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LUDIMILA CALHEIRA LAURINDO

REPRODUÇÃO DE ESPONJAS DE ÁGUAS CONTINENTAIS COMO MODELO PARA ESTUDO DE ECOTOXICOLOGIA E DISTRIBUIÇÃO

Recife
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Tese apresentada ao Programa de Pós-Graduação em Biologia Animal da Universidade Federal de Pernambuco, como requisito parcial para a obtenção do título de Doutor em Biologia Animal.

Área de concentração: Taxonomia dos grupos recentes.

Orientador: Profº. Dr. Ulisses dos Santos Pinheiro.
Coorientador: Profº. Dr. Emilio Lanna.

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"Quem nunca cometeu um erro,

nunca tentou algo novo".

(autor desconhecido)

RESUMO

A maioria das esponjas de águas continentais se reproduz assexuadamente, por gêmulas, e sexuadamente, por larvas parenquimela. As gêmulas são estruturas que desempenham uma função dupla, servindo tanto como dispositivos de dispersão quanto como corpos de resistência. Até o momento, apenas os aspectos morfológicos das gêmulas foram considerados para entender o padrão de distribuição das espécies. Ainda não foi testado se a capacidade de eclosão da gêmula pode influenciar na distribuição das esponjas. Dessa forma, o presente trabalho teve como objetivo investigar o desenvolvimento das esponjas a partir das gêmulas. No capítulo 1, é apresentada a descrição dos estágios de desenvolvimento de duas espécies de esponjas de águas continentais, *Heteromeyenia cristalina* e *Radiospongilla inesi*, com base em características fenotípicas. Os resultados mostraram diferenças nos estágios de desenvolvimento entre as espécies estudadas, sugerindo que tal processo é espécie-específico. Além disso, ao comparar o desenvolvimento dessas esponjas com espécies da região temperada, foram verificadas diferenças no tempo dos estágios, sendo que as espécies tropicais se desenvolvem mais rapidamente que as temperadas. No capítulo 2, foi testado o efeito de alguns metais pesados (cobre, cádmio e mercúrio), na eclosão das gêmulas e no início do desenvolvimento de *Radiospongilla inesi*. Como resultado, foi verificado que todos os metais pesados testados afetaram o desenvolvimento de *R. inesi*. No capítulo 3, é apresentado o primeiro registro do desenvolvimento sexuado e de larvas parenquimela para *Radiospongilla inesi*. Também foi observado que a espécie se reproduzia assexuadamente (produção de gêmulas) e sexuadamente (embriões e larvas) ao mesmo tempo. No capítulo 4, foi comparada a capacidade de eclosão das gêmulas de duas esponjas, *Radiospongilla inesi* (amplamente distribuídas na região neotropical), e *Spongilla alba* (conhecido apenas em ambientes mesohalinos), para testar se suas distribuições estão relacionadas à capacidade de eclosão das gêmulas em diferentes ambientes. Os resultados sugerem que as gêmulas das duas espécies têm distintas plasticidade adaptativa para lidar com diferentes tipos de águas continentais. Por fim, no capítulo 5, foi testada em diferentes tipos de águas continentais a capacidade de eclosão de quatro espécies com diferentes morfologia gemular. Como resultados, foi observado que as gêmulas das espécies estudadas apresentaram diferença na taxa de eclosão e, aparentemente, a distribuição dessas espécies está mais relacionada com a capacidade de eclosão e colonização de novos ambientes do que com a morfologia da gêmula.

Palavras-chave: Corpos de resistência. Larva. Metais Pesados. Porifera.

ABSTRACT

Most freshwater sponges reproduce asexually, through the production of gemmules, and sexually, releasing a parenchymella larvae. Gemmules are asexual structures that perform a double functional role, as dispersal devices and resting bodies. Until now, only the morphological aspects of the gemmules are being considered to understand the distribution pattern of freshwater sponges. However, whether the hatching capacity of gemmules influence the distribution of the species was not yet tested. Thus, this thesis aims to investigate the development of sponges from gemmules. In chapter 1, developmental stages of two neotropical freshwater sponges, *Heteromeyenia cristalina* e *Radiospongilla inesi*, are described based on phenotypic characteristics. The results showed differences in the developmental stages among the studied species, suggesting that development is species-specific. Moreover, when comparing the development of the tropical freshwater sponges with temperate species, differences in the timing of the stages were observed, being the tropical species faster than the temperate ones. In chapter 2, the effects of the heavy metals copper, cadmium and mercury on gemmules hatching and early development of the *Radiospongilla inesi* was tested. All tested heavy metals affected the development of *R. inesi*. In chapter 3, the sexual development and the parenchymella larvae of *Radiospongilla inesi* is reported for the first time. Also, this species was observed reproducing asexually (gemmules production) and sexually (embryos and parenchymella larvae) at the same time. In chapter 4, the hatchability of the gemmules of two sponges, *Radiospongilla inesi* (widely distributed in the neotropical region) and *Spongilla alba* (restricted to mixohanile environment) was compared to test if their distributions are related with the hatchability of the gemmules in different environments. Results suggest that the gemmules of the two studied species have distinct adaptive plasticities to deal with different types of inland waters. Finally, in chapter 5, the hatchability of gemmules of four species with different gemmular morphology was tested and compared in different kinds of inland waters. Gemmules of each tested species have different hatchability rates and, apparently, the distribution of these species is more related to the capacity of hatching and colonize new environments rather than the gemmule morphology.

Keywords: Resistant bodies. Larva. Heavy metals. Porifera.

SUMÁRIO

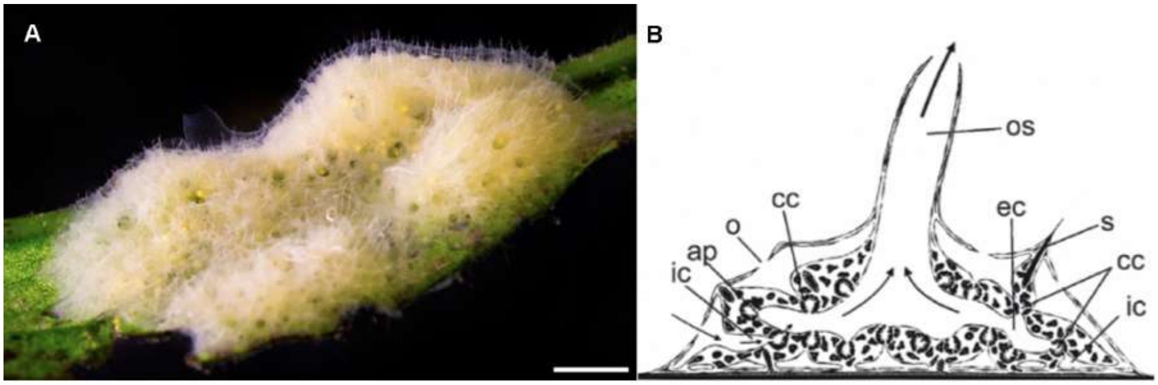
1 INTRODUÇÃO

O filo Porifera, popularmente conhecido como esponjas, reúne invertebrados exclusivamente sésseis, filtradores e aquáticos, que são considerados os animais viventes mais antigos, com registro fóssil de 760 milhões de anos atrás (YIN et al., 2015). Esses organismos são membros dominantes da comunidade bentônica e podem ser encontrados em locais bem diversos, como no continente Antártico, em recifes de corais, no lago Baikal, e do entremarés às profundezas oceânicas (MURICY; HAJDU, 2006; HAJDU et al., 2011).

O nome Porifera faz referência ao termo esponjoso e, apesar de dar ideia de macio, não há apenas esponjas flexíveis e algumas espécies são bem rígidas (HAJDU et al., 2011). As esponjas são muito diversificadas morfologicamente, podendo apresentar milímetros de comprimento até metros de diâmetros, além de exibirem grande variedade de formas, a depender da natureza e densidade dos seus componentes esqueléticos (HAJDU et al., 2011; VAN SOEST et al., 2012). Os esqueletos das esponjas, quando presentes, são secretados por células especializadas e formados por elementos minerais, chamados de espículas, constituídos de sílica ou carbonato de cálcio, que podem ser complementados ou substituídos por fibras de colágeno ou espongina (HAJDU et al., 2011; VAN SOEST et al., 2012). Todas essas características morfológicas são consideradas confiáveis pelos taxonomistas para descrição e identificação desses organismos a nível de espécie (HOOPER; VAN SOEST, 2002; MURICY; HAJDU, 2006). Contudo, estudos apontaram que esponjas submetidas a águas contaminadas com metais pesados podem apresentar modificações morfológicas (MYSING-GUBALA; POIRRIER, 1981; CEBRIAN; URIZ, 2007).

As esponjas possuem um plano corporal bastante simples, formado por três camadas: a pinacoderme, constituída por pinacócitos, com função de revestimento; a coanoderme, camada onde se localizam os coanócitos, células flageladas responsáveis pela circulação da água através do sistema aquífero da esponja; e o mesoílo, localizado entre a pinacoderme e a coanoderme e composto por elementos esqueléticos e por tipos celulares responsáveis pela digestão, reprodução e regeneração (Figura 1) (HAJDU et al., 2011; HOOPER; VAN SOEST, 2002; MURICY; HAJDU, 2006). O sistema aquífero é considerado a principal característica do grupo e permite que ocorra troca de materiais entre a esponja e o ambiente, processo fundamental para a realização das principais atividades fisiológicas do animal, como a reprodução, respiração e alimentação (BERGQUIST, 1978; MALDONADO; RIESGO, 2008).

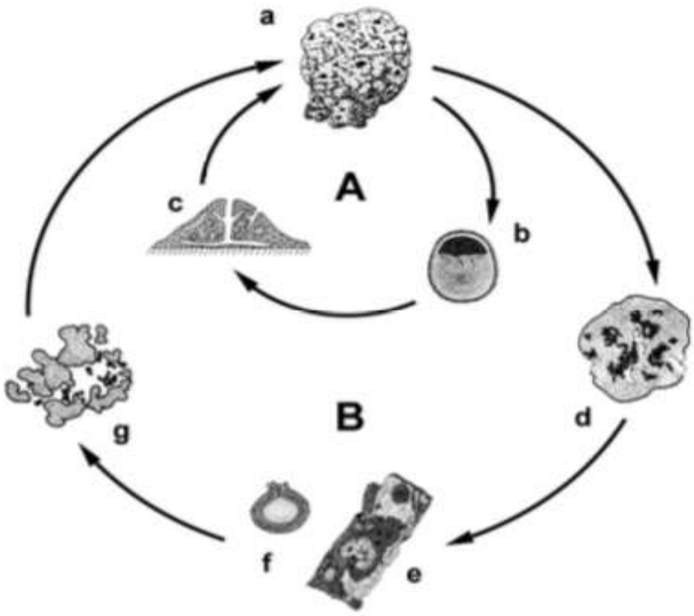
Figura 1. Estrutura corporal da esponja. **A**, Esponja de águas continentais *in situ*; **B**, Desenho esquemático de uma esponja juvenil com sistema aquífero. *ap* apófilo, *cc* câmara coanocitária, *ec* canal exalante, *ic* canal inalador, *o* óstio, *os* ósculo, *s* espícula. Escala: A = 4 mm.



Fonte: Pinheiro et al. (2015); Weissenfels (1975).

A reprodução das esponjas pode ocorrer de forma sexuada, com a liberação das larvas, ou assexuada, por fragmentação, brotos e gêmulas (MALDONADO; BERGQUIST, 2002; MALDONADO; RIESGO, 2008) (Figura 2). Na reprodução sexuada, em relação à produção de gametas, as esponjas podem ser hermafroditas ou gonocóricas (MALDONADO; RIESGO, 2008). Quanto ao desenvolvimento embrionário, as esponjas podem ser ovíparas, quando o desenvolvimento ocorrer externamente, ou vivíparas, quando há desenvolvimento interno (MALDONADO; RIESGO, 2008). De acordo com a origem embrionária e as características histológicas, pelo menos oito tipos de morfologia da larva (anfiblástula, calciblástula, triquimela, cinctoblástula, clavablástula, parenquimela, disférula, e hoplitomela) são conhecias para o filo Porifera (Figura 3) (MALDONADO; BERGQUIST, 2002; MALDONADO, 2006; ERESKOVSKY, 2010). Todas as larvas são lecitotróficas e de tempo de vida relativamente curto com duração variando entre as espécies (MALDONADO; BERGQUIST, 2002).

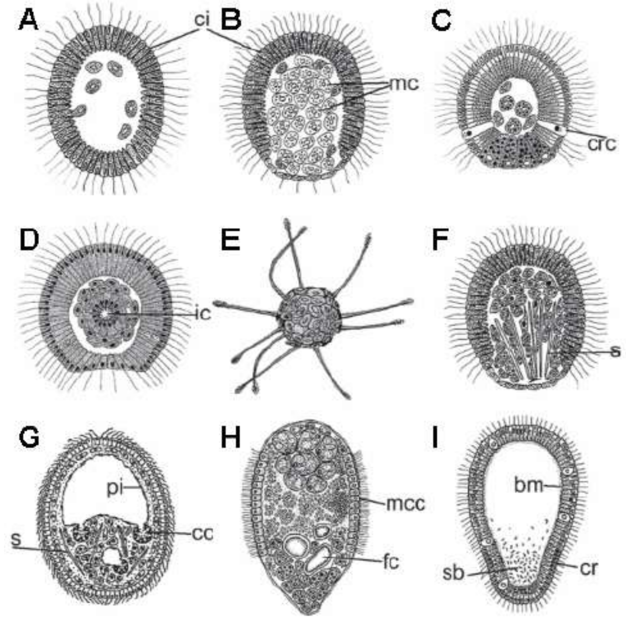
Figura 2. Ciclo de vida de esponjas de águas continentais que produzem gêmulas. **A**, Reprodução sexuada; **B**, Reprodução assexuada.



a Esponja ativa, **b** Larva parenquimela, **c** Estágio pós-larval, **d** Degeneração da esponja e gemulação, **e** Gêmulas em estágio de repouso, **f** Morfologia da gêmula, **g** Eclosão da gêmula e uma nova esponja é formada.

Fonte: Manconi; Pronzato (2016).

Figura 3. Desenho esquemático dos tipos de larvas de esponjas. **A**, Calciblástula; **B**, Clavablástula; **C**, Anfiblástula; **D**, Disférula; **E**, Hoplitomela; **F**, Parenquimela (espécies marinhas); **G**, Parenquimela (espécies de águas continentais); **H**, Triquimela; **I**, Cinctoblástula. *bm* membrana basal, *cc* câmara coanocitária, *ci* células ciliadas, *cr* células com cristaloides intranuclear, *crc* células em cruz, *fc* câmaras flageladas, *mc* células maternais, *ic* câmeras internas, *mcc* células multiciliares, *pi* pinacoderme da larva, *s* espículas, *sb* bactérias simbióticas.



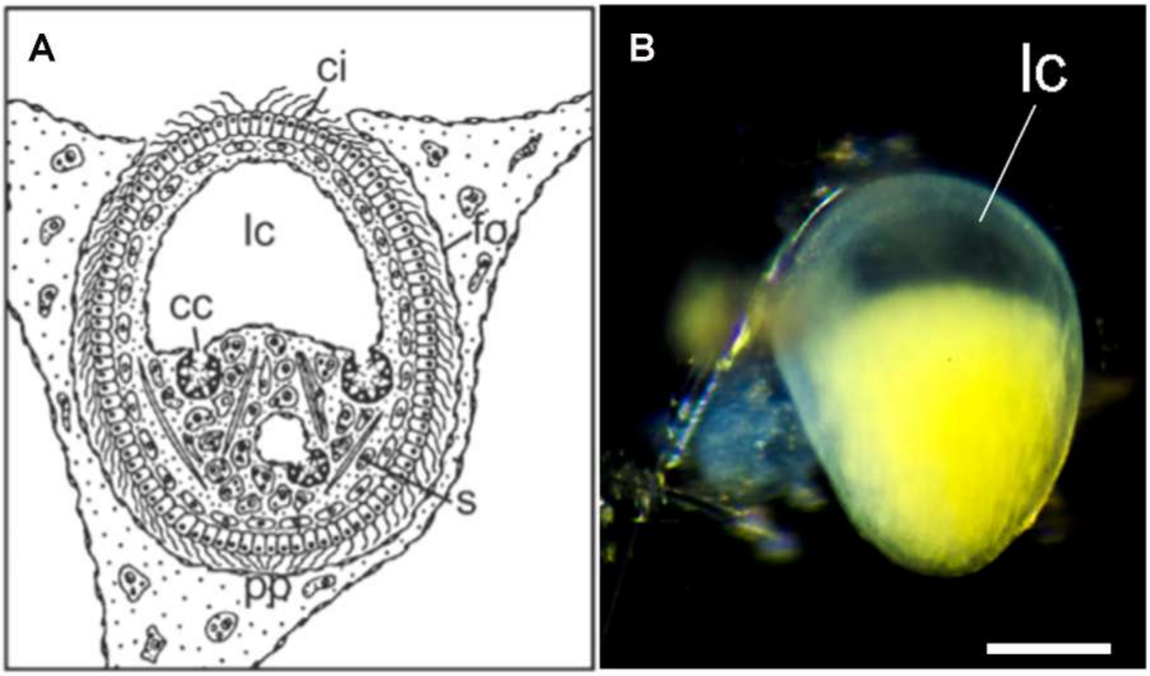
Fonte: Figura modificada de Ereskovsky (2010).

Atualmente, o filo Porifera inclui cerca de 9.200 espécies válidas, distribuídas em quatro classes: Hexactinellida Schmidt, 1870, Calcarea Bowerbank, 1862, Homoscleromorpha Bergquist, 1978 e Demospongiae Sollas, 1885 (VAN SOEST et al., 2020). Essa última é a mais diversa, com cerca de 85% das espécies atuais, e a mais amplamente distribuída em todos os ambientes aquáticos, incluindo os de águas continentais (MORROW; CÁRDENAS, 2015; MURICY; HAJDU, 2006; PAWLIK, 2003; HOOPER; VAN SOEST, 2002). Espécies de águas continentais pertencem a ordem Spongillida Manconi & Pronzato, 2002 e representam aproximadamente 3% de toda diversidade do filo (MANCONI; PRONZATO, 2007; PINHEIRO et al., 2015). Essa baixa diversidade já foi relacionada com a escassez de taxonomistas no grupo e a dificuldade de realização de inventários nesses ambientes (MANCONI; PRONZATO, 2007; PINHEIRO et al., 2015).

Esponjas da ordem Spongillida são caracterizadas pela presença de megascleras, espículas responsáveis pela estruturação das esponjas, que formam feixes pauci- a multiespiculares, juntamente com espongina (MANCONI; PRONZATO, 2002). As microscleras, quando presentes, estão espalhadas pelo corpo da esponja ou distribuídas pela superfície (MANCONI; PRONZATO, 2002). Por fim, as gemoscleras têm como função de revestir a gêmulas (corpos de resistência e propágulos assexuados contendo células totipotentes) e são utilizadas como principal caráter diagnóstico morfológico para identificação a nível de espécie (MANCONI; PRONZATO, 2002). Algumas famílias (Malawispongiidae Manconi & Pronzato, 2002, Lubomirskiidae Rezvoi, 1936 e Metschnikowiidae Czerniavsky, 1880) não produzem gêmulas e apenas a reprodução vivípara é conhecida, com a produção de larvas denominadas de parenquimela (MANCONI; PRONZATO, 2002).

As larvas parenquimela de Spongillida são morfologicamente mais complexas do que todas as outras larvas de Demospongiae (SIMPSON, 1984). Isso porque, além de apresentarem superfície total e uniformemente ciliada e o esqueleto em forma de leque, possuem câmaras de coanócitos e uma cavidade larval (Figura 4) (ERESKOVSKY, 2010). Essa cavidade aparentemente auxilia no controle da flutuação e, por estar presente apenas nas esponjas de águas continentais, é plausível considerá-la como um órgão com função osmorregulatória (MALDONADO, 2006; ERESKOVSKY, 2010).

Figura 4. Larva parenquimela de esponja de água doce. **A**, Desenho esquemático da larva em um folículo; **B**, larva vista sob microscópio estereoscópico. *lc* cavidade larval. Escala: B = 50 µm.



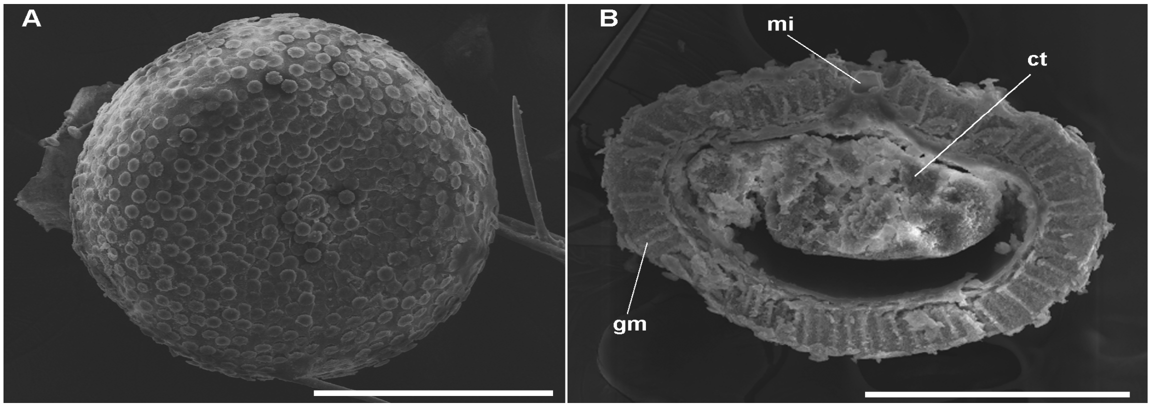
Fonte: Ereskovsky (2010); O autor (2020).

O ambiente de águas continentais difere do marinho principalmente pela distribuição espacial de suas águas e pelo fato de possuírem alta probabilidade de secar (PRONZATO; MANCONI, 1994). Águas continentais sofrem grande influência da variação sazonal, caracterizada pelas flutuações no nível da água, a exemplo de inundações, congelamentos e secas, que são ausentes no ambiente marinho (PRONZATO; MANCONI, 1994). Além disso, enquanto o mar é um habitat contínuo, o habitat de águas continentais é fragmentado, constituindo um empecilho para a dispersão larval (PRONZATO; MANCONI, 1994). Em razão disso, é crucial que espécies que habitam as águas continentais desempenhem estratégias de dispersão (VAN LEEUWEN et al., 2017), e é comum que a fauna desse ambiente possua também estratégias adaptativas de sobrevivência (CÁCERES, 1997). No caso das esponjas, a estratégia para sobreviver nessas condições abióticas desfavoráveis é a produção de gêmulas (PRONZATO; MANCONI, 1994).

As gêmulas são estruturas assexuadas que desempenham dupla função, tanto como dispositivos de dispersão quanto como corpos de resistência (MANCONI; PRONZATO, 2007). A dispersão da gêmulas pode ocorrer por meio da corrente d’água, pelo vento, e por animais

(MANCONI; PRONZATO, 2016), por exemplo, peixes (VOLKMER-RIBEIRO; GROSSER, 1981; MOREIRA; ZUANON, 2002) e aves (VAN LEEUWEN et al., 2017). Essas estruturas possuem células totipotentes, os tesócitos (arqueócitos em repouso), que geralmente são revestidas por espículas especializadas (gemoscleras) e camadas de espongina (Figura 5) (MANCONI; PRONZATO, 2002). O processo de germinação se inicia quando as células internas migram através de uma abertura (denominada micrópila) e começam a se diferenciar para formar uma nova esponja (SIMPSON, 1984). As gêmulas podem ser encontradas em espécies pertencentes a três das seis famílias viventes da ordem Spongillida: Spongillidae Gray, 1867, Metaniidae Volkmer-Ribeiro, 1986 e Potamolepidae Brien, 1967.

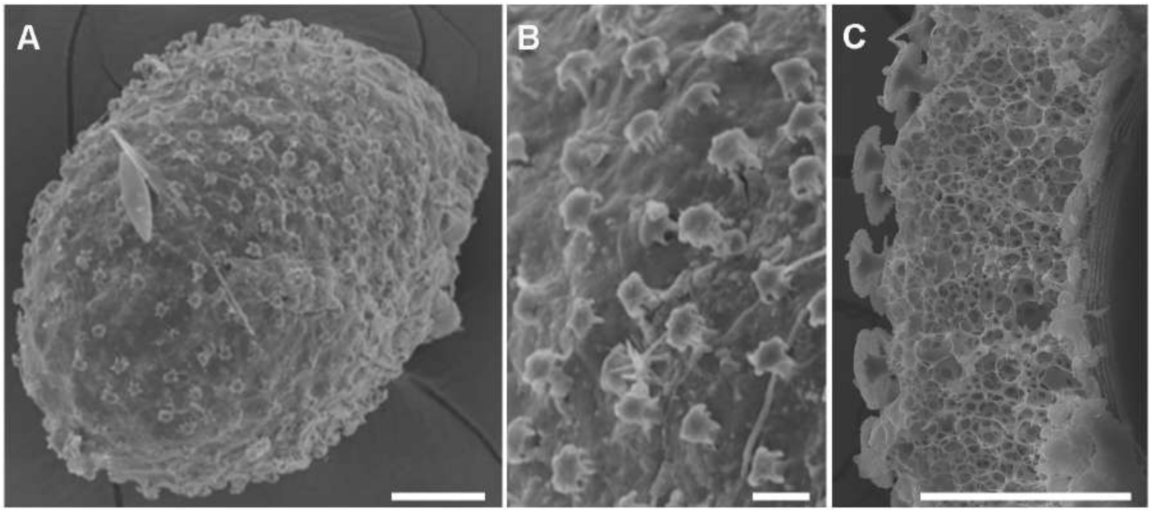
Figura 5. Gêmula vista sob microscopia eletrônica de varredura. **A**, Gêmula inteira; **B**, corte transversal de uma gêmula mostrando suas parte. *ct* células totipotentes, *gm* gemosclera, *m* micrópila. Escala: A, B = 300 µm.



Fonte: O autor (2020).

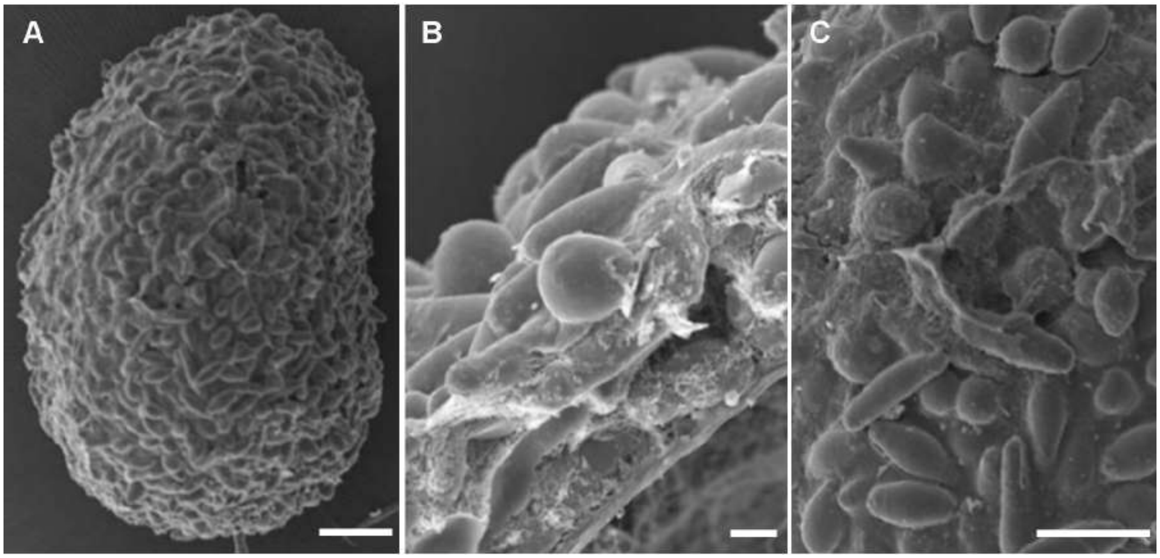
As gêmulas exibem diferentes níveis de complexidade morfológica (MANCONI; PRONZATO, 2007). Nas famílias Spongillidae e Metaniidae, particularmente, as gêmulas são consideradas morfologicamente complexas por apresentarem uma camada interna pneumática revestida por uma capa externa de gemoscleras completamente espinadas (Figura 6) (MANCONI; PRONZATO, 2007). A camada pneumática desenvolvida confere à gêmula a possibilidade de flutuar e realizar dispersão a jusante dos corpos d’águas, e as gemoscleras espinadas são capazes de se aderir a outros animais e serem transportadas (MANCONI; PRONZATO, 2007). Além disso, as gêmulas de Spongillidae e Metaniidae estão distribuídas por todo o corpo da esponja (MANCONI; PRONZATO, 2007). Por outro lado, as espécies da família Potamolepidae, por exemplo, possuem morfologia gemular simples com camada pneumática ausente e gemoscleras lisas e depositam suas gêmulas apenas na interface esponja-substrato (Figura 7) (MANCONI; PRONZATO, 2007).

Figura 6. Morfologia da gêmula complexa vista sob microscopia eletrônica de varredura. **A**, Gêmula inteira; **B**, Gemoscleras espinadas; **C**, Camada pneumática desenvolvida. Escala: A, C = 50 µm; B = 10 µm.



Fonte: Figura modificada de Pinheiro (2007); O autor (2020).

Figura 7. Morfologia da gêmula simples vista sob microscopia eletrônica de varredura. **A**, Gêmula inteira; **B**, Ausência de camada pneumática; **C**, Gemoscleras lisas. Escala: A = 100 µm; B = 10 µm; C = 50 µm.



Fonte: Figura modificada de Pinheiro (2007).

A presença ou ausência de gêmulas, bem como os níveis de complexidade gemular variam entre as famílias de Spongillida e têm sido considerados de grande impacto no sucesso da dispersão dos táxons de águas continentais (MANCONI; PRONZATO, 2002; EPENBECK et al., 2011). Manconi; Pronzato (1996; 2007) sugeriram que a distribuição das esponjas continentais está relacionada com a eficiência das gêmulas em atuar como dispositivo de dispersão. Como as famílias que não possuem gêmulas apresentam uma distribuição geográfica extremamente restrita (MANCONI; PRONZATO, 2002), parece que a ausência dessas estruturas está relacionada à uma condição de endemismo, por exemplo as esponjas que ocorrem no lagos antigos (MANCONI; PRONZATO, 2007). Nesses lagos, principalmente no Baikal e no Tanganica, estudos com abordagens moleculares têm sido realizados com o objetivo de analisar os padrões de radiação das espécies e a história evolutiva dessa fauna de esponjas (EPENBECK et al., 2011; ITSKOVICH et al., 2015). Contudo, mesmo compartilhando gêmulas complexas, existe divergência entre a distribuição ampla das espécies de Spongillidae e o padrão Circumtropical das de Metaniidae, que apresentam faixa geográfica semelhante à de Potamolepidae (MANCONI; PRONZATO, 2007). Até os dias atuais, apenas morfologia das gêmulas estão sendo consideradas para entender o padrão de distribuição das espécies de águas continentais.

O conhecimento sobre os estágios de desenvolvimento durante o processo de germinação tem sido usado como modelo para entender a fisiologia e biologia das esponjas de águas continentais (IMSIECKE et al., 1995; WINDSOR; LEYS, 2010; RIVERA et al., 2013), porém esses estudos foram realizados apenas em um grupo restrito de esponjas da Região Temperada. Sendo assim, estudo mais detalhado descrevendo os estágios de desenvolvimento das esponjas de água doce, especialmente pertencentes a outras linhagens e localidades, são necessários para entender como as esponjas são formadas a partir de gêmulas.

1.1 OBJETIVOS

1.1.1 Objetivo Geral

Investigar o desenvolvimento das esponjas de águas continentais a partir das gêmulas, verificando sua capacidade de eclosão frente a diferentes condições ambientais.

1.1.2 Objetivos Específicos

- ✓ Descrever os estágios de desenvolvimento de duas espécies de esponjas de águas continentais da Região Neotropical, *Heteromeyenia cristalina* Batista, Volkmer-Ribeiro & Melão, 2007 e *Radiospongilla inesi* Nicacio & Pinheiro, 2011;
- ✓ Comparar os estágios de desenvolvimento entre as duas espécies esponjas de águas continentais da Região Neotropical, e entre as espécies da Região Temperada;
- ✓ Verificar o efeito de metais pesados (Cd, Hg e Cu) no início do desenvolvimento de *Radiospongilla inesi*;
- ✓ Relatar a reprodução sexuada da esponja de águas continentais da Região Neotropical, *Radiospongilla inesi*;
- ✓ Verificar se as esponjas juvenis desenvolvidas a partir da larva diferem daquelas desenvolvidas a partir de gêmulas.
- ✓ Testar a distribuição de duas espécies de esponjas de águas continentais pertencentes à família Spongillidae Gray, 1867, ambas com morfologia gemular complexa – *Radiospongilla inesi*, amplamente distribuída na Região Neotropical, e *Spongilla alba* Carter, 1849 (Volkmer-Ribeiro & Machado, 2007), também amplamente distribuída, mas conhecida apenas em ambientes mixohalinos;
- ✓ Testar e comparar a capacidade de eclosão das gêmulas, em laboratório, de quatro espécies de esponjas de águas continentais da Região Neotropical: *Tubella variabilis* (Bonetto & Ezcurra de Drago, 1973) (Spongillidae) versus *Drulia ctenosclera* Volkmer & Mothes, 1981 (Metaniidae) e *Oncosclera navicella* (Carter, 1881) (Potamolepidae) versus *Radiospongilla inesi* Nicacio & Pinheiro, 2011 (Spongillidae);
- ✓ Avaliar se a capacidade de eclosão das gêmulas influencia na distribuição geográfica das esponjas.

2 TROPICAL FRESHWATER SPONGES DEVELOP FROM GEMMULES FASTER THAN THEIR TEMPERATE-REGION COUNTERPARTS

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3 EFFECTS OF HEAVY METALS ON EARLY DEVELOPMENT OF *RADIOSPONGILLA INESI* NICACIO & PINHEIRO, 2011 (PORIFERA: SPONGILLIDAE)

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Effects of heavy metals on early development of *Radiospongilla inesi* Nicacio & Pinheiro, 2011 (Porifera: Spongillidae)

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ABSTRACT. Aquatic environments have long been used as disposal areas of wastes contaminated by metals that produce harmful effects in aquatics organisms. Among the aquatic fauna, sponges are particularly more susceptible to heavy metal pollution due to high water filtering and heavy metals accumulation capacities. The effects of heavy metals accumulation in sponges have been evaluated at different organization levels and stages of the sponge's life cycle. Even so, data regarding the toxicity of heavy metals on early developmental phases of the sponge's life cycle are, in general, scarce. We studied for first time the effects of the heavy metals (copper, cadmium and mercury) in gemmules hatching and early development of the freshwater sponge, *Radiospongilla inesi*. Our results indicate that cadmium and mercury exposures can affect not only hatching gemmules, but also the development of fundamental structures in early stages of juvenile *Radiospongilla inesi* sponges, and subsequent colonization of new habitats. Although cooper exposure has not influenced in gemmules hatching, a delay occurs at the start of each stage, and at the highest concentrations tested, there was no development of juvenile sponges.

Keywords: Ecotoxicology, Gemmules, Sponge, Sublethal effects.

1. Introduction

Among the 94 natural environmental elements, 70 are metals, including some that are essential to life (Wood, 2012). On the other hand, when organisms are exposed to elevated levels of these metals, they can become potentially toxicant. Aquatic environments have long been used as disposal areas of wastes contaminated by metals that produce harmful effects in aquatics organisms due to their bioaccumulation and toxicity (Wood, 2012).

In the last decades, significant amounts of pollutants have been released in aquatic environments (Cebrian & Uriz, 2007a), including the metals copper (Cu), cadmium (Cd) and mercury (Hg). Under natural conditions, these three metals are commonly found in the water, but anthropogenic sources that increase their concentrations can make them become environmental pollutants (Wood, 2012). The main anthropogenic sources of copper, cadmium and mercury are industrial effluents, mining wastes, fossil-fuel combustion, antifouling, and fertilizers (Grosell, 2012; Kidd & Batchelar, 2012; McGeer et al., 2012). The excess of these pollutants in the water and extended exposure of aquatic animals to elevated concentrations of them can cause severe consequences, from cell mortality, alterations in the food chain and, in last instance, the mortality of aquatic species (Cebrian & Uriz, 2007b; Kidd & Batchelar, 2012; McGeer et al., 2012). Among these pollutants, copper is one of the most abundant, and its a metal essential for life, but the excess copper in the living cells causes toxicity (Grosell, 2012). Cadmium is another relevant pollutant that high concentrations can build up in the sediment and enter the food chain of bentonic organisms (McGeer et al., 2012). Mercury is largely known as a significant cause of mortality of several aquatic species (Cebrian & Uriz, 2007b).

Within the aquatic fauna, benthic invertebrates are especially susceptible to heavy metal pollution due to their reduced motility and filter-feeding habit (Cebrian & Uriz, 2007b). Besides that, these animals can accumulate metals and are efficient in trophic transfer of metals through the food chains (Wood, 2012). Among benthos, sponges have high water filtering and heavy metals accumulation capacities, being considered promising environmental biomonitors (Richelle-Maurer et al., 1994; Hansen et al., 1995; Cebrian et al., 2003; Perez et al., 2004; Cebrian et al., 2006; Ferrante et al., 2018). Sponges are exclusively aquatic organisms, including ca. 9200 valid species (van Soest et al., 2020), with about 3% distributed in freshwater environments (Pinheiro et al., 2015). Some of the freshwater sponges are able to produce gemmules (Manconi & Pronzato, 2015; 2016), i.e. asexual structures that work as resistant bodies and dispersal devices (Manconi & Pronzato, 2007). These structures contain specialized cells, called thesocytes, and armed by specialized spicules, called gemmuloscleres (Manconi & Pronzato, 2007). Completely formed gemmules show low metabolic rates and may become dormant, until hatching occurs (Reiswig et al., 2009). The process of germination begins when internal cells migrate through the opening of the micropyle, and start to differentiate to form a new functional sponge (Simpson, 1984).

The effects of heavy metals accumulation in sponges have been evaluated at different organization levels (molecular, cellular, and population) and stages of the sponges life cycle (larvae, settlers, juveniles, and adults). At a cellular level, Cebrian & Uriz (2007b) showed that metals exposure can cause morphological cellular changes and affect cell aggregation thus affecting several cellular functions, such as motility and phagocytosis. At a molecular level, Schroder et al. (2006) verified that the exposure of Baikalian sponges to heavy metals induced the production of DNA single-strand breaks in the sponges tissue. In addition, Francis & Harrison (1988) proposed that granule formation probably is a more effective detoxifying mechanism for metals among higher animals than sponges, and that there were no apparent differences in concentrations of metallothionein-like protein among experimental cultures. Metallothionein is a low molecular weight protein, whose synthesis can be induced for metals, its has a protective, detoxifying effect in cells (Wood, 2012).

Bioaccumulation of metals in freshwater sponges has been studied in both laboratory and field. Richelle et al. (1995) showed that, in the natural habitat, sponges could grow at higher metal concentrations than the threshold concentration measured in the laboratory. Additionally, the sensitivity thresholds measured in the laboratory differ among the different metals and species. In experimental laboratory study, Mysing-Gubala & Poirrier (1981) verified that heavy metals could cause malformations on spicules, also causing the death of the sponge when exposed to high concentrations of metals. Other studies reported different effects of bioaccumulation in sponges, depending on the exposure time, the studied species, and the life-cycle stage (Richelle-Maurer et al., 1994; Hansen et al., 1995; Cebrian et al., 2003; Perez et al., 2004; Cebrian et al., 2006; Ferrante et al., 2018). Cebrian et al. (2003) showed effects on behavioral and physiological responses such as changes in shape, growth rates and reproduction. Cebrian & Uriz (2007a) verified different effects on larval settlement depending on the studied species. Even so, data regarding the toxicity of heavy metals on early developmental phases of the sponges life cycle are, in general, scarce. Moreover, sublethal effects of metals may influence biological processes of the organisms that affect the structure and dynamics of the populations, thus drastically acting in an ecological level (Cebrian & Uriz, 2007b). In this sense, the sensitivity of early developmental stages to low levels of pollution may largely determine a subtle decline and even the disappearance of sponges populations in polluted environments.

In this way, here we tested the effects of the heavy metals copper (Cu), cadmium (Cd) and mercury (Hg), in gemmules hatching and early development of the freshwater sponge *Radiospongilla inesi* Nicacio & Pinheiro, 2011.

2. Methodology

2.1. Specimens collection and gemmules preparation

Specimens with gemmules of the freshwater sponge *Radiospongilla inesi* were collected manually between 0.1–0.5 m deep at fish farming tanks at the Universidade Federal Rural de Pernambuco, UFRPE (Recife, Pernambuco, northeastern Brazil; 8°1’9.40"S, 34°56'39.93"W). These tanks are supplied with water from Prata River. *Radiospongilla inesi* produce gemmules during the whole year, your gemmules hatch preferentially with water from its collection site and can develop under laboratory conditions (Calheira et al., 2019).

Sponges were individually packed in a container with water from the tanks and then transported to the laboratory. Additional water from the tanks was also collected and stored in plastic bottles (20L). Physico-chemical parameters of the water measured for this study were: temperature (°C); dissolved oxygen (DO mg/L); and conductivity (µS/cm); pH. These parameters were measured using a multiparameter (YSI–Professional Plus) (Table 1).

In the laboratory, the collected water was filtered and the gemmules were removed of the sponge tissue using tweezers. The external side of the gemmules was sterilized by soaking the whole gemmules in 1% hydrogen peroxide (H2O2) for 3 min. To remove all H2O2, gemmules were then washed several times with distilled water (Funayama et al., 2005).

Table 1. Physicochemical characteristics of the waters used in the treatments

Waters	Temperature (°C)	Dissolved Oxygen (DO mg/L)	Conductivity (uS/cm)	Salinity (g/L)	pH
R. inesi collection site	24.8	5.3	73.5	0.03	7.6

2.2. Preparation of metal solutions and Exposure

A primary 0.25 gL^{-1} stock solution was prepared for each metal copper chloride 98% pure (Sigma–Aldrich, Steinheim, Germany); cadmium chloride 98% pure (Sigma–Aldrich, Steinheim, Germany); and mercury chloride 98% pure (Sigma–Aldrich, Steinheim, Germany), dissolved in deionized water. Gemmules were exposed to a range of six concentrations for each heavy metal (1, 10, 50, 100, 500 and 1000), expressed as µg Cu L^{-1}, µg Cd L^{-1} and µg Hg L^{-1}. Three triplicates of 12 gemmules were exposed period of 30 days, and the test solutions were renewed every 2 days. Gemmules were allocated in plates with 24 spots of 3 ml, each spot with one gemmule. The gemmules were observed under a stereomicroscope to evaluate developmental stages.

2.3. Evaluation of Developmental stages

Development stages were analysed daily based on the description made by Calheira et al. (2019). The following stages were used: (Stage 0), the first stage or the resting gemmule right after gemmule obtention; (Stage 1), reached typically from day 3 onwards, after cells migrate from the gemmules through the micropyle; (Stage 2), typically from day 4 onwards is characterized by the formation of the spicules; (Stage 3), typically at day 5 is characterized by the formation of choanocyte chambers; (Stage 4), typically from day 7 onwards is characterized by the formation of osculum and is here considered as a functional juvenile sponge.

The low observed effect concentrations (LOEC) and no observed effect concentrations (NOEC) were derived from the effect observed in the stages. The NOEC was the highest tested concentration at which metal was observed to have no statistically significant toxic effect when compared to control (Crane & Newman, 2000). The LOEC was the lowest tested concentration at which metal was observed to have a statistically significant toxic effect when compared to control (Crane & Newman, 2000).

The following procedures were applied to all individuals monitored during the experiments. All individuals in the different treatments were individually monitored, and their hatch and progression along the different stages of development known to *Radiospongilla inesi* were identified and recorded at the day each stage was reached. The percentage of individuals reaching each stage at the end of the exposure was calculated for each replica.

2.3. Statistical analyses

Differences between treatments were analyzed by means of one-way ANOVA followed by the Dunnett's test. Data was first checked for normality and homoscedasticity by the Shapiro-Wilk test. The significance level of 5 % was adopted. Sigmaplot software version 14 (Jandel Scientific, Erkrath, Germany) was used for all statistical analyses.

3. Results

3.1 Progression of development in Controls

All five stages of development known to freshwater sponges were identified in the control group and were used to investigate the toxic effects of gemmules exposing to cadmium (Cd), copper (Cu) and mercury (Hg) (Fig. 1). In the control group, the first hatching (Stage 1) occurred only after four days of the experiment beginning. At the fifth day, 42% of gemmules had hatched, the first spicules were secreted (Stage 2) and there was the formation of choanocyte chambers (Stage 3). With 21 days, 56% of the gemmules reached the Stage 2 and in the follow day, 53% reached the Stage 3. The first juvenile sponges (Stage 4) was formed in the seventh day, and reached the high rates at 23 day with 44%. The hatching rate at the end of the experiment (21 days) was 61% (Fig. 2).

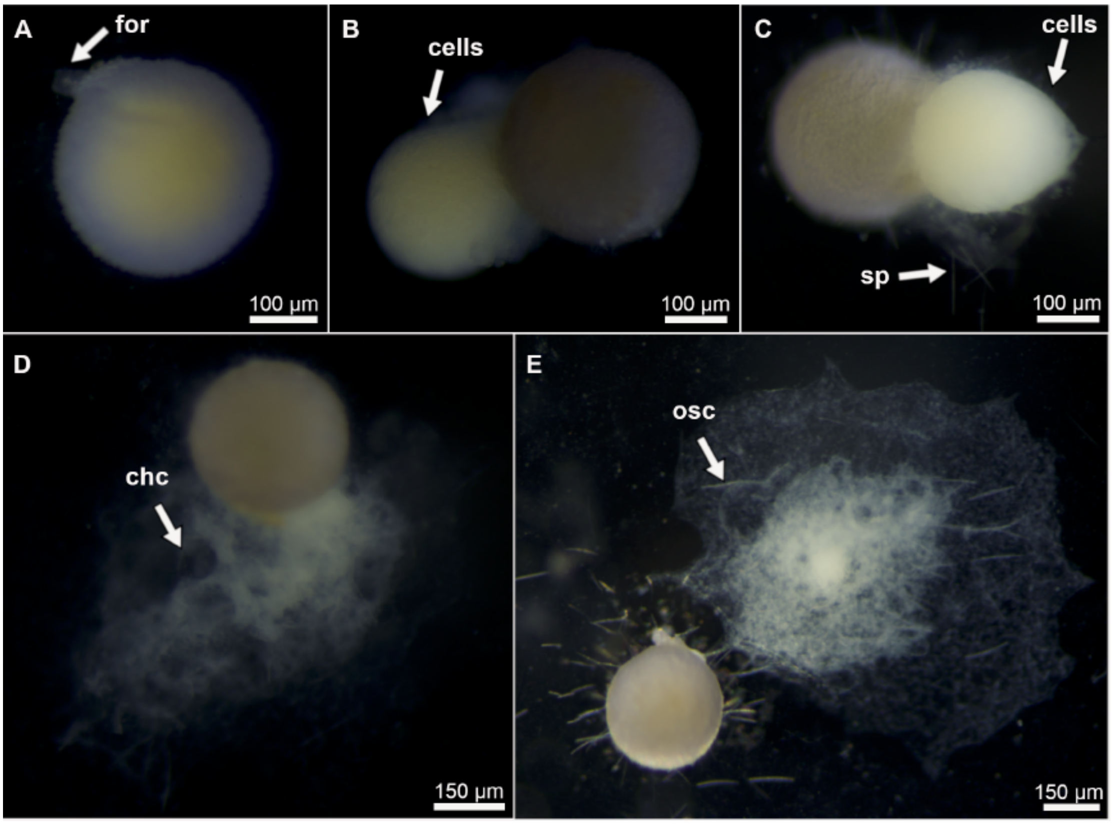


Fig. 1. Developmental stages of the germination of *Radiospongilla inesi*. **A** Stage 0 (resting gemmules), **B** Stage 1 (hatching gemmules), **C** Stage 2 (formation of the spicules), **D** Stage 3 (formation of the choanocyte chambers), **E** Stage 4 (developed juvenile sponge).

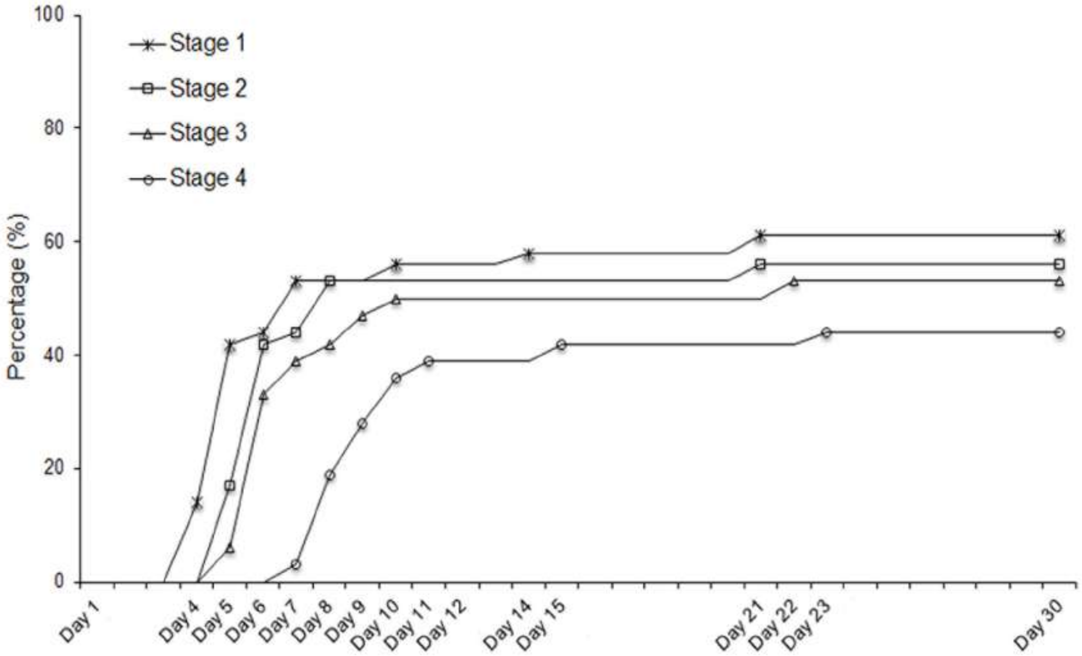


Fig. 2. Progression and percentage of individuals reaching the different developmental stages in the control group along the 30 days of the experiment. Percentage of gemmules of the control group in each stage at the end of the experiment (30 days).

3.2 % of individuals reaching stage 1 at 30 days – Hatching gemmules

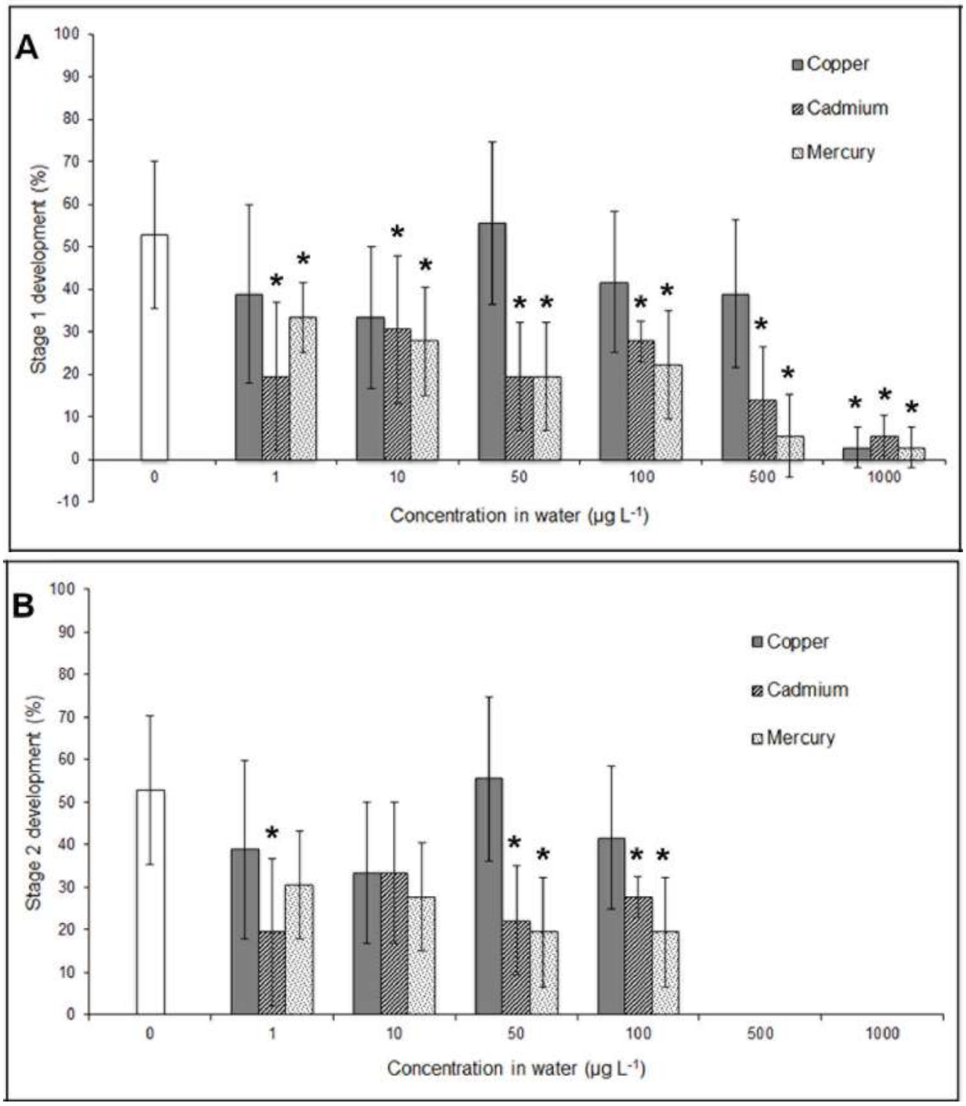
Copper (Cu): There was not observed effect concentration; at all tested concentration the mean response did not significantly ($p > 0.05$) differ from control group (Table 2; Fig. 3A).

Cadmium (Cd): The LOEC-Stage 1 (1 μgL^{-1}) was the lowest test concentration having a mean response that significantly ($p < 0.05$) differed when compared to control (Table 2; Fig. 3A).

Mercury (Hg): The LOEC-Stage 1 (1 μgL^{-1}) was the lowest test concentration having a mean response that significantly ($p < 0.05$) differed when compared to control (Table 2; Fig. 3A).

Table 2. One-way ANOVAs for metals concentrations (control, 1, 10, 50, 100, 500 and 1000 µg L^{-1}) effect on hatching of gemmules (Stage 1)

Variable	Factor	DF	F	*p*
Copper	Concentrations	6	3.171	<0.402
	Error	14		
Cadmium	Concentrations	6	3.764	0.001
	Error	14		
Mercury	Concentrations	6	6.269	0.000
	Error	14		



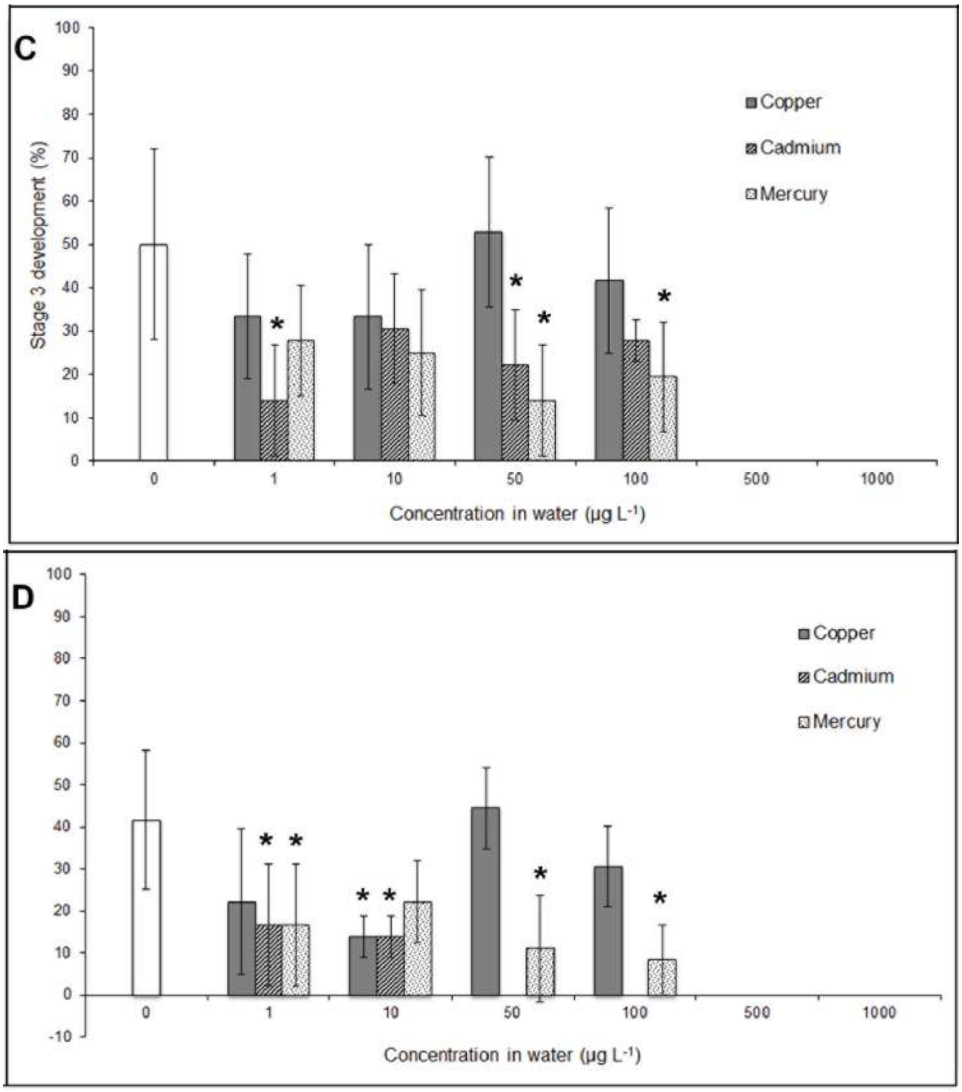


Fig. 3. Effect of the metals concentrations (control, 1, 10, 50, 100, 500 and 1000 µg L^{-1}) on each developmental stage after gemmules hatching at the end of the experiment. **A** % of individuals reaching stage 1, **B** % of individuals reaching stage 2, **C** % of individuals reaching stage 3, **D** % of individuals reaching stage 4. (*) Statistically different relative to control.

3.3 % of individuals reaching stage 2 at 30 days – formation of spicules

Copper (Cu): The LOEC-Stage 2 (500 μgL^{-1}) was the lowest test concentration having a mean response that significantly ($p < 0.05$) differed when compared to control, because from this concentration the gemmules that hatched did not reach the Stage 2.

Cadmium (Cd): The LOEC-Stage 2 (1 µgL^{-1}) was the lowest test concentration having a mean response that significantly ($p < 0.05$) differed when compared to control (Table 3; Fig. 3B). However, in the concentration 10 µgL^{-1} there was no significant difference ($p > 0.05$) in the percentage of spicules formation when compared to the control.

Mercury (Hg): The LOEC-Stage 1 (50 µgL^{-1}) was the lowest test concentration having a mean response that significantly ($p < 0.05$) differed when compared to control (Table 3; Fig. 3B).

Table 3. One-way ANOVAs for metals concentrations (control, 1, 10, 50, 100, 500 and 1000 µg L^{-1}) effect on formation of juvenile sponges (Stage 2)

Variable	Factor	DF	F	*p*
Copper	Concentrations	6	6.671	<0.002
	Error	14		
Cadmium	Concentrations	6	6.848	0.000
	Error	14		
Mercury	Concentrations	6	7.496	0.000
	Error	14		

3.4% of individuals reaching stage 3 at 30 days – formation of choanocyte chambers

Copper (Cu): The LOEC-Stage 3 (500 µgL^{-1}) was the lowest test concentration having a mean response that significantly ($p < 0.05$) differed when compared to control, because from this concentration the gemmules that hatched did not reach the Stage 3 (Table 4; Fig. 3C).

Cadmium (Cd): The LOEC-Stage 3 (1 µgL^{-1}) was the lowest test concentration having a mean response that significantly ($p < 0.05$) differed when compared to control (Table 4; Fig. 3C). However, in the concentration 10 and 100 µgL^{-1} there was no significant difference ($p > 0.05$) in the percentage of formation of the choanocyte chambers when compared to the control.

Mercury (Hg): The NOEC and LOEC were 10 and 50 µgL^{-1}, respectively (Table 4; Fig. 3C). The NOEC-Stage 3 (10 µgL-1) was highest tested concentration at which the mean response did not significantly ($p > 0.05$) differ from control group (Table 2; Fig. 3A). And the LOEC-Stage 1 (50 µgL^{-1}) was the lowest test concentration having a mean response that significantly ($p < 0.05$) differed when compared to control.

Table 4. One-way ANOVAs for metals concentrations (control, 1, 10, 50, 100, 500 and 1000 µg L^{-1}) effect on formation of choanocyte chambers (Stage 3)

Variable	Factor	DF	F	*p*
Copper	Concentrations	6	6.493	<0.002
	Error	14		
Cadmium	Concentrations	6	6.721	0.001
	Error	14		
Mercury	Concentrations	6	5.399	0.004
	Error	14		

3.5% of individuals reaching stage 4 at 30 days – Juvenile sponges

No juvenile sponges were seen in the highest concentrations tested of all three metals (500 and 1000 µg/L^{-1}).

Copper (Cu): Only in the concentration 10 µgL^{-1} there was significant difference ($p < 0.05$) in the percentage of formation of the juvenile sponges when compared to the control.

Cadmium (Cd): The LOEC-Stage 4 (1 µgL^{-1}) was the lowest test concentration having a mean response that significantly ($p < 0.05$) differed when compared to control (Table 5; Fig. 3D).

Mercury (Hg): The LOEC-Stage 4 (1 µgL^{-1}) was the lowest test concentration having a mean response that significantly ($p < 0.05$) differed when compared to control (Table 5; Fig. 3D). However, in the concentration 10 µgL^{-1} there was no significant difference ($p > 0.05$) in the percentage of formation of the juvenile sponge when compared to the control.

A summary of the values of NOEC and LOEC to each heavy metals tested is provided in Table 6.

Table 5. One-way ANOVAs for metals concentrations (control, 1, 10, 50, 100, 500 and 1000 µg L^{-1}) effect on formation of juvenile sponges (Stage 4)

Variable	Factor	DF	F	*p*
Copper	Concentrations	6	8.882	<0.000
	Error	14		
Cadmium	Concentrations	6	10.045	0.000
	Error	14		
Mercury	Concentrations	6	5.495	0.002
	Error	14		

Table 6. Values of the no observed effect concentrations (NOEC) and low observed effect concentrations (LOEC) that were obtained from the effect observed of the metals concentrations on each developmental stage when compared to control

	Copper		Cadmium		Mercury	
	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC
Stage 1	500	1000	ND	1	ND	1
Stage 2	100	500	ND	1	10	50
Stage 3	100	500	ND	1	10	50
Stage 4	1	10	ND	1	ND	1

ND = not determined.

4. Discussion

Here we provide, for the first time, results regarding the effect of the heavy metals copper, mercury and cadmium, in the early development of freshwater sponges, i.e. since gemmules hatching until the formation of juveniles sponges. Our results suggest that all tested heavy metals affect the development of *Radiospongilla inesi*.

Gemmules hatching were not influenced in the present study only by the metal copper (Cu). A similar pattern was seen on the early stages of the marine sponges, low concentrations of Cu had no effect on larval settlement for *Crambe crambe* and *Scopalina lophyropoda* (Cebrian & Uriz, 2007b). It has been reported for other invertebrates; Ng and Keough (2003) found that the Cu accelerate the attachment of bryozoan larvae; Gao et al. (2017) verified that, in low Cu concentration (1 mgL^{-1}), the number of hatched snails was higher than in the control group; Gama-Flores et al. (2007) showed that treatments containing low Cu levels and short exposure times stimulates reproduction in rotifers. In laboratory, Richelle et al. (1995)

demonstrated that the toxic effect of metals varies among species of the freshwater sponges and types of metal. When exposed the sponges to the same concentration of Cu, Richelle et al. (1995) found that it was weakly toxic for *Ephydatia fluviatilis* and *Ephydatia muelleri* (Lieberkühn, 1856) and not toxic for *Spongilla lacustris* (Linnaeus, 1759) that had a normal development.

Probably the non-effect of copper on the early development of *Radiospongilla inesi* can be related to metallothionein production. Although, here, there was no attempt to determine metal-binding affinity with the metallothionein. This detoxification mechanism has already been observed in freshwater sponges (Francis & Harrison, 1988). Furthermore, a large number of Cu exposure studies have revealed elevated metallothionein expression and protein levels in target organs for Cu accumulation (Grosell, 2012).

The copper also plays an essential biochemical function in sponges and it is possible that some species maintain high levels of heavy metals as a defense mechanism against predation or fouling (Webster et al., 2001). Marine sponges have the capacity to accumulate high levels of copper (Ferrante et al., 2018), although this can cause sublethal effects on some species (Cebrian et al., 2003; Cebrian et al., 2006; Cebrian & Uriz, 2007a). Here it was possible to evaluate the effect of copper concentrations on different stages of development. At high Cu concentrations (500 and 1000 mgL^{-1}), there was no sponge development, in agreement with previous studies that show that high copper concentration in the water can cause serious adverse effects on aquatic organisms (Gama-Flores et al., 2007; Gao et al., 2017).

Whereas marine sponges accumulate high levels of Cu, lower levels of cadmium are tolerated by these animals (Ferrante et al., 2018). In our study we found that low concentrations of Cadmium (1 mgL^{-1}), severely affected *R. inesi* in the percentage of hatched gemmules, non-formation of spicules and choanocytes chamber. A delay in the stages of development also was verified, thus affecting the survival of juveniles sponges. Olesen and Weeks (1994) verified that cadmium caused an immediate reduction in the sponge filtration rate, indicating a direct toxic effect on the choanocyte cells. Nevertheless, Cebrian and Uriz (2007b) verified that, like copper, cadmium does not influence on larval settlement for marine sponges.

Cebrian and Uriz (2007a) studied the effects of heavy metals (copper, cadmium and mercury) on sponge cell behavior and found out that the effect of copper and cadmium appear to be harmful for invertebrate cells because they inhibit cytoskeleton production. Mercury seems to be the most toxic to cytoskeleton alteration causing reduced cell mobility (Cebrian & Uriz, 2007a). Here, the cadmium seems to be more toxic than mercury, although they have similar effects on early stages of development of *Radiospongilla inesi*. It has corroborates with Richelle et al. (1995) that the toxicity of metals varies with the species and the metal. Our results show that the order of toxicity was as follow: Cd > Hg > Cu. This order of toxicity was inverse to that found by Liu and Chen (1987) for larval stages of crustaceans.

As marine sponges, freshwater sponges also have the capacity of accumulate heavy metals (Richelle-Maurer et al., 1994; Hansen et al., 1995), due the nature of the mineral or organic skeleton and biological composition. This accumulation can cause sublethal and lethal effects to the sponges (Mysing-Gubala & Porrier, 1981; Richelle et al., 1995; Hansen et al., 1995; Cebrian et al., 2003; Cebrian & Uriz, 2007a; Cebrian & Uriz, 2007b). Although the development of the sponge exposed to heavy metal appears normal, there may be sublethal effects, for example spicules malformations (Mysing-Gubala & Porrier, 1981; Richelle et al., 1995). Mysing-Gubala and Porrier (1981) exposed *Ephydatia fluviatilis* to different concentrations of mercury and cadmium in order to analyse the toxicity of these metals on the sponge development. In high concentrations, sponges could not survive and in low concentrations the inhibition of the gemmules formation or malformations in the gemmuloscleres were observed (Mysing-Gubala & Porrier, 1981). In the present work, no spicules malformations were seen on the juvenile sponge formed from gemmules of *Radiospongilla inesi*. This result indicates the reliability of the spicules morphology as a taxonomic character to identify this species.

In summary, the freshwater sponge *Radiospongilla inesi* appears to be a suitable biomonitor of metal contamination due to its developmental responses, such as alterations in percentage of hatching gemmules and changes in production of spicules and choanocyte chambers. All these changes can be easily monitored, as showed here. Our results indicate that cadmium and mercury exposures can affect not only hatching gemmules, but also the development of fundamental structures in early stages of juvenile sponges of *R. inesi*, and subsequent colonization of new habitats. Although cooper exposure has not significant difference in hatching percentage, a delay occurs at the start of each stage, and at the highest concentrations tested (500 and 1000 µg / L^{-1}), there was no development of juvenile sponges. The effect of cadmium, copper and mercury on the formation of a juvenile sponge from hatching gemmules of *R. inesi* may represent an important risk for populations in areas with both sources of contamination.

Compliance with Ethical Standards

This study is part of L. Calheira's PhD thesis through the Graduate Program in Animal Biology (Programa de Pós-Graduação em Biologia Animal) of the Departamento de Zoologia, UFPE. This work was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001, Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The authors declare that they have no conflict of interest and they agree to the submission of the manuscript and the corresponding author has been authorized by all co-authors. This article does not contain any studies with human participants or animals performed by any of the authors. Necessary permits for the field studies, including sampling and laboratory procedures, were obtained (Permit Number 18100-1 SISBIO/Instituto Chico Mendes de Conservação da Biodiversidade). Reported localities do not include protected areas.

References

Calheira, L., Lanna, E., Pinheiro, U., 2019. Tropical freshwater sponges develop from gemmules faster than their temperate-region counterparts. Zoomorphology 12p. https://doi.org/10.1007/s00435-019-00458-0

Cebrian, E., Martí, R., Uriz, M.J., Turon, X., 2003. Sublethal effects of contam- ination on the mediterranean sponge *Crambe crambe*: metal accumulation and biological responses. Marine Pollution Bulletin 46, 1273-1284.

Cebrian, E., Agell, G., Martí, R., Uriz, M.J., 2006. Response of the Mediterranean sponge *Chondrosia reniformis* Nardo to copper pollution. Environmental Pollution 141, 452-458.

Cebrian, E., Uriz, M.J. 2007a. Contrasting Effects of Heavy Metals on Sponge Cell Behavior. Arch Environ Contam Toxicol. 53, 552-558.

Cebrian, E., Uriz, M.J. 2007b. Contrasting effects of heavy metals and hydrocarbons on larval settlement and juvenile survival in sponges. Aquatic Toxicology 81, 137-143.

Crane, M., Newman, M.C. 2000. What level of effect is a no observed effect?. Environmental Toxicology and Chemistry 19, 516-519.

Ferrante, M., Vassallo, M., Mazzola, A., Brundo, M.V., Pecoraro, R., Grasso, A., Copat, C. 2018. In vivo exposure of the marine sponge *Chondrilla nucula* Schmidt, 1862 to cadmium

(Cd), copper (Cu) and lead (Pb) and its potential use for bioremediation purposes. Chemosphere 193, 1049-1057.

Francis, J.C., Harrison, F.W. 1988. Copper and Zinc Toxicity in *Ephydatia fluviatilis* (Porifera: Spongillidae). Transactions of the American Microscopical Society 107, 67-78.

Funayama, N., Nakatsukasa, M., Hayashi, T., Agata, K. 2005. Isolation of the choanocyte in the freshwater sponge, *Ephydatia fluviatilis* and its lineage marker, Ef annexin. Development, Growth & Differentiation 47, 243-253.

Gama-Flores, J.L., Castellanos-Paez, M.E., Sarma, S.S.S., Nandini, S. 2007. Effect of pulsed exposure to heavy metals (copper and cadmium) on some population variables of *Brachionus calyciflorus* Pallas (Rotifera: Brachionidae: Monogononta). Hydrobiologia 593, 201-208.

Gao, L., Doan, H., Nidumolu, B., Kumar, A, Gonzago, D. 2017. Effects of copper on the survival, hatching, and reproduction of a pulmonate snail (*Physa acuta*). Chemosphere 185, 1208-1216.

Grosell, M. 2012. Copper. Pp 53-133. *In*: Wood C.M., Farrell A.P. and Brauner C.J. (eds) Homeostasis and toxicology of essential metal. Fish physiology series 31A, Copyright Elsevier and Academic Press, Amsterdam, Boston, Heidelberg, London, New York, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo.

Hansen, I.V., Weeks, J.M., Depledge, M.H. 1995. Accumulation of copper, zinc, cadmium and chromium by the marine sponge *Halichondria panicea* Pallas and the implications for biomonitoring. Mar Pollut Bull. 31, 133-138.

Kidd, K., Batchelar, K. 2012. Mercury. Pp 237-295. *In*: Wood C.M., Farrell A.P. and Brauner C.J. (eds) Homeostasis and toxicology of essential metal. Fish physiology series 31B, Copyright Elsevier and Academic Press, Amsterdam, Boston, Heidelberg, London, New York, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo.

Liu, P.C., Cheng, J.C., 1987. Effects of Heavy Metals on the hatching rates of Brine Shrimp *Artemia salina* Cysts. Journal of the World Aquaculture Society 18, 78-83.

Manconi, R. & Pronzato, R. 2007. Gemmules as a key structure for the adaptive radiation of freshwater sponges: a morphofunctional and biogeographical study. Pp 61-77. *In*: Custódio M.R., Lôbo-Hajdu G., Hajdu E. and Muricy G. (eds.) Porifera research: biodiversity, innovation and sustainability. Série Livros 28, Museu Nacional, Rio de Janeiro.

Manconi, R., Pronzato, R. 2015. Chapter 3: Phylum Porifera. *In*: Thorp J, Rogers DC (eds) Keys to Nearctic Fauna: Thorp and Covich's Freshwater Invertebrates, 4th edn. Elsevier, San Diego, pp 39-83.

Manconi, R., Pronzato, R. 2016. How to survive and persist in temporary freshwater? Adaptive traits of sponges (Porifera: Spongillida): A review. Hydrobiologia 782:11-22.

McGeer, J.C., Niyogi, S., Smith., D.S. 2012. Cadmium. Pp 125-184. *In*: Wood C.M., Farrell A.P. and Brauner C.J. (eds) Homeostasis and toxicology of essential metal. Fish physiology series 31B, Copyright Elsevier and Academic Press, Amsterdam, Boston, Heidelberg, London, New York, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo.

Mysing-Gubala, M., Poirrier, M.A. 1981. The effects of cadmium and mercury on gemmule formation and gemmosclere morphology in *Ephydatia fluviatilis* (Porifera: Spongillidae). Hydrobiologia 76, 145-148.

Ng, T.Y.-T., Keough, M.J., 2003. Delayed effects of larval exposure to Cu in the bryozoan *Watersipora subtorquata*. Marine Ecology Progress Series 257, 77-85.

Olesen, T.M.E., Weeks, J.M., 1994. Accumulation of Cd by the marine sponge *Halichondria panicea* Pallas: effects upon filtration rate and its relevance for biomonitoring. Bulletin Environmental Contamintion and Toxicology 52, 722-728.

Perez, T., Vacelet, J., Rebouillon, P., 2004. In situ comparative study of several Mediterranean sponges as potential biomonitors for heavy metals. Boll. Mus. Ist. Biol. Univ. Genova 517-525.

Pinheiro, U., Calheira, L., Hajdu, E. 2015. A new species of freshwater sponge, *Heteromeyenia barlettai* sp. nov. from an aquarium in São Paulo, Brazil (Spongillida: Spongillidae). Zootaxa 4034, 351-363.

Reiswig, H.M., Frost, T.M., Ricciardi, A. 2009. Porifera. *In*: Thorp, J.H., Covich, A.P. (Eds.), Ecology and classification of North American freshwater invertebrates, 4th edn. Elsevier, New York, pp 91-123.

Richelle-Maurer, E., Degoudenne, Y., Van de Vyver, G. 1994. Some Aspects of Heavy Metal Tolerance in Freshwater Sponges. *In*: Van Soest, R.W.M., Van Kempen, T.M.G., Braekman, J.-C. (Eds.), Sponges in Time and Space, Proceedings of the 4th International Porifera Congress. A. Balkema, Rotterdam, pp. 351-354.

Richelle, E., Degoudenne, Y., Dejonghe, L., Van de Vyver, G. 1995. Experimental and field studies on the effect of selected heavy metals on three freshwater sponge species: *Ephydatia fluviatilis*, *Ephydatia muelleri* and *Spongilla lacustris*. Hydrobiologia 135, 209-231.

Schroder, H.C., Efremova, S.M., Margulis, B.A., Guzhova, I.V., Itskovich, V.B., Muller, W.E.G. 2006. Stress response in Baikalian sponges exposed to pollutants. Hydrobiologia 568, 277-287.

van Soest, R.W.M., Boury-Esnault, N., Hooper, J.N.A., Rtzler, K., De Voogd, N.J., Alvarez, B., Hajdu, E., Pisera, A.B., Manconi, R., Schoenberg, C., Janussen, D., Tabachnick, K.R., Klautau, M., Picton, B., Kelly, M., Vacelet, J., Dohrmann, M., Diaz, C.M., Cardenas, P. 2019. World Porifera Database. http://www.marinespecies.org/porifera/. Accessed 23 July 2019.

Webster, N.S., Webb, R.I., Ridd, M.J., Hill, R.T., Negri, A.P. 2001. The effects of copper on the microbial community of a coral reef sponge. Environmental Microbiology 3, 19-31.

Wood, C.M. 2012. An introduction to metals in fish physiology and toxicology: basic principles. Pp 1-51. *In*: Wood C.M., Farrell A.P. and Brauner C.J. (eds) Homeostasis and toxicology of essential metal. Fish physiology series 31A, Copyright Elsevier and Academic Press, Amsterdam, Boston, Heidelberg, London, New York, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo.

4 PRODUCTION OF GEMMULES AND LARVAE AT THE SAME TIME BY A NEOTROPICAL FRESHWATER DEMOSPONGE (PORIFERA)

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Production of gemmules and larvae at the same time by a neotropical freshwater demosponge (Porifera)

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Production of gemmules and larvae at the same time by a neotropical freshwater demosponge (Porifera)

Most freshwater sponges reproduce asexually, by the production of gemmules, and sexually, releasing parenchymella larvae. Gemmules are asexual structures that act as dispersal devices and resting bodies, while parenchymella larvae of freshwater sponges is the most morphologically complex larvae of all sponges. This larvae swim for a period before settling and starting the metamorphosis to form a juvenile sponge. Gemmule structure and development was better investigated if compared to larvae of freshwater sponges. For instance, the studies with the spongillids parenchymella larvae are restricted to only a few genera found especially in temperate zone. Here we report, for the first time, the sexual development and the parenchymella larvae of the freshwater sponge *Radiospongilla inesi* from Brazil. We also verified if the juvenile sponges developed from larvae differs from the juvenile developed from gemmules. We observed that *R. inesi* reproduces asexually (gemmules production) and sexually (embryos and parenchymella larvae) at the same time. The species is hermaphroditic and viviparous. Moreover, there was no difference in the timing to form a juvenile sponge of *R. inesi* from larvae attachment or hatching gemmules, contrasting with previous studies with temperate sponges that found that the juvenile development from larvae is faster than from gemmules. This strategy might seem important to improve the chances colonize unstable waters.

Keywords: parenchymella; *Radiospongilla inesi*; Spongillida; asexual reproduction.

Introduction

The order Spongillida (Demospongiae; Porifera) currently consists of six families of sponges exclusively found in freshwater environments all around the world (Manconi and Pronzato 2002; 2016). In this order, most species reproduce both sexually and asexually. The sexual reproduction culminates in the release of parenchymella larvae, while asexual reproduction involves the production of gemmules (Gaino et al. 2003). Gemmules are structures that perform a double functional role as dispersal devices and as resting bodies (Manconi and Pronzato 2007). Gemmules have totipotent cells (thesocytes) encapsulated within a collagenous coat that may also have specialized spicules (gemmuloscleres) embedded in its structure (Manconi and Pronzato 2002). Gemmule seems to be a very important adaptation for the survival of freshwater sponges, as it is widespread in the order. Only less than 10% of the species of Spongillida (families: Lubomirskiidae Weltner, 1895; Malawispongiidae Manconi & Pronzato, 2002; and Metschnikowiidae Czerniavsky, 1880) do not produce gemmules during their life cycles.

While the gemmules serves as both a resting body against harsh conditions of the environment and for asexual reproduction, freshwater sponges also rely on the production of new individuals through sexual reproduction. In this case, the adults are usually gonochoric, with the females brooding the embryo until it is released as a fully ciliated parenchymella larva (Manconi and Pronzato 2002; Riesgo et al. 2013). The Spongillida's parenchymella is considered the most morphologically complex sponge larvae (Simpson 1984), as it presents an anteriorly positioned cavity that is surrounded by a larval pinacoderm, as well as the presence of non-functional choanocyte chambers (Ereskovsky 2010). The role of the larval cavity is still uncertain, but it was suggested that it either aid in flotation or in osmoregulation (Maldonado 2006; Ereskovsky 2010). Additionally, the surface of the Spongillida parenchymella is completely ciliated and its skeleton is fan-shaped at cross-section, thus differing from the parenchymella of all other demosponges (Ereskovsky 1998; 2010). The choanocyte chambers were found in the larvae of some species of Lubomiskiidae, Malawispongiidae, and Spongillidae, being absent in Potamolepidae (Ereskovsky 2010). It is still unknown what is exactly the function of the choanocyte chambers in the larvae of these sponges, but even though they seem to form an incipient aquiferous system (as they are connected with some canals, Saller 1988; Maldonado and Bergquist 2002), they are considered to not be functional at this stage (Maldonado and Bergquist 2002).

Sponges larvae swim for a while before settling and start the metamorphosis to form a juvenile sponge (Leys and Degnan 2001). The direction of swimming is controlled by longer cilia, while the rotation is provided by the beating of short cilia that extend from the monociliated epithelial layer (Leys and Degnam 2005). Usually, swimming larvae rotate clockwise (Leys and Degnan 2005). Sponge larvae respond behaviorally to some physical factors, such as light, water flow and water temperature, despite the absence of sensory organs or nervous system (Maldonado and Young 1996). Light seems to be the main factor for to direct the swimming of the larvae (Maldonado and Young 1996). In parenchymella larvae, for example, they swim towards the bottom for settlement, guided by negative phototaxis (Maldonado 2006).

Little is known regarding the reproduction and the life cycle stages of freshwater sponges, especially from the Neotropical Region. However, studies with sponges from Temperate Europe found interesting results. Manconi and Pronzato (2016) found that, along a latitudinal gradient, Italian populations of *Ephydatia fluviatilis* (Linnaeus, 1759) showed different timing of life cycle phases, even though the sexual and asexual phases never overlap. On the other hand, specimens of *E. fluviatilis* and *Spongilla lacustris* (Linnaeus, 1759) from Germany were reproducing sexually (parenchymella larvae) and asexually (production gemmules) at the same time (Saller 1988). Gaino et al. (2003) showed that a population in an artificially controlled lake in Italy (Lake Piediluco) reproduces only sexually, being the production of gemmules absent in this area. Interestingly, in demosponges (and in many marine invertebrates) there is an alternation in the production of sexual and asexual propagules (Fell 1993; Ereskovsky et al. 2017). One of the explanations for this alternation is the competition for totipotent cells (mostly archaeocytes) used to produce both gametes and the asexual propagule (either buds or gemmules) (Ereskovsky et al. 2017). Consequently, previous reports on the coocurrence of sexual and asexual reproduction in freshwater sponges seems to be unexpected. Is this trait more widespread in freshwater sponges than earlier expected? What are the consequences of this strategy for the survival of the population in freshwater environments?

Very little is known about the biology of Neotropical spongillids (Calheira et al. 2019). To the best of our knowledge, there is no information about their gametogenesis, embryogenesis or the parenchymella larvae. We previously reported how new sponge juveniles develop from gemmules in two Neotropical freshwater sponges: *Radiospongilla inesi* Nicacio & Pinheiro, 2011 and *Heteromeyenia cristalina* Batista, Volkmer-Ribeiro & Melão, 2007 (Calheira et al. 2019). During that study, we had the opportunity to find specimens of *R. inesi* undergoing sexual reproduction. Therefore, the aim of the current work is to report, for the first time, the gametogenesis, embryogenesis, and the parenchymella larva of the neotropical freshwater sponge *R. inesi*, and to verify if the juvenile sponges developed from larva differ from those one developed from gemmules.

Materials and Methods

Samples of the freshwater sponge *Radiospongilla inesi* were collected at fish farming tanks from Universidade Federal Rural de Pernambuco, UFRPE (Recife, Pernambuco State, northeastern Brazil; 8°1’9.40"S, 34°56'39.93"W), in September 2018, at 0.5-1 m depth.

Specimens were placed in a container with water from the tanks and then transported to the laboratory (Calheira et al. 2019).

In the laboratory, the sponges were transferred to sterile Petri dish containing water from the tanks and, under a stereomicroscope, the occurrence of larvae was verified. To observe metamorphosis, larvae were allowed to settle on Petri dish containing water from the tanks and with some coverslips at the bottom. Images and videos were recorded with a digital camera (Nikon D5100) coupled in stereomicroscope (Zeiss Stemi 508). Fragments of the adult tissue containing gametes and embryos were fixed in Formol-Acetic-Alcohol solution, containing 10 mL of formaldehyde 40%, 5 mL of acetic acid 100%, and 85 mL ethanol 80% (for each 100 mL of fixative solution) for 2h. These fragments were preserved in ethanol 97% (Calheira et al. 2019). For light microscopy, they were immersed in a solution of 5% hydrofluoric acid (HF) for 6 h to remove the spicules. Later, the samples were washed to remove the excess of HF and subsequently, they were dehydrated in an ethanol series (50, 70, 80, 90 and 100%), cleared in xylene, and embedded in paraffin. Thin sections (5 µm) were mounted on glass slides and stained using Harris's hematoxylin and eosin (Lanna et al. 2018a).

Results and Discussion

While almost all analyzed specimens were reproducing asexually, with gemmules scattered throughout the sponges body, only a single specimen was sexually reproductive active (Figure 1(a)), containing several embryos in development (Figures 1(b)) and mature larvae in the basal portion of the sponge (Figure 1(c)).

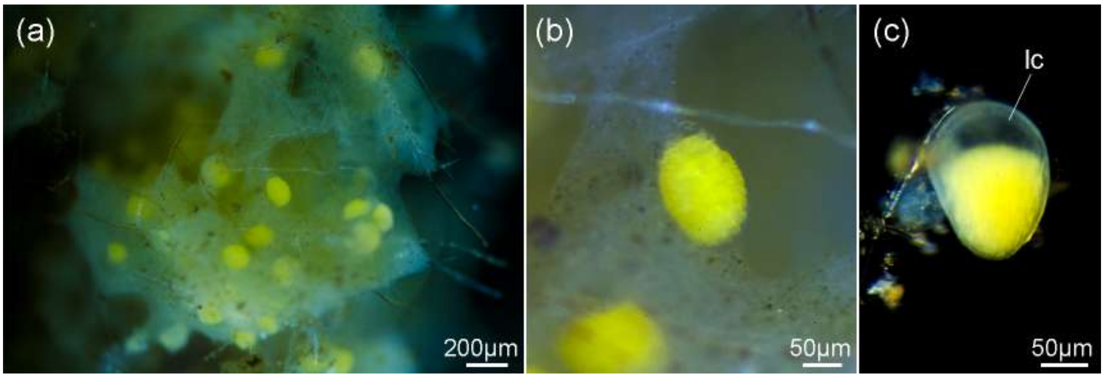

Figure 1. Basal portion of the *R. inesi* in the sexual reproductive phase (a) with embryo (b) and larva (c). (lc) larval cavity.

We found both types of gametes (spermatozoa and oocytes) being produced simultaneously in the mesohyl of the sponge, rendering *R. inesi* as a hermaphroditic species. We found at least three different stages of spermatogenesis (likely the primary spermatocyte, spermatids and spermatozoa, Figure 2a-c), always occurring inside spermatic cysts (ca. 35 μm in diameter) delimited by a follicle formed by thin and elongated cells (Figure 2a-c). The stage of spermatogenesis was almost synchronous within the spermatic cyst, but asynchronous in the specimen (Figure 2a). Oocytes were found also scattered in the mesohyl, usually surrounded by a group of nurse cells. These somatic cells were amoeboid and initially their cytoplasm was not granulated (Figure 2d). The previtellogenic oocyte measured ca, 30 μm in diameter, had a large nucleolated nucleus and a few granulated cytoplasm (Figure 2d). Oocytes started to phagocyte eosinophilic nurse cells that were found in the mesohyl and to accumulate them in their cytoplasm (Figure 2e). Later, the oocyte grew until reach ca. 110 μm and was fully granulated (Figure 2f). The number of gametes observed was relatively low, probably related to a late stage of sexual reproduction, as evidenced by the number of embryos in the mesohyl. Anyways, they resemble the gametes described previously to other spongillids (Saller 1988; Gaino et al. 2003) and deserves further investigation in both light and electron microscopy, especially because the fertilization process was not found and is still a mystery for freshwater sponges (Saller 1988).

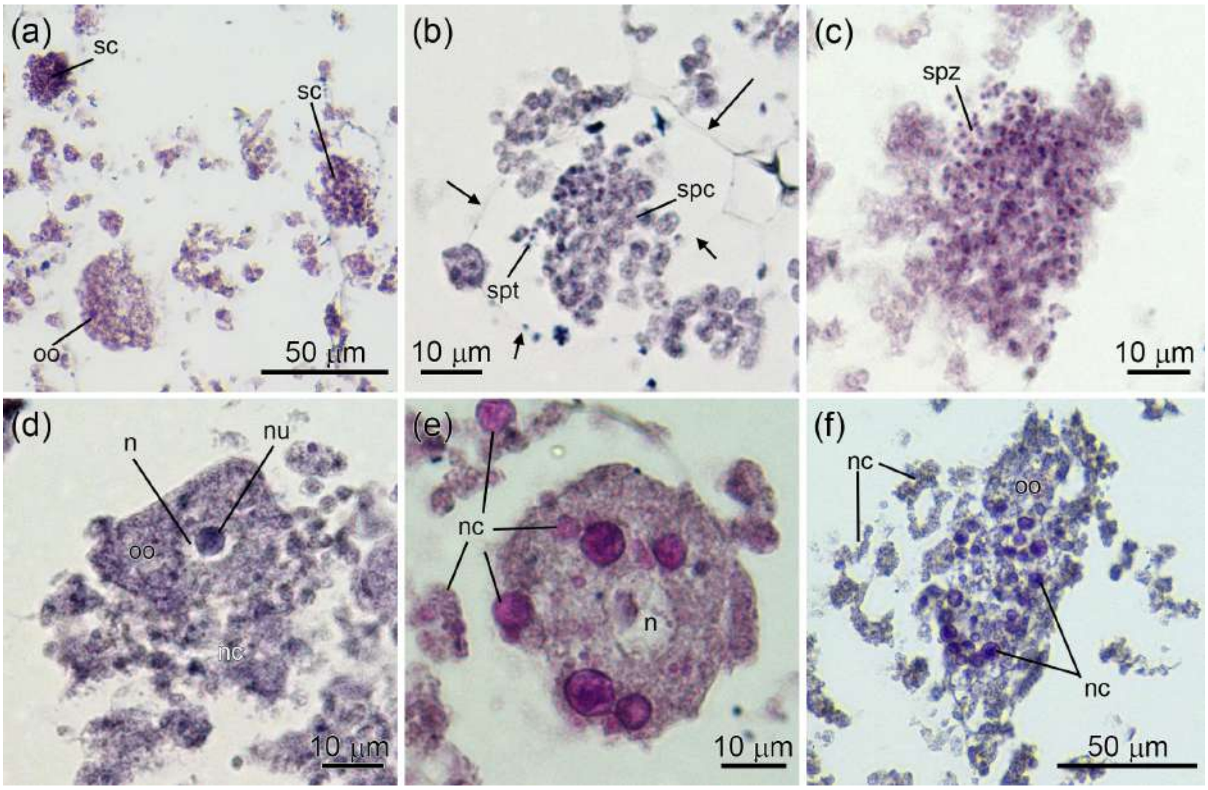

Figure 2. Light microscopy of the gametogenesis of *R. inesi*. (a) Overview of the choanosome showing oocytes (oo) and spermatic cysts (sc). (b) Detail of a spermatic cyst comprising spermatocytes (spc) and spermatids (spt). (c) Late spermatic cyst with spermatozoa (spz). (d) Previtellogenic oocyte (oo) with large nucleus (n) and nucleolus (nu) close to nurse cells (nc). (e) Vitellogenic oocytes (oo) with eosinophilic nurse cells (nc) (n – nucleus). (f) Late oocyte with the cytoplasm full of nurse cells (nc).

Radiospongilla inesi is a viviparous sponge, as it incubates embryos in its mesohyl. We observed early embryos with the blastomeres undergoing the first divisions to an advanced morula stage (Figure 3). All embryos were observed surrounded by a very thin follicle of elongated cells (Figure 3b). The size of the blastomeres decreased, as expected, during the development (Figure 3b). In the morula stage, blastomeres had a similar size and we could not detect early cell segregation (Figure 3d). We could not observe the multilayered embryo leading to the larvae in our histological preparations. On the other hand, we observed the presence of parenchymella *in vivo* with the specimens of *R. inesi* brought to the laboratory, confirming the occurrence of sexual and asexual reproduction at the same time. The parenchymella was entirely ciliated, with a large larval cavity (Figure 1c). One of the main characteristics of the parenchymellae of freshwater sponges (Maldonado 2006), choanocyte chambers, was not detected here, as we could not prepare histological slides of the tissue containing mature larvae or with the free-living larvae. However, apparently, the observed larvae of *R. inesi* seem to agree with the complex morphology already described to other parenchymellae of spongillids (e.g., Saller 1988).

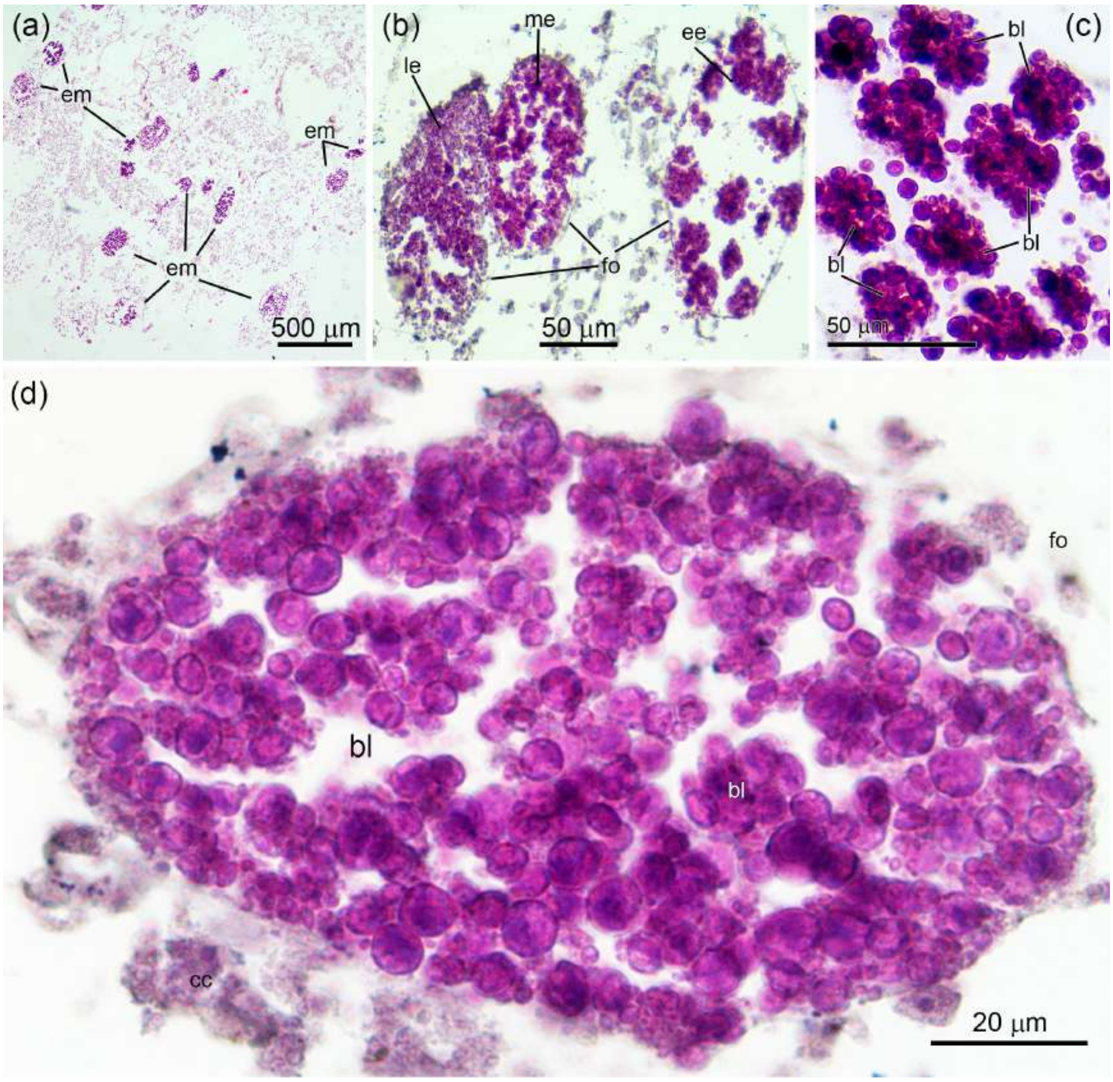

Figure 3. Light microscopy of the embryogenesis of *R. inesi.* (a) Overview of the

choanosome of the sponge showing several embryos in different stage of development. (b) Detail of three embryos in different stages of development: early cleavage (ee), mid-stage cleavage (me) and late stage cleavage (le). All embryos were surrounded by a thin follicle (fo). (c) Detail of the blastomeres (bl) during the early cleavage stage. (d) Detail of mid stage embryo with blastomeres (bl) of different sizes.

The rotation pattern known for most sponge larvae is clockwise rotation (Leys and Degnan 2005). However, the larvae of *R. inesi* swam rotating in counter-clockwise direction. This rotation pattern was described for parenchymella larvae of marine demosponges (Leys and Degnam 2001), and for cinctoblatula larvae from Homoscleromorpha (Maldonado 2006).

After 6 hours of swimming, larvae of *R. inesi* attached to the bottom of the Petri dish and started the metamorphosis (Figure 4). This time is within the range found in other demosponges, including 3-48 h in *Ephydatia fluviatilis*, freshwater sponges (Wielsputz and Saller 1990) and 2-48 h in *Amphimedon queenslandica*, a marine demosponge (Leys and Degnan 2005). In *R. inesi*, the metamorphosis was complete with the formation of a juvenile sponge after 24 h after attaching to the substrate, contrasting with the larvae of *A. queenslandica* and *E. fluviatilis* in which the juvenile sponge is formed 5-7 days and 72 h after the attachment, respectively (Leys and Degnan 2005; Wielsputz and Saller 1990). Therefore, the timing for the attachment, metamorphosis, and juvenile formation of the sponges larvae is apparently a species-specific characteristic.

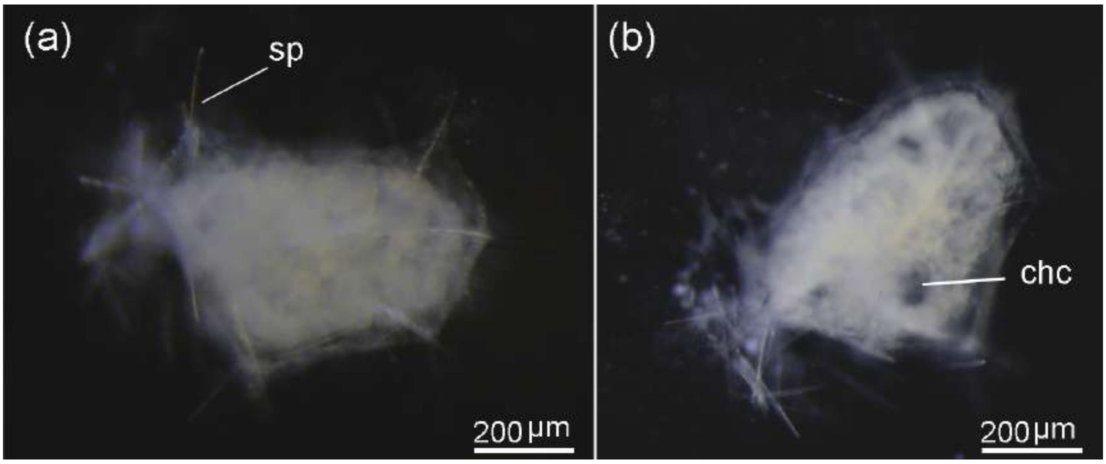

Figure 4. Metamorphosis of the larvae of *R. inesi*. (chc) choanocyte chamber, (sp) spicule.

Recently, Calheira et al. (2019) described the development of *R. inesi* during gemmule germination and observed that a functional juvenile sponge was formed 24 h after hatching. Here, we observed that there is no difference in the timing to form a juvenile sponge of *R. inesi* from larval attachment and hatching gemmules (Calheira et al. 2019). These results are contrasting with the ones reported to the temperate species *E. fluviatilis* (Wielsputz and Saller 1990). In *E. fluviatilis*, the juvenile sponge developing from larvae is formed faster than that formed from gemmules, which requires 96-156 h. In this sense, it seems that species from neotropical region (Calheira et al. 2019; present study) form a juvenile sponge faster than those from temperate regions (e.g., Wielsputz and Saller 1990).

Despite the results here presented, proper investigations including the gametes and embryogenesis of freshwater sponges from the Neotropical Region are still needed to better understand the patterns and process of reproduction in this region. As pointed by Lanna et al. (2018b), more studies on morphology (comparative and descriptive analysis of any stage of development and gametogenesis) are recommended to improve the knowledge of sponges reproduction and development.

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References

Calheira L, Lanna E, Pinheiro U. 2019. Tropical freshwater sponges develop from gemmules faster than their temperate-region counterparts. Zoomorphology. 1–12.

Ereskovsky A. 1998. Development of Haplosclerid sponges (Demospongiae, Ceractinomorpha). Russian Journal of Marine Biology. 25:361–371.

Ereskovsky AV. 2010. The comparative embryology of sponges. Berlin: Springer Science & Business Media.

Ereskovsky AV, Geronimo, A, Perez, T. 2017. Asexual and puzzling sexual reproduction of the Mediterranean sponge Haliclona fulva (Demospongiae): life cycle and cytological structures. Invertebrate Biology. 136:403–421. DOI: 10.1111/ivb.12195

Fell PE. 1993. Porifera. In: Adiyodi KG, Adiyodi RG, editors. Reproductive biology of invertebrates. VI Asexual propagation and reproductive strategies. Wiley, Chichester.

Gaino E, Rebora, M, Corallini C, Lancioni T. 2003. The life-cycle of the sponge *Ephydatia fluviatilis* (L.) living on the reed *Phragmites australis* in an artificially regulated lake. Hydrobiologia. 495:127–142.

Lanna E, Cajado B, Santos-da-Silva C, Hora J, Porto U, Vasconcellos V. 2018a. Is the Orton’s rule still valid? Tropical sponge fecundity, rather than periodicity, is modulated by temperature and other proximal cues. Hydrobiologia. 815:187–205.

Lanna E, Cajado B, Santos D, Cruz F, Oliveira F, Vasconcellos V. 2018b. Outlook on sponge reproduction science in the last ten years: are we far from where we should be? Invertebr. Reprod. Dev. 10:1–10.

Leys SP, Degnan BM. 2001. The cytological basis of photoresponsive behavior in a sponge larva. Biol. Bull. 201:323–338.

Leys SP, Degnan BM. 2005. Embryogenesis and metamorphosis in a haplosclerid demosponge: gastrulation and transdifferentiation of larval ciliated cells choanocytes. Invertebrate Biology. 121(3):171–189.

Maldonado M, Young CM. 1996. Effects of physical factors on larval behavior, settlement and recruitment of four tropical demosponges. Mar. Ecol. Prog. Ser. 138:169–180.

Maldonado M, Bergquist P. 2002. Phylum Porifera. In: Young CM, Sewell MA, Rice ME, editors. Atlas of marine invertebrates larvae. San Diego, San Francisco, New York, Boston, London, Sydney, Tokyo: Academic Press; p. 21–50.

Maldonado M. 2006. The ecology of the sponge larva. Can. J. Zool. 84:175–194.

Manconi R, Pronzato R. 2002. Suborder Spongillina subord. nov.: Freshwater sponges. In: Hooper JNA, Van Soest RWM, editors. Systema Porifera. A guide to the classification of sponges. New York, Boston, Dordrecht, London, Moscow: Kluwer Academic/ Plenum Publishers; p. 921–1020.

Manconi R, Pronzato R. 2007. Gemmules as a key structure for the adaptive radiation of freshwater sponges: a morphofunctional and biogeographical study. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G, editors. Porifera research – biodiversity, innovation and sustainability. Rio de Janeiro: Museu Nacional - Série Livros; p. 61–77.

Manconi R, Pronzato R. 2016. How to survive and persist in temporary freshwater? Adaptive traits of sponges (Porifera: Spongillida): A review. Hydrobiologia. 782:11–22.

Riesgo A, Novo M, Sharma PP, Peterson M, Maldonado M, Giribet G. 2013. Inferring the ancestral sexuality and reproductive condition in sponges (Porifera). Zoologica Scripta. 43:101–117.

Saller U. 1988. Oogenesis and larval development of *Ephydatia fluviatilis* (Porifera, Spongillidae). Zoomorphology. 108:23–28.

Simpson TL. 1984. The cell biology of sponges. New York (NY): Springer.

Wielsputz C, Saller U. 1990. The metamorphosis of the parenchymula-larva of *Ephydatia fluviatilis* (Porifera, Spongillidae). Zoomorphology. 109:173–177.

5 CAN THE HATCHABILITY OF GEMMULES DETERMINE THE DISTRIBUTION OF FRESHWATER SPONGES?

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Title: Can the hatchability of gemmules determine the distribution of freshwater sponges?

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Additional keywords: dormant structure, mixohaline, physiology, Porifera, Spongillidae.

Running Title: Hatchability define distribution of sponges

Abstract. Freshwater sponges produce gemmules, dormant structures that represent dispersal propagules and resting bodies. It is known that the hatchability of gemmules is different depending on the type of water. However, the relationship between the physiology of gemmules hatching and the distribution of the species was not yet tested. Here, we choose two species, *Radiospongilla inesi* (widely distributed) and *Spongilla alba* (restricted to mixohanile environment), to test if their distributions are related with the hatchability of the gemmules in different environments. Three treatments were tested (T): T1 (water of *R. inesi* collection site); T2 (mineral water) and T3 (water of *S. alba* collection site). For each treatment, three replicates with 30 gemmules were used. The gemmules were observed daily during 30 days. Data analysis was performed using ANOVA and a *posteriori* Tukey test. Our results showed that there was a significant difference among treatments ($P < 0.001$) and between species ($P < 0.001$). Gemmules of *R. inesi* hatched in all treatments: T1 (78%), T2 (26%), T3 (40%), while *S. alba* ones hatched only in T1 (2%) and T3 (56%). The gemmules of *R. inesi* developed into juvenile sponges in T1 and T2, and gemmules of *S. alba* developed juvenile sponges only in the water of species collection site (T3). Our results suggest that the gemmules of the two species have a distinct adaptive plasticity to cope with different kinds of inland waters. Although *S. alba* has a wide world distribution, in Brazil it is restricted to mixohaline waters. Thus, it is crucial to understand the physiology of the gemmules hatching and juvenile formation of freshwater sponges to explain its distribution patterns.

1. INTRODUCTION

The main differences between freshwater and marine environments are related with the high probability of dry-up and with the spatial distribution of waters (Pronzato & Manconi, 1994). Inland waters are strongly driven by seasonal fluvial and/or precipitation, characterized by water level fluctuations such as floods, freezes and droughts that are usually absent in marine habitats (Pronzato & Manconi, 1994). Thus, it is common to freshwater organisms to show adaptive strategies for survival and persistence (Cáceres, 1997). Moreover, the sea is a continuous habitat, while inland waters are mainly isolated areas, preventing active larval dispersal (Pronzato & Manconi, 1994). In this sense, it is crucial to species inhabiting inland waters to develop dispersal strategies (van Leeuwen, Lovas-Kiss, Ovegard, & Green, 2017), carried by biological and/or physical vectors, for example birds (zoochory), or wind (anemochory) (Manconi & Pronzato, 2016).

The majority of freshwater sponges (Porifera) produce dormant structures called gemmules (Manconi & Pronzato, 2002). These structures have work as dispersal propagules and as resting bodies, sometimes originated as a response to stressful environmental conditions (Manconi & Pronzato, 2007). Gemmules are asexually produced reproductive bodies composed of binucleated stem cells (thesocytes, a resting form of archeocytes), enveloped in a coat composed of peculiar specialized spicules called gemmuloscleres (Reiswig, Frost & Ricciardi, 2009). During gemmules hatching, the thesocytes are activated to become archeocytes, that start to differentiate to form a new functional sponge (Reiswig et al., 2009). Gemmules display different levels of morphological complexity, and their morphological features have been used as diagnostic at the family, genus and species levels (Manconi & Pronzato 2007).

Manconi and Pronzato (2007) suggested that the distribution of freshwater sponges is related to the efficiency of gemmules as dispersal devices. According to these authors, species with morphological complex gemmules (with well-developed pneumatic layer and spiny gemmuloscleres) show higher dispersal capacity with a tendency to cosmopolitism. On the other hand, species with morphologically simple gemmules (without pneumatic layer and spiny gemmuloscleres), the dispersal capacity is lower, resulting in a more restricted distribution (Manconi & Pronzato, 2007).

Calheira, Santos and Pinheiro (in press) showed that two species from the same environment have different ability to hatch from their gemmules. However, the studied species have wide distributions, preventing to answer whether the physiology of gemmules was related with the species distributions in different environments or not. In this sense, here we choose two species of freshwater sponges belonging to the family Spongillidae GRAY 1867, both with morphological complex gemmules – *Radiospongilla inesi* NICACIO & PINHEIRO 2011, widely distributed in the Neotropical Region, and *Spongilla alba* CARTER 1849, (Volkmer-Ribeiro & Machado, 2007), also widely distributed, but known only from mixohaline environments – to test whether their distributions are related with the gemmules hatchability in different environments.

2. METHODS

2.1 Studied species

Radiospongilla inesi has a wide distribution, originally was described from Pernambuco State, (Nicacio, Severi, & Pinheiro, 2011) and also recorded from Piauí State (UFPERPOR 2268), São Paulo (UFPEPOR 2153), and Paraná States (Parolin, Volkmer-Ribeiro, & Leandrini, 2010. The last record misidentified the species as *Radiospongilla amazonensis* VOLKMER & MACIEL 1983); which define this species has a wide distribution in the Brazil.

Spongilla alba is cosmopolitan, being recorded from Africa, Asia, Oceania and America (Muricy et al., 2011). However, it is likely that is a complex of species and should be revised (Muricy et al., 2011). In Brazil, the sponges was recorded from Pernambuco, Alagoas and Rio de Janeiro States (Volkmer-Ribeiro & Machado, 2007; Pinheiro, Nicacio, & Muricy, 2015), occurring at the ponds from coastal areas, typical examples of mesohaline environments (Volkmer-Ribeiro & Machado, 2007).

2.2 Specimen collection

Specimens with gemmules of the *Radiospongilla inesi* were manually collected, in depths of 0.1–0.5 m, at fish farming tanks from the Universidade Federal Rural de Pernambuco, UFRPE. These tanks are supplied with water from Prata river, Recife, Pernambuco State, Northeastern Brazil (8°1′9.40″S, 34°56′39.93″W) (Calheira et al., 2019). Specimens of *Spongilla alba* were collected by freediving, in depths of 1.5-2.0 m, at Carapebus pond, in the Restinga de Jurubatiba National Park, Carapebus, Rio de Janeiro State, Southeastern Brazil (22°13'S, 41°37'W). All specimens were placed in a container with water from collection sites and then transported to the laboratory. Additional water of these localities also were also collected and stored in plastic bottles (6 L), and samples of mineral water from Emerald fountain were also used due to its purity.

Physico-chemical parameters of the water samples measured for this study include: temperature (°C); dissolved oxygen (DO mg / L); conductivity (µS / cm); and pH. These parameters were measured using a multiparameter (YSI–Professional Plus). Salt concentration was obtained from conductivity values (Table 1).

Table 1. Physicochemical characteristics of the waters used in the treatments

Waters	Temperature (°C)	Dissolved Oxygen (DO mg/L)	Conductivity (uS/cm)	Salinity (g/L)	pH
R. inesi collection site (T1)	24.5	8.1	92.4	0.03	8.3
Mineral water (T2)	24.4	6.4	107.0	0.04	7.7
S. alba collection site (T3)	24.5	6.1	2891.0	1.48	7.4

2.3 Gemmules and treatments preparation

In the laboratory, gemmules were removed from the sponges tissue using tweezers. Then, gemmules were sterilized by soaking the whole gemmule in 1% hydrogen peroxide (H_2O_2) for 3 min. To remove all H_2O_2, the gemmules were washed several times with distilled water (Funayama, Nakatsukasa, Hayashi & Agata, 2005).

After that, the gemmules were allocated in 24 well (3 mL) plates, containing a single gemmule in each well, and maintained at room temperature (ca., 25 °C on average). Three treatments (T) were tested: T1 (water from the fish farming tanks); T2 (mineral water) and T3 (Carapebus pond water). For each treatment, three replicates with 30 gemmules were used. The gemmules were observed daily during 30 days under a stereomicroscope to verify the gemmules hatching development to form a juvenile sponges.

2.4 Statistical analyses

Data was first checked to homogeneity of variances using the Bartlett test. A factorial ANOVA was performed to test if there were differences in the average of hatching gemmules among treatments and between species, followed by the *a posteriori* Tukey test. All statistical analyses were performed using the STATISTICA v.7 software, and a 95% confidence interval was adopted.

Pilot tests were carried out to infer the number of repetitions for the experiments, observing the amount of gemmules that hatched in different types of water. The value attributed to the minimum difference between the means of the treatments was 30, and with three repetitions. In addition, pilots were made to observe whether there was a significant difference between specimens from the same population, and whether the location where the collected specimen could influence (for example, shallower or deeper location), and in both tests there was no significant difference.

3. RESULTS

The results showed that there were significant differences among treatments ($F_{2;\,12} = 76.3$, $P < 0.001$) and between species ($F_{1;\,12} = 137.8$, $P < 0.001$). Gemmules of *Radiospongilla inesi* hatched in all treatments and showed different percentage of hatching. The water of *R. inesi* collection site (T1) yielded higher hatching rates with 78%, whereas in the others treatments (T2 e T3) only 26% and 40% of gemmules hatched, respectively. On the other hand, gemmules of *Spongilla alba* did not hatch when submitted to mineral waters (T2), and showed a low hatching percentage of 2% in the water from the fish farming tanks (T1). However, when submitted to water where it was collected (T3), 56 % of the gemmules hatched (Figure 1 and Figure 2A).

The gemmules from *R. inesi* developed juvenile sponges only in treatments T1 and T2, with 46% and 47% of individuals reaching this stage, respectively. In *Spongilla alba*, only in the water of species collection site (T3), 30% of the gemmules developed in juvenile sponges (Figure 2B and Figure 3).

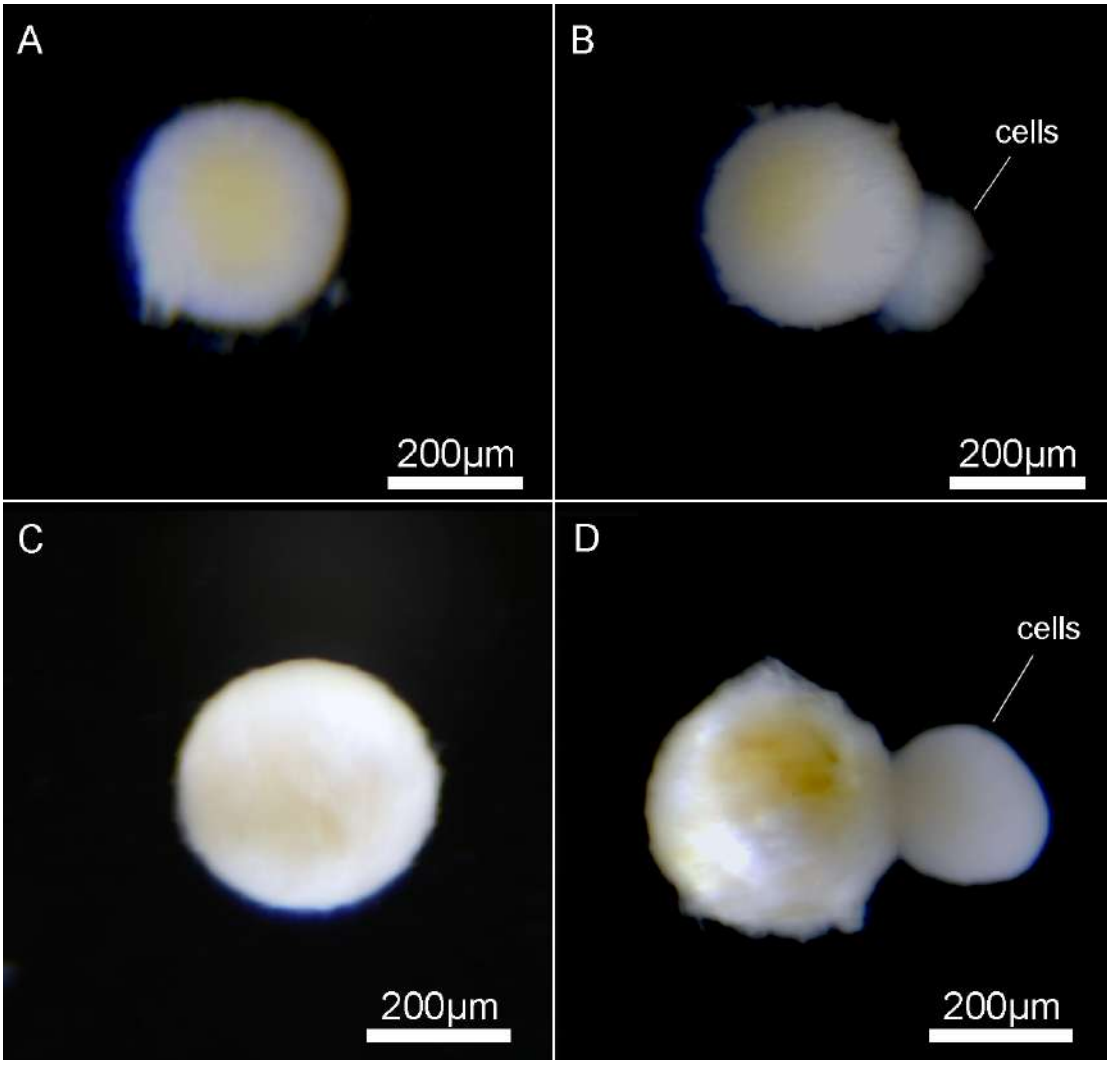

Figure 1. Gemmules from *Radiospongilla inesi* NICACIO & PINHEIRO 2011 and *Spongilla alba* CARTER 1849. **A.** Resting gemmule of *R. inesi*. **B.** Hatching gemmule of *R. inesi*. **C.** Resting gemmule of *S. alba*. **D.** Hatching gemmule of *S. alba*.

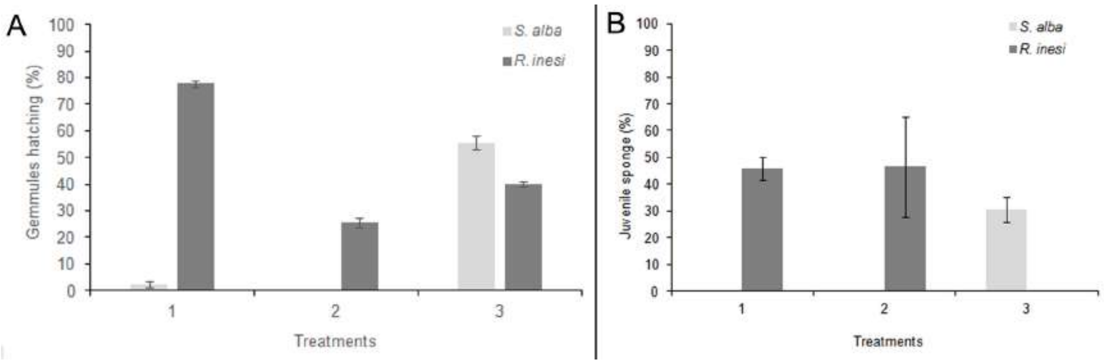

Figure 2. Percentage of gemmules hatching from *Spongilla alba* CARTER 1849 and *Radiospongilla inesi* NICACIO & PINHEIRO 2011. **A.** Gemmules submitted to treatments: T1. water of *R. inesi* collection site, Recife, PE; T2. mineral water; T3. water of *S. alba* collection site, Carapebus, RJ. **B.** Percentage of developed sponges from gemmules hatching.

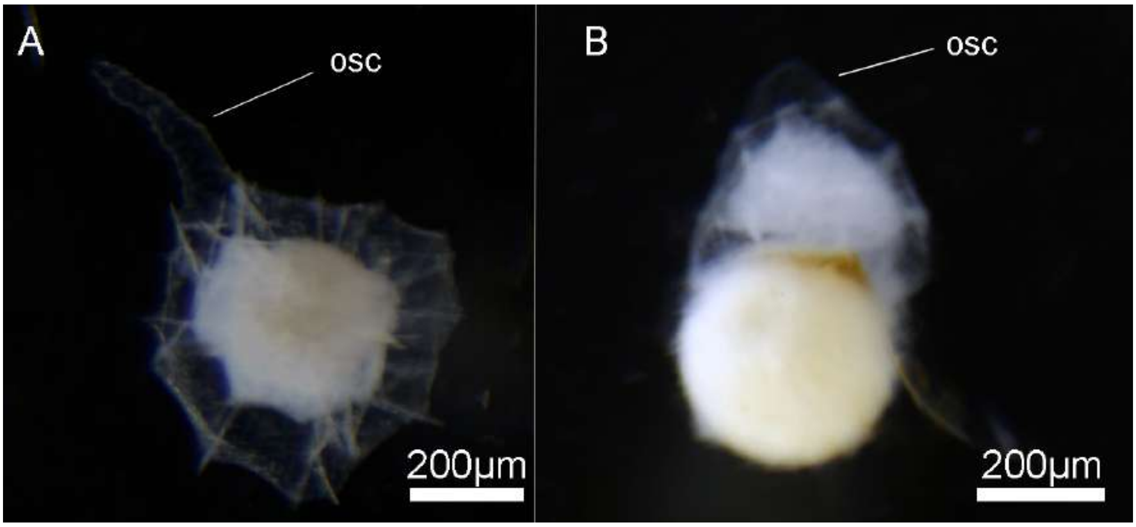

Figure 3. Juvenile sponges developed from gemmules hatching. **A.** *Radiospongilla inesi* NICACIO & PINHEIRO 2011. **B.** *Spongilla alba* CARTER 1849. *osc* osculum.

4. DISCUSSION

Calheira et al. (in press) showed that two species of spongillids from the same locality had different gemmules hatchability potentials when experimentally submitted to the same conditions. In the present study, we also demonstrated that two spongillids species have different ability of gemmules hatching, but here the species tested were from different types of environments.

Our results suggest that the gemmules of the two species studied here have distinct adaptive plasticities to deal with different kinds of inland waters. Although *Spongilla alba* is worldwide distributed, in Brazil it is restricted to mixohaline waters (Volkmer-Ribeiro & Machado, 2007). Here, this species produced juvenile sponges only in the water collected in the ponds where they are usually found, suggesting that even it is dispersed to others kinds of inland waters, probably the sponge will not develop and thus not colonize the new environment. In the case of *S. alba*, it is more likely that the distribution of the species is related to the hatching capacity rather than to its dispersal mechanisms. On the other hand, gemmules of *Radiospongilla inesi* hatched in all tested waters, without forming a juvenile sponge only in the mixohaline waters (Table 1). This finding is in accordance with the results of Calheira et al. (in press), which found that gemmules of *R. inesi* were not able to hatch after exposure to high salinity waters.

Potentially, hatchability might be affected by many factors, including salinity (Kim & Hagiwara, 2011). Few experimental studies regarding the salinity tolerance of freshwater sponges were done. Fell (1990) showed that gemmules of *Eunapius fragilis* (LEIDY 1851) hatched in high salinity waters (5% NaCl). It appears that the gemmules of this species can tolerate high ranges of salinity, although this species has not been encountered in brackish water (Poirrier, 1969). Controversially, higher salinity can result in inhibition of gemmules hatching in *Spongilla lacustris* (LINNAEUS 1759) (Simpson & Vaccaro, 1973). Francis, Poirrier and LaBiche (1982) found a significant negative effect of the salinity on the growth rate of *Ephydatia fluviatilis* (LINNAEUS 1759). The effect of increased salinity was also observed in other freshwater invertebrates. For instance, studies with freshwater branchiopods (Crustacea) from arid regions demonstrated that increases in salinity of the pond inhibit eggs hatching (Brown & Carpelan, 1971).

Reiswig et al. (2009) proposed that the distribution of freshwater sponge species is influenced by biogeographic factors. In smaller scales, the species distributions are largely constrained by physical environmental. Water chemistry (e.g., pH, salinity, silica) seems to play a major role in determining which species will be present in a particular lake or river within a region (Reiswig et al., 2009). Our results not only reinforce this proposal, as also indicate that the influence of the species' physiology (i.e. gemmules hatching and juvenile formation) are not only restricted to regional distribution patterns and probably the physico-chemical environmental variables play a major role in wide spread populations, as those of *R. inesi* and *S. alba*. In this sense, our results suggest that understand the physiology of gemmules hatching and juvenile formation of the species is crucial to explain its distribution patterns.

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References

Brown, L. R., & Carpelan, L. H. (1971). Egg Hatching and Life History of a Fairy Shrimp Branchinecta Mackini Dexter (Crustacea: Anostraca) in a Mohave Desert Playa (Rabbit Dry Lake). *Ecological Society of America*, *52*, 41-54.

Cáceres, C. E. (1997). Dormancy in invertebrates. *Invertebrate Biology*, *116*, 371-383.

Calheira, L., Santos, P. J. P., & Pinheiro, U. (2020). Hatchability of gemmules of two Neotropical freshwater sponges (Porifera: Spongillida). *Iheringia, Série Zoologia*, *110*, e2020001, 10 Jan. https://dx.doi.org/10.1590/1678-4766e2020001

Fell, P. E. (1990). Environmental factors affecting dormancy in the freshwater sponge *Eunapius fragilis* (Leidy). *Invertebrate Reproduction & Development*, *18*, 213-219. http://dx.doi.org/10.1080/07924259.1990.9672147

Francis, J. C., Poirrier, M. A., & LaBiche, R. A. (1982). Effects of calcium and salinity on the growth rate of *Ephydatia fluviatilis* (Porifera: Spongillidae). *Hydrobiologia*, *89*, 225-229.

Funayama, N., Nakatsukasa, M., Hayashi, T., & Agata, K. (2005). Isolation of the choanocyte in the freshwater sponge, *Ephydatia fluviatilis* and its lineage marker, Ef annexin. *Development, Growth & Differentiation, 47,* 243-253. https://doi.org/10.1111/j.1440-169X.2005.00800.x

Kim, H. J., & Hagiwara, A. (2011). Effect of salinity during resting egg formation and hatching on descendent reproduction in the rotifer *Brachionus rotundiformis* Tschugunoff. *Journal of Plankton Research*, *33*, 1033-1042.

Manconi, R., & Pronzato, R. (2002). Sub-order Spongillina subord. nov.: Freshwater sponges. In J. N. A. Hooper, & R. W. M. van Soest (Eds.), *Systema Porifera: A guide to the*

Classification of Sponges (pp. 921-1019). New York, EUA: Kluwer Academic/Plenum Publ.

Manconi, R., & Pronzato, R. (2007). Gemmules as a key structure for the adaptive radiation of freshwater sponges: a morphofunctional and biogeographic study. In M. R. Custódio, E. Hajdu, G. Lobo-Hadju & G. Muricy (Eds.), *Porifera Research: Biodiversity, Innovation and Sustainability* (pp. 61-77). Rio de Janeiro, BR: Museu Nacional.

Manconi, R., & Pronzato, R. (2016). How to survive and persist in temporary freshwater? Adaptive traits of sponges (Porifera: Spongillida): A review. *Hydrobiologia, 782,* 11-22. https://doi.org/10.1007/s10750-016-2714-x

Muricy, G., Lopes, D. A., Hajdu, E., Carvalho, M. S., Moraes, F. C., Klautau, M., Menegola, C., & Pinheiro, U. (2011). *Catalogue of Brazilian Porifera*. Rio de Janeiro, BR: Museu Nacional.

Nicacio, G., Severi, W., & Pinheiro, U. (2011). New species of *Radiospongilla* (Porifera: Spongillidae) from Brazilian inland waters. *Zootaxa, 3132,* 56-63. http://dx.doi.org/10.11646/zootaxa.3132.1.2

Parolin, M., Volkmer-Ribeiro, C., & Leandrini, J. A. (2010). *Abordagem ambiental interdisciplinar em bacias hidrográficas no Estado do Paraná*. Campo Mourão, BR: Editora da Fecilcam.

Pinheiro, U., Nicacio, G., & Muricy, G. (2015). An example of the importance of labels and fieldbooks in scientific collections: A freshwater sponge misunderstood for a marine new genus and species. *Zootaxa*, *3974*, 447-45.

Poirrier, M. A. (1969). Louisiana fresh-water sponges: taxonomy. ecology and distribution. Ph.D. Thesis, *Louisiana State University*. 173pp.

Pronzato, R., & Manconi, R. (1994). Adaptive strategies of sponges in inland waters. *Boll. Zool.*, *61*, 395-401. https://doi.org/10.1080/11250009409355912

Reiswig, H. M., Frost, T. M., & Ricciardi, A. (2009). Porifera. In J. H. Thorp & A. P. Covich (Eds.), *Ecology and Classification of North American Freshwater Invertebrates* (pp. 91-123). New York, NY: Elsevier.

Simpson, T. L., & Vaccaro, C. A. (1973). The role of intragemmular osmotic pressure in cell division and hatching of gemmules of the fresh-water sponge *Spongilla lacustris* (Porifera). *Z. Morph.*, *76*, 339-357.

van Leeuwen, C. H. A., Lovas-Kiss, A., Ovegard, M., & Green, A. J. (2017). Great cormorants reveal overlooked secondary dispersal of plants and invertebrates by piscivorous waterbirds. *Biol. Lett.*, *13*, 1-5. http://dx.doi.org/10.1098/rsbl.2017.0406

Volkmer-Ribeiro, C., & Machado, V. S. (2007). Freshwater sponges (Porifera, Demospongiae) indicators of some coastal habitats in South America: redescriptions and key to identification. *Iheringia, Série Zoologia*, *97*, 157-167.

6 WHAT'S MORE IMPORTANT TO DISTRIBUTION OF FRESHWATER SPONGES: HATCHABILITY OR MORPHOLOGY OF GEMMULES?

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Title: What's more important to distribution of freshwater sponges: hatchability or morphology of gemmules?

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Additional keywords: asexual propagule, cryptobiosis, hatchability, Porifera, Spongillida.

Running Title: Does morphology explain the distribution?

Abstract. Gemmules are asexual propagules containing totipotent cells that can be armed by special spicules: gemmuloscleres. This structure exhibits different levels of morphological complexity. Until now, only the morphological aspects of the gemmules are being considered to understand the distribution pattern of freshwater sponges. Here, we tested and compared the hatchability of gemmules of four species (complex gemmules (*Drulia ctenosclera*) vs complex gemmules (*Tubella variabilis*) and simple gemmules (*Oncosclera navicella*) vs complex gemmules (*Radiospongilla inesi*)), in different kinds of inland waters. Our results showed that gemmules of each species have different hatchability rate, and suggested that the distribution of these species in continental waters is more related to the capacity of hatching and colonize new environments rather than the gemmule morphology. Therefore, it seems that the gemmular morphology is not a limiting factor to the distribution of *O. navicella*. In the sense, the gemmular morphology is not factor crucial to distribution of species.

1. INTRODUCTION

The colonization of freshwater environments seems to be strictly related to the cryptobiosis phenomenon represented by resistant bodies (Cáceres, 1997). In freshwater sponges, these resistant bodies are represented by gemmules (Manconi & Pronzato, 2007). Gemmules are asexual propagules containing totipotent cells (thesocytes), and can be armed or not by special types of spicules, the so-called gemmuloscleres (Manconi & Pronzato, 2002). Completely formed gemmules exhibit a low metabolism that allows sponges to survive extreme environmental conditions (Reiswig, Frost, & Ricciardi, 2009; Pronzato & Manconi, 1995). The gemmules of some freshwater sponges are resistant to desiccation; they have been found to be capable of germination after dry storage for 10 to 25 years (Fell & Bazer, 1990). Upon germination, the totipotent cells move through the micropyle, and then differentiate to originate an active sponge (Reiswig et al., 2009). Gemmules are characteristic of species belonging to three of the six living families of the Order Spongillida MANCONI & PRONZATO 2002: Spongillidae GRAY 1867, Metaniidae VOLKMER-RIBEIRO 1986, Potamolepidae BRIEN, 1967. In the three others families, Malawispongiidae MANCONI & PRONZATO 2002, Lubomirskiidae REZVOI 1936 and Metschnikowiidae CZERNIAVSKY 1880, gemmules are absent.

Gemmules exhibit different levels of morphological complexity (Manconi & Pronzato, 2007). Those from families, Metaniidae and Spongillidae exhibit a complex morphology, with a well-developed pneumatic layer to float and dispersal downstream, and spiny spicules that are able to hook efficiently onto the carrier (Manconi & Pronzato, 2007). Also, gemmules from these families are distributed overall on the sponge body (Manconi & Pronzato, 2007). On the other hand, gemmules of potamolepids are only found at the basal portion of the sponge or strictly adhering to the substratum (Manconi & Pronzato, 2007). These gemmules do not have a pneumatic layer and the gemmuloscleres are smooth (Manconi & Pronzato, 2007).

Manconi and Pronzato (1996) suggested that migratory birds were responsible for the disjunct distribution of *Sanidastra yokotonensis* VOLKMER-RIBEIRO & WATANABE 1983, because their distribution coincided with the migratory routes of shorebirds. However, only recently, van Leeuwen, Lovas-Kiss, Ovegard and Green (2017) reported, for the first time, bird-mediated dispersal of *Ephydatia fluviatilis* (LINNAEUS 1759) through of gemmules. Anyways, the distribution of freshwater sponges is typically related to the efficiency of the gemmules as dispersal devices (Manconi & Pronzato 2007; 1996). Once sponges of families without gemmules show extremely restricted geographic ranges (Manconi & Pronzato, 2002), these relationships are not unlikely. Additionally, the dispersal capacity is considered to be low in species with gemmules that exhibits simple morphology (i.e., Potamolepidae, with restrict distribution), than in those with complex gemmules (i.e., Metaniidae and Spongillidae, with a tendency to cosmopolitism) (Manconi & Pronzato, 2007). Interestingly, although sharing gemmules with complex morphologies, Spongillidae and Metaniidae show different distribution patterns and the latter family is known from circuntropical regions, as Potamopelidae species that exhibit gemmules with simple morphology (Manconi & Pronzato, 2007). Besides that, at least one Potamolepidae species, *Oncosclera navicella*, is widely distributed in the Neotropical Region (Nicacio & Pinheiro, 2015).

Reiswig et al. (2009) highlighted that the restricted distribution of some species of freshwater sponges may involve adaptations to climate conditions that can influence the regional distribution patterns. There are few species whose occurrence is determined mainly by a single environmental factor; for example, *Spongilla alba* CARTER 1849 that is restricted to brackish water habitats (Volkmer-Ribeiro & Machado, 2007). Besides that, the restricted distribution of this species may be related to the ability of their gemmules to hatch and form sponge only in mixohaline waters (See Chapter 4). Calheira, Santos and Pinheiro (in press) tested the hatchability of gemmules of two spongillids from the same locality and found that these species have different potential of adaptation to different environments.

Here we tested and compared the hatchability of gemmules of four freshwater sponges species (*Tubella variabilis* (BONETTO & EZCURRA DE DRAGO 1973) (Spongillidae) vs *Drulia ctenosclera* VOLKMER & MOTHES 1981 (Metaniidae) and *Oncosclera navicella* (CARTER 1881) (Potamolepidae) vs *Radiospongilla inesi* NICACIO & PINHEIRO 2011 (Spongillidae)), in different kinds of inland waters to answer two questions: (1) are there differences among the hatching rates of species collected in different locals under the same experimental conditions?; and (2) what is more important, gemmular morphology or the ability to adapt to the environment? Our hypotheses are that the species show different hatchability potential of their gemmules; and that the potential of adaptation to different environments is more crucial than morphology of gemmules.

2. METHODS

2.1 Specimens Collection and Preparation

Specimens containing gemmules of *Radiospongilla inesi* and *Tubella variabilis* were collected at Universidade Federal Rural de Pernambuco (UFRPE), Recife, Pernambuco State, northeastern Brazil (08°19’40”S, 34°56’39.93”W). The first species was collected at fish farming tanks, and the latter at artificial channels that conducts water from Prata River to these fish tanks. Specimens of *Oncosclera navicella* were collected at Formoso River, Iretama, Paraná State, south Brazil (24°18'37"S, 52°05'56"O). Specimens of *Drulia ctenosclera* were collected at São Francisco River, Penedos, Alagoas State, northeastern Brazil. (10°20’14”S 36°34’4”W).

Sponges were individually packed in a container with water of their own environment and transported to the laboratory. Additionally, the tank water was also collected and stored in a glass container (6 L), and samples of mineral water from Emerald fountain were also used due to its purity. Physico-chemical parameters of the water samples measured for this study include: temperature (°C); dissolved oxygen (DO mg / L); conductivity (µS / cm); and pH. These parameters were measured using a multiparameter sensor (YSI–Professional Plus). Salt concentration was obtained from conductivity values (Tables 1,2).

In the laboratory, under a stereomicroscope, gemmules were removed from sponges. Then, gemmules were sterilized by soaking the whole gemmules in 1% hydrogen peroxide (H_2O_2) for 3 min. To remove all H_2O_2, the gemmules were washed several times with distilled water (Funayama, Nakatsukasa, Hayashi & Agata, 2005).

Table 1. Physicochemical characteristics of the waters used in the treatments

Waters	Temperature (°C)	Dissolved Oxygen (DO mg/L)	Conductivity (uS/cm)	Salinity (g/L)	pH
D. ctenosclera collection site (T1)	27.4	4.0	95.4	0.04	7.7
T. variabilis collection site (T2)	27.9	4.2	73.3	0.03	8.1
Mineral water (T3)	25.4	7.4	78,2	0.03	6.6

Table 2. Physicochemical characteristics of the waters used in the treatments

Waters	Temperature (°C)	Dissolved Oxygen (DO mg/L)	Conductivity (uS/cm)	Salinity (g/L)	pH
R. inesi collection site (T1)	28.6	8.5	76	0.03	6.8
Mineral water (T2)	24.6	7.1	72.2	0.03	7.4
O. navicella collection site (T3)	25.5	9.12	108	0.04	8.9

2.2 Cultivation

For cultivation, the gemmules were transferred to 24 well (3 mL) plates, containing a single gemmule in each well. Two experiments were performed: 1) Spongillidae vs Metaniidae, with three treatments – T1 (São Francisco River water), T2 (water from the fish farming tanks) and T3 (mineral water); 2) Spongillidae vs Potamolepidae, also with three treatments –T1 (water from the fish farming tanks), T2 (mineral water) and T3 (Formoso River water). For each treatment, three replicates with 30 gemmules were used. The gemmules were observed daily during 30 days under a stereomicroscope to register the number of hatching gemmules. The culture was maintained at room temperature (ca., 25 °C on aver- age).

2.3 Statistical analyses

Bartlett test was used to test the homogeneity of variances. A factorial ANOVA was used to test the significance of hatching average differences among the different assays and between species. The *a posteriori* Tukey test was used to compare pairs of averages between the assays. The significance level of 5% was adopted.

Pilot tests were carried out to infer the number of repetitions for the experiments, observing the amount of gemmules that hatched in different types of water. The value attributed to the minimum difference between the means of the treatments was 30, and with three repetitions. In addition, pilots were made to observe whether there was a significant difference between specimens from the same population, and whether the location where the collected specimen could influence (for example, shallower or deeper location), and in both tests there was no significant difference.

3. RESULTS

3.1 Experiment 1: Spongillidae vs Metaniidae

Gemmules of both species hatched in all treatments (Figure 1). However, *Tubella variabilis* showed higher hatching rates in all treatments, with 92% in T1, 92% in T2, and 99% in T3, while *Drulia ctenosclera* presented only 32%, 22% and 32%, respectively for the same treatments (Figure 2A). The hatching rates observed in this experiment presented significant differences among treatments ($F_{2;\,12} = 7.1$, $p < 0.009$) and between species ($F_{1;\,12} = 1305.0$, $p < 0.000$). There was no significant difference between the species in the treatments ($p > 0.05$).

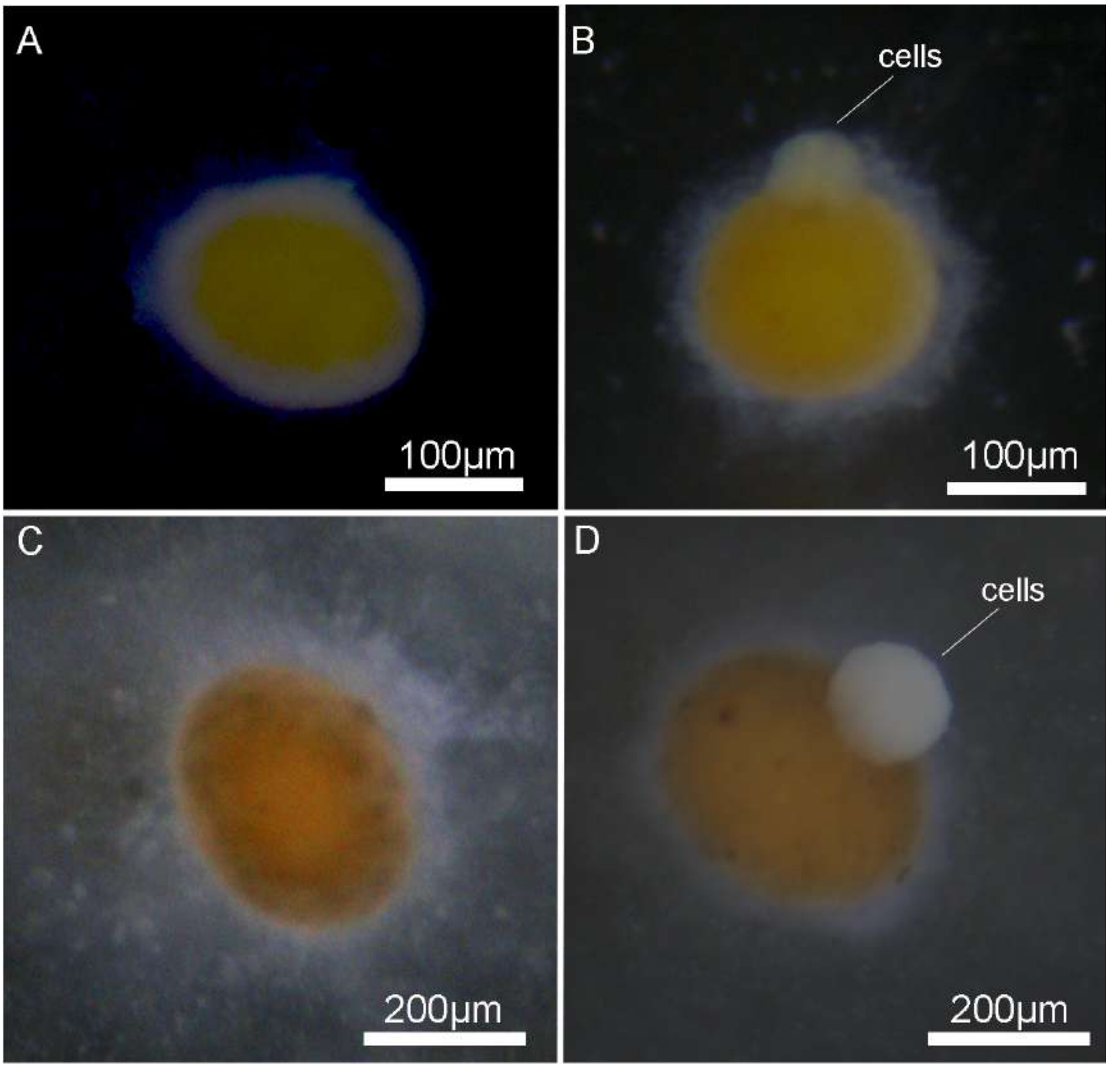

Figure 1. Gemmules from *Tubella variabilis* (BONETTO & EZCURRA DE DRAGO 1973) and *Drulia ctenosclera* VOLKMER & MOTHES 1981. **A.** Resting gemmule of *T. variabilis*. **B.** Hatching gemmule of *T. variabilis*. **C.** Resting gemmule of *D. ctenosclera*. **D.** Hatching gemmule of *D. ctenosclera*.

3.2 Experiment 2: Spongillidae vs Potamolepidae

Gemmules of both species hatched in all treatments (Figure 3). Gemmules of *Radiospongilla inesi* obtained a percentage of hatching of 70% in T1, 49% in T2 and 48% in T3, while gemmules of *Oncosclera navicella* showed hatching percentage of 38% in T1, 53 % in T2 and 66% in T3 (Figure 2B). Results showed that there was no difference among treatments ($P > 0.05$) and neither between species ($P > 0.05$). However, there was difference between the species in the treatments ($F_{2;\ 12} = 9.47$, $P < 0.003$). The Tukey test showed that the significant difference was in T1 ($P = 0.02$). Additionally, both *R. inesi* and *O. navicella* showed the higher hatching rates in treatments with waters from its collections sites (Figure 2B).

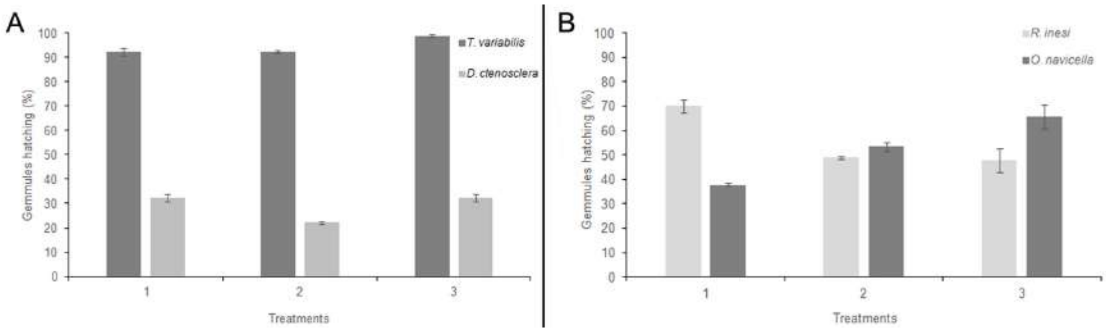

Figure 2. Percentage of gemmules hatching. **A.** Gemmules from *Tubella variabilis* (BONETTO & EZCURRA DE DRAGO 1973) and *Drulia ctenosclera* VOLKMER & MOTHES 1981, submitted to treatments: T1. water of *D. ctenosclera* collection site, Penedo, AL; T2. water of *T. variabilis* collection site, Carapebus, RJ; T3. mineral water. **B.** Gemmules from *Radiospongilla inesi* NICACIO & PINHEIRO 2011 and *Oncosclera navicella* (CARTER 1881), submitted to treatments: T1. water of *R. inesi* collection site, Recife, PE; T2. mineral water; T3. water of *O. navicella* collection site, Iretama, PR.

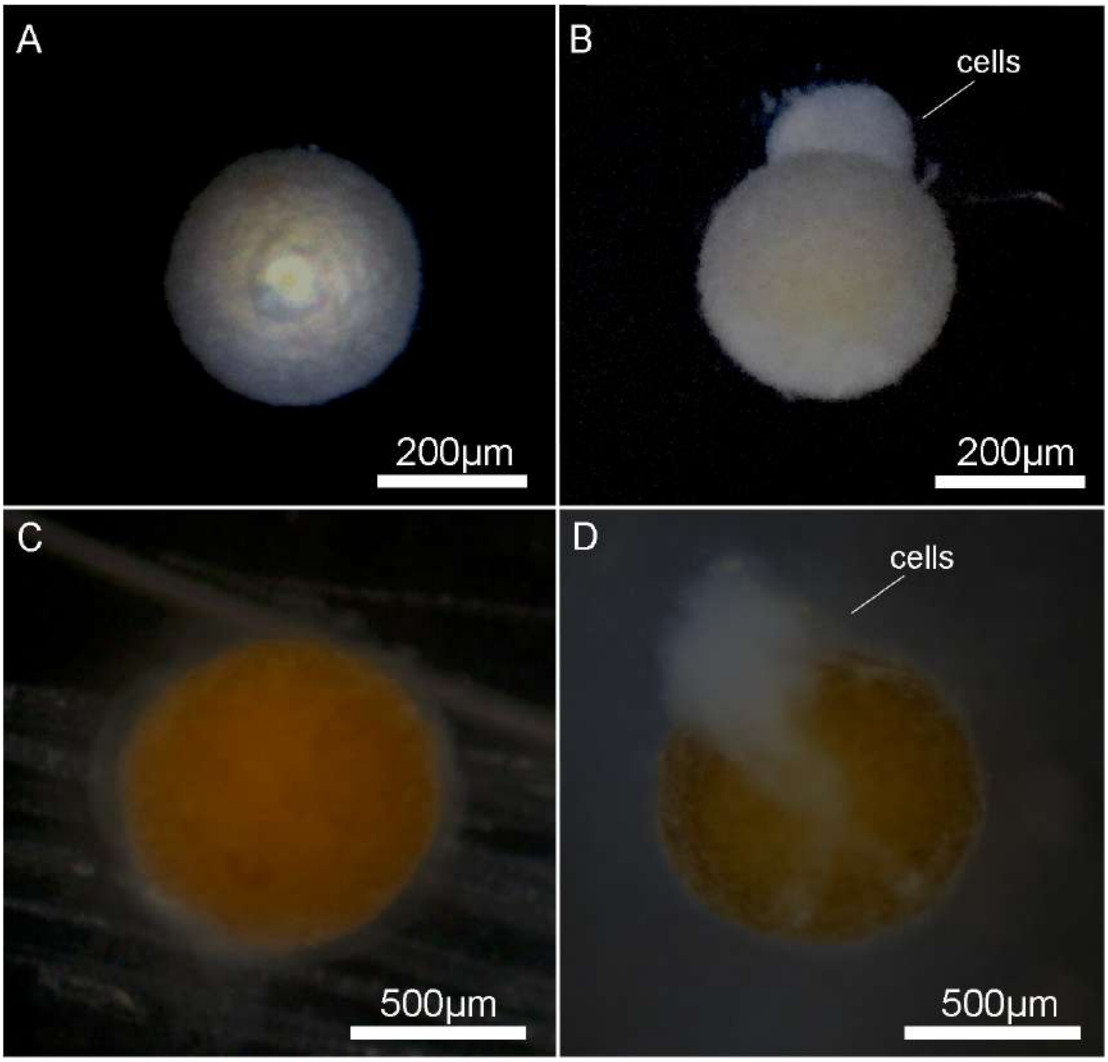

Figure 3. Gemmules from *Radiospongilla inesi* NICACIO & PINHEIRO 2011 and *Oncosclera navicella* (CARTER 1881). **A.** Resting gemmule of *R.inesi*. **B.** Hatching gemmule of *R. inesi*. **C.** Resting gemmule of *O. navicella*. **D.** Hatching gemmule of *O. navicella*.

4. DISCUSSION

4.1 Hatchability potential of the gemmules

Our results showed that each species has a different capacity of hatching, which corroborate the results from Calheira et al. (in press), as the four species here studied showed different responses to the treatments.

Gemmules of *Tubella variabilis* showed a high hatching potential in all waters (over 90%), followed by *Radiospongilla inesi*, *Oncosclera navicella* and *Drulia ctenosclera*, respectively (Figure 2). Among these species, *T. variabilis* has the smallest gemmules (average of 260 µm in diameter), apparently suggesting that the gemmules size may be related with the speed and success of hatching. Barbeau, Reiswig and Rath (1989) observed that the smaller gemmules of the freshwater sponge *Ephydatia mulleri* (LIEBERKÜHN 1856) hatched slightly faster than larger ones, but the gemmules size showed no influence on hatching rates.

As the sponges used in our experiments are from different localities, it is necessary to consider the condition of the habitat that each specimen was collected. *Drulia ctenosclera* and *Oncosclera navicella* were collected in two natural environments with different physico-chemical features (Tables 1,2). *Tubella variabilis* and *Radiospongilla inesi* were collected in two artificial environments, but the first was from a stream and the latter from a standing freshwater environment. Then, it can be that these environmental conditions influence the hatchability of each species.

The comparison between our results regarding *Radiospongilla inesi* with data available in Calheira et al. (in press) indicate that there is some intraspecific variation. Hatching rates of *R. inesi* at its own collection water and mineral water found here was 80% and 79%, respectively; Calheira et al. (in press) reported, in the same conditions, rates of 70%

in its own collection water and 49% in mineral water. These variations can be related with the concentration of organic matter in the environment that was high in the present study due the rainfall that carries a large amount of organic matter to the fish farming tanks. Melão and Rocha (1996) found that the amount of organic matter can influence hatching rates of gemmules of *Metania spinata* (CARTER, 1881), somewhat corroborating our results. However, the relation of hatching rates and organic matter was not tested here and further investigations are needed to better understand the influence of organic matter in gemmules hatching.

4.2 Gemmular morphology and distribution of species

Our results indicate that the species distribution seems to be more related to the gemmules hatching ability than to the morphology of the gemmules, contrasting to what was suggested previously by Manconi and Pronzato (2007; 1996). The comparison of the hatching rates of two species with complex gemmules, *Drulia ctenosclera* and *Tubella variabilis*, showed differences in the hatching rates between the species and the treatments (Figure 2A). Our results also indicate that if gemmules of *T. variabilis* are carried to a new environment, it are three times more likely to hatch and originate a new sponge than gemmules of *D. ctenosclera*. These findings reinforce the importance of the gemmules hatchability in the distribution of freshwater sponges.

We also observed that gemmules of simple morphology of *Oncosclera navicella* had similar hatching capacity as those with complex morphology from *Radiospongilla inesi* (Figure 2B). This suggests that the wide distribution of these species in continental waters is more related to the ability gemmules hatching. Therefore, it appears that the gemmular morphology is not a limiting factor for the distribution of *O. navicella*. Moreover, other organisms with resistance bodies that also exhibit simple morphology (e.g., with smooth surfaces and without hooks), such as bryozoans statoblasts, dormant egg of tardigrades and cysts of branchiopods, were transported, and colonized new locations by passive dispersal (Cáceres, 1997). It is important to notice, however, that the morphological complexity of the gemmules can potentialize the distribution of species, as suggested by Manconi and Pronzato (2007), as the species has the physiological plasticity (i.e., ability of the gemmules to hatch and originate a new sponge in several environmental conditions) to colonize a new environment.

Our results showed that freshwater sponges have different gemmules hatching rates in different types of inland waters. Additionally, the gemmular morphology itself is not a crucial factor to the distribution of these species. It is noteworthy that, other questions related to the gemmules physiology, as the factors that induce the formation of this dormant stages and control hatching gemmule (Reiswig et al., 2009), still need to be elucidated.

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References

Cáceres, C. E. (1997). Dormancy in invertebrates. *Invertebrate Biology*, *116*, 371-383.

Calheira, L., Santos, P. J. P., & Pinheiro, U. (2020). Hatchability of gemmules of two Neotropical freshwater sponges (Porifera: Spongillida). *Iheringia, Série Zoologia*, *110*, e2020001, 10 Jan. https://dx.doi.org/10.1590/1678-4766e2020001

Fell, P. E., & Bazer, L. J. (1990). Survival of the gemmules of *Anheteromeyenia ryderi* (Potts) following aerial exposure during winter in New England. *Hydrobiologia*, *190*, 241-246.

Funayama, N., Nakatsukasa, M., Hayashi, T., & Agata, K. (2005). Isolation of the choanocyte in the freshwater sponge, *Ephydatia fluviatilis* and its lineage marker, Ef annexin. *Development, Growth & Differentiation, 47,* 243-253. https://doi.org/10.1111/j.1440-169X.2005.00800.x

Manconi, R., & Pronzato, R. (1996). Geographical distribution and systematic position of *Sanidastra yokotonensis* (Porifera: Spongillidae). *Bulletin del Institut Royal des Sciences Naturelles de Belgique*, *66*, 219-225.

Manconi, R., & Pronzato, R. (2002). Sub-order Spongillina subord. nov.: Freshwater sponges. In J. N. A. Hooper, & R. W. M. van Soest (Eds.), *Systema Porifera: A guide to the Classification of Sponges* (pp. 921-1019). New York, EUA: Kluwer Academic/Plenum Publ.

Manconi, R., & Pronzato, R. (2007). Gemmules as a key structure for the adaptive radiation of freshwater sponges: a morphofunctional and biogeographic study. In M. R. Custódio, E. Hajdu, G. Lobo-Hadju & G. Muricy (Eds.), *Porifera Research: Biodiversity, Innovation and Sustainability* (pp. 61-77). Rio de Janeiro, BR: Museu Nacional.

Melão, M. G. G., & Rocha, O. (1996). Germinação de gêmulas de *Metania spinata* (Porifera, Metaniidae). *Anais do VII Seminário Regional de Ecologia*, *7*, 81-85.

Pronzato, R., & Manconi, R. (1995). Long-term dynamics of a freshwater sponge population. *Freshwater Biology*, *33*, 485-495.

Reiswig, H. M., Frost, T. M., & Ricciardi, A. (2009). Porifera. In J. H. Thorp & A. P. Covich (Eds.), *Ecology and Classification of North American Freshwater Invertebrates* (pp. 91-123). New York, NY: Elsevier.

van Leeuwen, C. H. A., Lovas-Kiss, A., Ovegard, M., & Green, A. J. (2017). Great cormorants reveal overlooked secondary dispersal of plants and invertebrates by piscivorous waterbirds. *Biol. Lett.*, *13*, 1-5. http://dx.doi.org/10.1098/rsbl.2017.0406

Volkmer-Ribeiro, C., & Machado, V. S. (2007). Freshwater sponges (Porifera, Demospongiae) indicators of some coastal habitats in South America: redescriptions and key to identification. *Iheringia, Série Zoologia*, *97*, 157-167.

7 CONSIDERAÇÕES FINAIS

A principal hipótese do presente trabalho era: se a fisiologia das gêmulas das esponjas de águas continentais poderia influenciar na distribuição das espécies. E para entendermos a formação de uma esponja juvenil a partir da gêmulas, fornecemos, pela primeira vez, uma descrição completa dos estágios de desenvolvimento a partir da eclosão das gêmulas de duas esponjas da região Neotropical, *Radiospongilla inesi* e *Heteromeyenia cristalina*. Com esse estudo foi possível constatar que os estágios de desenvolvimento das esponjas a partir das gêmulas de espécies da região Tropical é mais rápido que de espécies da região Temperada. Embora a temperatura possa influenciar na capacidade de eclosão das gêmulas, no presente estudo foi constatado que não há relação entre a temperatura e o tempo gasto pela gêmula para formar uma esponja juvenil. Assim, aparentemente, outros fatores, além da temperatura, podem estar influenciando no desenvolvimento.

Uma vez estabelecido o modelo de estágios de desenvolvimento da esponja a partir da gêmulas foi possível verificar os efeitos letais e subletais do metais pesados. Os resultados a respeito do efeito dos metais pesados (cobre, cádmio e mercúrio) sobre o início do desenvolvimento de *Radiospongilla inesi* mostraram que todos os metais testados afetam o desenvolvimento dessa espécie. Além disso, constatamos que *Radiospongilla inesi* tem um ótimo potencial para ser utilizado como espécie biomonitora de ambientes aquáticos contaminados por metais, devido as respostas obtidas nesse estudo, como alterações na taxa de eclosão das gêmulas, na produção de espículas e câmaras coanocitárias.

Durante esse estudo, tivemos a oportunidade de encontrar espécimes de *Radiospongilla inesi* em reprodução assexuada (com produção de gêmulas) e sexuada (com produção de embriões e larvas). Portanto, o primeiro registro da gametogênese, embriogênese e da larva parenquimela para essa esponja foi apresentado. Os resultados sobre a reprodução mostraram que *R. inesi* é hermafrodita e vivípara. Também verificamos o tempo das esponjas juvenis se desenvolverem a partir da larva que foi igual ao tempo quando desenvolvidas a partir de gêmulas (Calheira et al. 2019).

No presente trabalho, experimentos foram feitos a fim de responder questões relacionadas a distribuição das esponjas: (1) A distribuição das espécies está relacionada ao tipo de ambiente ou à dispersão? (2) O que seria mais crucial para determinar o padrão de distribuição das esponjas, a morfologia gemular ou a fisiologia da gêmula?. Para responder essa primeira questão escolhemos duas espécies da família Spongillidae, *Radiospongilla inesi* (ampla distribuição no Brasil) e *Spongilla alba* (apesar de apresentar uma ampla distribuição, ela é restrita ao ambiente), ambas com gêmulas complexas. Como resultados observamos que gêmulas de *R. inesi* não são capazes de eclodir quando expostas a alta salinidade. E a distribuição geográfica da *S. alba* está mais relacionada com a capacidade de eclosão das suas gêmulas e de colonizar novos ambientes do que com seus mecanismos de dispersão.

A presença ou ausência de gêmulas, bem como os níveis de complexidade gemular variam entre as famílias de Spongillida e têm sido considerados de grande impacto no padrão distribuição das espécies de águas continentais. Manconi; Pronzato (1996; 2007) sugeriram que a distribuição das esponjas continentais está relacionada com a eficiência das gêmulas em atuar como dispositivo de dispersão. Então, para entendermos o q seria mais crucial para explicar o padrão de distribuição das esponjas de águas continentais, escolhemos 4 espécies: *Drulia ctenosclera*, pertencente a família Metaniidae (gêmulas complexas) vs *Tubella variabilis*, família Spongillidae (gêmulas complexas) e *Oncosclera navicella*, família Potamolepidae (gêmulas simples) vs *Radiospongilla inesi*, família Spongillidae (gêmulas complexas). O estudo sobre eclosão de gêmulas mostrou que cada espécie de esponja têm diferentes capacidades de eclosão em diferentes tipos de água. Os resultados mostraram que a distribuição das espécies parece estar mais relacionada com a capacidade de eclosão das gêmulas do que com a sua morfologia.

REFERÊNCIAS

BERGQUIST, P. R. **Sponges**. Los Angeles: University of California Press, 1978.

BOWERBANK, J. S. On the Anatomy and Physiology of the Spongiadae. Part II. **Philosophical Transactions of the Royal Society**, v. 152, n. 2, p. 747-829, 1862.

BRIEN, P. Éponges du Luapula et du Lac Moero. In: SYMOENS, J. J. (Ed.). **Exploration hydrobiologique du Lac Bangweoloet du Luapula: Résultats Scientifiques**. Cercle hydrobiologique de Bruxelles, 1967, p. 1–52.

CÁCERES, C. E. Dormancy in invertebrates. **Invertebrate Biology**, v. 116, p. 371–383, 1997.

CEBRIAN, E.; URIZ, M. J. Contrasting Effects of Heavy Metals on Sponge Cell Behavior. **Arch Environ Contam Toxicol**, v. 53, p. 552–558, 2007.

ERPENBECK, D.; WEIER, T.; VOOGD, N. J.; WÖRHEIDE, G.; SUTCLIFFE, P.; TODD, J. A.; MICHEL, E. Insights into the evolution of freshwater sponges (Porifera: Demospongiae: Spongillina): Barcoding and phylogenetic data from Lake Tanganyika endemics indicate multiple invasions and unsettle existing taxonomy. **Molecular Phylogenetics and Evolution**, v. 61, p. 231–236, 2011.

ERESKOVSKY, A. V. **The comparative embryology of sponge**. Springer, 2010.

GRAY, J. E. Notes on the Arrangement of Sponges, with the Descriptions of some New Genera. **Proceedings of the Zoological Society of London**, v. 2, p. 492–558, pls. XXVII–XXVIII, 1867.

HAJDU, E.; PEIXINHO, S.; FERNANDEZ, J. C. C. **Esponjas marinhas da Bahia**: guia de campo e laboratório. Rio de Janeiro: Museu Nacional. [Série Livros, no 45.], 2011. 276 p.

HOOPER, J. N. A.; VAN SOEST, R. W. M. Class Demospongiae Sollas, 1885. In: **Systema Porifera**. New York: Kluwer Academic/Plenum Publishers, 2002. p. 15–51.

IMSIECKE, G.; STEFFEN, R.; CUSTODIO, M.; BOROJEVIC, R.; MULLER, W. Formation of spicules by sclerocytes from the freshwater sponge *Ephydatia muelleri* in short-term cultures in vitro. **In Vitro Cell Dev Biol Anim**, v. 31, p. 528–535, 1995.

ITSKOVICH, V.; KALUZHNAYA, O.; VEYNBERG, Y.; ERPENBECK, D. Endemic Lake Baikal sponges from deep water. 1: Potential cryptic speciation and discovery of living species known only from fossils. **Zootaxa**, v. 3990, n. 1, p. 123–137, 2015.

MALDONADO, M. The ecology of the sponge larva. **Canadian Journal of Zoology**, v. 84, n. 2, p. 175–194, 2006.

MALDONADO, M., BERGQUIST, P. Phylum Porifera In: YOUNG C. M.; SEWELL M. A.; RICE M. E. (Eds). **Atlas of marine invertebrates 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, 2008.

MANCONI, R.; PRONZATO, R. Geographical distribution and systematic position of Sanidastra yokotonensis (Porifera: Spongillidae). **Bulletin de l'Institut Royal des Sciences Naturelles de Belgique. Biologie**, v. 66, p. 219–225, 1996.

MANCONI, R.; PRONZATO, R. Sub-order Spongillina subord. nov.: Freshwater sponges. In: HOOPER, J. N. A.; van SOEST, R. W. M. (Eds.). **Systema Porifera: A guide to the Classification of Sponges.** New York: Kluwer Academic/Plenum Publishers, 2002, p. 921–1019.

MANCONI, R.; PRONZATO, R. Gemmules as a key structure for the adaptive radiation of freshwater sponges: a morphofunctional and biogeographical study. In: CUSTÓDIO, M. R.; LÔBO-HAJDU, G., HAJDU, E.; MURICY, G. (Eds.). **Porifera research: biodiversity, innovation and sustainability**. Rio de Janeiro: Museu Nacional. [Série Livros, no 45.], 2007, p. 61–77.

MANCONI, R.; PRONZATO, R. How to survive and persist in temporary freshwater? Adaptive traits of sponges (Porifera: Spongillida): A review. **Hydrobiologia**, v. 782, p. 11–22, 2016.

MOREIRA, S. S.; ZUANON, J. Dieta de *Retroculus lapidifer* (Perciformes: Cichlidae), um peixe reofílico do Rio Araguaia, estado do Tocantins, Brasil. **Acta Amazonica**, v. 34, n. 4, p. 691–705, 2002.

MORROW, C.; CÁRDENAS, P. Proposal for a revised classification of the Demospongiae (Porifera). **Frontiers in Zoology**, v. 12, n. 7, p. 1–27, 2015.

MURICY, G.; HAJDU, E. **Porifera Brasilis – Guia de identificação das esponjas marinhas mais comuns do sudeste do Brasil**. Rio de Janeiro: Museu Nacional, (Série Livros, v.17), 2006, 104 p.

MYSING-GUBALA, M.; POIRRIER, M. A. The effects of cadmium and mercury on gemmule formation and gemmosclere morphology in *Ephydatia fluviatilis* (Porifera: Spongillidae). **Hydrobiologia**, v. 76, p. 145–148, 1981.

PAWLIK, J. Invertebrate Zoology – Sorting Sponges. **Science**, v. 299, p. 1846–1847, 2003.

PINHEIRO, U. S. **Contribuições a Taxonomia e Biogeografia das Esponjas de Águas Continentais Brasileiras**. 2007. 220 f. Tese (Doutorado em Zoologia) – Universidade Federal do Rio de Janeiro/Museu Nacional, Rio de Janeiro.

PINHEIRO, U.; CALHEIRA, L.; HAJDU, E. A new species of freshwater sponge, *Heteromeyenia barlettai* sp. nov. from an aquarium in São Paulo, Brazil (Spongillida: Spongillidae). **Zootaxa**, v. 4034, p. 351–363, 2015.

PRONZATO, R.; MANCONI, R. Adaptive strategies of sponges in inland waters. **Bollettino di Zoologia**, v. 61, p. 395–401, 1994.
RIVERA, A.; WINTERS, I.; RUED, A.; DING, S.; POSFAI, D.; CIENIEWICZ, B.; CAMERON, K.; GENTILE, K.; HILLA, A. The evolution and function of the Pax/Six regulatory network in sponges. **Evol Dev**, v. 15, p. 186–196, 2013.

SCHMIDT, O. Grundzüge einer Spongien-Fauna des atlantischen Gebietes. **Wilhelm Engelmann**: Leipzig, v. 3, n. 5, p. 1-88, 1870.

SOLLAS, W. J. A Classification of the Sponges. **Annals and Magazine of Natural History**, v. 16, n. 95, p. 395, 1885.

SIMPSON, T. L. **The cell biology of sponges**. Springer, New York, 1984.

VAN LEEUWEN, C. H. A.; LOVAS-KISS, A.; OVEGARD, M.; GREEN, A. J. Great cormorants reveal overlooked secondary dispersal of plants and invertebrates by piscivorous waterbirds. **Biol. Lett.**, v. 13, p. 1–5, 2017.

VAN SOEST, R. W. M.; BOURY-ESNAULT, N.; VACELET, J.; DOHRMANN, M.; ERPENBECK, D.; DE VOOGD, N. J.; SANTODOMINGO, N.; VANHOORNE, B.; KELLY, M.; HOOPER, J. N. A. Global Diversity of Sponges (Porifera). **PLOS ONE**, v. 7, n. 4, p. e35105, 27 abr. 2012.

VAN SOEST, R. W. M.; BOURY-ESNAULT, N.; HOOPER, J. N. A.; RÜTZLER, K.; DE VOOGD, N. J.; ALVAREZ DE GLASBY, B.; HAJDU, E.; PISERA, A. B.; MANCONI, R.; SCHOENBERG, C.; JANUSSEN, D.; TABACHNICK, K. R.; KLAUTAU, M.; PICTON, B.; KELLY, M.; VACELET, J.; DOHRMANN, M.; DÍAZ, M. C.; CÁRDENAS, P. **World Porifera Database**. Disponível em: <http://www.marinespecies.org/porifera/index.php>. Acesso em: 10 jan. 2020.

VOLKMER-RIBEIRO, C. Evolutionary study of the freshwater sponge genus *Metania* Gray, 1867 : 3. Metaniidae, new family. **Amazoniana**, v. 9, n. 4, p. 493–509, 1986.

VOLKMER-RIBEIRO, C.; GROSSER, K. M. Gut contentes of *Leporinus obtusidens* “sensos) Von Hering (Pisces, Characoidei) used in a survey for freshwater sponges. **Rev. Brasil. Biol**, v. 41, n. 1, p. 175–183, 1981.

VOLKMER-RIBEIRO, C.; REITNER, J. Renewed Study of the Type Material of Palaeospongilla chubutensis Ott and Volkheimer (1972). In: REITNER, J.; KEUPP, H. (Eds). **Fossil and Recent Sponges**. Springer-Verlag: Berlin, Heidelberg & New York, 1991, p. 121–133.

WELTNER, W. Spongillidenstudien III. Katalog und Verbreitung der bekannten Süsswasserschwämme. **Archiv für Naturgeschichte**, v. 61, n. 1, p. 114–144, 1895.

WINDSOR, P. J.; LEYS, S. P. Wnt signaling and induction in the sponge aquiferous system: evidence for an ancient origin of the organizer. **Evol Dev**, v. 12, p. 484–493, 2010.

YIN, Z.; ZHUA, M., DAVIDSON, E. H.; BOTTJER, D. J.; ZHAO, F.; TAFFOREAU, F. Sponge grade body fossil with cellular resolution dating 60 Myr before the Cambrian. **PNAS**, v. 112, p. 1–8, 2015.

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All papers should demonstrate a high level of novelty, originality and uniqueness. The following sections and subject fields are included:

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This section will publish manuscripts dealing with fundamental processes in the environment that are related to the behavior, fate and alteration of organic and inorganic contaminants of environmental concern. This sections focuses on the dynamics of contaminants in environmental compartments such as water, soil, sediment, organisms, dust and air their interactions with the biosphere. This section also includes all scientific aspects of persistent organic pollutants (POPs), including exposure studies in the environment and people, toxicology, epidemiologic investigations, risk assessment and processes that generate or attenuate these pollutants. Only studies that are of significance to an international audience, including case studies of particular global interest, or lend themselves to interpretation at the global level should be submitted. Papers on climate change are not considered.

Specific topics of interest include:

- Emerging contaminants, such as pharmaceuticals, pesticides, flame retardants, other industrial chemicals, POPs, endocrine disruptors, etc.
- Trace metals, organometals, metalloids and radionuclides
- Environmental fate studies including transport, biodegradation, bio-accumulation and/or deposition, atmospheric (photo)chemical processes, hydrolysis, adsorption/desorption
- Transformation and mineralisation of chemicals, e.g. by bio- and photo degradation, redox processes and hydrolysis
- Soil and water chemistry focused on interaction, degradation and speciation aspects of environmental contaminants
- Novel environmental analytical methods including case studies
- Development and application of environmental modelling and quantitative structure-activity relationships to study fate and environmental dynamics
- Monitoring studies presenting new strategies, report of novel contaminants, findings or interpretations of general interest for an international readership.
- Non-target and suspect screening (e.g. effect-directed analysis)
- Marine toxins

Toxicology and Risk Assessment

The section on Environmental Toxicology and Risk Assessment covers all aspects of toxicology, i.e., the science of adverse effects of chemicals and toxic substances on living organisms including humans, and the scientific assessment of the risk that such adverse effects may occur.

Specific topics of interest include:

- Adverse effects of chemicals in environmental, aquatic and terrestrial, organisms
- Epidemiological studies on effects of chemicals in humans
- Biochemical studies related to mechanisms of adverse effects
- Toxicokinetics and metabolic studies on chemicals related to adverse effects
- Development and validation of testing methods based on living organisms or biological materials
- Effects of nanoparticles, nanocomposites and microplastics in the environment
- Endocrine disruption

- High-throughput screening
- Mechanistic toxicology
- Fish toxicology
- DNA and protein adducts
- In vitro assays and omics techniques
- Phytotoxicity

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This section focuses on technologies that manage and/or reduce environmental contaminants, including reuse and recycling processes. The technology must be beyond a basic laboratory study or have obvious implications for current or potential treatment or remediation technologies. As an example, manuscripts focusing on fundamental (bio)adsorption studies or metal extraction by plant species should be submitted to a more suitable journal. The results of studies of a routine nature should not be submitted for review. For example, for oxidation processes, the intermediates and/or the extent of mineralization of the targeted compound(s) and wastes must be quantified in addition to target compound attenuation.

Specific topics that are encouraged for publication include:

- Advanced water and wastewater treatment processes and sludge management
- Remediation (including phytoremediation) employing novel strategies, findings, or interpretations
- Hazardous waste industrial chemicals
- Hydraulic fracturing and produced water
- Electrochemical methods for water and solids treatment
- Nanotechnology
- Advanced oxidation processes
- Photolysis and photocatalysis
- Natural treatment systems (riverbank filtration, aquifer recharge and recovery)
- Characterization and fate of natural and effluent organic matter

Not considered are studies that focus on the synthesis of new materials to be used in waste water purification or remediation. Studies focusing on the removal of single contaminants are often less competitive for publication in Chemosphere.

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Environmental scientists, chemical engineers, biologists, toxicologists.

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

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