,50E-04	1,78E-02
f__Sedimenticolaceae	-82.237	22.765	0.00030333	0.017947

Supplementary table 3: Number of KEGG pathways mainly associated to each treatment after analysis with DESeq2 algorithm identify significant differentially abundant KO enriched between healthy and bleached corals.

KO	Healthy	KO	Bleached
ko01100	Metabolic pathways (10)	ko01110	Biosynthesis of secondary metabolites (1)
ko01110	Biosynthesis of secondary metabolites (9)	ko02024	Quorum sensing (1)
ko00860	Porphyrin and chlorophyll metabolism (7)	ko01100	Metabolic pathways (1)
ko01120	Microbial metabolism in diverse environments (5)	ko01503	Cationic antimicrobial peptide (CAMP) resistance (1)
ko01220	Degradation of aromatic compounds (3)	ko00900	Terpenoid backbone biosynthesis (1)
ko00361	Chlorocyclohexane and chlorobenzene degradation (2)	ko02020	Two-component system (1)
ko01501	beta-Lactam resistance (2)	ko01200	Carbon metabolism (1)
ko00040	Pentose and glucuronate interconversions (2)	ko00680	Methane metabolism (1)
ko02024	Quorum sensing (1)	ko01120	Microbial metabolism in diverse environments (1)
ko04974	Protein digestion and absorption (1)	ko05120	Epithelial cell signaling in *Helicobacter pylori* infection (1)
ko04721	Synaptic vesicle cycle (1)		
ko04724	Glutamatergic synapse (1)		
ko01240	Biosynthesis of cofactors (1)		
ko02020	Two-component system (1)		
ko00053	Ascorbate and aldarate metabolism (1)		
ko00052	Galactose metabolism (1)		
ko00540	Lipopolysaccharide biosynthesis (1)		
ko00660	C5-Branched dibasic acid metabolism (1)		
ko01200	Carbon metabolism (1)		
ko00906	Carotenoid biosynthesis (1)		
ko00680	Methane metabolism (1)		
ko00362	Benzoate degradation (1)		
ko05120	Epithelial cell signaling in Helicobacter pylori infection (1)		
ko00622	Xylene degradation (1)		
ko00900	Terpenoid backbone biosynthesis (1)		
ko00520	Amino sugar and nucleotide sugar metabolism (1)		
ko00790	Folate biosynthesis (1)		
ko00640	Propanoate metabolism (1)		
ko01503	Cationic antimicrobial peptide (CAMP) resistance (1)		
ko00601	Glycosphingolipid biosynthesis - lacto and neolacto series (1)		

6 CONCLUSÕES GERAIS

Os recifes de coral representam um importante componente tanto ecológico quanto econômico, e por isso cada vez mais estão sujeitos a medidas de conservação. Em Alagoas, as principais formações coralíneas estão protegidas pela Área de Proteação Ambiental Costa dos Corais, cujas principais medidas baseiam-se no zoneamento dos recifes de coral em diversas categorias com níveis diferentes de permissibilidade de acesso e execução de atividades antrópicas.

Apesar disso, são cada vez mais frequentes relatos sobre o branqueamento dos corais. Métodos moleculares como o PhyloChip™, cuja proposta está mais direcionada para a avaliação ambiental, constituem uma importante ferramenta para o monitoramento dessas regiões. Como foi visto, dois dos rios que deságuam nessas áreas apresentam, com base em dados microbiológicos e físico-quimicos, características que indicam impactos antrópicos significativos. A previsão precoce do estresse do ecossistema torna-se cada vez mais crítica para uma implementação eficaz de estratégias de gerenciamento e restauração locais em locais de recife ameaçados. Fatores como a presença do agronegócio e o lançamento de esgoto ao longo dos rios devem ser avaliados regularmente, já que ambos são possíveis fontes de contaminação ambiental. Com isso, faz-se necessário estender medidas de conservação para além do ambiente recifal e incluir intervenções que minimizem os impactos causados pela baixa qualidade das águas dos rios presentes na região.

De forma geral, não foi possível observar um estado claramente disbiótico entre exemplares branqueados, conforme relatado anteriormente em outros estudos. Ainda, as mudanças observadas foram mais significativas quanto a estação do ano em que as amostras foram coletadas do que a condição de saúde. A presença de um rio que deságua em um dos recifes de coral aparentemente levou a uma menor dissimilaridades entre as comunidades bacterianas amostradas na região, principalmente durante a estação chuvosa, destacando ainda mais a relevância dos ambientes no entorno dos recifes de coral. É provável que o branqueamento tenha surgido em decorrência de

mudanças ambientais sazonais, que como consequência afeta a fisiologia dos corais, causando um desequilíbrio principalmente em grupos “raros” da comunidade que também se refletiu no perfil funcional predito, que apresentou menos grupos procarióticos e vias metabólicas relacionadas à aquisição de nutrientes. Diferenciar a situação de branqueamento resultante de estressores locais e sazonais de eventos de branqueamento resultantes de estresse termal pode contribuir para entender como as as complexas mudanças na microbiota contribuem para a homeostase do coral hospedeiro.