INVERTEBRATE BIOLOGY

REVISTA DE Biología Tropical

https://doi.org/10.15517/rev.biol.trop..v73i1.60485

Reproduction of the sea cucumber *Holothuria* (*Halodeima*) *grisea* (Holothuriida: Holothuriidae) from Santa Catarina coast, southern Brazil

Yara Aparecida Garcia-Tavares[1]; https://orcid.org/000-0002-4190-8029
Yara Nantes-Vasconcelos[1]; https://orcid.org/0009-0001-6038-2288
Guilherme Sabino-Rupp[2]; https://orcid.org/0000-0002-5476-9689
Pablo Damian Borges-Guilherme[1]; https://orcid.org/0000-0001-7471-6907
Adriano Weidner Cacciatori-Marenzi[3]; https://orcid.org/0000-0002-8154-5867

1. Programa de Pós-Graduação em Ambientes Costeiros e Insulares (PALI), Universidade Estadual do Paraná (UNESPAR) campus de Paranaguá, Paranaguá, Paraná, Brasil; yara.tavares@unespar.edu.br (*Correspondence), yara.nantesv@gmail.com, pablo.borges@unespar.edu.br
2. Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI), Centro de Desenvolvimento em Aquicultura e Pesca, Florianópolis, Santa Catarina, Brasil; rupp@epagri.sc.gov.br
3. Universidade do Vale do Itajaí, Itajaí, Santa Catarina, Brasil; marenzi@univali.br

Received 05-VII-2024. Corrected 25-II-2025. Accepted 21-V-2025.

ABSTRACT

Introduction: The sea cucumber, *Holothuria* (*Halodeima*) *grisea*, is a common species in intertidal rocky shores along the Brazilian coast. Due to its abundance, it is considered a potential resource for aquaculture development.

Objective: To characterize some reproductive features of *H.* (*H.*) *grisea* in southern Brazil, including sex ratio, gonadosomatic and maturity indices, and macro and microscopical observations of gonad tubules.

Methods: Adult individuals were randomly collected in August and October 2019; February, March, May, and October 2020 at the intertidal region during low tides on the coast of Santa Catarina, Brazil. The individuals were transferred to the laboratory, where they were subject to biometry, dissection, and preservation to further macro and microscopic examinations.

Results: The sex ratio was 1 : 1 and the gonad indexes were similar in both sexes and between months. Two main cohorts (25.3 and 85.3 µm) and three cytometric intervals (CI) were established. Based on histology for males, and mainly cohorts and CI for females, the individuals were categorized into five stages of the gonadal development scale (GDS) in tubules: growing, premature, mature, spawning and post spawning. Maturation and spawning events were frequent in all months, mainly February, March, and May 2020. Seven colors of the tubules were presented (transparent, transparent-pink, white, transparent-white, creamy-white, cream, and pink) with a large overlap between sex and GDS.

Conclusions: This study is the first to describe the reproductive behavior of *H.* (*H.*) *grisea* on the coast of Santa Catarina and this population exhibits a continuous reproductive pattern with reduction in winter season. Our results can offer a baseline for future research, providing various qualitative and quantitative description tools for greater effectiveness in understanding the maturity cycle of populations and managing the sea cucumber fishery in Brazil.

Key words: Holothuroidea; reproduction; gametogenesis; gonad index; maturity index; oocyte size.

RESUMEN

Reproducción del pepino de mar *Holothuria (Halodeima) grisea* (Holothuriida: Holothuriidae) de la costa de Santa Catarina, sur de Brasil

Introducción: El pepino de mar, *Holothuria* (*Halodeima*) *grisea*, es una especie común en las costas rocosas intermareales de la costa brasileña. Debido a su abundancia, se considera un recurso potencial para el desarrollo de la acuicultura.
Objetivo: Caracterizar algunos rasgos reproductivos en el sur de Brasil, incluyendo proporción de sexos, índices gonadosomáticos y de madurez y observaciones macro y microscópicas de los túbulos gonadales.
Métodos: Los individuos adultos fueron recolectados al azar en agosto y octubre de 2019; febrero, marzo, mayo y octubre de 2020 en la región intermareal durante las mareas bajas de la costa de Santa Catarina, Brazil. Los individuos se transfirieron al laboratorio, donde fueron objeto de biometría, disección y preservación para posteriores exámenes macro y microscópicos.
Resultados: La proporción de sexos fue de 1 : 1 y el índice gonadal resultó similar en cada sexo y entre los meses. Se establecieron principalmente dos cohortes (25.3 y 85.3 µm) y tres intervalos citométricos (IC). Basándose en la histología de los machos, y principalmente en las cohortes y los IC en hembras, se categorizaron cinco estadios de desarrollo gonadal (GDS) de los túbulos: en crecimiento, prematuro, maduro, desove y postdesove. Los eventos de maduración y desove fueron frecuentes en todos los meses, principalmente en febrero, marzo y mayo de 2020. Se presentaron siete colores de los túbulos (transparente, transparente-rosado, blanco, transparente-blanco, blanco-crema, crema y rosa) con un gran solapamiento entre sexo y GDS.
Conclusiones: Este estudio es el primero en describir el comportamiento reproductivo de *H.* (*H.*) *grisea* en la costa de Santa Catarina, cuya población exhibe un patrón reproductivo continuo con reducción en la estación invernal. Nuestros resultados pueden ofrecer una línea base para futuras investigaciones, proporcionando diversas herramientas de descripción cualitativa y cuantitativa para una mayor eficacia en la comprensión del ciclo de madurez de las poblaciones y el manejo de la pesquería del pepino de mar en Brasil.

Palabras clave: Holothuroidea; reproducción; gametogénesis; índice gonadosomático; índice de madurez; tamaño del ovocito.

INTRODUCTION

Sea cucumbers (Holothuroidea) are echinoderms found in all regions of the oceans, from intertidal regions to the deep-sea and from polar regions to the tropics. With approximately two thousand species they represent important components of marine ecosystems (Belbachir & Mezali, 2018; Marquet et al., 2017; Purcell et al., 2016; Tolon & Engin, 2019). In addition to their ecological significance, the body walls of sea cucumbers are considered highly nutritional and valuable seafood (bêche-de-mer or trepang) that is particularly demanded by Asian consumers (Hamel et al., 2001; Purcell et al., 2013; Ramofafia et al., 2003; Robinson & Lovatelli, 2015), reaching high prices and considered a commodity in the international seafood market (Benítez-Villalobos et al., 2013; Purcell & Eriksson, 2015). Moreover, holothurians possess a wide range of bioactive compounds that can be used in the production of pharmaceutical, nutraceutical and cosmetics products (Egloso & Delantar, 2018; Marrugo-Negrete et al., 2021; Mezali et al., 2014; Purcell, 2014). The strong demand for these organisms has depleted stocks worldwide, and has led to the collapse of natural populations, classifying some species as threatened (Carleton et al., 2013; Diupotex-Chong et al., 2022; Laguerre et al., 2020; Lewerissa et al., 2021; Purcell et al., 2012; Santos et al., 2015; Shedrawi et al., 2019; Tolon & Engin, 2019).

Holothuria (*Halodeima*) *grisea* Selenka, 1867 is one of approximately 120 large and conspicuous sea cucumbers species of the genera that occur in shallow tropical or subtropical waters (Hamel et al., 2001). It has been recognized as amphi-Atlantic species, from the Gulf of Mexico to Brazil and found in the Eastern Atlantic off West Africa (Pawson et al., 2010). Along the Brazilian coast *H.* (*H.*) *grisea* represents a very common sea cucumber usually, living under rocks or burrowing into sandy substrata, inhabiting the intertidal and subtidal habitats protected from wave action (de Lima-Bueno et al., 2018; Sabino-Rupp et al., 2024).

A fundamental barrier to improvements in the management of sea cucumber fisheries is a lack of data regarding the basic biological parameters of the most exploited species (Laguerre et al., 2020; Ramos-Miranda et al., 2017; Tolon & Engin, 2019; Venâncio et al., 2022). Studies on the reproduction of marine invertebrates are an important source of information for the potential sustainable exploitation of these resources for those species already exploited, not only for proposing management plans and closed seasons for commercial catches but also to analyze the potential use of this species in an aquaculture production (de Lima-Bueno et al., 2015; Muthiga et al., 2009; Sabino-Rupp et al., 2021). The knowledge of the reproductive characteristics of *H.* (*H.*) *grisea* is essential because this species is already subject of indiscriminate catch and commercialization in Brazil (Zacagnini-Amaral et al., 2008; Rodrigues-Ponte & Vieira-Feitosa, 2019; Sabino-Rupp & Cacciatori-Marenzi, 2021).

Southern Brazil is the high latitude limit of geographic distribution of *H. (H.) grisea* along with two other holothuroids: *Isostichopus badionotus* and *Parathyone braziliensis* (de Lima-Bueno et al., 2018; Sabino-Rupp et al., 2023; Slivak et al., 2022; Ramos-Xavier, 2010). Due to its great abundance (Mendes et al., 2006; Sabino-Rupp et al., 2023; Sabino-Rupp et al., 2024) and potential for aquaculture development (Sabino-Rupp et al., 2021) this work aimed to study for the first time the reproductive biology *H.* (*H.*) *grisea* in Santa Catarina coast. Aiming to generate information about its reproductive characteristics and to contribute to aquaculture development, this study focuses on important aspects such as sex ratio, gonad (tubule development) and maturity indices, macro and microscopical analysis of the tubules in different periods of the year.

MATERIALS AND METHODS

Study site: South Brazil is in a subtropical region, which is considered a transition area between the tropical marine biogeographic province and the warm-temperate province (Briggs & Bowen, 2013). Santa Catarina coastline comprises around 550 km, which includes hundreds of beaches, several coastal islands, mangroves, coastal lagoons, bays and estuaries (Sabino-Rupp et al., 2023). Large cities are also located in the coastal zone holding a population of more than 1 million people, which is increasing pressure on marine ecosystems (Ventura-de Souza et al., 2022).

The regional climate is dominated by the Atlantic Tropical air mass, with average monthly temperatures between 15 °C and 18 °C in winter and between 24 °C and 26 °C in summer. Average monthly rainfall varies between 100 and 350 mm, with a tendency for rain to be concentrated in the spring and summer months, and relative humidity can reach up to 85 % (Rodrigues-Filho et al., 2016). The prevailing winds are Northeasterly throughout the year and Southwesterly in winter (de Araújo et al., 2006). The water masses that occur near the continental shelf are the Tropical Water (summer and fall) influenced by the Brazil Current and, eventually, the Central Water of the South Atlantic (ACAS), in summer, in the lower layers of the water column in coastal areas (Resgalla-Jr & França-Schettini, 2006; Sabino-Rupp et al., 2005)

Armação do Itapocoroy Bay is placed on the North-central coast of Santa Catarina State (Penha municipality) in Southern Brazil (26º47'S & 48º 36'W). It is a sheltered bay with low-hydrodynamic wave action, gently sloping bedrock, and a sandy substrate consisting of coarse grain-sized sediment (Mendes et al., 2006). Along the coast *H. (H.) grisea* was common in lower intertidal rocky shores in the Northern and central portions of the littoral (Sabino-Rupp et al., 2023).

Sampling: Twenty adult individuals per month (the largest sizes observed in the field) were randomly collected by hand from a rocky-sandy shore where the intertidal region was enlarged and had low slope. Specimens were sampled for over one year (August and October 2019; February, March, May and October 2020). This study was carried out under a license (number 68215-1) to collect native fauna from the Brazilian Biodiversity Authorization (Instituto Chico Mendes de Conservação da Biodiversidade, 2007) and Information System and registration (number A61927E) of the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (Alves et al., 2018).

All individuals were transported to the Centro de Maricultura of CTTMar/UNIVALI laboratory where they were placed in buckets with 7 % magnesium chloride in seawater to relax until processing. Gonads were sexed through visual observation of the color and subsequent confirmation by histological examination. Gonads that could not be sexed macroscopically were recorded as indeterminate.

Measurements of total fresh weight (g) and total length (cm) were recorded with Mark M223 digital analytical balance (0.01 g) and a manual vernier caliper (0.02 mm) After that an incision was made along the ventral surface to remove the organs, including gonads; the gonad wet weight (g ± 0.01) was also recorded. Gonad indexes (GI) were calculated using the ratio between the gonad weight (GW), total wet weight (TWW) and gutted body wet weight (GBW) for comparisons of their effectiveness as proposed by the literature (Arsad et al., 2017; Gaudron et al., 2008; Tahri et al., 2019): GI_1 (GW × 100) / TW) and GI_2 (GW × 100) / GBW.

Histological analysis: The gonads were immediately fixed in a buffered 4 % formalin solution for 48 h and subsequently transferred to Davidson solution or formalin solution until the histological procedures. The samples were then processed with sequential submersions in graded ethanol for dehydration followed by xylene for clarification and impregnation with paraffin wax at 60 °C. After the gonad samples were embedded in 100 % (v / v) paraffin, they were cut with a thickness of 7 µm using a manual rotary microtome (Minot type) and stained with Harris' Haematoxylin solution and alcoholic Eosin Y (yellowish) solution.

Only a section of a gonad tubule was used to obtain definitive preparations. Digital images of the histological sections were captured for sex diagnosis and the gonadal development scale (GDS) under a light optical microscope (Olympus CX43) coupled with a camera (Olympus EP50). GDS was classified for each sex were based on the most outstanding histological characteristics specified for the stages used in other following previous works for the genera (Benítez-Villalobos et al., 2013; de Lima-Bueno et al., 2015; Leite-Castro et al., 2016; Ramofafia et al., 2003; Navarro et al., 2012; Venâncio et al., 2022).

To quantify reproductive status the maturity index (MI) is also calculated by the formula:

$$MI = \Sigma\ (ni \times si)\ /\ N$$

Where *ni* is the number of sea cucumbers at each tubule development stage (*i.e.* GDS), *si* is the numerical score attributed to that stage, and *N* is the total number of sea cucumbers collected monthly (Arsad et al., 2017; Benítez-Villalobos et al., 2013; Venâncio et al., 2022). Each MI value indicates its respective GDS throughout the collecting period.

Oocyte size: Measurements of female gametes (i.e. non-vitellogenic 'primary/secondary' and vitellogenic oocytes) were performed along the major axis and only those whose nucleus was visible. The diameter of all oocytes (per female) was recorded at least five areas in each histological preparation using Feret's diameter measurement (the longest distance between any 2 points along the selection perimeter) (Benítez-Villalobos et al., 2013) with the image analysis package ImageJ 2 (Rueden et al., 2017). The absolute frequencies of all oocyte diameters were grouped into size ranges each month.

The intervals of the size classes of the diameter average (Da) of the oocyte were determined by the Sturges' rule (Sturges, 1926), the main cohorts of the oocytes were determined using the Bhattacharya method (Bhattacharya, 1967). The development of oogenesis was analyzed through the evolution of oocyte size in relation to time. Bhattacharya's (1967) method was utilized to separate oocyte cohorts (Benítez-Villalobos et al., 2013; Stakowian et al., 2020).

Data analysis: A theoretical 1 : 1 sex ratio (female : male) was tested using Chi-square test (χ^2) considering the total sample over the study period. Differences in Gonad Index (GI) and mature oocyte diameter by months were analyzed using Kruskal–Wallis non-parametric test. All differences were considered statistically significant at the significance level of 5 % (p-value < 0.05). All statistical analyses were performed in R (R Core Team, 2021). Additional packages used included MASS (Venables & Ripley, 2002), vegan (Oksanen et al., 2020) and tidyverse (Wickham et al., 2019).

RESULTS

Sex ratio: A total of 83 *H.* (*H.*) *grisea* were sampled for histological analyses, with an average length (± standard deviation) of 13.7 ± 2.5 cm (from 8.8 to 20.2 cm) and an average weight of 52.2 ± 22.1 g (from 28.9 to 200.9 g). In total, 45 males (54.2 %), 36 females (43.4 %) and 2 undetermined (2.4 %) were sampled. The sex ratio of *H.* (*H.*) *grisea* not differs significantly from the 1:1 ratio. Macroscopic sexing is based on visual analysis of the gonads diagnosed correctly 85.5 % of the total organisms sexed by histological analysis and most of the misdiagnosed individuals were those visually classified as indeterminate, due to the absence of gonad coloration.

Gonad indexes: GI_1 values ranged from 2.76 ± 1.81 to 5.83 ± 5.12 and GI_2 from 4.12 ± 2.46 to 10.77 ± 15.46 with no significant differences detected between sexes or months (GI_1: χ^2 = 0.09, d.f. = 5, p = 0.999; GI_2: χ^2 = 0.23, d.f. = 5, p = 0.998) (SMF1A, SMF1B). The maximum values were recorded in October 2019 and 2020 (spring) and the lowest GI mean were pointed in February and May 2020 (summer and autumn months).

Gametogenic events: Histological diagnosis of gametogenic events were described and were made by some features as tubules and lumen aspects, degree of development of gametes and presence of nutritive phagocytes (SMT1, SMF2). Qualitative and/or quantitative analysis allowed us to characterize five different stages of gametogenic development: growing, premature, mature, spawning and post-spawning).

Biometric oocyte analysis: A total of 5 860 oocytes with diameter average (Da) ranging from 5.3 to 135.3 μm were measured. The Sturges' method distributed the population into 14 size classes of 10 μm mm o μm / class. Bhattacharya's method identified 2 main cohorts and then established three cytometric intervals for oocyte population: (1) growing: Da < 2 5.3 μm, (2) premature: 25.3 < Da < 85.3 μm and (3), mature: Da > 85.3 μm. Vitellogenic oocytes (completely mature), were observed with average diameters from 65.0 to 85.3 μm.

Throughout the study period, most oocyte population consisted of premature interval (65 %) followed completely mature (19 %) and growing (16 %) (SMF3). The latter were observed in August 2019 (35 %), an important gonial trigger, and in October 2019 (16 %) and October 2020 (23 %). Premature gametes were observed in all months with most cells measured in February/March and all in May 2020. Complete cell maturation made up 25 to 30 % of the cells in March and October 2020.

The females were categorized in four stages based on size ranges: growing, when an remarkable amount of primary oocytes was observed (1 / 3 of cells were above to 25.3 μm) and 2 / 3 are premature (in the process of vitellogenesis); mature, when 1 / 3 of oocytes were up to 85.3 μm and 2 / 3 are premature; spawning with proliferation when primary, premature and mature cells are in 1 : 2 : 1 ratio; and depletion or post spawning when all cells (the lowest quantities observed over the period) were only in intermediated diameter (SMF4).

Kruskal-Walli's test comparisons of mature (vitelogenic) oocytes (Da > 65 mm) showed significant differences (H = 266.73, d.f.= 5 / 1677, p = 0.0001) between October 2019 (Da = 85.3 µm; n = 757) and February, March and May 2020 that were grouped with a mean oocyte diameter ranging from 71.0 to 75.8 µm (17 > n > 447) (SMF5). Averages values close to 80 µm were recorded in August 2019 (n = 67) and October 2020 (n = 310).

Tubule development stages (GDS) frequency and Maturity Index (MI): Growing and premature stages were usually observed in August 2019 and October 2019 (winter and spring months) (SMF6). Maturation and spawning occurred in February, May and October 2020 (summer, fall and spring months). Post spawning stage was observed mainly in February 2020. The maximum MI values were associated with maturation and spawning stages from March to May 2020.

In males, the growing stage was not observed. Premature stage was observed in August 2019, October 2019 and 2020 (winter and spring) but growing cells could be seen in the same individual. Maturation and spawning events were frequent in all months mainly February, March and May 2020 (summer, autumn and spring). The maximum MI values were associated with spawning stage in March and May 2020.

A summary of the macro-microscopic (sex, GDS and tubules colors) diagnosis monthly records was presented with seven colors categories: transparent, transparent-pink, white, transparent-white, creamy-white, cream and pink with a large overlap between sex and GDS (SMT2).

DISCUSSION

The reproductive pattern of *H. (H.) grisea* from Santa Catarina population was characterized by a constancy of mature and releasing gametes for most part of the year. The females of this population showed a period of great investment in cell growth (winter and spring months) which allowed the development of multiple cohorts of oocytes observed in subsequent seasons and thus supported the great reproductive period in practically three seasons of the year (spring, summer and autumn). Post spawning stage observed mainly in summer end probably reflects a greater effort to release gametes. It is speculated that favorable conditions for the supply of energy resources from feeding during the winter months were responsible for this scenario.

The highest intensity of the reproductive events occurring between the spring and summer months was also observed by de Lima-Bueno et al. (2015) in a population from the coast of Paraná located around 100 km North from Armação do Itapocoroy, in both locations the months with lowest temperatures were characterized by a reduction in reproductive activity: growing (autumn) and post-spawning (winter). The presence of underdeveloped gonads or almost non-existent gonads described by de Lima-Bueno et al. (2015) probably refers to a large reduction in the size of the organ. This process of resorption and disappearance of gonads is considered a common phenomenon in species of the order Aspidochirotida during post-spawning stage (Hamel et al., 1993; Hoareau & Conand, 2001; Ramofafia et al., 2000; Rasolofonirina et al., 2005).

In comparison to Paraná population, it is agreed that winter may be a recovering period after the last reproductive effort during autumn. Although both locations are geographically close and within the same Spalding´s ecoregion (Spalding et al., 2007). The identification of a longer period of reproductive activity in the present study may be due to interpopulation variations (Arsad et al., 2017; Marquet et al., 2017; Pasquini et al., 2022; Tahri et al., 2019) where a series of environmental parameters that are acting successively or in combination to orchestrate spawning in the sea cucumbers (Marquet et al., 2017; Tolon & Engin, 2019) or even inter-annual variation (Marquet et al., 2017; Venâncio et al., 2022) as observed in this study with the difference frequency of stages between October 2019 and 2020.

Another factor to be considered is the low sampling frequency (de Lima-Bueno et al., 2015), as pointed by Leite-Castro et al. (2016) which can compromise the diagnosis of gametogenic stages and possibly explain why only three stages of tubules development were found in Paraná. We also believe that a major point to be discussed is how to quantify gonad maturity, since tubule recruitment growth model can occur (see Smiley, 1988). If no synchronization was observed in tubules development in each gonad, we could expect a high variability among the same individual (Hamel et al., 1993; Marquet et al., 2017; Mercier & Hamel, 2009; Rasolofonirina et al., 2005).

The present study reveals that the spring and summer seasons are the mainly reproductive period over Brazilian coast. Besides small differences between Southern (present study) and Northeast populations (Leite-Castro et al., 2016) of *H.* (*H.*) *grisea* along to Brazilian coast, individuals have a long reproductive period (mainly spring-summer) which suggests the existence of a similar pattern between these tropical and subtropical environments. *Holoturia* species have a wide geographical range and when exposed to different environmental factors follow a biannual or continuous reproductive pattern with variations in the onset of gametogenesis and spawning (Abdel-Razek et al., 2005; Dissanayake & Stefansson, 2010; Mercier et al., 2007; Muthiga & Kawaka, 2008; Muthiga et al., 2009; Rogers et al., 2018).

A review of publications on reproduction in sea cucumbers indicates that several species of *Holothuria* both near the equator and at higher latitudes showed reproductive periodicity at least a half of the year with patterns which reflect the seasonal differences in the environment they inhabit (Benítez-Villalobos et al., 2013) (SMT3). Generally, the size of the organism, in particular body length and weight, can be associated with gonad growth and in *Holothuria*, the body size is highly variable (SMT3). In the present study the sizes of *H.* (*H.*) *grisea* can be considered intermediate to small, compared to the other species of the same genus.

In the present study fluctuation of both gonadal indices (GI_1 and GI_2) could be associated with the longer period of highest variability of cells ratios in the females (immature and mature in oocytes) and the continuous reproductive activity of males during the year. Although GI_1 has been widely used as a good indicator of gonad maturity of sea cucumber (Arsad et al., 2017; Bahida et al., 2022; Gaudron et al., 2008; Rogers et al., 2018; Tolon & Engin, 2019), many times this index could be influenced by gonad recovery period (Benítez-Villalobos et al., 2013) or by resorption process (Drumm & Loneragan, 2005; Rasolofonirina et al., 2005).

We also agree with the use of GI relationship with the wet weight of the body wall consider a more reliable parameter for sea cucumber size for some authors (Muthiga et al., 2009; Ramofafia et al., 2000; Rasolofonirina et al., 2005). Furthermore, in reproductive season large-sized individuals could have less developed gonads or, inversely, or even gonads not developed at all or missing. This phenomenon in holothuroids could be also influenced by evisceration and subsequent viscera regeneration (Ramos-Miranda et al., 2017).

For most benthic marine species with complex life cycles, the larval stage is the dominant dispersal stage, this being directly correlated to the temporal extension of the larval phase (Rakaj et al., 2018). Moran et al. (2013), Stakowian et al. (2020) and Venâncio et al. (2022) pointed out that development time is inversely proportional to egg size. Larval period of *H.* (*H.*) *grisea* in laboratory conditions was about 20 days, but it may be influenced by temperature and diet conditions (Sabino-Rupp et al., 2021).

This reproductive strategy matches what we observed in *Holothuria* species that presents an intermediate egg size-less than 200 **μm** (Benítez-Villalobos et al., 2013; Huang et al., 2018; Navarro et al., 2012; Rasolofonirina et al., 2005; Rogers et al., 2018) with *H.* (*H.*) *grisea* turned out to be considerably smaller than that reported for other congeneric species which might also reflect its rapid larval development as a life history strategy (Rakaj et al., 2018). In laboratory condition carried out by Sabino-Rupp et al. (2021) at Armação do Itapocoroy the vitellogenic oocytes had an average diameter of 135.8 μm (± 6.7 μm) for this species and it may reach 160 μm (Rupp *pers. obs.*). Thus, it is important to bear in mind that in studies where histological techniques are used, we are referring to a gamete that has undergone retraction after the fixation techniques and is not considered a cell under fresh conditions.

The allocation of reproductive energy into a large *versus* a small number of offspring based on egg size is an important life-history strategy for any species. When comparing the three Brazilian populations of sea cucumbers of *H.* (*H.*) *grisea* we can hypothesize that the smaller size ranges of the oocytes in southern Brazil would be associated with differences in the metabolic demands of each population under local environmental conditions.

The present study integrated qualitative (macroscopical diagnosis and microscopic assessment) and quantitative tools (oocyte size scale and gonad and maturity indexes) to evaluate the reproductive activity and the gamete (oocyte) investment of *H.* (*H.*) *grisea* from Santa Catarina population. Integrative use of quantitative and qualitative analyses for gonadal developmental stage determination proved to be insightful, since qualitative methods alone impair an adequate comparison due to the lack of standardization. Quantitative tools such as oocyte diameter distribution, tubule development and maturity index provided strong evidence to indicate that the species exhibits a continuous reproductive pattern with a reduction in winter season.

Most studies on the reproduction of holothuroids use the coloration of the gonads for sexing and the degree of development of the gonad (tubules). It is common to indicate that creamy-colored gonads are testes and that pink-colored gonads are ovaries (Arsad et al., 2017; de Lima-Bueno et al., 2015; Muthiga et al., 2009; Navarro et al., 2012; Pasquini et al., 2022; Rogers et al., 2018; Santos et al., 2015; Venâncio et al., 2022). In our study, the visual assessment of sexual identification was mostly correct but inconsistent for diagnosing the animal maturity. Although no photographic records of the colors were made in this study, recommended that these are taken for further studies.

Aquaculture and fisheries remarks: As sea cucumber fisheries along the Brazilian coast is an unreported and unregulated activity (Rodrigues-Ponte & Vieira-Feitosa, 2019; Sabino-Rupp & Cacciatori-Marenzi, 2021), it is urgent to carry out biological and ecological studies to develop effective management measures before the overexploitation of sea cucumber populations. In Brazil**,** aquaculture efforts have been attempted in the Northeast region (Souza-Junior et al., 2017) and more recently in the South (Santa Catarina). The availability of *H.* **(***H.***)** *grisea* natural stocks is one of the main reasons for the selection of this species for aquaculture studies (Sabino-Rupp et al., 2021; Sabino-Rupp et al., 2023; Sabino-Rupp et al., 2024). The information from this study may support spawning induction by knowing when to capture broodstock but it does not support larval culture or juvenile production. Therefore, it is suggested that the activities to induce spawning of *H.* (*H.*) *grisea* should not be attempted during late autumn and winter months. It is also recommended to carry out further studies on growth, recruitment and survivorship on longer time-scales, on determination of the first sexual maturity and on fecundity per size distribution. Such features would allow a better understanding of the population ecology and the development of aquaculture and stock management tools, as well as fishing regulations based on precise information for conservation of the Santa Catarina ecosystems.

In fact, this study is the first to describe the reproductive behavior of *H.* (*H.*) *grisea* on the coast of Santa Catarina, and specifically in Armação de Itapocoroy. This can offer a baseline for future research, providing various qualitative and quantitative description tools for greater effectiveness in understanding the maturity cycle of populations and managing the sea cucumber fishery in Brazil. Finally, it is suggested that additional reproductive studies be carried out over a longer period of years, as inter-annual variation is likely to occur, and ongoing climate change, along with an above historical average increase in rainfall and heat in Southern Brazil make this recommendation imperative.

Ethical statement: The authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

See supplementary material
A34v73n1-suppl1

REFERENCES

Abdel-Razek, F. A., Abdel-Rahman, S. H., El-shimy, N. A., & Omar, H. A. (2005). Reproductive biology of the tropical sea cucumber *Holothuria atra* (Echinodermata: Holothuroidea) in the Red Sea coast of Egypt. *Egyptian Journal of Aquatic Research*, *31*(2), 383–402.

Alves, R. J. V., Weksler, M., Oliveira, J. A., Buckup, P. A., Pombal-Jr, J. P., Santana, H. R. G., Peracchi, A. L., Kellner, A. W. A., Aleixo, A., Langguth, A., de Almeida, A. M. P., Albernaz, A. L., Ribas, C. C., Zilberberg, C., Grelle, C. E. V., Rocha, C. F. D., Lamas, C. J. E., Haddad, C. F. B., Bonvicino, C. R., ... Caramaschi, U. (2018). Brazilian legislation on genetic heritage harms Biodiversity Convention goals and threatens basic biology research and education. *Anais da Academia Brasileira de Ciências*, *90*(2), 1279–1284. https://doi.org/10.1590/0001-37652018201800460

Arsad, N. A., Othman, R., Muhamad-Shaleh, S. R., Abdullah, F. C., Matsumoto, M. M., Mustafa, S., & Senoo, S. (2017). Reproductive pattern of sea cucumber, *Holothuria scabra* at two different sites in Sabah, Malaysia. *Journal of Natural Sciences Research*, *7*(20), 67–78.

Bahida, A., Abrehouche, A., Nhhala, H., Chadli, H., Nhhala, I., & Erraioui, H. (2022). Reproductive activity of three sea cucumber species *Holothuria forskali*, *Holothuria sanctori* and *Holothuria tubulosa* in WSW Alboran Sea, M'diq Bay, Morocco. *Egyptian Journal of Aquatic Biology & Fisheries*, *26*(4), 77–196. https://doi.org/10.21608/ejabf.2022.249163

Belbachir, N. E., & Mezali, K. (2018). Food preferences of four aspidochirotid holothurians species (Holothuroidea: Echinodermata) inhabiting the *Posidonia oceanica* meadow of Mostaganem area (Algeria). *SPC Beche-de mer Information Bulletin*, *38*, 55–59.

Benítez-Villalobos, F., Avila-Poveda, O. H., & Gutiérrez-Méndez, I. S. (2013). Reproductive biology of *Holothuria fuscocinerea* (Echinodermata: Holothuroidea) from Oaxaca, Mexico. *Sexuality and Early Development in Aquatic Organisms*, *1*, 13–24. https://doi.org/10.3354/sedao00003

Bhattacharya, P. K. (1967). Efficient estimation of a shift parameter from grouped data. *Annals of Mathematical Statistics*, *38*(6), 1770–1787. https://doi.org/10.1214/aoms/1177698611

Briggs, J. C., & Bowen, B. W. (2013). Marine shelf habitat: Biogeography and evolution. *Journal of Biogeography*, *40*(6), 1023–1035. https://doi.org/10.1111/jbi.12082

Carleton, C., Hambrey, J., Govan, H., Medley, P., & Kinch, J. (2013). Effective management of sea cucumber fisheries and the beche-de-mer trade in Melanesia. *SPC Fisheries Newsletter*, *140*, 24–42.

de Araújo, S. A., Haymussi, H., Reis, F. H., & Silva, F. E. (2006). Caracterização climatológica do município de Penha, SC. In J. O. Branco, & A. Marenzi (Eds.), *Bases ecológicas para um desenvolvimento sustentável: Estudo de caso em Penha* SC (pp. 11–28). SC. Editora da UNIVALI.

de Lima-Bueno, M. L., dos Santos-Alitto, R. A., Borges-Guilherme, P. D., Di-Domenico, M., & Borges, M. (2018). Guia ilustrado dos Echinodermata da porção sul do embaiamento sul Brasileiro. *Pesquisa e Ensino em Ciências Exatas e da Natureza*, *2*(2), 169–237. https://doi.org/10.29215/pecen.v2i2.1071

de Lima-Bueno, M. L., Garcia-Tavares, Y. A., Di Domenico, M., & Borges, M. (2015). Gametogenesis and weight change of body organs of the sea cucumber *Holothuria* (*Halodeima*) *grisea* (Aspidochirotida: Holothuriidae) in southern Brazil. *Revista de Biología Tropical*, *63*(Supplement 2), 285–296. https://doi.org/10.15517/rbt.v63i2.23163

Dissanayake, D. C. T., & Stefansson, G. (2010) Reproductive biology of the commercial sea cucumber *Holothuria atra* (Holothuroidea: Aspidochirotida) in the northwestern coastal waters of Sri Lanka. *Invertebrate Reproduction & Development*, *54*(2), 65–76. https://doi.org/10.1080/07924259.2010.9652318

Diupotex-Chong, M. E., Solís-Marín, F. A., & Laguarda-Figueras, A. (2022). Some aspects to oogenesis from sea cucumber *Cucumaria californica* (Semper, 1868) (Echinodermata: Holothuroidea) of the mexican Pacific. *Brazilian Journal of Animal and Environmental*

Research, *5*(1), 564–580. https://doi.org/10.34188/bjaerv5n1-044

Drumm, D. J., & Loneragan, N. R. (2005). Reproductive biology of *Holothuria leucospilota* in the Cook Islands and the implications of traditional fishing of gonads on the population. *New Zeland Journal of Marine and Freshwater Research*, *39*(1), 141–146. https://doi.org/10.1080/00288330.2005.9517297

Egloso, N. L., & Delantar, F. V. (2018, June 14-15). *Acceptability of sea cucumber (*Holothuria nobilis*) food chips and its nutritional value: Inputs to income generating project* [Conference session]. 9th International Conference on Environmental, Chemical, Biological and Medical Sciences, Cebu, Phillippines. https://doi.org/10.17758/ERPUB1.ER0618215

Gaudron, S. M., Kohler, S. A., & Conand, C. (2008). Reproduction of the sea cucumber *Holothuria leucospilota* in the Western Indian Ocean: Biological and ecological aspects. *Invertebrate Reproduction & Development*, *51*(1), 19–31. https://doi.org/10.1080/07924259.2008.9652253

Hamel, J. F., Conand, C., Pawson, D. L., & Mercier, A. (2001). The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): Its biology and exploitation as Beche-de-mer. *Advances in Marine Biology*, *41*, 129–223. https://doi.org/10.1016/S0065-2881(01)41003-0

Hamel, J. F., Himmelman, J. H., & Dufresne, L. (1993). Gametogenesis and spawning of the sea cucumber *Psolus fabricii* (Duben and Koren). *Biological Bulletin*, *184(2)*, 125–143. https://doi.org/10.2307/1542223

Hoareau, T., & Conand, C. (2001). Sexual reproduction of *Stichopus chloronotus*, a fissiparous sea cucumber, on Reunion Island, Indian Ocean. *SPC Beche-de mer Information Bulletin*, *15*, 4–12.

Huang, W., Huo, D., Yu, Z., Ren, C., Jiang, X., Luo, P., Chen, T., & Hu, C. (2018). Spawning, larval development and juvenile growth of the tropical sea cucumber *Holothuria leucospilota*. *Aquaculture*, *488*, 22–29. https://doi.org/10.1016/j.aquaculture.2018.01.013

Instituto Chico Mendes de Conservação da Biodiversidade. (2007). *Sistema de Autorização e Informação em Biodiversidade (SISBIO)*. ICMBio. https://www.icmbio.gov.br/sisbio/

Laguerre, H., Raymond, G., Plan, P., Améziane, N., Bailly, X., & Le Chevalier, P. (2020). First description of embryonic and larval development, juvenile growth of the black sea-cucumber *Holothuria forskali* (Echinodermata: Holothuroidea), a new species for aquaculture in the north-eastern Atlantic. *Aquaculture*, *521*, 734961. https://doi.org/10.1016/j.aquaculture.2020.734961

Leite-Castro, L. V., de Souza-Junior, J., Salmito-Vanderley, C. S. B., Ferreira-Nunes, J., Hamel, J. F., & Mercier, A. (2016). Reproductive biology of the sea cucumber *Holothuria grisea* in Brazil: Importance of social and environmental factors in breeding coordination. *Marine Biology*, *163*, 67. https://doi.org/10.1007/s00227-016-2842-x

Lewerissa, Y. A., Ongkers, O. T. S., Pattikawa, J. A., Tetelepta, J. M. S., & Divinubun, A. (2021). The fishery of sea-cucumber Holothuridae in Kilbat Village, Central Maluku Regency, Indonesia. *IOP Conference Series: Earth and Environmental Science*, *797*, 012008. https://doi.org/10.1088/1755-1315/797/1/012008

Marquet, N., Conand, C., Power, D. M., Canário, A. V. M., & González-Wangüemert, M. (2017). Sea cucumbers, *Holothuria arguinensis* and *H. mammata*, from the southern Iberian Peninsula: Variation in reproductive activity between populations from different habitats. *Fisheries Research*, *191*, 120–130. https://doi.org/10.1016/j.fishres.2017.03.007

Marrugo-Negrete, J., Pinedo-Hernández, J., Marrugo-Madrid, S., Navarro-Frómeta, E., & Diez, S. (2021). Sea cucumber as bioindicator of trace metal pollution in coastal sediments. *Biological Trace Element Research*, *19*(5), 2022–2030. https://doi.org/10.1007/s12011-020-02308-3

Martins, A. D., & Rezende-Ventura, C. (2013). Ciclo gametogênico do pepino-do-mar *Holothuria* (*Halodeima*) *grisea* (Echinodermata: Holothuroidea). *Proceedings of the 2th Latin American Congress of Echinoderms*, *61*, 63–106.

Mendes, F. M., Cacciatori-Marenzi, A. W., & Di Domenico, M. (2006). Population patterns and seasonal observations on density and distribuition of *Holothuria grisea* (Holothuroidea: Aspidochirotida) on the Santa Catarina coast, Brazil. *SPC Beche-de mer Information Bulletin*, *23*, 5–16.

Mercier, A., & Hamel, J. F. (2009). *Advances in marine biology: Endogenous and exogenous control of gametogenesis and spawning in echinoderms* (Vol. 55). Academic Press. https://doi.org/10.1016/S0065-2881(09)55001-8

Mercier, A., Ycaza, R. H., & Hamel, J. F. (2007). Long-term study of gamete release in a broadcast-spawning holothurian: Predictable lunar and diel periodicities. *Marine Ecology Progress Series*, *329*, 179–189.

Mezali, K., Soualili, D. L., Neghli, L., & Conand, C. (2014). Reproductive cycle of the sea cucumber *Holothuria* (*Platyperona*) *sanctori* (Holothuroidea: Echinodermata) in the southwestern Mediterranean Sea: interpopulation variability. *Invertebrate Reproduction & Development*, *58*(3), 179–189. https://doi.org/10.1080/07924259.2014.883337

Moran, A. L., McAlister, J. S., & Whitehill, E. A. G. (2013). Eggs as energy: Revisiting the scaling of egg size and energetic content among echinoderms. *The Biological Bulletin*, *224*(3), 184–191. https://doi.org/10.1086/BBLv224n3p184

Muthiga, N. A. & Kawaka, J. (2008, July 7–11th). *The effects of temperature and light on the gametogenesis and spawning of four sea urchin and one sea cucumber species on coral reefs in Kenya*. In B. Riegl, & R. E. Dodge (Eds.), 11th International coral reef symposium proceedings (pp. 356–360). ICRS Conference Proceedings.

Muthiga, N. A., Kawaka, J. A., & Ndirangu, S. (2009). The timing and reproductive output of the commercial sea cucumber *Holothuria scabra* on the Kenyan coast. *Estuarine, Coastal and Shelf Science*, *84*(3), 353–360. https://doi.org/10.1016/j.ecss.2009.04.011

Navarro, P. G., García-Sanz, S., & Tuya, F. (2012). Reproductive biology of the sea cucumber *Holothuria sanctori* (Echinodermata: Holothuroidea). *Scientia Marina*, *76*(4), 741–752. https://doi.org/10.3989/scimar.03543.15B

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., … Borman, T. (2020). *vegan: Community ecology package* (Version 2) [Software]. https:// 10.32614/CRAN.package.vegan

Pasquini, V., Porcu, C., Marongiu, M. F., Follesa, M. C., Giglioli, A. A., & Addis, P. (2022). New insights upon the reproductive biology of the sea cucumber *Holothuria tubulosa* (Echinodermata, Holothuroidea) in the Mediterranean: Implications for management and domestication. *Frontiers in Marine Science*, *9*, 1029147. https://doi.org/10.3389/fmars.2022.1029147

Pawson, D. L., Pawson, D. J., & King, R. A. (2010). A taxonomic guide to the Echinodermata of the South Atlantic Bight, USA: 1. Sea cucumbers (Echinodermata: Holothuroidea). *Zootaxa*, *2449*, 1–48. https://doi.org/10.5281/zenodo.195134

Purcell, S. W. (2014). Value, market preferences and trade of beche-de-mer from Pacific Island sea cucumbers. *PloS ONE*, *9*(4), e95075. https://doi.org/10.1371/journal.pone.0095075

Purcell, S. W., & Eriksson, H. (2015). Echinoderms piggybacking on sea cucumbers: Benign effects on sediment turnover and movement of hosts. *Marine Biology Research*, *11*(6), 666–670. https://doi.org/10.1080/17451000.2014.962544

Purcell, S. W., Conand, C., Uthicke, S., & Byrne, M. (2016). Ecological roles of exploited sea cucumbers. In R. N. Hughes, D. J. Hughes, I. P. Smith, & A. C. Dale (Eds.), *Oceanography and Marine Biology* (pp. 375–394). CRC press.

Purcell, S. W., Hair, C. A., & Mills, D. J. (2012). Sea cucumber culture, farming and sea ranching in the tropics: Progress, problems and opportunities. *Aquaculture*, *368–369*, 68–81. https://doi.org/10.1016/j.aquaculture.2012.08.053

Purcell, S. W., Mercier, A., Conand, C., Hamel, J. F., Toral-Granda, M. V., Lovatelli, A., & Uthicke, S. (2013). Sea cucumber fisheries: global analysis of stocks, management measures and drivers of overfishing. *Fish and Fisheries*, *14*(1), 34–59. https://doi.org/10.1111/j.1467-2979.2011.00443.x

Rakaj, A., Fianchini, A., Boncagni, P., Scardi, M., & Cataudella, S. (2018) Artificial reproduction of *Holothuria polii*: A new candidate for aquaculture. *Aquaculture*, *498*, 444–453. https://doi.org/10.1016/j.aquaculture.2018.08.060

Ramofafia, C., Battaglene, S. C., Bell, J. D., & Bryne, M. (2000). Reproductive biology of the commercial sea cucumber *Holothuria fuscogilva* in the Solomon Islands. *Marine Biology*, *136*, 1045–1056. https://doi.org/10.1007/s002270000310

Ramofafia, C., Byrne, M., & Battaglene, S. C. (2003). Reproduction of the commercial sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea) in the Solomon Islands. *Marine Biology*, *142*, 281–288. https://doi.org/10.1007/s00227-002-0947-x

Ramos-Miranda, J., del Río-Rodríguez, R., Flores-Hernández, D., Rojas-González, R. I., Gómez-Solano, M., Cu-Escamilla, A. D., Gómez-Criollo, F., Sosa-López, A., Torres-Rojas, Y. E., & Juárez-Camargo, P. (2017). Reproductive cycle of the sea cucumber *Holothuria floridana* in the littorals of Campeche, Mexico. *Fisheries Science*, *83*, 699–714. https://doi.org/10.1007/s12562-017-1100-6

Ramos-Xavier, L. A. (2010). Inventário dos equinodermos do estado de Santa Catarina, Brasil. *Brazilian Journal of Aquatic Science and Techonology*, *14*, 73–78.

Rasolofonirina, R., Vaitilingon, D., Eeckhaut, I., & Jangoux, M. (2005). Reproductive cycle of edible echinoderms from the Southwestern Indian Ocean II. The Sandfish *Holothuria scabra* (Jaeger, 1833). *Western Indian Ocean Journal of Marine Science*, *4*(1), 61–76. https://doi.org/10.4314/wiojms.v4i1.28474

R Core Team (2021). R*: A language and environment for statistical computing.* (Version 4.0.4) [Software]. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Resgalla-Jr, C., & França-Schettini, C. A. (2006). Características e variação do seston da enseada da Armação do Itapocoroy, Penha, SC. In J. O. Branco, & A. W. Cacciatori-Marenzi (Eds.), *Bases ecológicas para um desenvolvimento sustentável: Estudo de caso em Penha, SC* (pp. 107–120). Editora da UNIVALI.

Robinson, G., & Lovatelli, A. (2015). Global sea cucumber fisheries and aquaculture FAO's inputs over the past few years. *FAO Aquaculture Newsletter*, *53*, 55–57.

Revista de Biología Tropical, ISSN: 2215-2075 Vol. 73: e60485, enero-diciembre 2025 (Publicado Jun. 10, 2025)

Rodrigues-Filho, J. L., Guerreiro-Couto, E. C., Barbieri, E., & Branco, J. O. (2016). Ciclos sazonais da carcinofauna capturada na pesca do camarão-sete-barbas, *Xiphopenaeus kroyeri* no litoral de Santa Catarina. *Boletim do Instituto de Pesca*, *42*(3), 648–661. https://doi.org/10.20950/1678-2305.2016v42n3p648

Rodrigues-Ponte, I. A., & Vieira-Feitosa, C. V. (2019) Evaluation of an unreported and unregulated sea cucumber fishery in eastern Brazil. *Ocean & Coastal Management*, *167*, 1–8. https://doi.org/10.1016/j.ocecoaman.2018.09.016

Rogers, A., Hamel, J. F., & Mercier, A. (2018). Population structure and reproductive cycle of the commercial sea cucumber *Holothuria mexicana* (Echinodermata: Holothuroidea) in Belize. *Revista de Biologia Tropical*, *66*(4), 1629–1648. http://dx.doi.org/10.15517/rbt.v66i4.32551

Rueden, C. T., Schindelin, J., Hiner, M. C., Dezonia, B. E., Walter, A. E., Arena, E. T., & Eliceiri, K. W. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics*, *18*, 529. https://doi.org/10.1186/s12859-017-1934-z

Sabino-Rupp, G., & Cacciatori-Marenzi, A. W. (2021). Holotúrias do litoral de Santa Catarina (Brasil): Captura ilegal e potencial para a aquicultura. In M. De Donato, N. González-Henríquez, M. Rey-Méndez, P. M. Baltazar-Guerrero, J. Sonnenholzner, J. J. Alió-Mingo, & C. Lodeiros (Eds.), *Foro Iberoamericano de los recursos marinos y la acuicultura: Sinergia entre ciencia e industria para el desarrollo y la sostenibilidad* (pp. 607–618). Ediciones AFRIMAR-AFIRMA.

Sabino-Rupp, G., Cacciatori-Marenzi, A., Ventura de Souza, R., & Schroeder, R. (2024). Population survey of *Holothuria* (*Halodeima*) *grisea* (Aspidochirotida: Holothuriidae) at its limit of geographic distribution in the Western South Atlantic. *Revista de Biologia Tropical*, *72*(Supplement 1), e58623. http://dx.doi.org/10.15517/rev.biol.trop..v72is1.58623

Sabino-Rupp, G., Cacciatori-Marenzi, A. W., Ventura de Souza, R., & Martins, L. (2023). Sea cucumbers (Echinodermata: *Holothuroidea*) from Santa Catarina coast, Southern Brazil, with notes on their abundance and spatial distribution. *Journal of Shellfish Research*, *42*(1), 143–153. https://doi.org/10.2983/035.042.0115

Sabino-Rupp, G., da Costa, R. C., Cacciatori-Marenzi, A. W., Manzoni, G. C., & da Silva, I. S. (2021) Reprodução e larvicultura de *Holothuria (H.) grisea* Selenka, 1867 (Holothuroidea: Aspidochirotida) em laboratório: Resultados iniciais no sul do Brasil. *AquaTechinica*, *3*(3), 133–143. https://doi.org/10.5281/zenodo.5758271

Sabino-Rupp, G., Parsons, G. J., Thompson, R. J., & de Bem, M. M. (2005). Influence of environmental factors, season and size at deployment on growth and retrieval of postlarval lion's paw scallop *Nodipecten nodosus* (Linnaeus, 1758) from a subtropical environment. *Aquaculture*, *243*(1–4), 195–216. https://doi.org/10.1016/j.aquaculture.2004.10.007

Santos, R., Dias, S., Pinteus, S., Silva, J., Alves, C., Tecelao, C., Pedrosa, R., & Pombo, A. (2015). Sea cucumber *Holothuria forskali*, a new resource for aquaculture? Reproductive biology and nutraceutical approach. *Aquaculture Research*, *47*(7), 2307–2323. https://doi.org/10.1111/are.12683

Shedrawi, G., Kinch, J. P., Halford, A. R., Bertram, I., Molai, C., & Friedman, K. J. (2019). CITES listing of sea cucumber species provides opportunities to improve management of the beche-de-mer trade. *SPC Fisheries Newsletter*, *159*, 6–8.

Slivak, N. N., Lindner, A., & Picolli-Romanowski, H. (2022.) Echinoderms from Santa Catarina, southern Brazil: an update on biodiversity and distribution. *Papéis Avulsos de Zoologia*, *62*, e202262004. https://doi.org/10.11606/1807-0205/2022.62.004

Smiley, S. (1988). The dynamics of oogenesis and the annual ovarian cycle of *Stichopus californicus* (Echinodermata: Holothuroidea). *Biological Bulletin*, *175*(1), 79–93.

Souza-Junior, J., Ponte, I., Melo-Coe, C., Lobo-Farias, W. R., Veiera-Feitosa, C., Hamel, J. F., & Mercier, A. (2017). Sea cucumber fisheries in Northeast Brazil. *SPC Beche-de-mer Information Bulletin*, *37*, 43–47.

Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Nick, D., Ferdaña, Z. A., Finlayson, M., Halpern, B. S., Jorge, M. A., Lombana, A., Lourie, S. A., Martin, K. D., Mcmanus, E., Molnar, J., Recchia, C. A., & Robertson, J. (2007). Marine ecoregions of the world: Abioregionalization of coastal and shelf areas. *BioScience*, *57*(7), 573–583.

Stakowian, N., Guilherme, P. D. B., Ferreira, A. M., de Lima-Bueno, M., & Tavares, Y. A. G. (2020). Reproductive investment of *Perna perna* (Mytilida: Mytilidae) in subtropical regions: Combining several methods. *Pan-American Journal of Aquatic Sciences*, *15*(3), 178–194.

Sturges, H. A. (1926). The choice of a class interval. *Journal of the American Statistical Association*, *21*(153), 65–66. https://doi.org/10.1080/01621459.1926.10502161

Tahri, Y., Dermeche, S., Chahrour, F., & Bouderbala, M. (2019). The reproduction cycle of the sea cucumber *Holothuria* (*Holothuria*) *tubulosa* Gmelin, 1791 (Echinodermata: Holothuroidea: Holothuriidae) in Oran coast, Algeria. *Biodiversity Journal*, *10*(2), 159–172. https://doi.org/10.31396/Biodiv.Jour.2019.10.2.159.172

Tolon, M. T., & Engin, S. (2019). Gonadal development of the holothurian *Holothuria polii* (Delle Chiaje, 1823) in spawning period at the Aegean Sea (Mediterranean Sea). *Ege Journal of Fisheries and Aquatic*

Sciences, *36*(4), 379–385. https://doi.org/10.12714/egejfas.36.4.09

Venables, W. N., & Ripley, B. D. (2002*). Modern applied statistics with S* (4th ed.). Springer.

Venâncio, E., Félix, P. M., Brito, A. C., Azevedo-e Silva, F., Simões, T., Sousa, J., Mendes, S., & Pombo, A. (2022). Reproductive biology of the sea cucumber *Holothuria mammata* (Echinodermata: Holothuroidea). *Biology*, *11*(5), 622. https://doi.org/10.3390/biology11050622

Ventura-de Souza, R., Moresco, V., Miotto, M., Marques-Souza, D. S., & de Campos, C. J. A. (2022). Prevalence, distribution and environmental effects on faecal indicator bacteria and pathogens of concern in commercial shellfish production areas in a subtropical region of a developing country (Santa Catarina, Brazil). *Environmental Monitoring and Assessment*, *194*, 286. https://doi.org/10.1007/s10661-022-09950-5

Wickham, H., Averick, M., Bryan, J., Chang, W., D' Agostino-Mcgowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Lin-Pedersen, T., Miller, E., Milton-Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D. P., Spinu, V., …Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Zacagnini-Amaral, A. C., Volkmer-Ribeiro, C., Dreher-Mansur, M. C., dos Santos, S. B., Paiva-Avelar, W. E., Mattews-Cascon, H., Pereira-Leite, F. P., Schmidt-de Melo, G. A., Alves-Coelho, P., Buckup, G. B., Buckup, L., Rezende-Ventura, C. R., & Gonçalves-Tiago, C. G. (2008). A situação de ameaça dos invertebrados aquáticos no Brasil. In A. B. Monteiro-Machado, G. Moreira-Drummond, & A. Pereira-Paglia (Eds.), *Livro vermelho da fauna brasileira ameaçada de extinção* (pp. 156–351). Ministério do Meio Ambiente, Brasília.