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Efeitos do aquecimento e acidificação dos oceanos em esponjas calcareas (Porifera)

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Biodiversidade e Biologia Evolutiva, pela Universidade Federal do Rio de Janeiro, como parte dos requisitos necessários para obtenção do título de Mestra em Biodiversidade e Biologia Evolutiva.

Orientadora: Prof[a]. Dr[a]. Michelle Klautau

Coorientador: Dr. Flávio da Costa Fernandes

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RESUMO

Organismos calcificadores podem estar potencialmente ameaçados frente às mudanças climáticas, no entanto, nada se sabe sobre os efeitos do aquecimento e da acidificação dos oceanos em esponjas calcareas. Com isso, o presente estudo teve como objetivos: (1) Avaliar possíveis corrosões e variações na forma, tamanho e composição química (Mg/Ca) das espículas em condições de maior temperatura e acidez; (2) Avaliar a capacidade das esponjas de sintetizar espículas submetidas a essas condições e; (3) Analisar possíveis mudanças na comunidade bacteriana associada às esponjas nos diferentes tratamentos. Foram feitos experimentos em aquário utilizando quatro combinações de temperatura (22 e 26^{o}C) e pH (8.1 e 7.6), retratando condições atuais e projeções futuras dos oceanos. Ao final de 10 dias de experimento, as espículas não apresentaram corrosão. A ausência de corrosão pode estar relacionada a uma proteção promovida pela capa orgânica que envolve as espículas das esponjas calcareas, pelo menos no tempo do experimento. As esponjas foram capazes de sintetizar espículas normais em todos os tratamentos, apresentando somente algumas espículas danificadas, o que poderia indicar um possível mecanismo adaptador de regulação do pH no sítio de calcificação. A proporção de espículas danificadas aumentou significativamente como aumento da temperatura, mas ainda assim não representou um problema real para as esponjas. A razão de Mg/Ca se mostrou constante ao longo do ano em esponjas *in situ* e também nos experimentos em aquário. Com relação à comunidade bacteriana, não houve nenhuma diferença significativa em relação aos tratamentos, contudo, foram observadas tendências, tais como o aumento do gênero *Staphylococcus*. Apesar desse gênero ser bem reconhecido como um patógeno para humanos, não podemos associar esse aumento a nenhum impacto negativo para o hospedeiro, já que não foi observada necrose nem aumento de mortalidade. Um core bacteriano foi identificado em todas as esponjas analisadas, com domínio do gênero *Ruegeria*, o que pode estar associado a *quorum sensing*, promovendo benefícios para as esponjas. Sendo assim, nossos resultados indicam que as esponjas Calcareas podem não estar tão vulneráveis em relação às projeções das mudanças climáticas como previamente proposto, porém, trabalhos de longa duração são necessários para se confirmar esses resultados.

Palavras-chave: Mudanças Climáticas, *Sycettusa hastifera*, Carbonato de Cálcio, Comunidade Bacteriana.

ABSTRACT

Calcifying organisms may be potentially threatened by climate change, however, nothing is known about the possible effects of ocean warming and acidification on calcareous sponges. Thereby, the objective of this study was: (1) To evaluate possible corrosion and variations in shape, size and chemical composition (Mg/Ca) of the spicules under conditions of higher temperature and acidity; (2) Evaluate the ability of the sponges to synthesize spicules subjected to these conditions and; (3) To analyze possible changes in the bacterial community associated to these sponges under different treatments. Experiments were performed in aquaria using four combinations of temperature (22 and 26^{o}C) and pH (8.1 and 7.6), representing current conditions and future projections of the oceans. At the end of 10 days of experiment, the spicules showed no corrosion. The absence of corrosion may be related to a protection promoted by the organic cover that surrounds the spicules of the calcareous sponges, at least in the time of the experiment. The sponges were able to synthesize normal spicules in all treatments, presenting only a few deformed ones, which could indicate a possible adapter mechanism of pH regulation at the calcification site. The proportion of deformed spicules increased significantly as the temperature increased, but still did not represent a real problem for the sponges. The Mg/Ca ratio was shown to be constant throughout the year in sponges *in situ* and also in aquaria experiments. Regarding the bacterial community, there was no significant difference in relation to the treatments, however, trends were observed, such as the increase of the genus *Staphylococcus*. Although this genus is well recognized as a pathogen for humans, we cannot associate this increase with any negative impact on the host, since no necrosis or increased mortality was observed. A bacterial core was identified in all sponges analyzed, with dominance of the genus *Ruegeria*, which may be associated with *quorum sensing*, promoting benefits for sponges. Thus, our results indicate that calcareous sponges may not be as vulnerable to climate change projections as previously proposed, but long-term studies are needed to confirm these results.

Keywords: Climate Change, *Sycettusa hastifera*, Calcium Carbonate, Bacterial Community.

SUMÁRIO

1. INTRODUÇÃO EXPANDIDA

1.1 Panorama Histórico-Político das Mudanças Climáticas

Em 1979 foi realizada a primeira Conferência Mundial sobre o Clima (WCC) e devido às crescentes evidências científicas relativas à interferência humana no sistema climático, juntamente com o aumento da preocupação pública sobre questões ambientais, os governos viram a necessidade de criar um órgão imparcial e independente para lidar com isso (Blodel *et al*., 2006). Assim, foi criado em 1988 o Painel Intergovernamental de Mudanças no Clima (IPCC), com o objetivo de compilar uma revisão abrangente sobre o estado do conhecimento da ciência das mudanças climáticas, fornecer recomendações sobre os impactos sociais e econômicos e identificar possíveis estratégias de resposta. É com base nesse relatório que a Convenção-Quadro das Nações Unidas sobre Mudança no Clima (UNFCCC), criada em 1992, define obrigações básicas aos países aderidos para enfrentar e combater as mudanças climáticas desde o âmbito global até local, representando o fórum internacional mais importante sobre o tema (Blodel *et al*., 2006). Entrou em vigor em 2005 o Protocolo de Quioto, que complementa a Convenção, comprometendo legalmente os países desenvolvidos a reduzirem suas emissões de gases do efeito estufa entre o período de 2008 – 2012 (UNFCCC, 2007). Em 2015 foi adotado um novo acordo climático, o Acordo de Paris, que tem como premissa principal manter o aumento da temperatura global menor que 2 ^{o}C em relação aos níveis pré-industriais, além de incluir elementos mais abrangentes, como estratégias de mitigação, adaptação, informações sobre perdas e danos, finanças, dentre outros, entrando em vigor no final de 2016. No quinto relatório de avaliação do IPCC (AR5), lançado em 2014, foram descritos quatro possíveis cenários para o final do século XXI, relacionados às emissões e concentrações de gases do efeito estufa na atmosfera, caracterizados como RCP - *Representative Concentration Pathways* (IPCC, 2014; Tabela 1).

Tabela 1: Possíveis cenários de emissões de CO_2 para o final do século XXI de acordo com o Painel Intergovernamental de Mudanças no Clima.

RCP	Temperatura (^{o}C)	CO_2 (ppm)	Status das Emissões de CO_2
RCP2.6	2	430 - 530	Diminuem pela metade até 2050
RCP4.5	≥2	530 - 720	Estabilizam em metade dos níveis atuais até 2080
RCP6.0	>2	720 - 1000	Aumentam até 2080 e, então, diminuem
RCP8.5	>4	>1000	Continuam aumentando nos níveis atuais

1.2 Dióxido de Carbono e Mudanças Climáticas

O dióxido de carbono (CO_2) é um dos principais gases do efeito estufa, responsável não somente pelo aquecimento, mas também pela acidificação dos oceanos. A concentração desse gás na atmosfera aumentou cerca de 45% desde a Revolução Industrial, iniciada em 1750, devido sobretudo à queima de combustíveis fósseis e desmatamento, ambas provocadas pelo ser humano (VMO, 2017). A taxa de aumento do CO_2 atmosférico nos últimos 70 anos é quase 100 vezes maior do que no final da última era glacial e, atualmente, a concentração desse gás na atmosfera chegou a 400 ppm (VMO, 2017). Metade do gás emitido por essas atividades antrópicas é absorvida pelos oceanos e florestas, que representam os principais sumidouros de CO_2, enquanto o restante permanece na atmosfera por centenas a milhares de anos (Sabine *et al*., 2004; Pan *et al*., 2011).

O efeito estufa é um fenômeno natural causado não somente pelo CO_2, mas também por outros gases que absorvem e reemitem radiação infravermelha, como Metano (CH_4) e Óxido Nitroso (NO_2), entre outros. Com isso, o aumento da concentração desses gases na atmosfera resulta em mais calor retido na atmosfera e, consequentemente, em um aumento da temperatura média global da superfície da terra e dos oceanos. No entanto, os oceanos têm absorvido mais CO_2 do que o sua capacidade de suporte, acarretando em desequilíbrios, tais como a acidificação dos oceanos. O CO_2 absorvido pelos oceanos reage com a água do mar (H_2O), gerando ácido carbônico (H_2CO_3). Este por sua vez se dissocia, liberando íons hidrogênio (H^+) e íons bicarbonato (HCO_3^-) que também se dissociam em H^+ e íons carbonato (CO_3^{-2}), tal como representado no esquema a seguir (Doney *et al*., 2009):

$$\mathbf{CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow H^+ + CO_3^{-2}}$$

Esse sistema carbonato ocorre naturalmente e tende a ficar em equilíbrio, funcionando como um tampão para manter o pH da água do mar dentro de uma curta variação. Se algum dos componentes aumenta ou diminui, a equação se desloca afim de reestabelecer o equilíbrio. Com isso, quanto maior a concentração de CO_2, maior a liberação de íons H^+ e, consequentemente, a redução do pH, aumentando a acidez na água do mar. Em contrapartida, os íons CO_3^{-2} tendem a formar HCO_3^- novamente, já que representam uma forma mais estável, afim de restaurar o equilíbrio da reação. Essa redução na concentração de íons CO_3^{-2}

prejudica a formação do carbonato de cálcio ($CaCO_3$), já que é essencial em sua formação junto aos íons cálcio (Ca^{+2}), como representado no esquema a seguir:

$$\mathbf{CO_3^{-2} + Ca^{+2} \leftrightarrow CaCO_3}$$

As taxas de formação e dissolução do $CaCO_3$ variam de acordo com o estado de saturação (Ω) do mineral, onde de maneira geral $\Omega > 1$ ocorre formação do mineral e $\Omega < 1$ ocorre dissolução, como definido pela reação a seguir:

$$\mathbf{\Omega = [Ca^{+2}]\,[CO_3^{-2}] / \mathit{K}'_{sp}}$$

O K'_{sp} é um produto de solubilidade estequiométrica influenciado pela temperatura, pressão, salinidade e fase mineral do carbonato de cálcio. No ambiente natural esse mineral pode se cristalizar em diferentes fases minerais, tais como: aragonita, calcita ou magnésio-calcita (sendo o magnésio presente em baixas ou altas concentrações). Essas fases minerais apresentam taxas de formação e solubilidade diferentes, sendo que magnésio-calcita-enriquecido representa a fase mineral mais solúvel, seguido por aragonita e calcita (Busemberg & Plummer, 1989).

1.3 Efeitos das Mudanças Climáticas em Organismos Marinhos

1.3.1 Calcificadores

Os organismos calcificadores são aqueles que utilizam o carbonato de cálcio ($CaCO_3$) na constituição do seu corpo ou parte dele, como conchas, tecas e esqueletos. Sua susceptibilidade em relação às mudanças climáticas pode variar em função da fase mineral da sua constituição, bem como com a presença e extensão de uma camada orgânica protetora desse esqueleto/concha. As respostas dos organismos marinhos às mudanças de temperatura e pH nos oceanos variam de espécie para espécie. Alguns são mais susceptíveis, enquanto outros possuem ou desenvolvem ferramentas que auxiliam sua perseverança em condições de estresse. Ries e colaboradores (2009) fizeram um extenso trabalho sobre os efeitos da acidificação dos oceanos em organismos calcificadores. Eles verificaram que a camada orgânica protetora do esqueleto/concha, a capacidade de regular o pH no sítio de calcificação

e a associação com organismos fotossintetizantes influencia mais na susceptibilidade às mudanças climáticas do que a própria fase mineral. Os crustáceos, por exemplo, mesmo com composição de magnésio-calcita-enriquecido, não apresentaram dissolução, mesmo em condições de pH extremamente baixo (~7.3). Na verdade, apresentaram maior taxa de calcificação quando submetidos a essas condições. Isso poderia ser explicado pela presença de uma epicutícula relativamente grossa que envolve sua carapaça e pela habilidade de transportarem bicarbonato para o sítio de calcificação e o converterem em carbonato, eliminando os íons hidrogênio liberados nesse processo, mantendo assim o pH básico (Cameron, 1985; Fig. 1).

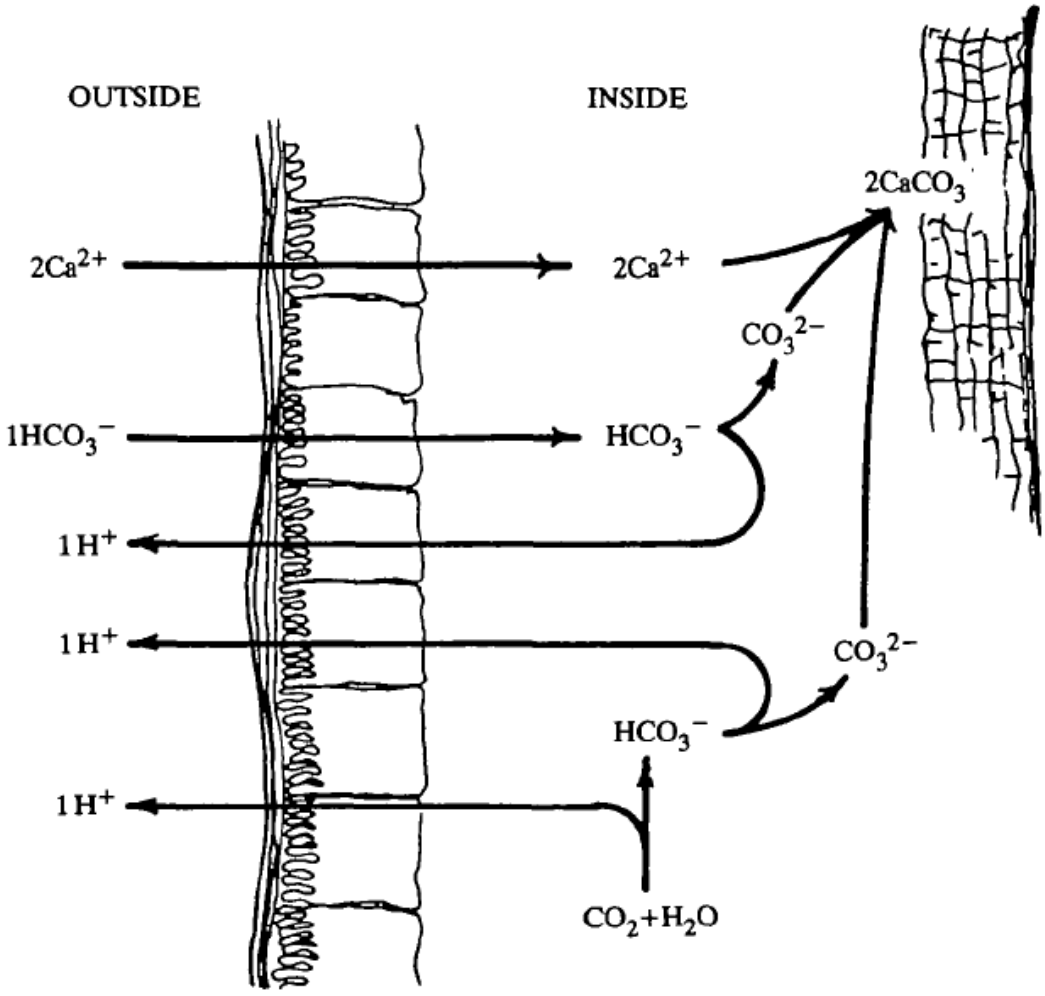

Figura 1: Esquema do transporte de Ca^{+2}, HCO_3^- e H^+ na pós muda do caranguejo-azul (*Callinectes sapidus*). Retirado de Cameron (1985).

Em contrapartida, existem vários outros trabalhos que mostram a grande vulnerabilidade dos calcificadores frente às mudanças climáticas. Foster e colaboradores

(2016) viram que o esqueleto de juvenis de corais da espécie *Acropora spicifera* crescia totalmente deformado quando submetido à redução do pH. Em contrapartida, com o aumento da temperatura e a redução do pH, o esqueleto ainda apresentava deformidades, mas em menores proporções. Isso mostra que de certa forma, os dois estressores juntos se equilibram, pelo menos em relação aos efeitos para essa espécie. Já para algas coralinas da espécie *Porolithon onkodes*, Diaz-Pulido e colaboradores (2012) verificaram que os efeitos do aumento da temperatura e redução do pH agiam de forma sinérgica, intensificando a mortalidade e a dissolução dessas algas. Para outra espécie de alga coralina, a *Lithophyllum cabiochae*, foi visto similarmente o processo de efeito sinérgico entre as duas variáveis, temperatura e pH, intensificando necrose, mortalidade e dissolução (Martin & Gattuso, 2009). Já para alga coralina *Lithothamnion glaciale*, a redução do pH enfraquece a sua estrutura, mas ainda assim ela consegue crescer sob condições de pH baixo, mesmo que com taxas mais lentas (Ragazzola *et al*., 2012).

1.3.2 Esponjas

As esponjas pertencem ao filo Porifera e compreendem o grupo de animais mais antigo ainda existente no mundo. Esses organismos são sésseis, filtradores e possuem uma estrutura corporal simples, baseada em comunicação celular (Bergquist, 1978). As esponjas realizam filtração por meio de um sistema de canais inalantes e exalantes, chamado de sistema aquífero, no qual células flageladas (coanócitos) geram uma corrente de água unidirecional através do seu corpo (Bergquist, 1978). É por meio dessa corrente de água que as esponjas obtêm seu alimento, fazem trocas gasosas e liberam suas excretas (Hooper *et al*., 2002). Seu sistema de filtragem é muito eficiente, onde em média um quilo de esponja filtra aproximadamente 24.000 litros de água por dia (Vogel, 1977). Essa eficiência permite que as esponjas desempenhem papel fundamental para o bom funcionamento do ambiente marinho, fazendo a conexão bento-pelágica, pelo *sponge loop*, ou seja, transformando matéria orgânica dissolvida (MOD) em particulada (MOP) e participando na ciclagem de nutrientes, tais como o Carbono, Nitrogênio, Fósforo e a Sílica (Maldonado *et al*., 2012; De Goeij *et al*., 2013). Além disso, são importantes na consolidação e bioerosão de substratos e também atuam servindo de alimento e refúgio para outros organismos marinhos (Randall & Hartman, 1968; Wulff, 2001; Cerrano *et al*., 2006). Atualmente, o filo é dividido em quatro classes viventes: Demospongiae, Calcarea, Hexactinellida e Homoscleromorpha, totalizando mais de 8.800

espécies descritas consideradas válidas (Van Soest *et al*., 2018). O esqueleto das esponjas, quando presente, pode ser constituído por espículas inorgânicas livres ou fusionadas e, em geral, é constituído por espículas de sílica (SiO_2), sendo a classe Calcarea a única que possui espículas de carbonato de cálcio ($CaCO_3$).

As esponjas, de uma maneira geral, aparentam ter baixa sensibilidade às mudanças climáticas e, por isso, poderiam passar a dominar alguns recifes de coral no cenário futuro dos oceanos (Bell *et al*., 2013). Goodwin e colaboradores (2014) verificaram que ao longo de um gradiente de redução de pH, a comunidade de esponjas também diminuía, exceto pela esponja *Crambe crambe*. Essa espécie apresentava maior área de cobertura em pH 7.8 do que em 8.1, provavelmente devido à redução dos competidores por espaço. Houve um *trade-off* entre defesa química e taxa de crescimento, fazendo com que em um ambiente com menos competidores por espaço, fosse mais vantajoso investir no seu crescimento, já que é uma espécie com características oportunistas. Em um evento de branqueamento em massa seguido de alta mortalidade de corais, devido ao aumento de temperatura ocasionado pelo El Niño, a esponja *Chondrilla* cf. *nucula* aumentou sua área de cobertura, também provavelmente devido à redução da competição por espaço com os corais (Aronson *et al*., 2002). Ambas as espécies mostram, assim, resistência aos estressores, como temperatura e pH.

A associação com microrganismos também pode influenciar o desempenho das esponjas em relação aos fatores de estresse. Em algumas espécies, por exemplo, a habilidade de reestruturar seu microbioma contribui para sua sobrevivência em condições de pH baixo (Ribes *et al*., 2016). *Dysidea avara* teve alta aquisição de bactérias via transmissão horizontal e não apresentou problemas na sua taxa de crescimento em relação à redução do pH. Em contrapartida, a espécie *Chondrosia reniformis* não teve mudança no seu microbioma e sofreu severos problemas na sua taxa de crescimento. Morrow e colaboradores (2015) detectaram que as esponjas *Cinachyra* sp. e *Coelocarteria singaporensis* eram mais abundantes em locais com pH mais baixo e que, além disso, apresentavam uma maior quantidade de cianobactérias associadas a elas, se comparado aos locais de pH normal. Essa associação com cianobactérias provavelmente estava fornecendo benefício nutricional extra e permitindo então seu desenvolvimento em condições adversas. Por isso, esponjas heterotróficas respondem diferentemente das fototróficas, em relação aos efeitos da temperatura e do pH, como observado pela Bennett e colaboradores (2016). Nas espécies heterotróficas, a redução do pH e o aumento da temperatura agem sinergicamente, potencializando a porcentagem de necrose

nos indivíduos. Enquanto em espécies fototróficas, a redução do pH ameniza o aumento da temperatura, reduzindo tanto a necrose quanto o branqueamento. Assim, a associação com organismos fototróficos estaria favorecendo o desempenho das hospedeiras em condições de estresse, já que foi visto, por exemplo, que a cianobactéria *Synechococcus* aumenta suas taxas de crescimento e fotossintética sob condições de baixo pH e alta temperatura (Fu *et al*., 2007). Além disso, algumas espécies apresentam um limite de tolerância em relação à temperatura, como é o caso da esponja *Rhopaloiedes odorabile* (Webster *et al*., 2008). Submetida a diferentes temperaturas, a composição da comunidade bacteriana e sua saúde não sofreram alteração em 27, 29 e 31 ^{o}C. Contudo, a 33 ^{o}C, em menos de 24 horas já havia grandes alterações na composição bacteriana, seguida de necrose celular dentro de três dias. Isso nos mostra que os efeitos e susceptibilidades a estressores são intrínsecos a cada holobionte, fazendo-se necessária uma avaliação pelo menos ao nível de espécie.

Embora existam diversos estudos acerca dos efeitos do aumento da temperatura e da redução do pH em esponjas, eles são relacionados majoritariamente à classe Demospongiae. Smith e colaboradores (2013) levantaram o questionamento sobre a vulnerabilidade das esponjas Calcareas em um cenário de mudanças climáticas, sugerindo que poderiam não ser bem sucedidas devido à composição do seu esqueleto. No entanto, em um trabalho sobre bioincrustação foi verificado que, a abundância da esponja calcarea *Leucosolenia* sp. aumentou com a redução do pH, mas nenhum tipo de análise do esqueleto, fisiológica ou microbiológica foi realizada (Peck *et al*., 2015). Com isso, a necessidade de análises mais detalhadas ainda se faz necessária, a fim de avaliar a possível vulnerabilidade dessas esponjas no cenário futuro dos oceanos.

OBJETIVOS

O presente estudo teve como objetivo geral verificar os efeitos do aquecimento e acidificação dos oceanos em esponjas calcareas, utilizando a esponja *Sycettusa hastifera* como modelo. Os objetivos específicos foram:

1. Avaliar possíveis corrosões e variações na forma, no tamanho e composição química (Mg/Ca) das espículas submetidas a condições de alta temperatura e acidez;

2. Avaliar se as esponjas são capazes de sintetizar espículas sob essas condições;

3. Analisar potenciais mudanças na comunidade bacteriana associada aos indivíduos submetidos ao aumento da temperatura e redução do pH.

Calcareous sponges might not be as threatened by climate changes as previously thought

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ABSTRACT

The aim of the present study was to verify the possible effects of ocean warming and acidification on the skeleton of calcareous sponges and in their bacterial community, using Sycettusa hastifera *as a model. At the end of 10 days of experiment, the spicules showed no corrosion, even under low pH. The absence of corrosion may be related to a protection promoted by the organic cover that surrounds the spicules of the calcareous sponges, at least in the time of the experiment. The sponges were able to synthesize normal spicules in all treatments, presenting only a few deformed ones, which could indicate a possible adapter mechanism of pH regulation at the calcification site. The proportion of deformed spicules increased significantly as the temperature increased, but still did not represent a real problem for the sponges. The Mg/Ca ratio was shown to be constant throughout the year in sponges* in situ *and also in aquaria experiments. A trend of higher* Staphylococcus *sp. abundance in elevated temperature treatments was identified, but this shift could not be associated with a negative impact on the host, as it was not observed necrosis or high mortality. The genus* Ruegeria *spp. dominated the bacterial core and its presence could be related to quorum sensing and thus to benefits to the host. Thus, our results suggest that calcareous sponges may not be as vulnerable to climate change projections as previously proposed, but long-term studies are needed to confirm these results.*

Keywords: Climate Change, *Sycettusa hastifera*, Calcium Carbonate, Bacterial Community.

INTRODUCTION

In the last 260 years since pre-industrial times, the concentration of carbon dioxide (CO_2) in the atmosphere has increased by 45% due to anthropogenic activities (VMO, 2017). This is one of the most important greenhouse gases, responsible for ocean warming (OW) and acidification (OA). According to the worst-case scenario (RPC 8.5) predicted by the Intergovernmental Panel on Climate Change (IPCC), the temperature is expected to increase up to 4.8 ^{o}C and the pH to reduce 0.3 – 0.5 units, what will represent 170% of acidity increase by the end of the XXI century (IPCC, 2014). As the CO_2 in the atmosphere absorbs part of the infrared radiation maintaining the earth warm, the non-natural increase in its concentration enhances global warming and consequently ocean warming. Besides, the oceans absorb the remaining atmospheric CO_2 that reacts with the seawater forming carbonic acid (H_2CO_3), which dissociates by losing hydrogen ions (H^+) into bicarbonate (HCO_3^-) and then in carbonate ions (CO_3^{-2}). The increase in H^+ lowers the pH and the concentration of CO_3^{-2}, being the latter also responsible for reducing the calcium carbonate saturation state ($CaCO_3$; $\Omega = [Ca^+]\,[CO_3^{-2}] / K'sp$) (Doney *et al*., 2009). Temperature can also influence the calcium carbonate saturation state (Ω), since $K'sp$ is a stoichiometric solubility product that along with the low concentration of CO_3^{-2} changes the rates of formation and dissolution of $CaCO_3$ (Doney *et al*., 2009). This is an essential mineral for the calcifying organisms and may be crystalized mainly in three mineral phases: calcite, aragonite and magnesium-calcite. These polymorphs of $CaCO_3$ have different solubility rates, being pure calcite less soluble than aragonite, while the solubility increases with increasing Mg concentrations, characterizing high-Mg calcite as the most soluble mineral phase of $CaCO_3$ and, therefore, making the calcifying organisms with this composition more vulnerable to climate changes (Andersson *et al*., 2008).

Ocean acidification has already been shown to be the major problem for calcifying organisms (Hofmann *et al*., 2010) causing deleterious impacts, such as weakening and structural deformities, sometimes leading to death (Hall-Spencer *et al*., 2008; Ragazzola *et al*., 2012; Li *et al*., 2014; Fitzer *et al*., 2016; Foster *et al*., 2016). OW also affects skeletal mineralogy, increasing magnesium concentration and thus increasing dissolution in acidified waters (Swezey *et al*., 2017). On the other hand, in conditions of high-CO_2 the Mg concentration decreases, but changes in the structural properties may lead to reduced elasticity and functionality (Ragazzola *et al*., 2016). In some cases, OW and OA act synergistically,

intensifying the rates of mortality and dissolution of the organisms (Diaz-Pulido *et al*., 2012). Besides that, climate changes also may shift the microbial community in marine organisms in general, regardless of being a calcifier (Webster *et al*., 2012; Morrow *et al*., 2015). Nonetheless, reef-building corals are the main focus of climate change research while other calcifying organisms, such as calcareous sponges, are usually neglected.

Sponges (phylum Porifera) are sessile and very efficient filter-feeding animals, capable of making the trophic connection of the benthic-pelagic zone (sponge loop), cycling nutrients and inorganic compounds, and participating on the consolidation and bioerosion of marine substrates (Rützler, 1975; Vogel, 1977; Bergquist, 1978; Wulff, 2001; Maldonado *et al*., 2012; De Goeij *et al*., 2013; Rix *et al*., 2016). They are considered reservoirs of biodiversity, providing food and refuge for some organisms, increasing the three-dimensionality and complexity of benthic communities, and consequently favouring an increase in local abundance and biodiversity (Pearse, 1950; Cerrano *et al*., 2006; Padua *et al*., 2013). Besides the ecological interactions with macro-organisms in marine environments (Wulff, 2006; Ribeiro *et al*., 2016), they are also intrinsically associated with microorganisms, what makes them holobionts, leading to cases of co-evolution (Webster & Taylor, 2012).

Currently, sponges are divided into four living classes, being the class Calcarea the only one with spicules of calcium carbonate, crystalized as high-Mg calcite (Jones & Jenkins, 1970). The class Calcarea comprises more than 730 species in the world, representing the second best-known class of the phylum Porifera (Van Soest *et al*., 2018). Smith and collaborators (2013) raised concern about the possible threatened future of calcareous sponges in a high-CO_2 world, but no experimental work was done to answer this question. Peck and collaborators (2015) in a biofouling work tagged the calcareous species *Leucosolenia* sp. as a winner in low pH, contrasting most of the previous information and expectation about calcifying organisms in future acidic oceans. Sponges in general appear to have low sensitivity to climate changes, being considered potential organisms to dominate some coral reefs in the future oceans, since they have already been seen increasing their area coverage due to the decrease in spatial competition with the reduction of coral cover (Bell *et al*., 2013, 2015).

There are several studies concerning the effects of climate changes on sponges, regarding their ecology (Fabricius *et al*., 2011; Guihen *et al*., 2012; Goodwin *et al*., 2014), microbiology (Webster *et al*., 2008a; Cebrian *et al*., 2011; Morrow *et al*., 2015; Ramsby *et al*.,

2018), physiology (Riisgard *et al*., 1993; Duckworth *et al*., 2012; Massaro *et al*., 2012; Bennett *et al*., 2016), biomineralization (Vicente *et al*., 2015), bioerosion (Wisshak *et al*., 2012, 2013, 2014; Fang *et al*., 2013; Stubler *et al*., 2014, 2015; Schönberg *et al*., 2017) and gene expression (López-Legentil *et al*., 2008; Pantile & Webster, 2011; Webster *et al*., 2013; Guzman & Conaco, 2016). Nonetheless, more than 75% of these works are exclusively on a single climate change stressor (OW or OA), making necessary to increase the studies combining both stressors in order to have a more realistic projection on the effects of warming and acidification in the future oceans.

In a recent publication about the effects of high temperature and low pH on sponges, it was verified that not a single work took care of these effects on calcareous sponges (Carballo & Bell, 2017). Hence, we still need more data about the possible vulnerability of these sponges in the future oceans. The present work is the first experimental climate change study dedicated exclusively to calcareous sponges. The major aim of this work is to verify the effects of ocean warming and acidification on adults of the calcareous sponge *Sycettusa hastifera* (Row, 1909), regarding their skeleton and microbiota: (1) Evaluate possible corrosion, variation in size, shape and chemical composition (Calcium and Magnesium) of the spicules in conditions of higher temperature and acidity; (2) Evaluate if the sponges can regenerate and synthesize normal spicules under these different conditions; and (3) Analyse potential shifts in the associated bacterial community in high temperature and low pH conditions.

MATERIALS AND METHODS

- **Sampling**

Adult individuals of *Sycettusa hastifera* were collected at approximately 1 m depth by snorkelling in a mussel farm at Arraial do Cabo, Rio de Janeiro state, Brazil (22^{o}58’02.9’’S, 42^{o}00’25.3’’W). All specimens were transferred to the facilities of the Marine Aquarium Research Centre of Rio de Janeiro (AquaRio) in 20 L recipients with aquarium aerator.

- **Experimental Treatments**

At AquaRio we developed a semi-open system of aquaria to perform a short-term experiment of ocean warming and acidification (Fig. 1). The system comprised four aquaria of 80 L each, with constant water flow and renewal of 10% of the total aquarium per hour (8

L/hour). Temperature and pH values were tested based on present and future conditions, following the worst-case scenario (RCP8.5) for 2100 of the Intergovernmental Panel on Climate Changes (IPCC, 2014). Four treatments were performed: Control (22 ^{o}C and pH 8.1), High Temperature (26 ^{o}C and pH 8.1), Low pH (22 ^{o}C and pH 7.6), and Combined Effects (26 ^{o}C and pH 7.6) with three temporal replicas of each treatment. Each replica lasted 10 days, being the first 24 hours of acclimation (Control conditions: 22 ^{o}C and pH 8.1). The control temperature of 22 ^{o}C was chosen because it is the mean temperature of the sample site. After the acclimation period, sponges were distributed into treatment aquaria (N = 5 per aquarium; N = 15 per treatment) and temperature was gradually ramped 1 ^{o}C per hour and pH levels decreased 0.1 unit per hour. All aquaria were fitted with aerators (porous stones) to keep the seawater flowing. To reach the target temperatures, chillers (Mundo Sub – MS3000) and heaters (Glass Heater ViaAqua and Super Aquatic – 200W) were used. The reduction of pH was done by injecting CO_2 through an aquarium diffuser coupled to a pump (Sarlo Better 1000) to maximize the dissolution of the gas in the seawater. Besides, a pH controller (Analytical Instruments - Model PH-301) was used to maintain the low pH (*i.e.* pH 7.6). Temperature, pH (Analyser – Model PH 300M), Salinity (Hand-Held Analog Refractometer) and Alkalinity (Gran Method; Camourze-Mod, 1994) were measured daily and the other parameters of the carbonate system were calculated using the software CO2Calc (Table 1; Table S1; Robbins *et al*., 2010).

The experiments were short-term due to two factors: (1) The seawater at AquaRio is treated with ozone and ultraviolet filters, eliminating all bacteria and thus the principal component of sponges diet, and (2) Spicules synthesis and microbial changes in sponges occur within less than 10 days, which means that we had sufficient time to run the experiments (Jones, 1959; Webster *et al*., 2008).

- **Skeleton**

Sponge individuals (N = 3 per treatment) were fixed in ethanol 93%. A fragment of each individual was dissolved in sodium hypochloride, isolating the spicules from the organic part. The spicules slides were prepared following standard procedures (Klautau & Valentine, 2003). Measurements of length and width of the actines (20 spicules per individual/replica) were performed in an optical microscope (Nikon Eclipse E200). To analyse the shape of the spicules, after isolation with sodium hypochloride they were washed in distilled water,

transferred to ethanol 100% and placed on stubs with carbon conductive tape and coverslip. They were then dried and sputter coated with gold for Scanning Electron Microscopy (SEM - Jeol 6510). To evaluate the proportion of deformed spicules between treatments, 100 spicules were randomly classified in normal or deformed, using the same spicules slides used for measurements, in the optical microscope (Nikon Eclipse E200).

For quantitative chemical composition analyses of calcium and magnesium, spicules were pulverized and analysed by Atomic Absorption Spectroscopy technique (AAS) using a Shimadzu (AA-6800) Spectrophotometer operated with air-acetylene flame atomizer. The wavelength of calcium and magnesium are 422.7 nm and 285.2 nm, respectively. The absorbances read were compared with a calibration curve in order to determine the content of each chemical component in the samples. Beside the samples submitted to the treatments, Mg/Ca ratios were also measured in sponges collected in the sea throughout the year. These measurements were performed to verify if there was aquaria effect in the Mg/Ca ratios. To evaluate if the sponges were able to synthesize spicules under experimental conditions, half of the oscular region was cut off on the third day of experiment by hand with a scalpel (N = 6 per treatment). The regeneration was monitored daily with photographs using a Canon Powershot D20. By the end of the experiments, a tangential section of the cortex of the regenerated area was analysed by SEM with the same preparation described above for spicules analysis.

A non-metric Multidimensional Scaling (nMDS) was used to visually compare the spicules measurements of the treatments with temperature and pH as factors. Two-way permutational analysis of variance (PERMANOVA; Anderson, 2001) using 9,999 permutations with Euclidean distance matrices and temperature and pH as factors was used to determine if the differences in the spicules measurements among treatments were statistically significant. These analyses were performed in the software PAST version 3. A two-way ANOVA with logit transformation (Warton & Hui, 2011) using temperature and pH as factors was used to determine if the differences among treatments in spicules proportion (normal x deformed) and Mg/Ca ratios were statistically significant. This analysis was performed in the software R version 3.4.4 ® for Windows (R Development Core Team, 2018), using the package *car* (Fox & Weisberg, 2011). All graphs were made in Microsoft Excel.

- **Microbiota**

To assess possible shifts in the microbiota across treatments, sponges were frozen in liquid nitrogen and then macerated with a pestle. DNA extraction was then performed (N = 3 sponges per treatment and field sample) using DNeasy PowerSoil Kit (Qiagen), following the manufacturer's protocol. DNA was quantified using Qubit Fluorometer (Invitrogen). The V4 hypervariable region of the 16S rRNA gene was sequenced in the Illumina MiSeq platform using the bacterial primers 515F/806R (Caporaso *et al*., 2011) at the Argonne National Laboratory (Lemont, IL, USA). Sequence data was processed using Qiime2 (https://qiime2.org/). Briefly, raw sequences were de-multiplexed, quality-filtered and trimmed in the 5' end with a maximum length of 218 bp. Reads were denoised, amplicon sequence variants and chimerism were determined using the plugin DADA2 (Callahan *et al*., 2016; 2017), being the latter excluded from the further analysis. Representative sequences of a taxonomic group were determined and taxonomic assignments were performed based on SILVA 119 database (https://www.arb-silva.de/documentation/release-119/). Sequences matching chloroplast, mitochondrial, archaea and eukaryote sequences were discarded. Pivot tables were made to visualize and condense the ASV table by taxonomic levels in Microsoft Excel. Alpha diversity indices (Shannon H' and Chao1) were calculated and plotted using the software PAST version 3 and the Excel, respectively. A two-way ANOVA with temperature and pH as factors was used to determine if the differences between treatments in alpha diversity were statistically significant. For beta diversity, a non-metric Multidimensional Scaling (nMDS) was used to visually compare the bacterial communities of the treatments with temperature and pH as factors. A two-way permutational analysis of variance (PERMANOVA; Anderson, 2001) using 9,999 permutations with Bray-Curtis distance matrices and temperature and pH as factors was used to determine if the differences in the bacterial community between treatments were statistically significant. Similarity percentage (SIMPER) was used to determine which sequences most contributed for the communities differences. A one-way ANOVA and a PERMANOVA were used to determine if the differences between field and aquaria samples were statistically different. Graphs were made in Excel and Venny 2.1.0 and statistical analyses were performed in the software PAST version 3.

Table 1: Summary of seawater chemistry parameters measured (*) and calculated (**) of the ocean warming and acidification experiments, represented as the mean and the standard deviation (SD) of measurements taken daily (N = 8 sampling days) of the three experimental replica.

Treatment	Temp (^{o}C)*	pH (Total)*	Salinity*	Alkalinity (µmol kg $^{-1}$)*	pCO_2 (µatm)**	Ω Calcite**
Control	22.09 (0.13)	8.14 (0.03)	34.52 (0.99)	2311 (48)	301 (30)	5.67 (0.31)
High Temperature	27.06 (1.32)	8.16 (0.03)	34.91 (0.95)	2317 (47)	278 (28)	6.78 (0.51)
Low pH	22.24 (0.15)	7.63 (0.04)	34.96 (0.93)	2308 (44)	1182 (119)	2.13 (0.19)
Combined Effects	26.35 (1.02)	7.64 (0.05)	34.78 (1.04)	2315 (52)	1156 (167)	2.54 (0.25)

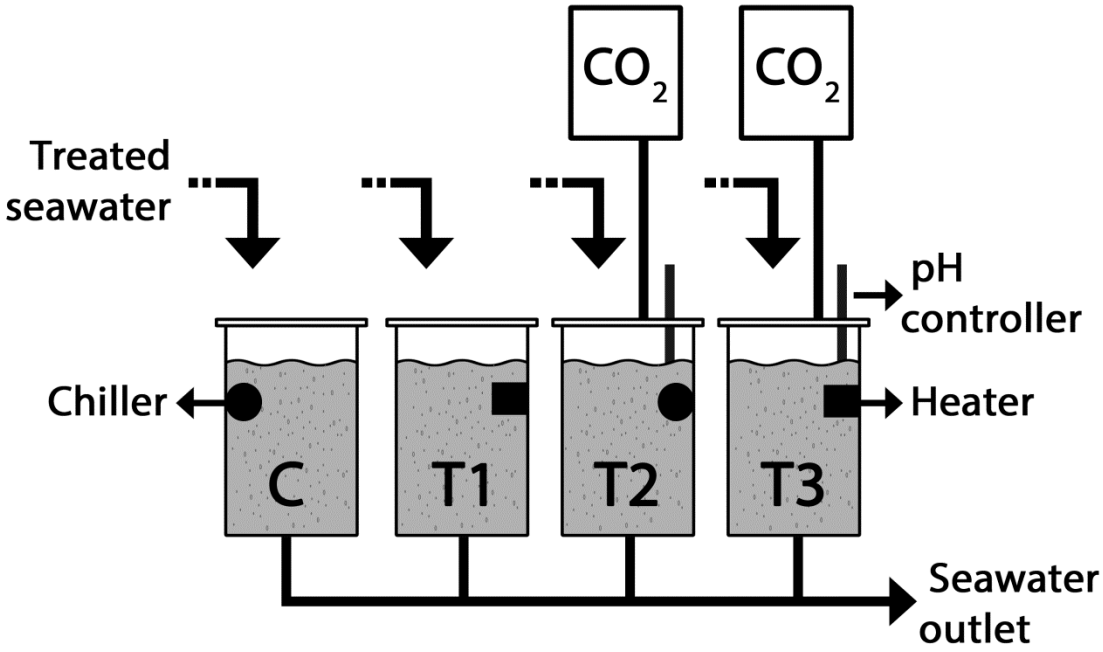

Figure 1: Semi-open system of aquaria for ocean warming and acidification experiments. (C) Control: pH 8.1 / 22 ^{o}C; (T1) High Temperature: pH 8.1 / 26 ^{o}C; (T2) Low pH: pH 7.6 / 22 ^{o}C; and (T3) Combined Effects: pH 7.6 / 26 ^{o}C.

RESULTS

- **Skeleton**

At the end of the experiments, the spicules of *S. hastifera* individuals presented no signs of visual corrosion in both light and electronic microscopies in any of the treatments (Fig. 2). About their size, the two-way PERMANOVA showed no significant differences (Table 2A; Table S2) and a non-metric Multi-Dimensional scaling (nMDS) plot revealed overlap among treatments (Fig. 3A-B). We found a significantly higher number of deformed

spicules in sponges submitted to high temperature, although yet the amount in general was low (High Temperature and Combined Effects treatments; Two-way ANOVA, P = 0.0467; Max of 15% deformed) (Fig. 4-5; Table 3A). The Mg/Ca ratios in individuals in the sea were constant throughout the year (Fig. 6 Seasonal field samples) and the same was observed for the aquarium samples mean after the treatments, with no significant differences between them (Fig. 6 Aquarium samples; Table 3B). However, the specimen with higher proportions of deformed spicules also presented higher Mg/Ca ratio (Fig. 5). Differently from what we had expected in the regenerated parts, the sponges were able to synthesize normal spicules under all treatments, presenting only a few deformed ones (Fig. 7).

- **Microbiota**

A total of 4,320 amplicon sequence variants (ASVs) was identified, being 951 (22%) associated only with field samples and 2,887 (66.8%) only with aquarium samples (Fig. 8). Both presented an exclusive core and the field core represents the natural bacterial community of this species (Fig. 12; Table S4A-B). The whole bacterial community of *S. hastifera* was distributed in 33 phyla and candidate phyla, being Proteobacteria the dominant phylum (especially Alpha- and Gammaproteobacteria), comprising more than 50% of the sequences in the field and treatments (Fig. 9). Of the total ASVs identified, only 0.14% was detected in all sponges analyzed (N = 15) representing the bacterial community core, with no significant differences between field and aquarium (PERMANOVA, P = 0.1159) (Fig. 8, 10). The core comprises only the phylum Proteobacteria, dominated by *Ruegeria* spp. and the class JTB23 (Fig. 10; Table S4C).

The bacterial alpha-diversity (Shannon and Chao1) was similar in field and aquarium treatments, with no significant differences between them (ANOVA, Shannon P = 0.4462; Chao1 P = 0.7467; Fig. 11; Table 4). However, beta-diversity was significantly different in field and aquarium treatments, including the control (PERMANOVA, P = 0.0026). SIMPER analysis comparing field and aquarium treatments revealed that *Candidatus* Branchiomonas (class Betaproteobacteria) and *Staphylococcus* sp. (phylum Firmicutes) were the taxa that most contributed to this difference, representing more than 20% of the variation and the mean abundance for both of them was higher in the field (Table 5). However, the higher mean abundance of *Staphylococcus* sp. in the field has resulted of an outlier in the third field replicate, representing more than 45% of the ASVs in the specimen (Fig. 9; Table S3). Other

important ASVs contributing at least 0.5% to this differentiation included members of Cyanobacteria, Alpha and Gammaproteobacteria.

In the aquaria, 685 ASV (20.3%) was associated only with the Control, 501 (14.9%) with High Temperature, 522 (15.5%) with Low pH and 484 (14.4%) with Combined Effects (Fig. 13). All treatments presented an exclusive core, with the exception of Combined Effects (Table S4D-F). The Control core presented one ASV of the genus *Parvularcula* sp. (class Alphaproteobacteria) and the High Temperature another one of the order Myxococcales, family 0319-6G20 (class Deltaproteobacteria) (Table S4D-E). The Low pH core comprised 11 ASVs, dominated by the phyla Bacteroidetes and Proteobacteria (Table S4F). At the genus level, one ASV of the genus *Gilvibacter* sp., *Ulvibacter* sp., *Lewinella* sp., *Planctomyces* sp., *Nannocystis* sp. and of the OM27 clade was found associated only with the individuals of Low pH treatment.

A two-way PERMANOVA revealed no significant differences in the bacterial community related to temperature and pH treatments (Table 2B). However, in the nMDS plot, temperature treatments revealed a trend for splitting the samples (Fig. 3C-D). Although not statistically significant, it seemed to have a tendency of increase in Firmicutes and decrease in Bacteroidetes in higher temperature (High Temperature and Combined Effects) (Fig. 9). SIMPER analysis comparing the bacterial communities between temperature and pH treatments revealed *Rhodobacteraceae*-affiliated bacterium and *Staphylococcus* sp. as the taxa that most contributed to this dissimilarity, representing 16% of the variation (Table 6; Fig. 14). The mean abundance of Rhodobacteraceae sequences was lower in 26 ^{o}C treatments, while for *Staphylococcus* sp. was higher. Other important ASVs contributing at least 0.5% to this differentiation included members of Bacteroidetes, JTB23, Alpha and Gammaproteobacteria.

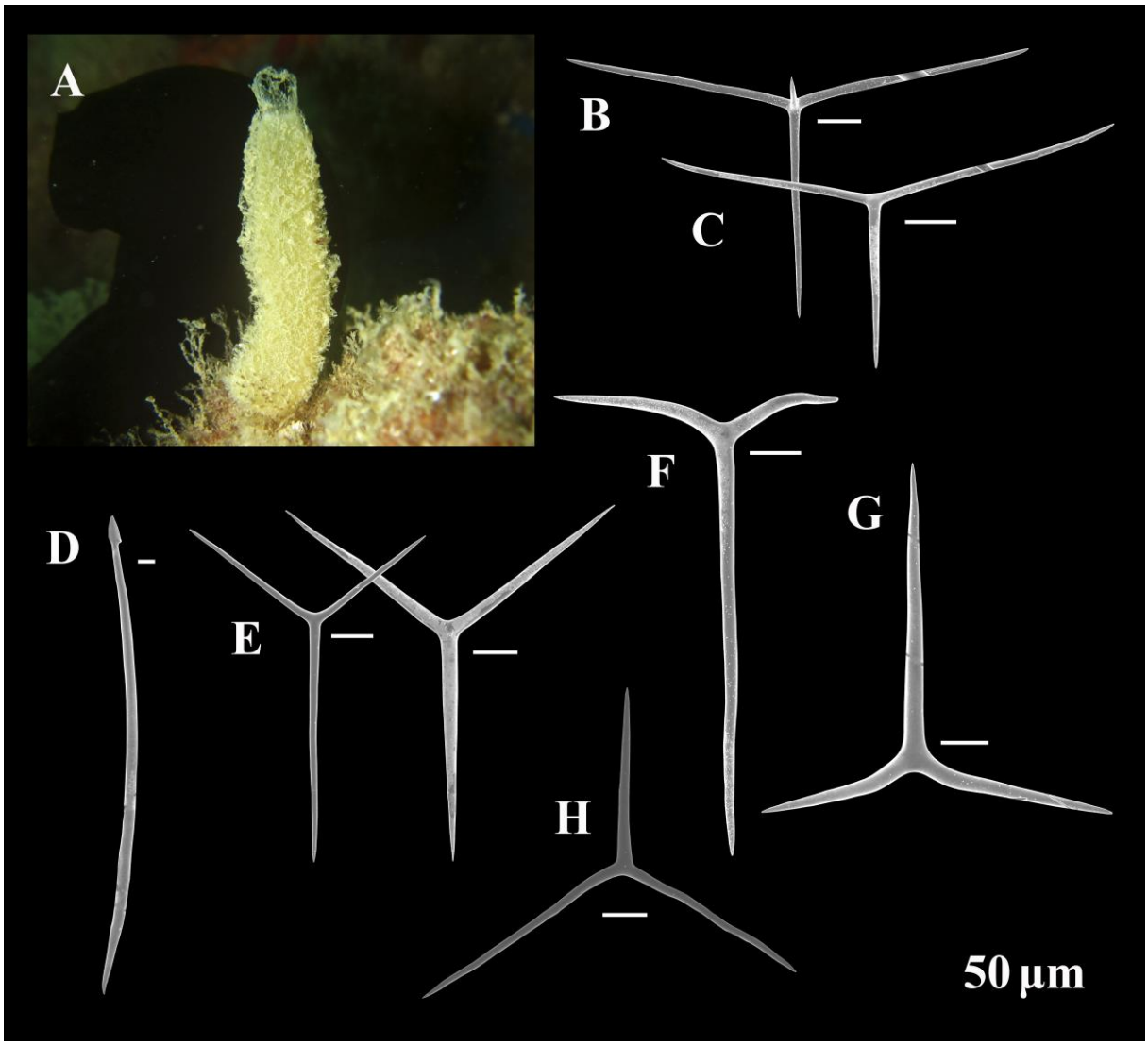

Figure 2: *Sycettusa hastifera* morphology. (A) *In vivo* specimen; (B) Tetractine of the crown; (C) Triactine of the crown; (D) Diactine; (E) Cortical triactines; (F) Subcortical triactine; (G) Subatrial triactine; and (H) Atrial triactine.

Table 2: Two-way PERMANOVA of spicules measurements (Euclidean matrix) and bacterial community (Bray-Curtis matrix) in response to the fixed factors Temperature, pH and their interaction with 9,999 permutations.

A) Spicules Measurements					**B) Bacterial Community**		
Source	***df***	**MS**	**Pseudo-*F***	***P***	**MS**	**Pseudo-*F***	***P***
Temperature	1	12342	0.60408	0.5484	0.2677	1.594	0.1134
pH	1	78822	3.858	0.0673	0.1490	0.887	0.5139
Temperature x pH	1	2728,7	0.13356	0.9408	0.1460	0.869	0.5449
Residual	8	20431			0.1680		

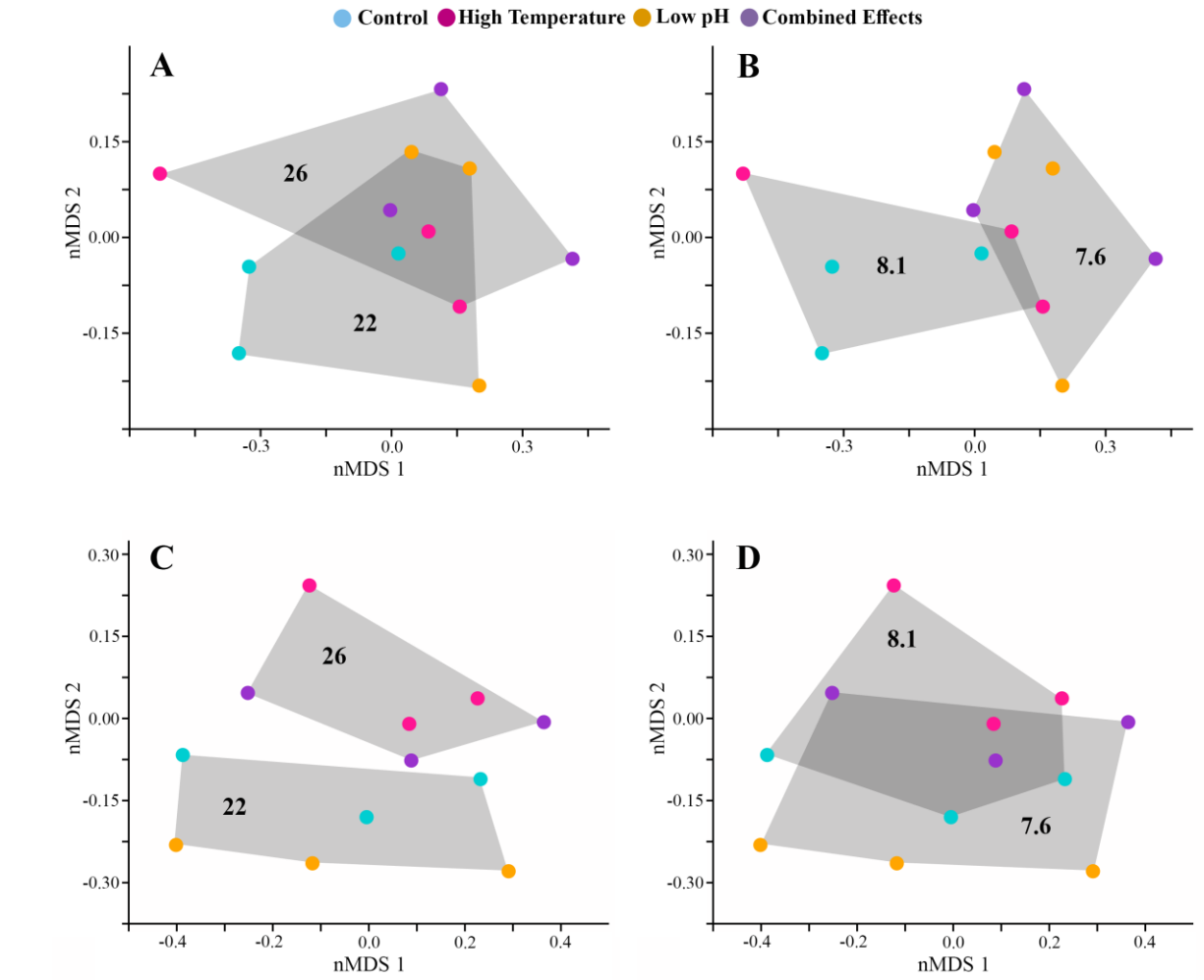

Figure 3: Non-metric Multi-Dimensional Scaling (nMDS) ordination in two dimensions. (A-B) Spicules measurements (Euclidean matrix; Stress = 0.06) and (C-D) Bacterial microbiome (Bray-Curtis matrix; Stress = 0.124). Each dot represents one replica.

Table 3: Two-way ANOVA with logit transformation of spicules proportion (Deformed x Normal) and Mg/Ca ratio in response to the fixed factors Temperature, pH and their interaction.

A) Spicules Proportion					**B) Mg/Ca**		
Factor	***df***	**MS**	**F**	***P***	**MS**	**F**	***P***
Temperature	1	3.760	5.519	0.0467	0.0438	1.734	0.224
pH	1	0.443	0.650	0.4435	0.0845	3.345	0.105
Temperature x pH	1	0.023	0.034	0.8578	0.0201	0.795	0.399
Residual	8	0.681			0.0253		

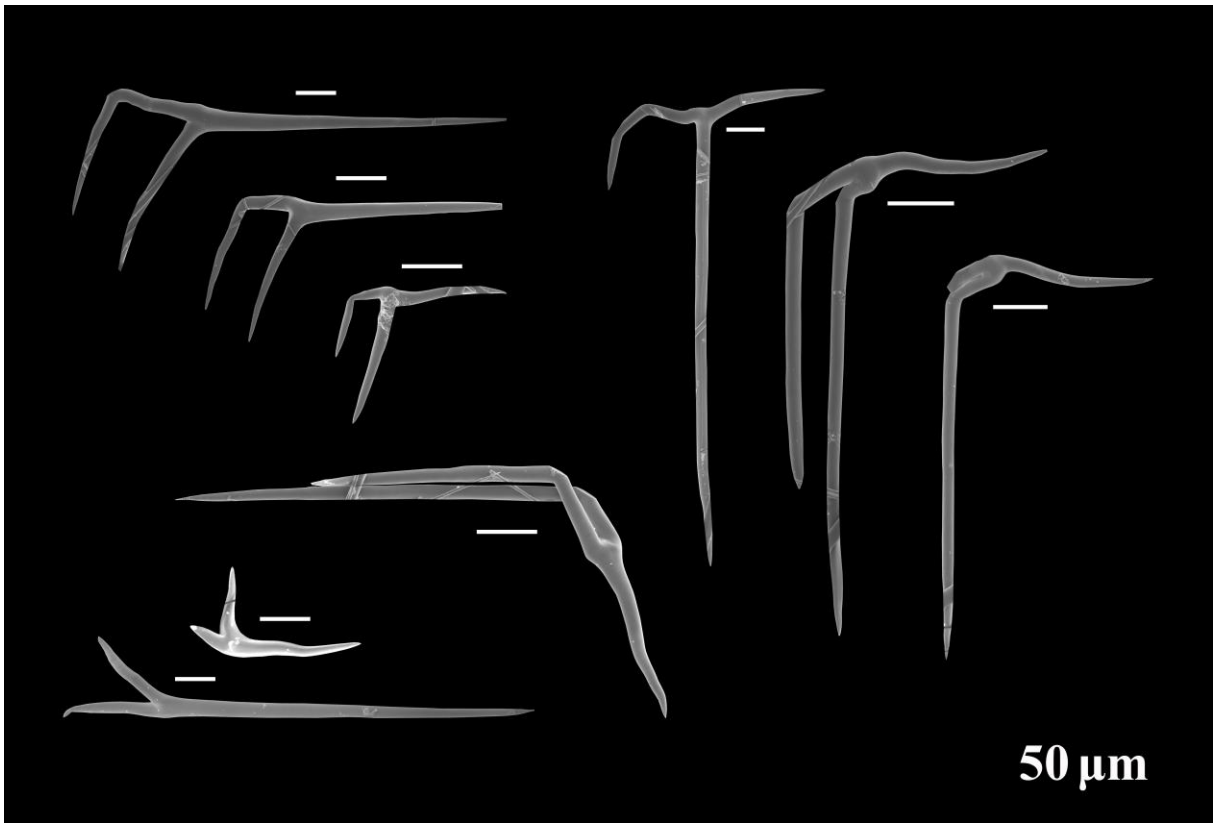

Figure 4: Deformed spicules of *Sycettusa hastifera*.

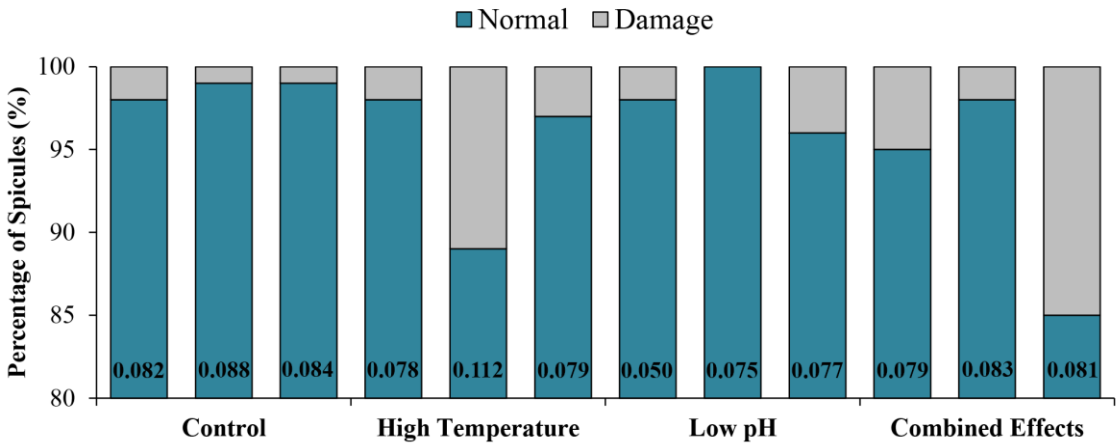

Figure 5: Proportion of Deformed *versus* Normal spicules from each individual in the aquarium treatments (N = 20 spicules per individual; N = 60 spicules per treatment). Numbers in the bottom of each column represents the Mg/Ca ratio of each individual.

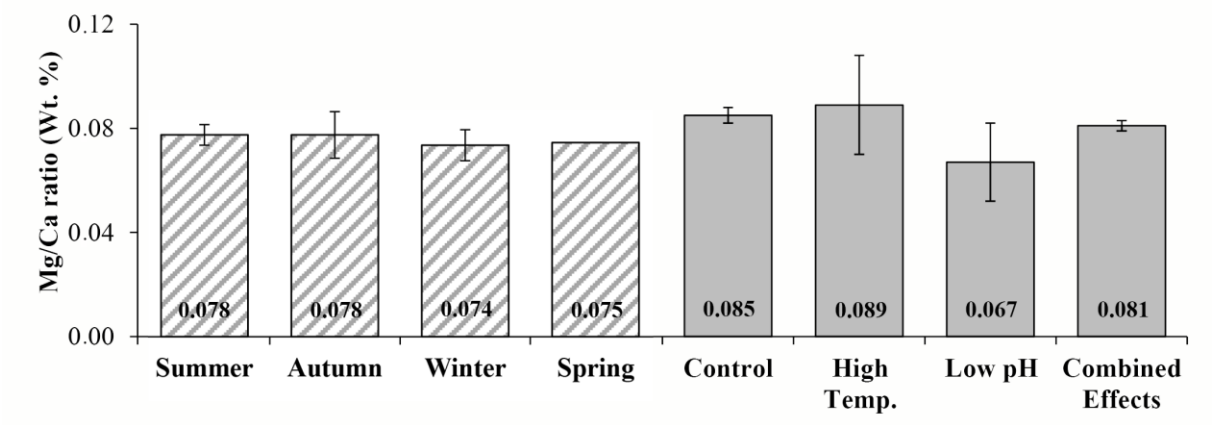

Figure 6: Mean Mg/Ca ratio (±SD) in weight percent (Wt. %) of seasonal field samples: Summer (N = 2), Autumn (N = 2), Winter (N = 3) and Spring (N = 1); and aquarium samples: Control, High Temperature, Low pH and Combined Effects (N = 3 for each treatment). Numbers in the bottom of each column represents the mean Mg/Ca ratio.

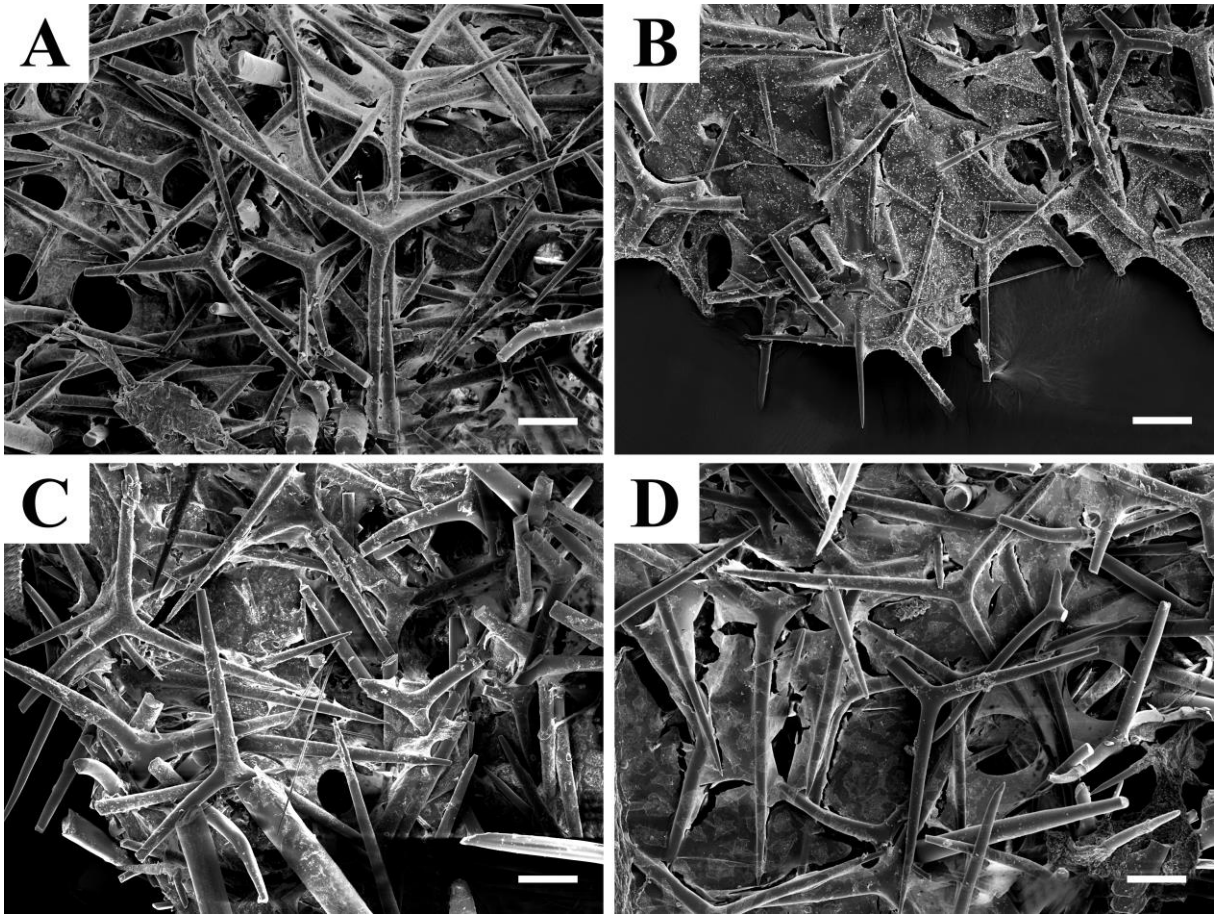

Figure 7: Tangential section of the cortex of the regenerated area. (A) Control; (B) High Temperature; (C) Low pH and; (D) Combined Effects. White bar scale of 50 μm.

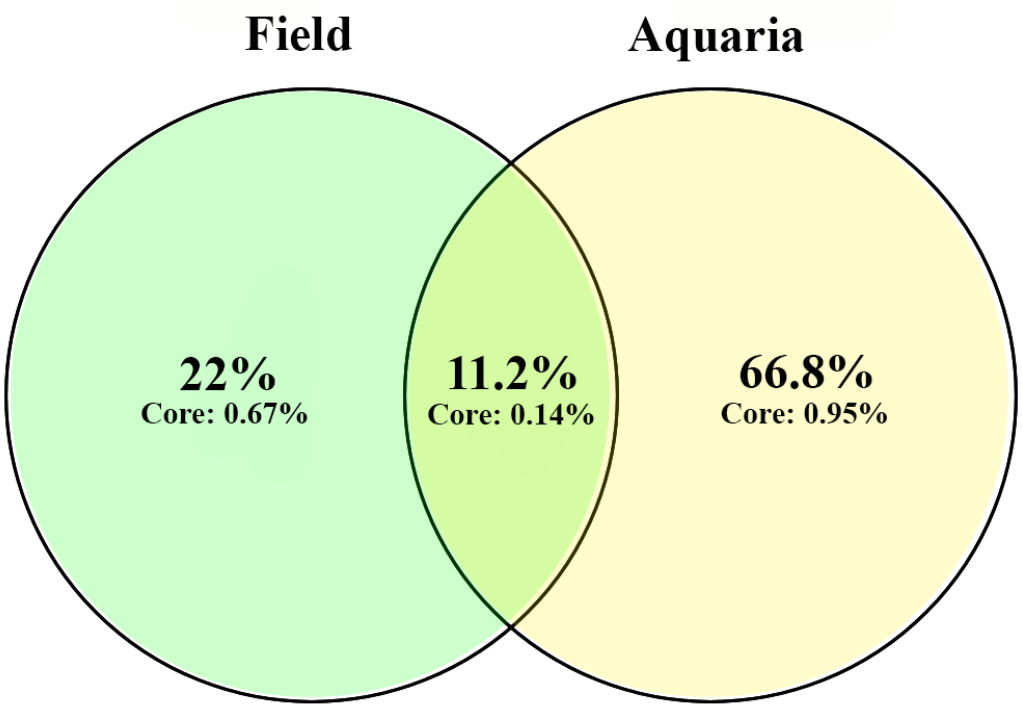

Figure 8: Percentage of amplicon sequence variants (ASV) of *Sycettusa hastifera* bacterial community distributed in field and aquaria samples with the respective core percentages.

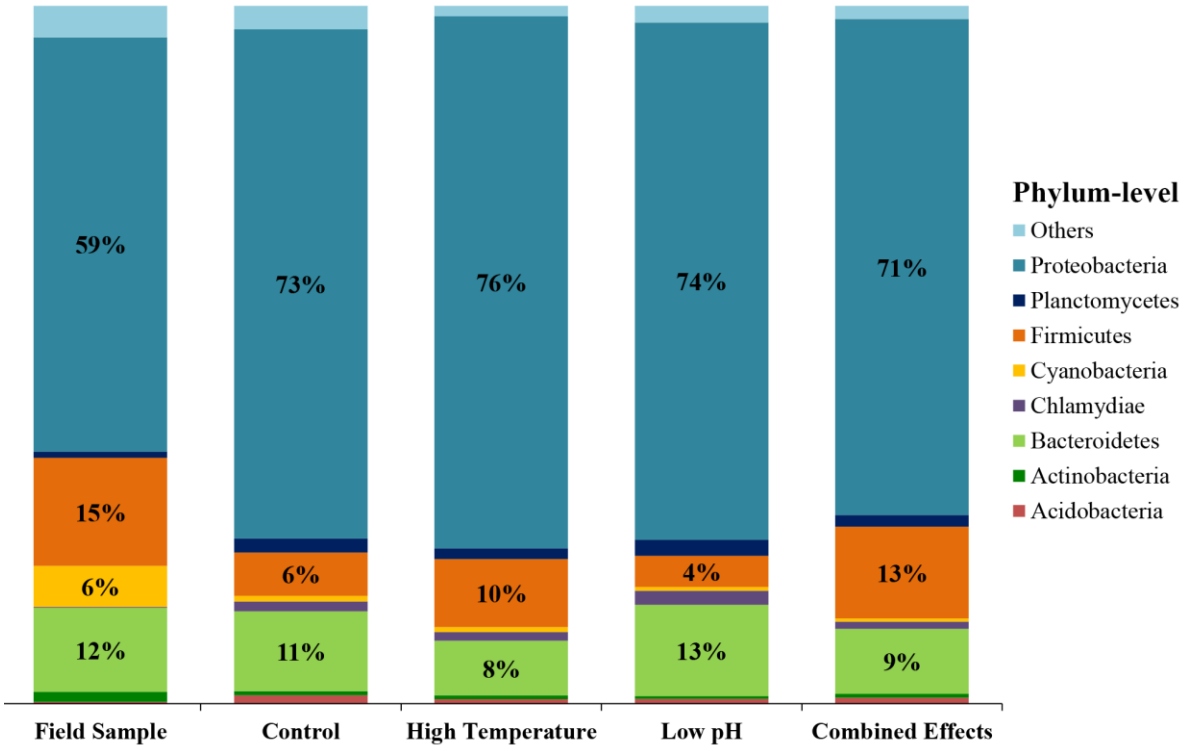

Figure 9: Mean relative abundance of the most dominant phyla in *Sycettusa hastifera* bacterial microbiome across all replicas (N = 3 per treatment).

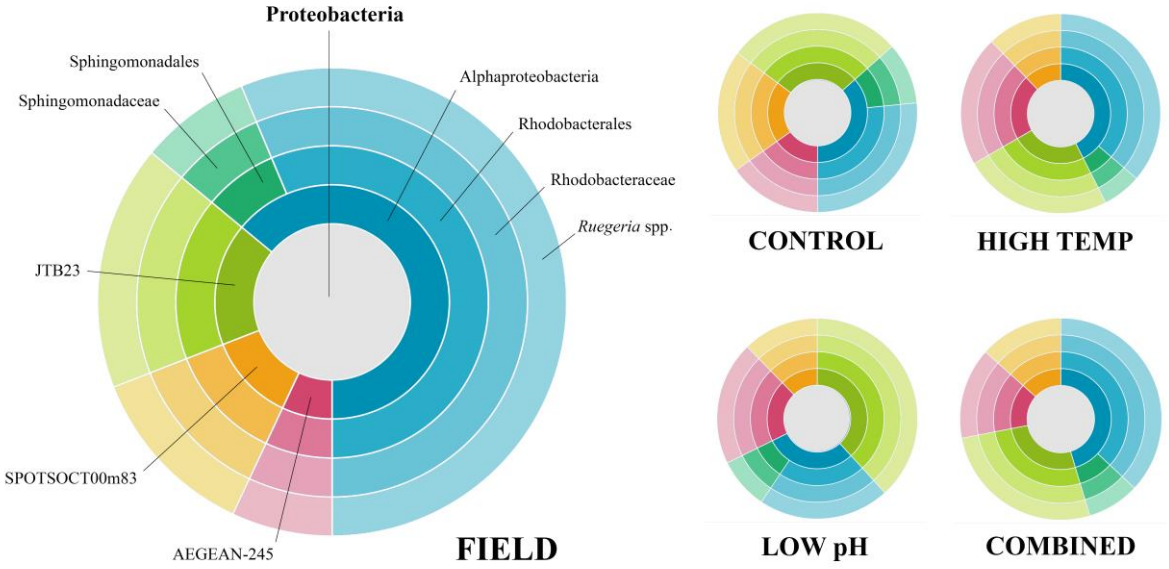

Figure 10: Bacterial core present in 100% of the sponges in the field and treatments samples. Not tagged spaces are "unclassified". Phylum in bold.

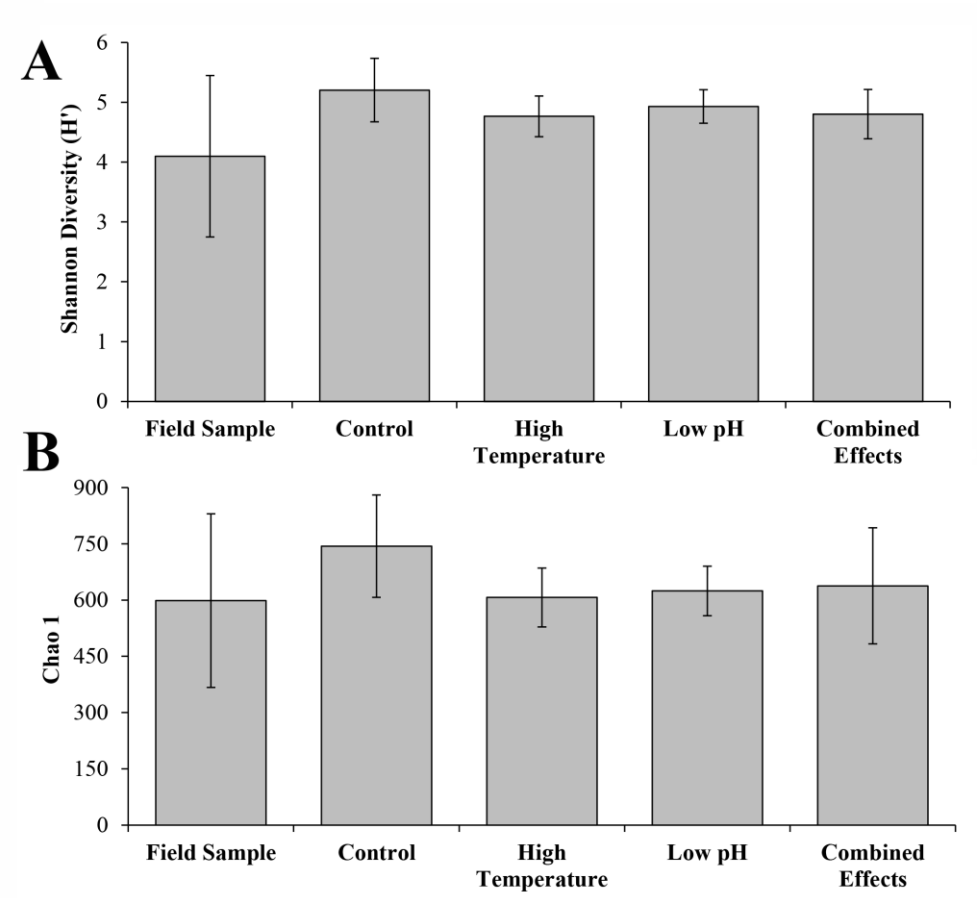

Figure 11: Bacterial community alpha-diversity of each treatment (Mean + Standard Deviation). (A) Shannon index and; (B) Chao1.

Table 4: Two-way ANOVA of bacterial community alpha-diversity (Shannon and Chao1) of *Sycettusa hastifera* in response to the fixed factors Temperature, pH and their interaction.

A) Shannon Index					**B) Chao1**		
Factor	***df***	**MS**	**F**	***P***	**MS**	**F**	***P***
Temperature	1	0.2423	1.499	0.2557	11408,3	0.859	0.3812
pH	1	0.0419	0.259	0.6244	5896,33	0.444	0.5241
Temperature x pH	1	0.0719	0.445	0.5235	16875	1.270	0.2924
Residual	8	0.1616			13287		

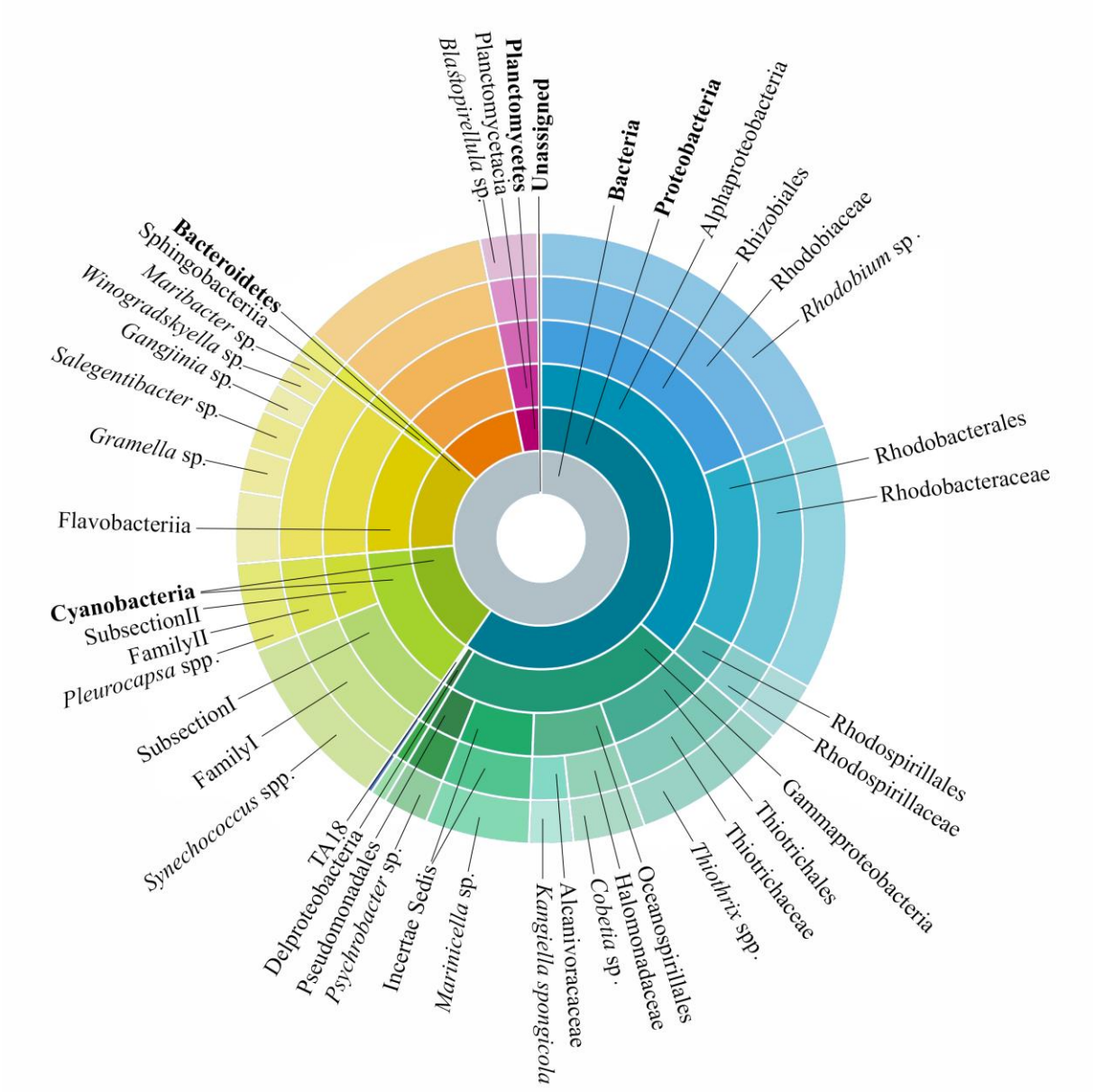

Figure 12: Field core. Complete microbial taxonomy in the supplementary table (Table S4C) Phylum in bold.

Table 5: Similarity Percentage Analysis (SIMPER) of the ASVs that contributed at least 0.5% to dissimilarity between field samples and aquarium treatments. Percentage contribution (Contr. %) and mean abundance across replicas (N = 3 individuals per group). Legend: FI = Field; CO = Control; HT = High Temp.; LPH = Low pH and CEF = Combined Effects.

ASV	Contr. %	FI	CO	HT	LPH	CEF	Microbial Taxonomy
ASV2047	10.37	7572	3	2	6	6	Proteobacteria, Betaproteobacteria, Nitrosomonadales, Nitrosomonadaceae, *Candidatus* Branchiomonas
ASV3352	10.02	6248	2591	4051	1745	5319	Firmicutes, Bacilli, Bacillales, Staphylococcaceae, *Staphylococcus*
ASV2518	4.32	0	4801	1080	4652	2862	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae
ASV0112	3.47	2678	0	0	0	0	Proteobacteria, Alphaproteobacteria
ASV1722	2.12	0	1400	2149	1232	1578	Proteobacteria, Alphaproteobacteria, OCS116 clade
ASV0342	1.84	0	726	3598	137	987	Proteobacteria, Gammaproteobacteria, Oceanospirillales, Oceanospirillaceae, *Neptuniibacter*
ASV2583	1.51	1134	10	34	16	69	Proteobacteria, Alphaproteobacteria
ASV4292	1.46	0	1125	1372	471	1470	Proteobacteria, Gammaproteobacteria, Oceanospirillales, Oceanospirillaceae, *Neptuniibacter*
ASV2806	1.45	0	570	1544	1450	738	Proteobacteria, Gammaproteobacteria, Oceanospirillales, Oceanospirillaceae, *Marinobacterium*
ASV1867	0.86	0	467	1139	243	754	Proteobacteria, Gammaproteobacteria, Oceanospirillales, Oceanospirillaceae, *Neptuniibacter*
ASV1118	0.79	601	0	0	5	4	Cyanobacteria, Cyanobacteria, SubsectionI, FamilyI, *Synechococcus*
ASV2157	0.76	570	4	0	0	0	Cyanobacteria, Cyanobacteria, SubsectionI, FamilyI, *Synechococcus*
ASV2024	0.74	0	357	660	766	477	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae
ASV1197	0.72	0	585	529	382	691	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae
ASV2203	0.71	528	5	2	3	4	Bacteria
ASV0047	0.69	0	314	1129	327	279	Proteobacteria, Gammaproteobacteria, Alteromonadales, Alteromonadaceae, *Glaciecola*
ASV1255	0.62	0	248	302	1143	149	Proteobacteria, Gammaproteobacteria, Oceanospirillales, Oceanospirillaceae, *Marinobacterium*

ASV0606	0.53	0	218	177	769	345	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae
ASV2315	0.52	389	0	0	0	0	Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhodobiaceae, *Rhodobium*
ASV2690	0.51	0	251	685	194	392	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae, *Tropicimonas*
ASV0926	0.51	37	401	497	394	357	Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae

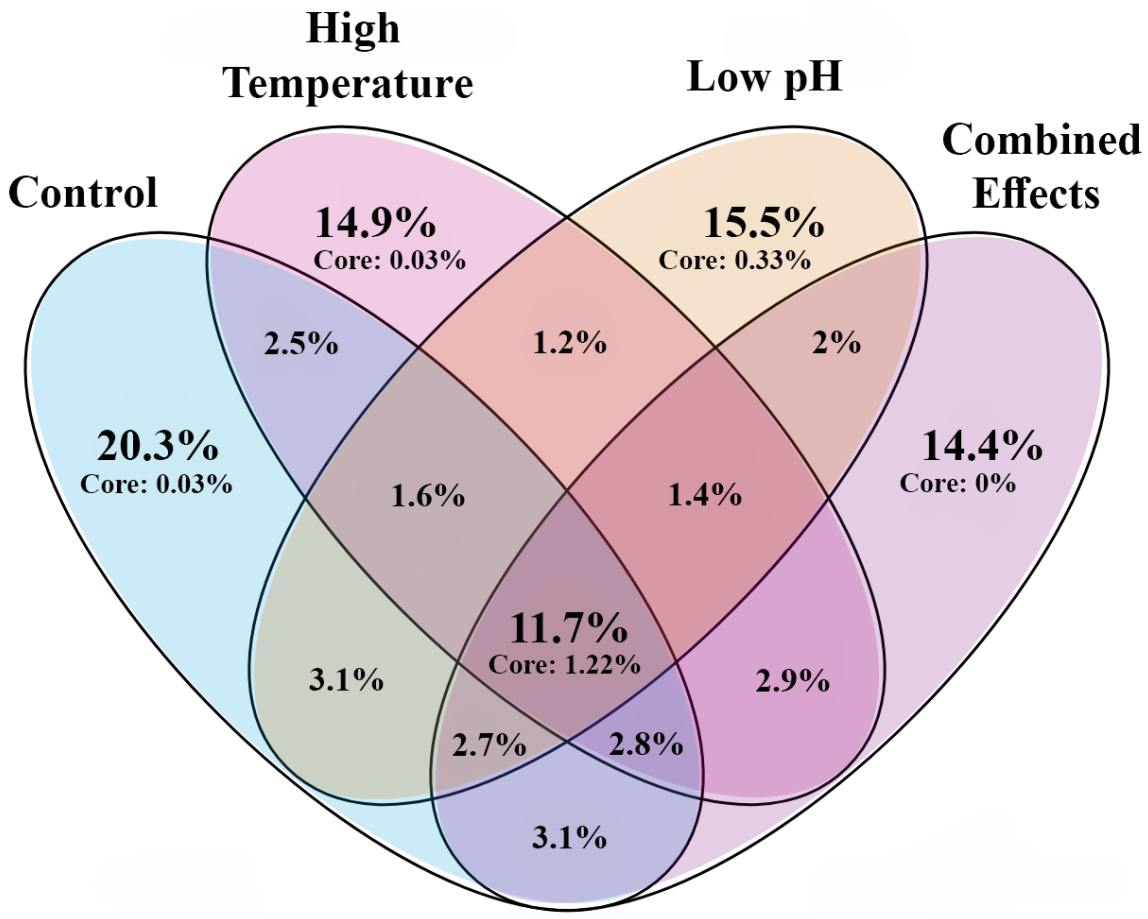

Figure 13: Percentage of amplicon sequence variants (ASV) of *Sycettusa hastifera* bacterial community distributed in aquarium treatments with the respective core percentage.

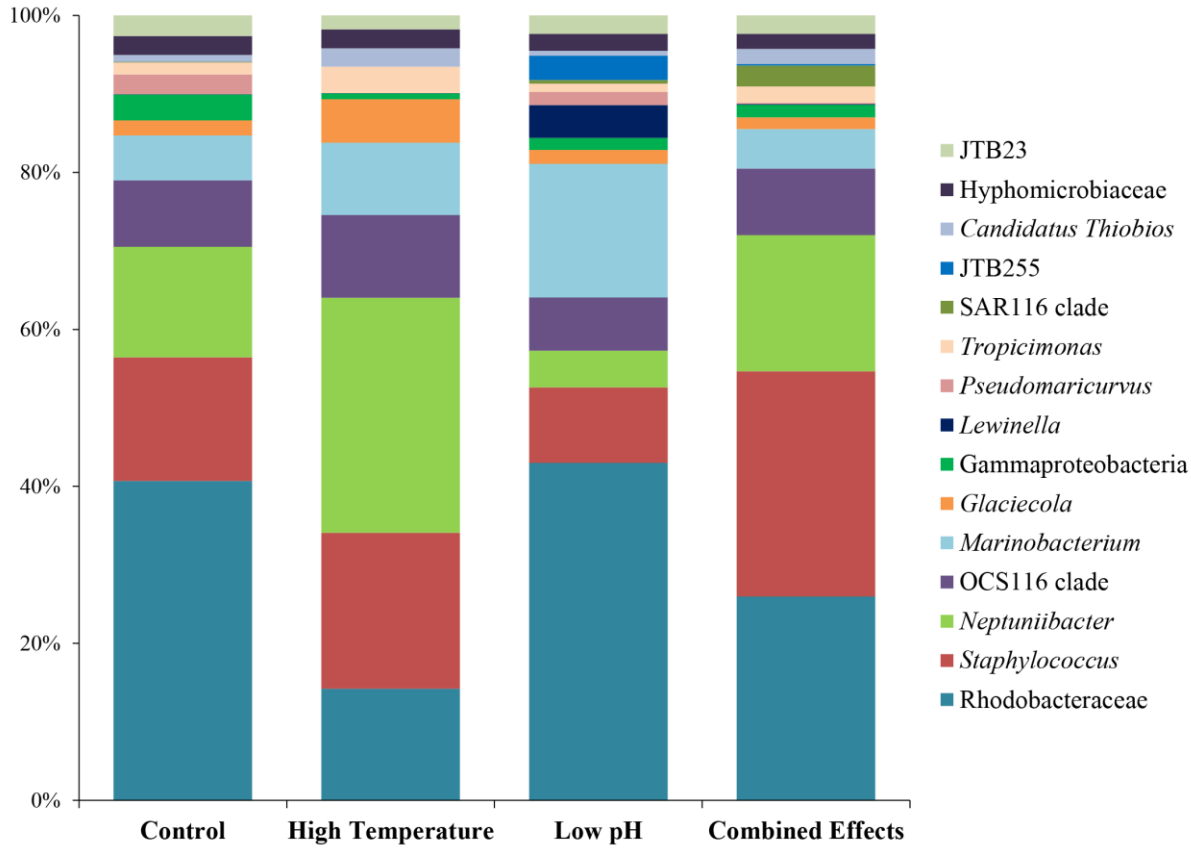

Figure 14: Mean relative abundance of the taxa that contribute at least 0.5% in dissimilarity between temperature and pH treatments according to SIMPER analysis (N = 3 per treatment).

Table 6: Similarity Percentage Analysis (SIMPER) of the ASVs that contributed at least 0.5% to dissimilarity between aquarium treatments related to temperature. Percentage contribution (Contr. %) and with mean abundance across replicas (N = 3 individuals per treatment). Legend: CO = Control; HT = High Temp.; LPH = Low pH and CEF = Combined Effects.

ASV	Contr. %	CO	HT	LPH	CEF	Microbial taxonomy
ASV2518	8.29	4801	1080	4652	2862	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae
ASV3352	8.09	2591	4051	1745	5319	Firmicutes, Bacilli, Bacillales, Staphylococcaceae, *Staphylococcus*
ASV0342	3.98	726	3598	137	987	Proteobacteria, Gammaproteobacteria, Oceanospirillales, Oceanospirillaceae, *Neptuniibacter*
ASV4292	1.98	1125	1372	471	1470	Proteobacteria, Gammaproteobacteria, Oceanospirillales, Oceanospirillaceae, *Neptuniibacter*
ASV1722	1.71	1400	2149	1232	1578	Proteobacteria, Alphaproteobacteria, OCS116 clade

ASV2806	1.64	570	1544	1450	738	Proteobacteria, Gammaproteobacteria, Oceanospirillales, Oceanospirillaceae, *Marinobacterium*
ASV1867	1.39	467	1139	243	754	Proteobacteria, Gammaproteobacteria, Oceanospirillales, Oceanospirillaceae, *Neptuniibacter*
ASV1255	1.23	248	302	1143	149	Proteobacteria, Gammaproteobacteria, Oceanospirillales, Oceanospirillaceae, *Marinobacterium*
ASV0047	1.02	314	1129	327	279	Proteobacteria, Gammaproteobacteria, Alteromonadales, Alteromonadaceae, *Glaciecola*
ASV2024	0.97	357	660	766	477	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae
ASV3202	0.95	543	152	274	296	Proteobacteria, Gammaproteobacteria
ASV0606	0.85	218	177	769	345	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae
ASV1976	0.80	7	8	758	20	Bacteroidetes, Sphingobacteriia, Sphingobacteriales, Saprospiraceae, *Lewinella*
ASV2674	0.68	412	0	304	22	Proteobacteria, Gammaproteobacteria, Alteromonadales, Alteromonadaceae, *Pseudomaricurvus*
ASV2690	0.68	251	685	194	392	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae, *Tropicimonas*
ASV3826	0.63	308	191	475	288	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae
ASV0271	0.63	13	0	86	490	Proteobacteria, Alphaproteobacteria, Rickettsiales, SAR116 clade
ASV1197	0.62	585	529	382	691	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae
ASV3063	0.58	123	32	494	44	Proteobacteria, Gammaproteobacteria, Oceanospirillales, Oceanospirillaceae, *Marinobacterium*
ASV3009	0.58	0	0	557	32	Proteobacteria, Gammaproteobacteria, Xanthomonadales, JTB255
ASV1133	0.58	146	475	120	364	Proteobacteria, *Candidatus* Thiobios
ASV0926	0.55	401	497	394	357	Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae
ASV1247	0.55	269	78	360	63	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae
ASV1797	0.51	430	361	423	432	Proteobacteria, JTB23
ASV1758	0.50	165	190	406	92	Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae

DISCUSSION

- **Skeleton**

Different from what was expected for a high Mg-calcite calcifier, the spicules of *S. hastifera* did not present signs of corrosion, even under low pH. A possible explanation for this could be the presence of an organic layer covering the spicules. Many calcifying organisms produce organic layers that protect their skeleton/shells from the ambient seawater and calcareous sponges also have this kind of protection (Jones, 1955; Kopp *et al*., 2011; Rossi *et al*., 2014). The class Calcarea, different from all the other classes in the phylum Porifera, does not present an axial filament to guide spicules synthesis. Then, the sclerocytes (cells responsible for the spiculogenesis) secret this organic sheath that covers the whole spicule and probably guides biomineralization (Jones, 1955; Ledger & Jones, 1977; Sethmann & Wörheide, 2008; Kopp *et al*., 2011; Rossi *et al*., 2014). Ries and collaborators (2009) showed that organisms with higher organic cover extent, despite their mineral phase, did not present dissolution in a 60-days experiment, even under extremely low pH (pCO_2 2856 ppm ~ pH 7.31). Thereby, it is most possible that the organic sheath that surrounds the whole spicule, although thin, protects *S. hastifera* skeleton, at least within the experiment time.

In addition, individuals of *S. hastifera* were also capable of synthesizing normal spicules in all treatments, even though they presented higher proportions of deformed spicules in high temperature treatments. Recently, Voigt and collaborators (2017) showed that the calcareous sponge *Sycon ciliatum* expresses SLC4 proteins in its biomineralization process. These proteins are known to be bicarbonate transporters and pH regulators at the calcification site (Romero *et al*., 2013). If the bicarbonate was transported at the calcification site and transformed into carbonate, the decrease in carbonate ions concentration due to an increase in pCO_2 could not represent a problem for these sponges. This ability has already been seen in calcifying organisms, such as foraminifera and crustaceans (Cameron, 1985; De Nooijer *et al*., 2009) and could enhance their survival and resilience under acidic conditions, at least at a threshold. In fact, Ries and collaborators (2009) observed that crustaceans showed even greater calcification rates under extremely low pH (~7.3), and coralline red algae, limpets, purple urchins and green algae showed greater calcification rates under intermediary pH (~7.8), while in the extremely low pH (~7.3) the calcification rates decreased again. This whole scenario suggests that even with the ability to regulate pH at the calcification site, the responses and tolerance are variable. Therefore, as *S. hastifera* synthesized normal spicules

even under low pH (~7.6), it is possible that this species also has a pH regulatory system. It also agrees with the abundance increase of the calcareous sponge *Leucosolenia* sp. under low pH in a 100-days experiment, showing functionality even submitted to stressors (Peck *et al*., 2015). Although high temperatures raised the proportion of deformed spicules, the percentage of those spicules was still low (2 – 15%). Even in regenerated areas, for example, where new spicules were certainly synthesized, very few deformed spicules were found. However, it is possible that the observed percentage of deformed spicules was due to the small area analyzed in each specimen. The presence of deformed spicules does not seem to represent a real problem for the sponges. The only problem that we can suppose is to taxonomy, since characters such as shape and size of the spicules are important for the morphological identification of species.

The Mg/Ca ratios observed in the field in different seasons were constant and the same was observed in the aquaria, regardless of the treatment. However, one of the specimens submitted to the High Temperature treatment (26 ^{o}C / pH 8.1) presented a higher proportion of deformed spicules and also the highest Mg/Ca ratio (0.112), while the specimen submitted to the Combined Effect (26 ^{o}C / pH 7.6) that also presented a higher proportion of deformed spicules, presented the Mg/Ca ratio similar to the other treatments (0.081). The Mg/Ca ratio in other organisms growing under these stressors frequently increases in high temperature and reduces in low pH, equilibrating each other when combined (Spero *et al*., 2015; Glandon *et al*., 2018). Although, the increase in Mg proportions in calcareous organisms enhances the solubility and this might be crucial to survival in future conditions of the oceans (Berner, 1975; Ragazzola *et al*., 2016; Swezey *et al*., 2017).

- **Microbiota**

Knowledge on calcareous sponges microbiota is still very scarce, with only few works regarding molecular identification of their bacterial community (*e.g.* Flemer *et al*., 2012; Fromont *et al*., 2016). Taking into account Porifera as a whole, Proteobacteria is known to be the dominant phylum of bacterial community (Thomas *et al*., 2016) and the same was observed in our study for *S. hastifera*. Considering only calcareous sponges, we found a very similar phylum composition between our study species and *Leucetta prolifera*, with dominance also of Cyanobacteria and Bacteroidetes (Fromont *et al*., 2016).

The presence of Cyanobacteria is interesting because calcareous sponges are mostly found in shaded habitats, and yet, five species presented cyanobacteria association: *Clathrina* sp., *Sycon* sp., *Leucetta prolifera*, *Leucetta* sp. and *Pericharax heteroraphis* (Feldmann, 1933; Wilkinson, 1979, 1980; Díaz *et al*., 2007; Fromont *et al*., 2016). In the field samples of the present study, this association was especially with *Synechococcus* spp. and *Pleurocapsa* spp., being the former one of the most common cyanobacteria associated with sponges in general, while the latter was only found, so far, associated with *Leucetta prolifera* (Fromont *et al*., 2016). The genus *Pleurocapsa* was retrieved only with next-generation sequencing (NGS) and then this association can be underestimated as well as the whole cyanobacterial diversity in sponges (Konstantinou *et al*., 2018). Interestingly, individuals kept in the aquaria (even the control specimens) presented a heavy loss of cyanobacteria association, especially of the genus *Synechococcus*, indicating that *Pleurocapsa* might be more resistant to different environmental conditions. This loss was also seen in corals kept in aquaria, suggesting an adaptation of the bacterial community to different environmental conditions (Kooperman *et al*., 2007). The association between sponges and cyanobacteria possibly provides benefits to the sponges by nitrogen fixation, production of secondary metabolites and nutrients, and all these benefits would be mostly lost in aquaria (Carpenter & Foster, 2002).

Besides, the taxa that most contributed to field and aquarium differences were *Candidatus* Branchiomonas (class Betaproteobacteria) and *Staphylococcus* sp. (phylum Firmicutes), both presenting higher concentrations in the field samples. The former has already been seen associated with the coral *Siderastrea siderea* and the latter with sponges and corals, for example, including coral larvae (Li *et al*., 2006; Flemer *et al*., 2012; Sharp *et al*., 2012; Saurav *et al*., 2016; Bonthond *et al*., 2018). *Candidatus* Branchiomonas cysticola, specifically, is a common pathogenic agent of epitheliocystis in sea-farmed Atlantic salmon (Toenschoff *et al*., 2012). However, the effects of *Candidatus* Branchiomonas in sponges are not known and the present study revealed that this bacterium is sensitive to aquarium conditions, as its mean abundance reduced drastically in the aquaria. Despite being widely known as a human pathogen, the genus *Staphylococcus* was only found, so far, associated with one disease in sea cucumber (Deng *et al*., 2009) and has even some strains with positive characteristics, such as production of melanin and biosurfactants (Eddouaouda *et al*., 2012; Sánchez *et al*., 2017; Vijayan *et al*., 2017). Therefore, it is not possible to consider the increase of *Staphylococcus* as a negative factor, as there is no strong evidence for it even in

literature. In fact, *Staphylococcus* has already been found associated with both healthy and diseased corals (Chiou *et al*., 2010; Beneleva *et al*., 2015; Hadaidi *et al*., 2016). In *Mussismilia hispida*, for example, *Staphylococcus warneri* was found only in healthy corals, while *S. saccharolyticus* was found only in unhealthy corals, nonetheless, it was not the etiological agent of the disease (Castro *et al*., 2010). Thereby, the presence of *Staphylococcus* sp. in *S. hastifera* bacterial community cannot be associated with any disease, but rather it could be an opportunistic bacterium, not necessarily pathogenic. It is important, however, to highlight that there was an outlier field sample that presented more than 45% of the sequences associated with *Staphylococcus* sp., which increased the mean abundance of Firmicutes-phylum in the field samples. This predominance could reveal a dysbiosis process in this outlier, since it is very difficult to access visually calcareous sponges health due to their frequent small size and white color. In addition, since the lowest taxonomic level was genus, we cannot discard the possibility of human influence in the bacterial community of the calcareous sponges in the field, since the sponges were collected in a mussel farm. Then, high concentrations of *Staphylococcus* sp. in the field could not be a rule, but rather an exception of a more vulnerable individual, leading or not to a negative impact on the host.

Considering only the aquaria experiments, our results indicate that ocean warming is possibly more important than acidification for driving shifts in the bacterial community of *S. hastifera*. Although these changes in high temperature treatments, especially the reduction of *Rhodobacteraceae*-affiliated bacterium (class Alphaproteobacteria) and the increase of *Staphylococcus* sp. (phylum Firmicutes), were not statistically significant probably due to discrepant differences in the replicates. As already seen in the field, this scenario could be revealing that some individuals might be more sensitive and respond faster to changes as others. When submitted to environmental stress, such as warming and acidification, the healthy holobiont equilibrium dynamic might resist or be disrupted, leading to dysbiosis and thus disease (Pita *et al*., 2018). In previous higher temperature experiments, it was observed in some sponges an increase of Firmicutes, associated with bacterial community dominance shifts, and decrease of Alphaproteobacteria (Webster *et al*., 2008a; Fan *et al*., 2013). These shifts have already been associated with diseases, as already seen for the sponges *Aplysina aerophoba* and *Geodia baretti* (Webster *et al*., 2008b; Luter *et al*., 2017). However, in the present study we do not have evidences, such as necrosis or high mortality, that the calcareous sponges were facing disease or even a dysbiosis process in the higher temperature

experiments. The genus *Staphylococcus* was already identified in the calcareous sponge *Leucosolenia* sp. and no signs of harm were related (Flemer *et al*., 2012). In fact, in *Leucosolenia* sp. this genus presented even antifungal activity, which could be used for the benefit of the sponge itself.

The genus *Ruegeria* dominated the bacterial community core of *S. hastifera*, being present in all specimens analyzed. This genus has already been seen associated with many marine organisms, such as corals, sponges, ascidians and algae (Menezes *et al*., 2010). *Ruegeria* strains are known to produce cyclic dipeptides that play a key role in quorum sensing system, allowing cell-cell communication and regulating bacterial-sponge interactions (Mitova *et al*., 2004; Mohamed *et al*., 2008). They are capable of controlling flagellar biosynthesis and motility, regulating population density and biofilm formation, trying to maintain a healthy bacterial community (Zan *et al*., 2012, 2013). In the present study, *Ruegeria* strains abundance presented no significant differences between treatments, and the maintenance of these strains could be beneficial for the sponges principally under stressed conditions. In corals, for example, the abundance of *Ruegeria* decreased under low pH and high temperature for *A. millepora* but for *T. reniformis* it remained the same (Grottolli *et al*., 2018). Thereby, the shifts or stability in microbial communities are related to the species studied and the loss or prevalence of some strains submitted to environmental stressors also is related to host demand.

CONCLUSION

Here we provided the first study on how the skeleton and the bacterial community of a calcareous sponge may respond to climate changes. In our conclusions, the calcareous sponge *Sycettusa hastifera* may not be as threatened by climate change as previously thought. The spicules presented no signs of corrosion even under low pH and could be being protected by the organic layer that covers the spicules. Even under stressed conditions, these sponges were able to synthesize normal spicules, perhaps indicating a pH regulatory system at calcification site. The proportions of deformed spicules increased under high temperature treatments, but this change does not seem to represent a problem for the sponges. The Mg/Ca ratio in the individual with high proportions of deformed spicules increased under high temperature treatment, reinforcing that these deformed spicules were synthesized during the experiment. The increase in Mg concentration could increase the solubility of the spicule, impairing

survival and fitness of these sponges under future oceans conditions. The bacterial community changed mainly related to the increase of *Staphylococcus* sp. under high temperature treatments. However, this increase could not be addressed to a negative impact on the sponges, since there is no evidence for it as necrosis and/or high mortality. *Ruegeria* spp. were present in all sponges analyzed and may be related to quorum sensing, trying to maintain the bacterial community healthy. Thereby, although similar studies with other species are necessary to extrapolate our results, calcareous sponges could face problems under future oceans conditions but on the other hand, they presented some indications that could also make them thrive.

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SUPPLEMENTARY MATERIALS

Table S1: Summary of replicate tank seawater parameters. Mean and standard deviation (between brackets).

Treatment	Tank Replica	Temp (°C)*	pH (Total)*	Salinity*	Alkalinity (µmol kg $^{-1}$)*	pCO_2 (µatm)**	Ω Calcite**
Control	1	22.11 (0.08)	8.13 (0.03)	34.29 (1.11)	2369 (25)	321 (33)	5.68 (0.34)
	2	22.06 (0.21)	8.16 (0.03)	34.00 (0.53)	2288 (23)	284 (27)	5.77 (0.34)
	3	22.10 (0.09)	8.14 (0.03)	35.25 (0.89)	2275 (23)	298 (20)	5.57 (0.26)
High Temperature	1	26.81 (0.26)	8.16 (0.02)	34.86 (1.21)	2359 (14)	287 (29)	6.81 (0.32)
	2	27.88 (1.36)	8.18 (0.03)	34.63 (0.92)	2310 (55)	265 (25)	7.07 (0.50)
	3	26.50 (1.63)	8.15 (0.03)	35.25 (0.71)	2282 (24)	284 (28)	6.46 (0.55)
Low pH	1	22.30 (0.16)	7.64 (0.03)	35.00 (1.15)	2364 (14)	1169 (89)	2.24 (0.14)
	2	22.23 (0.16)	7.66 (0.02)	34.63 (0.92)	2283 (13)	1090 (64)	2.22 (0.11)
	3	22.20 (0.14)	7.59 (0.04)	35.25 (0.71)	2278 (23)	1287 (110)	1.94 (0.16)
Combined Effects	1	26.94 (0.50)	7.60 (0.04)	34.86 (1.21)	2380 (26)	1329 (135)	2.42 (0.26)
	2	26.25 (1.13)	7.67 (0.03)	34.38 (1.19)	2286 (16)	1069 (74)	2.61 (0.17)
	3	25.88 (1.09)	7.67 (0.04)	35.13 (0.64)	2279 (26)	1058 (115)	2.60 (0.30)

Table S2: Spicules measurements of *Sycettusa hastifera* from each treatment. Minimum, mean, standard deviation and maximum values for length and width of each actine.

		Control								
		Lenght (µm)				**Width (µm)**				
Spicule	Actine	Min	Mean	SD	Max	Min	Mean	SD	Max	N
Diactines	_	260	733	333.67	1610	10	22.33	5.16	30	60
Cortical triactines	Paired	155	232.17	32.84	305	12.5	15.21	1.74	20	60
	Unpaired	160	265.75	37.31	345	15	18	2.38	22.5	60
Subcortical triactines	Paired 1	210	423.83	117.1	670	10	16.04	3.57	20	60
	Paired 2	120	205.92	38.25	280	10	15.71	3.32	22.5	60
	Unpaired	90	136.33	23.52	200	10	16.38	3.17	20	60
Subatrial triactines	Paired	110	237.67	64.17	420	10	21.92	5.41	32.5	60
	Unpaired	185	398.75	101.23	630	10	23.63	5.88	35	60
Atrial triactines	Paired	95	191.42	36.6	275	7.5	9.83	1.29	12.5	60
	Unpaired	125	193.75	34.33	275	10	11.79	1.67	15	60

		High Temperature								
		Lenght (µm)				Width (µm)				
Spicule	Actine	Min	Mean	SD	Max	Min	Mean	SD	Max	N
Diactines	_	250	669.33	298.45	1550	10	20.5	5.02	30	60
Cortical triactines	Paired	160	236.5	38.45	315	12.5	16.38	2.23	22.5	60
	Unpaired	165	265.75	41.6	350	15	19.13	2.72	27.5	60
Subcortical triactines	Paired 1	220	393.92	117.26	640	10	15.83	3.21	25	60
	Paired 2	110	211.67	48.68	310	7.5	15.25	3.18	20	60
	Unpaired	85	137.08	28.62	200	10	16.17	3.33	22.5	60
Subatrial triactines	Paired	110	216.5	45.99	310	7.5	20.21	5.09	30	60
	Unpaired	220	399.58	94.19	630	7.5	21.83	6.21	35	60
Atrial triactines	Paired	115	183.17	41.08	280	5	9.54	2.46	15	60
	Unpaired	105	191.08	40.75	290	7.5	12.04	2.66	17.5	60

		Low pH								
		Lenght (µm)				Width (µm)				
Spicule	Actine	Min	Mean	SD	Max	Min	Mean	SD	Max	N
Diactines	_	250	559.5	208.71	1260	10	19.83	3.18	25	60
Cortical triactines	Paired	120	207.75	38.87	315	12.5	14.04	1.67	20	60
	Unpaired	145	234.33	42.83	330	15	16.63	1.83	22.5	60
Subcortical triactines	Paired 1	180	399.83	132.62	800	10	13.29	2.37	17.5	60
	Paired 2	85	176.58	45.87	290	7.5	13.29	2.5	17.5	60
	Unpaired	80	124.5	22.82	180	10	13.63	2.7	20	60
Subatrial triactines	Paired	115	213.08	52.08	320	10	19.83	5.23	30	60
	Unpaired	245	406.75	95.48	640	10	21.67	6.29	35	60
Atrial triactines	Paired	135	193.17	25.48	250	7.5	11.63	1.77	15	60
	Unpaired	105	190.33	30.28	260	7.5	9.92	1.12	12.5	60

		Combined Effects								
		Lenght (µm)				Width (µm)				
Spicule	Actine	Min	Mean	SD	Max	Min	Mean	SD	Max	N
Diactines	_	260	534.67	173.93	990	10	17.58	3.62	25	60
Cortical triactines	Paired	125	230.17	42.52	310	10	16.25	2.32	20	60
	Unpaired	150	251.17	43.56	360	15	19.71	2.69	27.5	60
Subcortical triactines	Paired 1	185	363.08	100.9	600	10	15.83	2.94	20	60
	Paired 2	105	200.83	38.95	290	10	15.54	2.95	20	60
	Unpaired	75	128.42	27.44	190	10	16.08	2.74	20	60
Subatrial triactines	Paired	95	196.42	54.36	305	7.5	19.43	4.84	30	53
	Unpaired	125	367.45	117.39	630	7.5	22.45	5.38	35	53
Atrial triactines	Paired	110	188.92	34.51	250	5	9.88	1.75	12.5	60
	Unpaired	110	188.25	36.07	280	7.5	12.38	1.92	17.5	60

Table S3: Amplicon sequence variant (ASV) abundance associated to *Sycettusa hastifera* bacterial community at phylum level. Raw abundance per replica and mean abundance ± standard deviation (R = replica).

	FIELD					CONTROL					HIGH TEMPERATURE					LOW pH					COMBINED EFFECTS				
Phylum	**R 01**	**R 02**	**R 03**	**Mean**	**SD**	**R 01**	**R 02**	**R 03**	**Mean**	**SD**	**R 01**	**R 02**	**R 03**	**Mean**	**SD**	**R 01**	**R 02**	**R 03**	**Mean**	**SD**	**R 01**	**R 02**	**R 03**	**Mean**	**SD**
Acidobacteria	29	284	44	119	143	261	775	494	510	257	317	269	218	268	50	84	347	386	272	164	253	537	266	352	160
Actinobacteria	382	1112	288	594	451	273	378	143	265	118	303	311	95	236	122	175	153	176	168	13	385	228	92	235	147
Armatimonadetes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	1
Bacteroidetes	3961	8180	2850	4997	2812	4203	6065	4430	4899	1016	4009	3592	2340	3314	869	4364	4424	6993	5260	1501	4180	4953	2382	3838	1319
BD1-5	92	173	203	156	57	89	18	20	42	40	22	20	16	19	3	68	36	12	39	28	43	19	5	22	19
Candidate division BRC1	0	6	0	2	3	16	54	30	33	19	13	7	13	11	3	11	0	20	10	10	9	35	18	21	13
Candidate division KB1	0	0	0	0	0	0	0	5	2	3	0	0	6	2	3	0	0	0	0	0	0	0	7	2	4
Candidate division OD1	3	60	54	39	31	7	4	47	19	24	9	16	6	10	5	8	0	16	8	8	30	9	0	13	15
Candidate division SR1	0	81	9	30	44	0	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Candidate division TM7	2	0	0	1	1	0	0	2	1	1	0	2	0	1	1	0	0	0	0	0	0	0	0	0	0
Candidate division WS3	0	0	0	0	0	9	30	9	16	12	9	10	10	10	1	6	8	24	13	10	6	13	4	8	5
Chlamydiae	60	74	25	53	25	1237	464	125	609	570	392	1134	56	527	552	1887	477	88	817	947	652	528	118	433	279
Chlorobi	11	20	7	13	7	7	14	20	14	7	0	4	0	1	2	0	12	5	6	6	9	19	0	9	10
Chloroflexi	141	366	121	209	136	99	150	166	138	35	45	86	14	48	36	7	113	80	67	54	77	68	43	63	18
CKC4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	1
Cyanobacteria	2343	3982	1007	2444	1490	233	646	161	347	262	149	669	84	301	321	127	419	100	215	177	170	313	64	182	125
Deferribacteres	15	29	8	17	11	57	74	44	58	15	23	70	40	44	24	21	48	79	49	29	57	46	11	38	24
Deinococcus-Thermus	0	23	10	11	12	0	0	0	0	0	5	6	0	4	3	0	6	0	2	3	0	13	5	6	7
Fibrobacteres	0	9	0	3	5	0	17	14	10	9	0	0	0	0	0	0	11	13	8	7	0	14	18	11	9
Firmicutes	270	80	18965	6438	10849	38	1988	5955	2660	3015	15	6815	5571	4134	3621	254	72	5045	1790	2820	25	8098	8157	5427	4678
Fusobacteria	0	8	0	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gemmatimonadetes	0	94	3	32	53	61	236	133	143	88	58	134	17	70	59	84	148	217	150	67	102	155	9	89	74
Lentisphaerae	0	0	0	0	0	0	16	0	5	9	0	0	0	0	0	0	0	3	1	2	0	0	0	0	0

Nitrospirae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	0	6	10	0	0	0	0	0
NPL-UPA2	0	5	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planctomycetes	273	693	155	374	283	807	1095	626	843	237	912	545	505	654	224	867	936	985	929	59	824	848	398	690	253
Proteobacteria	30300	28060	15645	24668	7894	38451	30786	24548	31262	6964	35957	31258	29626	32280	3287	38412	30332	20739	29828	8847	38756	28940	20375	29357	9198
SHA-109	12	0	0	4	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spirochaetae	26	10	10	15	9	26	72	13	37	31	0	20	11	10	10	0	25	31	19	16	2	23	346	124	193
TA06	0	0	0	0	0	0	0	2	1	1	0	0	0	0	0	0	0	2	1	1	0	0	0	0	0
Tenericutes	0	0	0	0	0	0	2	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TM6	0	4	0	1	2	390	185	62	212	166	153	253	25	144	114	292	108	46	149	128	143	93	23	86	60
Verrucomicrobia	78	295	91	155	122	512	340	26	293	246	76	35	26	46	27	14	119	71	68	53	52	118	15	62	52
Blank	1853	974	722	1183	594	508	509	294	437	124	286	257	113	219	93	405	438	283	375	82	181	237	246	221	35

Table S4: Microbial taxonomic classification of the cores.

A) FIELD CORE

ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV4283	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Gangjinia*	unclassified
ASV2333	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Gramella*	unclassified
ASV0916	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Maribacter*	unclassified
ASV1575	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Salegentibacter*	unclassified
ASV0198	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Winogradskyella*	unclassified
ASV1843	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified	unclassified
ASV2407	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified	unclassified
ASV1107	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Saprospiraceae	unclassified	unclassified
ASV0091	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	*Synechococcus*	unclassified
ASV1679	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	*Synechococcus*	unclassified
ASV3921	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	*Synechococcus*	unclassified

ASV0588	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	*Pleurocapsa*	unclassified
ASV3157	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI	FamilyI	*Pleurocapsa*	unclassified
ASV3430	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	*Blastopirellula*	unclassified
ASV2315	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodobiaceae	*Rhodobium*	unclassified
ASV0001	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified	unclassified
ASV0055	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified	unclassified
ASV0227	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified	unclassified
ASV2277	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	unclassified	unclassified
ASV1856	Bacteria	Proteobacteria	Deltaproteobacteria	GR-WP33-30	unclassified	unclassified	unclassified
ASV0431	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Alcanivoracaceae	*Kangiella*	*K. spongicola*
ASV2673	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Halomonadaceae	*Cobetia*	unclassified
ASV3873	Bacteria	Proteobacteria	Gammaproteobacteria	Incertae Sedis	Incertae Sedis	*Marinicella*	N2yML2
ASV4221	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	*Psychrobacter*	unclassified
ASV3015	Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	*Thiothrix*	unclassified
ASV3037	Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae	*Thiothrix*	unclassified
ASV2388	Bacteria	Proteobacteria	TA18	unclassified	unclassified	unclassified	unclassified
ASV2051	Bacteria	unclassified	unclassified	unclassified	unclassified	unclassified	unclassified
ASV1373	Unassigned						

B) AQUARIUM CORE

ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV0047	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Glaciecola*	unclassified
ASV0164	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	unclassified	unclassified
ASV0606	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified	unclassified
ASV0908	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified	unclassified
ASV0957	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	*Marinobacterium*	unclassified
ASV0999	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified	unclassified

ASV1059	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified	unclassified
ASV1133	Bacteria	Proteobacteria	unclassified	unclassified	unclassified	*C.* Thiobios	unclassified
ASV1176	Bacteria	Chlamydiae	Chlamydiae	Chlamydiales	Simkaniaceae	unclassified	unclassified
ASV1197	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified	unclassified
ASV1255	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	*Marinobacterium*	unclassified
ASV1303	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*Defluviicoccus*	unclassified
ASV1651	Bacteria	Planctomycetes	OM190	unclassified	unclassified	unclassified	unclassified
ASV1722	Bacteria	Proteobacteria	Alphaproteobacteria	OCS116 clade	unclassified	unclassified	unclassified
ASV1728	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Marinobacter*	unclassified
ASV1758	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified	unclassified
ASV1844	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	*Bdellovibrio*	unclassified
ASV1867	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	*Neptuniibacter*	unclassified
ASV2024	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified	unclassified
ASV2690	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Tropicimonas*	unclassified
ASV2775	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Filomicrobium*	unclassified
ASV2806	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	*Marinobacterium*	unclassified
ASV2824	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified	unclassified
ASV2889	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Saprospiraceae	*Lewinella*	unclassified
ASV2945	Bacteria	Proteobacteria	Deltaproteobacteria	Sh765B-TzT-29	unclassified	unclassified	unclassified
ASV3117	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	unclassified	unclassified
ASV3119	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Spongiibacter*	unclassified
ASV3173	Bacteria	Cyanobacteria	Melainabacteria	Obscuribacterales	unclassified	unclassified	unclassified
ASV3349	Bacteria	Proteobacteria	Gammaproteobacteria	unclassified	unclassified	*Marinicella*	unclassified
ASV3366	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	*Pseudohaliea*	unclassified
ASV3528	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Saprospiraceae	unclassified	unclassified
ASV3821	Bacteria	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	*Nitrosomonas*	unclassified
ASV3826	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified	unclassified
ASV3833	Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae	*Cycloclasticus*	unclassified

ASV3850	Bacteria	Proteobacteria	unclassified	unclassified	unclassified	unclassified	unclassified
ASV4117	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	unclassified	unclassified	unclassified
ASV4195	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	unclassified	unclassified	unclassified
ASV4197	Bacteria	Proteobacteria	Deltaproteobacteria	Sh765B-TzT-29	unclassified	unclassified	unclassified
ASV4227	Bacteria	Proteobacteria	Skagenf62	unclassified	unclassified	unclassified	unclassified
ASV4230	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	C1-B045	unclassified
ASV4292	Bacteria	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	*Neptuniibacter*	unclassified

C) CORE

ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV1634	Bacteria	Proteobacteria	AEGEAN-245	unclassified	unclassified	unclassified	unclassified
ASV2599	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Ruegeria*	unclassified
ASV3405	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	*Ruegeria*	unclassified
ASV4036	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	unclassified	unclassified
ASV1797	Bacteria	Proteobacteria	JTB23	unclassified	unclassified	unclassified	unclassified
ASV2262	Bacteria	Proteobacteria	SPOTSOCT00m83	unclassified	unclassified	unclassified	unclassified

D) CONTROL CORE

ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV0569	Bacteria	Proteobacteria	Alphaproteobacteria	Parvularculales	Parvularculaceae	*Parvularcula*	unclassified

E) HIGH TEMPERATURE CORE

ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV2119	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	0319-6G20	unclassified	unclassified

F) LOW pH CORE

ASV	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASV3319	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	unclassified	unclassified
ASV2296	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Gilvibacter*	unclassified
ASV0108	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Ulvibacter*	unclassified
ASV2895	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	unclassified	unclassified
ASV2655	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Saprospiraceae	*Lewinella*	unclassified
ASV1280	Bacteria	Gemmatimonadetes	Gemmatimonadetes	BD2-11 terrestrial	unclassified	unclassified	unclassified
ASV3148	Bacteria	Planctomycetes	OM190	unclassified	unclassified	unclassified	unclassified
ASV1106	Bacteria	Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	*Planctomyces*	unclassified
ASV2609	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	unclassified	unclassified
ASV3174	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	OM27 clade	unclassified
ASV0037	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Nannocystaceae	*Nannocystis*	unclassified

CONCLUSÃO

O presente trabalho trouxe informações inéditas acerca dos efeitos do aquecimento e acidificação dos oceanos no esqueleto e comunidade bacteriana de uma esponja Calcarea. De uma maneira geral, a espécie *Sycettusa hastifera* se mostrou resistente aos estressores, pelo menos dentro do período dos experimentos (10 dias). As espículas não apresentaram corrosão, nem mesmo sob efeito de pH baixo e poderiam, por isso, estar sendo protegidas pela capa orgânica que envolve as espículas das esponjas calcareas. Tão pouco apresentaram variações significativas em relação ao tamanho das espículas. Mesmo submetidas ao aumento de temperatura e redução do pH, as esponjas foram capazes de sintetizar espículas normais, apresentando somente algumas danificadas, indicando um possível mecanismo adaptador para isso, como a regulação do pH ou mesmo a conversão de bicarbonato em carbonato no sítio de calcificação. A proporção de espículas danificadas aumentou nos tratamentos de alta temperatura, mas ainda assim não representou um problema real para as esponjas. A razão de Mg/Ca se mostrou constante ao longo do ano em esponjas *in situ* e também nos experimentos em aquário. Entretanto, um indivíduo que teve maior proporção de espículas danificadas quando submetido à temperatura alta também obteve a maior razão Mg/Ca. Isso reforça que essas espículas tenham sido sintetizadas ao longo do experimento e mostra uma relação positiva entre aumento da temperatura e aumento da razão Mg/Ca em organismos crescendo sob essas condições de estresse. Esse aumento de Mg acarretaria em uma maior solubilidade do mineral, o que poderia gerar problemas quanto ao cenário futuro dos oceanos. Contudo, a proteção orgânica de organismos calcificadores influencia mais na resposta às variáveis climáticas do que a própria fase mineral do carbonato de cálcio. E como foi visto que as espículas de fato podem estar sendo protegidas por essa capa orgânica, é possível que o aumento do Mg não acarrete um problema real para as esponjas. Em relação à comunidade bacteriana, houve uma diferença significativa em relação às amostras de campo, com as de aquário. Isso já era de se esperar, já que o aquário gera efeitos na microbiota dos organismos, por isso também a necessidade de um tratamento controle, para que os resultados sejam comparáveis de forma real. Entre os tratamentos de aquário, não foram encontradas diferenças significativas na comunidade bacteriana, contudo foram observadas tendências, como o aumento do gênero *Staphylococcus*. No entanto, apesar de ser um patógeno bem reconhecido para humanos, sua influência no ambiente marinho tem mais vieses positivos do que negativos. De toda forma, não tivemos evidências reais, tais como necrose ou aumento de

mortalidade para associar o aumento de *Staphylococcus* com algum impacto negativo para o hospedeiro. Um core bacteriano foi identificado em todas as esponjas analisadas e apresentava domínio do gênero *Ruegeria*. Esse gênero está relacionado com atividades de *quorum sensing*, o que traria benefícios para as esponjas com sua permanência na comunidade bacteriana. Sendo assim, nossos resultados indicam que as esponjas Calcareas talvez não estejam tão vulneráveis em relação às projeções das mudanças climáticas como previamente proposto. Apesar de terem o esqueleto de carbonato de cálcio, elas talvez possuam habilidades e ferramentas para serem bem sucedidas em condições estressoras, o que as tornaria possíveis vencedoras frente às mudanças climáticas.

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