Programa de Pós-Graduação em Biodiversidade e Evolução
Universidade Federal da Bahia

Ciclo reprodutivo da esponja *Amphimedon viridis* (Demospongiae, Haplosclerida) em uma região entre-marés do Atlântico tropical

Beatriz Santos de Brito

Salvador
2025

Beatriz Santos de Brito

Ciclo reprodutivo da esponja *Amphimedon viridis* (Demospongiae, Haplosclerida) em uma região entre-marés do Atlântico tropical

Dissertação apresentada ao Instituto de Biologia da Universidade Federal da Bahia para a obtenção do Título de Mestre em Biodiversidade e Evolução pelo Programa de Pós-Graduação em Biodiversidade e Evolução.

Orientador: Emilio de Lanna Neto

Salvador

2025

Dados internacionais de catalogação-na-publicação (SIBI/UFBA/Biblioteca Universitária Reitor

Brito, Beatriz Santos de.
Ciclo reprodutivo da esponja Amphimedon viridis (Demospongiae, Haplosclerida) em uma região entre-marés do Atlântico tropical / Beatriz Santos de Brito. - 2025.
95 f.: il.

Orientador: Prof. Dr. Emilio de Lanna Neto.
Dissertação (mestrado) - Universidade Federal da Bahia, Instituto de Biologia, Salvador, 2025.

1. Biologia marinha. 2. Invertebrados marinhos - Brasil, Nordeste. 3. Invertebrados marinhos - Re produção. 4. Esponja - Reprodução. I. Lanna Neto, Emilio. II. Universidade Federal da Bahia. Instituto de Biologia. III. Título.

CDD - 593.40981
CDU - 593.4(81)

Comissão julgadora

Ciclo reprodutivo da esponja *Amphimedon viridis* (Demospongiae, Haplosclerida) em uma região entre-marés do Atlântico tropical

Beatriz Santos de Brito

Orientador: Emilio de Lanna Neto

Dissertação de Mestrado submetida ao Programa de Pós-Graduação em Biodiversidade e Evolução da Universidade Federal da Bahia como parte dos requisitos necessários à obtenção do título de Mestre na área de Biodiversidade e Evolução.

Aprovada por: Em: 20 de fevereiro de 2025.

_____

Prof. Dr.: Wagner Ferreira Magalhães

_____

Prof. Dr.: Sérgio Nascimento Stampar

_____

Prof. Dr.: Emilio de Lanna Neto

Orientador

A minha família por todo apoio, incentivo e amor.

Dedico

O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínimo fará coisas admiráveis.

José de Alencar

Agradecimentos

Começo este agradecimento refletindo sobre esses dois anos de mestrado. Nessa caminhada, vivi muitas experiências novas e importantes ao lado de pessoas especiais. Mas, sobretudo, tive o apoio incondicional da minha família, mesmo com a distância. Gostaria de agradecer aos meus pais, Antonio e Zenilda, por todo o cuidado e preocupação. Aos meus irmãos, Priscila e Cleiton, por todo amor. E a Diones por toda a parceria nesses anos. O apoio de vocês sempre significou muito para mim. Vocês são minha base, e tudo o que faço é pensando em nós. Amo muito vocês.

Agradeço ao meu orientador, Emilio Lanna, por todos os ensinamentos, incentivos e correções. Emilio me apresentou às esponjas e me direcionou durante todo esse período. Além disso, acreditou em mim quando eu mesma não acreditava e me incentivou inúmeras vezes quando eu estava desanimada. Lembro-me de que, no início do mestrado, a parte de bancada estava dando tudo errado. Eu não tinha nenhuma experiência, e aquilo me causava muitas inseguranças, até que um dia as lâminas deram certo. Empolgada, mostrei para ele, e ele disse: "Aê, Bia, as esponjas já gostam de você" (rsrs). Naquele dia, me senti abraçada e tive a certeza de que as coisas dariam certo – e deram. Desde então, Emilio nunca hesitou em me ajudar, ensinar, tirar dúvidas e até mesmo desenhar quando eu não estava entendendo algo (kkk). Obrigada por tanto, Emilio. Hoje, olho para trás, reflito sobre tudo o que aprendi e devo muito a você.

Não poderia deixar de agradecer aos colegas de apartamento (Léo, Adrian e Ray), que, de forma individual, foram muito importantes nesse meu período em Salvador. Foram com eles que dividi algumas angústias, mas, sobretudo, alegrias. Inclusive as noites infinitas de jogos de War, que eu e Adrian sempre queríamos parar no meio do caminho (kkkkk). Também as noites de filmes antigos e ruins, mas muito bons. Ah, e as partidas de baralho, que eu não sabia jogar, mas a competitividade nos rendia boas risadas. Em especial, gostaria de agradecer a Adrian, que, nos últimos tempos, foi com quem mais dividi minha vida e pude mostrar minhas fraquezas.

Agradeço aos colegas de laboratório por todas as risadas, conversas e contribuições. Em especial as comemorações de aniversário, que sempre significaram muito para mim. Estar longe de casa era difícil, mas vocês sempre tornavam esse dia mais leve. Também agradeço pelos perrengues e bons momentos que vivemos, principalmente nas coletas e nos congressos. Em especial, agradeço à Ju, com quem criei uma conexão muito bonita e

genuína. E um carinho especial pelas professoras e pelos colegas da pós, com quem vivi momentos incríveis.

Agradeço à FAPESB pela bolsa, que foi fundamental para minha manutenção em Salvador.

Por último, mas não menos importante, agradeço a Deus por sua bondade e misericórdia. Foi nele que me apoiei nos momentos sensíveis e felizes, e foi nele que encontrei forças para me reerguer e continuar.

Índice

Resumo 01

Abstract 02

Introdução Geral 03

Filo Porifera Grant, 1836 03

Classe Demospongiae e ordem Haplosclerida 06

Reprodução na classe Demospongiae 07

Reprodução sexuada na ordem Haplosclerida 10

Amphimedon viridis *Duchassaing & Michelotti, 1864* 11

Influência do ambiente na reprodução das esponjas 13

Objetivos 15

Capítulo Único. Reproductive cycle of the sponge *Amphimedon viridis* (Demospongiae, Haplosclerida) in an intertidal region of the tropical Atlantic 16

Abstract 17

1 Introduction 18

2 Material and methods 21

2.1.*Study location* 21

2.2.*Reproduction data acquisition* 22

2.3.*Acquisition of environmental data* 23

2.4.*Data analysis* 24

3 Results 25

3.1.*Reproductive elements* 27

3.2.*Reproductive Dynamics* 29

3.3.*Dynamics of the environmental factors* 33

3.4.*Influence of the environmental factors on the reproduction* 35

4 Discussion 39

4.1.*Reproductive cycle of* Amphimedon viridis 39

4.2.*Influence of exogenous factors on the reproduction and surface area of* Amphimedon viridis 45

Conclusions 49

Acknowledgements 51

References 52

Conclusão 67

Referências 68

Anexos 74

Resumo

Este estudo analisou o ciclo reprodutivo de *Amphimedon viridis* em uma região entre-marés do Atlântico tropical entre abril de 2023 a agosto de 2024. Também investigamos como os fatores ambientais poderiam explicar a dinâmica reprodutiva da espécie. A reprodução foi contínua, com desenvolvimento assíncrono dos elementos reprodutivos. A espécie apresentou baixo esforço reprodutivo, com apenas uma pequena porcentagem da população envolvida na reprodução ao longo do estudo. Observamos que *A. viridis* é vivípara, e durante os 17 meses de pesquisa, foi registrada apenas uma larva (parenquimela). Durante o período analisado, foram identificados ovócitos em 14 meses, cistos espermáticos em 5 meses e embriões em 10 meses. Embora os ovócitos tenham sido mais frequentes, sua densidade foi menor (0,01 ± 0,01 oócitos.mm^{-2}) em comparação aos cistos espermáticos (0,51 ± 1,14 cistos espermáticos.mm^{-2}). Não encontramos diferença significativa entre os meses do estudo para o tamanho dos ovócitos e cistos espermáticos; no entanto, os embriões foram maiores durante os meses da estação seca. Entre os fatores ambientais analisados, a temperatura do ar (mês atual e com um mês de atraso), marés baixas (mês atual), clorofila-a e a precipitação (mês atual e com um a dois meses de atraso) influenciaram significativamente a reprodução de *A. viridis*, afetando principalmente a densidade de ovócitos. O tamanho médio dos indivíduos da população foi de 195,64 ± 230,60 cm², mostrando variações significativas entre os meses, mas sem diferença de tamanho entre indivíduos reprodutivos e não reprodutivos. Além disso, tanto a temperatura do ar quanto a temperatura da água do mar impactaram negativamente a área superficial da espécie, especialmente durante os períodos com marés baixas, quando os indivíduos eram maiores. Esses resultados indicam que fatores climáticos e oceanográficos desempenham um papel fundamental no ciclo reprodutivo de *A. viridis*, influenciando tanto o esforço reprodutivo quanto o desenvolvimento morfológico.

Palavras-chave: Gametogênese, Invertebrados, Porifera

Abstract

This study analyzed the reproductive cycle of *Amphimedon viridis* in an intertidal region of the tropical Atlantic between April 2023 and August 2024. We also investigated how the environmental factors could explain the reproductive dynamics of the species. Reproduction was continuous, with asynchronous development of reproductive elements. The species exhibited low reproductive effort, with only a small percentage of the population involved in reproduction throughout the study. It was observed that *A. viridis* is viviparous, and over the 17 months of research, only one larva (parenchymella) was recorded. During the analyzed period, oocytes were identified in 14 months, spermatic cysts in 5 months, and embryos in 10 months. Although oocytes were more frequent, their density was lower (0.01 ± 0.01 oocytes.mm^{-2}) compared to spermatic cysts (0.51 ± 1.14 spermatic cysts.mm^{-2}). No significant difference was found among the months of the study for the size of oocytes and spermatic cysts; however, embryos were larger during the dry season months. Among the environmental factors analyzed, air temperature (current month and one-month lag), low tide (current month), chlorophyll-a, and precipitation (current month and with one to two-month lags) significantly influenced the reproduction of *A. viridis*, mainly affecting oocyte density. The average individual size of the population was 195.64 ± 230.60 cm², showing significant variations among months but no difference of size between reproductive and non-reproductive individuals. Additionally, both air temperature and seawater temperature negatively impacted the surface area of the species, especially during periods with lower tides when individuals were larger. These results indicate that climatic and oceanographic factors play a fundamental role in the reproductive cycle of *A. viridis*, influencing both reproductive effort and morphological development.

Keywords: Gametogenesis, Invertebrates, Porifera

Introdução Geral

Filo Porifera Grant, 1836

O filo Porifera Grant, 1836, representa o filo mais antigo ainda existente e desempenha um papel fundamental na compreensão da evolução dos animais, por ser considerado um possível grupo irmão dos metazoários (Redmond & McLysaght, 2023). Com base em registros fósseis e em análises de relógio molecular, sabe-se que as esponjas existem há aproximadamente 700 milhões de anos, e vêm se diversificando desde então (Moraes, 2011; Gold et al. 2016; Dohrmann & Wörheide, 2017; Turner, 2021). Atualmente, o filo Porifera possui cerca de 9.721 espécies descritas, distribuídas em quatro classes monofiléticas (De Voogd et al. 2024) (Fig. 1). A classe Demospongiae Sollas, 1885, é a mais diversa do filo, representando cerca de 81% de suas espécies, com aproximadamente 8.050 espécies descritas (Lavrov et al. 2023; De Voogd et al. 2024). Essas esponjas possuem um esqueleto mineralizado sustentado por fibras de espongina e apresentam diferentes formas reprodutivas. A classe Hexactinellida Schmidt, 1879, conhecida popularmente como esponjas de vidro, é comumente encontrada em águas profundas e apresenta um esqueleto composto por espículas silicosas, contando com aproximadamente 708 espécies descritas (Henkel, Borkenhagen & Janussen, 2015; De Voogd et al. 2024). A classe Homoscleromorpha Bergquist, 1978 possui cerca de 136 espécies, geralmente pequenas, habitando ambientes crípticos, com um esqueleto formado por espículas silicosas ou ausentes. Além disso, apresenta uma membrana basal formada por colágeno tipo IV e junções celulares especializadas entre coanócitos e pinacócitos (Domingos, Lage & Muricy, 2016; Lage et al. 2019; De Voogd et al. 2024). Por fim, a classe Calcarea Bowerbank, 1862 é caracterizada por um esqueleto composto de carbonato de cálcio ($CaCO_3$) e é predominantemente encontrada em águas rasas, apresentando atualmente cerca de 827 espécies descritas (Wörheide et al. 2012; De Voogd et al. 2024).

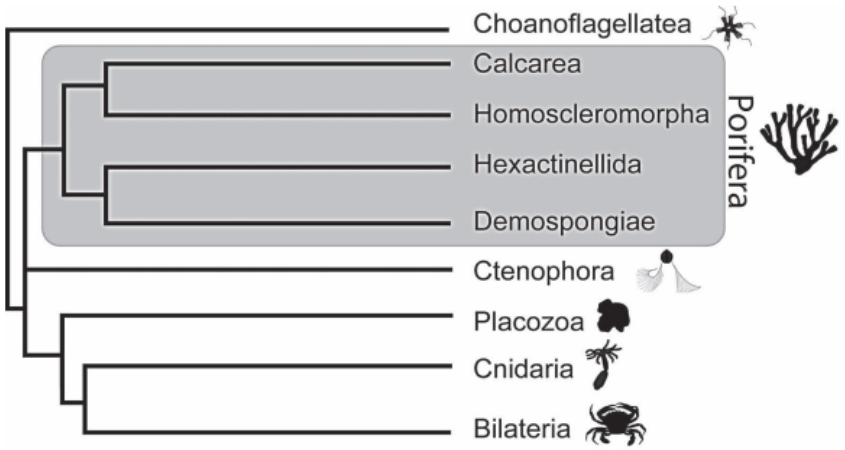

Figura 1 - Relação filogenética das quatro classes de Porifera e outros metazoários. (Fonte: Lanna et al. 2024).

As esponjas são organismos multicelulares, exclusivamente aquáticos, sésseis e bentônicos. Seu tamanho varia de milímetros a metros de diâmetro e possuem uma enorme variedade de formas. Quando adultos apresentam hábito filtrador, capazes de reter até 80% das partículas em suspensão, alimentando-se principalmente de matéria orgânica particulada e fitoplâncton (Stabili et al. 2006; Hadas, Shpigel & Ilan, 2009; De Goeij et al. 2013). São organismos principalmente marinhos, embora também sejam encontrados em água doce. No ambiente marinho são encontrados desde regiões entre-marés até águas profundas. Sua distribuição geográfica abrange zonas tropicais, temperadas e polares, evidenciando sua capacidade de adaptação a diferentes condições ambientais (Varijakzhan et al. 2021).

Por serem organismos sésseis produzem metabólitos secundários como forma de defesa contra predadores (Kelman et al. 2001; Perdicaris, Vlachogianni & Valavanidis, 2013). Esses compostos bioativos apresentam um grande interesse econômico, por serem farmacologicamente importantes. Os metabólitos permitem que as esponjas possam ser utilizadas em uma grande variedade de atividades para a saúde humana, principalmente por apresentarem propriedades anti-inflamatórias, antitumorais, antifúngicas, antivirais e antibacterianas (Varijakzhan et al. 2021).

A organização corporal das esponjas é considerada simples e é composta por três camadas principais: a pinacoderme, que atua como revestimento externo; o mesoílo, uma matriz extracelular contendo células móveis e os elementos esqueléticos (fibras de espongina e espículas); e a coanoderme, onde se localizam os coanócitos. Essa estrutura é organizada em torno de um sistema aquífero que é exclusivo do filo, formado por canais inalantes e exalantes e câmaras coanocitárias com o mesoílo sendo suporte deste sistema (Fig. 2, Carrier et al. 2022). Atualmente, são classificados seis tipos de sistemas aquíferos no filo Porifera, o asconoide, siconoide, sileibde, solenoide, leuconoide e cladonoide (Fig. 3) (Cavalcanti &

Klautau, 2011; Lopes & Klautau, 2023). Em esponjas com sistema aquífero leuconoide, a água do meio externo passa pelos poros dérmicos (óstios), canais inalantes, prosópila, câmaras coanocitárias, apópila, canais exalantes, átrio e finalmente o ósculo (Fig. 2, Carrier et al. 2022). Por meio do sistema aquífero, as esponjas realizam processos essenciais, como alimentação, trocas gasosas e a absorção ou liberação de gametas.

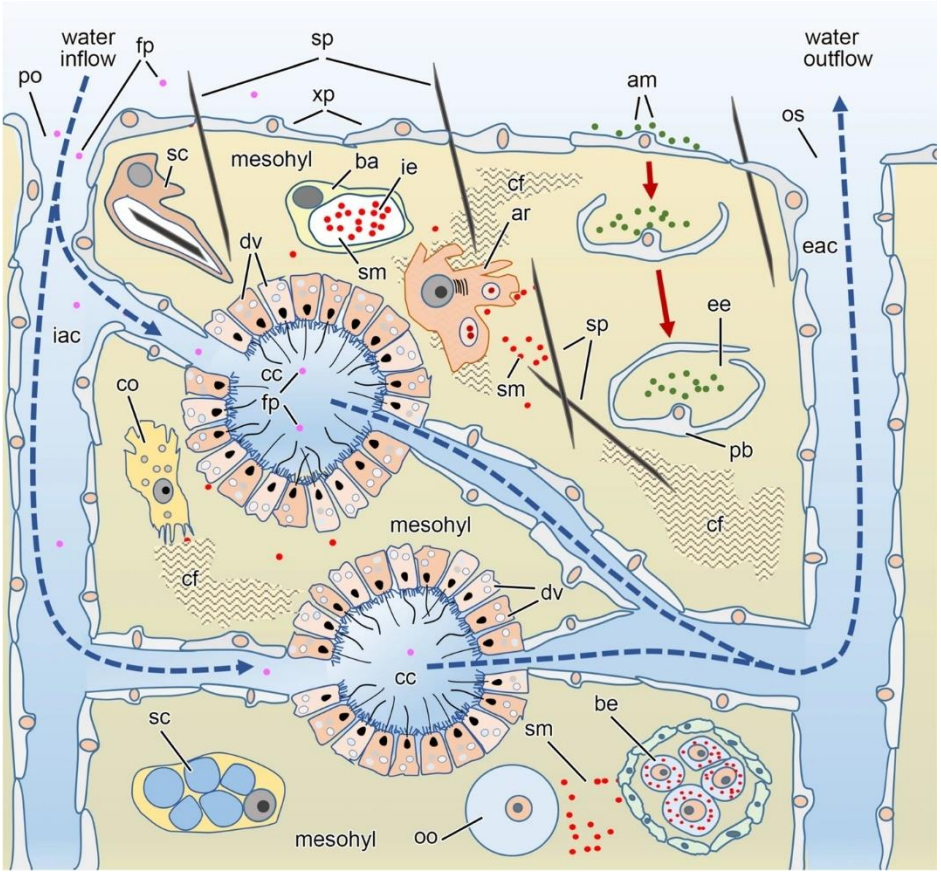

Figura 2 - Ilustração da morfologia interna do corpo das esponjas da classe Demospongiae. Canais aquíferos exalantes (eac); canais aquíferos inalantes (iac); câmaras coanocitárias (cc); célula deixa o epitélio para entrar no mesoílo (setas vermelhas); colêncitos (co); embriões incubados (be); esclerócitos (sc); espículas (sp); exopinacócitos (xp); fibrilas de colágeno (cf); fluxo da água (setas azuis); micróbios de vida livre (am); micróbios simbióticos de vida livre (sm); ósculo (os); ovócitos (oo); partículas em suspensão (fp); poros (po); vesículas de digestão (dv); ambiente extracelular (ee); ambiente intracelular (ie); bacteriócitos (ba); bacteriócito de bolso (pb). (Fonte: Carrier et al. 2022).

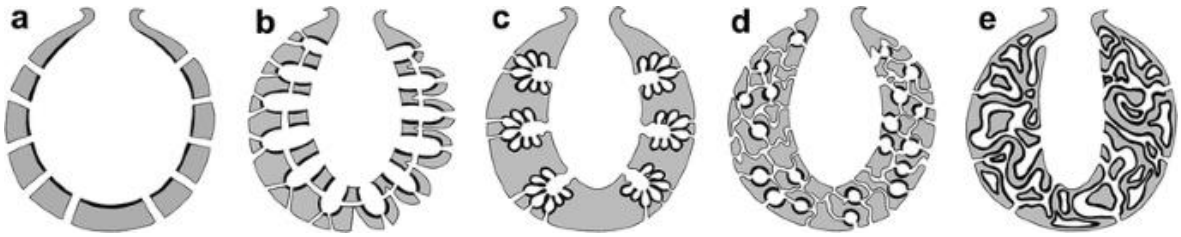

Figura 3 - Diferentes tipos de sistemas aquíferos encontrados em Porifera. (a) asconoide. (b) siconoide. (c) sileibde. (d) leuconoide. (e) solenoide. (Fonte: Cavalcanti & Klautau, 2011).

Classe Demospongiae e ordem Haplosclerida

A classe Demospongiae e suas subclasses são reconhecidas como monofiléticas (Morrow & Cárdenas, 2015). As esponjas desta classe possuem um esqueleto orgânico composto por fibras de espongina e/ou espículas silicosas, que podem ser classificadas como megascleras e microscleras. Esses organismos não formam estruturas sinciciais, apresentando, portanto, elementos celulares discretos (Hooper & Van Soest, 2002). O sistema aquífero é predominantemente do tipo leuconoide, caracterizado pelo aumento do número de câmaras coanocitárias, redução do tamanho dessas câmaras no mesoílo e pelo desenvolvimento de canais inalantes e exalantes (Ereskovsky, 2010; Cavalcanti & Klautau, 2011) (Figs. 2, 3D). Atualmente, a classe conta com 22 ordens, incluindo a ordem Haplosclerida Topsent, 1928.

A ordem Haplosclerida distingue-se pela presença de um esqueleto ectossomal e coanossomal, bem como um esqueleto isodictial formado por megascleras (Fig. 4A-B, Hajdu et al. 2011). Seus organismos possuem espículas óxeas, de forma pontiaguda e fusiforme, facilmente diferenciadas das espículas de outras ordens da classe Demospongiae (Fig. 4C, Hajdu et al. 2011). Esta ordem abriga uma grande diversidade de esponjas marinhas, que podem atingir tamanhos significativos (Van Soest & Hooper, 2002). Os indivíduos podem apresentar variadas formas corporais, como incrustantes, tubulares, maciças, arborescentes, lobadas ou escavadoras, e sua consistência pode variar entre macia, dura ou quebradiça (Van Soest & Hooper, 2002). Esta é uma ordem bastante diversa, mas a maioria das famílias pertencentes à ordem Haplosclerida não são consideradas monofiléticas (Morrow & Cárdenas, 2015).

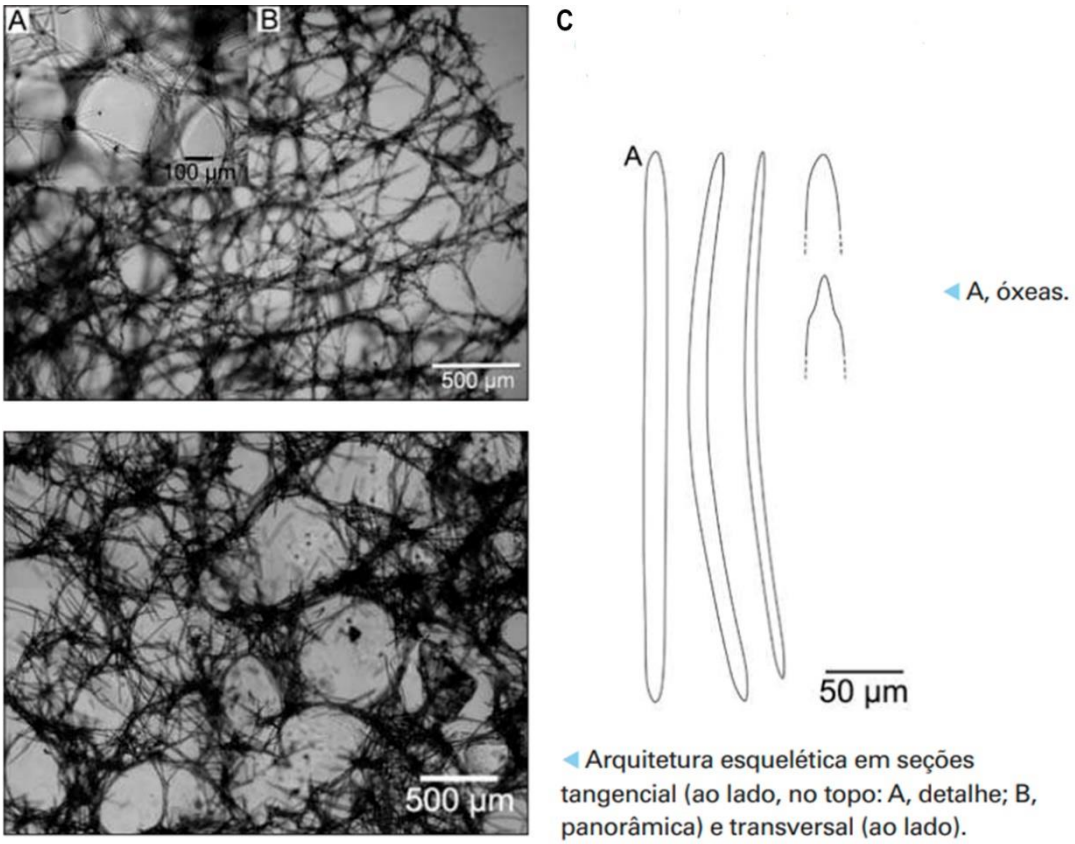

Figura 4 - Estrutura esquelética da *Xestospongia muta* (Schmidt, 1870). (A-B) Esqueleto ectossomal. (C) Espicula óxeas. (Fonte: Hajdu et al. 2011).

Reprodução na classe Demospongiae

As esponjas da classe Demospongiae se reproduzem tanto de forma assexuada quanto sexuada. A reprodução assexuada acontece por três mecanismos: brotamento, gemulação e fragmentação (Ereskovsky, 2010). A reprodução assexuada por brotamento ocorre através da formação de um broto na parede do corpo da esponja, que se desprende e dá origem a um novo indivíduo. Na reprodução por gemulação, há a produção interna de gêmulas, por meio de células totipotentes no mesoílo. Essas gêmulas *a posteriori* são liberadas para o meio externo sendo transportadas pelo vento e/ou por animais. Na reprodução por fragmentação, há a regeneração de um fragmento que se rompeu da esponja parental (Maldonado & Riesgo, 2009; Ereskovsky, 2010).

A reprodução sexuada, por sua vez, envolve células pluripotentes, como coanócitos e arqueócitos, que se diferenciam em gametas (5A) (Zarrouk et al. 2013; Funayama, 2018; Brusca et al. 2018). A reprodução sexuada é caracterizada pela gametogênese e embriogênese. Na espermatogênese, os espermatozoides são formados em cistos espermáticos, que se originam a partir de coanócitos presentes nas câmaras coanocitárias (5B, Gaino, Frine & Giuseppe, 2010). Quando os cistos espermáticos atingem a maturidade,

eles migram para regiões próximas aos canais exalantes, permitindo a liberação dos espermatozoides na água circundante (Ereskovsky, 2010). Os ovócitos, por sua vez, desenvolvem-se a partir de arqueócitos e passam pelo processo de vitelogênese, durante o qual acumulam vitelo para sustentar o embrião em desenvolvimento (5C, Gaino, Frine & Giuseppe, 2010). Uma característica notável dos ovócitos é sua capacidade de fagocitar células nutridoras, que fornecem energia e nutrientes adicionais (5D) (Gaino, Frine & Giuseppe, 2010; Maldonado & Riesgo, 2009; Ereskovsky, 2010).

Após a maturação dos gametas masculinos e femininos, há a fecundação, que pode ser interna, dentro do corpo da esponja (viviparidade), ou externa, na coluna d'água (oviparidade), dependendo da espécie (Pechenik, 2016; Brusca et al. 2018). Na classe Demospongiae, a viviparidade foi favorecida como a condição ancestral e a oviparidade adquirida de forma independente (Riesgo et al. 2014). Além disso, os indivíduos podem ser hermafroditas, produzindo ambos os tipos de gametas, ou gonocóricos, produzindo apenas um tipo de gameta (Fromont & Bergquist, 1994; Maldonado & Riesgo, 2009; Brusca et al. 2018). Após a fecundação, inicia-se a embriogênese (5E, Gaino, Frine & Giuseppe, 2010), marcada pelas clivagens do zigoto, que podem ser iguais ou desiguais. O processo culmina na formação de uma larva livre-natante, que é liberada pelo ósculo ou, em alguns casos, rompe o tecido da esponja para ser liberada. A larva fixa-se no substrato e desenvolve-se até alcançar a fase adulta. Essas larvas são lecitotróficas, utilizando reservas energéticas armazenadas no vitelo, possuem um tempo de vida relativamente curto e nadam intermitentemente em períodos curtos, inicialmente com uma natação mais rápida e tornando-se mais lenta (Maldonado & Bergquist, 2002; Chu & Reiswig, 2014; Lanna & Riesgo 2020). Embora no filo Porifera sejam observados diferentes tipos de larvas (Fig. 6, Ereskovsky, 2010), na classe Demospongiae, a larva parenquimela é predominante (Ereskovsky, 2010).

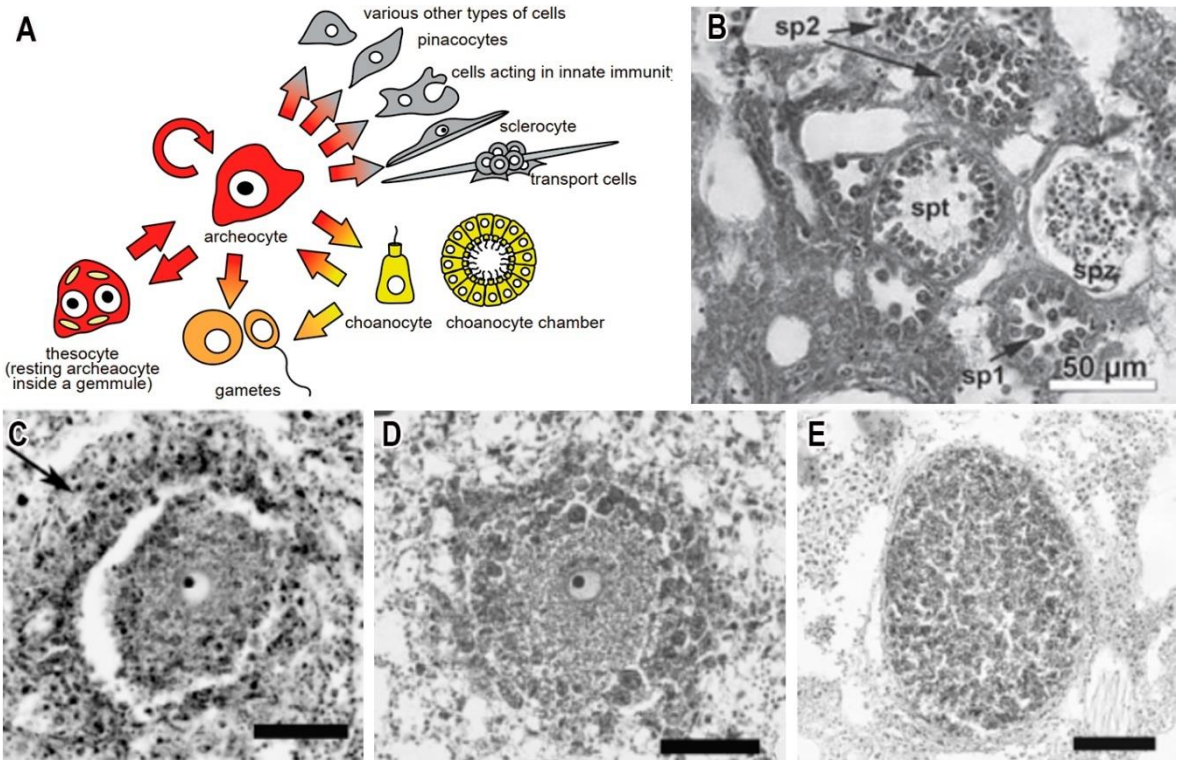

Figura 5 - Esquema de diferenciação celular e cortes histológicos de estruturas reprodutivas. (A) Modelo do sistema de diferenciação celular em Demospongiae. (B) Cistos espermáticos de *Hippospongia communis* (Lamarck, 1814). (C) Ovócito em estágio de vitelogênese rodeado por células nutridoras (barra= 30 µm). (D) Ovócito fagocitando células nutridoras (barra= 50 µm) e (E) embrião da *Hymeniacidon perlevis* (Montagu, 1814) (barra= 50 µm). Cistos espermáticos primários (sp1); Cistos espermáticos secundários (sp2); Espermátides (spt); Espermatozóides (spz). (Gaino, Frine & Giuseppe, 2010; Zarrouk et al. 2013).

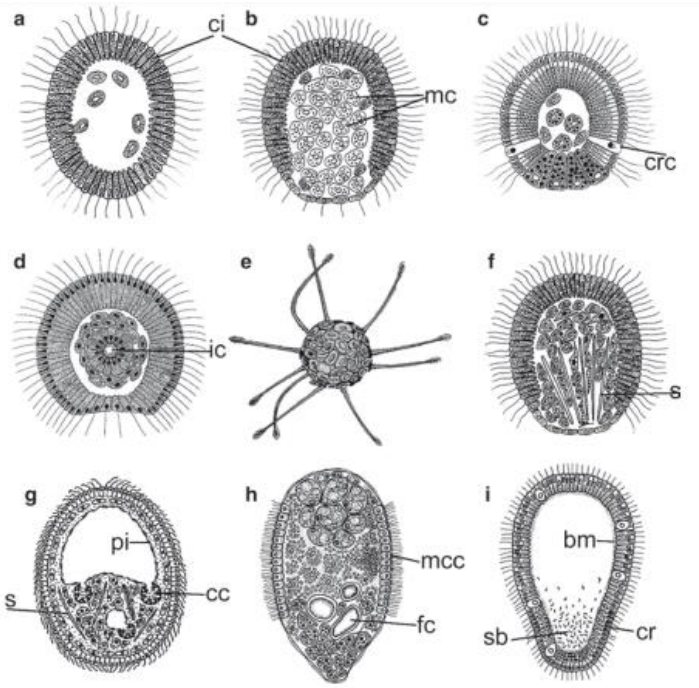

Figura 6 - Diferentes tipos de larvas encontradas no filo Porifera. (a) Calciblastula. (b) Pseudoblastula. (c) Amphiblastula. (d) Disphaerula. (e) Hoplitomella. (f) Parenchymella – Poecilosclerida. (g) Parenchymella – Spongiliida. (h) Trichimella. (i) Cinctoblastula. Bactérias simbióticas (sb); câmara de coanócitos larval (cc); câmara flagelada (fc); câmara interna (ic); células ciliadas (ci); células com cristaloides intranucleares (cr); células em cruz (crc); células maternas (mc); células multiciliadas (mcc); espículas larvais (s); membrana basal (bm); pinacoderme larval (pi). (Fonte: Ereskovsky, 2010).

Reprodução sexuada na ordem Haplosclerida

A viviparidade é característica das famílias da ordem Haplosclerida, embora também sejam encontradas algumas famílias ovíparas (Morrow & Cardenas 2015). As esponjas ovíparas liberam gametas de forma sincronizada com as populações locais, garantindo uma reprodução coordenada. Na ordem Haplosclerida, também são observados indivíduos hermafroditas (simultâneos, sucessivos ou alternantes) e gonocóricos (Sarà, 1993). O processo de gametogênese na ordem assemelha-se ao que é observado e descrito para a classe Demospongiae. A espermatogênese, por exemplo, ocorre nos cistos espermáticos (Fig. 7A, Ilan & Loya, 1990), estruturas localizadas no mesoílo, cuja quantidade e tamanho podem variar dentro de uma mesma população (Ereskovsky, 2010). O desenvolvimento dos espermatócitos dentro de cada cisto é geralmente síncrono; no entanto, diferentes estágios de desenvolvimento podem ser observados entre os cistos de uma mesma espécie. Ao final da espermatogênese, os espermatozoides maduros são liberados na coluna d'água através dos canais exalantes.

Os ovócitos, por sua vez, são originados a partir de arqueócitos e passam pelo processo de vitelogênese (Fig. 7B, Ilan & Loya, 1990). Durante a ovogênese, uma característica marcante é a presença de células nutridoras ao redor dos ovócitos nos estágios iniciais, que posteriormente são fagocitadas, fornecendo nutrição para o gameta (Leys & Degnan, 2002). Especificamente, os ovócitos dessas esponjas são classificados como isolécitos ou oligolécitos (Reiswig, 1976). O desenvolvimento embrionário em Haplosclerida é caracterizado por clivagens totais, desiguais e assíncronas (Fig. 7C, Simpson, 1984; Ereskovsky, 2010). Após as clivagens, forma-se a mórula, que passa por um processo de diferenciação celular, dando origem às estruturas larvais do tipo parenquimela (Fig. 7D, Ilan & Loya, 1990). Quando maduras, essas larvas são liberadas no ambiente (Simpson, 1984; Fromont, 1994).

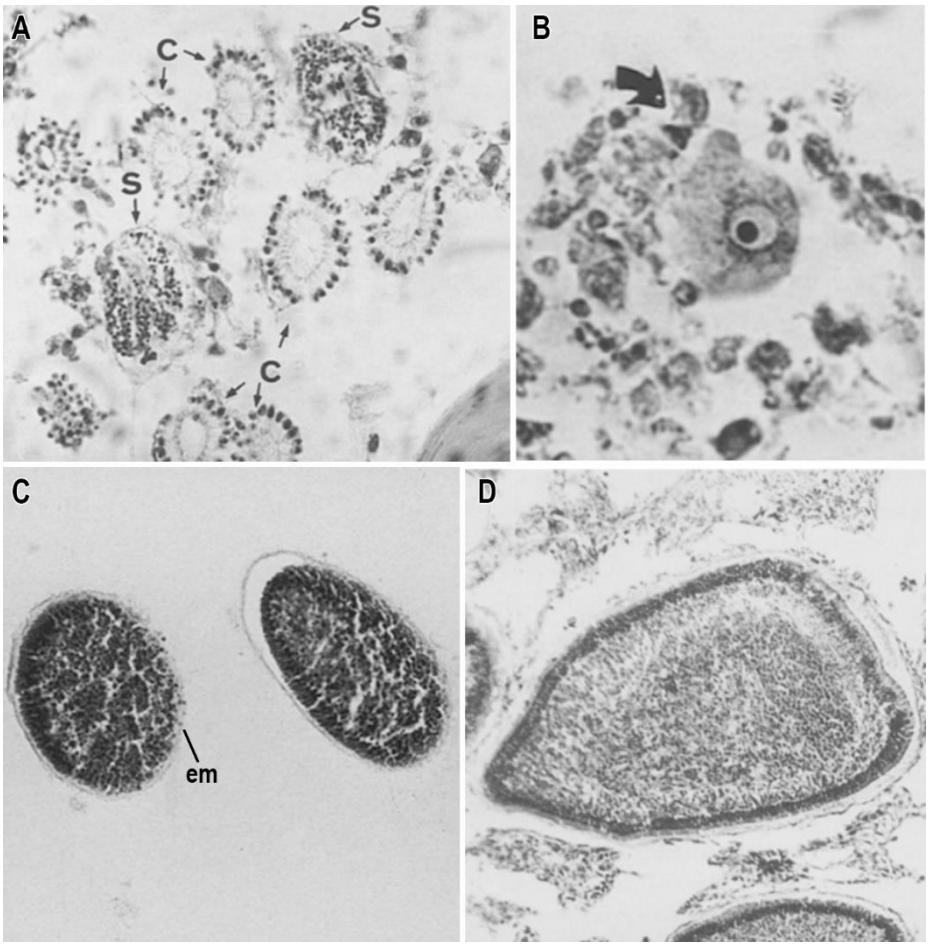

Figura 7 - Microscopia dos elementos reprodutivos da ordem Haplosclerida. (A) Cistos espermáticos e (B) Ovócito em estágio inicial da espécie *Chalinula sp*. (C) Embrião da *Xestospongia bergquistia* Fromont, 1991. (D) larva parenquimela da espécie *Chalinula sp.* Cisto espermático (s); câmaras coanocitárias (c); embrião (em). (Fonte: Ilan & Loya, 1990; Ereskovsky, 2010).

Amphimedon viridis *Duchassaing & Michelotti, 1864*

Amphimedon viridis (Demospongiae, Haplosclerida, Niphatidae) é uma esponja amplamente distribuída geograficamente, ocorrendo desde os Estados Unidos até o Brasil. No território brasileiro, sua presença é registrada em cinco estados: Pernambuco, Alagoas, Bahia, Rio de Janeiro e São Paulo (Wells et al. 1960; Muricy et al. 2011; De Voogd et al. 2024). Em Salvador, *A. viridis* é uma espécie abundante nos afloramentos rochosos, sendo encontrada tanto em regiões entre-marés quanto em áreas mais profundas, atingindo até 15 metros de profundidade. Essa esponja apresenta tamanho corporal considerável, com indivíduos medindo de 8 cm² a 836,4 cm² na região entre-marés de Salvador (BA). *Amphimedon viridis* pode habitar tanto ambientes eutrofizados quanto águas mais limpas, demonstrando alta adaptabilidade. É uma espécie que possui relevância socioeconômica como recurso biotecnológico, destacando-se pela produção de metabólitos secundários bioativos, como a halitoxina e anfitoxina, com toxicidade seletiva contra microrganismos

(Berlinck et al. 1996; Lee, Cho & Tran, 2021).

Amphimedon viridis pertence ao gênero *Amphimedon* Duchassaing & Michelotti, 1864, que apresenta cerca de 50 espécies distribuídas ao redor do mundo. Cinco delas ocorrem no Brasil: *Amphimedon compressa* Duchassaing & Michelotti, 1864; *Amphimedon viridis*; *Amphimedon erina* (de Laubenfels, 1936); *Amphimedon estelae* Santos, Docio & Pinheiro, 2014 e *Amphimedon complanata* (Duchassaing, 1850), sendo que *A. viridis* e *A. estelae* ocorrem no Estado da Bahia. *Amphimedon viridis* possui formato maciço incrustante, ósculos abundantes e distribuídos por todo corpo (Fig. 8A). É uma esponja que possui arquitetura ectossomal e esqueleto coanossomal (Fig. 9A, Hajdu et al. 2011), espículas óxeas (Fig. 9B, Hajdu et al. 2011) e presença de espongina no esqueleto (Hajdu, 2011; Santos, 2016; Cerqueira et al. 2020). Além disso, apresenta sistema aquífero leuconoide, composto por canais e câmaras coanocitárias abundantes e esféricas (Fig. 8B-D).

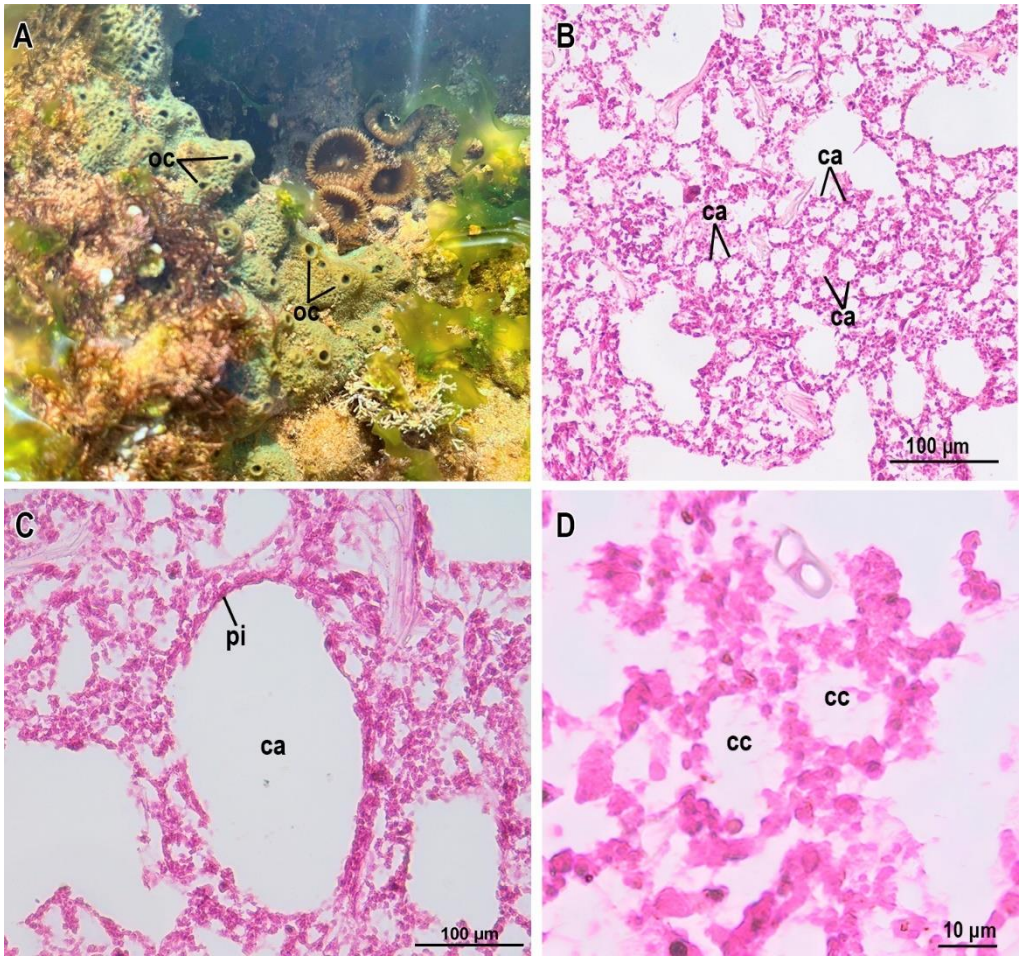

Figura 8 - Anatomia interna e morfologia externa de *Amphimedon viridis*. (A) *Amphimedon viridis* in situ. (B) Imagem histológica do sistema aquífero leuconoide. (C) Detalhe do canal e (D) das câmaras coanocitárias. Canais (ca); câmaras coanocitárias (cc); ósculos (oc); pinacoderme (pi).

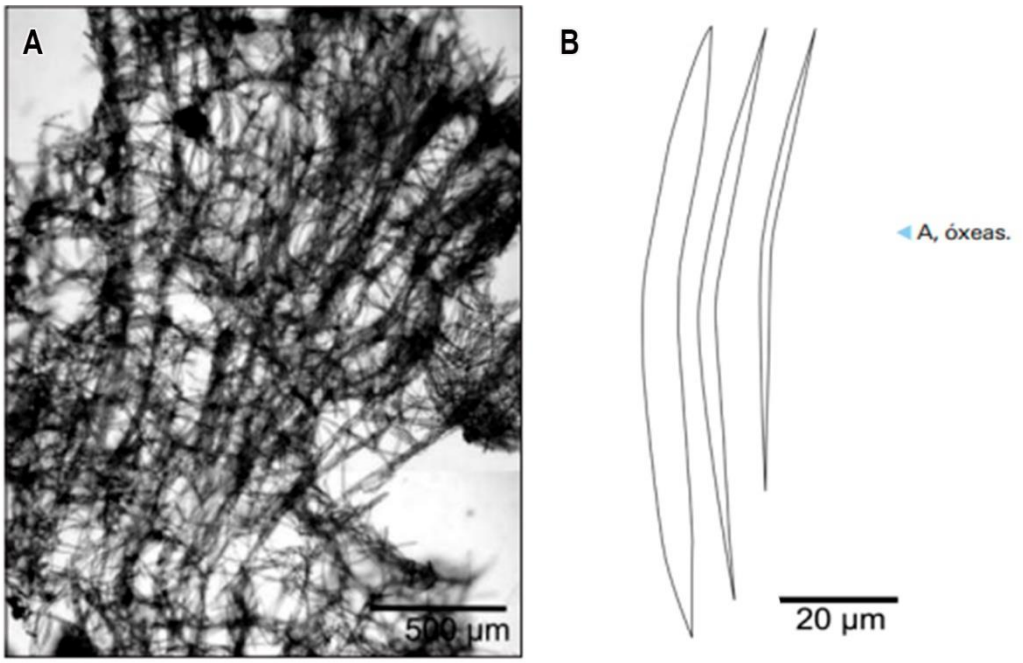

Figura 9 - Estrutura esquelética da *Amphimedon viridis*. (A) arquitetura ectossomal. (B) Espículas óxeas. (Fonte: Hajdu et al. 2011).

Influência do ambiente na reprodução das esponjas

A região entre-marés, localizada na interface entre o ambiente aquático e terrestre, é caracterizada por oscilações diárias causadas pela descida e subida da maré pelo menos uma vez ao dia. Este ambiente representa um lugar desafiador para viver, pois os organismos dessas regiões sofrem com as constantes flutuações nas condições físico-químicas. Os invertebrados marinhos que se adaptaram a esses locais enfrentam estresses cíclicos, sejam eles mecânicos, térmicos ou químicos, e.g.: o hidrodinamismo, aumento da temperatura, dessecamento e variação da salinidade (Leeuwis & Gamperl, 2022). A região entre-marés embora represente uma pequena parte do ambiente marinho, é um dos mais bem estudados (Pereira & Soares, 2009).

Historicamente as esponjas são reconhecidas como animais altamente plásticos (Hill & Hill, 2002) capazes de responder de maneira diversa às variações ambientais, incluindo alterações nos padrões reprodutivos (Schönberg, 2016). Dependendo das condições ambientais que as esponjas se encontram podem apresentar padrões reprodutivos diferentes (contínuo ou sazonais) (Riesgo, 2007; Lanna et al. 2018). Esponjas de regiões tropicais tendem a se reproduzir de forma contínua com um baixo esforço reprodutivo, enquanto espécies de regiões temperadas se reproduzem sazonalmente com alto esforço reprodutivo. Fatores ambientais, principalmente a temperatura, influenciam nessas estratégias. Esponjas da região entre-marés expostas a estresses ambientais cíclicos, por exemplo, apresentam uma

quantidade de larvas reduzida, mas estas são grandes, o que favorece seu rápido assentamento e crescimento da esponja (Ayling, 1980). Além disso, algumas espécies conseguem alternar o modo reprodutivo em sexuado e assexuado, como estratégia para maximizar seu sucesso reprodutivo (Vasconcellos & Lanna, 2022). Nesse sentido, estudar o ciclo reprodutivo de *Amphimedon viridis*, em combinação com a influência do ambiente, é crucial para compreendermos a influência dos fatores ambientais sobre os aspectos reprodutivos da espécie. Considerando que o conhecimento sobre a reprodução das espécies da ordem Haplosclerida ainda é limitado, investigações mais detalhadas como essa são fundamentais para ampliar nossa compreensão sobre a ecologia e a biologia reprodutiva das esponjas.

Objetivos

Objetivo Geral

Investigar o ciclo reprodutivo de *Amphimedon viridis* na praia da Pituba: Salvador, BA.

Objetivos específicos

I. Determinar o período reprodutivo de *Amphimedon viridis;*
II. Investigar a variação temporal no esforço reprodutivo e no tamanho dos elementos reprodutivos de *Amphimedon viridis;*
III. Investigar a influência de fatores endógenos e exógenos no ciclo e no esforço reprodutivo da espécie;
IV. Investigar a influência dos fatores ambientais na área de superfície de *Amphimedon viridis.*

Capítulo único

Título: Reproductive cycle of the sponge *Amphimedon viridis* (Demospongiae, Haplosclerida) in an intertidal region of the tropical Atlantic

Autores: Beatriz Santos de Brito e Emilio Lanna

A ser submetido para: Invertebrate Biology **(ISSN: 1744-7410)**

Abstract

This study analyzed the reproductive cycle of *Amphimedon viridis* in an intertidal region of the tropical Atlantic between April 2023 and August 2024. We also investigated how the environmental factors could explain the reproductive dynamics of the species. Reproduction was continuous, with asynchronous development of reproductive elements. The species exhibited low reproductive effort, with only a small percentage of the population involved in reproduction throughout the study. It was observed that *A. viridis* is viviparous, and over the 17 months of research, only one larva (parenchymella) was recorded. During the analyzed period, oocytes were identified in 14 months, spermatic cysts in 5 months, and embryos in 10 months. Although oocytes were more frequent, their density was lower (0.01 ± 0.01 oocytes.mm^{-2}) compared to spermatic cysts (0.51 ± 1.14 spermatic cysts.mm^{-2}). No significant difference was found among the months of the study for the size of oocytes and spermatic cysts; however, embryos were larger during the dry season months. Among the environmental factors analyzed, air temperature (current month and one-month lag), low tide (current month), chlorophyll-a, and precipitation (current month and with one to two-month lags) significantly influenced the reproduction of *A. viridis*, mainly affecting oocyte density. The average individual size of the population was 195.64 ± 230.60 cm², showing significant variations among months but no difference of size between reproductive and non-reproductive individuals. Additionally, both air temperature and seawater temperature negatively impacted the surface area of the species, especially during periods with lower tides when individuals were larger. These results indicate that climatic and oceanographic factors play a fundamental role in the reproductive cycle of *A. viridis*, influencing both reproductive effort and morphological development.

Keywords: Gametogenesis, Invertebrates, Porifera

1. INTRODUCTION

The influence of the environment on the life cycle of marine invertebrates is well recognized. However, to what extent is the reproductive cycle of sponges (Porifera) shaped by the environmental conditions in which they live? Reproduction, though a fundamental biological activity for the maintenance and perpetuation of species, can be affected by changes in the physicochemical conditions of the environment. Sponges, for instance, are sessile organisms without specialized organs, and they are particularly sensitive to environmental fluctuations (Riesgo & Maldonado, 2008; Lanna et al., 2018). Thus, understanding the physiological responses of sponges to environmental factors, especially in the context of their reproductive cycles, is crucial for estimating how climate change might influence the survival of these organisms. To address this knowledge gap, we selected the sponge *Amphimedon viridis* Duchassaing & Michelloti, 1864 as a model species, given the absence of studies on its reproductive cycle and the potential impacts of climate change. Moreover, this species belongs to the same order as *Amphimedon queenslandica* Hooper & van Soest, 2006, one of the main models for evolutionary developmental biology in Porifera (Maritz et al., 2010).

The sponge *A. viridis* belongs to the class Demospongiae Sollas, 1885 order Haplosclerida Topsent, 1928, with a broad geographic distribution ranging from Florida to São Paulo (Muricy et al., 2011; de Voogd et al., 2024). This species inhabits intertidal zones and depths of up to fifteen meters. It has socioeconomic relevance as a potential biotechnological resource for obtaining pharmacologically active substances (Berlinck et al., 1996). It stands out as a major producer of secondary metabolites (Lee et al., 2021). The selective toxicity of its extracts against microorganisms is attributed to the presence of highly bioactive alkaloids, such as halitoxin and amphitoxin. Due to these metabolites, this species has been the subject of various studies on its antimicrobial activity. However, until now, its

reproductive aspects have been unknown (Kelman et al., 2001). Additionally, *A. viridis* plays a significant ecological role, providing shelter and protection for associated fauna and serving as a food source for other organisms (Huang, 2007; Archer et al., 2020).

Along Brazil's northeastern coast, *A. viridis* is frequently observed in the intertidal zone, a region at the interface between aquatic and terrestrial environments. This zone is characterized by exposure during low tide and submersion during high tide. With the rise and fall of the tides, organisms living in these regions are subjected to cyclic stresses, including mechanical, thermal, and chemical challenges, such as hydrodynamics, temperature increase, desiccation, salinity variation, and air exposure at least once daily (Leeuwis & Gamperl, 2022). Despite representing only a small fraction of the marine environment, the intertidal zone is undoubtedly the most extensively studied of all ecosystems due to its unique location (Pereira & Soares, 2009). The dynamic conditions of this zone, combined with large-scale climate changes such as the El Niño phenomenon, can impact sponge biology (Jones et al., 2010). While *A. viridis* has adaptations that enable its survival in this environment, it was not yet understood how its gametogenesis and embryogenesis respond to the adverse conditions of the intertidal zone. It is known, however, that significant fluctuations in environmental conditions can compromise reproductive success, reduce fecundity, and negatively impact the survival of marine invertebrates (Brazeau & Lasker, 1992).

Sponges, despite lacking gonads, have pluripotent cells such as choanocytes and archaeocytes, which differentiate into gametic cells, enabling sexual reproduction (Funayama, 2018). In the class Demospongiae, various reproductive strategies are observed, with viviparity being predominant, although oviparity is also present in some species. Hermaphroditism and gonochorism are also observed in this class (Fromont & Bergquist, 1994). Documented strategies include viviparity and gonochorism (Fell, 1974; Elvin, 1976;

Fromont, 1994), viviparity and hermaphroditism (Ilan & Loya, 1990; Leys & Degnan, 2002; Ilan et al., 2004), and oviparity and gonochorism (Fromont & Bergquist, 1994; Maldonado & Riesgo, 2009a; Ereskovsky et al., 2017). Specifically, in the genus *Amphimedon* Duchassaing & Michelotti, 1864, individuals are viviparous and hermaphroditic (either simultaneous or sequential), with internal fertilization and the development of parenchymella larvae (Ilan & Loya, 1988, 1990; Maldonado & Riesgo, 2009a; Maritz et al., 2010).

The density, duration, and reproductive period of sponges can be influenced by exogenous factors, related to environmental aspects, and endogenous factors, such as body size (Stearns, 1976; Vasconcellos & Lanna, 2022; Oliveira & Lanna, 2022). Among the exogenous factors, temperature stands out as the main regulator of the sponge reproductive cycle (Riesgo & Maldonado, 2008; Ereskovsky, 2000; Lanna et al., 2018). However, in tropical regions, reproduction is strongly influenced by rainfall and food availability, which play critical roles in the triggering and maintenance of the reproductive cycle (De Goeij et al., 2008; Lanna et al., 2015; 2021). This relationship is associated with the increased food supply, allowing more energy to be allocated to reproduction. Regarding endogenous factors, some sponges exhibit a positive correlation between fecundity and body size, with larger individuals generally producing more gametes. However, reproduction does not depend exclusively on organism size (Fromont & Bergquist, 1994; Maldonado & Riesgo, 2008b; Lanna & Klautau, 2016; Calazans & Lanna, 2019). Interestingly, the opposite can also occur, as the strategy of delaying sexual maturation to invest in growth may compromise the population success of intertidal sponges (Fromont, 1994; Whalan et al., 2007).

Most studies on the reproductive aspects of intertidal sponges have focused on temperate regions (Lanna et al., 2018). However, in tropical areas, factors such as temperature, rainfall, and chlorophyll-a significantly influence the reproductive cycle of sponges (Calazans &

Lanna, 2019; Lanna et al., 2021; Vasconcellos & Lanna, 2022). In this context, we aimed to investigate how these and other environmental factors affect the reproductive cycle of *A. viridis*. To this end, we posed the following questions: How is the reproductive cycle of *A. viridis*? When is the species' reproductive period? Do reproductive effort, reproductive element size, and surface area of *A. viridis* vary between rainy and dry months? How do endogenous and exogenous factors impact the reproductive cycle of *A. viridis*? Based on previous findings, we hypothesize that *A. viridis* reproduces continuously; there is no significant difference in the size or quantity of reproductive elements throughout the study period, given Salvador's relatively stable weather despite its two distinct seasons; but the environmental factors can influence the reproductive effort and specimen size.

2. MATERIAL AND METHODS

2.1 Study location

To investigate the reproductive dynamics of *Amphimedon viridis,* we collected biological material from Pituba Beach (-13.00 S, -38.45 W) in Salvador, Bahia, Brazil (Fig. 1). Pituba Beach features a linear area of approximately 200 meters of rocky outcrop, with tide pools exposed at low tide of the semidiurnal tide regimes of the region. Salvador is a city with stable weather throughout the year, historically, with average temperatures ranging from 22 °C to 31 °C. The region has two recognized seasons: the rainy season (March-August) and the dry season (September-February) (Lessa et al., 2009; Lanna et al., 2018). *Amphimedon viridis* is abundant and commonly found in the study area, found attached to the substrate and covered by a thin layer of sand. In addition, *A. viridis* have a wide distribution, extending from the USA to southern Brazil (Wells et al., 1960; Muricy & Ribeiro, 1999).

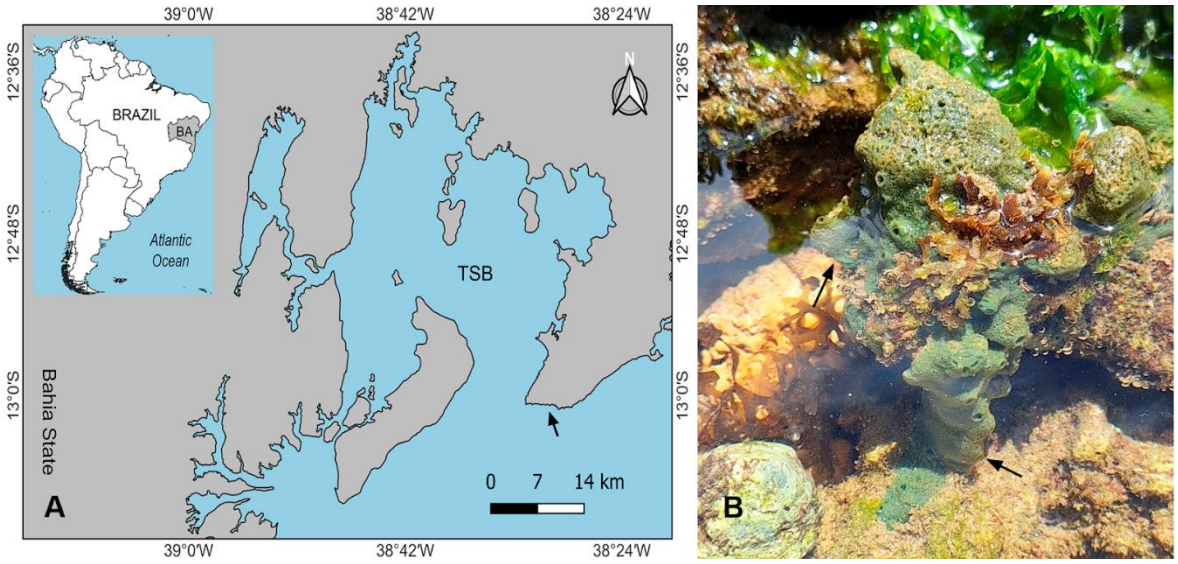

FIGURA 1 Geographical location of the study area (A). Todos os Santos Bay (TSB). Atlantic Ocean. Pituba Beach, Salvador, Bahia *(seta)*, and a photograph of *Amphimedon viridis* in *situ (seta)*, (B).

2.2 Acquisition of Reproduction data

Specimens of *Amphimedon viridis* were collected monthly from April 2023 to August 2024 (17 months) in this intertidal zone during low tides. Each month, a tissue fragment was collected from each of the 10 *A. viridis* specimens (n= 170 sponges collected during the study). Before collecting, we measured the width and length of the specimens with a measuring tape to calculate the sponge's surface area. The surface area of *A. viridis* was calculated using the formula for the area of a rectangle: Area = width × length. With the help of a pair of scissors, a fragment of approximately 1 cm³ was cut from each specimen and fixed in 4% formalin in seawater for 24 hours. After 24 hours, the fragments were removed from the formalin and washed with distilled water. Subsequently, the fragments were placed in 5% hydrofluoric acid for 24 hours for demineralization, then washed with distilled water and dehydrated in a series of ethanol (50%, 70%, 80%, 90%, and 100%). The fragments were then cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned to 5 μm thickness using a rotary microtome. The sections were, then, stained with Harris hematoxylin and eosin (Lanna et al., 2018). For each of the ten samples, two histological slides were prepared to verify the presence of reproductive elements (spermatic cysts,

oocytes, embryos, and larvae) using a light microscope. Additionally, fifteen pictures of each specimen were taken randomly (final magnification of 200x) using the charge-coupled device (CCD) camera of the microscope, totaling approximately 4 mm^2 sponge area for each specimen. With the obtained images, we used ImageJ software to calculate the size and average density (± SD) per mm^2 of the reproductive elements of each specimen (Abramoff et al., 2004). Then, we calculated the average density of the reproductive elements for the population in that month (Lanna & Klautau, 2018; Vasconcellos & Lanna, 2022).

2.3 Acquisition of environmental data

We investigated the effect of eight environmental factors (air temperature, rainfall, seawater surface temperature, salinity, low tide height, chlorophyll-a concentration (phytoplankton), and particulate organic matter) to investigate if they could be influencing on different data of the reproductive cycle of the species (Lanna et al., 2018). The data on air temperature (°C) and rainfall ($mm.month^{-1}$) were obtained through the Brazilian National Institute of Meteorology (INMET, https://portal.inmet.gov.br/, last accessed September 2024); seawater surface temperature (°C) and salinity (ppm) through the Brazilian Coast Monitoring System (SiMCosta, https://simcosta.furg.br/home, last accessed September 2024); low tide height (m) through the Tide Table website (https://tabuademares.com/br/bahia/salvador, last accessed September 2024); and to obtain the daily concentration of chlorophyll-a ($mg.m^3$), which is a proxy indicative of microalgae (Perez et al., 2006), and particulate organic matter ($mg.m^3$), we used satellite images from the SeaWifs website (https://oceancolor.gsfc.nasa.gov/cgi/browse.pl, last accessed September 2024). The images were obtained daily and analyzed using the SeaDAS software (https://seadas.gsfc.nasa.gov/) to obtain the concentration of chlorophyll-a and particulate organic matter in the pixel of the study area. We used the average monthly values of the

environmental factors, except rainfall, which we considered as the total accumulated rainfall for each month. The environmental factors were collected from April 2023 to August 2024.

2.4 Data analysis

The data were tested for normality and homoscedasticity before the statistical tests. The statistical tests were carried out to answer three different questions. 1) Is there variation on the reproduction during the studied period? To answer that, we analyzed the variation in sponge area, density, and size of reproductive elements (spermatic cysts, oocytes, and embryos) among the months of the study applying ANOVA tests to assess differences among the studied months, followed by Tukey's post hoc tests with Bonferroni correction to identify significant pairwise differences. 2) Is the area of reproductive sponges different from those that were not reproducing? This difference between the area of sponges that were reproducing and the area of sponges that were not reproducing was analyzed through a T-test. 3) Could the environmental factors influence the reproduction and surface área of the species? To investigate this issue, we applied Generalized Linear Models (GLM) to verify the effect of environmental factors in the reproduction of *Amphimedon viridis*. The dataset, which includes area, density of reproductive elements, and environmental factors with different scales, was first transformed into a scale using the 'scale' function in R. The delayed effect of environmental factors on the reproduction of *A. viridis* was included in the models (Lanna et al. 2018). The current month was denoted by the number (0) and the delays were denoted by minus signs preceding the number; for instance, rainfall (−1) means a delay of 1 month and rainfall (−2) means a delay of 2 months. The purpose is to understand the effect of environmental factors from previous months on the growth and reproduction of *A. viridis*, as sponges may take time to respond to environmental changes (Riesgo & Maldonado, 2008;

Lanna et al., 2021). After that, we calculated the full model comprising all seven environmental factors and their respective delays (0, 1 and 2 months) for the area of the sponge and for each reproductive element density and size. We used the Gaussian family and the link function to fit the global models for continuous variables. We then used the *dredge* function in the 'MuMIn' library in R (Bartón 2024) to investigate all the possible combinations with the different environmental factors. Based on the multi-model theory, all models that are within the ΔAICc <2 were considered to be as explanatory as the others (Burnham & Anderson, 2002). Consequently, we calculated the averaged model to obtain the mean coefficient and the confidence interval for all the variables included in the best models. The environmental factors in which the confidence interval did not overlap zero were considered as having a significant influence on the model explaining the response variable. All analyses were conducted using R (RStudio Team, 2022).

3. RESULTS

We observed that *Amphimedon viridis* is commonly found on the walls of tide pools, under sandstone reefs, and rocky substrates. The average surface area (size) of individuals in the *A. viridis* population was 195.64 ± 230.60 cm². The highest average surface area of the population occurred between the rainy and dry seasons, from August to October 2023 (Figure 2A).

The *A. viridis* population was reproductively active continuously during the 17 months of the study. Based on an exploratory analysis of the 170 studied specimens, 78 were observed reproductively active. We considered reproductively active individuals as those engaged in gametogenesis or incubating embryos and larvae. Among the 78 reproductively active individuals, 41 (52.56%) specimens had oocytes, six (7.69%) had spermatic cysts, and

14 (17.94%) had embryos. Interestingly, eight individuals (10.25%) presented both oocytes and spermatic cysts at the same time, five individuals (6.41%) presented both oocytes and embryos at the same time, while three specimens (3.84%) had oocytes, spermatic cysts, and embryos, and one specimen (1.28%) had oocytes, spermatic cysts, embryos and larva. We observed the co-occurrence of male and female elements in 21.79% of the reproductively active population. Based on this, we consider *A. viridis* to be a simultaneous hermaphrodite. Individuals with only oocytes were randomly found in 14 months (82.35%) during the study period, while individuals with spermatic cysts were found in five months (29.41%), followed by individuals with embryos in ten months (58.82%), and individuals with more than one reproductive element simultaneously in nine months (52.94%). Although reproductive individuals of *A. viridis* were continuously observed in the population; their number was usually low. The period of the study when the number of reproductive individuals was higher occurred between August 2023 and January 2024 (Figure 2B).

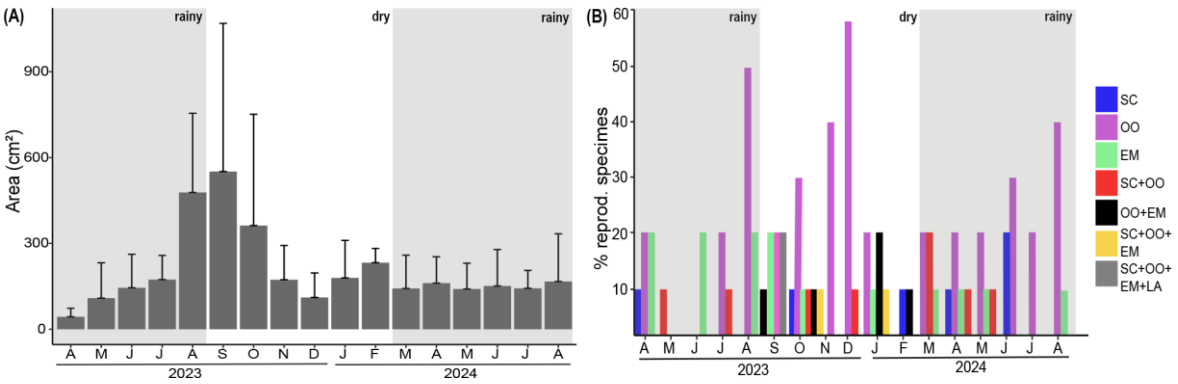

FIGURE 2 Population and reproductive dynamics of *Amphimedon viridis* from April 2023 to August 2024 from Pituba Beach, Salvador, Bahia, Brazil. (A) Average surface area of *Amphimedon viridis* during the study period. (B) Percentage of specimens with spermatic cysts, oocytes, embryos, oocytes and spermatic cysts simultaneously, oocytes and embryos simultaneously; and spermatic cysts, oocytes, and embryos simultaneously. Abbreviations: sc, spermatic cysts; oo, oocytes; em, embryos.

3.1 Reproductive elements

In *A. viridis*, spermatogenesis occurred in spermatic cysts, forming clusters of basophilic spermatogenic cells. These cysts were rounded and distributed throughout the mesohyl. We observed synchrony in the maturation within the cysts, but this was not true when considering different cysts, as they usually exhibited different stages of maturation (Figure 3A). We observed two stages of development of the spermatic cysts. In the first stage, the cells (spermatogonia) were larger, and fewer cells were within the cysts. In the second stage, the cells (spermatids) were smaller and more abundant within the cysts (Figure 3B e 3C). The diameter of the sperm cysts ranged from 11.46 μm to 42.96 μm, with an average of 23.44 ± 5.35 μm (n = 1,097). The largest spermatic cysts were recorded in September 2023 and December 2024 (42.96 μm and 37.25 μm, respectively).

The oocytes of *Amphimedon viridis* were found scattered throughout the mesohyl, exhibiting a rounded or oval shape. The nucleus of the cells was central, and distinct, with a rounded nucleolus located peripherally in the nucleus. During maturation, the oocytes were surrounded by a monolayer of elongated follicular cells, which remained until full maturation, acting as nurse cells (Figure 3D). In the previtellogenic stage, the small oocytes were irregular due to the low amount of yolk and their ability to move within the mesohyl (Figure 3D). As vitellogenesis progressed, a noticeable increase in oocyte size occurred due to greater yolk quantity (Figure 3E). It was observed that some oocytes seemed to phagocytize nurse cells, contributing to the increase in yolk (Figure 3F). The diameter of the oocytes ranged from 25.85 μm to 129.43 μm, with an average of 48.91 ± 18.09 μm (n = 73). The largest oocytes were recorded in June and July 2024 (129.43 μm and 120.47 μm, respectively). These oocytes were the ones found with phagocytized nurse cells.

We could not observe fertilization, but as the embryo and larva were brooded inside the mesohyl of the specimens, we can assume that fertilization is internal and, consequently,

the species is viviparous. During the early stages of embryogenesis, the embryos of *A. viridis* undergo successive chaotic cleavages, increasing the number of cells, and constantly being surrounded by a monolayer of nurse cells. In the early stage of embryogenesis, we observed large, spaced, and scarce spherical cells surrounded by a monolayer of nurse cells (Figure 3G). In the more advanced stage, the embryos assumed a rounded shape and considerable size, being composed of abundant spherical cells. The embryo was still surrounded by a thin layer, which resulted from a reduction in the number and size of the nurse cells. The mature embryos were observed to be distributed throughout the mesohyl (Figure 3H). The diameter of the embryos ranged from 75.78 μm to 444.58 μm, with an average of 235.38 ± 82.14 μm (n = 64). The largest embryos were recorded in June and September 2023 (375.4 μm and 444.58 μm, respectively).

After the embryogenesis, we could observe the presence of parenchymella larva in the mesohyl of *A.viridis*. The larva was elongated and composed of three distinct layers, each formed by different cell types, being the external layer of the larva composed of ciliated cells (Figure 3I). The diameter of the single observed larva was 180.844 μm. Finally, we observed that the production of spermatic cysts, oocytes, and embryos was asynchronous at both the population and individual levels.

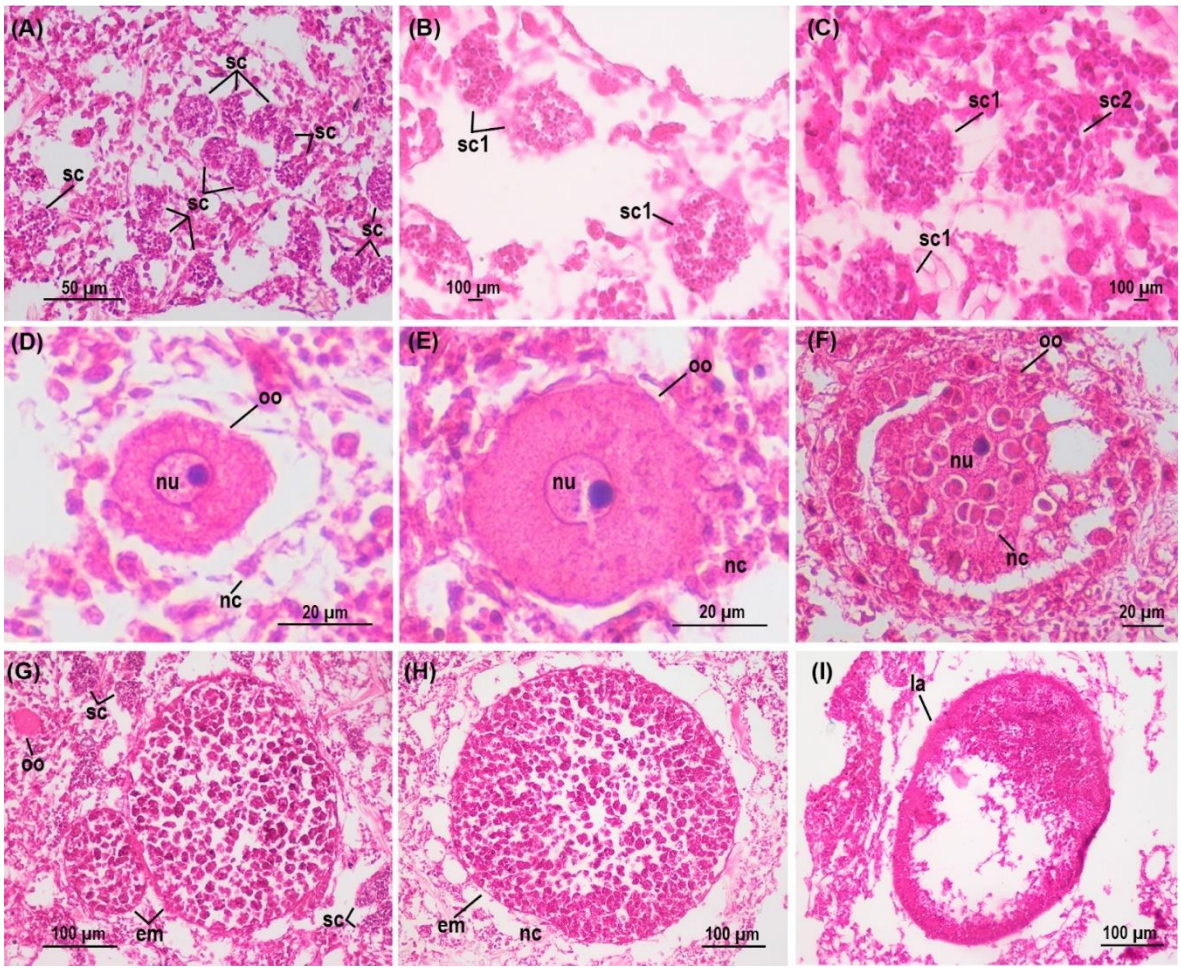

FIGURE 3 Reproductive elements of *Amphimedon viridis* from Pituba Beach, Salvador, Bahia, Brazil. (A-C) Spermatic cysts at different stages of maturation (sc). (A) cysts distributed in the mesohyl (sc). (B-C) cysts in early stage (sc 1) and advanced stage (sc 2) of spermatogenesis. (D-F) Oocytes at different stages of maturation (oo). (D) Pre-vitellogenic oocyte surrounded by nurse cells (nc). (E) Vitellogenic oocyte surrounded by nurse cells (nc). (F) Post-vitellogenic oocyte with phagocytosed nurse cells (nc). (G-H) Embryos (em) at different stages of maturation. (G) Two embryos in early stage of development. (H) Embryo in advanced stage of development. (I) Larva of the parenchymella type.

3.2 Reproductive dynamics

From the quantification, individuals involved in spermatogenesis showed an average density of 0.51 ± 1.14 spermatic cysts.mm^{-2} throughout the entire study period, with a higher concentration during the dry season (Figure 4A). The density of cysts was lower in April and May 2023, especially in May 2023 (0.43 ± 0.4 spermatic cysts.mm^{-2}), and higher in September 2023 (40 ± 24.40 spermatic cysts.mm^{-2}). However, we could not find any significant difference in the density of spermatic cysts among the months (Table 1). The average diameter of spermatic cysts was larger in September 2023 (26.12 ± 5.53 µm) and

smaller in February 2024 (20.05 ± 3.62 μm), both in the dry season (Figure 4D). However, we found no significant differences in the average size of spermatic cysts among the different months (Table 1).

From the quantification, individuals involved in oogenesis were observed in almost all months (except June 2023 and February 2024), with an average density of 0.01 ± 0.01 oocytes.mm^{-2} for the whole studied period. The average oocyte density was lowest in April 2023 (0.09 ± 0.06 oocytes.mm^{-2}) and highest in August 2023 (0.8 ± 0.23 oocytes.mm^{-2}), both during the rainy season (Figure 4B). It increased slightly at the end of the rainy season in 2023 and 2024. However, we did not observe significant differences in the average of oocyte density among the different months (Table 1). The average size of oocytes varied very little during the study period, but there was an increase at the end of the rainy season in 2024, from June to August 2024, with the highest values in July 2024 (100.62 ± 21.37 μm) and the lowest in April 2024 (40.2 ± 8.47 μm) (Figure 4E). Nevertheless, we did not observe significant variations in the average oocyte size among the months (Table 1).

From the quantification, individuals involved in embryogenesis were observed over ten months during the study period, with an average density of 0.01 ± 0.03 embryos.mm^{-2} per month. The average embryo density was higher during the dry season, in September 2023 (0.94 ± 0.45 embryos.mm^{-2}) and lower in June 2023 (0.14 ± 0.08 embryos.mm^{-2}) and March 2024 (0.14 ± 0.12 embryos.mm^{2}) (Figure 4C). However, no significant difference in embryo density was found among the different months (Table 1). The average diameter of embryos was larger in June 2023 (288.82 ± 100.72 μm) and smaller in August 2023 (161.77 ± 39.79 μm), both in the rainy season (Figure 4F). We found a significant difference in the average size among the different months during the study period. (Table 1). In addition, we found through the Tukey test that the significant variation in embryo size was mainly related to density of embryos observed in September 2023. The presence of larvae was not

quantified during the study period, although one individual with larva was observed (Figure 3).

The surface area of *A. viridis* was smaller in April 2023 (43.5 ± 29.9 cm²) and larger in September 2023 (551.17 ± 517.77 cm²), (Figure 2A). A significant difference in the average surface area was observed among the different months (Figure 2A; Table 1). During the study period, we did not find any significant difference in the average surface area between reproductive and non-reproductive individuals for all the reproductive elements (Table 2).

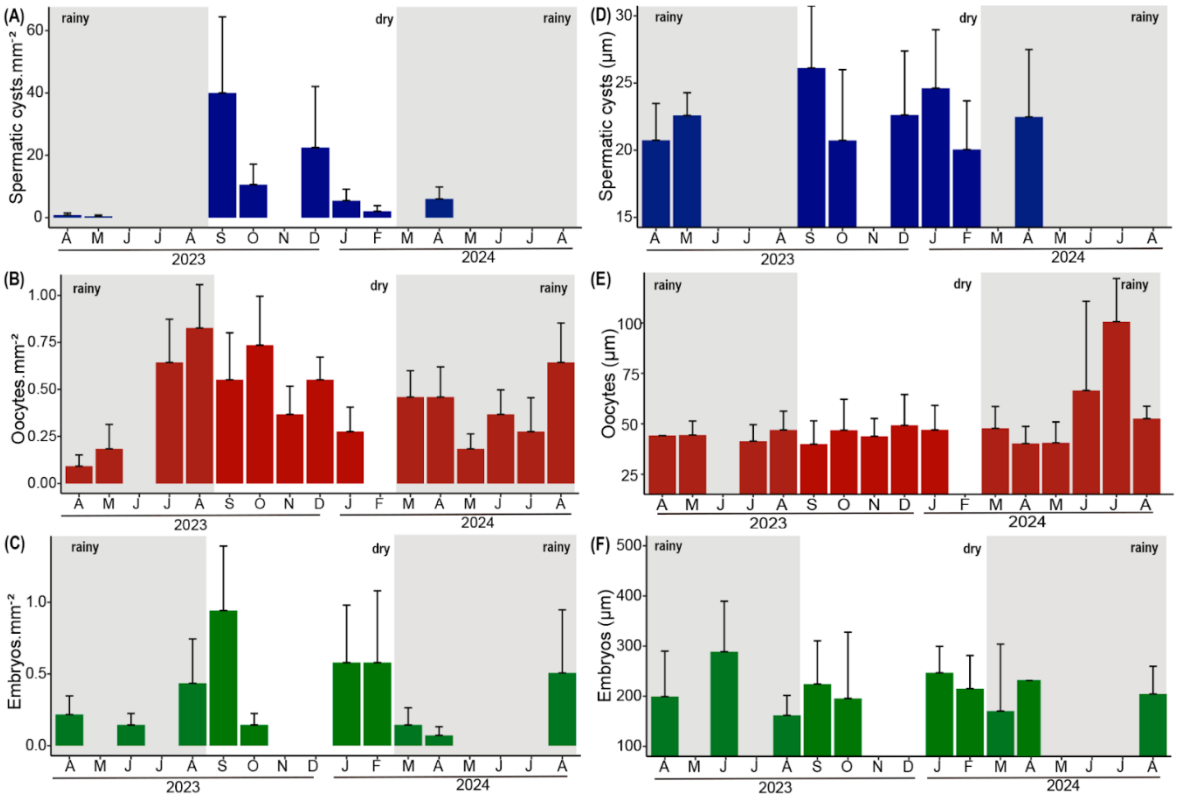

FIGURE 4 Reproductive dynamics of *Amphimedon viridis* during the study period in Pituba Beach, Salvador, Bahia, Brazil. (A-C) Monthly average density (bars) and standard deviation (lines) of the reproductive elements per mm² (A) Oocyte, (B) Spermatic cysts, and (C) Embryos. (D-F) Monthly average size of the reproductive elements of *A. viridis* during the study period (D) Oocyte. (E) Spermatic cyst. (F) Embryo.

TABLE 1 Summary of statistical results from ANOVA comparing the density and size of reproductive elements among the months of the study. Significant: *P < 0.05, ***P<0.001. Abbreviations: df, Degrees of Freedom; F value, F-statistic; Mean Sq, Mean Square; Pr(>F), P-value for F-test; Sum Sq, Sum of Squares.

	df	Sum Sq	Mean Sq	F value	Pr (>F)
Density of spermatic cysts					
Month	17	18210	1071.2	1.321	0.186
Residuals	152	123248	810.8		
Size of spermatic cysts					
Month	17	746	43.87	1.263	0.224
Residuals	152	5279	34.73		
Density of oocytes					
Month	17	6.28	0.3691	0.993	0.469
Residuals	152	56.48	0.3716		
Size of oocytes					
Month	17	9506	559.2	1.048	0.41
Residuals	152	81076	533.4		
Density of embryos					
Month	17	14.1	0.8293	1.143	0.319
Residuals	152	110.3	0.7255		
Size of embryos					
Month	17	1777234	10426	1.943	0.0183 *
Residuals	152	815778	5367		
Surface area					
Month	17	2982994	175470	4.473	<0.00107 ***
Residuals	149	5844694	39226		

TABLE 2 Summary of statistical results from the T-test comparing the average size (surface area) between reproductive and non-reproductive individuals. Abbreviations: SWO, Surface area of individuals with oocytes; SLO, Surface area of individuals lacking oocytes; SWC, Surface area of individuals with spermatic cysts; SLC, Surface area of individuals lacking spermatic cysts; SWE, Surface area of individuals with embryos; SLE, Surface area of individuals lacking embryos.. Significant: *P < 0.05.

	t	df	P-value
SWO and SLO	1.303	53.397	0.196
SWC and SLC	0.087	13.485	0.931
SWE and SLE	0.086	25.586	0.931

3.3 Dynamics of the environmental factors

We obtained data for seven environmental factors in different databases during the study period (February 2023 to August 2024). During this period, we calculated the monthly average for each factor, except for rainfall, for which we obtained the accumulated total of rain in each month. The monthly average of low tides varied during the study period, with the highest low tides observed in May 2023 at 0.62 (± 0.23) m and in July 2023 at 0.62 (± 0.20) m, while the lowest low tides were recorded in December 2023 at 0.42 (± 0.31) m (Figure 5A).

Salinity, as expected, was higher from September 2023 to February 2024 (dry season), ranging from 33.62 (± 0.23) psu in February 2023 to 37.07 (± 0.66) psu in January 2024 (Figure 5B). The accumulated monthly rainfall in Salvador ranged from 25 mm in October 2023 to 794.4 mm in May 2024, with the highest rainfall occurring during the rainy season, from March to August (Figure 5C). The water temperature varied slightly throughout the study period, with the lowest temperature recorded in July 2023 at 26.12 (± 0.35 °C) and the highest in March 2024 at 29.23 (± 0.79 °C) (Figure 5D).

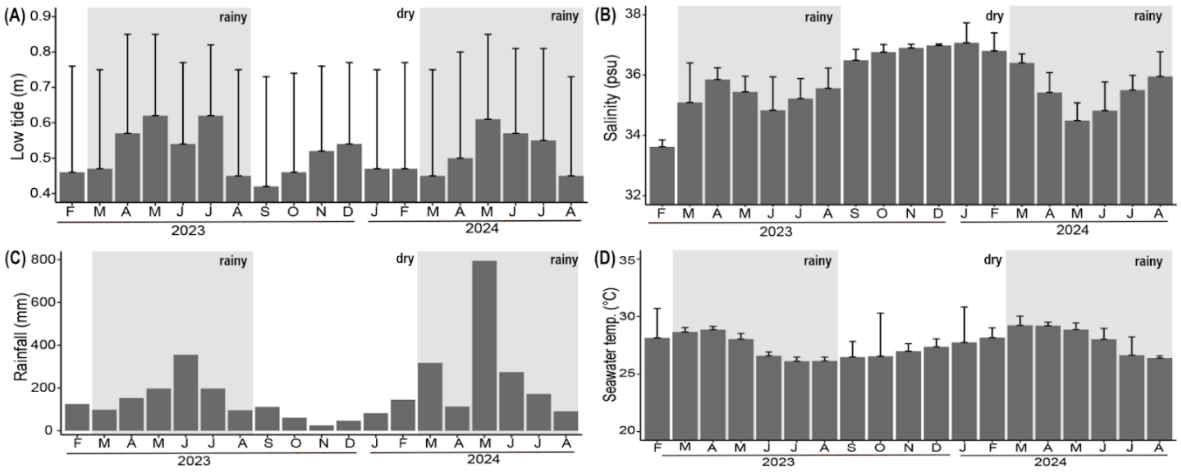

FIGURE 5 Dynamics of environmental factors during the study period (February 2023 to August 2024), from Pituba Beach, Salvador, Bahia, Brazil. (A) Low tide, (B) Salinity, (C) Rainfall, and (D) Seawater temperature. The bars show the average values and the lines indicate the standard deviations, except in C, where the accumulated monthly Rainfall is shown.

Air temperature varied over the months, with higher temperatures observed during the dry season and the beginning of the rainy season (December 2023 to March 2024), with March 2024 being the month with the highest air temperature at 28.48 (± 2.04) °C and July 2023 having the lowest temperature at 24.33 (± 1.60) °C (Figure 6A).

The average Chlorophyll-a (a proxy for phytoplankton) varied from 0.51 (± 0.19) mg/m³ in March 2024 to 7.65 (± 10.62) mg/m³ in June 2024; however, the chlorophyll-a concentration was considered relatively low for a large part of the study period, with chlorophyll-a levels greater than 2.00 mg/m³ occurring in only seven months (Figure 6B). The particulate organic matter (POM) varied over the months, with a peak concentration of 330.29 (± 112.87) g/m³ in June 2024 and the lowest concentration in February 2024 at 104.83 (± 21.14) g/m³ (Figure 6C).

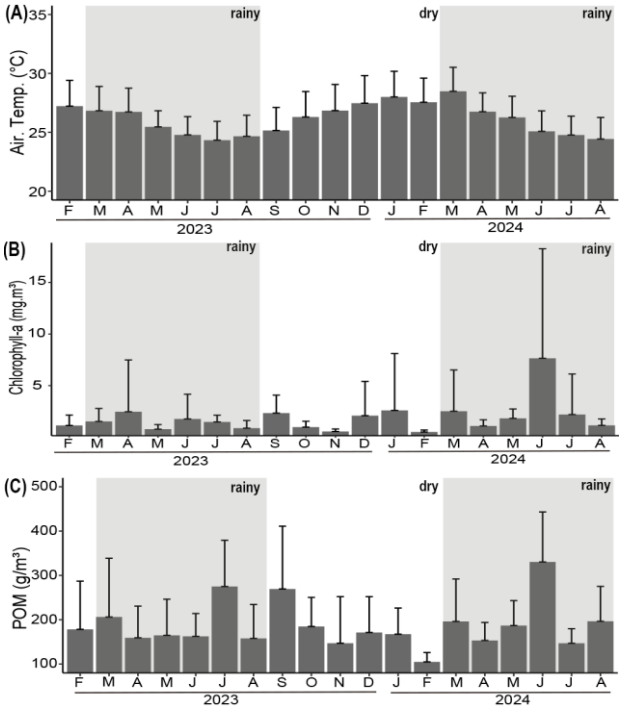

FIGURE 6 Dynamics of environmental factors during the study period (February 2023 to August 2024) from Pituba Beach, Salvador, Bahia, Brazil. (A) Air temperature, (B) Chlorophyll-a, and (C) Particulate Organic Matter. The bars show the average values and the lines indicate the standard deviations.

3.4 Influence of the environmental factors on the reproduction

We found seven explanatory models for the variation in the density of spermatic cysts, with the variables and their lags (the numbers indicate a lag of 0, -1, or -2 months): chlorophyll-a (-2), low tides (0 and -2), particulate organic matter (0, -1 and -2), rainfall (-2) seawater temperature (-1 and -2) and air temperature (-1). However, none of the variables contributed significantly to the average model (Figure 7A, Table 3). We retrieved two models to explain the variation in the size of spermatic cysts with the variables: chlorophyll-a (0), particulate organic matter (-2), rainfall (-2), salinity (0, and -2), seawater temperature (0, -1 and -2), low tides (-2), and air temperature (0). However, only chlorophyll-a (0) was positively significant for the average model (Figure 7B, Table 3).

Oocytes were the reproductive elements with most environmental factors related to their reproductive dynamics. However, we found only three models to explain the density of oocytes, with the variables: chlorophyll-a (-1 and -2), low tides (0), rainfall (0 and -2), seawater temperature (-2), air temperature (0 and -1), and salinity (0) with the chlorophyll-a

(-2), low tides (0), rainfall (0 and -2) and air temperature (0) being positively significant for the average model, while seawater temperature (-2) and air temperature (-1) were negatively significant for the average model (Figure 7C, Table 3). For the size of oocytes, we retrieved five explanatory models with the variables: chlorophyll-a (-1), low tides (-2), particulate organic matter (0), rainfall (-2), water temperature (-1), and air temperature (-2). Among these variables, chlorophyll-a (-1) and particulate organic matter (0) were positively significant for the average model (Figure 7D, Table 3).

We identified five explanatory models for embryo density with the variables: chlorophyll-a (-1), low tides (0 and -2), rainfall (-2), and particulate organic matter (0). Among these variables, only low tides (0) were negatively significant for the average model (Figure 6E, Table 3). For the variation in embryo size, we found seven explanatory models consisting of the variables: rainfall (0), low tides (0), particulate organic matter (0), salinity (0, -1 and -2), and air temperature (0). However, none of the variables contributed significantly to the average model (Figure 7E, Table 3).

We obtained two explanatory models for the variation in the surface area of *Amphimedon viridis* (Figure 8, Table 3). The models were composed of the variables: chlorophyll-a (-2), low tides (0), rainfall (-1), seawater temperature (0 and -2), and air temperature (0). Among the environmental factors, only rainfall (-1) was positively significant for the average model (Figure 8, Table 3), while chlorophyll-a (-2), low tides (0), water temperature (-2), and air temperature (0) were negatively significant for the average model (Figure 8, Table 3).

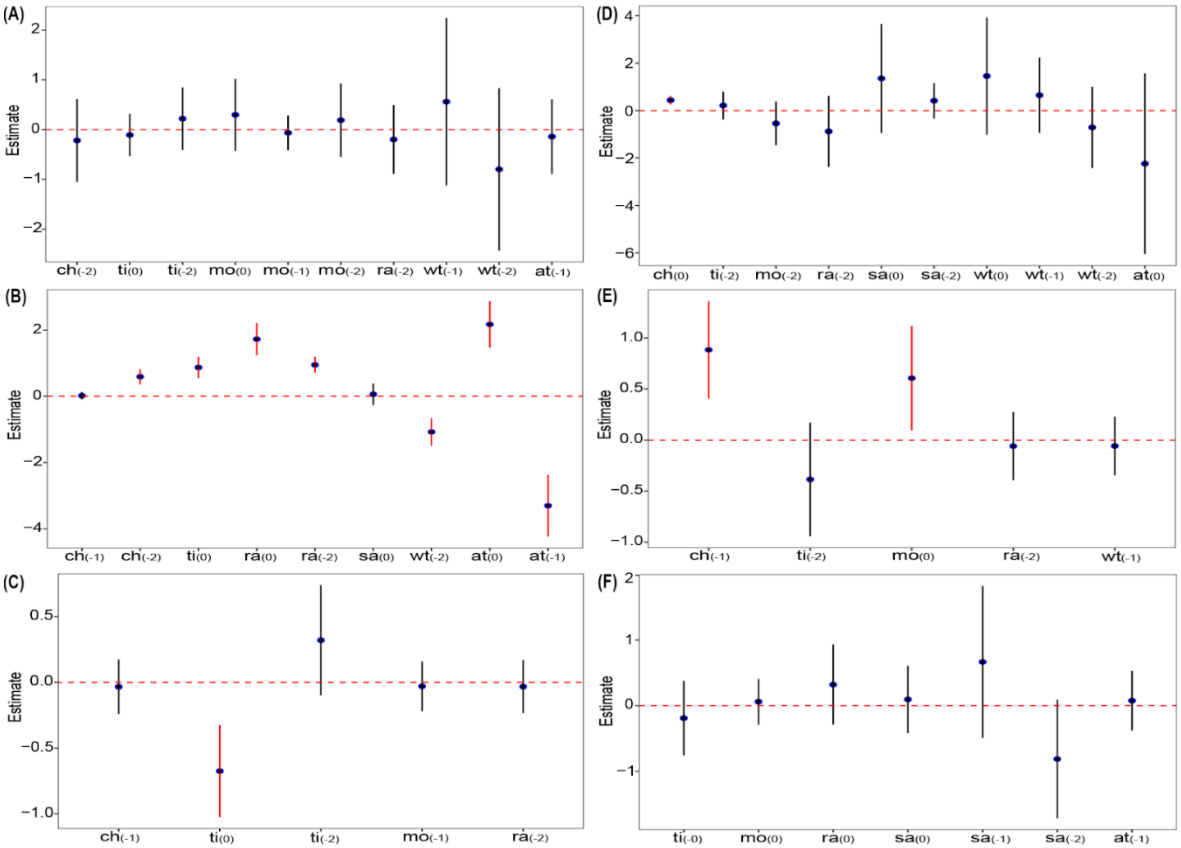

FIGURE 7 Multi-model averaged variables coefficients (points) and 95% confidence intervals (lines) for the monthly average density and size of the reproductive elements of *Amphimedon viridis*, from Pituba Beach, Salvador, Bahia, Brazil. (A-C) Density of the reproductive elements per mm² (A) Spermatic cysts, (B) Oocyte, and (C) Embryos. (D-F) Size of the reproductive elements of *A. viridis* during the study period (D) Spermatic cyst, (E) Oocyte, and (F) Embryos. Variables with significant contributions to the models are marked in red. Environmental factors: at, air temperature; ch, chlorophyll; mo, particulate organic matter; ra, rainfall; sa, salinity; ti, low tides; wt, water temperature. Subscript numbers indicate a lag of 0, 1, or 2 months.

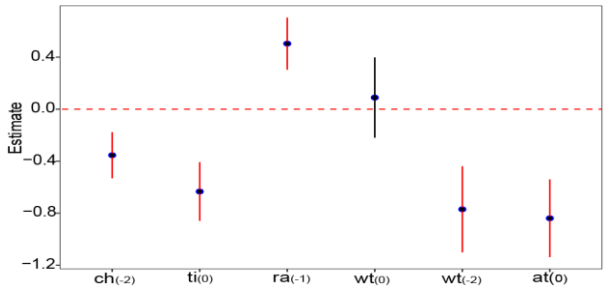

FIGURE 8 Multi-model averaged variables coefficients (points) and 95% confidence intervals (lines) for the monthly avearage surface area of *Amphimedon viridis*, from Pituba Beach, Salvador, Bahia, Brazil. Legend as in Figure 7.

TABLE 3 Top models with the environmental factors that explain the variation in the monthly average of the density and size of spermatic cysts, oocytes, and embryos of *Amphimedon viridis* and its surface area in Pituba Beach, Salvador, Bahia, Brazil. Environmental factors: at, air temperature; ch, chlorophyll; mo, particulate organic matter; ra, rainfall; sa, salinity; ti, low tides; wt, water temperature. The numbers in parentheses indicate a lag of 0, 1, or 2 months. Abbreviations: AICc, Corrected Akaike Information Criterion; Delta, Difference between a reference value and a value of interest; Df, Degrees of freedom; LogLink, Log-likelihood.

Models	**df**	**logLik**	**AICc**	**delta**	**weight**
Density - spermatic cysts					
ch(2)+ti(0)+mo(2)	5	-15.72	46.90	0.00	0.23
ti(2)+mo(0)+ra(2)+wt(1)+wt(2)	7	-10.63	47.71	0.81	0.15
wt(2)	3	-20.02	47.88	0.98	0.14
ti(2)+mo(1)+wt(1)+wt(2)+at(1)	7	-10.76	47.97	1.07	0.13
mo(0)+ra(2)	4	-18.33	47.98	1.08	0.13
mo(0)+wt(2)	4	-18.34	48.01	1.11	0.13
ti(2)+mo(0)+wt(1)+wt(2)	6	-14.15	48.71	1.81	0.09
Size - spermatic cysts					
ch(0)+mo(2)+ra(2)+sa(0)+sa(2)+wt(0)+at(0)	9	0.07	43.58	0.00	0.58
ti(2)+wt(1)+wt(2)	5	-14.40	44.25	0.66	0.42
Density - oocytes					
ch(2)+ti(0)+ra(0)+ra(2)+wt(2)+at(0)+at(1)	9	3.48	36.75	0.00	0.74
ch(2)+ti(0)+ra(0)+ra(2)+sa(0)+wt(2)+at(0)+at(1)	10	8.28	40.11	3.36	0.14
ch(1)+ch(2)+ti(0)+ +ra(0)+ra(2)+wt(2)+at(0)+at(1)	10	8.18	40.32	3.56	0.12
Size - oocytes					
ch(1)+ti(2)+mo(0)	5	-14.75	44.96	0.00	0.42
ch(1)+ti(2)+mo(0)+wt(1)	6	-13.03	46.45	1.49	0.20
ch(1)+mo(0)	4	-17.50	46.34	1.39	0.21
wt(1)+at(2)	4	-17.55	46.43	1.47	0.16

ch(1)+ti(2)+mo(0)+ra(2)	6	-13.20	46.81	1.85	0.17
Density - embryos					
ti(0)+ti(2)	4	-14.36	40.06	0.00	0.39
ti(0)	3	-17.01	41.87	1.81	0.16
ch(1)+ti(0)+ti(2)	5	-13.26	41.98	1.92	0.15
ti(0)+ti(2)+ra(2)	5	-13.27	42.00	1.94	0.15
ti(0)+ti(2)+mo(1)	5	-13.30	42.04	1.99	0.15
Size - embryos					
ra(0)+sa(1)+sa(2)	5	-16.83	49.12	0.00	0.20
ti(0)+ra(0)+sa(1)+sa(2)	6	-14.53	49.46	0.34	0.17
sa(1)+sa(2)	4	-19.16	49.65	0.53	0.15
mo(0)+ra(0)+sa(1)+sa(2)	6	-14.72	49.84	0.72	0.14
ra(0)+sa(0)+sa(2)	5	-17.24	49.93	0.81	0.13
ti(0)+sa(2)+at(1)	5	-17.38	50.22	1.09	0.11
ti(0)	3	-21.30	50.45	1.33	0.10
Surface area					
ch(2)+ti(0)+ra(1)+wt(2)+at(0)	7	1.92	22.60	0.00	0.68
ch(2)+ti(0)+ra(1)+wt(0)+wt(2)+at(0)	8	4.93	24.14	1.54	0.32

4. DISCUSSION

4.1 Reproductive cycle of Amphimedon viridis

The reproductive aspects of *Amphimedon viridis* were similar to those observed in other species of its order, Haplosclerida (Fell, 1976; Wapstra & van Soest, 1987; Fromont, 1994). The species exhibited continuous reproduction, associated with low reproductive

effort. Viviparity was identified, as well as the asynchronous development of reproductive elements within the same individual. Furthermore, our data suggest that the reproductive process is influenced by different environmental variables. *Amphimedon viridis* exhibited continuous reproduction, with oocytes present throughout almost the entire study period, while spermatic cysts and embryos were found in short periods. Although continuous reproduction is observed in several species of the class Demospongiae, it is not a phylogenetically inherited characteristic but rather influenced by environmental factors. In tropical regions, where the climate is more stable, sponges and other marine invertebrates tend to reproduce continuously (Lanna & Klautau, 2016; Lanna et al., 2018; 2021), making this strategy expected for *A. viridis*. Although it is more common in organisms from tropical regions, continuous reproduction can also be observed in species from temperate regions (Riesgo et al., 2007).

Despite the continuous reproduction of *A. viridis*, its reproductive effort was low, with a small percentage of the population involved in reproduction during the study period. This low investment was expected, considering the environmental stresses characteristic of the intertidal zone (Gaino et al., 2010), as gamete formation in any invertebrate demands a high energy cost (Harri et al., 2002). This pattern of low reproductive effort is also observed in another species of the genus, *Amphimedon chloros* Ilan, Gugel & van Soest, 2004, studied in shallow waters of the Red Sea (Ilan et al., 2004). However, the opposite was observed in *Amphimedon queenslandica* Hooper & van Soest, 2006, another species of the same genus, which exhibited a higher reproductive effort in reefs of Shark Bay, at Heron Island Reef, southern Great Barrier Reef (Maritz et al., 2010). These results highlight that reproductive effort can be directly influenced by environmental conditions, as three species of the same genus exhibited distinct patterns of reproductive investment depending on the environments

they inhabited. Nonetheless, how and which environmental factors influence reproductive effort of *A. viridis* will be further addressed in the next section.

The species *Cladocroce caelum* Santos, Da Silva, Alliz & Pinheiro, 2014, belonging to the order Haplosclerida and studied in a submerged area in Salvador, exhibited reproductive characteristics similar to those of *A. viridis*. *Cladocroce caelum* displayed continuous reproduction, low reproductive effort, and a higher frequency of oocytes in the mesohyl (Lanna et al., 2018). Continuous reproduction and low reproductive effort were also observed in *Tedania ignis* (Duchassaing & Michelotti, 1864), belonging to the order Poecilosclerida, studied in Salvador. In contrast, *Haliclona cymaeformis* (Esper, 1806) from the order Haplosclerida, but studied in the Great Barrier Reef under different environmental conditions, exhibited reproductive activity only during the summer, with abundant spermatic cysts in the mesohyl and the presence of larvae (Fromont, 1994).

The viviparity observed in *A. viridis* is an ancestral condition in Demospongiae (Riesgo et al., 2014) maintained in species of Haplosclerida (Fromont & Bergquist, 1994; Maldonado & Bergquist, 2002). Additionally, the simultaneous presence of male and female reproductive elements in the same individual indicates that *A. viridis* is a simultaneous hermaphrodite, consistent with observations in other phylogenetically related species (Ilan & Loya, 1988, 1990; Maldonado & Riesgo, 2009b; Maritz et al., 2010; Riesgo et al., 2014). The reproductive elements of *A. viridis* were found dispersed in the mesohyl, as observed in other species of the order (Ilan et al., 2004; Leong & Pawlik, 2011). However, in some species, such as *A. queenslandica*, the reproductive elements are incubated in specialized chambers (Leys & Degnan, 2002; Maritz et al., 2010). It is worth noting that *A. viridis* and *A. queenslandica*, although named as the same genus, belong to different phylogenetic lineages of Haplosclerida, leading to a non-monophyletic *Amphimedon.* As *A. chloros* is found in the same lineage of *A. viridis*, the existence of the specialized chambers in *A.*

queenslandica is likely an exclusive characteristic of this Australian species (Fromont, 1994; Ilan & Loya, 1988, 1990; Redmond et al., 2011).

We observed asynchronous reproductive stages in the Pituba Beach population of *A. viridis*, with spermatic cysts, oocytes, and embryos at different stages of development. This asynchronous development is common in Demospongiae and represents an advantageous strategy for avoiding self-fertilization and ensuring genetic variability in offspring (Leys & Degnan, 2002; Degnan et al., 2005). It also allows the release of reproductive elements at different times throughout the year, promoting greater reproductive success and reducing competition for resources (Degnan et al., 2008). This strategy could ensure the continuous release of larvae, increasing the likelihood of these larvae encountering favorable conditions for settlement in the intertidal zone.

The reproductive effort of *A. viridis* supports the hypothesis that viviparous sponges tend to have a lower density of oocytes than oviparous ones (Witte & Barthel, 1994; Lanna et al., 2015). The density of oocytes in *A. viridis* during the study was low, ranging from 0.09 to 0.8 oocytes.mm^{-2}. Interestingly, a lower density of oocytes was observed when compared to spermatic cysts in the current studied species, although the frequency of oocytes was higher. This pattern is common among sponges (Simpson, 1984; Mercurio et al., 2007). The lower density of oocytes in *A. viridis* can be attributed to the high energy demand required for oogenesis, while the higher frequency of oocytes is related to the extended incubation period, as they undergo the vitellogenesis process. Ereskovsky et al. (2013) observed that specimens of *Oscarella lobularis* (Schmidt, 1862) subjected to food limitation displayed a higher proportion of males compared to females, likely due to the lower energy demand required for spermatogenesis. This explanation should apply to *A. viridis*, too.

Embryogenesis also demands a significant amount of energy for the sponges. We observed that embryo size was larger during the dry season, characterized by higher solar

incidence. This higher solar incidence may have favored increased photosynthetic rates in phytoplankton, an important food source for sponges. Thus, we believe that during the dry season, *A. viridis* had greater food availability, allowing more energy allocation to embryo growth. We will discuss in more detail the influence of solar incidence on the greater food availability for the sponge in the next session.

Regarding larval development, our results showed that *A. viridis* produces parenchymella-type larvae, a larval type common in Demospongiae lineages, including the order Haplosclerida. (Ereskovsky, 1999; Leys & Degnan, 2002; Degnan et al., 2008; Worheide et al., 2012; Lanna et al., 2024). Surprisingly, the presence of larvae in *A. viridis* was very rare, with only one larva recorded during the study period. In contrast, larvae are more commonly observed in other species in Demospongiae lasting two to three months being brooded in the mesohyl of the sponges (Ayling, 1980; Ettinger-Epstein et al., 2007). The prolonged presence of larvae is also observed in other species of the genus, such as *A. queenslandica* and *A. compressa*, and represents a response to the asynchronous development of embryos (Maritz et al., 2010; Leong & Pawlik, 2011; Stephens et al., 2012). The duration of larval development in sponges is still poorly understood. However, the study by Maritz and collaborators (2010), conducted in a submerged environment, suggests that *A. queenslandica* takes approximately three weeks from fertilization to reach the mature larval stage. For *A. viridis*, we believe that both larval development and release occur over a short period, possibly as a response to the specific conditions of the intertidal environment. Nevertheless, it is still unclear whether this characteristic reflects an adaptation to the environmental stresses typical of the intertidal zone in Salvador, considering that *Spongia ceylonensis* (Dendy, 1905), studied in a tropical intertidal region of Penghu, Taiwan, exhibited larvae for a period of five months. Similarly, Gaino et al. (2010) observed a higher frequency of larvae in *Hymeniacidon perlevis* (Montagu, 1814), in the intertidal zone

compared to the sublittoral, in a study conducted in the Mar Piccolo di Taranto (Ionian Sea, Apulia). This difference was attributed to the presence of macroalgae, which reduced desiccation in the intertidal zone while causing anoxic episodes in species fully submerged.

The size and morphology of the reproductive elements of *A. viridis* showed similarities with those of other species in the order Haplosclerida. The morphology of the reproductive elements of *A. viridis* was similar to that observed in *Chalinula sp.* (Grant, 1841), *Xestospongia bergquistia* (Laubenfels, 1932), *Haliclona fulva* (Topsent, 1893), and *A. chloros* (Ilan & Loya, 1990; Ereskovsky, 2010). The average size of the spermatic cysts in *A. viridis* was comparable to that of *Haliclona amboinensis* (Lévi, 1961), while the oocytes resembled those of *H. cymiformis*, and *H. fulva*. However, the average size of the spermatic cysts and oocytes in *A. viridis* was smaller compared to *A. chloros*, a species of the same genus (Ilan et al., 2004). The average larval size, in turn, was similar to that of *Haliclona loosanoffi* (Hartman, 1958), (Fell, 1976; Fromont, 1994; Ereskovsky et al., 2017). These comparisons indicate that, although the characteristics of reproductive elements are widely shared within the order Haplosclerida, they may undergo minor variations due to geographic and environmental differences.

Body size was not a limiting factor for the onset of reproduction in *A. viridis*, as reproductive elements were found in specimens ranging from 8 to 836.4 cm^2. This suggests that the species reaches sexual maturity early, likely as a strategy to increase its chances of propagation in the inhospitable intertidal zone. Similar results were observed by Maldonado & Riesgo (2008a) in *Corticium candelabrum* (Schmidt, 1862) and by Fromont & Bergquist (1994) in *Xestospongia bergquistia* (Fromont, 1991), *Xestospongia exigua* (Kirkpatrick, 1900), and *Xestospongia testudinaria* (Lamarck, 1815). This pattern is also observed in cnidarians, such as *Galaxea fascicularis* (Linnaeus, 1767) and *Seriatopora hystrix* (Dana, 1846) (Rapuano et al., 2023). However, some sponges in the class Demospongiae exhibit

distinct reproductive patterns, such as *Rhopaloeides odorabile* (Thompson, Murphy, Bergquist & Evans, 1987), which initially invests energy in growth, reaching a minimum size before starting reproduction as a strategy to reduce mortality (Whalan et al., 2007). This differs from certain species that concentrate most of their energy on a single reproductive event before dying (Lanna et al., 2007).

4.2 Influence of exogenous factors on the reproduction and surface area of Amphimedon viridis

We identified different explanatory models for the dynamics of each reproductive element. In this study, we observed that air temperature (current month and one-month-lag), low tide (current month), chlorophyll-a and rainfall (current month, with a one- and two-month lag) were the main environmental drivers of the reproduction of *Amphimedon viridis*. Chlorophyll-a was used as a proxy for the density of phytoplankton, an important food source for sponges (Ribes et al., 1999; Maldonado et al., 2012). Our results showed that the chlorophyll-a concentration positively influenced the size of spermatic cysts. This finding aligns with previous studies also in the region of Salvador (Lanna et al., 2018; Cajado & Lanna, 2021), that demonstrated that greater food availability leads to increased investment in cyst production.

Chlorophyll-a, with a two-month lag (-2), showed a positive influence on oocyte density, while chlorophyll-a with a one-month lag (-1) and particulate organic matter POM (0) were positively associated with oocyte size. Considering that oogenesis requires a high energy input, the availability of food in the environment is essential for the production and growth of these gametes (Elvin, 1976). In this context, chlorophyll-a was used as an indicator of phytoplankton abundance, as mentioned, and reflects food availability, while POM was recognized as a crucial nutritional source for sponges (De Goeij et al., 2008). The

simultaneous association of these food resources indicates that an increase in energy availability might promote the increase in size of the oocytes and more intense gamete production (Riesgo et al., 2015; Lanna et al., 2024).

In addition to chlorophyll-a, the month's low tides (without lag) showed a positive relationship with oocyte density, which was expected since reduced exposure during low tides decreases susceptibility to environmental stress. Interestingly, we observed that the month's low tides (without lag) showed a negative relationship with embryo density, a pattern also reported by Calazans & Lanna (2019) for *Heteropia glomerosa* (Bowerbank, 1873), at the Terminal Turístico Náutico da Bahia, in Salvador. This negative relationship in *A. viridis* may be associated with the fertilization period, which likely occurred during times of higher low tides, resulting in an increased number of embryos during lower tide periods. As the average tide level rises again, embryos may be rapidly released as larvae. Similar phenomena have been described in other intertidal marine invertebrates, whose reproduction and larval release are synchronized with spring tides (Gupta, 2017; Tamaki et al., 2018).

Rainfall (current month and two-month lag) positively influenced oocyte density. Although rainfall can affect water salinity, causing stress, particularly for sponges in intertidal pools, it also increases food availability in coastal areas by transporting organic matter and nutrients (Arianoutsou, 1989; Fabricius, 2005). Our results contrast with findings in *Aplysina insularis* (Duchassaing & Michelotti, 1864), *Desmapsamma anchorata* (Carter, 1882), *Tedania ignis*, and *Cladocroce caelum*, where rainfall negatively influenced reproduction (Lanna et al., 2018; Oliveira & Lanna, 2022). In those cases, the large amount of sediment transported during the rainy season likely affected sponge filtration (Edmunds & Lasker, 2016; Wahab et al., 2014). For *A. viridis*, the sediment brought by rainfall apparently did not affect reproduction, as observed in Lanna et al. (2018) and Oliveira &

Lanna (2022), but we hypothesize that the food carried out from the coast to the pools might have been essential for oogenesis.

An unexpected result was the positive correlation of the month's air temperature with oocyte density, once air temperature can impact the dehydration of the exposed organisms in intertidal regions, or - at least - increase the salinity and decrease the oxygen available in the water pools. Nonetheless, the increase in the water temperature is constantly associated to the reproductive effort of several species of sponges in different regions of the world (Lanna et al., 2018) An example is the case of *Geodia cydonium* (Linnaeus, 1767), studied in the Bay of Porto Cesareo, which concentrates its reproductive efforts in the summer, a period when the water temperature increases, reaching values above 25 °C (Mercurio et al., 2007). Putting together our findings with the previous ones, we can highlight that both air and water temperatures could drive oocyte production. Although *A. viridis* inhabits a tropical region with minimal climate variation, temperature fluctuations can still positively influence its reproduction. However, air temperature (one-month lag) and water temperature (two-month lag) negatively correlated with oocyte density. While such negative relationships are uncommon, they may be explained by stress on sponges. Increased air and water temperatures in intertidal pools affect oxygen solubility and salinity through evaporation (Vinagre et al., 2019). Despite sponges' tolerance to oxygen and salinity variations (Knudby et al., 2013; Lee & Riding, 2018), these cyclic environmental changes in the intertidal region, combined with the high energy demand of oogenesis, explain the overall reduced oocyte numbers observed in this species.

Global climatic events, such as El Niño, can impact intertidal marine invertebrates. However, the effects of this phenomenon on marine sponges remain underexplored. Previous studies indicated that reef sponges demonstrated higher tolerance to water warming during El Niño events (Kelmo, et al., 2013; Nava, García-Madrigal & Carballo, 2019). In contrast,

our results indicated that the increase in air and water temperatures associated with ENSO had a negative effect on the reproductive dynamics of *A. viridis*. However, the true influence of ENSO on *A. viridis* reproduction remains uncertain, as this study was conducted exclusively during an ENSO event. Thus, it is possible that ENSO is either masking or amplifying the effects of temperature on the species reproduction. Nonetheless, the negative effect of the temperature on reproduction suggests that ENSO may impact the reproductive biology of some species, particularly on oocyte density. Further studies investigating sponge reproductive cycles both during and outside ENSO events are essential for a better understanding of the impacts of this phenomenon.

Interestingly, we also observed that both air temperature and seawater temperature negatively affected the surface area of *A. viridis*. One can infer that the influence of the temperature on the reproduction of *A. viridis*, as discussed above, could be indirect, meaning that the proper negative relationship of temperatures could be with the size of the sponges. Nonetheless, we observed that the size of the sponge did not influence in the reproductive effort of *A. viridis*. These findings highlight the importance to investigate multiple drivers of the reproduction at the same time. The negative influence of the temperature on the size of the specimens of *A. viridis* contrasts with other studies showing a positive relationship between temperature and growth rate (Duckworth & Battershill, 2003; Page et al., 2005; Koopmans & Wijffels, 2008). The intertidal zone, characterized by daily thermal fluctuations experiences high evaporation rates, leading to elevated desiccation levels. Although sponges are tolerant of these fluctuations, increased temperatures intensify environmental stresses, causing tissue damage in these organisms (Guzman & Conaco, 2016). Furthermore, higher temperatures increase the vulnerability of marine invertebrates to pathogens (Garren et al., 2016). In this context, sponges tend to redirect their energy toward maintenance and survival, reducing investment in growth. On the other hand, rainfall,

with a one-month lag, showed a positive relationship with the surface area of *A. viridis*. We believe this relationship is associated with the influence of rainfall in lowering temperatures in the intertidal zone, as well as increasing food availability carried by water flow. In contrast, chlorophyll-a, with a two-month lag, showed a negative relationship with surface area, differing from expectations and commonly observed patterns (Duckworth et al., 2004; Koopmans & Wijffels, 2018). We attribute this relationship to the period during which the sponge directs its energy toward reproduction rather than growth. This pattern reflects an energy trade-off strategy, where resources are alternately allocated between growth and reproduction (Elvin, 1976).

Finally, we observed a negative influence of the month's low tides on the surface area of *A. viridis*, meaning that when the tides were lower the individuals were larger. Previous studies in the sublittoral zone have shown that low tides were positively related to the growth of *Aplysina solangeae* Pinheiro, Hajdu & Custódio, 2007 and *Aplysina fulva* (Pallas, 1766), likely due to increased water volume and greater food availability (Oliveira & Lanna, 2022). However, little is known about the effects of low tides on the size of intertidal sponges. In *A. viridis*, we believe that this effect is related to the time when the sponge allocates its energy for reproduction rather than growth. We observed that larger individuals during low tides had fewer oocytes and more embryos. As embryo incubation demands a large amount of energy, the sponge may allocate its resources mainly to this process, prioritizing incubation over growth.

Conclusions

We found that the reproduction of *A. viridis* in the intertidal zone is continuous, with low reproductive effort. The species is viviparous and a simultaneous hermaphrodite, and its gametogenesis, embryogenesis, and larval type were similar to those observed in other

species of its order, Haplosclerida. However, the larval density of *A. viridis* was very low, likely due to the specific environmental conditions of the intertidal zone, although this aspect is not yet fully understood. The surface area, density, and size of the reproductive elements were influenced by various environmental factors, as conditions in tropical regions like Salvador tend to be more stable throughout the year. We found no significant correlations between the species surface area and the density of reproductive elements. Although the study was conducted entirely during the ENSO event 2023-2024, we observed a negative effect of water temperature on oocyte density, suggesting that ENSO may impact the reproduction of this species. Finally, the reproductive strategies in *Amphimedon viridis* seems to be determined by both phylogenetic and environmental factors.

Acknowledgements

We thank our colleagues from the Laboratory of Evolutionary and Developmental Biology for their support in obtaining biological material throughout the study, with special acknowledgment to Laura Oliveira. We also express our gratitude for the financial support provided by the National Institute of Science and Technology in Interdisciplinary and Transdisciplinary Studies in Ecology and Evolution (INCT IN-TREE), the Bahia State Research Support Foundation (FAPESB), and the National Council for Scientific and Technological Development (CNPq). The collection of sponge specimens was conducted in compliance with Brazilian environmental regulations, under authorization from the Chico Mendes Institute for Biodiversity Conservation (ICMBio, License No. 269772).

References

Abramoff, M. D., Magalhaes, P. J., & Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics International, 11*, 36–42.

Archer, S. K., Kahn, A. S., Thiess, M., Law, L., Leys, S. P., Johannessen, S. C., Layman, C. A., Burke, L., & Dunham, A. (2020). Foundation species abundance influences food web topology on glass sponge reefs. *Frontiers in Marine Science*, *7*, 549478. https://doi.org/10.3389/fmars.2020.549478

Arianoutsou, M. (1989). Atmospheric deposition of nutrients in a coastal maquis ecosystem of northeastern Greece. *International Journal of Biometeorology*, *33*, 124–130. https://doi.org/10.1007/BF01686289

Ayling, A. L. (1980). Patterns of sexuality, asexual reproduction, and recruitment in some subtidal marine Demospongiae. *The Biological Bulletin,* 158(3), 271–282. https://doi.org/10.2307/1540854

Bartoń, K. (2024). *MuMIn: Multi-Model Inference* [Pacote R]. Recuperado de https://CRAN.R-project.org/package=MuMIn.

Berlinck, R. G., Ogawa, C. A., Almeida, A. M., Sanchez, M. A. A., Malpezzi, E. L., Costa, L. V., Hajdu, E., & De Freitas, J. C. (1996). Chemical and pharmacological characterization of halitoxin from *Amphimedon viridis* (Porifera) from the southeastern Brazilian coast. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology, 115*(2), 155-163. https://doi.org/10.1016/S0742-8413(96)00107-7

Brazeau, D. A., & Lasker, H. R. (1992). Reproductive success in the Caribbean octocoral *Briareum asbestinum*. *Marine Biology, 114*, 157-163. https://doi.org/10.1007/BF00350865

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach*. Springer.

Cajado, B., & Lanna, E. (2021). How can environmental variables affect the sexual reproduction of a tropical symbiotic sponge? *Marine Biodiversity, 51*(2), 23. https://doi.org/10.1007/s12526-021-01167-y

Calazans, V. P., & Lanna, E. (2019). Influence of endogenous and exogenous factors on the reproductive output of a cryptogenic calcareous sponge. *Marine Biodiversity, 49*(6), 2837-2850. https://doi.org/10.1007/s12526-019-01013-2

Chung, I. F., Huang, Y. M., Lee, T. H., & Liu, L. L. (2010). Reproduction of the bath sponge *Spongia ceylonensis* (Dictyoceratida: Spongiidae) from Penghu, Taiwan. *Zoological Studies, 49*(5), 601–607. https://zoolstud.sinica.edu.tw/Journals/49.5/601.pdf.

De Goeij, J. M., Moodley, L., Houtekamer, M., Carballeira, N. M., & Van Duyl, F. C. (2008). Tracing 13C-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca caerulea*: Evidence for DOM-feeding. *Limnology and Oceanography, 53*(4), 1376-1386. https://doi.org/10.4319/lo.2008.53.4.1376

De Voogd, N. J., et al. (2024). World Porifera Database. Retrieved December 10, 2025, from https://www.marinespecies.org/porifera/

Degnan, B. M., Leys, S. P., & Larroux, C. (2005). Sponge development and antiquity of animal pattern formation. *Integrative and Comparative Biology, 45*(2), 335–341. https://doi.org/10.1093/icb/45.2.335

Degnan, B., Adamska, M., Craigie, A., Degnan, S., Fahey, B., Gauthier, M., Hooper, J., Larroux, C., Leys, S., Lovas, E., & Richards, G. (2008). The demosponge *Amphimedon queenslandica*: Reconstructing the ancestral metazoan genome and

deciphering the origin of animal multicellularity. *Cold Spring Harbor Protocols*. https://doi.org/10.1101/pdb.emo108

Duckworth, A. R., Battershill, C. N., & Schiel, D. R. (2004). Effects of depth and water flow on growth, survival, and bioactivity of two temperate sponges cultured in different seasons. *Aquaculture*, *242*(1–4), 237–250. https://doi.org/10.1016/j.aquaculture.2004.08.046

Duckworth, A., & Battershill, C. (2003). Sponge aquaculture for the production of biologically active metabolites: The influence of farming protocols and environment. *Aquaculture, 221*(1–4), 311–329. https://doi.org/10.1016/S0044-8486(03)00070-X

Edmunds, P. J., & Lasker, H. R. (2016). Cryptic regime shift in the benthic community structure of shallow reefs in St. John, U.S. Virgin Islands. *Marine Ecology Progress Series, 559*, 1–12. https://doi.org/10.3354/meps11900

Edmunds, P. J., Coblentz, M., & Wulff, J. (2020). A quarter-century of variation in sponge abundance and community structure on shallow reefs in St. John, U.S. Virgin Islands. *Marine Biology, 167*, 1–17. https://doi.org/10.1007/s00227-020-03740-8

Elvin, D. V. (1976). Seasonal growth and reproduction of an intertidal sponge *Haliclona permolis* (Bow.). *Biological Bulletin*, *151*, 108–125. https://doi.org/10.2307/1540709

Ereskovsky, A. V. (1999). Development of sponges of the order Haplosclerida. *Russian Journal of Marine Biology*, *25*, 361–371. https://www.researchgate.net/profile/Alexander-Ereskovsky/publication/286061571_Development_of_sponges_of_the_order_haplosclerida/links/5de0ed22a6fdcc2837f43bd7/Development-of-sponges-of-the-order-haplosclerida.pdf

Ereskovsky, A. V. (2000). Reproduction cycles and strategies of the cold-water sponges *Halisarca dujardini* (Demospongiae, Halisarcida), *Myxilla incrustans* and *Iophon piceus* (Demospongiae, Poecilosclerida) from the White Sea. *The Biological Bulletin, 198*(1), 77-87. https://doi.org/10.2307/1542805

Ereskovsky, A. V., Dubois, M., Ivanišević, J., Gazave, E., Lapébie, P., Tokina, D., & Pérez, T. (2013). Pluri-annual study of the reproduction of two Mediterranean *Oscarella* species (Porifera, Homoscleromorpha): Cycle, sex ratio, reproductive effort, and phenology. *Marine Biology, 160*, 423–438. https://doi.org/10.1007/s00227-012-2100-9

Ereskovsky, A. V., Geronimo, A., & Pérez, T. (2017). Asexual and puzzling sexual reproduction of the Mediterranean sponge *Haliclona fulva* (Demospongiae): Life cycle and cytological structures. *Invertebrate Biology, 136*(4), 403–421. https://doi.org/10.1111/ivb.12195

Ettinger-Epstein, P., Whalan, S. W., Battershill, C. N., & de Nys, R. (2007). Temperature cues gametogenesis and larval release in a tropical sponge. *Marine Biology, 153*, 171–178. https://doi.org/10.1007/s00227-007-0793-y

Fabricius, K. E. (2005). Effects of terrestrial runoff on the ecology of corals and coral reefs: Review and synthesis. *Marine Pollution Bulletin, 50*(2), 125–146. https://doi.org/10.1016/j.marpolbul.2004.11.028

Fell, P. E. (1974). Diapause in the gemmules of the marine sponge *Haliclona loosanoffi*, with a note on the gemmules of *Haliclona oculata*. *Biological Bulletin, 147*, 333–351. https://doi.org/10.2307/1540453.

Fell, P. E. (1976). The reproduction of *Haliclona loosanoffi* and its apparent relationship to water temperature. *The Biological Bulletin, 150*(2), 200–210. https://doi.org/10.2307/1540468

Fromont, J. (1994). Reproductive development and timing of tropical sponges (Order Haplosclerida) from the Great Barrier Reef, Australia. *Coral Reefs, 13*(2), 127–133. https://doi.org/10.1007/BF00300773.

Fromont, J., & Bergquist, P. R. (1994). Reproductive biology of three sponge species of the genus *Xestospongia* (Porifera: Demospongiae: Petrosida) from the Great Barrier Reef. *Coral Reefs, 13*, 119-126. https://doi.org/10.1007/BF00300772

Funayama, N. (2018). The cellular and molecular bases of the sponge stem cell systems underlying reproduction, homeostasis and regeneration. *International Journal of Developmental Biology, 62*(6-8), 513-525. https://doi.org/10.1387/ijdb.180016nf

Gaino, E., Frine, C., & Giuseppe, C. (2010). Reproduction of the intertidal sponge *Hymeniacidon perlevis* (Montagu) along a bathymetric gradient. *The Open Marine Biology Journal, 4*(1). https://doi.org/10.2174/1874450801004010047

Garren, M., Son, K., Tout, J., Seymour, J. R., & Stocker, R. (2016). Temperature-induced behavioral switches in a bacterial coral pathogen. *The ISME Journal, 10*(6), 1363–1372. https://doi.org/10.1038/ismej.2015.216

Gupta, N. J. (2017). Rhythms in marine invertebrates: Managing intertidal life alongside daily program. *Examines in Marine Biology and Oceanography, 1*(1), 17–21. https://doi.org/10.31031/EIMBO.2017.01.000504

Guzman, C., & Conaco, C. (2016). Gene expression dynamics accompanying the sponge thermal stress response. *PLOS ONE, 11*(10). https://doi.org/10.1371/journal.pone.0165368

Hill, M. S. (1996). Symbiotic zooxanthellae enhance pumping rates in the tropical sponge *Haliclona caerulea*. *Marine Biology, 125*(4), 591–596. https://doi.org/10.1007/BF00349246

Hill, M., & Hill, A. (2002). Morphological plasticity in the tropical sponge *Haliclona* sp.: Responses to predators and wave energy. *The Biological Bulletin*, *202*(1), 86–95. https://doi.org/10.2307/1543225

Hoffmann, F., Larsen, O., Thiel, V., Rapp, H. T., Pape, T., Michaelis, W., & Reitner, J. (2005). An anaerobic world of sponges. *Geomicrobiology Journal*, *22*(1–2), 1–10. https://doi.org/10.1080/01490450590922505

Huang, J. P., McClintock, J. B., Amsler, C. D., & Huang, Y. M. (2008). Mesofauna associated with the marine sponge *Amphimedon viridis*. Do its physical or chemical attributes provide a prospective refuge from fish predation? *Journal of Experimental Marine Biology and Ecology*, *362*(2), 95-100. https://doi.org/10.1016/j.jembe.2008.06.007

Ilan, M., & Loya, Y. (1988). Reproduction and settlement of the coral reef sponge *Niphates* sp. (Red Sea). *Proceedings of the Sixth International Coral Reef Symposium, 2*, 745-749.

Ilan, M., & Loya, Y. (1990). Sexual reproduction and settlement of the coral reef sponge *Chalinula* sp. from the Red Sea. *Marine Biology, 105*, 25-31. https://doi.org/10.1007/BF01344267

Ilan, M., Gugel, J., & Van Soest, R. (2004). Taxonomy, reproduction and ecology of new and known Red Sea sponges. *Sarsia: North Atlantic Marine Science, 89*(6), 388-410. https://doi.org/10.1080/00364820410002659

Jablonski, D., & Bottjer, D. J. (1990). The ecology of evolutionary innovations: The fossil record. In M. H. Nitecki (Ed.), *Evolutionary Innovations* (pp. 253–288). University of Chicago Press.

Johnson, J. A. (1979). Aspects of the reproductive cycles and population dynamics of intertidal sponges. *Journal of Experimental Marine Biology and Ecology, 36*(1), 1–17.

Kelman, D., Kashman, Y., Rosenberg, E., Ilan, M., Ifrach, I., & Loya, Y. (2001). Antimicrobial activity of the reef sponge *Amphimedon viridis* from the Red Sea: Evidence for selective toxicity. *Aquatic Microbial Ecology, 24*(1), 9-16. https://doi.org/10.3354/ame024009

Kelmo, F., Bell, J. J., & Attrill, M. J. (2013). Tolerance of sponge assemblages to temperature anomalies: Resilience and proliferation of sponges following the 1997–8 El Niño Southern Oscillation. *PLOS ONE, 8*(10). https://doi.org/10.1371/journal.pone.0076441

Knudby, A., Kenchington, E., & Murillo, F. J. (2013). Modeling the distribution of *Geodia* sponges and sponge grounds in the Northwest Atlantic. *PLOS ONE, 8*(12). https://doi.org/10.1371/journal.pone.0082306

Koopmans, M., & Wijffels, R. H. (2008). Seasonal growth rate of the sponge *Haliclona oculata* (Demospongiae: Haplosclerida). *Marine Biotechnology, 10,* 502–510. https://doi.org/10.1007/s10126-008-9086-9

Lanna, E. (2007). Life cycle of *Paraleucilla magna* Klautau, Monteiro, and Borojevic, 2004 (Porifera, Calcarea). In M. R. Custódio, G. Lôbo-Hajdu, E. Hajdu, & G. Muricy (Eds.), *Porifera Research: Biodiversity, Innovation, and Sustainability* (pp. 413–441). Museu Nacional, Rio de Janeiro.

Lanna, E., & Klautau, M. (2016). Some aspects of the oogenesis of three species of clathrinid sponges (Calcarea, Porifera). *Journal of the Marine Biological Association of the United Kingdom, 96*(2), 529-539. https://doi.org/10.1017/S0025315415001290

Lanna, E., Cajado, B., Silva, C. S., Hora, J., Porto, U., & Vasconcelos, V. (2018). Is the Orton's rule still valid? Tropical sponge fecundity, rather than periodicity, is modulated by temperature and other proximal cues. *Hydrobiologia, 815*(1), 187-205. https://doi.org/10.1007/s10750-018-3562-7

Lanna, E., Paranhos, R., Paiva, P. C., & Klautau, M. (2015). Environmental effects on the reproduction and fecundity of the introduced calcareous sponge *Paraleucilla magna* in Rio de Janeiro, Brazil. *Marine Ecology, 36*(4), 1075-1087. https://doi.org/10.1111/maec.12202

Lanna, E., Riesgo, A., Koutsouveli, V., & Leys, S. P. (2024). Physiology of reproduction in Porifera. In *Frontiers in Invertebrate Physiology: A Collection of Reviews* (pp. 1-63). Apple Academic Press. https://doi.org/10.1201/9781003403319-1

Lanna, E., Santos, D., Carvalho, V., Vilela, B., Santos, G. G., Skinner, L. F., Segal, B., Carraro, J. L., & Pinheiro, U. (2021). Different environmental drivers influence the reproduction of a tropical sponge (*Tedania ignis*) along a latitudinal gradient. *Marine Ecology Progress Series, 667*, 25-41. https://doi.org/10.3354/meps13712

Lee, J. H., & Riding, R. (2018). Marine oxygenation, lithistid sponges, and the early history of Paleozoic skeletal reefs. *Earth-Science Reviews, 181*, 98–121. https://doi.org/10.1016/j.earscirev.2018.04.003

Lee, Y. J., Cho, Y., & Tran, H. N. K. (2021). Secondary metabolites from the marine sponges of the genus *Petrosia*: A literature review of 43 years of research. *Marine Drugs, 19*(3), 122. https://doi.org/10.3390/md19030122

Leeuwis, R. H., & Gamperl, A. K. (2022). Adaptations and plastic phenotypic responses of marine animals to the environmental challenges of the high intertidal zone. In *Oceanography and Marine Biology* (pp. 625-679). CRC Press. https://doi.org/10.1201/9781003288602-13

Leong, W., & Pawlik, J. R. (2011). Comparison of reproductive patterns among seven Caribbean sponge species does not reveal a resource trade-off with chemical defenses. *Journal of Experimental Marine Biology and Ecology, 401*(1–2), 80–84. https://doi.org/10.1016/j.jembe.2011.02.043

Lessa, G. C., Cirano, M., Genz, F., Tanajura, C. A. S., & Silva, R. D. (2009). Oceanografia física. *Baía de Todos os Santos: aspectos oceanográficos, 1*, 68–119. Salvador: EDUFBA.

Leys, S. P., & Degnan, B. M. (2002). Embryogenesis and metamorphosis in a haplosclerid demosponge: gastrulation and transdifferentiation of larval ciliated cells to choanocytes. *Invertebrate Biology, 121*(3), 171-189. https://doi.org/10.1111/j.1744-7410.2002.tb00058.x

Maldonado, M. (2006). The ecology of the sponge larva. *Canadian Journal of Zoology, 84*(2), 175–194. https://doi.org/10.1139/z05-177

Maldonado, M., & Bergquist, P. R. (2002). Phylum Porifera. In C. M. Young, M. A. Sewell, & M. E. Rice (Eds.), *Atlas of Marine Invertebrate Larvae* (pp. 21–50). Academic Press.

Maldonado, M., & Riesgo, A. (2008a). Reproduction in the phylum Porifera: A synoptic overview. *Tissue and Cell, 40*(5–6), 387–426.

Maldonado, M., & Riesgo, A. (2008b). Reproductive output in a Mediterranean population of the homosclerophorid *Corticium candelabrum* (Porifera, Demospongiae), with notes on the ultrastructure and behavior of the larva. *Marine Ecology, 29*, 189-205. https://doi.org/10.1111/j.1439-0485.2008.00244.x

Maldonado, M., & Riesgo, A. (2009a). Gametogenesis, embryogenesis, and larval features of the oviparous sponge *Petrosia ficiformis* (Haplosclerida, Demospongiae). *Marine Biology, 156*, 2181-2197. https://doi.org/10.1007/s00227-009-1248-4

Maldonado, M., & Riesgo, A. (2009b). Reproduction in the phylum Porifera: A synoptic overview. *Treballs de la Societat Catalana de Biologia, 59*, 29–49.

Maldonado, M., & Young, C. M. (1998). Limits on the bathymetric distribution of keratose sponges: A field test in deep water. *Marine Ecology Progress Series, 174*, 123–139. https://doi.org/10.3354/meps174123

Maldonado, M., Ribes, M., & van Duyl, F. C. (2012). Nutrient fluxes through sponges: Biology, budgets, and ecological implications. *Advances in Marine Biology, 62*, 113–182. https://doi.org/10.1016/B978-0-12-394283-8.00003-5

Maritz, K., Calcino, A., Fahey, B., Degnan, B., & Degnan, S. M. (2010). Remarkable consistency of larval release in the spermcast-mating demosponge *Amphimedon queenslandica*. *The Open Marine Biology Journal*, *4*(1), 5-7. https://doi.org/10.2174/1874450801004010057

McMurray, S., Blum, J., & Pawlik, J. (2008). Redwood of the reef: Growth and age of the giant barrel sponge *Xestospongia muta* in the Florida Keys. *Marine Biology, 155*(2), 159–171. https://doi.org/10.1007/s00227-008-1014-z

Mercurio, M., Corriero, G., & Gaino, E. (2007). A 3-year investigation of sexual reproduction in *Geodia cydonium* (Jameson 1811) (Porifera, Demospongiae) from a semi-enclosed Mediterranean bay. *Marine Biology, 151*, 1491–1500. https://doi.org/10.1007/s00227-006-0584-x

Muricy, G., & Ribeiro, S. M. (1999). Shallow-water Haplosclerida (Porifera, Demospongiae) from Rio de Janeiro State, Brazil (Southwestern Atlantic). *Beaufortia, 49*(9), 83–108. https://repository.naturalis.nl/pub/504686/BEAU1999049009001.pdf

Nava, H., García-Madrigal, C. A. E., & Carballo, J. L. (2019). Relationships between boring sponge assemblages and the availability of dead coral substrate on Mexican Pacific

coral reefs. *Journal of the Marine Biological Association of the United Kingdom, 99*(4), 795–805. https://doi.org/10.1017/S0025315418000899

Oliveira, F. S., & Lanna, E. (2022). Population dynamics of three species of the genus *Aplysina* (Porifera, Demospongiae, Verongiida) in a tropical bay in the Western Atlantic Ocean. *Marine Biodiversity, 52*(2), 20. https://doi.org/10.1007/s12526-021-01257-x

Page, M. J., Northcote, P. T., Webb, V. L., Mackey, S., & Handley, S. J. (2005). Aquaculture trials for the production of biologically active metabolites in the New Zealand sponge *Mycale hentscheli* (Demospongiae: Poecilosclerida). *Aquaculture, 250*(1–2), 256–269. https://doi.org/10.1016/j.aquaculture.2005.04.069

Pérez, T., & Maldonado, M. (2019). Biology and ecology of sponge larvae. In J. Becerro, et al. (Eds.), *Advances in Marine Biology: Sponges as Key Components of Marine Ecosystems* (Vol. 85, pp. 89–145). Academic Press.

Perez, T., Perrin, B., Carteron, S., Vacelet, J., & Boury-Esnault, N. (2006). Celtodoryx girardae gen. nov. sp. nov., a new sponge species (Poecilosclerida: Demospongiae) invading the Gulf of Morbihan (North East Atlantic, France). *Cahiers de Biologie Marine, 47*(2), 205.

Pile, A. J., Patterson, M. R., & Savarese, M. (1997). Trophic effects of sponge feeding within Lake Baikal's littoral zone. *Limnology and Oceanography, 42*(1), 171–178. https://doi.org/10.4319/lo.1997.42.1.0178

Rapuano, H., Shlesinger, T., Roth, L., Bronstein, O., & Loya, Y. (2023). Coming of age: Annual onset of coral reproduction is determined by age rather than size. *iScience, 26*(5). https://doi.org/10.1016/j.isci.2023.106533

Redmond, N. E., Raleigh, J., van Soest, R. W. M., Kelly, M., & Travers, S. A. A. (2011). Phylogenetic Relationships of the Marine Haplosclerida (Phylum Porifera). *PLOS ONE*. https://doi.org/10.1371/journal.pone.0024344

Reiswig, H. M. (1971). In situ pumping activities of tropical Demospongiae. *Marine Biology, 9*, 38–50. https://doi.org/10.1007/BF00348816

Reiswig, H. M. (1973). Particle feeding in natural populations of three marine Demosponges. *Biological Bulletin, 145*(2), 302–312. https://doi.org/10.2307/1540270

Ribes, M., Coma, R., & Gili, J. M. (1999). Heterotrophic feeding in benthic suspension feeders: The natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dictyoceratida). *Marine Ecology Progress Series, 176*, 179–190. https://doi.org/10.3354/meps176179

Ricciardi, A., & Bourget, E. (1999). Global patterns of macroinvertebrate biomass in marine intertidal communities. *Marine Ecology Progress Series, 185*, 21–35. https://doi.org/10.3354/meps185021

Riesgo, A., & Maldonado, M. (2008). Differences in reproductive timing among sponges sharing habitat and thermal regime. *Invertebrate Biology, 127*, 357-367. https://doi.org/10.1111/j.1744-7410.2008.00128.x

Riesgo, A., Maldonado, M., & Durfort, M. (2007). Dynamics of gametogenesis, embryogenesis, and larval release in a Mediterranean homosclerophorid demosponge. *Marine and Freshwater Research, 58*(4), 398–417. https://doi.org/10.1071/MF06052

Riesgo, A., Novo, M., Sharma, P. P., Peterson, M., Maldonado, M., & Giribet, G. (2014). Inferring the ancestral sexuality and reproductive condition in sponges (Porifera). *Zoologica Scripta, 43*(1), 101–117. https://doi.org/10.1111/zsc.12031

Riesgo, A., Taboada, S., Sanchez-Vila, L., Sola, J., Bertran, A., & Avila, C. (2015). Some like it fat: Comparative ultrastructure of the embryo in two demosponges of the genus *Mycale* (order Poecilosclerida) from Antarctica and the Caribbean. *PLOS ONE, 10*(3). https://doi.org/10.1371/journal.pone.0118805

Rützler, K. (1975). The role of burrowing sponges in bioerosion. *Oecologia, 19*(3), 203–216. https://link.springer.com/article/10.1007/BF00345306

Schmahl, G. (1985). Cytological studies on the reproduction and development of the Caribbean coral reef sponge *Tethya actinia* (Porifera, Demospongiae). *Zoomorphology, 105*, 377–383. https://doi.org/10.1007/BF00345306

Simpson, T. L. (1984). *The Cell Biology of Sponges*. Springer-Verlag.

Stearns, S. C. (1976). Life-history tactics: A review of the ideas. *The Quarterly Review of Biology, 51*(1), 3-47. https://doi.org/10.1086/409052

Stephens, K. M., Galvin, J., Lawless, A., & McCormack, G. P. (2012). Reproduction of *Haliclona indistincta* (Bowerbank, 1866) (Phylum Porifera: Order Haplosclerida). *Journal of the Marine Biological Association of the United Kingdom*. doi:10.1017/S0025315412001300.

Tamaki, A., Itoh, J. I., Hongo, Y., Takeuchi, S., & Takikawa, T. (2018). Normal delayed establishment of a semilunar brooding and larval release cycle in the course of the reproductive season of the ghost shrimp population on a warm temperate intertidal sandflat. *Journal of Shellfish Research, 37*(3), 529–570. https://doi.org/10.2983/035.037.0309

Turon, X., Galera, J., & Uriz, M. J. (1997). Clearance rates and aquiferous systems in sponges lacking choanocyte chambers: Redescription of *Calyx nicaensis* Schmidt (1870) (Porifera, Demospongiae). *Scientia Marina, 61*(4), 443–455.

https://doi.org/10.1002/(SICI)1097-010X(19970501)278:1<22::AID-JEZ3>3.0.CO;2-8

Van Soest, R. W. M., & Hooper, J. N. A. (2002). *Systema Porifera: A guide to the classification of sponges*. *Integrative and Comparative Biology*, *42*(3), 762–763.

Vasconcellos, V., & Lanna, E. (2022). Reproductive cycles of the oviparous sponges *Cinachyrella apion* (Spirophorida) and *Tethya maza* (Tethyida) in a tropical intertidal environment. *Invertebrate Biology, 141*(4), e12386. https://doi.org/10.1111/ivb.12386

Vinagre, C., Dias, M., Cereja, R., Abreu-Afonso, F., Flores, A. A. V., & Mendonça, V. (2019). Upper thermal limits and warming safety margins of coastal marine species: Indicator baseline for future reference. *Ecological Indicators, 102*, 644–649. https://doi.org/10.1016/j.ecolind.2019.03.030

Wahab, M. A. A., De Nys, R., Abdo, D., Webster, N., & Whalan, S. (2014). The influence of habitat on post-settlement processes, larval production and recruitment in a common coral reef sponge. *Journal of Experimental Marine Biology and Ecology, 461*, 162–172. https://doi.org/10.1016/j.jembe.2014.08.006

Wapstra, M., & Van Soest, R. W. M. (1987). Sexual reproduction, larval morphology and behaviour in demosponges from the southwest of the Netherlands. In *Taxonomy of Porifera: From the NE Atlantic and Mediterranean Sea* (pp. 281–307). Berlin, Heidelberg: Springer Berlin Heidelberg.

Webster, N. S., & Taylor, M. W. (2012). Marine sponges and their microbial symbionts: Love and other relationships. *Environmental Microbiology, 14*(2), 335–346. https://doi.org/10.1111/j.1462-2920.2011.02460.x

Wells, H. W., Wells, M. J., & Gray, I. E. (1960). Marine sponges of North Carolina. *Journal of the Elisha Mitchell Scientific Society, 76*(2), 200–245. http://www.jstor.org/stable/24334280

Whalan, S., Battershill, C., & de Nys, R. (2007). Sexual reproduction of the brooding sponge *Rhopaloeides odorabile*. *Coral Reefs, 26,* 655–663. https://doi.org/10.1007/s00338-007-0236-8

Witte, U., & Barthel, D. (1994). Reproductive cycle and oogenesis of *Halichondria panicea* (Pallas) in Kiel Bight. *Sponges in Time and Space*, 297–305. A.A. Balkema Publishers.

Wörheide, G., Dohrmann, M., Erpenbeck, D., Larroux, C., Maldonado, M., Voigt, O., Borchiellini, C., & Lavrov, D. V. (2012). Deep phylogeny and evolution of sponges (Phylum Porifera). *Advances in Marine Biology*, 61, 1–78. https://doi.org/10.1016/B978-0-12-387787-1.00007-6

Conclusão

Os resultados deste estudo indicam que *Amphimedon viridis* na zona entre-marés apresenta reprodução contínua, caracterizada por um baixo esforço reprodutivo. A espécie é vivípara e hermafrodita simultânea, compartilhando características reprodutivas comuns a outras esponjas da ordem Haplosclerida, incluindo gametogênese, embriogênese e o tipo larval (parenquimela). A densidade larval de *A. viridis* foi baixa, possivelmente devido às condições ambientais específicas da zona entre-marés, embora esse aspecto ainda não esteja totalmente compreendido. A densidade de ovócitos foi menor em comparação aos cistos espermáticos. E não encontramos diferença significativa entre os meses do estudo para o tamanho dos ovócitos e cistos espermáticos; no entanto, os embriões foram maiores durante os meses da estação seca. Observamos que a área de superfície, densidade e tamanho dos elementos reprodutivos foram influenciados por diversos fatores ambientais, sendo que, em regiões tropicais como Salvador, essas condições tendem a ser mais estáveis ao longo do ano. Entre os fatores ambientais analisados, a temperatura do ar, maré baixa, concentração de clorofila-a e precipitação foram os que mais influenciaram a reprodução e a área de superfície da espécie. O tamanho médio da população foi de 195,64 ± 230,60 cm^2, mostrando variações significativas entre os meses, no entanto, não foram encontradas correlações significativas entre a área de superfície e a densidade dos elementos reprodutivos. Embora o estudo tenha sido realizado integralmente durante o evento El Niño-Southern Oscillation (ENSO) 2023-2024, foi observado um efeito negativo da temperatura da água na densidade de ovócitos, sugerindo que esse fenômeno climático pode impactar a reprodução da espécie. Por fim, os dados sugerem que as estratégias reprodutivas de *Amphimedon viridis* são determinadas tanto por fatores filogenéticos quanto ambientais.

Referências Bibliográficas

AYLING, A. L. Patterns of sexuality, asexual reproduction, and recruitment in some subtidal marine Demospongiae. *The Biological Bulletin*, v. 158, n. 3, p. 271–282, 1980.

BERLINCK, R. G. et al. Chemical and pharmacological characterization of halitoxin from *Amphimedon viridis* (Porifera) from the southeastern Brazilian coast. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, v. 115, n. 2, p. 155-163, 1996.

BERLINCK, R. G. S. et al. Antimicrobial activity of the reef sponge *Amphimedon viridis* from the Red Sea: evidence for selective toxicity. *Aquatic Microbial Ecology*, v. 24, n. 1, p. 9-16, 2001.

BRUSCA, R. C.; MOORE, W.; SHUSTER, S. M. *Invertebrados*. 3. ed. Rio de Janeiro: Editora Guanabara Koogan Ltda, 2018.

CAJADO, B.; LANNA, E. How can environmental variables affect the sexual reproduction of a tropical symbiotic sponge? *Marine Biodiversity*, v. 51, n. 2, p. 23, 2021.

CARRIER, T. J. et al. Symbiont transmission in marine sponges: reproduction, development, and metamorphosis. *BMC Biology*, v. 20, n. 1, p. 100, 2022.

CAVALCANTI, F. F.; KLAUTAU, M. Solenoid: a new aquiferous system to Porifera. *Zoomorphology*, v. 130, p. 255-260, 2011.

CERQUEIRA, W. R. P. et al. Registro de petróleo em poríferos e cnidários durante o impacto agudo de derramamento no Nordeste brasileiro em 2019. *Scientia Plena*, v. 16, n. 8, 2020.

CHU, J. W.; REISWIG, H. M. Mechanisms of propagule release in the carnivorous sponge *Asbestopluma occidentalis*. *Invertebrate Biology*, v. 133, n. 2, p. 109-120, 2014.

CINAR, M. E. et al. Macro-benthic invertebrates associated with the black sponge *Sarcotragus foetidus* (Porifera) in the Levantine and Aegean Seas, with special emphasis on alien species. *Estuarine, Coastal and Shelf Science*, v. 227, p. 106306, 2019.

DE GOEIJ, J. M. et al. Surviving in a marine desert: the sponge loop retains resources within coral reefs. *Science*, v. 342, p. 108-110, 2013.

DE VOOGD, N. J. et al. World Porifera Database. Available at: https://www.marinespecies.org/porifera/. Accessed on: 5 Feb. 2024.

DOHRMANN, M.; WÖRHEIDE, G. Dating early animal evolution using phylogenomic data. *Scientific Reports*, v. 7, n. 1, p. 3599, 2017.

DOMINGOS, C.; LAGE, A.; MURICY, G. Overview of the biodiversity and distribution of the Class Homoscleromorpha in the Tropical Western Atlantic. *Journal of the Marine Biological Association of the United Kingdom*, v. 96, n. 2, p. 379-389, 2016.

ERESKOVSKY, A. V. *The comparative embryology of sponges*. Springer Science & Business Media, 2010.

FITT, W. Florida manatees *Trichechus manatus latirostris* actively consume the sponge *Chondrilla caribensis*. *PeerJ*, v. 8, p. 8443, 2020.

FROMONT, J. Reproductive development and timing of tropical sponges (Order Haplosclerida) from the Great Barrier Reef, Australia. *Coral Reefs*, v. 13, n. 2, p. 127-133, 1994.

FROMONT, J.; BERGQUIST, P. R. Reproductive biology of three sponge species of the genus *Xestospongia* (Porifera: Demospongiae: Petrosida) from the Great Barrier Reef. *Coral Reefs*, v. 13, p. 119-126, 1994.

FUNAYAMA, N. The cellular and molecular bases of the sponge stem cell systems underlying reproduction, homeostasis and regeneration. *International Journal of Developmental Biology*, v. 62, n. 6-8, p. 513-525, 2018.

GOLD, D. A. et al. Sterol and genomic analyses validate the sponge biomarker hypothesis. *Proceedings of the National Academy of Sciences*, v. 113, p. 2684-2689, 2016.

HADAS, E.; SHPIGEL, M.; ILAN, M. Particulate organic matter as a food source for a coral reef sponge. *Journal of Experimental Biology*, v. 212, n. 22, p. 3643-3650, 2009.

HAJDU, E.; PEIXINHO, S.; FERNANDEZ, J. C. C. *Esponjas marinhas da Bahia: guia de campo e laboratório*. 1. ed. Rio de Janeiro: Museu Nacional, 2011.

HELMUTH, B. et al. Climate change and latitudinal patterns of intertidal thermal stress. *Science*, v. 298, n. 5595, p. 1015-1017, 2002.

HELMUTH, B. From cells to coastlines: how can we use physiology to forecast the impacts of climate change? *Journal of Experimental Biology*, v. 212, n. 6, p. 753-760, 2009.

HENKEL, D.; BORKENHAGEN, K.; JANUSSEN, D. Phylogeny of the Hexactinellida: phylogenetic reconstruction of the subclass Hexasterophora based on morphological characters. *Journal of the Marine Biological Association of the*

United Kingdom, v. 95, n. 7, p. 1365-1369, 2015.

HILL, M. S.; HILL, A. L. Morphological plasticity in the tropical sponge *Anthosigmella varians*: responses to predators and wave energy. *The Biological Bulletin*, v. 202, n. 1, p. 86-95, 2002.

HOOPER, J. N.; VAN SOEST, R. W. Class Demospongiae Sollas, 1885. In: Systema Porifera: a guide to the classification of sponges. 15-51, 2002.

ILAN, M.; LOYA, Y. Sexual reproduction and settlement of the coral reef sponge *Chalinula* sp. from the Red Sea. *Marine Biology*, v. 105, p. 25–31, 1990.

KELMAN, D. et al. Antimicrobial activity of the reef sponge *Amphimedon viridis* from the Red Sea: evidence for selective toxicity. *Aquatic Microbial Ecology*, v. 24, n. 1, p. 9-16, 2001.

LAGE, A. et al. Taxonomy of *Plakina* (Porifera: Homoscleromorpha) from Aegean submarine caves, with descriptions of three new species and new characters for the genus. *Marine Biodiversity*, v. 49, p. 727-747, 2019.

LANNA, E. et al. Is the Orton's rule still valid? Tropical sponge fecundity, rather than periodicity, is modulated by temperature and other proximal cues. *Hydrobiologia*, v. 815, n. 1, p. 187-205, 2018.

LANNA, E. et al. Physiology of reproduction in Porifera. In: Frontiers in Invertebrate Physiology: A Collection of Reviews. Apple Academic Press, p. 1-63, 2024.

LANNA, E.; RIESGO, A. Sponge larvae do not swim that fast: a reply to Montgomery et al. (2019). *Journal of the Marine Biological Association of the United Kingdom*, v. 100, n. 1, p. 181-183, 2020.

LAVROV, D. V. et al. Phylomitogenomics bolsters the high-level classification of Demospongiae (phylum Porifera). *bioRxiv*, 2023.

LEE, Y. J.; CHO, Y.; TRAN, H. N. K. Secondary metabolites from the marine sponges of the genus *Petrosia*: A literature review of 43 years of research. *Marine Drugs*, v. 19, n. 3, p. 122, 2021.

LEE, Y.; LEE, J.; LEE, H. Microbial symbiosis in marine sponges. *Journal of Microbiology*, v. 39, n. 4, p. 254-264, 2001.

LEEUWIS, R. H.; GAMPERL, A. K. Adaptações e respostas fenotípicas plásticas de animais marinhos aos desafios ambientais da zona intermareal alta. In: CRC Press, *Oceanografia e Biologia Marinha*, p. 625–679, 2022.

LEYS, S. P.; DEGNAN, B. M. Embryogenesis and metamorphosis in a haplosclerid demosponge: gastrulation and transdifferentiation of larval ciliated cells to

choanocytes. *Invertebrate Biology*, v. 121, n. 3, p. 171-189, 2002.

LOPES, M. V.; KLAUTAU, M. Phylogeny and revision of *Leucaltis* and *Leucettusa* (Porifera: Calcarea), with new classification proposals and description of a new type of aquiferous system. *Zoological Journal of the Linnean Society*, v. 198, p. 691-746, 2023.

MALDONADO, M.; BERGQUIST, P. Phylum Porifera. In: YOUNG, C. M.; SEWELL, M. A.; RICE, M. E. (Eds.), *Atlas of marine invertebrate larvae*. Academic Press, p. 630, 2002.

MALDONADO, M.; RIESGO, A. Reproduction in the phylum Porifera: a synoptic overview. *Treballs de la Societat Catalana de Biologia*, v. 59, p. 29-49, 2009.

MORAES, F. C. *Esponjas das ilhas Oceânicas Brasileiras*. Rio de Janeiro: Museu Nacional, 2011.

MORROW, C.; CÁRDENAS, P. Proposal for a revised classification of the Demospongiae (Porifera). *Frontiers in Zoology*, v. 12, p. 7, 2015.

MURICY, G.; LOPES, D. A.; HAJDU, E.; CARVALHO, M. S.; et al. *Catalogue of Brazilian Porifera*. Rio de Janeiro: Museu Nacional, 2011.

PECHENIK, J. A. *Biologia dos Invertebrados*. 7. ed. Porto Alegre: Editora AMGH Ltda, 2016.

PERDICARIS, S.; VLACHOGIANNI, T.; VALAVANIDIS, A. Bioactive natural substances from marine sponges: new developments and prospects for future pharmaceuticals. *Natural Products Chemistry and Research*, v. 1, n. 3, p. 2329-6836, 2013.

PEREIRA, R. C.; SOARES-GOMES, A. *Biologia marinha*. Rio de Janeiro: Interciência, v. 1, p. 382, 2002.

REDMOND, A. K.; MCLYSAGHT, A. Reply to: Available data do not rule out Ctenophora as the sister group to all other Metazoa. *Nature Communications*, v. 14, n. 1, p. 710, 2023.

RESWIG, H. M. Natural gamete release and oviparity in Caribbean Demospongiae. In: HARRISON, F. M.; COWDEN, R. R. (Eds.), *Aspects of sponge biology*. Academic Press, New York, 1976.

RIESGO, A. et al. Ancestral sexuality and reproductive condition in sponges (Porifera). *Zoologica Scripta*, v. 43, n. 1, p. 101-117, 2014.

RIESGO, A. Sexual reproduction in demosponges: ecological and evolutive implications. PhD thesis, Departamento de Biología Celular, Universidad de Barcelona,

Barcelona, 2007. 266 p.

SANTOS, G. J. G. *Taxonomia das esponjas marinhas do Estado da Paraíba*. Tese de Doutorado - Universidade Federal de Pernambuco, Recife, 2016.

SARÀ, M. Porifera. In: ADIYODI, K. G.; ADIYODI, R. G. (Eds.), *Reproductive biology of invertebrates*, vol. 5. Wiley, Chichester, 1993.

SCHÖNBERG, C. H. L. Happy relationships between marine sponges and sediments – a review and some observations from Australia. *Journal of the Marine Biological Association of the United Kingdom*, v. 96, n. 2, p. 493-514, 2016.

SIMPSON, T. L. *The Cell Biology of Sponges*. Springer-Verlag, 1984.

SINGH, A.; THAKUR, N. L.; SHEIKH, F. Differential tolerance capacity of intertidal organisms (sponge and zoanthid) to the environmental stresses: Preliminary findings from a rockpool transplantation experiment. *Journal of Earth System Science*, v. 131, n. 1, p. 1, 2022.

SOMERO, G. N. Thermal physiology and vertical zonation of intertidal animals: optima, limits, and costs of living. *Integrative and Comparative Biology*, v. 42, n. 4, p. 780-789, 2002.

STABILI, L. et al. Filtering activity of *Spongia officinalis* var. *adriatica* (Schmidt) (Porifera, Demospongiae) on bacterioplankton: implications for bioremediation of polluted seawater. *Water Research*, v. 40, n. 16, p. 3083-3090, 2006.

TURNER, E. C. Possible fossils of poriferous bodies in early Neoproterozoic microbial reefs. *Nature*, v. 596, p. 87-91, 2021.

VAN SOEST, R. W.; HOOPER, J. N. Order Haplosclerida Topsent, 1928. In: HOOPER, J. N.; VAN SOEST, R. W. (Eds.), *Systema Porifera: a guide to the classification of sponges*, p. 831-832, 2002.

VARIJAKZHAN, D. et al. Bioactive compounds from marine sponges: fundamentals and applications. *Marine Drugs*, v. 19, n. 5, p. 246, 2021.

VASCONCELLOS, V.; LANNA, E. Reproductive cycles of the oviparous sponges *Cinachyrella apion* (Spirophorida) and *Tethya maza* (Tethyida) in a tropical intertidal environment. *Invertebrate Biology*, v. 141, n. 4, p. e12386, 2022.

WELLS, H. W.; WELLS, M. J.; GRAY, I. E. Marine sponges of North Carolina. *Journal of the Elisha Mitchell Scientific Society*, v. 76, n. 2, p. 200-245, 1960.

WÖRHEIDE, G. et al. Deep phylogeny and evolution of sponges (phylum Porifera). *Advances in Marine Biology*, v. 61, p. 1-78, 2012.

WÖRHEIDE, G. et al. World Porifera Database. Retrieved from:

https://www.marinespecies.org/porifera/, 2024.

Anexos

Link: https://onlinelibrary.wiley.com/page/journal/17447410/homepage/forauthors.html

Invertebrate Biology

AUTHOR GUIDELINES

SECTIONS

1. Submission
2. Aims and Scope
3. Manuscript Categories and Requirements
4. Preparing the Submission
5. Editorial Policies and Ethical Considerations
6. Author Licensing
7. Publication Process After Acceptance
8. Post-Publication
9. Editorial Office Contact Details

1. SUBMISSION

Invertebrate Biology is transferring to a new publisher for 2025.

From **1st November 2024** please go to https://www.amicros.org/journal for information on how to submit.

2. AIMS AND SCOPE

Invertebrate Biology invites papers describing original, significant research focused on understanding any aspect of the biology of invertebrate animals (metazoans), including morphology and ultrastructure; genetics, phylogenetics, and evolution; physiology and ecology; neurobiology and behavior; biomechanics; reproduction and development; and cell and molecular biology. Although the journal has a significant history of publishing articles on protozoans and other organisms (as Transactions of the American Microscopical Society), since 1995 the title and the taxonomic focus of the journal has shifted to invertebrate animals.

The journal focuses on studies of the biology of invertebrates in nature, or studies that use laboratory methods to understand invertebrate diversity and adaptations. Studies of invertebrates used in medicine, agriculture, or aquaculture (especially studies of species that are mainly associated with human industrial activity) should have a significant component that is related to the biology of the study organisms in nature (or their adaptations for life). Authors of studies that have a purely applied context (e.g., behavior or physiology of agricultural organisms in a purely agricultural setting) are encouraged to submit those manuscripts to a journal focused on applied research.

We encourage authors to support the society that publishes *Invertebrate Biology*, and receive the journal in which their manuscript appears, by becoming a member of the American Microscopical Society. (See also Wiley Online Library.)

3. MANUSCRIPT CATEGORIES

Submissions to Invertebrate Biology fall into four main types: Research Article, Review Article, Book Review, and Description of a New Method.

Research Articles must be based on complete studies that represent a substantive advance in knowledge of the organisms.

Review Articles and Book Reviews are welcomed. Authors are encouraged to consult the editor-in-chief before submitting a manuscript in these categories.

Descriptions of a New Method may be considered for publication. Such manuscripts are expected to fulfill two criteria: first, the new method must be shown to work; second, the new method must either satisfy a problem of broad general application in research on invertebrates, or the new method must be applied to the study of some specific aspect of the biology of the study organism(s). Authors of manuscripts that describe a new method are encouraged to explain in a cover letter how the manuscript fulfills those criteria.
New species descriptions and taxonomic revisions may form a secondary component of a research article in which the primary focus of the study is some aspect of the biology (rather than the classification) of the organisms. Authors of alpha taxonomy studies are encouraged to submit instead to one of the appropriate journals focused on species descriptions and classification.

4. PREPARING THE SUBMISSION

A manuscript must adhere to Invertebrate Biology guidelines before it will be approved and sent out for review.

Parts of the Manuscript
The manuscript should be submitted in separate files: main text file; figures; tables; supporting information files.
Submitted manuscripts may be rejected by the editor-in-chief without this information. The corresponding author must provide an ORCID identifier; coauthors are encouraged to provide ORCID identifiers as well.

Main Text File

Manuscripts can be uploaded either as a single document (containing the main text, tables and figures), or with figures and tables provided as separate files. Should your manuscript reach revision stage, figures and tables must be provided as separate files. The main manuscript file can be submitted in Microsoft Word (.doc or .docx) or LaTex (.tex) format.

LaTex Guidelines for Submission

LaTex users must use the File Designation "Main Document" from the dropdown box. For reviewing purposes, you should upload to the journal's system (e.g. ScholarOne) a single .pdf that you have generated from your compiled source files.

Should your manuscript reach revision stage, figures and tables must be provided as separate files. The main manuscript file can be submitted in LaTex (.tex) format.

When submitting your revision, you must still upload a single .pdf that you have generated from your revised source files. You must use the File Designation "Main Document" from the dropdown box. In addition, you must upload your TeX source files.

For all your source files you must use the File Designation "Supplemental Material not for review". Previous versions of uploaded documents must be deleted.

If your manuscript is accepted for publication, we will use the files you upload to typeset your article within a totally digital workflow

The text file should be uploaded and presented in the following order:

i. Cover Page and keywords;
ii. Abstract;
iii. A short informative title containing the major key words. The title should not contain abbreviations (see Wiley's best practice SEO tips);
iv. The full names of the authors;
v. The author's institutional affiliations where the work was conducted, with a footnote for the author's present address if different from where the work was conducted;
vi. Main text;
vii. Acknowledgements;
viii. References;
ix. Illustrations (Table, each table complete with title and footnotes, Figures, etc.);
x. Figure Legends;
xi. Supporting information files (if relevant).

Figures and supporting information must be supplied as separate files.

Authorship
Please refer to the journal's Authorship policy in the Editorial Policies and Ethical Considerations section for details on author listing eligibility.

Conflict of Interest Statement
Authors will be asked to provide a conflict of interest statement during the submission process. For details on what to include in this section, see the 'Conflict of Interest' section in the Editorial Policies and Ethical Considerations section below. Submitting authors should ensure they liaise with all co-authors to confirm agreement with the final statement.

Cover Page
The first manuscript page should be a cover page that includes the manuscript title; full names and institutional affiliations for each author; full contact information for the corresponding author.

Keywords
On the Cover Page, include five additional keywords not in the title.

Abstract
The second page should include an Abstract that summarizes the main findings, conclusions, and significance of the work in a concise and informative way. After acceptance, the editors will invite authors to add a second Abstract translated into an additional language. Authors are encouraged (but not required) to improve the accessibility of published research findings by adding a second-language Abstract. The second-language Abstract will not be copy edited, so authors should ensure that the content and meaning of the Abstract are the same in both languages.

Headings
Four main headings of the manuscript are numbered sequentially: 1. INTRODUCTION; 2. METHODS; 3.

RESULTS; 4. DISCUSSION. Subheadings are numbered sequentially within each main heading (e.g., 2.1 Study location; 2.1.2 Sample sites). Subheadings should be no more than 40 characters.

Main Text

Manuscripts must be written in clear, concise English. Manuscript text files should be double-spaced, with margins of 2.5 cm. Please use SI and metric units throughout: hr, hour; min, minute; s, second; ms, millisecond; L, liter; ml, milliliter; °C, degrees Celsius (please use the degree symbol, not a superscript "o"). For ratio units use a slash (e.g., "mg/kg"), but for compound ratios use superscripts (e.g., "mg kg^{-1} hr^{-1}"). Use the unit of measurement as the abbreviation, not the name of the variable (e.g., "hr" or "min" for time, not "t"). Authors are asked to avoid using slash phrases such as "and/or". Latin abbreviations such as "e.g.,", "i.e.," and "etc." should be used in parenthetical phrases only.

Acknowledgments

Contributions from anyone who does not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgments section. Financial and material support should also be mentioned. Thanks to anonymous reviewers are not appropriate.

References

The references should be prepared according to the ***Publication Manual of the American Psychological Association*** (APA) (7th edition, 2020). This means in-text citations should follow the author-date style in which the author's last name and the year of publication for the source should appear in the text, for example, (Jones, 1998). Articles with one or two authors include all names in every in-text citation; articles with three or more authors abbreviate to the first author name plus et al. Include the year in the indirect repeated citations, for example, Several studies (Smith & John, 2005a, 2005b; Smith 2003a, 2003b).
If two references with same year shorten to the same form, cite the surnames of the first authors and of as many of the subsequent authors as necessary to distinguish the two references, followed by a comma and et al., for example, Bradley, Ramirez, and Soo (1994) and Bradley, Soo, et al. (1994).
If two references with six or more authors shorten to the same form, cite the surnames of the first authors and of as many of the subsequent authors as necessary to distinguish the two references, followed by a comma and et al. For example if references 'Barrett, Koenig, Cave, Tang, Lane, and Gabriel (1996)' and 'Barrett, Koenig, Wood, Kengman, Mosy, and Daly (1996)' appears in list, then these can be cited, respectively, as follows: Barrett, Koenig, Cave, et al. (1996) and Barrett, Koenig, Wood, et al. (1996).

The complete reference list should be sorted alphabetically by name and appear at the end of the manuscript. Please note that for journal articles, issue numbers are not included unless each issue in the volume begins with page 1, and a DOI should be provided for all references where available. For more information about APA referencing style, please refer to the **APA FAQ**.

Reference examples follow:

Journal Article:

Audrain-McGovern, J., Lerman, C., Wileyto, E. P., Rodriguez, D., & Shields, P. G. (2004). Interacting effects of genetic predisposition: Depression on adolescent smoking progression. *American Journal of Psychiatry, 161*, 1224–1230.

Note: delete issue numbers for journals with consecutive pagination within a volume.

Surnames and initials for up to 20 authors should be provided in the reference list. If there are more than 20 authors, then list 19 authors, with ellipses, followed by last author:

Pegion, K., Kirtman, B. P., Becker, E., Collins, D. C., LaJoie, E., Burgman, R., Bell, R., DelSole, R., Min, D., Zhu, Y., Li, W., Sinsky, E., Guan, H., Gottschalck, J., Metzger, E. J., Barton, N. P., Achuthavarier, D., Marshak, J., Koster, R., . . . Kim, H. (2019). The subseasonal experiment (SubX): A multimodel subseasonal prediction experiment. *Bulletin of the American Meteorological Society, 100*(10), 2043–2061.

Book:

The publisher location is no longer included in the reference.

Covey, S. R. (2013). *The 7 habits of highly effective people: Powerful lessons in personal change*. Simon & Schuster.

Book Chapter:

Harrison, P. L. (2011). Sexual reproduction of scleractinian corals. In Z. Dubinsky & N. Stambler (Eds.), *Coral reefs: An ecosystem in transition* (pp. 59–85). Springer.

Illustrations

Whenever practical, data should be presented in graphs rather than tables. Authors are encouraged to design tables and figures with the final journal page size in mind: each item should be formatted so that it is at least 85 mm wide (the width of a single column in the two-column journal layout); no more than 175 mm wide (the full-page width); and no more than 230 mm high (the full-page height). All elements of each table or figure (letters, structures, labels, symbols) must be large enough (at least 1.5 mm high) to be clear and readable at final size.

Tables must be submitted as text (not as an image, or a PDF). Authors are asked to use the Microsoft Word table formatting tools (or other similar format). Please do not make tables out of tab-delimited text. Each table should be numbered sequentially in the order in which it is referred to in the main text.

Tables should be self-contained and complement, not duplicate, information contained in the text. Legends should be concise but comprehensive – the table, legend, and footnotes must be understandable without reference to the text.

Figures

Invertebrate Biology now accepts embedded rich media files (video, audio) as figures. Video/audio files should be submitted along with the article itself, the transcript, and any other files at point of submission. The maximum file size for submitting audio or video content is 300 MB with a maximum duration of 5 minutes (note: the combined manuscript files for a submission, including video, audio, tables, figures, and text must not exceed 350 MB). Please refer to the embedded rich media author submission guidelines here for the complete instructions.

Although authors are encouraged to send the highest-quality figures possible, for peer-review purposes, a wide variety of formats, sizes, and resolutions are acceptable. Click here for the basic figure requirements for figures submitted with manuscripts for initial peer review, as well as the more detailed post-acceptance figure requirements.

Figures must be submitted in publication-ready form. Each figure should be numbered sequentially in the order in which it is referred to in the main text. Each figure must be uploaded as an individual electronic file, one file per numbered figure. Additionally, figures may be inserted into the main text file if desired (reviewers often find it helpful to see the figure alongside the relevant text). Please use the RGB color palette for color figures; please save figures without color content as grayscale (to reduce file sizes and upload times).

Figures composed as pixel-based images (e.g., photographs, micrographs, drawings) can be submitted as bitmaps in Tagged Image File Format (.tif), PNG (.png), or JPEG (.jpeg).TIFF images should have dimensions of at least 85 mm wide, up to 175 mm wide and up to 230 mm high, with a resolution at those dimensions of at least 300 dpi (for RGB color) or 500 dpi (for grayscale images). Micrographs, maps, anatomical drawings, and similar images should each include a scale bar in the figure and its value in the figure or the legend; please do not use expressions of magnification. Please also refer to the figure preparation guidelines here for complete intstructions and submission checklist.

Maps or charts should not be based on Google Earth, Google Maps, or other copyright materials or data without express consent of the copyright holder. Authors are encouraged to draw maps or charts of study areas by using open-source tools and data, or using maps and charts that are in the public domain.

Figures composed as line art or data illustrations (e.g., scatterplots, bar graphs, box-and-whisker plots, regressions) should be submitted as vector graphics in Encapsulated PostScript (.eps) format or in PDF format. Data graphics with color content should use the RGB color palette. It is acceptable but less desirable to submit line art as TIFF images; for line art submitted in TIFF format, the minimum acceptable resolution is 1200 dpi at final published size (at least 85 mm wide, up to 175 mm wide, up to 230 mm high).

Figures composed from multiple parts or panels must be submitted as a single electronic file (not as separate panels or parts). Each panel should be sequentially labeled with an upper-case letter in parentheses (e.g., "(A)", "(B)", etc.). Figures composed from both color and grayscale images should be submitted at the higher resolution (i.e., 500 dpi); figures composed from both line art and bitmaps (e.g., data graphics plus micrographs) should be submitted as a TIFF bitmap at the highest resolution (i.e., 1200 dpi). Authors are encouraged to avoid placing a frame or box around individual panels in multipart figures, except where the frame adds information (e.g., frames that show latitude and longitude on maps or charts, or gutters that separate histological images from each other).

Wherever possible, authors should adopt a uniform style for labeling panels in multipart figures, structures in micrographs or anatomical drawings, locations on maps, or differences among treatment groups in data graphics (e.g., upper-case letters for panels in the upper-right corner of each panel; scale bars for each panel in the lower left corner). To avoid confusion between labels for panels and labels for other elements, please use lower-case letters or abbreviated phrases for anatomical structures and other elements (e.g., "a" or "ant"), or to indicate differences among treatment groups (e.g., "a", "b", etc.); use upper-case letters ("A", "B", etc.) for panel labels only.

Full-page multipanel figures with high-resolution bitmap images may be large files. LZW compression (e.g., in Photoshop) can be used to reduce file size without affecting image quality. Further information on preparing electronic figures is available at Wiley Author Services. Authors can also consult the editor-in-chief for advice on preparing figures.

Authors are encouraged to submit additional, unlabeled photographs or drawings as possible cover illustrations.

For each species studied, the complete scientific name with taxonomic authority and date should be given; for example,*Ostrea edulis* LINNAEUS 1758 for a species and its original taxonomic description; or *Pisaster brevispinus* (STIMPSON 1857), for a species with a revised taxonomic description or genus assignment. Use SMALL CAPS for the taxonomic authority, and do not use a comma to separate the taxonomic authority from the year; this formatting helps to distinguish the taxonomic authority from the names of authors of cited reference works. For marine invertebrates, authors are encouraged to use the World Register of Marine Species as an authoritative source for up-to-date species names, taxonomic authorities, and classification.

The complete species name and taxonomic authority for the species studied should be given either at the first mention in the main text of the manuscript or in 2. METHODS, but not in the title or abstract. Use the full binomial (*Ostrea edulis*) at the first mention in each section of the paper, and then abbreviate (*O. edulis*, not *Ostrea* unless referring to the genus). The scientific name of any taxon is capitalized and treated as a singular noun, not a plural or an adjective (e.g., "*Cancer productus* is a wide-spread species", not "*Cancer productus* are widespread."). In particular, authors should avoid using taxon names as adjective phrases (e.g., "the chelae of *C. productus*", not "*C. productus* chelae").

Figure Legends

Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define or explain all abbreviations and units of measurement.

Color figures

Figures submitted in colour may be reproduced in color online free of charge. Please note, however, that it is preferable that line figures (e.g. graphs and charts) are supplied in black and white so that they are legible if printed by a reader in black and white.

Additional Files

Supporting Information files

Supporting information files are not essential to the article, but provide greater depth and background. They are hosted online and appear without editing or typesetting. They may include tables, figures, videos, datasets, etc.

Files containing supporting information can be uploaded to a third-party repository (e.g., Dryad, FigShare, GitHub) or can be uploaded with the other manuscript files as Supporting Information files and hosted with the full-text article on Wiley Online Library. Please cite digital object identifiers (DOI) or accession numbers in 2. METHODS (or elsewhere as appropriate) for files uploaded to a third-party repository. Authors are encouraged to consult repository-vetting sites such as re3data.org or fairsharing.org for help in identifying registered and certified data repositories relevant to any specific discipline or subject area.

For files to be hosted on Wiley Online Library and accessed via the full-text article, please add an additional section following the References and before the Figure legends, using the formatting shown below. Please include the name of each supporting information item and a brief description or caption. Each item should be numbered sequentially in the order in which it is cited in the main text (e.g., Appendix S1, etc.). At the first mention in the main text of any supporting information file, please use the full name (e.g., "(see Supporting Information Figure S1)", and for all subsequent instances use just the name of each item (e.g., "(see Figure S1)"). Links to each item in the list will be added to the full-text version of the article. No specific format is prescribed for supporting information files; authors are encouraged to adopt a clear and consistent style for the organization and presentation of data and other supplementary information.

Example:

Additional Supporting Information may be found online in the supporting tab for this article.

FIGURE S1 Brief text description of this figure

FIGURE S2 Brief text description of this figure

TABLE S1 Brief text description of this table

APPENDIX S1 Brief text description of this dataset or text file

VIDEO S1 Brief text description of this video

Click here for Wiley's FAQs on supporting information.

Note: if data, scripts, or other artefacts used to generate the analyses presented in the paper are available via a publicly available data repository, authors should include a reference to the location of the material within their paper.

General Style Points
The following points provide general advice on formatting and style.

- **Abbreviations:** In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Initially, use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation only.
- **Units of measurement:** Measurements should be given in SI or SI-derived units. Visit the Bureau International des Poids et Mesures (BIPM) website for more information about SI units.
- **Numbers:** numbers under 10 are spelt out, except for: measurements with a unit (8 mmol/l); age (6 weeks old), or lists with other numbers (11 arthropods, 9 annelids, 4 molluscs).
- **Trade Names:** Chemical substances should be referred to by the generic name only. Trade names should not be used. Drugs should be referred to by their generic names. If proprietary drugs have been used in the study, refer to these by their generic name, mentioning the proprietary name and the name and location of the manufacturer in parentheses.

Wiley Author Services

Manuscript Preparation Tips: Wiley has a range of resources for authors preparing manuscripts for submission available here. In particular, we encourage authors to consult Wiley's best practice tips on Writing for Search Engine Optimization.

Article Preparation Support
Wiley Editing Services offers expert help with English Language Editing, as well as translation, manuscript formatting, figure illustration, figure formatting, and graphical abstract design – so you can submit your manuscript with confidence.

Also, check out our resources for Preparing Your Article for general guidance about writing and preparing your manuscript.

5. EDITORIAL POLICIES AND ETHICAL CONSIDERATIONS

Peer Review and Acceptance
The acceptance criteria for all papers are the quality and originality of the research and its significance to journal readership. Papers will only be sent to review if the Editor-in-Chief determines that the paper meets the appropriate quality and relevance requirements.

Wiley's policy on the confidentiality of the review process is available here.

Animal Studies
A statement indicating that the protocol and procedures employed were ethically reviewed and approved, as well as the name of the body giving approval, must be included in the Methods section of the manuscript. Authors are encouraged to adhere to animal research reporting standards, for example

the ARRIVE guidelines for reporting study design and statistical analysis; experimental procedures; experimental animals and housing and husbandry. Authors should also state whether experiments were performed in accordance with relevant institutional and national guidelines for the care and use of laboratory animals:

• US authors should cite compliance with the US National Research Council's Guide for the Care and Use of Laboratory Animals, the US Public Health Service's Policy on Humane Care and Use of Laboratory Animals, and Guide for the Care and Use of Laboratory Animals.

• UK authors should conform to UK legislation under the Animals (Scientific Procedures) Act 1986 Amendment Regulations (SI 2012/3039).

• European authors outside the UK should conform to Directive 2010/63/EU.

Species Names
Upon its first use in the title, abstract, and text, the common name of a species should be followed by the scientific name (genus, species, and authority) in parentheses. For well-known species, however, scientific names may be omitted from article titles. If no common name exists in English, only the scientific name should be used.

Genetic Nomenclature
Sequence variants should be described in the text and tables using both DNA and protein designations whenever appropriate; *gene names should be italicized*; protein names should be in plain type face. Sequence variant nomenclature must follow the current HGVS guidelines; see varnomen.hgvs.org, where examples of acceptable nomenclature are provided.

Sequence Data
Nucleotide sequence data can be submitted in electronic form to any of the three major collaborative databases (DDBJ, EMBL, GenBank). Genome or transcriptome sequences should be uploaded to a suitable data archive (e.g., Sequence Read Archive); assemblies may also be uploaded to an online repository (e.g., Transcriptome Shotgun Assembly Database). The suggested wording for referring to accession-number information is: "These sequence data have been submitted to the GenBank databases under accession number U12345".

Structural Data
For papers describing structural data, atomic coordinates and the associated experimental data should be deposited in the appropriate databank (see below). **Please note that the data in databanks must be released, at the latest, upon publication of the article.** We trust in the cooperation of our authors to ensure that atomic coordinates and experimental data are released on time.

Organic and organometallic compounds: Crystallographic data should not be sent as Supporting Information, but should be deposited with the Cambridge Crystallographic Data Centre (CCDC) at ccdc.cam.ac.uk/services/structure%5Fdeposit.

Inorganic compounds: Fachinformationszentrum Karlsruhe (FIZ; fiz-karlsruhe.de).

Proteins and nucleic acids: Protein Data Bank (rcsb.org/pdb).

NMR spectroscopy data: BioMagResBank (bmrb.wisc.edu).

Conflict of Interest
The journal requires that all authors disclose any potential sources of conflict of interest. Any interest or relationship, financial or otherwise that might be perceived as influencing an author's objectivity is

considered a potential source of conflict of interest. These must be disclosed when directly relevant or directly related to the work that the authors describe in their manuscript. Potential sources of conflict of interest include, but are not limited to: patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. The existence of a conflict of interest does not preclude publication. If the authors have no conflict of interest to declare, they must also state this at submission. It is the responsibility of the corresponding author to review this policy with all authors and collectively to disclose with the submission ALL pertinent commercial and other relationships.

Funding
Authors should list all funding sources in the Acknowledgments section. Authors are responsible for the accuracy of their funder designation. If in doubt, please check the Open Funder Registry for the correct nomenclature: https://www.crossref.org/services/funder-registry/

Authorship
The list of authors should accurately illustrate who contributed to the work and how. Only individuals who meet all four of the criteria below qualify for authorship (and all individuals who fulfill the criteria should be included as authors). Each author should have:

1. Made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; and
2. Been involved in drafting the manuscript or revising it critically for important intellectual content; and
3. Given final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content; and
4. Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Contributions from anyone who does not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgments section (for example, to recognize contributions from people who provided technical help, collation of data, writing assistance, acquisition of funding, or a department chairperson who provided general support). Prior to submitting the article all authors should agree on the order in which their names will be listed in the manuscript.

Data Sharing and Data Accessibility
Please review Wiley's policy here. This journal encourages data sharing.

Authors are strongly encouraged to share the data, scripts, code, and other artefacts supporting the results in the paper by archiving them in an appropriate public repository. Authors may provide a data availability statement, including a link to the repository and persistent identifier(s), in order that this statement can appear in the published paper. Shared data should be cited.

Sample statements are available here. If published, statements will be placed in the heading of your manuscript.

Publication Ethics
This journal follows the core practices of the Committee on Publication Ethics (COPE) and handles cases of research and publication misconduct accordingly (https://publicationethics.org/core-practices)"

Note this journal uses iThenticate's CrossCheck software to detect instances of overlapping and similar text in submitted manuscripts. Read Wiley's Top 10 Publishing Ethics Tips for Authors here. Wiley's Publication Ethics Guidelines can be found here.

ORCID
As part of the journal's commitment to supporting authors at every step of the publishing process, the journal encourages the submitting author (only) to provide an ORCID iD when submitting a manuscript. Find more information here.

6. AUTHOR LICENSING

Authors of accepted articles will be required to sign a Copyright Transfer Agreement (CTA) form. The CTA will allow the publisher to publish the article, to administer rights, and to follow up on any infringements of copyright.
After each article is accepted, the corresponding author for the paper will receive an email prompt to login to Author Services and use the Wiley Author Licensing Service (WALS) to complete the license agreement on behalf of all authors of the paper.

Authors may choose to publish under the terms of the journal's standard copyright agreement, or OnlineOpen under the terms of a Creative Commons License.

General information regarding licensing and copyright is available here. To review the Creative Commons License options offered under OnlineOpen, please click here. (Note that certain funders mandate a particular type of CC license be used; to check this please click here.)

For authors signing the copyright transfer agreement:
If the OnlineOpen option is not selected, the corresponding author will be presented with the CTA to sign. The terms and conditions of the CTA can be previewed in the samples associated with the Copyright FAQs.

For authors choosing OnlineOpen:
OnlineOpen is available to authors of primary research articles who wish to make their article available to non-subscribers on publication, or whose funding agency requires grantees to archive the final version of their article. With OnlineOpen, the author, the author's funding agency, or the author's institution pays a fee to ensure that the article is made available to non-subscribers upon publication via Wiley Online Library, as well as deposited in the funding agency's preferred archive.

Authors are not asked or encouraged to indicate a preference for OnlineOpen before acceptance of the manuscript for publication. All manuscripts are peer reviewed in the same way and accepted or rejected based on the merits of the research, not based on the payment of the open-access fee.

If the OnlineOpen option is selected the corresponding author will have a choice of the following Creative Commons License Open Access Agreements (OAA):

Creative Commons Attribution License (CC-BY) OAA

Creative Commons Attribution Non-Commercial License (CC-BY-NC) OAA

Creative Commons Attribution Non-Commercial -NoDerivs License (CC-BY-NC-ND) OAA

General information regarding licensing and copyright is available on the **Wiley Author Services** and the **Wiley Open Access** websites.

For more information on this journal's APCs, please see the Open Access page.

7. PUBLICATION PROCESS AFTER ACCEPTANCE

Accepted Article Received in Production
When an accepted article is received by Wiley's production team, the corresponding author will receive an email asking them to login or register with Wiley Author Services. The author will be asked to sign a publication license at this point.

Accepted Articles
Accepted manuscripts go through two stages before publication: 1) final edit by journal staff and the EIC for content-related issues and general formatting; 2) copyediting and typesetting by the publisher's production staff. Authors have an opportunity to review, approve, and edit the accepted article at both stages. Authors will receive a content-edited version of their manuscript within approximately 1 month after acceptance. Authors will have 1 week to upload their final version of the manuscript based on changes directed by the content editor and the EIC. Manuscripts will not be assigned to an issue until the final version has been received.

The journal offers Wiley's Accepted Articles service for all manuscripts. This service ensures that accepted 'in press' manuscripts are published online shortly after acceptance, prior to copy-editing or typesetting. Accepted Articles are published online a few days after final acceptance, appear in PDF format only, and are given a Digital Object Identifier (DOI), which allows them to be cited and tracked. After publication of the final version article (the article of record), the DOI remains valid and can still be used to cite and access the article.

Proofs
Authors will receive an e-mail notification with a link and instructions for accessing HTML page proofs online. Page proofs should be carefully proofread for any copyediting or typesetting errors. Online guidelines are provided within the system. No special software is required, most common browsers are supported. Authors should also make sure that any renumbered tables, figures, or references match text citations and that figure legends correspond with text citations and actual figures. Proofs must be returned within 48 hours of receipt of the email. Review and correction of a PDF (rather than HTML) proof, and return of that proof via e-mail, is possible in the event that the online system cannot be used or accessed.

ELocators
This journal now uses eLocators (not page numbers) for individual articles. eLocators are unique identifies for an article that serve the same function page numbers have traditionally served in the print world. When citing the published article, please insert the eLocator in place of the page number. For more information, please visit the Author Services eLocator page here.

Programa de Pós-Graduação em
BIODIVERSIDADE E EVOLUÇÃO
UNIVERSIDADE FEDERAL DA BAHIA – INSTITUTO DE BIOLOGIA
Rua Barão de Geremoabo, s/n. Ondina – Salvador – Bahia – CEP. 40.170-000

ATA DA SESSÃO PÚBLICA DO COLEGIADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM BIODIVERSIDADE E EVOLUÇÃO - INSTITUTO DE BIOLOGIA – UFBA

Título de Dissertação: **Ciclo reprodutivo da esponja *Amphimedon viridis* (Demospongiae, Haplosclerida) em uma região entre-marés do Atlântico tropical**

Mestranda: **Beatriz Santos de Brito**
Orientador: **Emilio de Lanna Neto**

De acordo com o regimento geral da UFBA e com o regimento interno deste programa de pós-graduação, foram iniciados os trabalhos da Comissão Examinadora, composta pelos professores **Dr. Emilio de Lanna Neto** (Presidente), **Dr. Sérgio Nascimento Stampar** e **Dr. Wagner Ferreira Magalhães** às **09:00** horas do dia **20 de fevereiro de 2025**. A mestranda fez a apresentação oral de sua dissertação durante 31 minutos. Após o encerramento das arguições, às 11:00 horas, a Comissão Examinadora pronunciou-se pela sua aprovação conforme parecer em anexo. Esta Ata será assinada pelos membros da Comissão Examinadora e deste Colegiado de curso, para compor o processo de emissão do diploma.

Salvador, 20 de fevereiro de 2025.

COMISSÃO EXAMINADORA

gov.br Documento assinado digitalmente
EMILIO DE LANNA NETO
Data: 20/02/2025 11:08:00-0300
Verifique em https://validar.iti.gov.br

_____
Membro: Dr. Emilio de Lanna Neto
Universidade Federal da Bahia

gov.br Documento assinado digitalmente
SERGIO NASCIMENTO STAMPAR
Data: 20/02/2025 11:13:12-0300
Verifique em https://validar.iti.gov.br

_____
Membro: Dr. Sérgio Nascimento Stampar
Universidade Estadual Paulista “Júlio de Mesquita Filho”

gov.br Documento assinado digitalmente
WAGNER FERREIRA MAGALHAES
Data: 20/02/2025 11:21:03-0300
Verifique em https://validar.iti.gov.br

_____
Membro: Dr. Wagner Ferreira Magalhães
Universidade Federal da Bahia

Programa de Pós-Graduação em
BIODIVERSIDADE E EVOLUÇÃO
UNIVERSIDADE FEDERAL DA BAHIA – INSTITUTO DE BIOLOGIA
Rua Barão de Geremoabo, s/n. Ondina – Salvador – Bahia – CEP. 40.170-000

Título de Dissertação: **Ciclo reprodutivo da esponja *Amphimedon viridis* (Demospongiae, Haplosclerida) em uma região entre-marés do Atlântico tropical**

Mestranda: **Beatriz Santos de Brito**
Orientador: **Emilio de Lanna Neto**

BANCA EXAMINADORA

_____
Membro: Dr. Emilio de Lanna Neto
Universidade Federal da Bahia

_____
Membro: Dr. Sérgio Nascimento Stampar
Universidade Estadual Paulista "Julio de Mesquita Filho"

_____
Membro: Dr. Wagner Ferreira Magalhães
Universidade Federal da Bahia

PARECER DA BANCA

A Comissão Examinadora considera o trabalho de conclusão de curso:
[x]Aprovado
[ ]Reprovado

Os itens abaixo deverão ser modificados conforme sugestão da banca:

[ ] Introdução

_____

_____

_____

_____

[ ] Revisão Bibliográfica

_____

[ ] Metodologia

[ ] Resultados Obtidos

[ ] Conclusões

Para assinatura do orientador **APENAS APÓS A EFETUAÇÃO DAS MODIFICAÇÕES SUGERIDAS**

Declaro, para fins de homologação, que as modificações, sugeridas pela banca examinadora, acima mencionada, foram cumpridas integralmente.

Prof. Dr. Emilio de Lanna Neto
Orientador