**INSTITUTO DE BIOCIÊNCIAS**
**PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA ANIMAL**

VERÔNICA KREIN

# IMPACTOS DO AUMENTO DA TEMPERATURA DO OCEANO NA REPRODUÇÃO DO CORAL-CÉREBRO-DA-BAHIA (*Mussismilia braziliensis* (Verrill, 1868))

PORTO ALEGRE
2022

VERÔNICA KREIN

# IMPACTOS DO AUMENTO DA TEMPERATURA DO OCEANO NA REPRODUÇÃO DO CORAL-CÉREBRO-DA-BAHIA (*Mussismilia braziliensis* **(Verrill, 1868))**

Dissertação apresentada ao Programa de Pós-graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Mestre em Biologia Animal.

Área de concentração: Biologia e Comportamento Animal.

Orientador(a): Prof. Dr. Leandro Cesar de Godoy

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Aprovada em 12 de setembro de 2022.

BANCA EXAMINADORA

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Dra. Cristiane Francisca da Costa Sassi

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Dr. Guilherme Henrique Pereira Filho

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Dra. Júlia Giora

"Foi o tempo que dedicaste à tua rosa
que a fez tão importante"

**Antoine de Saint-Exupéry**

## AGRADECIMENTOS

Em primeiro lugar gostaria de agradecer ao meu orientador Dr. Leandro Godoy que aceitou o desafio de me orientar no mestrado e me acolheu de braços abertos. Desde que conheci o Projeto ReefBank me apaixonei e tenho grande admiração por ele. Fazer parte do projeto é uma grande realização pessoal, pois estou trabalhando com três coisas que eu sempre quis: corais, mudanças climáticas e conservação. Agradeço ao professor Leandro por ter acreditado em mim quando nem eu mesma acreditava e por todas as palavras, ensinamentos e conselhos neste percurso do mestrado, levarei pra sempre em meu coração.

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**RESUMO**

O aumento da temperatura do oceano, decorrente das mudanças climáticas, é o principal responsável pelos eventos de branqueamento em corais. Em temperaturas elevadas, os corais rompem a simbiose com as algas unicelulares (simbiodínios), o que deixa seu esqueleto de carbonato de cálcio visível abaixo do tecido. Se a simbiose não se reestabelecer, os corais branqueados podem morrer devido a falta de nutrientes provenientes da fotossíntese dos simbiontes. *Mussismilia braziliensis* é uma espécie de coral endêmica do Brasil, é hermafrodita e reproduz através da liberação de pacotes de gametas, contendo oócitos e espermatozoides, para que ocorra a fecundação externa cruzada. A presente dissertação teve como objetivo compreender como o aumento da temperatura da água do mar afeta os espermatozoides e a reprodução da *M. braziliensis*. O estudo foi realizado em fevereiro de 2020 durante a estação reprodutiva da espécie, na base de pesquisa do Projeto Coral Vivo em Arraial d'Ajuda, com 40 colônias coletadas no Recife de Fora. Os pacotes de gametas foram coletados com pipetas Pasteur e após sua dissociação realizou-se a separação de oócitos e espermatozoides. Os espermatozoides foram submetidos a diferentes temperaturas de acordo com as projeções do IPCC 2014, conforme os cenários RCP2.6 e RCP8.5, que correspondem a um aumento de 1,7ºC e 4,8ºC na temperatura do oceano, respectivamente, além da temperatura ambiente do Recife de Fora no momento da desova (28 ºC). Os espermatozoides foram avaliados quanto a sua motilidade e morfologia, nos diferentes tratamentos de temperatura e ao longo do tempo. Além disso, em 2019 ocorreu o maior evento de branqueamento já registrado para o Atlântico Sul, afetando as colônias de *M. braziliensis* no seu período de oogênese. Dessa foram, também foram analisados os impactos do branqueamento de 2019 na reprodução da espécie em 2020. Apenas 10% das colônias observadas desovaram, liberando em média 57 pacotes com $2,94 \times 10^7 \pm 5,99 \times 10^6$ espermatozoides e 73,20 ± 60,55 oócitos. Alguns pacotes de gametas não apresentavam espermatozoides vivos. As taxas de motilidade apresentaram diferença significativa ($p < 0,05$) no decorrer do tempo nas três temperaturas em que os espermatozoides foram expostos. As anomalias observadas para os espermatozoides foram: cabeça solta, cabeça degenerada, macrocefalia, microcefalia, gota distal, flagelo enrolado, flagelo quebrado e células espermáticas aglutinadas. Não apenas o branqueamento impacta na reprodução da espécie, como também a exposição direta dos gametas às altas temperaturas. Ambas as situações afetam diretamente o sucesso da fertilização e comprometem a perpetuação e manutenção da espécie.

**Palavras-chave:** mudanças climáticas, corais pétreos zooxantelados, reprodução sexuada, resiliência, espermatozoides, Atlântico Sul.

**ABSTRACT**

The increase in the ocean temperature, due to climate changes, is the main responsible for bleaching events in corals. At high temperatures, corals break the symbiosis with the unicellular algae (symbiodiniaceans), leaving their carbonate skeleton apparent. If the symbiosis does not reestablish the bleached corals may die due to lack of nutrients from photosynthesis of the symbiont. *Mussismilia braziliensis* is an endemic species from Brazil, is hermaphrodite and reproduces through the release of gamete bundles, containing oocytes and spermatozoa, for external cross-fertilization. The present dissertation aimed to understand how the increase in seawater temperature affects the spermatozoa and the reproduction of *M. braziliensis*. The study was conducted February 2020 during the species' breeding season, on the research base of Coral Vivo Project in Arraial d'Ajuda, with 40 colonies collected from Recife de Fora. The gamete bundles were collected with Pasteur pipettes and after their dissociation the separation of oocytes and spermatozoa was performed. The spermatozoa were subjected to different temperatures (IPCC 2014 projections) according to RCP2.6 and RCP8.5 scenarios, which correspond to an increase of 1.7ºC e 4.8ºC on the seawater temperature, respectively, besides to the ambient temperature of Recife de Fora at the time of spawning (28 ºC). The spermatozoa motility and morphology were evaluated on the different temperature treatments and over the time. Furthermore, in 2019 the largest bleaching event ever recorded for the South Atlantic occurred, affecting *M. braziliensis* colonies in their oogenesis period. In this way, the impacts of the 2019 bleaching event on the reproduction of species in 2020 were also analyzed. Only 10% of observed colonies spawned, releasing an average of 57 bundles with $2.94 \times 10^7 \pm 5.99 \times 10^6$ spermatozoa and 73.20 ± 60.55 oocytes. Some gamete bundles did not contain live spermatozoa. The motility rates showed a significant difference ($p < 0,05$) over the time on the three different temperature which spermatozoa were exposed. The anomalies observed to the spermatozoa were: free head, degenerated head, macrocephaly, microcephaly, distal droplet, coiled flagellum, broken flagellum and agglutinated of cells. Not only does bleaching impact the reproduction of the species, but also the direct exposure of gametes to high temperatures. Both situations directly affect the success of fertilization and compromise the perpetuation and maintenance of the species.

**Keywords:** climate change, reef-building coral, sexual reproduction, resilience, spermatozoa, South Atlantic

## ESTRUTURA DA DISSERTAÇÃO

Esta dissertação está estruturada em “Introdução Geral”, “Objetivos”, “Síntese dos Resultados”. “Capítulo I”, “Capítulo II” e “Conclusão”. A Introdução Geral inclui uma revisão bibliográfica acerca do tema abordado nesta dissertação, abordando questões como mudanças climáticas, impactos do aumento da temperatura do oceano nos recifes de coral do mundo e no Brasil, eventos de branqueamento e aspectos da biologia e ecologia da espécie *Mussismilia braziliensis* (Verrill, 1868), seguido dos objetivos do presente trabalho e de uma síntese geral dos resultados encontrados. Em seguida, são apresentados dois capítulos no formato de artigos científicos. O capítulo I caracteriza a reprodução da espécie *M. braziliensis* no ano de 2020 que foi intensamente afetada pelo maior evento de branqueamento já registrado para o Atlântico Sul, em 2019. O capítulo II avalia os impactos do aumento da temperatura do oceano na viabilidade dos espermatozoides de *M. braziliensis.* Desta forma, o Capítulo I está formatado de acordo com o periódico *Global Change Biology*, ao qual que será submetido, enquanto que o Capítulo II está formatado para ser submetido no periódico *Coral Reefs.* Por fim, são apresentadas as principais conclusões de ambos os artigos.

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| Nome da Instituição: UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL | CNPJ: 92.969.856/0001-98 |

### Cronograma de atividades

| # | Descrição da atividade | Início (mês/ano) | Fim (mês/ano) |
|---|---|---|---|
| 1 | Coleta de colonias matrizes para obtenção dos gametas (1° Fase) | 01/2018 | 02/2018 |
| 2 | Redação e submissão de artigos científicos | 04/2018 | 09/2020 |
| 3 | Coleta de colonias matrizes para obtenção dos gametas (2° Fase) | 01/2019 | 02/2019 |
| 4 | Devolução das colonias matrizes ao seu recife de coral de origem | 05/2020 | 05/2020 |
| 5 | evolução das colonias matrizes ao seu recife de coral de origem | 05/2019 | 05/2019 |
| 6 | Manutenção das colonias matrizes nos viveiros / coleta dos gametas / Experimentos de congelamento | 01/2020 | 05/2020 |
| 7 | Coleta de colonias matrizes para obtenção dos gametas (3° Fase) | 01/2020 | 02/2020 |
| 8 | Manutenção das colonias matrizes nos viveiros / coleta dos gametas / Experimentos de resfriamento | 01/2018 | 05/2018 |
| 9 | Devolução das colonias matrizes ao seu recife de coral de origem | 05/2018 | 05/2018 |
| 10 | Relatório final | 12/2020 | 12/2020 |
| 11 | Manutenção das colonias matrizes nos viveiros / coleta dos gametas / Experimentos de toxicidade | 01/2019 | 05/2019 |

### Equipe

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| 4 | NAYARA OLIVEIRA DA CRUZ | Colaboradora técnica/científica, Execução dos experimentos | 010.902.712-47 | Brasileira |

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| | |
|---|---|
| 1 | O pesquisador somente poderá realizar atividade de campo após o término do estado de emergência devido à COVID-19, assim declarado por ato da autoridade competente. |
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### Locais onde as atividades de campo serão executadas

| # | Descrição do local | Município-UF | Bioma | Caverna? | Tipo |
|---|---|---|---|---|---|
| 1 | Parque Natural Municipal do Recife de Fora | BA | Marinho | Não | Fora de UC Federal |

### Atividades

| # | Atividade | Grupo de Atividade |
|---|---|---|
| 1 | Coleta/transporte de espécimes da fauna silvestre in situ | Fora de UC Federal |
| 2 | Coleta/transporte de amostras biológicas ex situ | Atividades ex-situ (fora da natureza) |
| 3 | Manutenção temporária (até 24 meses) de invertebrados silvestres em cativeiro | Atividades ex-situ (fora da natureza) |

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### Atividades X Táxons

| # | Atividade | Táxon | Qtde. |
|---|---|---|---|
| 1 | Manutenção temporária (até 24 meses) de invertebrados silvestres em cativeiro | Mussismilia braziliensis | - |
| 2 | Coleta/transporte de amostras biológicas ex situ | Mussismilia braziliensis | - |
| 3 | Coleta/transporte de espécimes da fauna silvestre in situ | Mussismilia braziliensis | 30 |

### Materiais e Métodos

| # | Tipo de Método (Grupo taxonômico) | Materiais |
|---|---|---|
| 1 | Amostras biológicas (Invertebrados Aquáticos) | Outras amostras biológicas(Gametas) |
| 2 | Método de captura/coleta (Invertebrados Aquáticos) | Captura manual |

### Destino do material biológico coletado

| # | Nome local destino | Tipo destino |
|---|---|---|
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| Táxon* | Qtde. | Tipo de Amostra | Qtde. | Data |
|---|---|---|---|---|
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* Identificar o espécime do nível taxonômico possível.

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Prefeitura Municipal de Porto Seguro - BA
**Secretaria Municipal de Meio Ambiente**
*Diretoria de Oceanografia e Gerenciamento Costeiro*

# AUTORIZAÇÃO PARA ATIVIDADES COM FINALIDADE CIENTÍFICA NA ÁREA DO PARQUE NATURAL MUNICIPAL DO RECIFE DE FORA

**PESQUISADOR** **Autorização nº** 01/2019

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**TÍTULO DO PROJETO**

**Projeto ReefBank: reprodução *in vitro* e congelamento de gametas como ferramentas tecnológicas para conservação dos corais brasileiros.**

TIPO DE PESQUISA: [ ] Socioambiental [ X ] Biológica [ ] Outra

Material Biológico: [ X ] Com coleta [ ] Sem coleta

Destino do Material Biológico: Manutenção temporária nos viveiros do Projeto Coral Vivo, Arraial D'Ajuda, BA

Previsão de Coleta de Material Biológico: [ X ] Sim [ ] Não

| Taxon: | Quantidade: |
|---|---|
| *Mussismilia harttii* | 40 colônias |
| *Mussismilia hispida* | 40 colônias |
| *Mussismilia braziliensis* | 40 colônias |

LOCAL DE COLETA: Recife de Fora e cabeços nos arredores.

PERÍODO DE EXECUÇÃO: Fevereiro de 2019 a fevereiro de 2021.

**RESUMO DO PROJETO**

Os recifes de coral estão entre os ecossistemas de maior diversidade do planeta. Cerca de 25% de toda vida marinha está a eles associada, desempenhando papel fundamental na renovação dos estoques pesqueiros que sustentam milhares de comunidades. Porém, impactos antrópicos relacionados às mudanças climáticas têm se tornado preocupantes. O estresse térmico causado pelo aumento da temperatura do oceano rompe a simbiose entre as microalgas que vivem no tecido dos corais e que dão ao coral seu alimento, tornando-os brancos (branqueamento). Dependendo da intensidade do estresse, os corais não se recuperam e morrem.

Os únicos recifes de águas rasas do Atlântico Sul estão ao longo da costa brasileira e abrigam 16 espécies de corais pétreos, com cinco delas endêmicas do Brasil. Os corais-cérebro *Mussismilia harttii*, *M. hispida* e *M. braziliensis* estão entre os principais construtores dos recifes brasileiros.

Enquanto práticas de conservação *in situ* como áreas marinhas protegidas podem ajudar a diminuir os impactos locais, os impactos antrópicos como o aquecimento global continuarão causando declínios populacionais. Grandes mortalidades sem recuperação levam a diminuição na diversidade genética das populações de corais e a possibilidade de extinção de espécies é alta.

Uma maneira segura de conservar a existência de corais é através da preservação de seus gametas (espermatozoide e oócito) em baixas temperaturas (criopreservação), permitindo o uso futuro dessas células para a formação de novos corais. Embora seja um método comprovado para manutenção de células e conservação de espécies, estudos com gametas de coral são recentes e limitados na literatura. Espermatozoides de três espécies de corais australianos e um caribenho foram congelados com sucesso, sendo eficientes na fecundação dos oócitos e gerando larvas saudáveis.

Nosso projeto traz uma proposta inédita no Brasil, buscando aplicar ferramentas tecnológicas em prol da conservação dos corais-cérebro. Nós iremos conhecer a biologia dos gametas dos corais, avaliar sua sensibilidade ao resfriamento e aos crioprotetores. Essas etapas serão cruciais para estabelecermos um protocolo de congelamento que permita estocagem dos gametas por tempo indeterminado. A viabilidade dos gametas descongelados será avaliada e a capacidade de fecundação verificada pelo estabelecimento da tecnologia de reprodução in vitro. A prole gerada

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terá o desenvolvimento acompanhado, certificando ter recrutas dos corais-cérebro aptos para povoar recifes degradados. A avaliação da diversidade genética da prole dará suporte à criação do primeiro banco de gametas de coral do Atlântico Sul.

Local: Porto Alegre

Data: 18 de dezembro de 2018

Eu, Leandro Cesar de Godoy, responsável pelo projeto de pesquisa, me comprometo a enviar relatório das atividades de campo no prazo de até 30 dias após o término das saídas.

Prof. Dr. Leandro Cesar de Godoy
Departamento de Zootecnia
Universidade Federal do Rio Grande do Sul

Autorizo, em 11/01/ 2019.

Diretoria de Oceanografia e Gerenciamento Costeiro

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## INTRODUÇÃO GERAL

Recifes de coral são ecossistemas formados predominantemente por corais construtores pertencentes a ordem Scleractinia. Apesar desses ecossistemas compreenderem apenas 0,1% do fundo oceânico, pelo menos 25% da biodiversidade marinha depende desses ambientes para sobreviver (alimentação, reprodução, nidificação, proteção) (Mulhall 2009; Wilkinson 2008). Além disso, os recifes de coral fornecem importantes serviços ecossistêmicos, como alimento por meio da pesca, turismo e lazer, proteção costeira e a ampla diversidade associada aos recifes, por sua vez, é rica em compostos químicos que podem ser utilizados na indústria farmacêutica (Hoegh-Guldberg 2007; Castro et al. 2016; Hugues et al. 2017). Os corais escleractíneos realizam uma importante simbiose com algas unicelulares dinoflageladas (Muscatine & Porter, 1977). Nessa relação, os corais fornecem proteção, dióxido de carbono e outros compostos inorgânicos provenientes da sua digestão aos simbiontes, enquanto os simbiontes fornecem matéria orgânica (alimento) e oxigênio aos corais através da fotossíntese (Stanley 2006; Lesser 2007). Essa é a principal fonte de obtenção de energia para os corais construtores de recife (Stanley 2006; Lesser 2007).

O aumento da temperatura da água do mar, decorrente das mudanças climáticas que se intensificaram devido as atividades antropogênicas nas últimas décadas (principalmente o aumento da emissão de gases do efeito estufa), tem ocasionado severos prejuízos aos recifes de coral, como os eventos de branqueamento (Hoegh-Guldberg 2007; IPCC 2014; Cheng et al. 2019). Com o aumento da temperatura do oceano, a relação de simbiose entre corais e as algas dinoflageladas fica comprometida. As algas simbiontes passam a ter seus processos de fotossíntese e respiração celular alterados, produzindo um excesso de espécies reativas de oxigênio (EROs) que são consideradas tóxicas para o coral, além de perderem seus pigmentos fotossintetizantes e morrerem devido as altas temperaturas (Iglesias-Prieto et al. 1992; Lesser 2006; Roth 2014). As EROs causam estresse oxidativo aos corais, e danificam lipídeos, proteínas e o DNA (Iglesias-Prieto et al. 1992; Lesser 2006; Roth 2014). Como mecanismo de defesa contra o estresse oxidativo, o coral tende a romper a simbiose com as algas presentes em seus tecidos, tornando visível o o seu esqueleto de carbonato de cálcio, esse conjunto de alterações dá nome ao fenômeno de branqueamento de corais (Brown 1997; Stanley 2006). Caso a simbiose não se reestabeleça, os corais podem acabar morrendo. A ausência de simbiontes interfere nas atividades metabólicas do coral, compromete a sua habilidade de reproduzir-se, sua habilidade de heterotrofia, as taxas de crescimento e suas taxas de

calcificação (Stanley 2006; Lesser 2007; Leão 2009).

Eventos de branqueamento tem se tornado frequentes no mundo todo. No Atlântico Sul, o Brasil é o único país em que ocorrem recifes, ao longo de 3000 km da costa (Leão et al. 2016) e a frequência dos eventos de branqueamento tem crescido nas últimas décadas, com relatos de branqueamento em corais escleractíneos registrados para os anos de 1993-1994 (Castro & Pires 1999), 1996 (Costa et al. 2001), 1998, 2003, 2005, 2008-2009 (Costa-Sassi et al. 2014; Sassi et al. 2015), 2010 (Leão et al. 2016; de Oliveira Soares et al. 2019) 2016 (Sabadini 2017; Teixeira et al. 2019), 2019 (Banha et al. 2019; Duarte et al. 2020; Ferreira et al. 2021), 2020 (Pereira et al. 2022). Tem se estimado que ao menos 40% dos recifes mundiais já tenham sido perdidos (Heron et al. 2016; Hughes et al. 2018, 2019) e que os recifes que ainda existem estarão em situação crítica dentro dos próximos 20 anos (Wilkinson 2008; Castro et al. 2016).

A espécie *Mussismilia braziliensis* (Verrill, 1868), conhecida popularmente como coral-cérebro-da-bahia, é uma das principais construtoras de recifes em Abrolhos e tem a sua ocorrência restrita aos recifes de coral que ocorrem no estado da Bahia (Castro & Pires 2001). *M. braziliensis* é uma espécie hermafrodita com desenvolvimento dos gametas (oócitos e espermatozoides) ocorrendo em um mesmo mesentério (Pires et al. 1999). Durante o período reprodutivo da espécie, entre março e metade de maio e junto da lua nova, os pacotes de gametas são liberados na coluna da água para que ocorra a fertilização cruzada (Pires et al. 1999; Castro et al., 2016). Após a fertilização, os embriões darão origem á larvas plânulas que vão se assentar ao substrato e dar início a novas colônias, expandindo o substrato dos recifes baianos.

O aumento da temperatura da água do oceano e os consequentes eventos de branqueamento têm sido responsáveis por inúmeros impactos na reprodução sexuada dos corais ao redor do mundo. Alguns impactos já observados na reprodução de corais são atraso e interrupção no processo de gametogênese (Szmant & Gassman 1990; Ward et al. 2000; Mendes & Woodley 2002), redução no número de pacotes liberados por colônia (Ward et al. 2000), redução na concentração de gametas (Ward et al. 2000; Paxton et al. 2015; Johnston et al. 2020), comprometimento da motilidade e tempo de vida dos espermatozoides (Henley et al. 2021), aumento do número de espermatozoides anormais (Finzi et al. 1995), diminuição no tamanho dos oócitos e redução na fecundidade (Hirose & Hidaka, 2000; Ward et al., 2000; Howells et al., 2016; Johnston et al., 2020; Godoy et al., 2021) e comprometimento no sucesso de fertilização (Hirose & Hidaka 2000; Omori et al., 2001; Paxton et al., 2015). Como os gametas de *M. braziliensis* são liberados na coluna d'água, eles acabam ficando mais expostos e consequentemente mais vulneráveis ao estresse ambiental, o que pode reduzir a viabilidade dos

espermatozoides e oócitos (Harrison 2011; Pires et al. 2016).

Estudos que avaliam a influência dos fatores ambientais, como o aumento de temperatura, na reprodução de corais brasileiros ainda são escassos. Até o momento não há dados sobre como as mudanças climáticas impactam a espécie *Mussismilia braziliensis* e a sua reprodução. Uma vez que a espécie é endêmica do Brasil e uma importante construtora dos recifes de Abrolhos, é muito importante conhecer sua biologia reprodutiva para gerar conhecimentos que possam auxiliar futuras estratégias de conservação. Considerando as projeções climáticas para o aumento de temperatura dos oceanos até 2100, de acordo com o IPCC 2014, pode ser que nos reste pouco tempo para salvar a espécie de uma catástrofe ambiental. Assim, esse estudo buscou compreender pela primeira vez como eventos de branqueamento e como o aquecimento da temperatura da água do mar pode influenciar na reprodução da espécie *M. brasiliensis*.

## OBJETIVOS

### Objetivo geral

Avaliar o efeito da temperatura da água do oceano na viabilidade dos gametas e da reprodução do coral-cérebro-da-bahia *Mussismilia braziliensis* (Verrill, 1869).

### Objetivos específicos

*Capítulo I*

Avaliar o impacto do evento de branqueamento mais severo já registrado para o Atlântico Sul (2019), na reprodução do coral-cérebro-da-bahia *Mussismilia braziliensis* (Verrill, 1869), através do número de colônias que desovaram, número de pacotes de gametas desovados, motilidade e concentração espermática, tempo de vida dos espermatozoides, concentração e tamanho dos oócitos e taxas de fertilização.

*Capítulo II*

Avaliar o efeito do aumento da temperatura da água do mar na taxa de motilidade, tempo de vida e morfologia dos espermatozoides do coral-cérebro-da-bahia *Mussismilia braziliensis* (Verrill, 1869).

## SÍNTESE DOS RESULTADOS

*Capítulo I*

Apenas 10% das colônias de *Mussismilia braziliensis* observadas desovaram em fevereiro de 2020. Cada colônia liberou em média 57 pacotes de gametas. Os pacotes de gametas desovados apresentaram em média $2,94 \times 10^7 \pm 5,99 \times 10^6$ espermatozoides e 73,20 ± 60,55 oócitos. Os oócitos mediam 481,64 ± 58,87 µm de diâmetro. O tempo de vida dos espermatozoides de *M. braziliensis* foi de 8-9h. Alguns pacotes de gametas ao desovarem não apresentavam espermatozoides vivos. A viabilidade embrionária observada durante a reprodução de 2020 foi de 3,75 ± 3,76%.

*Capítulo II*

Os espermatozoides de *M. braziliensis* possuem cabeça de 0,03 ± 0,01 µm de diâmetro e um flagelo que chega a 5,8 ± 1,21 µm de comprimento.

As taxas de motilidade apresentaram diferença significativa $p < 0.0001$, $F (4, 120) = 24.35$ no decorrer do tempo (2 h 30 min, 5 h e 7 h após a desova) nas três temperaturas em que os espermatozoides foram expostos 28ºC (temperatura ambiente Recife de Fora), 29,7ºC (cenário RCP2.6) e 32,8ºC (cenário RCP8.5). Quanto maior a temperatura, menores as taxas de motilidade observadas dentro de um mesmo tempo de análise após a desova. Sete horas após a desova, em todos os tratamentos, os espermatozoides já não apresentavam qualquer motilidade.

Ao analisar os efeitos do tempo e da temperatura sobre a morfologia espermática, não foram observadas diferenças significativas na porcentagem de espermatozoides sem anomalias. As anomalias observadas foram: cabeça solta, cabeça degenerada, macrocefalia, microcefalia, gota distal, flagelo enrolado, flagelo quebrado e células espermáticas aglutinadas. Anomalias relacionadas ao flagelo foram mais frequentes conforme a temperatura aumentava.

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# CAPÍTULO I

**The price of resilience: impacts on sexual reproduction of the coral *Mussismilia braziliensis* (Verrill, 1868) after the most severe bleaching event of the Southwestern Atlantic.**

**Running Title:** *The price of resilience on coral reproduction*

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**Abstract:** Coral reefs are home to at least 25% of all marine biodiversity and provide important ecosystem services to human society. The increase in ocean temperature and acidification, resulting from anthropogenic activities, have been responsible for numerous impacts on coral reefs in recent decades. The bleaching phenomenon, which occurs due to the rupture of the symbiosis between dinoflagellates of the Symbiodiniaceae family and scleractinian corals, is a major current concern. Bleaching causes the coral to lose its main source of nutrition, compromising its physiological activities and obtaining energy. If symbiosis is not reestablished, corals eventually die. Little is known about the impacts of bleaching on the sexual reproduction of reef-building corals. In Brazil, *Mussismilia braziliensis* is an endemic species,

with occurrence restricted to Bahia and one of the most important reef-builder in Abrolhos. This study evaluated the impact of the most severe bleaching event ever recorded for the South Atlantic on the reproduction of *M. braziliensis*. The species recovered quickly from bleaching, prioritizing its energy stores and heterotrophy to keep colonies alive. Bleaching coincided with the beginning of gametogenesis, compromising reproduction in several ways. Only 10% of colonies spawned, there was a marked reduction (67%) in the number of bundles released per colony when compared to years without bleaching, and embryo viability was 3.75 ± 3.76%. In addition, some gamete bundles showed all spermatozoa dead soon after spawning. This is the first time that the reproduction of *Mussismilia braziliensis* has been evaluated. Impairment of sexual reproduction leads to impairment of reef cover, therefore studies with this approach should be prioritized in times of drastic environmental changes.
**Keywords:** Climate change, Gametogenesis, Brazilian reefs, Coral spawning.

## 1 | INTRODUCTION

Coral reefs are mainly formed by colonies of scleractinian corals. Although these ecosystems correspond to only 0.1% of the ocean floor, at least 25% of marine species directly depend on these environments to feed, reproduce, nest and protect themselves (Mulhall, 2009). In addition, coral reefs provide important ecosystem services used by humans, such as coastal protection, food, chemical compounds used in the pharmaceutical industry, tourism and leisure (Mulhall, 2009; Hughes et al., 2017). Despite their importance, coral reefs have become constantly threatened by global climate change (NOAA, 2021), resulting from anthropogenic activities. The significant increase mainly in the concentration of $CO_2$ intensifies the greenhouse effect, retaining heat in the Earth's atmosphere and consequently in the oceans (Baker, 2008; IPCC, 2014; Cheng et al., 2019).

Scleractinian corals have an important symbiotic relationship with photosynthetic dinoflagellates from the family Symbiodiniaceae (Muscatine & Porter, 1977). While coral provides carbon dioxide, inorganic products from coral digestion, and protection for symbiodiniaceans, they provide organic matter and oxygen to corals through photosynthesis (Muscatine & Porter, 1977; Stanley, 2006; Lesser, 2007), and serve as the main source of energy for the corals (Stanley, 2006; Lesser, 2007). With the increase in seawater temperature, endosymbiotic symbiodiniaceans have their photosynthesis process and cellular respiration altered, producing an excess of reactive oxygen species (ROS), which is considered toxic for

corals (Iglesias-Prieto et al., 1992; Lesser, 2006; Roth, 2014). As a defense mechanism against oxidative stress (see Lesser, 2006), coral tends to expel the algae from its tissues, leaving the transparent tissue and its carbonate skeleton visible, a process known as coral bleaching (Brown 1997; Stanley 2006).

Disruption of the symbiosis interferes with the metabolic activities of the coral, thus decreasing its ability to reproduce, its linear growth and its calcification rates (Stanley, 2006; Lesser, 2007). With lower density of symbiodiniaceans in their tissue, some corals can obtain energy through their energy stores or heterotrophic feeding (Grotolli et al., 2006; Mies et al. 2018), but this alternative is usually insufficient to meet the daily metabolic demands of corals (Grotolli et al., 2006; Mies et al., 2018). Therefore, very high temperatures or prolonged periods of exposure to above-normal temperatures can lead to coral mortality (Ayre & Hughes, 2004; Underwood et al. 2009), directly affecting local coral cover rates. At least one third of the world's coral reefs have been exposed to the phenomenon of bleaching in recent decades, due to ocean warming (Heron et al. 2016). Between 2009 and 2018, there was a 14% loss of coral cover (GCRMN, 2021) and it is estimated that approximately 40% of the world's reefs have already been lost (Hughes et al. 2018). If there is no reduction in greenhouse gas emissions, mass bleaching events will become even more frequent and intense and, consequently, the chances of corals to recover and survive will be lower (Hughes, 2020).

In the South Atlantic, the only biogenic coral reefs occur in Brazil, along 3.000 km of coast, and bleaching events have been recorded since 1996 (Leão et al., 2016). The Brazilian coral reefs have been less affected by bleaching, due to the lower incidence of thermal anomalies and the environmental and biological characteristics of the coral reef associated fauna (Magris et al, 2015; Mies et al., 2020). Corals along the Brazilian coast are reported to be highly resistant to some stressful environmental conditions, such as high rates of turbidity and low variations in seawater temperature (Leão, 2005). However, in February 2019, Brazilian reefs faced the largest bleaching event ever record (Banha et al., 2019; NOAA Coral Reef Watch, 2021), causing unprecedented damages and locally coral mortality rates. Some species affected by bleaching were *Millepora alcicornis*, *Mussismilia braziliensis*, *M. harttii*, and *M. hispida* (Banha et al. 2019, Duarte et al., 2020; Ferreira et al., 2021; Soares et al., 2021). Despite bleaching events, it has already been observed that Brazilian corals belonging to the genus *Mussismilia* have a good energy supply by heterotrophy (Pires et al., 2011; Mies et al., 2018; Ribeiro et al., 2018; Marangoni et al., 2019). However, the bleached corals need to invest their limited energy stores and the energy acquired by heterotrophy in the recovery and survival of

the colonies (Hall & Hughes, 1996). As a result, reproduction may not be prioritized, since investing in recovery and survival seems to be priorities.

Thus, the increase in water temperature and consequent bleaching have been responsible for numerous impacts on the sexual reproduction of corals around the world. Some known bleaching impacts are: the delay, incomplete or interruption of gametogenesis (Szmant & Gassman, 1990; Ward et al., 2000; Mendes & Woodley, 2002), asynchrony of spawning (Paxton et al., 2015), reduction in the number of bundles released by colonies (Ward et al., 2000), reduction in the concentration of gametes (Ward et al., 2000; Johnston et al., 2020), compromise of the sperm motility, and lifespan (Henley et al., 2021), smaller and/or decrease in the number of oocytes compared to breeding seasons where corals did not undergo bleaching and reduced overall fecundity (Hirose & Hidaka, 2000; Ward et al., 2000; Howells et al., 2016; Johnston et al., 2020; Godoy et al., 2021), and compromise the fertilization success (Hirose & Hidaka 2000; Omori et al., 2001; Paxton et al., 2015).

The impairment of sexual reproduction, apart from reduced calcification rates and, consequently, reduction in colony growth (Mendes & Woodley, 2002; Wilkinson, 2008) causes great long-term damage to the coral cover of reef ecosystems. However, approaches that investigate these impacts are still scarce for South Atlantic species. In this way, we evaluated the impacts of the largest bleaching event ever recorded in the Southwestern Atlantic (2019) on the reproduction of *Mussismilia braziliensis* (Verrill, 1868), a hermaphrodite and endemic species of the South Atlantic tropical turbid reefs (i.e. Abrolhos Bank, ~18ºS) where it is among the most important reef-builders (Pires et al., 1999; Castro & Pires, 2001; Kikuchi et al., 2003).

## 2 | MATERIALS AND METHODS

### 2.1 | Study site

The study was carried out in the Recife de Fora Natural Marine Park located in Porto Seguro, Bahia State, Brazil, (16°23'00″S; 38°58'00″W), 10.04 km from the coast (Porto Seguro, 2016). The park is a Brazilian integral protection conservation unit, covering a 19.68 km² area with shallow water reef formations (Porto Seguro, 2021). The marine park is characterized by a high coral richness, comprising all recorded species of scleractinian corals for Brazil (Castro & Pires, 2001). Regarding the conservation status, 19 species of corals and associated coral species, that occur in this environment are considered threatened (Porto Seguro, 2016)). Among the threatened species is the endemic *Mussismilia braziliensis*Castro & Pires, 2001; Leão et al.,

2016).

### 2.2 | Characterization of the thermal-stress event

To characterize the 2019-2020 thermal–stress event, daily values of Sea Surface Temperature (SST) and Degree Heating Week (DHW) were obtained from the Abrolhos Reefs Virtual Station (https://coralreefwatch.noaa.gov/product/vs/timeseries/brazil.php#abrolhos_reefs) using data collected from February 2019 to February 2020 via the NOAA Coral Reef Watch program, using CoralTemp Version 1.0 SST data product, with 5-km$^2$ resolution dataset. The Abrolhos Reefs station was selected to analyze the weather patterns due to its proximity to the sampling site. For Recife de Fora, location of the colonies of *M. braziliensis* were collected, there are no data recorded in the NOAA Coral Reef Watch program that we could more accurately assess.

The highest monthly mean temperature defines the Maximum Monthly Mean (MMM) for a specific coral reef site. When SST values surpass MMM value, corals start to getting stressed (see Glynn and D'Croz, 1990). Furthermore, 1 ºC above the MMM represents the Bleaching Threshold temperature (MMM + 1 ºC), regardless of location (Jokiel & Coles, 1990). When the SST is equal or warmer than the bleaching threshold temperature, corals can start experiencing impacts of heat stress (Goreau & Hayes, 1994; Montgomery & Strong, 1994).

The DHW is a measure to estimate how long the temperature (SST) had stayed above the MMM (Kayanne, 2017). A DHW of 1 ºC-week is equal to a week of SSTs 1 °C higher than MMM temperature (Donner et al., 2005). It is a standardized index to estimate thermal anomalies intensity and shows how much heat stress has accumulated in an area (Glesson & Strong, 1995; Kayanne, 2017). Results indicate that not only the intensity, but also the duration of elevated water temperature on a reef, can be an excellent predictor of the strength of a bleaching event (Glesson & Strong, 1995). When DHW value exceeds 4 ºC-weeks, we can expect to see significant coral bleaching, and when DHW exceeds 8 ºC-weeks or higher, we would likely see severe, widespread bleaching and significant coral mortality (Donner et al., 2005; Liu et al., 2005; Kayanne, 2017).

### 2.3 | Experimental animals

Colonies of *Mussismilia braziliensis* (n = 40) (7537.85 ± 3,959.74 cm² of diameter) were collected at the Recife de Fora Marine Park (≅ 1.5 m deep), under SISBIO license Nº 63368-1. Collections were performed in early February 2020, two weeks prior to the predicted

spawning period (Pires et al., 1999; Pires et al., 2016). Colonies were placed in two 1000-L semi-closed tanks connected to the ocean at the Coral Vivo Research Base.

### 2.5 | Egg-sperm bundles collection

Spawning monitoring and collects were carried out from February 18 to 27, during the spawning window period. To avoid gamete contamination, each broodstock colony was isolated in a plastic bowl and monitored during spawning. Egg-sperm bundles were collected from the water surface, counted and immediately transferred to 50-mL tubes (10 bundles per tube) containing 10 mL of seawater. After dissociation of the bundle, oocytes floating on top of the tube were separated using a Pasteur pipette and the sperm concentrated at the bottom was transferred to another 50-mL tube containing filtered (0.2 μm) seawater. Local seawater temperature was around 28 ºC at the time of spawning. We used a temporal series of spawning data recorded at Coral Vivo Research Base to make clear comparisons when possible.

### 2.6 | Gamete quality assessments

Oocytes from five spawned gamete-bundles were assessed under a stereomicroscope to measure their diameter and the concentration of oocytes per bundle. Oocyte diameter was accessed by photographs under a hemocytometer and were measured using the ImageJ with the java 1.8.0 172 software. Three samples of sperm (10 μL) were placed on histological glass slides and the motility rate was assessed (in triplicate) under a light microscope (40x magnification). The percentage of motile cells exhibiting forward movement was recorded at 1.5, 4, 6, 8, and 9h after spawning to determine spermatozoa lifespan, i.e., the full arrest of motility for all spermatozoa in the optical field (Godoy et al., 2021). To evaluate sperm concentration per bundle, 20 μL of sperm (n = 4) was fixed in 60 μL of 4% buffered formal saline solution (Hancoch, 1957). An aliquot of this dilution was placed by capillarity into a hemocytometer (n = 4) to analyze spermatozoa concentration.

### 2.7 | Fertilization and embryo viability

*In vitro* fertilization trials were conducted by adding 15 bundles from four different colonies into 400-mL glass aquaria (n = 3) and kept at 28 °C with constant aeration. Embryo viability was assessed 44 hours post-spawning by counting the number of swimming larvae, i. e., individuals that successfully transitioned from embryo to larva, in each aquarium under a

dissecting microscope. The following equation was used to determine embryo viability (EV):

$$EV = \frac{l}{b \times o} \times 100$$

where *l* is the number of developing larvae obtained, *b* is the number of bundles added to each aquarium and *o* is the average number of oocytes per bundle (Godoy et al., 2021).

### 2.8 | Statistical analyses

To describe the data on the number of packages released, sperm concentration and oocyte size, mean and standard deviation were used. To analyze the variation of time in relation to motility, the following tests were used: Normality and homogeneity of variance of all data were assessed using the Shapiro-Wilk and Levene tests, respectively. For both tests, whenever the P value was > 0.05, we used parametric tests, and when the P value was < 0.05 non-parametric tests were used (Zar, 1999). Motility was analyzed over time using Kruskal-Wallis following the Dunn test for average comparison. To investigate differences in spermatozoa concentration and embryo viability, non-parametric data were analyzed using Mann-Whitney's test. All tests were performed using the GraphPad Prism 7.0 software. Non-parametric data are presented in box plotted with median and maximum-minimum values.

## 3 | RESULTS

### 3.1 | The bleaching event at the Recife de Fora Marine Park in 2019

In 2019 both SST and DHW values broken records in South Atlantic (Duarte et al., 2020). The NOAA Coral Reef Watch (2019) emitted several bleaching warnings to Brazilian coral reefs in 2019, once values of DHW > 8 ºC-weeks signal "bleaching alert level 2" . The anomaly on the sea water temperature started by the end of January2019 (Ferreira et al., 2021), when SST exceeded the 28 ºC (MMM) and lasted until July 2019 (Figure 1). The peak of SST was observed on February (29.33 ºC), which has exceeded the local bleaching threshold. Occurrence of hotspots could be observed from February to May 20 (about 15 weeks of hotspots accumulation) (Figure 1). The DHW reached the maximum historical value (19.65 ºC-week) for Abrolhos between April and May 2019 (Figure 1) (Duarte et al., 2020; Ferreira et al., 2021). Indeed, *Mussismilia braziliensis* experienced a mass bleaching from April to July-August 2019 (Ferreira et al., 2021).

### 3.2 | Reproductive performance of *M. braziliensis* in 2020

The spawning took place only on the 22nd and 23rd of February. Among the 40 colonies monitored (7537.85 ± 3,959.74 cm² of diameter), only four (10%) spawned on day 22 and a single colony on day 23. Spawning on February 22 started at 7:50 pm and ended at 8:35 pm (UTC -3). Each colony released an average of 57 egg-sperm bundles. Analyzing the historical series of records from the Coral Vivo Research Base, it was possible to verify that the 2019 bleaching event promoted an average reduction of 67 percentage points in the number of bundles spawned per colony compared to previous years of spawning records for *M. braziliensis* (Figure 2).

### 3.3 | Motility and spermatozoa lifespan

The motility of *M. braziliensis* spermatozoa began to be evaluated 1.5 h after spawning on February 22, showing a motility rate of 49.44 ± 19.82%. There was no significative difference in the motility rate over the first 8 h after spawning $p > 0.9999$. However, after 9 h the spermatozoa motility significantly decreased $p = 0.0004$ (Figure 3). On the second spawning night (February 23), sperm from all bundles ($n = 5$) were analyzed, and none of the bundles had live sperm (0% motility).

### 3.4 | Gametes concentration and embryo viability

*Mussismilia braziliensis* colonies spawned bundles containing $2.94 \times 10^7 \pm 5.99 \times 10^6$ spermatozoa and 73.20 ± 60.55 oocytes. The oocytes measured 481.64 ± 58.87 μm in diameter. The embryo viability observed for *M. braziliensis* during this spawning event was 3.75 ± 3.76%.

## 4 | DISCUSSION

The resilience of *M. braziliensis* to the 2019 bleaching event, which could be observed by the high and fast recovery and low mortality rates (Ferreira et al. 2021), physiological processes that demand significant amounts of energy (Ramirez-Llodra, 2002) Species of the same genus, *M. harttii* and *M. hispida*, which underwent the same bleaching event in 2019, did not demonstrate the same resilience observed in *M. braziliensis*, but showed reproductive success according to Godoy et al. (2021). Spawning of these two species was recorded in September 2019, even with part of the colonies still bleached (Godoy et al., 2021), while *M. braziliensis* was already fully recovered in August (Abrolhos) and September (Recife de Fora) of the same year (Coral Vivo, 2022).

Although there is no data in the literature on the number of oocytes per spawned bundle for the species, the histological studies of Pires et al. (2011; 2016) pointed out that the maximum number of oocytes ever observed in the same polyp of *M. braziliensis* that did not undergo bleaching was 987, with an average fecundity of 339 ± 73 oocytes per polyp. In addition, Pires et al. (2011) observed that *M. braziliensis* invests considerably in reproductive effort compared to other massive coral species, which corroborates our observations and allows us to state that the number of oocytes found per bundle (73.20 ± 60.55) was severely reduced after the bleaching event. Regarding the oocyte's diameter, our finding cannot be compared due to the lack of data in the literature. Pires et al. (2016) observed *M. braziliensis* oocytes in histological sections measuring 392 μm in diameter when inside the polyp, before being spawned. When the bundles are released to the environment and dissociated, hydration of the oocytes occurs, which consequently increase in size, as observed by Valente et al. 2022 (under review).

When comparing with other species belonging to the genus *Mussismilia* (see Godoy et al., 2021), we could observe a lower concentration of spermatozoa per bundle, since healthy colonies of *M. hispida* contained 4.1 ± 5.3 x $10^7$ and *M. harttii* had 3.7 ± 2.0 x $10^7$ spermatozoa per bundle. This suggests that bleaching may have also negatively affected the spermatogenesis process. The sperm motility of *M. braziliensis* in the first evaluation after spawning (1 h 30 min later; 49.44 ± 19.82%), was close to that found for *Fungia scutaria* (37%) and *Montipora capitata* (40%) after the 2015 bleaching event in Kaneohe Bay (Hawaii) (Hagedorn et al., 2016). Godoy et al. 2021 did observe that sperm motility 3 h after spawning was 56.5 ± 11.5% for healthy and 64.2 ± 31.7% for bleached colonies of *M. hispida*, which demonstrates that sperm motility in *M. braziliensis* quickly decayed post-spawning. The observation of dead spermatozoa on the second spawning night indicates a reproductive failure for the species.

The high number of colonies that did not spawned in the 2020 breeding season could also be related to oocyte uptake and consequent interruption of gametogenesis. Some coral species, such as *Siderastrea siderea* and *Acropora formosa* absorb their oocytes in response to stress as a way to obtain the energy needed to survive and recover (Okubo et al., 2007; St. Gelais et al., 2016). However, we cannot conclude whether oocyte absorption actually occurred in *M. braziliensis*, as the coral reproductive tissues were not evaluated. It is also important to consider that the oogenesis requires a lot of energy (Hayward & Gillooly, 2011), especially during vitellogenesis (Ramirez-Llodra, 2002), therefore the energy restriction caused by the absence of symbiodiniaceans during bleaching may have negatively affected the production of oocytes and vitellogenic material. As a consequence, the impairment of oogenesis leads to a

reduction in the chances of fertilization and compromises embryonic development due to the lack of nutritional content (Levitan, 1996).

Levitan et al. (2014) observed a reduction in the number of colonies that spawned after bleaching events for the corals *Orbicella franksi* and *Orbicella annularis*. Different from what Szmant & Gassman (1990) observed for *Montastraea annularis*, in which colonies recovered from bleaching completed the gametogenesis process normally. What we observed for *M. braziliensis* is worrying, considering that all colonies when sampled were visually recovered and healthy. This indicates that either there was no gametogenesis in most colonies, as the species bleached weeks before initiating the oogenesis process and may have simply interrupted or ignored this physiological process, or it delayed the production of gametes and, in this assumption, part of the spawning would have occurred after our observations. Ward et al. (2000) and Johnston et al. (2020) suggest that the observation of color reestablishment by colonies after bleaching events is not enough to declare that the coral is recovered. Bleaching events reflect adverse and even lasting effects on coral reproduction, in addition to previously bleached colonies being more susceptible to future bleaching events (Ward et al., 2000; Hughes & Grottolli, 2013; Johnston et al., 2020).

Direct exposure of *M. braziliensis* gametes to environmental stressors, since the species performs external fertilization, may also be interfering with fertilization rates and embryo survival. The species' reproduction period in 2020 showed values of SST higher than the MMM and the bleaching threshold for the region (22 February), starting a new period of hotpots and indicating a possible alert to bleaching (NOAA, 2021). The seawater temperature at the time of reproduction (above 28 ºC) (NOAA, 2021) may have contributed to the low fertilization rate observed (3.75 ± 3.76%), compromising both oocyte and sperm physiology. The high local temperature at the time of spawning may also be responsible for compromising the offspring survival and being the main responsible for the observed lifespan for spermatozoa and for the impairment of sperm motility. Temperature is one of the main regulators of sperm motility and fertilization capacity in most species, as it directly influences the enzymatic activities that take place during flagellar movement (Alavi & Cosson, 2005).

So many impacts on sexual reproduction bring up another issue with regard to scleractinian corals, their perpetuation and maintenance of coral reefs. Unlike soft or branched corals that are able to reproduce asexually through fission or budding, as is the case of *Millepora alcicornis* (Edmunds, 1999), massive corals depend almost exclusively on sexual reproduction to propagate in the environment. In other words, successful sexual reproduction in reef-building

corals is the main way to maintain this ecosystem (Ward et al., 2000). Furthermore, in the South Atlantic, intense abiotic factors (storms, hurricanes, strong sea currents) capable of fragmenting massive colonies like *M. braziliensis* and favoring asexual reproduction are not observed, as occurs in other reefs (eg .: Caribe) with branched species (Edmunds, 1999). In addition to the low fertilization rate observed for *M. braziliensis*, it does not guarantee that all embryos formed will develop. Bleaching events in Australia's Great Barrier Reef have been shown to be responsible for a reduction in recruitment, caused by compromised sexual reproduction (Hughes et al. 2019).

The growth in bands (of high and low densities) observed in corals is a phenomenon that occurs due to endogenous processes, such as the reallocation of energy from growth to reproduction, which is mediated by exogenous factors (luminosity, nutrition, temperature), that play a secondary role in the process of growth (Wellington & Glynn, 1983). Linear growth rates of *M. braziliensis* colonies are extremely low, around 0.56 ± 0.03 cm/year according to Oliveira (2014) and 0.8 mm/year according to Leão (1982). This suggests that it takes years for a colony to reach size and sexual maturity to ensure its perpetuation through sexual reproduction. The data presented so far suggest that *Mussismilia braziliensis* prefers to invest in long-term survival rather than reproduction. However, the impacts that have been imposed on coral perpetuation could be irreparable if bleaching events continue to occur at such frequency and duration. The impairment of sexual reproduction compromises the genetic variability of the species, the formation of new individuals and colonies to restore the reefs, generates a collapse in larval recruitment, influences the loss of adult individuals and the ability to form future assemblages (Hughes et al., 2019). It is important to note that the effects of bleaching on reproduction can be experienced in the long term and, not just in the first breeding season following bleaching (Levitan et al., 2014). This suggests negative impacts on the maintenance of coral populations over time if bleaching events continue as predicted. Scientists need to pay attention to the effects of rising ocean water temperatures on the maintenance of coral cover and the sexual reproduction of reef-building corals before climate change becomes irreversible.

## 5 | CONCLUSIONS

Despite the high resilience of the species, being one of the fastest to completely recover its colonies from bleaching (visually), the lack of energy from the symbiotic algae compromised gametogenesis and consequently the sexual reproduction. We observed a low number of colonies spawning, fewer gamete-bundles spawned per colony compared to previous years, low

sperm motility, bundles with dead spermatozoa, low number of oocytes per bundle as well as low embryo viability. An alternative is that *M. braziliensis* may have reabsorbed its oocytes to obtain energy for survival and recovery from bleaching. This is the first time that the reproduction of *Mussismilia braziliensis* has been evaluated. With an alert to the frequency of bleaching events that has been increasing in quantity and intensity, studies like this one are important to assess the species' ability to reproduce after bleaching events. An impairment of sexual reproduction leads to impairment of the construction and maintenance of reefs in the South Atlantic, where *M. braziliensis* is one of the main reef-building species.

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**FIGURAS (CAPÍTULO I)**

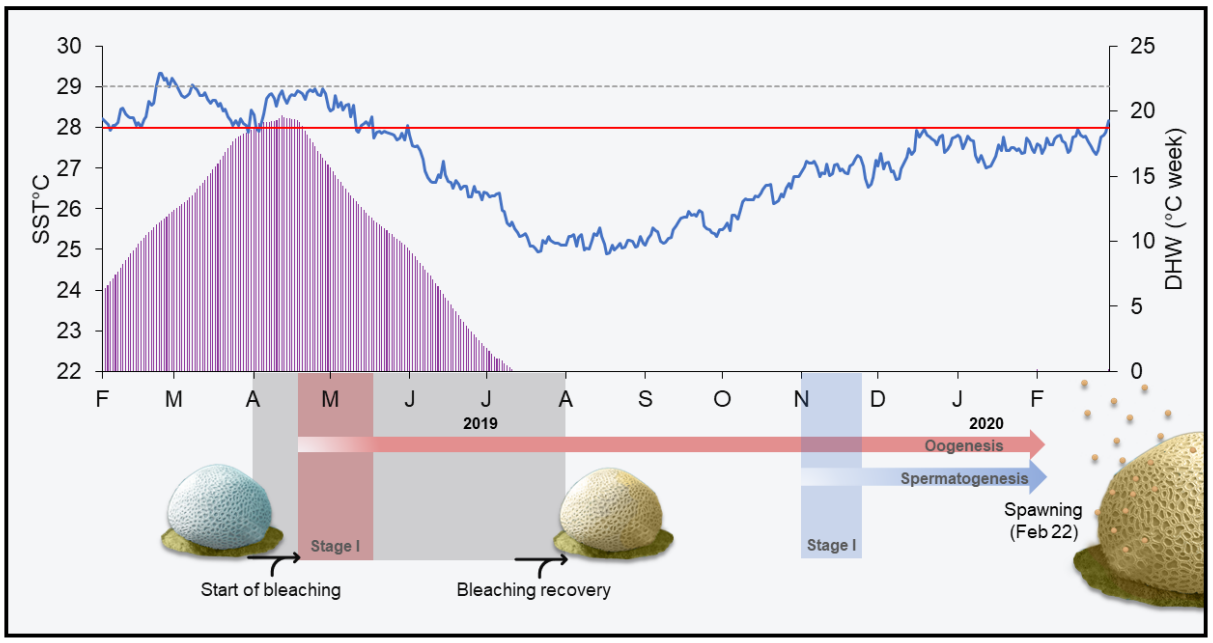



**FIGURE 1** Sea Surface Temperatures (SST; blue solid line) and Degree Heating Weeks (DHW; lilac vertical bars) from Abrolhos Reefs Virtual Station according to the NOAA Coral Reef Watch, using daily temperature readings from February 2019 to February 2020. The red solid horizontal line indicates local Maximum Monthly Mean (MMM), about 28ºC. The gray dashed horizontal line indicates the local bleaching threshold (MMM + 1°C). The lower gray rectangle indicates the bleaching period of *Mussismilia braziliensis* (April to July). The pink arrow represents the oogenesis period for the species. The dark pink column indicates the beginning of oocyte development (Stage I). The blue arrow represents the spermatogenesis period and blue column indicates the first stage of spermatogenesis. Spawning happened on 22 and 23 February (2020) when SST achieved the MMM once again.

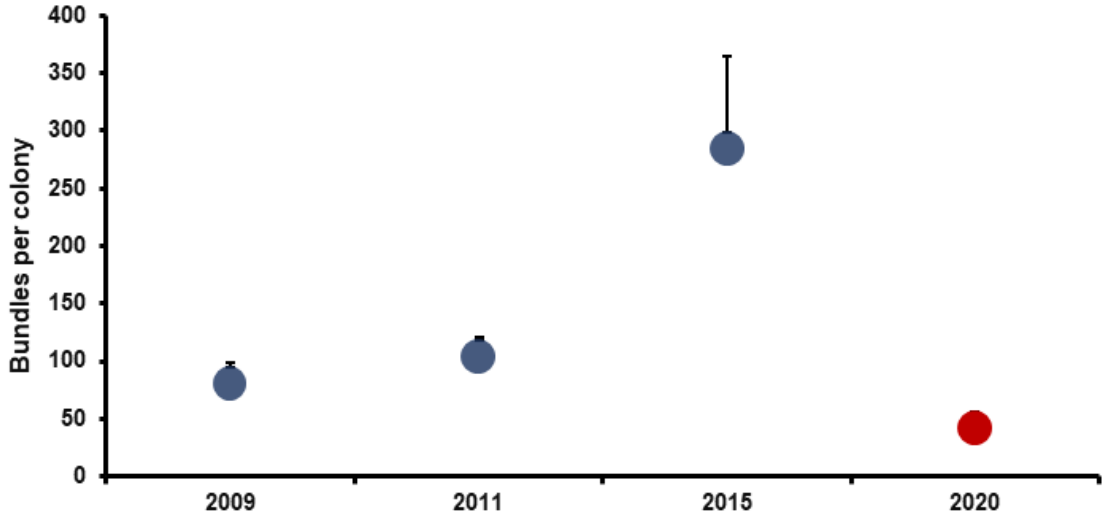



**FIGURE 2** Temporal variation on spawned bundles per colony of *Mussismilia braziliensis* in years without bleaching (blue circles) and after the most severe bleaching event of the Southwestern Atlantic (red circle). Data was recorded at the Coral Vivo Research Base.

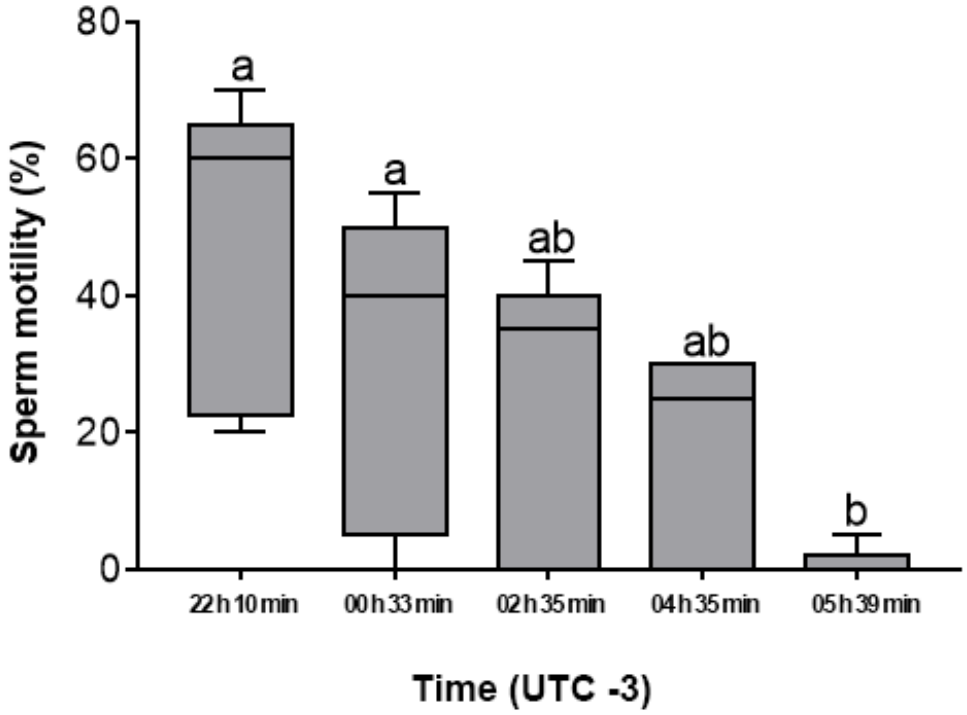



**FIGURE 3** Box and whisker plots of the sperm motility in *Mussismilia braziliensis* following spawning. The bottom and top of each box represent the lower (Q1) and upper (Q3) quartile, respectively, and the line inside each box represents the median. Whiskers above and below the box indicate the maximum and minimum sperm motility, respectively. Os dados foram submetidos ao teste Kruskall Wallis p = 0.0007, H = 19.28. Distinctive letters indicate statistical

diference ($P < 0.05$).

## CAPÍTULO II

**Influences of ocean warming on sperm viability of the Brazilian reef-building coral *Mussismilia braziliensis* (Verrill, 1868)**

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**Abstract:** In recent decades, climate changes resulting from anthropogenic activities, such as the high emission of greenhouse gases, have resulted in an increase in temperature in the Earth's atmosphere and consequently in the ocean. Coral reefs are highly sensitive to environmental variations, such as temperature increases. Considering that these ecosystems are home to at least 25% of all marine life, impacts on these environments can lead to irreversible damages. One of the biggest threats to corals has been the frequent bleaching events, caused by the breakdown of the symbiosis between reef-building corals and their symbionts. Little is known about how the increase in temperature impacts the reproduction of the Tropical South Atlantic corals under marginal turbid conditions. Thus, this study aimed to evaluate the impacts of the increase in water temperature on the sperm of the endemic coral, considered the main reef-building species in Abrolhos, *Mussismilia braziliensis*. Spermatozoa were exposed to different temperatures: 28ºC, Recife de Fora local temperature; 29.7ºC, considering the milder scenario RCP2.6 of IPCC 2014 climate projections; and 32.8ºC considering the most critical scenario RCP8.5. Analyzes were performed regarding sperm motility and morphology over time at different temperatures. It was found that the increase in temperature significantly decreases the rate of sperm motility. In addition, the increase in temperature was responsible for the increase in anomalies in the sperm flagellum over time. This is the first study to seek to understand the effects of ocean warming on coral sperm metabolism.

**Keywords:** Climate change, Endemism, Brain coral, Coral reproduction, Spermatozoa, South Atlantic

## Introduction

Climate change resulting from anthropogenic activities, which lead to an increase in the concentration of greenhouse gases that retain heat in the atmosphere and mainly in the oceans, such as carbon dioxide, methane, and nitrous oxide, have intensified since the Industrial Revolution (IPCC 2014). Studies show that the oceans retain the heat emitted by gases on Earth, which minimizes the effects on the Earth's surface, but is raising the temperature of the oceans much faster than predicted (Cheng et al. 2019). Global warming has contributed to increased rainfall, rising sea levels, melting glaciers and ice caps, declining oxygen levels in the oceans and reducing coral cover (IPCC 2014).

Coral reefs are environments rich in biodiversity, comprising at least 25% of the species that occur in the oceans (Mulhall 2009; Wilkinson 2008). In addition, coral reefs provide important ecosystem services for the organisms that live on them and for humans (Mulhall, 2009). These ecosystems are important food sources, refuge, and nesting sites for different marine species; food source for humans through fishing; recreation and tourism environments; suppliers of chemical compounds for the pharmaceutical industry; and to protecting coastal regions from the influence of waves and storms (Hoegh-Guldberg 2007; Castro et al. 2016; Hugues et al. 2017).

In the South Atlantic, the only biogenic reefs occur on Brazilian coast. The diversity of zooxanthellate corals in Brazilian reefs is low, with about 23 species, but with a high degree of endemism (Castro et al. 2016). Among the five endemic species, three belong to the genus *Mussismilia* (Cnidária: Anthozoa: Hexacoralli): *M. braziliensis* (Verrill 1868), *M. harttii* (Verrill, 1868), and *M. hispida* Verrill, 1901, and all are important reef-builders on the Brazilian coast (Castro and Pires 2001; Leão et al. 2016).

Coral reefs have been impacted by the world climate change, mainly due to the ocean retention of greenhouse gases that generate the warming and acidification of its waters (Hoegh-Guldberg 2007; Cheng et al. 2019). Warming sea water triggers a phenomenon called coral bleaching. Coral bleaching occurs when dinoflagellate algaethat live in symbiosis with corals are expelled from the host tissues, the coral tissue becomes transparent, and consequently showed its limestone skeleton (Brown 1997; Stanley 2006). The coral expels the symbiodiniaceans as a form of protection, since when the water temperature increases, the

photosynthetic process of the algae is altered and the uncontrolled production of unpaired oxygen molecules occurs, causing oxidative stress to the coral, damaging lipids, proteins, and the DNA (Iglesias-Prieto et al. 1992; Lesser 2006; Roth 2014). If the water temperature does not return to normal, the bleaching situation may be irreversible, and if the symbiosis between coral and symbiodiniaceans is not restored, the coral dies as it lacks nutrients. In recent decades, bleaching events have become increasingly frequent worldwide (Heron *et al.* 2016). In Brazil, bleaching events were recorded in 1993-1994 (Castro and Pires 1999), 1996, 1998, 2003, 2005, 2010 (Leão et al. 2016; de Oliveira Soares et al. 2019) 2016 (Sabadini 2017; Teixeira et al. 2019), 2019 (Banha et al. 2019; Duarte et al. 2020; Ferreira et al. 2021), 2020 (Pereira et al. 2022) in several reef-building species.

The anthropogenic impact on coral reefs goes far beyond the climate crisis. Local impacts such as intensive fishing, pollution, organic matter dump, contamination of water with industrial residues or pesticides and disorderly tourism, also affect the development and maintenance of coral reefs (Castro et al. 2016; Hugues et al. 2017). It is estimated that approximately 40% of the world's reefs have already been lost (Heron et al. 2016; Hughes et al. 2018; 2019) and that the remaining will be in critical condition in the next 20 years (Wilkinson 2008; Castro *et al.* 2016).

The Brazilian endemic species *Mussismilia braziliensis* (Verrill, 1868) is the main reef-builder in Abrolhos (Castro & Pires 2001; Wilkinson 2008) and its occurrence is restricted to reefs in the state of Bahia. *Mussismilia braziliensis* is a hermaphroditic species, with gamete development (oocytes and spermatozoa) occurring in the same mesentery (Pires et al. 1999). During the species' reproductive season between March and mid-May (Pires et al. 1999), synchronized with the new moon (Castro et al., 2016), gamete bundles are released into the water column for external and cross fertilization. After fertilization, the embryo will give rise to a planula larva. The planula settles on the substrate and give rise to new colonies, expanding the substrate of Bahian reefs. Sexual reproduction is responsible for the perpetuation and genetic variability of species. As gametes from *M. braziliensis* are released into the water column, they are more vulnerable to environmental stress than the animal itself, which can reduce the ability of sperm and oocytes to remain viable. (Harrison 2011; Pires et al. 2016).

Temperature is one of the main regulators of sperm motility and fertilization capacity in most species, as it directly influences the physiological functions and enzymatic activities that occur during the flagellar movement (Alavi & Cosson, 2005; Bradshaw and Holzapfel 2006). Studies show that an increase in seawater temperature by 2ºC accelerates the spawning period,

reduces the number of spermatozoa per bundle and the fecundity of the *Acropora digitifera* (Paxton et al. 2015). The reduction in sperm concentration and motility after a bleaching phenomenon has also been observed for the genus *Acropora* (Omori et al. 2001). Finzi et al. (1995) suggest that morphological anomalies could be a good predictor of heat stress. Furthermore, Boni (2019) showed that spermatozoa exposed to heat stress had a reduction of sperm concentration and sperm motility, increases the apoptosis, reduces its antioxidant capacity, harm mitochondrial structure and functions, and the chromatin condensation.

Some sperm quality indicators commonly used in fish, such as sperm motility rate, lifespan, plasma membrane integrity, and sperm morphology, can be applied to coral gametes, providing important subsidies to determine fertilization success. Studies evaluating the influence of environmental factors on sperm quality in corals are scarce. There are still no data on the impact of climate change on the species *Mussismilia braziliensis*. Since the species is endemic to Brazil, it is very important to know its reproductive biology in detail and generate knowledge that can help in strategies for the conservation of the species before its population is lost to an environmental catastrophe. In addition, as *M. braziliensis* is an important reef-builder, its loss can consequently result in the partial loss of the Brazilian reef ecosystem. Considering the climate projections of ocean temperature increase, according to the IPCC 2014, until 2100, it may be that we have a little time to assess the impacts of warming ocean waters on the species. Assessing how gametes behave in the face of environmental changes can provide crucial answers for the conservation of *M. braziliensis*.

Considering the severity of ocean warming and the lack of knowledge about the reproduction of this endemic species, the anthropogenic impacts may become irreversible in a short period of time and make conservation proposals unfeasible. Thus, the aim of this study was to evaluate the impacts of the increase in ocean temperature on the motility and sperm morphology. To carry out this assessment, two scenarios of climate projections from the IPCC 2014 were selected, a milder one (RCP2.6), which predicts an increase of 1.7ºC to for the ocean by 2100, and a more severe one (RCP8.5), which predicts an increase by 4.8ºC.

## Material and Methods

### Study site, experimental animals and permits

Visibly healthy colonies of *Mussismilia braziliensis* (n = 40) (7.537,85 ± 3.959,74 cm² of diameter) were collected by diving (≅ 1.5 m deep) on early February 2020 at the Recife de Fora

Natural Marine Park (16°23'00″S; 38°58'00″W), a Brazilian integral protection conservation unit. The marine park covers a 19.68 km² area with shallow water reef formations and it is characterized by a high degree of endemism (Castro and Pires 2001). The colonies were transferred to a 1000-L semi-closed tank connected to the ocean at the Coral Vivo Research Base (Arraial d'Ajuda, Bahia, Brazil). This research was authorized by the Chico Mendes Institute for Biodiversity Conservation (SISBIO Nº 63368-1) and by the Municipal Secretary of Environment from Porto Seguro (Authorization N° 01/2019).

**Sperm collection**

Collects of egg-sperm bundles were performed in late February during the spawning window period. The bundles (n = 93) were collected from the water surface by a Pasteur pipette and immediately transferred to 50-mL tubes (10 bundles per tube) containing 10 mL of seawater. After the natural dissociation of the bundle, eggs floating on top of the tube were separated and the sperm concentrated at the bottom was transferred to another 50-mL tube. The sperm was then diluted in filtered (0.2 µm) seawater to compose a pool (270 mL). Sperm pooling was performed to prevent individual colony effects, as sperm quality and concentration among colonies vary significantly (Godoy, *personal communication*).

**Experimental design**

To carry out the thermal variation experiment we prepared three aquariums with 20 L of filtered seawater, which were kept at different temperatures. The following thermal variations were used: 28ºC (control group), corresponding to the seawater temperature at Recife de Fora marine park on February 2020; 29.7ºC, corresponding to a 1.7ºC increase in the ocean's temperature according to the RCP2.6 scenario predicted by IPCC (2014); and 32.8ºC corresponding to the RCP8.5 scenario, which suggests an increasing up to 4.8ºC in ocean temperature (IPCC, 2014). Water temperatures were controlled using an automatic system (Reef Angel Controller), with 15.000 W heaters and temperature loggers (HOBO U22-001 Data Logger-).

The spermatozoa pool was randomly distributed in five 15-mL tubes (5-mL per tube) to each experimental treatment. The treatments were arranged in a 3 x 3 factorial scheme. The first factor referred to the three tested temperatures (28; 29.7, and 32.8 °C) and the second factor corresponded to the three post-spawning exposure time (2.5, 5, and 7 h). At each evaluation time, all the content available in the tubes were used: 0.5 mL was used for motility analysis and fixed in 4% buffered saline formalin (20 µL of semen: 40 µL of fixative solution) for

morphological studies; and 4.5 mL was distributed into three cryotubes (1.5 mL per cryotube) which were centrifuged and frozen in liquid nitrogen.

**Sperm motility assessment**

Sperm motility was assessed to determine the percentage of live cells exhibiting forward movement (Godoy et al. 2021). Samples (10 μL) were placed on a glass slide and observed (triplicate) through an optical microscope. It was recorded at 2.5, 5, and 7 h after spawning, i.e., the full arrest of motility for all spermatozoa in the optical field.

**Spermatozoa morphology**

To assess spermatozoa morphology 30-μL aliquots of the fixed samples were mixed with 30 μL of rose Bengal dye and a smear was prepared. The analysis was performed under an optical microscope (100x) (PRECISON P502). For each experimental unit we assessed three slides. The spermatozoa were classified as normal (without anomalies) or abnormal, with damages on their structure, according to described by Amaral et al. (2022).

**Statistical analyses**

Data were tested for normality by the Shapiro-Wilk test and for homogeneity by Levene test. When data were normal, treatments were compared using two-way analysis of variance and for non-parametric distribution, the Kruskal - Wallis test was used. Motility was analyzed over timeusing Two-Way ANOVA following Tukey's test to compare means. We consider value (P value $\leq 0.05$) for all statistical tests. Non-parametric data are presented in boxes plotted with median and maximum-minimum values and for standard data with normal distribution, the presented is done by mean ± deviation.. All were based on Zar (1999).

**Results**

**Sperm motility**

On the first evaluation time the sperm motility rate had significant difference $p < 0.0001$, $F(4, 120) = 24.35$ between all temperatures (Fig. 1). At 28ºC, ambient temperature of Recife de Fora, there was a significant decrease in motility over the time. The same occurred at 29.7ºC. Generally, the higher the water temperature, the lower the percentage of motile spermatozoa. When exposed to the highest temperature, spermatozoa showed a decrease of 16 percentage points in motility rate, compared to those kept at 28°C. After five hours, the sperm motility rate

of those kept at higher temperatures did not differ from each other (29.7°C = 4.9 ± 3%; and 32.8°C = 2.0 ± 1.5%), however, they were significantly lower $p < 0.0001$, $F(2, 120)=378$ than the rates found for spermatozoa kept at 28ºC (14.6 ± 4.6%). At 32.8ºC the drop in the motility rate was so rapid that no significant difference was observed between five and seven hours. Seven hours post-spawning, spermatozoa of all treatments did not present any motility rate $p = 0.4421$.

In addition to the negative effects of increasing seawater temperature, sperm lifespan also influenced the percentage of motile cells. This was evidenced at the lowest temperature (28ºC) at all times analyzed. However, the higher temperature had such harmful effects on sperm, that the spermatozoa motility rate at five hours was such lowest as to not differ from that observed at seven hours.

**Spermatozoa morphology**

*Mussismilia braziliensis* normal spermatozoon has a spherical head (0.03 ± 0.01 µm of diameter), a flagellum midpiece and one elongated flagellum which reaches 5.8 ± 1.21 µm of length, and that normally do not appear spiraled or coiled. The *M. braziliensis*' spermatozoa head varies to 0.023 from 0.037 µm of diameter, and the flagellum varies from 4.113 to 7.475 µm of length.

When analyzing the effects of time and temperature on sperm morphology, we did not observe a significant difference on the percentage of normal spermatozoa (Fig. 2; Fig. 3a). On the first time (2.5 h) of analysis we did observe a significative effect of rising temperature on sperm morphology. The percentage of normal spermatozoa was significantly lower at the highest temperatures (29.7ºC and 32.8 ºC). Despite we observed a reduction pattern on the number of normal spermatozoa with the increasing of temperature, it was not statistically significant ($p = 0.5617$).

The morphological anomalies found on *M. braziliensis*' spermatozoa were free head, degenerate head, macrocephaly, microcephaly, distal gout, coiled flagellum, broken flagellum, and agglutination of cells (Fig. 3; Table 1). Spermatozoa exposed to 28ºC showed a significant difference on the percentage of free head over the time (from 2.5 to 7 hours). At seven hours post-spawning a significative increase on spermatozoa with free head was found at such temperature. At 29.7ºC, coiled flagellum anomaly decreased significantly over the time. By analyzing the sperm morphology at different temperatures, we also observed a significant

increase on broken flagellum anomaly as the temperature increases.

We observed a positively correlation between flagellum damages and increase in seawater temperature (Fig. 4). The higher the seawater temperature the higher the anomalies found in the flagellum, consequently compromising sperm motility rates. This pattern was observed for all treatments.

**Discussion**

In this study, we described for the first time *Mussismilia braziliensis'* spermatozoa and its anomalies. We observed a significant decrease in motility rate of spermatozoa over the time and when exposed to different temperatures. The ambient temperature of Recife de Fora (28°C) on the spawning time was high enough to negatively affect the motility rates of *Mussismilia braziliensis*' spermatozoa over the time. We observed that in higher temperatures sperm motility drops rapidly, compromising the viability of spermatozoa right after spawning. Furthermore, as the temperature increased and time passed, we observed a drop in the number of normal spermatozoa and an increase on flagella damages. Other studies have shown the same pattern between increase in water temperature and decrease in motility rate for marine invertebrates and broadcast-spawning species, like polychaetas, oysters, and corals (*Acropora tenuis*) (eg. Kupriyanova and Havenhand 2005; Parker 2010; Albright and Mason 2013). In humans, the decrease in normal sperm morphology was also observed by Thijssen et al. (2014) when the temperature rises. Hurley et al. 2017 verify that extreme temperatures have the potential to impact sperm quality and function in a bird species (*Zebra finch*).

Temperature is one of the main regulators of sperm performance like motility, duration of sperm activity and fertilization success (Alavi and Cosson 2005; Kupriyanova and Havenhand 2005; Dorts et al., 2012). Increase on environmental temperature influences on the physiology and in the metabolic rates (enzymatic activities) of spermatozoa. The mitochondria produces the energy (ATP – adenosine triphosphate), through oxidative phosphorylation, which is stored (Chen 1988). Posteriorly, the stored energy is used for the flagellar beating (motility), and it is crucial for determine the sperm lifespan. When exposed to high temperatures, spermatozoa increase their motility rates, and since they have a limited energy store, consequently the motility duration (life span) is reduced (Alavi and Cosson 2005), and the fertilization success is impaired (Kupriyanova and Havenhand 2005). At high temperatures the axoneme needs more ATP to maintain the beating of flagellum, consequently the energy reserves of the spermatozoa are used up faster (Cosson 2010), and sperm lifetime decreases.

Nagy et al. (2016) observed in fish spermatozoa, that at high temperatures (30-40ºC) the

mitochondrial membrane potential changes, hyperpolarizing quickly in 10 minutes if compared to low temperatures, and depolarizing in 30 minutes. In the mussel *Mytilus galloprovincialis* the prolonged exposure to high temperature (28ºC) decreases sperm motility, and also was observed that mitochondrial membrane potential of spermatozoa first increases quickly, and then collapses (Boni et al. 2016; Boni 2019). At high temperatures cellular metabolism increases and consequent mitochondrial activities increases, compromising its capacity. The consequence of increased mitochondrial activity is an increase in the respiratory activity (oxidative phosphorylation) which leads to an increase in production of reactive oxygen species (ROS), generating oxidative stress, activating apoptosis mechanisms (Reinhardt 2007; Christen et al. 2018; Reinhardt et al. 2015, Boni 2019). Sperm cells are very susceptible to oxidative stress. Oxidative stress is responsible for degenerate the cell membrane, affects DNA and mitochondrial integrity, which impairs cell motility and fertilization ability (Reinhardt 2007; Tarín et al. 2000; Gallo et al. 2020). Boni et al. (2016) found that spermatozoa exposed to heat stress, quicky increase the lipid peroxidation, DNA fragmentation and showed abnormal spermatozoa morphologies.

The phenotype of gametes also influences the success of fertilization, and it is related from three sources and their interactions: the male nuclear genotype, the spermatozoa mitochondria, and the environment, which is related to exposure to environmental stressors on both, before and after gamete release (Marshall 2015; Reinhardt et al. 2015). The impact of environmental stressors can occur in two ways: colonies can be expose to high temperatures during the spermatogenesis and affect sperm morphology, or the sperm released into the water column itself may undergo phenotypic changes due to environmental conditions to which it was directly exposed (Marshall 2015). In this case, the phenotype observed to *M. braziliensis*' spermatozoa can be related to the environmental stressors which the colonies were exposed, like the severe bleaching event in 2019, or/and with directly exposure of sperm to the ocean water on ambient temperature of the Recife de Fora (28ºC) by the time of spawning. Mechanical damage to the cells also can be generated from high temperatures associated with the quick use of stored energy by spermatozoa (Greenwood and Bennett, 1981).

We observe that only 27% of the spermatozoa had normal motility at 28ºC and 2.5 h post spawning. Therefore, some observed anomalies are probably due to the impact of heat stress during spermatogenesis. The other spermatozoa damages come from the temperature which they were exposed, as the increase in anomalies was observed when temperature increases. At the ambient temperature of Recife de Fora (28°C), despite not showing a

statistically significant difference, we can observe an increase in free head spermatozoa as the temperature increases. This indicates that these spermatozoa lost their flagella over the time. The most severe anomaly is the absence of the flagellum (free head), as the sperm is not even able to progress its movement to fertilize an oocyte (Bem Khelifa et al. 2014). So, this damage completely compromises reproduction, as these spermatozoa no longer have motility. This observation corroborates with the observation on motility rates at 28°C that declined over the time. Broken flagellum significantly increases as the temperature increases for *M. braziliensis*' spermatozoa. Furthermore, coiled flagellum has also been shown to be an anomaly that appears more often as the temperature increases. However, at 29.7°C there was a significant decrease in the number of sperm with coiled flagellum over the time. This could be explained by the high number of free heads found at seven hours after spawning at such temperature. Probably coiled flagella were no longer observed due to loss of flagellum by sperm. Significative increasing in flagellum anomalies (mostly coiled flagellum) was also observed by Finzi et al. (1995) in rabbits when temperature increases. In addition, the positive correlation between flagellum damages and the temperature increasing demonstrates that in fact external factors, in this case temperature, are influencing the sperm phenotype. Not only at 28ºC, but also in the temperature projections estimated by the IPCC (2014) for subsequent years, we observed a significant increase in flagellum anomalies exposed to 29.7ºC and 32.8ºC.

Guillaume et al. (2016) observed that the environmental stressors to which *Galeolaria caespitosa* were subjected affected spermatozoa much more than oocytes, that is, the effect of the environment may be more intense with regard to male gametes. As well as the thermal history to which *Strongylocentrotus purpuratus* males were exposed, significantly impacted the number of successful fertilization events due to a reduced ability of sperm to reach oocytes for fertilization to occur (Leach et al. 2021). Thus, the great impairment observed in the reproduction of *M. braziliensis* in 2020 (Krein et al. in prep.) may be much more related to damage to sperm than to oocytes, considering the stress generated during gametogenesis in the 2019 bleaching event. As demonstrated by Krein et al. (*in prep.*) *M. braziliensis* colonies invested energy in bleaching recovery instead of investing in reproduction. Even if normal sperm production occurs under stressful temperatures, the impact can later be seen on sperm quality and performance (Boni et al. 2016; Johnstone et al. 2019).

Despite the sperm ageing naturally, the fact that they are exposed to higher temperatures contribute to accelerate this physiological and aging process (Reinhardt 2007). Spermatozoa (or cell) aging consist in the natural accumulation of damages on the cell structures which

declines cell function over the time. Oxidative stress, ATP depletion and the impact of temperature are examples of accumulated damages (Reinhardt 2007). In mammals the ageing of spermatozoa is associated to molecular changes, biochemical, and cellular changes which compromise some cellular functions involved in fertilization, such as sperm motility and the acrosomal reaction to fertilize the oocyte (Tarín et al. 2000). Other possibility is that in some cases the abnormalities observed for the flagella, like absence of flagellum (free head) and coiled flagellum can be associated with genetic mutations. These genetic mutations can occur at the sperm midpiece or at the flagellum, causing defects in the axoneme structure, peri-axonemal structure, defects in the centriole assembly and defects on the intraflagellar transport (IFT) complex, and in intracellular transport channels (Wang et al. 2019; Touré et al. 2020).

This is the first time that motility rate and morphology of *Mussismilia braziliensis'* spermatozoa were evaluated. Considering that temperature plays a fundamental role with regard to sperm motility and lifespan, the increase in ocean temperature, as projected and already demonstrated for the species *M. braziliensis* in 2020, directly affects the fertilization success of the species, compromising the perpetuation and maintenance of its population. Studies that seek to understand sperm metabolism and physiological compensation to stressful environmental events are necessary to understand how gamete-releasing organisms deal with reproduction and fertilization in a wide spectrum of ambient temperatures. The absence of studies that clarify the impacts of increased environmental temperature on sperm physiology and metabolism was a limiting factor in this study, in an attempt to explain what may have happened to the sperm analyzed here. Lewis and Ford (2012) have already mentioned the absence of studies that assess the impact of environmental issues and exposure to toxic elements on the morphology of coral spermatozoa, which are organisms strongly threatened by human actions. If the ocean temperature continues to increase as it has been and according to estimated projections, the compromise of the species' reproduction could become irreversible. This will lead to a drastic decrease in the genetic variability and population of the species.

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Council for Scientific and Technological Development (CNPq-Brazil).

**Data availability**

Data are available upon request.

**Conflict of interest**

On behalf of all authors, the corresponding author states that there is no conflict of interest.

**References**

This paper will be submitted on Coral Reefs:
https://www.springer.com/journal/338/submission-guidelines

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**FIGURAS E TABELAS (CAPÍTULO II)**

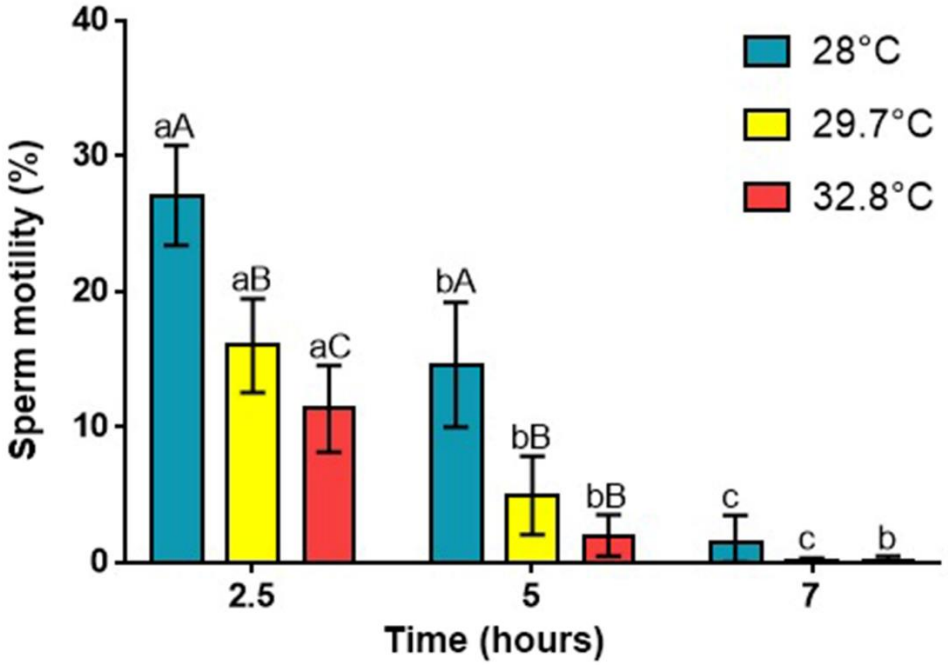



**Fig. 1** Sperm motility of *Mussismilia braziliensis* after exposure to ocean warming scenarios. Motility was accessed at 2.5-, 5- and 7-hours post-spawning and in three different temperatures: 28ºC, temperature of seawater in Recife de Fora; 29.7ºC according to RCP2.6 scenario of IPCC 2014; 32.8ºC according to RCP8.5 scenario of IPCC 2014 Anova Two-Way test, $p < 0.0001$, $F(4, 120) = 24.35$. Distinct lowercase letters represent significant difference in the same temperature over the time. Distinct capital letters represent significant difference between temperatures within the same time.

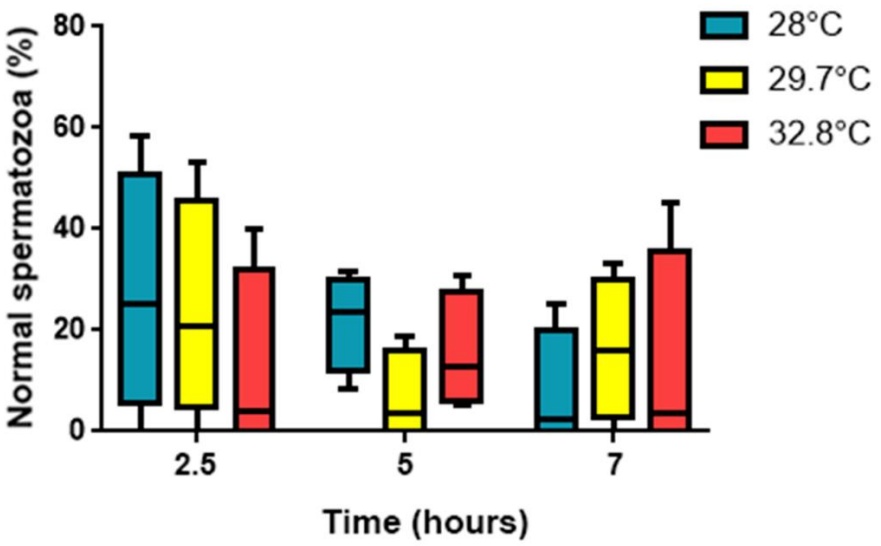



**Fig. 2** Percentage of normal spermatozoa of *Mussismilia braziliensis* at 2.5-, 5- and 7-hours post-spawning and in three different temperatures: 28ºC, temperature of seawater in Recife de Fora; 29.7ºC according to RCP2.6 scenario of IPCC 2014; 32.8ºC according to RCP8.5 scenario of IPCC 2014. The data were submitted to the Kruskal-Wallis test, p = 0.2536, H = 3.2.

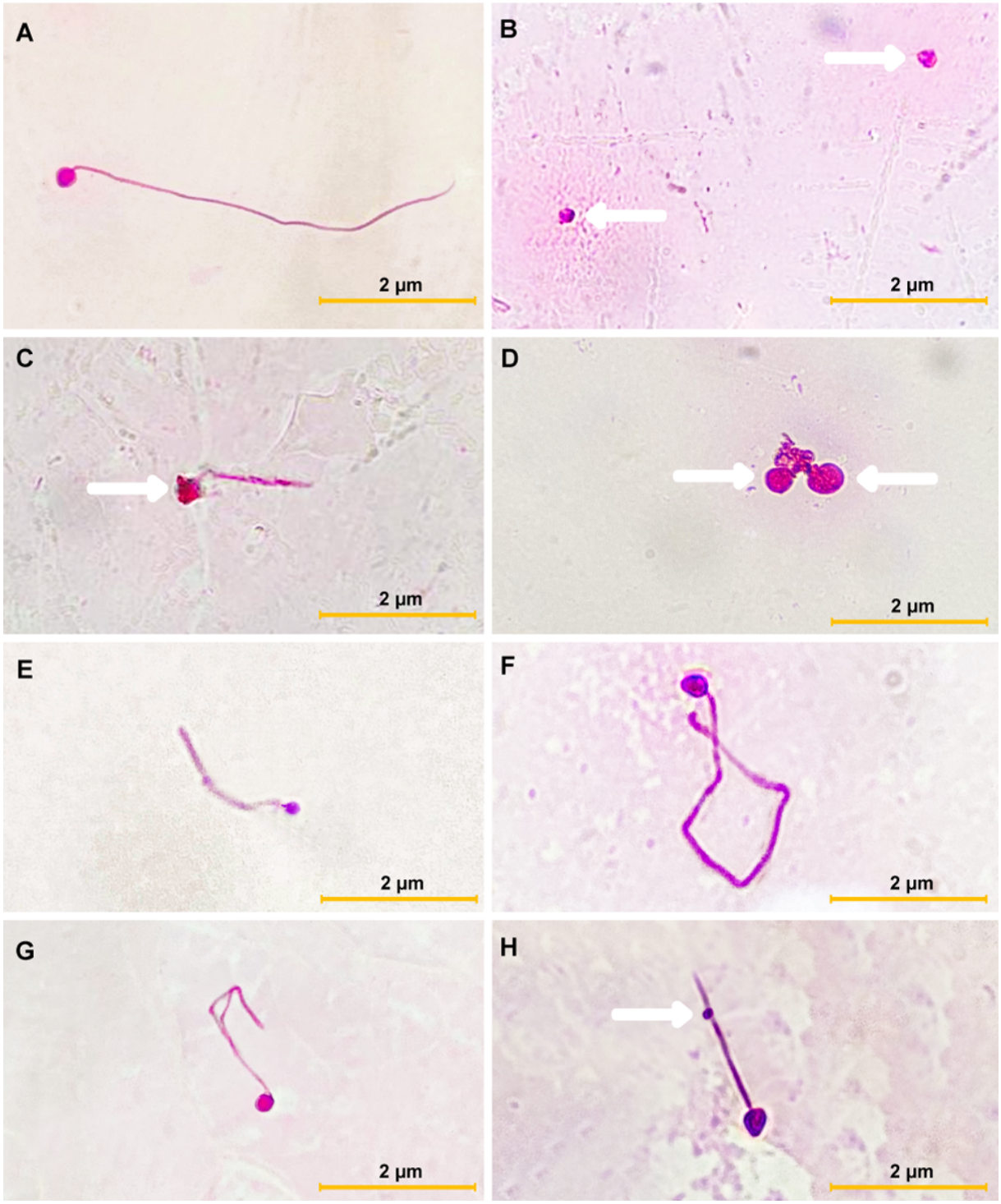



**Fig. 3** Photomicrographs of *Mussismilia braziliensis*' spermatozoa: **a)** normal spermatozoon; **b)** free head; **c)** degenerated head; **d)** macrocephaly; **e)** microcephaly; **f)** broken flagellum **g)** coiled flagellum; **h)** distal gout.

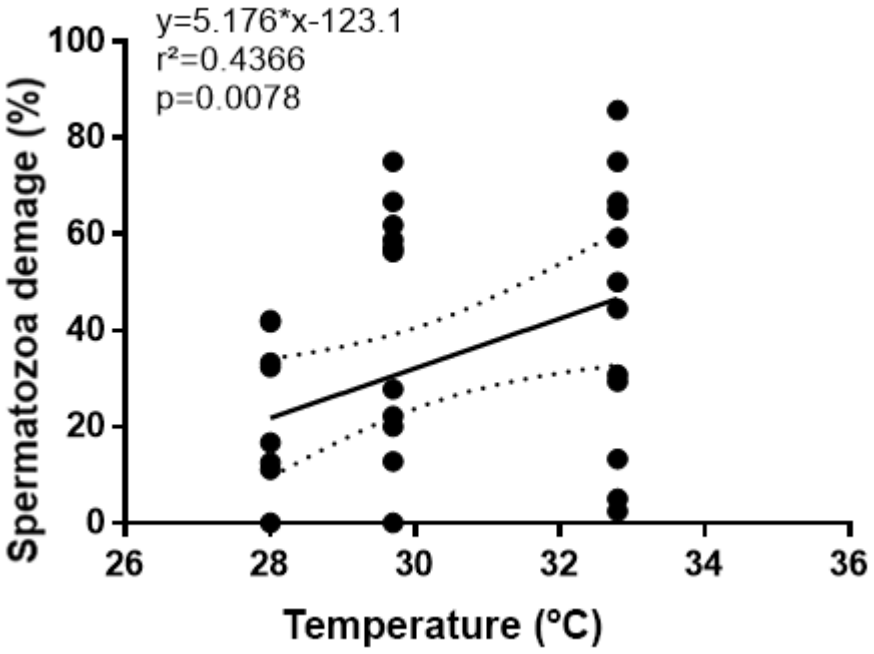



**Fig. 4** The relationship of *Mussismilia braziliensis'* spermatozoa damages with increase on temperature: 28ºC is the temperature of seawater in Recife de Fora; 29.7ºC according to RCP2.6 scenario of IPCC 2014; 32.8ºC according to RCP8.5 scenario of IPCC 2014 The data were submitted to the spearman correlation test.

**Table 1** Sperm morphology of *Mussismilia braziliensis* on February 2020 spawning. This data was accessed at 2.5-, 5- and 7-hours post-spawning and in three different temperatures: 28ºC, temperature of seawater in Recife de Fora; 29.7ºC according to RCP2.6 scenario of IPCC 2014; 32.8ºC according to RCP8.5 scenario of IPCC 2014. Data are presented as mean ± standard deviation. Different lowercase letters represent significant difference between different times on the same temperature. Capital letters represent significant difference between different scenarios of temperature over the time. *P value* ≤ 0.05 indicates statistical significance.

| **Temperature** | **28°C** | | | **29.7°C** | | | **32.8°C** | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time (h)** | **2.5** | **5** | **7** | **2.5** | **5** | **7** | **2.5** | **5** | **7** | ***P value*** |
| **Normal** | 27.2 ± 24 | 21.7 ± 9.7 | 7.5 ± 12 | 23.6 ± 22 | 6.4 ± 8.8 | 16.2 ± 14 | 12 ± 19 | 15.4 ± 11.5 | 13 ± 21.5 | P=0.5617 |
| **Free head** | 7.6 ± 7.6$^{b}$ | 17.2 ± 14.8$^{ab}$ | 51.9 ± 33.7$^{a}$ | 17.9 ± 5.7 | 12 ± 12.1 | 42.7 ± 17.1 | 28.7 ± 13.9 | 22.7 ± 15.7 | 27.4 ± 24.9 | *P=0.0246* |
| **Degenerate head** | 9.3 ± 12.5 | 20.1 ± 21.9 | 8.7 ± 10.8 | 8.9 ± 3.9 | 8 ± 11.8 | 11 ± 15.6 | 5.5 ± 11.1 | 7.5 ± 15 | 15.8 ± 17.1 | P=0.9536 |
| **Macrocephaly** | 26.1 ± 34 | 2.7 ± 5.5 | 0 ± 0 | 1.3 ± 2.7 | 1.5 ± 3.1 | 0 ± 0 | 0 ± 0 | 4.1 ± 8.3 | 3.7 ± 7.5 | P=0.4565 |
| **Microcephaly** | 7.6 ± 7.4 | 3.3 ± 4.1 | 18.7 ± 23.9 | 7.7 ± 9.6 | 23 ± 32.8 | 1.4 ± 2.9 | 5.6 ± 3.7 | 6.3 ± 8.3 | 0 ± 0 | P=0.7112 |
| **Broken flagellum** | 9.5 ± 19.1$^{B}$ | 10.9 ± 10.3 | 6.8 ± 13.7$^{B}$ | 18.4 ± 14$^{AB}$ | 22.4 ± 22.1 | 27.2 ± 21.9$^{A}$ | 25.2 ± 20.1$^{A}$ | 19.6 ± 16 | 27.4 ± 39.3$^{A}$ | *P=0.0010* |
| **Coiled flagellum** | 12.2 ± 15.5 | 21 ± 7.2 | 6.2 ± 7.2 | 20.6 ± 18$^{a}$ | 24.6 ± 20.9$^{a}$ | 1.1 ± 2.3$^{b}$ | 22.7 ± 11.3 | 24.1 ± 0.99 | 12.5 ± 25 | *P=0.0163* |
| **Distal droplet** | 0 ± 0 | 2.6 ± 0 | 0 ± 0 | 1.1 ± 2.3 | 1.7 ± 3.5 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | P=0.5330 |

## CONCLUSÃO

Essa foi a primeira vez que o impacto do aumento da temperatura do oceano na reprodução do coral-cérebro-da-bahia (*Mussismilia braziliensis*) é avaliado. O estudo trouxe dados sobre o impacto gerado pelo maior evento de branqueamento já registrado para o Atlântico Sul. Apesar da espécie ter se demonstrado altamente resiliente, sendo uma das primeiras espécies a demonstrar completa recuperação do branqueamento (visualmente), a sua reprodução foi altamente comprometida em 2020. O possível motivo do comprometimento com a reprodução está relacionado ao fato das colônias de *M. braziliensis* terem utilizado seus estoques energéticos e a energia proveniente da alimentação heterotrófica para investir em recuperação do branqueamento e manutenção das colônias existentes vivas, negligenciando assim a demanda de energia para a reprodução. Além disso foram observados impactos negativos na motilidade e morfologia dos espermatozoides de *Mussismilia braziliensis* que tiveram contato direto com a água do mar em altas temperaturas conforme as projeções climáticas mais branda (RCP2.6) e mais severa (RCP8.5) do IPCC 2014. Dessa forma, não apenas o branqueamento impacta na reprodução da espécie, como também a exposição direta dos gametas às altas temperaturas. Ambas as situações afetam diretamente o sucesso da fertilização e comprometem a perpetuação e manutenção da espécie. Com os frequentes alertas de eventos de branqueamento e se a temperatura do oceano continuar aumentando conforme as projeções estimadas, o comprometimento da reprodução sexuada (responsável pela variabilidade genética) da espécie *Mussismilia braziliensis* pode tornar-se irreversível. Consequentemente a construção e manutenção dos recifes baianos, onde *M. braziliensis* é uma das principais espécies construtora de recifes, poderão entrar em colapso e acarretar na perda de um recife inteiro, levando junto a sua biodiversidade local.