

## **Gametogenesis and weight change of body organs of the sea cucumber *Holothuria (Halodeima) grisea* (Aspidochirotida: Holothuriidae) in Southern Brazil**

Maristela de Lima Bueno<sup>1,2,3</sup>, Yara Aparecida Garcia Tavares<sup>3</sup>, Maikon Di Domenico<sup>1</sup>  
& Michela Borges<sup>1</sup>

1. Zoological Museum “Prof. Dr. Adão José Cardoso”, Institute of Biology, University of Campinas; Campinas, São Paulo, Brazil; maik2dd@gmail.com, borgesm@unicamp.br
2. Postgraduate degree in Animal Biology, Institute of Biology, University of Campinas; Campinas, São Paulo, Brazil; maristelabueno04@gmail.com
3. University of Paraná, campus Paranaguá, Department of Biological Sciences; Paranaguá, Paraná, Brazil; tavares.y@gmail.com

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**Abstract:** The sea cucumber *Holothuria (Halodeima) grisea* is a widely distributed and economically important species that has been harvested in Brazil for several years, where there are no studies on its reproduction. We studied a population near Guaratuba Bay in Southern Brazil. Four sampling campaigns were made, in February, April, June and October 2008. We examined the macroscopic and microscopic features of the gonads to describe the gametogenesis. In addition, we analyzed the weight change of body organs. A total of 94 individuals were examined and no evidence of external sexual dimorphism was found. In April there were more males than females. Biometrically the body wall and gonads are the most important organs. The body wall increases in the June and the gonads during October and February; there might be an exchange of energy between these organs. Mature gonads are elongated and thick (red in females, cream in males). There were mature individuals year round, with an increase of sexual activity in February when oocyte mature diameter ranges from 70 to 160  $\mu\text{m}$ . Spawning was in winter (June). Maturation period and body component variations were similar between sexes. Rev. Biol. Trop. 63 (Suppl. 2): 285-296. Epub 2015 June 01.

**Key words:** reproduction, sea cucumber, gonad index, histology, spawning, rocky shore.

Holothuroidea is a diverse class of worm-like and usually soft-bodied echinoderms. They are commonly found in tropical shallow waters from the intertidal zone to the deep ocean (Hadel, Monteiro, Ditadi, Tiago & Tommasi, 1999; Pawson, 2007) which can comprise up to 90 % of the total biomass of these environments (Pawson, 1966; Hendler, Miller, Pawson & Kier, 1995). In Brazilian coast, these organisms are found rather commonly grouped under rocky outcrops (Mendes, Marenzi & Di Domenico, 2006), usually in contact with the sandy floor, from which they feed during periods of immersion (Tommasi, 1969).

Most holothurians are large organisms, free-living, iteroparous and dioecious. They typically reproduce once a year by spawning their gametes into the sea where fertilization occurs (Hyman, 1955; Smiley, McEuen, Chaffee & Krishnan, 1991). The reproductive cycle in sea cucumbers is often very complex. Gametogenesis and spawning usually follow a distinct seasonal pattern, varying in relation to species and geographic locations (Drumm & Loneragan, 2005). Some abiotic factors such as seawater temperature and photoperiod (Smiley et al., 1991; Ramofafia, Byrne & Battaglione, 2000), lunar rhythms (Battaglione, Seymour,

Ramofafia & Lane, 2002; Mercier, Sun, Bailon & Hamel, 2011) and biotic factors such as food availability (Drumm & Loneragan, 2005) have been suggested as exogenous cues affecting the reproduction of the species.

*Holothuria (Halodeima) grisea* has been harvested in some areas of southeastern-south Brazilian coast (Hadel et al., 1999; A. Marenzi, personal communication, February 19, 2010) several years ago. A sustainable exploitation of this resource necessarily requires a description of the reproduction cycle of this potentially exploitable species to implement a harvest season and a closure during the spawning (Guzmán, Guevara & Hernandez, 2003; Abdel-Razek, Abdel-Rahman, El-Shimy & Omar, 2005).

Though *Holothuria (Halodeima) grisea* represents a very common sea cucumber along the Brazilian coast, its spatial and temporal distribution, beyond ecological aspects of this species are still poorly understood. In this investigation, the reproductive aspects of a population of *Holothuria (Halodeima) grisea* was evaluated by macroscopic and histological characteristics of gonads, as well as the participation of the body components in the gametogenesis process.

## MATERIALS AND METHODS

**Study site:** Specimens were collected from a rocky shore located nearby the mouth zone of Guaratuba Bay in the State of Paraná, Southern Brazil (25° 53' 22.73" S and 48° 33' 37.81" W). The climate is classified as mesothermic subtropical with a mean annual rainfall of 2.500 mm (maximum of 5.300 mm), an average annual temperature of 22 °C with a hot rainy summer season and mean air humidity around 85 % (Marone et al., 2004).

The main atmospheric disturbances are cold fronts from the SW-NE direction that originate in Southern America. A typical rainy season initiates in late spring and lasts during most of the summer while the dry season lasts from late autumn to late winter, but is usually interrupted by a short and weak rainy

period in early winter (Lana, Marone, Lopes & Machado, 2001). The tides are characterized by diurnal inequality and attain to maximum and minimum amplitudes of approximately 2 and 0.5 m respectively (Knoppers, Brandini & Thamm, 1987).

In the sampling campaigns the water temperature varied from 26 °C (February) to 19 °C (April and June) in the studied period. The seawater salinity remained relatively constant, approximately 30.

**Field sampling and lab routines:** *Holothuria (Halodeima) grisea* specimens were sampled in February, April, June and October 2008. About twenty to twenty-five specimens larger than 10 cm were randomly collected by hand in each month. Surface seawater temperature and salinity were recorded *in situ*. All individuals were transferred to the laboratory, relaxed and dissected. The total body drained weight following the opening of the body and the removal of coelomic fluid, the body wall, respiratory tree, haemal system, empty digestive tract and gonads were recorded with an electronic precision scale (0.0001 g). The removed gonads were macroscopically and microscopic examined in order to determinate sex and maturity stage and then fixed in 9 % formaldehyde (Despalatovic, Grubelic, Simunovic, Antolic & Zuljevic, 2004).

The gonadal macroscopic features (gonad color, opacity and consistency of the tubules) were presented on a scale adapted from Abdel-Razek et al. (2005) and Ghobadyan, Morovvati, Ghazvineh and Tavassolpour (2012). For the histological examinations, small sections of the gonad tubules were dehydrated, embedded in paraffin wax, sectioned (7 µm-thick) and stained with haematoxylin and eosin (H / E). The stages of gametogenesis were based on those used in previous studies of sea cucumber gonads (Purwati & Luong-Van, 2003; Drumm & Loneragan, 2005; Rasolofonirina, Vaitilingon, Eeckhaut & Jangoux, 2005; Shiell & Uthicke, 2006). The mature oocytes were measured by the median diameter axis [(length + width) x 0.5] with an ocular micrometer.

**Data Analysis:** Frequency distribution of the maturity scale in both sexes (growing, mature or post-spawning) was calculated at each month. Sex ratio was estimated considering the total sample over the study period and between months. Statistically significant difference from the expected 1:1 sex ratio was tested using  $\chi^2$  (Chi-square test) (Zar, 1996).

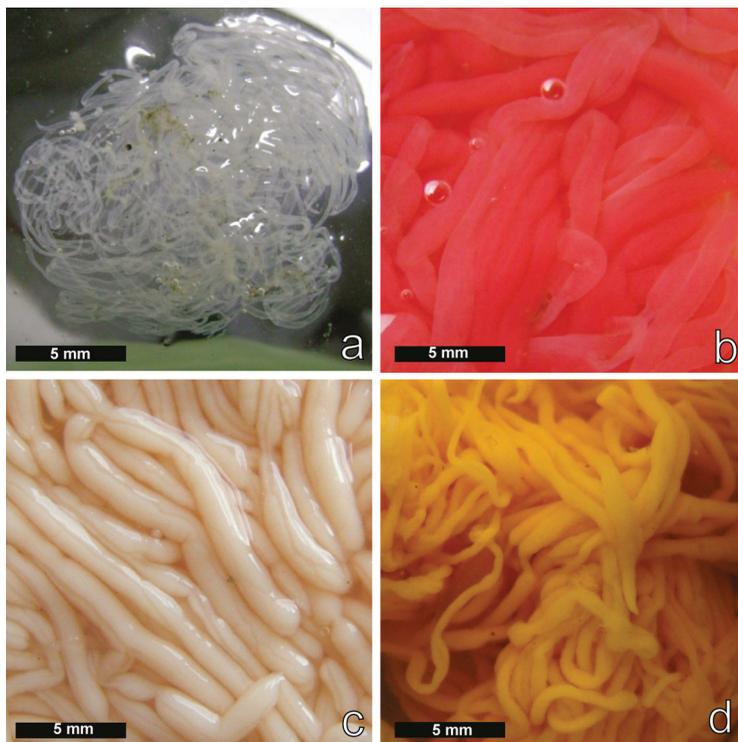
The relationship between total wet weight and organs (body wall, respiratory tree, haemal system, digestive tract, gonadal) is described by the linear function ( $y = a + bx$ ). To evaluate if the relationship between total wet weight and individual's organ weight among the months, we use analysis of covariance (ANCOVA) after logarithmic transformation of all the variables (Ebert, Hernandez & Russell, 2011). Regressions coefficients were tested for significant deviations from zero. Slopes of different regression equations were compared with each other and intercepts with the Y-axis with an ANOVA. Besides, we test a post-hoc SNK

test and the significance was tested at the 95 % confidence level.

## RESULTS

**Macroscopic features in gametogenesis stages (Fig. 1 a - d):** Gonads are a single structure, consisting of numerous filamentous tubules basically gathered into one tuft attached to the left side of the dorsal mesentery. The macroscopic appearance in relation to color, opacity and texture of the gonads between the months showed noticeable differences to the naked eye. However, during growth and depleted, no macroscopic morphological differences were observed between sexes.

*Growth:* The gonad was characterized as shorter, thinner, branched and with yellow-white color with an increase in opacity and consistency in both sexes (Fig. 1a).



**Fig. 1.** *Holothuria (Halodeima) grisea*. Macroscopic features of the gonads at different developmental stages. **a**, Growth. **b**, Mature female. **c**, Mature male. **d**, Depleted.

**Mature:** The maturing gonad was characterized by long, thick, branched maximum volume tubules and, with rare exceptions, the female gonads showed red color and the male gonads presented a marked cream tone. The structures had an opaque appearance and were quite swollen (Fig. 1 b - c).

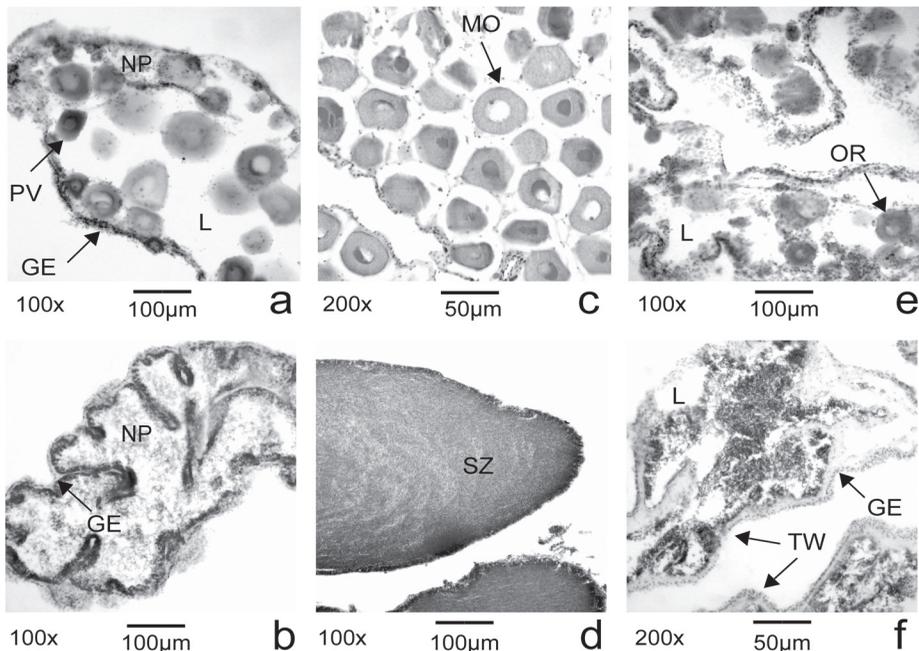
**Depleted:** After spawning, both sexes showed branched, thin, whitish, translucent tubules and with a less consistent texture (Fig. 1 d).

**Microscopic features of the gonad maturity stages (Fig. 2 a - f):** Based on the histological analyses, the maturation of the tubules was classified into three stages for females and males:

**Growing:** the gonad lumen was still filled with nutritive phagocytes. In females, pre and early vitellogenic oocytes had an irregular-shaped contour and appeared attached to the

tube wall. Some solitary vitellogenic oocytes moved away from the wall and occupied the center of tube lumen among nutritive phagocytes. The size of these germ cells varies according to the developmental stage, and the vitellogenic oocyte has, on average, a diameter of 75.56  $\mu\text{m}$  (SD = 17.69, n = 144) (Fig. 2 a). In males, primary sexual cells (spermatogonia and spermatocyte) occurred as a band close to the tube wall. Few or no spermatozoa occur at the center of the tube surrounded by nutritive phagocytes (Fig. 2 b).

**Maturation:** most of the ovarian lumen are filled with mature gametes of polygonal shape exhibiting a great number of cortical granules at the surface. Few primary oocytes are found and nutritive phagocytes cells are rare or absent; the size of the oocytes on average was 76.27  $\mu\text{m}$  (SD = 13.23, n = 75) and the maximum diameter found was 160  $\mu\text{m}$  (Fig. 2 c). In males, most of the tube lumens were filled with mature spermatozoa. A straight band



**Fig. 2.** *Holothuria (Halodeima) grisea*. Microphotographs of gonadal stages of the males and females at Guaratuba Bay. Females: **a** Growing, **c** Maturing and **e** Post-spawning; Males: **b** Growing, **d** Maturing and **f** Post-spawning (GE germinal epithelium; NP nutritive phagocytes; SZ spermatozoa; L lumen; TW tubule wall; PV pre vitellogenic oocytes; MO mature oocytes; OR residual oocyte).

of primary cells was still present, but nutritive phagocytes were absent (Fig. 2 d).

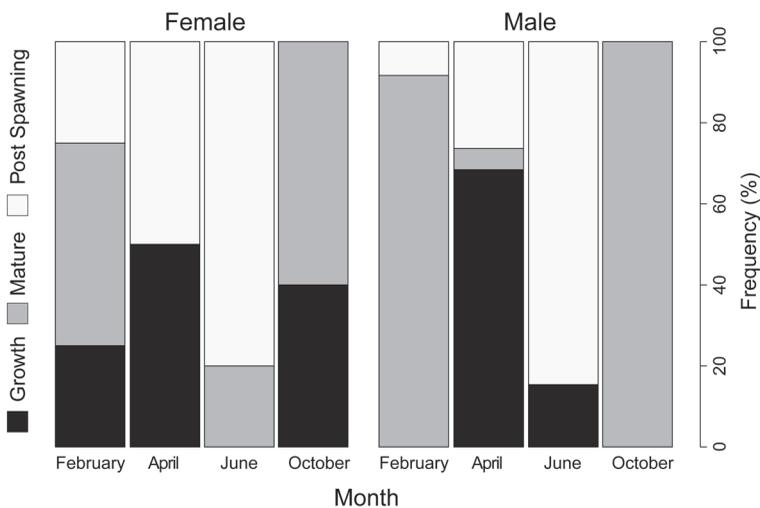
**Post-spawning:** A few relict oocyte (Fig. 2 e) or spermatozoa (Fig. 2 f) may be found in a reduced tube lumen within sparse nutritive phagocytes. Phagocytosis is evidenced by the presence of strong basophilic agglutination structures. In some male's gonads, the tubes showed a mixture of nutritive phagocytes, primary cells and spermatozoa (Fig. 2 f). The lumen of the tubules (females) may further include residual oocytes with an average diameter of 75.74  $\mu\text{m}$  (SD = 15.64, n = 61).

**Sex ratio and gametogenesis:** A total of 94 individuals of *Holothuria (Halodeima) grisea* were examined: 33 females, 55 males and 6 individuals without gonads. External sexual dimorphism was not observed. The results of the test statistic  $\chi^2$  no accepted the null hypothesis ( $H_0$ ) for the sex ratio equal to 1 ( $\chi^2 = 5.5$ ,  $p = 0.019$   $df=1$ ). However, analyzing each month the female to male ratio ranged, from February ( $\chi^2 0.04$   $df = 1$   $p = 0.841$ ), April ( $\chi^2 6.76$ ,  $df = 1$   $p = 0.009$ ), June ( $\chi^2 1.47$ ,  $df = 1$   $p = 0.225$ ) and October ( $\chi^2 = 1.19$ ,  $df = 1$   $p = 0.275$ ), showing sex ratio different from 1 only at April.

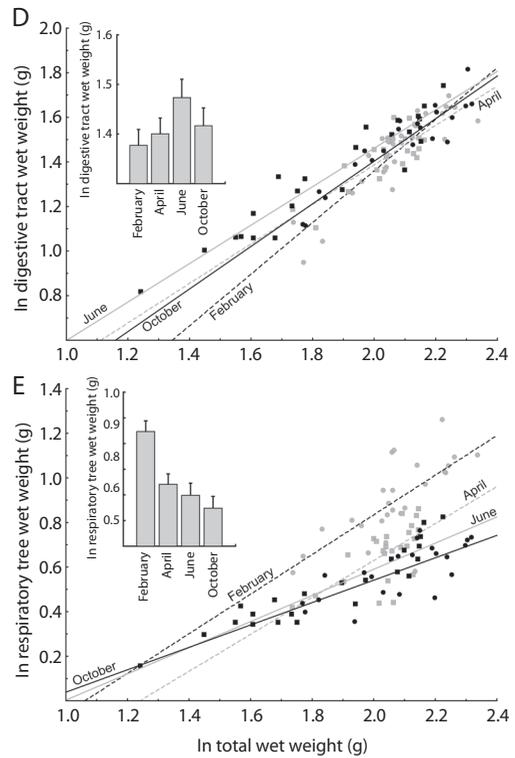
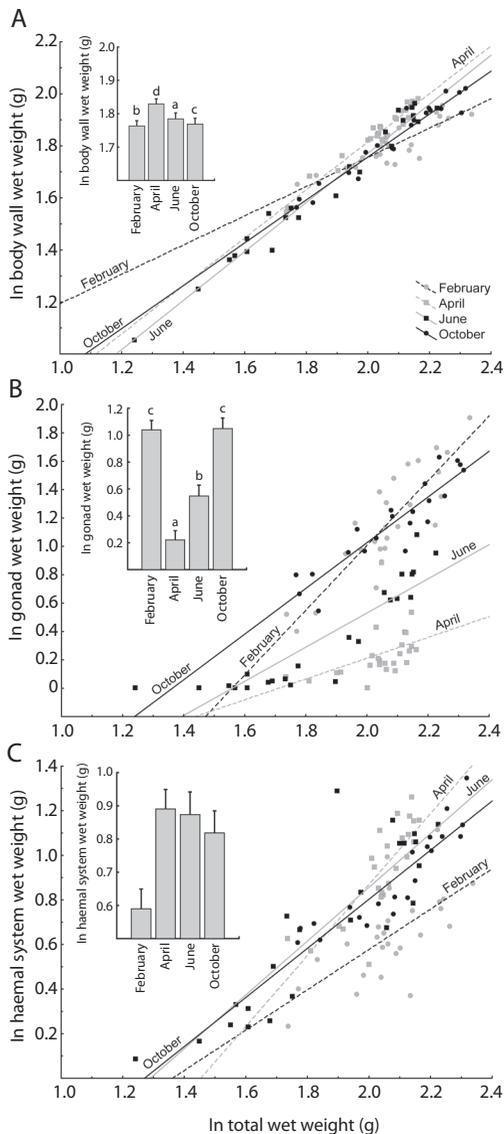
The analysis of gametogenesis of *Holothuria (Halodeima) grisea* could be characterized by continuous emission of gametes throughout the samplings, despite an increase of individuals during maturation in the February. For both sexes mature gonads were observed mainly in the February and October (summer and spring months), whereas in April (autumn) half of population showed their gonads in growing stage. In June (winter) the majority of individuals were characterized as post-spawning stage (Fig. 3). Individuals lacking the gonad appeared in April and June.

The ANCOVA analysis performed on body compartments data with total wet weight as the covariate, showed body wall and gonads as the most representative organs. Although there is high plasticity between months, this variation was different for each body compartments, but there was no difference in variation between sexes (ANCOVA:  $F_{10,170} = 1.076$ ,  $p = 0.382$ ).

The body wall of *Holothuria (Halodeima) grisea* represents almost the entire animal weight (~ 80 - 90 %) and showed differences between the samplings (ANCOVA:  $F_{3,87} = 14.258$ ,  $p < 0.001$ ), increasing in April (growth stage) and fall in the June (post-spawning stage) (Fig. 4 a, Table 1).



**Fig. 3.** Frequency distribution (%) of different maturity stages for males and females of *Holothuria (Halodeima) grisea* in Guaratuba Bay.



**Fig. 4.** *Holothuria (Halodeima) grisea*. Linear models ( $p < 0.001$ ) and Means ( $\pm$  IC, g), for the variation of the body organs, using the total wet weight as a covariate ( $N = 94$ ). Body wall **A**, gonads **B**, hemal system **C**, digestive tract **D** and respiratory tree **E**. The letters (a, b, c and d) show the differences of the SNK test from ANCOVA.

Gonads showed significant differences between the samplings (ANCOVA:  $F_{3,87} = 7.383$ ,  $p < 0.001$ ), increased tenfold of size during periods of maturation (October and February), decreasing in April (growth stage) and dropping too in June (post-spawning stage) (Fig. 4 b, Table 1).

Haemal system (ANCOVA:  $F_{3,87} = 2.024$ ,  $p > 0.05$ ) varied by 1 to 30 % of the total weight, although no differences among the slopes, showed a difference between the intercepts

( $t$  - value:  $- 2.047$ ,  $p > 0.005$ ), the organ is significantly smaller in the February (Fig. 4 c, Table 1).

Digestive tract (ANCOVA:  $F_{3,87} = 2.052$ ,  $p > 0.05$ ) varied by 2 to 4 % of the total weight and showed no difference between the slopes or intercepts (Fig. 4 d, Table 1).

Respiratory tree (ANCOVA:  $F_{3,87} = 1.495$ ,  $p > 0.05$ ) was the organ with lesser participation in relation to the total weight of the

TABLE 1

*Holothuria (Halodeima) grisea*. Variance of the body organs between months from Guaratuba Bay-Brazil in 2008, using summary statistics with raw data and ANCOVA with logarithmic data

	ln intercept		ln slope		r <sup>2</sup>	n	p
	Mean (SD)	p	Mean (SD)	p			
<b>Body Wall</b>							
February	0.629 (0.124)	0.000	0.562 (0.059)	0.000	0.786	25	0.000
April	- 0.030 (0.069)	0.753	0.923 (0.047)	0.000	0.943	25	0.000
June	- 0.115 (0.061)	0.074	0.943 (0.032)	0.000	0.975	23	0.000
October	0.107 (0.100)	0.286	0.824 (0.047)	0.000	0.942	21	0.000
<b>Gonads</b>							
February	- 3.557 (0.539)	0.000	2.282 (0.260)	0.000	0.762	25	0.000
April	- 1.249 (0.664)	0.072	0.730 (0.324)	0.034	0.180	25	0.034
June	- 1.877 (0.252)	0.000	1.203 (0.135)	0.000	0.791	23	0.000
October	- 2.195 (0.335)	0.000	1.610 (0.160)	0.000	0.841	21	0.000
<b>Haemal system</b>							
February	- 1.232 (0.430)	0.008	0.904 (0.207)	0.000	0.441	25	0.000
April	- 2.314 (0.491)	0.000	1.591 (0.240)	0.000	0.655	25	0.000
June	- 1.555 (0.262)	0.000	1.205 (0.140)	0.000	0.779	23	0.000
October	- 1.399 (0.245)	0.000	1.101 (0.117)	0.000	0.822	21	0.000
<b>Digestive tract</b>							
February	- 0.956 (0.245)	0.000	1.558 (0.118)	0.000	0.800	25	0.000
April	- 0.386 (0.277)	0.177	0.887 (0.135)	0.000	0.650	25	0.000
June	- 0.263 (0.115)	0.032	0.862 (0.061)	0.000	0.903	23	0.000
October	- 0.507 (0.182)	0.011	0.955 (0.087)	0.000	0.863	21	0.000
<b>Respiratory tree</b>							
February	- 0.943 (0.363)	0.016	0.888 (0.175)	0.000	0.516	25	0.000
April	- 1.028 (0.386)	0.013	0.829 (0.188)	0.000	0.456	25	0.000
June	- 0.582 (0.093)	0.000	0.586 (0.049)	0.000	0.868	23	0.000
October	- 0.463 (0.232)	0.060	0.502 (0.111)	0.000	0.518	21	0.000

individual (1 - 2 %), also did not change (Fig. 4 e, Table 1).

## DISCUSSION

Currently, it is known that the regulation of the reproductive cycle is not just concern with the timing of spawning, for this is only the climax of considerable preparatory activity, but encompasses all aspects of the organism's physiology and behavior (Giese, 1959).

Since the spatial and temporal distribution, beyond ecological aspects of *Holothuria (Halodeima) grisea* are still poorly understood in Paraná coast, we decided to reduce the sampling effort. Although we do not observe all stages gametogenesis due to lack of sample, we can characterize a reproductive pattern of *H. (H.) grisea* with continuous and synchronic emission of gametes across the months. The

increase in number of sexually mature individuals in February, may indicate a population strategy in maximize the reproductive synchrony at this time of the year.

Recent studies on the reproduction of *Holothuria (Halodeima) grisea* in Brazil (available only in meeting proceedings yet) reported that the sea cucumbers show only one annual spawning during the reproductive cycle. Martins and Ventura (2013) in Southeastern (Rio de Janeiro State, Lat. 23° S) observed that both sex are mature between October and January (spring and summer). Leite et al. (2014) in Northeastern (Ceará State, Lat. 3° S), observed mature individuals (with high gonadosomatic index) between December and April, and many individuals of indeterminate sex between June and July.

In spite of annual pattern of reproductive cycle be considered arbitrary (Holland, 1991), many studies describe only one

annual spawning during the reproductive cycle, as recorded for temperate species like *Psolus fabricii* (Düben & Koren, 1846) (Hamel, Himmelman & Dufresne, 1993) from eastern Canada and tropical species like *Isostichopus badionotus* (Selenka, 1867) (Lima, Ventura & Campos-Creasey, 2001) from southeast Brazil, *Holothuria (Mertensiothuria) leucospilota* (Brandt, 1835) (Druum & Loneragan, 2005) from Cooks Island and *Holothuria (Metriatyla) scabra* Jaeger, 1833 (Rasolofonirina et al., 2005) from south-west of Madagascar.

For Caribbean waters, *Holothuria (Halodeima) mexicana* Ludwig, 1875 and *Isostichopus badionotus* were reported in mature gonad stages throughout the year, suggesting that individuals may reproduce continuously or asynchronously several times during the year (Guzmán et al., 2003).

An important environmental factor influencing the cycle of this species is the water temperature, especially in sites with marked seasonality as the coast off Paraná (Lat. 25° S). Maximal reproduction was observed for *Holothuria (Halodeima) grisea* during the warmest part of the year, when the temperature was high. Similarly, the spawning of *Isostichopus badionotus* from Brazil occurs during the summer, when the temperature reaches its maximum of 30 °C (Lima et al., 2001).

The sex-ratio of the studied population was near 1:1 in almost all months, with the exception of April, where the number of males was higher than females. This unbalanced sex ratio is also observed in some species (Simunovic & Grubelic 1998; Shiell & Uthicke 2005), but deviations from unity in sex ratios among large aspidochirotetes are rare, and typically associated with species that undergo asexual reproduction (Harriott 1982; Uthicke et al. 1998, 1999). However, given that asexual reproduction was not observed for the *Holothuria (Halodeima) grisea*, and may be related to the low number of samples collected.

Hamel et al. (1993) studied the relation of organs (respiratory tree, intestine and gonads) with seasonal changes and increased metabolic demands for gametogenesis in *Psolus fabricii*

in Eastern Canada, found differences in body organs between males and females especially for gonadal index, that in males was more than twice that of females. For our results however body components variations were similar in males and females, which may indicate similarities in the metabolic demands for the species since in that case there is a greater requirement for sex.

The highest values observed for the development of gonads occurred in periods when there was lower participation of the body wall. Such opposition may indicate that there is an exchange of energy between these organs.

Prim, Lawrence and Turner (1976) and Giese (1976) suggested that the body wall of holothurians has a great storage potential, mainly for proteins, and according with Lawrence and Lane (1982) resorption of the body wall should enable echinoderms to maintain basic metabolism during periods of nutrient deprivation. This assumption is generally made for thick-skinned holothurian species (Lawrence, 1972; Prim et al., 1976), which eviscerate and then regenerate the gut within 1 to 3 months (Jespersen & Lutzen, 1971).

Unlike the temperate species, the body components of *Holothuria (Halodeima) grisea* such as respiratory tree, haemal system and digestive tract had little contribution to the weight of the animal. However, the gonads represent a large part of the weight of individual. The size of gonads was ten times higher in February than October collections. April and June showed small variation of the gonads size, which does not necessarily indicate a pattern of the species in the region. Other studies also showed significant differences in the gonad size during the year, as is *Holothuria (Mertensiothuria) leucospilota* (Drum & Loneragan, 2005) and *Psolus fabricii* (Hamel et al., 1993).

The percentage of individuals with mature gonads, as well as the gonad index, can be used to estimate the reproductive effort or energy invested in reproduction (Conand, 1993). Furthermore, the body weight could also be important for interpreting interspecific variations. We observed a strong antagonism between the

index of the body wall and the gonads. Though some authors as Chao, Chen and Alexander (1995) recognize that the gonad index is a reliable indicator to study the sexual reproduction of Holothuroidea. We do not recommend the index alone to determine the maturation for females since, as observed in this study, the constant presence of mature cells in the female individuals masked the interpretation of the index (without differences between samplings). For males the highest values of the gonadal index in October and February coincided with the maturation of individuals and the maximum drop of the index indicated that April to June are the most important periods for the issuance of gametes.

Gonads showed a great variation in size, stage of development and coloration, both in relation to species and between males and females from the same species. In this study the gonads of *Holothuria (Halodeima) grisea* had differences to color, opacity and texture in relation to reproductive condition between the sexes. Purwati and Luong-Van (2003) and Abdel-Razek et al. (2005) confirm the differences in staining pattern in mature individuals of *Holothuria (Mertensiothuria) leucospilota* and *Holothuria (Halodeima) atra* Jaeger, 1833, respectively. For other species as *Stichopus chloronotus* Brandt, 1835 (Hoareau & Conand, 2001) and *Cucumaria frondosa* (Gunnerus, 1767) (Hamel & Mercier, 1996), there were no such changes; therefore, histological examination is still the safest method to describe the reproductive cycle of holothurians.

For most echinoderms, life strategies are characterized by the energy requirement for one or both physiological trends, especially in the case of females, who use too much energy to produce larger gametes (Lawrence, 1987). However, this reproductive effort is strongly conditioned by life strategies developed by the population of a given environment. This hypothesis can be verified comparing the average diameter of mature oocytes of tropical and temperate species; e.g., Hamel et al. (1993) reported mature oocytes with diameter between 400-600 $\mu$  for the species *Psolus fabricii*. While

tropical species such as *Holothuria (Metriatyra) scabra* (Rasolofonirina et al., 2005), *Holothuria (Mertensiothuria) leucospilota* (Drumm & Loneragan, 2005) and *Holothuria (Halodeima) grisea* produce oocytes around 70 - 160  $\mu$ .

In conclusion, the period of maturation and variation in body organs was similar between the sexes. These similarities in the metabolic demands of the population could be confirm a strategy to maximize the reproductive synchrony. This species is important for the Brazilian coast, since it is becoming rare in some places and has the potential for commercial exploitation as a food source and for its extraction with pharmaceuticals purposes (Alvarado, Ortiz, Benavides & Toral-Granda, 2013). In spite the lack of information and sample efforts, our results represents the first official information about gametogenesis process of this species and contribute to sustainable management of this population.

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#### RESUMEN

**Aspectos reproductivos y la relación entre los órganos y la gametogénesis en *Holothuria (Halodeima) grisea* (Echinodermata: Holothuroidea).** El pepino de mar *Holothuria (Halodeima) grisea* es una especie ampliamente distribuida y económicamente importante que se ha recolectado en Brasil desde hace varios años. No hay estudios previos realizados sobre la reproducción de esta especie en Brasil. Además, son aún poco conocidos los

aspectos bio-ecológicos de esta especie. En este estudio, se investigaron algunos aspectos de la reproducción del pepino de mar *Holothuria (Halodeima) grisea* en una población cerca de la bahía de Guaratuba en el sur de Brasil. Se hicieron cuatro salidas de muestreo, en febrero, abril, junio y octubre de 2008, abarcando las cuatro estaciones. Examinamos las características macroscópicas y microscópicas de las gónadas para describir la gametogénesis, adicionalmente, se analizó el cambio de peso de cada componente de los órganos del cuerpo (pared corporal, gónadas, sistema haemal, tracto digestivo y árbol respiratorio). Se examinó un total de 94 individuos y no se encontraron evidencias de dimorfismo sexual externo. Los resultados del análisis estadístico de la prueba  $\chi^2$  rechazan la hipótesis nula (H0) con una relación de sexos igual a 1, sin embargo, el análisis de cada mes, esta diferencia de género se observó sólo en el mes de abril, cuando el número de los males es mucho mayor que la de las hembras. Las medidas biométricas mostraron que la pared del cuerpo y las gónadas son los órganos más importantes, con aumentos de la pared corporal en el junio y de las gónadas durante octubre y febrero, que pueden indicar que hay un intercambio de energía entre estos órganos. Macroscópicamente, las gónadas maduras muestran túbulos largos, gruesos y ramificados siendo que las hembras presentan coloración rojiza e los machos tonos de crema. Sin embargo, durante el crecimiento y agotado, no se observaron diferencias morfológicas macroscópicas entre los sexos. Microscópicamente, se observó la presencia constante de individuos maduros entre meses, con un aumento de la actividad sexual en febrero, cuando los ovocitos maduros tuvieron un diámetro oscila entre 70 a 160  $\mu$ . El periodo de desove ocurrió en los meses de invierno (junio). El periodo de maduración y la variación del cuerpo de los componentes fueron similares entre los sexos. La similitud en las demandas metabólicas de la población podría estar confirmando una estrategia para maximizar la sincronía reproductiva.

**Palabras clave:** Gametogénesis, índice gonadal, pepino de mar, desove, costa rocosa.

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