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Gonadal development and reproductive strategies of the tropical octopus (*Octopus insularis*) in northeast Brazil

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Abstract *Octopus insularis* inhabits shallow waters along the coast and oceanic islands of northeastern Brazil, where it is the main target of commercial fishing of octopuses. This study aims to investigate the hypothesis that the tropical *O. insularis* has a distinct gonadal development and reproductive features when compare to its congener *O. vulgaris* from the subtropical regions. In order to describe its reproductive development, 545 octopuses were collected in the Northeastern Brazil. A good correspondence was observed between the gonad morphology and its histological structure. Oocytes in different development stages were observed in mature females. Most female in early maturity stages had sperm stored in the spermathecae, indicating that females copulate when still immature. There was no correlation between testis weight and the Needham complex, suggesting a protracted period of spermatophore production. *Octopus insularis* has a general gonadal development pattern similar to *O. vulgaris*, however, some differences were observed, as maturation at a smaller size, probably associated to a shorter life, and lower fecundity. The distinct reproductive features of *O. insularis* seem to be related to less variable conditions in the tropical environments. Management should take into account the differences and establish specific rules for the Northeast Brazil octopus fisheries.

Keywords Maturity scales · Histology · Ovulation · Management · Brazil

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Introduction

In general, the coleiod cephalopods are semelpareous, short-living terminal spawners with direct development and parental care during embryogenesis (Boyle & Rodhouse, 2005). However, within the single breeding season, cephalopods species can show particularities at the reproductive strategies, involving the oocyte maturation process and the spawning mode. In the ovary, the oocytes may develop synchronously, in which all cells grows simultaneously; asynchronously, in which there are oocytes at all stages of development

and group-synchronous mode, when there are at least two distinct groups of oocytes developing (Sauer & Lipinski, 1990; Rocha et al., 2001).

The octopus species that passes through a planktonic phase before it settle on the substrate have small oocytes and high fecundity, while species that produce benthic paralarvae have larger eggs and lower fecundity (Mangold, 1987; Villanueva & Norman, 2008). Several commercially important species of genus *Octopus* have fecundity of several hundred thousand eggs (Mangold, 1987; Carvalho & Reis, 2003; Boyle & Rodhouse, 2005).

During the gonadal development occurs the processes of growth and differentiation of gonads and glands in the reproductive systems of both sexes. These processes are known as oogenesis in female and spermatogenesis in male, and differ significantly between species of cephalopods (Mangold, 1987). Thus, it is reasonable describe and compare the gonadal development between species, because it can reflect the differences among the reproductive strategies adopted (Arkhipkin, 1992). The sexual maturation in female octopuses involves the growth of eggs through the accumulation of large amounts of lipoproteins and yolk, as well as the increase in the accessory reproductive organ, the oviducal gland (O'Dor & Wells, 1978; Boyle & Rodhouse, 2005). In males, the mature spermatozoa are produced in the testis, packaged within complex spermatophores and then stored in the Needham's sac (Mangold, 1983; Norman, 2003). The process of gametogenesis has been widely discussed for several species of octopods, including *O. vulgaris* (Gonçalves et al., 2002; Rodríguez-Rúa et al., 2005; Idrissi et al., 2006; Jiménez-Badillo et al., 2008), *O. mimus* (Olivares-Paz et al., 2001), and *O. maya* (Avila-Poveda et al., 2009).

Based on the size and aspect of the oviducal gland and ovaries, for females, and the presence or absence of spermatophores in the Needham's sac, for males, different macroscopic scales of gonad stages have been developed, especially for *O. vulgaris*, main octopus fished in the world (Guerra, 1975; Mangold, 1987; Quetglas et al., 1998). The elaboration of reliable scale maturation requires a significant correspondence between the changes in the gonad morphology and the development of gametes observed by a histological approach (Olivares-Paz et al., 2001; Rodríguez-Rúa et al., 2005).

The tropical species *Octopus insularis* Leite et al. (2008) usually occurs on rocky and reef habitats, and feeds mainly on crustaceans, bivalves, gastropods and, less frequently, cephalopods and fish (Leite et al., 2009; Bouth et al., 2011). It presents distinct morphological characteristics of its congener *O. vulgaris*, such as short and stout arms, mantle and head with large reddish-brown and rough skin (Leite et al., 2008). This species occurs in a wide region of the South Atlantic, inhabiting shallow waters along the coast and oceanic islands of northeastern Brazil, where it is the main target of commercial fishing of octopuses (Leite et al., 2009).

The understanding of the reproductive process of a population subject of a fishery is important, because it can help to establish conservation and management strategies by providing information regarding the sizes and times of the year at which males and females are immature, mature, and spawned (Jennings et al, 2001). Because of its importance as a fishery resource in the North-Northeast of Brazil, the processes of gonadal development along the life cycle of *O. insularis* were studied in this article with emphasis into the micro and macroscopic characteristics of the gonads, their relationship with the maturity indices and the implications for its reproductive strategy. Furthermore, this study aims to investigate the hypothesis that the tropical *O. insularis* has a distinct gonadal development and reproductive features when compare to its congener *O. vulgaris* from the subtropical and temperate regions. This species has been used as the main biological model to management regulations in the whole Brazilian coast (Brazilian Fishery and Aquaculture Ministry, 2007).

Materials and methods

A total 257 males and 288 females were collected from the coastal region of Rio do Fogo (northeastern Brazil) (05°16′22″S and 35°22′58″W) between January 2010 and September 2011. The animals were measured (ML) and weighed and the gonads were removed. The mantle length and total weight (WT) were recorded for all the specimens.

In the laboratory, the gonads were fixed in 10% formalin and preserved in 70% alcohol. The following measures were recorded: the weight of ovary (OW); complex oviducal, oviducal glands, and oviducts (OCW); and the diameter of the oviducal gland (OGD) in females, and the weight of testis (TW), and

Needham's sac (NCW) in males. The measurements had an accuracy of 0.5 mm and weights of 0.001 g.

The macroscopic description of different stages of gonad development in males and females was performed based on the color, texture, and size of the gonads (Guerra, 1975; Barbosa et al., 1997; Quetglas et al., 1998).

The indices tested to determine the reproductive status throughout the gonadal development were: Gonadosomatic index (GSI: Otero et al., 2007): GSI = (NCW/WT − NCW) × 100 and maturation index (MI; Guerra, 1975): MI = (NCW/NCW + TW) × 100 for males; and GSI = (OW/WT − OW) × 100, MI = (OCW/OCW + OW) × 100, and oviducal gland index, OGI = (OGD/ML) (Rodríguez-Rúa et al., 2005) for females.

For the microscopic analysis of the maturation stages, six gonads fixed in Bouin solution were used (three males and three females) from each size class, considering intervals of 10 mm. Fragments of tissues were dehydrated with absolute ethyl alcohol and cleaned in xylene. The fragments, embedded in paraffin, were cut into sections of 5 μm with microtome and stained with hematoxylin–eosin following standard procedures (Gutiérrez, 1967). Photos of the histological slides were taken with a Canon 6.0 camera, coupled to Leica DM 500 microscope.

The fecundity of males was obtained by counting the number of spermatophores present in Needham's sac of 42 mature individuals. Moreover, the potential fecundity of *O. insularis* was obtained from the ovaries of five females immersed in Gilson solution for the dissociation and removal of connective tissue. From each ovary the oocytes present in three samples of 1 g each were counted and the fecundity was determined by extrapolation. Spermatophores lengths were obtained for 42 specimens of mature octopus. From each specimen, 10 spermatophores were measured without the filament. The diameter of 120 oocytes was measured on the ovaries of 35 females with support of photographs taken by a stereomicroscope and manipulated in the software Image Tool 3.0.

Analysis of variance (one-way ANOVA) and simple regression were used to analyze the variations in ML and WT, as well as the gonadosomatic indices, between different stages of maturation. The Tukey HSD test was used for posteriori analyses. The software packages Statistica 8.0 and Systat 12.0 were utilized for the statistical tests.

Results

Macroscopic description of gonadal maturation

Four macroscopic stages of gonadal maturation were identified in males (immature, maturing, mature, and post-mature) and five for females (immature, beginning of maturation, final maturation, mature, and spawned). However, only one spawned female was found, so this stage was not described (ML = 122 mm; WT = 900 g). The macroscopic descriptions for each of the stages of sexual maturation in males and females of *O. insularis* are summarized in Tables 1 and 2; the gonad images corresponding to each stage are shown in Fig. 1.

Oogenesis and spermatogenesis

Four maturity stages were histologically described for females based on the characteristics of the vitellogenic oocytes and pre-vitellogenic (immature, pre-vitellogenesis I, pre-vitellogenesis II, and vitellogenesis). Descriptions are summarized in Table 2 and the gonads corresponding to each stage are shown in Fig. 2. At the beginning of gonadal development in *O. insularis* females, the oogonia are connected to the germinal epithelium and throughout maturation they are modified in oocytes by a process called oogenesis (Fig. 2a). The oocytes have a rounded shape in the early stages and become more elongated in the later stages. In addition, they have basophilic cytoplasm (purple color) when immature, which becomes intensively eosinophilic (pink color) due to the yolk concentration in mature oocytes. In early stages, there is a double layer of follicle cells surrounding the oocyte (Fig. 2b). Throughout maturation, this layer invaginates into the cell (Fig. 2c). In the later stages, the cytoplasm accumulates yolk causing an increase in the oocyte diameter, which induces a displacement of follicular folds toward the cell periphery. Finally, the formation of chorion occurs (Fig. 2d).

Four microscopic stages of sexual maturation were described for males of *O. insularis*, based on processes of formation of mature spermatozoids within the seminiferous tubules (immature, maturing, mature, and post-mature). The results are shown in Table 1 and represented in Fig. 2. The spermatogenesis in males of *O. insularis* occurs within numerous seminiferous tubules present in the testes, which are separated from each other by a basement membrane (Fig. 2e).

Table 1 Macroscopic and microscopic stages of sexual maturation in *O. insularis* males, showing the range of the gonadosomatic index (GSI) relating to each stage of maturation

Stages	Macroscopic	Microscopic	GSI
I (Immature)	Needham's sac small and white, having only one spongy mass. Absence of spermatophores	The seminiferous tubules are small, rounded, and defined. Presence of spermatogonia near the wall of the tubule, primary and secondary spermatocytes. Next the central lumen is found spermatidia in differentiation	0.015–0.100
II (Maturing)	Testis larger than in stage I. Needham's sac with thin and white spermatophores not fully formed yet, surrounded by a spongy mass	Seminiferous tubules elongated and more clearly defined throughout the testis. At this stage, all cell types of spermatogenesis are present. Spermatozoa maturing in the lumen of each tubule	0.026–0.280
III (Mature)	Yellowish testis. Plenty of well-formed spermatophores inside the Needham's sac. The weight of the spermatophoric sac increases relative to the weight of the testis	The spaces between the seminiferous tubules, which undergo intense stretching, become greatly reduced. Presence of all cell types, with abundance of spermatozoa in the lumen	0.047–0.387
IV (Post-maturation)	Frequently minor testis compared to stage III. Needham's soft sac having a few well-formed spermatophores	Few cells are present in the seminiferous tubule. Large gaps in the central lumen with little spermatozoa present, indicating that they were expelled from the testis to be stored within the spermatophores in the Needham's sac	0.114–0.337

Throughout the maturation process, these tubules become larger and more elongated. The spermatozoids (spermatozoa) are concentrated in the lumen of each tubule (Fig. 2f, g). They present fine basophilic heads and long and thin eosinophilic tails. Then, the mature spermatozoa are expelled from the testes to be stored within the spermatophores (Fig. 2h).

The macroscopic and microscopic stages were highly correlated: $R^2 = 0.824$ ($n = 30$), for males and $R^2 = 0.938$ ($n = 37$) for females. For males, these were coincident in 87% of the specimens and for females in 92% (Table 3), which indicates validation of this sexual maturity scale.

The averages of ML and WT of the males were significantly different between the maturation stages (ML: $F = 45.92$; gl = 3; $P < 0.05$) (WT: $F = 39.72$; gl = 3; $P < 0.05$), with the same occurring for females (ML: $F = 71.64$; gl = 3; $P < 0.05$) (WT: $F = 111.07$; gl = 3; $P < 0.05$) (Posteriori Tukey HSD).

In both sexes, the averages of size and weight increased with the reproductive stage evolution (Table 4). However, the weight averages of the males in stages I and II did not present significant differences. Likewise, females in stages III and IV did not show any significant differences related to ML and WT.

The presence of spermatozoa in the oviducal glands of 72.73% of the analyzed females (Fig. 3) indicated that even immature females of *O. insularis* copulated and stored spermatozoa in structures present in the oviducal gland known as oviducal cisterns or spermathecae.

All of the females with <68 mm of ML presented empty spermathecae, while the majority of individuals with ML above of this value had sperm stored in the spermathecae (Fig. 4).

Fecundity

The number of spermatophores inside the Needham's sac of mature males ranged from 15 to 66, with an average of 39.23 ± 14.02. The production of spermatophores by individual body weight ranged from 0.02 to 0.15 (mean 0.05 ± 0.02) spermatophores/g. There were no significant relations between the number of spermatophores and ML and WT. An average of 13.87 ± 2.25 mm of spermatophore length (SL) was registered for *O. insularis*. The shorter length was 8.40 mm and the highest was 17.82 mm. The relations between the size of spermatophore with ML ($N = 42$, $F = 25.47$, gl = 1, $P < 0.05$) and WT ($N = 42$,

Table 2 Description of microscopic and macroscopic stages of sexual maturation in *O. insularis* females, showing the extent of ovarian diameter (OD), oviducal glands (OG), and the maturation index (MI) related to each stage of gonadal development

Stage	Macroscopic		Microscopic			
	Ovary	Oviducal glands	Ovary	OD (mm)	OG (mm)	MI
I (Immature/immature)	White and very small, with no signs of ovarian granulation through the membrane. Oocytes very small and round	Small and white. The diameter is slightly larger than the diameter of the oviduct	Several oogonia in the germinal epithelium. The oocytes are rounded, basophilic cytoplasm having a large nucleus and nucleolus near the nuclear membrane. The oocytes may be surrounded by a single layer of flattened follicle cells	4.00–12.00	0.90–3.85	0.63–0.24
II (Beginning of maturation/pre-vitellogenesis I)	White and small, showing signs of ovarian granulation through the membrane. Larger oocytes and somewhat longer	Larger, with two or three different stripes. It presents longitudinal lines. Begins the process of darkening of the gland	Presence of oogonia in the basophilic cytoplasm and germinal epithelium. Oocytes still rounded, become large with large nucleus, located in the most peripheral portion of the cell, having only a nucleolus. There is a double layer of follicle cells surrounding the oocyte: an external elongated layer and a internal with cuboidal shape	10.10–22.20	2.15–5.00	0.47–0.08
III (Final maturation/ pre-vitellogenesis II)	Large yellowish and possessing oocytes clearly visible. It still attached to the stalk elongated ovarian	Large with longitudinal lines and about a third is darkness	The oocytes increase considerably in diameter. The cytoplasm has a granular aspect and become eosinophilic due to the accumulation of yolk. The double layer of follicular cells invaginates into the oocyte. Not observed the presence of nucleoli	17.00–31.00	3.90–6.90	0.29–0.03
IV (Mature/ vitellogenesis)	Great with high abundance of oocytes, and less attached to the peduncle ovarian	Very large and almost entirely black with longitudinal lines	Intense proliferation of yolk within the oocyte, which causes a displacement of follicular folds toward the cell periphery. The cytoplasm is intensely eosinophilic and the nucleus is not displayed. Initiates the formation of the chorion	27.80–56.00	4.65–9.85	0.15–0.005

$F = 37.35$, gl = 1, $P < 0.05$) followed logarithmic models (Fig. 5), indicating that the SL stabilizes in a certain range of ML and WT.

The number of oocytes in the ovaries of *O. insularis* ranged from 68,502 to 120,166, with an average of 93,820 ± 19,003. The mean number of oocytes by gram of body weight was 97.48 ± 15.13 (80.59–121.38). The low number of ovaries available for the fecundity calculation limited the analysis of the relationship between the number of eggs with ML and WT.

Ovulation pattern

The mean diameters of the oocytes varied significantly between the maturation stages ($F = 3433.21$, gl = 3,

$P < 0.05$) (Table 5), increasing throughout the gonadal development (posteriori Tukey HSD).

In a single ovary, oocytes in more than one development stage were found (Fig. 6). Analyzing the relative frequencies of the oocyte diameters throughout the maturation stages, it was verified that in stages III and IV the oocytes with diameters characteristic of the initial stages were still found, indicating that even in the advanced stages there is still a considerable presence of oocytes undergoing differentiation.

Fig. 2 Ovaries and testis of *O. insularis* in different microscopic development stages. Females: **a** Stage I. Oocytes rounded off with large nucleus and some nucleoli (*arrow*). **b** Stage II. Oocytes surrounded by a double layer of follicular cells. **c** Stage III. Invagination of the follicular cells layer and yolk presence. **d** Stage IV. Oocyte with intense yolk accumulation. *GE* germinal epithelium, *OO* oocyte, *N* nucleus, *FC* follicular cells, *Y* yolk, *C* chorion. Males: **e** Stage I. Many spermatogonia next to the tubule wall (*arrow*). **f** Stage II. Spermatozoa maturing in the tubule lumen. **g** Stage III. Many mature spermatozoa. **h** Stage IV. Large empty spaces in the tubule lumen (*arrow*). *SS* spermatogonia, *SP* spermatidia, *SZ* spermatozoa. The bars represents 100 μm ▶

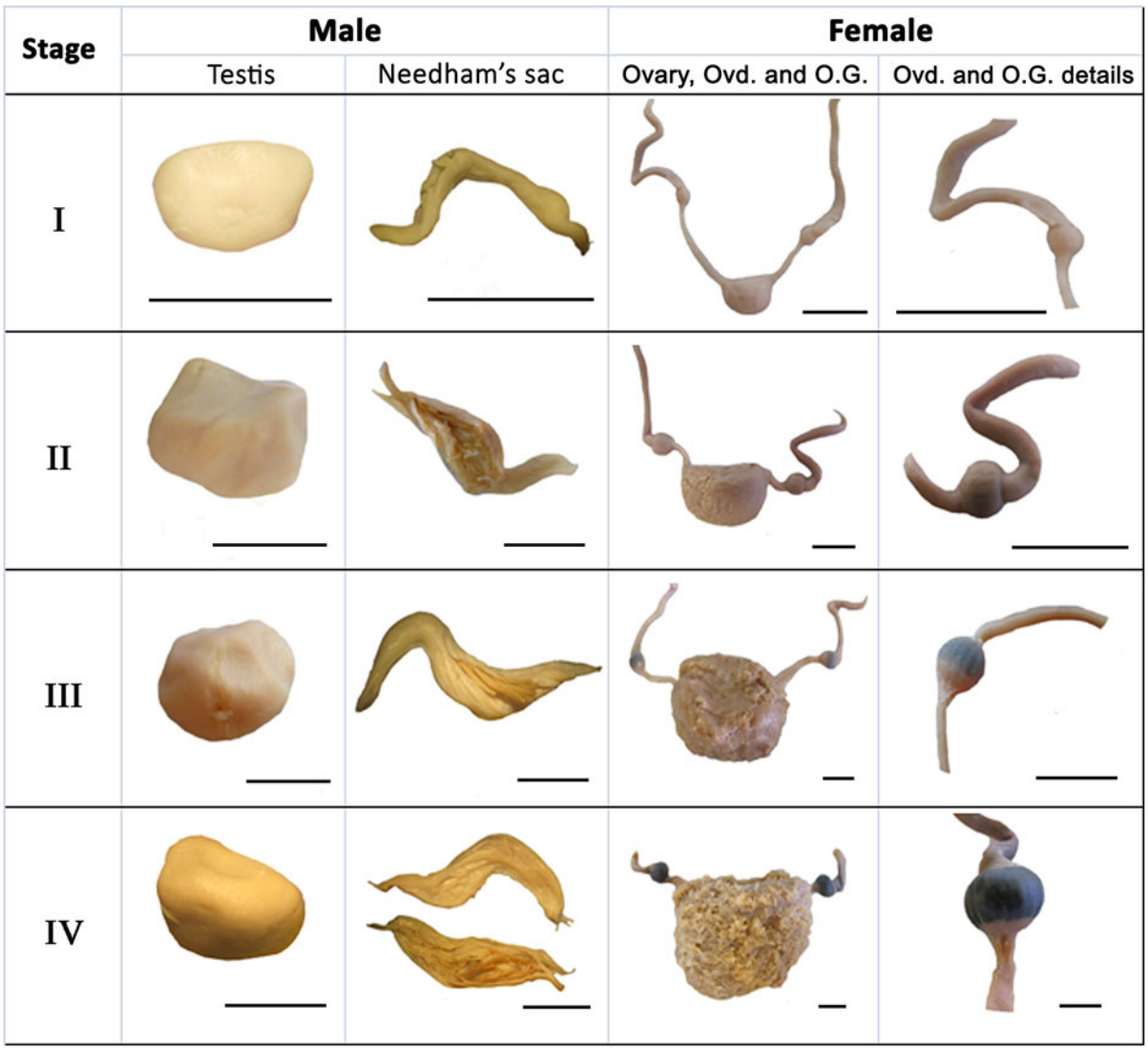

Fig. 1 Gonadal macroscopic maturity stages in both sexes of *Octopus insularis* showing testis, Needham's sac, ovaries, oviducts (Ovd.), and oviducal glands (O.G.). The bars represents 10 mm

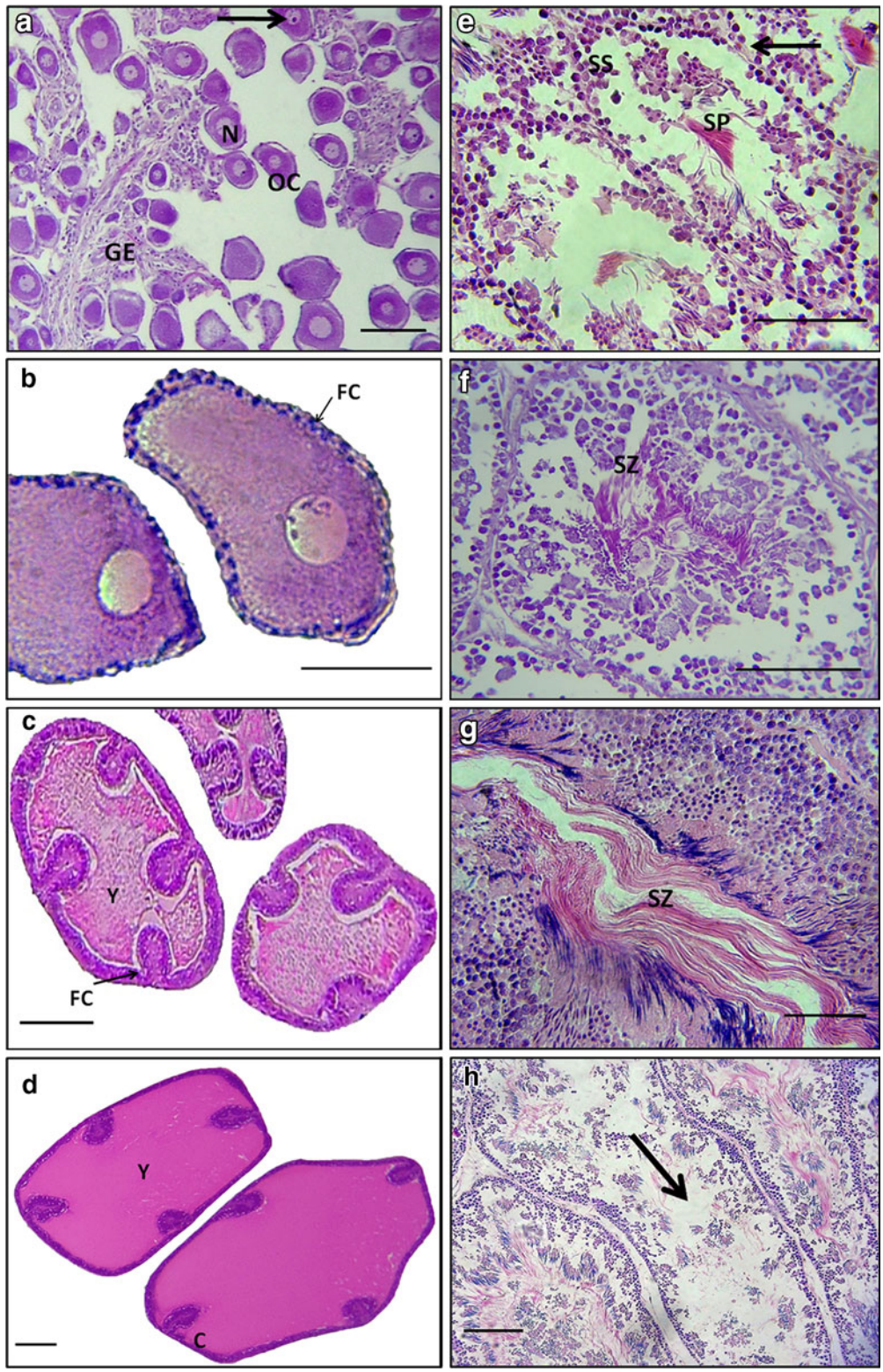
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Table 3 Relationship between macroscopic (IA–IVA) and microscopic (I–IV) gonadal maturation stages in males and females of *O. insularis*

Stages	Males				Females			
	I	II	III	IV	I	II	III	IV
IA	3	2			13			
IIA		4	1			9	2	
IIIA			14	1			7	1
IVA			2	3				5

Table 4 Range, mean, and standard deviation (SD) of the mantle length and total weight in each maturation stage of males and females of *O. insularis* at Rio do Fogo

Macroscopic stage	*N*	Mantle length (mm)			Total weight (g)		
		Range	Mean	SD	Range	Mean	SD
Male							
I	15	56–101	76.33 **a**	15.76	134–800	385.40 **a**	226.82
II	109	61–134	90.61 **b**	14.09	200–1,580	518.23 **a**	240.98
III	129	78–145	106.40 **c**	14.55	310–1,600	813.35 **b**	279.44
IV	9	101–152	125.89 **d**	16.43	875–1,580	1099.78 **c**	223.23
Female							
I	101	50–120	89.79 **a**	14.29	119–1,100	458.25 **a**	501.50
II	147	76–190	107.18 **b**	13.18	400–1,900	849.14 **b**	888.76
III	27	103–150	123.93 **c**	12.73	780–1,940	1202.96 **c**	1322.40
IV	13	110–170	130.46 **c**	20.53	600–1,900	1301.38 **c**	1544.81

Different letters indicate significant differences between the maturation stages

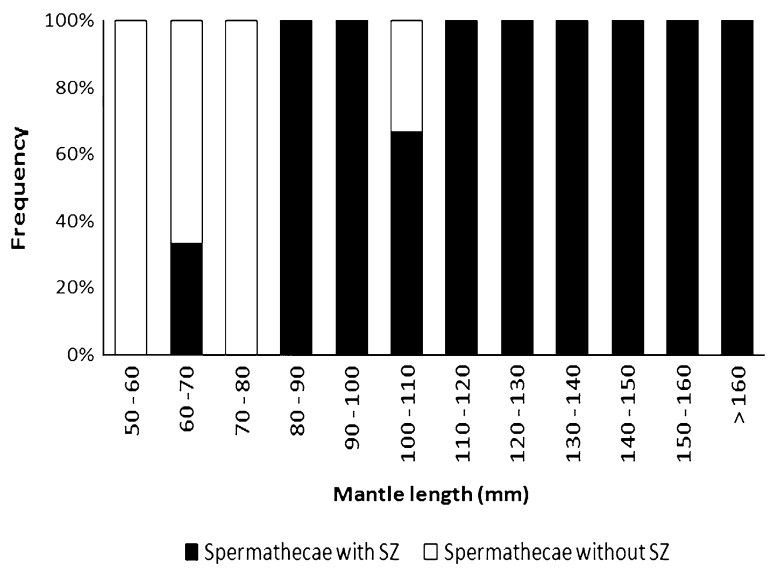

Fig. 3 Relative frequency of *O. insularis* females for size class (ML) that present spermatozoa (SZ) inside of the spermathecae. $N = 33$

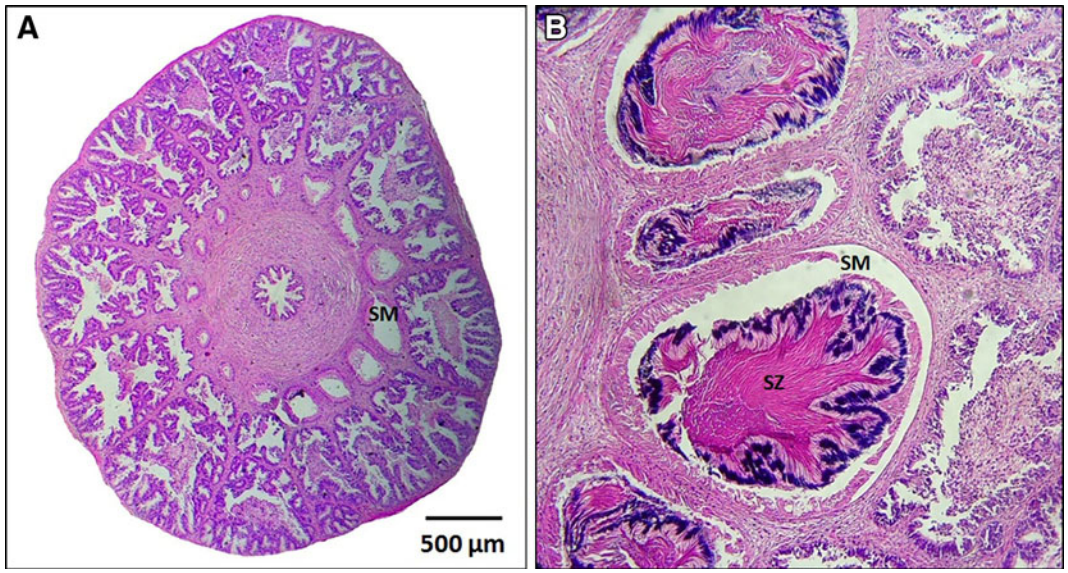

Fig. 4 Longitudinal cut of the oviducal glands of *O. insularis*, revealing the oviducal cisterns or spermathecae (SM) empty (**A**) or filled by spermatozoa (SZ) (**B**). ML (**A**) = 69.88 mm; ML (**B**) = 117.13 mm

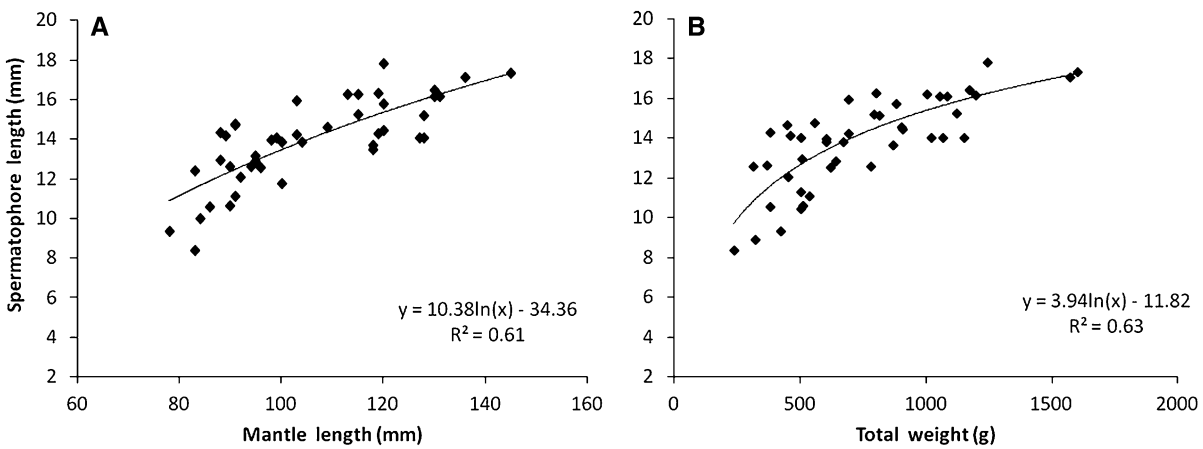

Fig. 5 Logarithmic relationships between the spermatophore length (SL) and the mantle length of *O. insularis* (**A**), and between the SL and the total weight (**B**)

Indicators of the reproductive status

Throughout the maturity stages of the males, a significant variation of the GSI was verified ($F = 34.97$, gl = 3, $P < 0.05$) (Fig. 7). The values of this index tended to increase with the maturation; however, there were no differences between the most advanced stages, III and IV. On the other hand, the MI, which relates the testis weight to the Needham's sac weight, did not show any significant differences between the male reproductive stages.

In females, a significant increase of the GSI was detected ($F = 81.42$, gl = 3, $P < 0.05$), as well as a significant reduction of the MI throughout the reproductive stages ($F = 115.06$, gl = 3, $P < 0.05$) (Fig. 8). The OGI also ranged throughout the maturation stages

Table 5 Mean, amplitude, and standard deviation (SD) of *O. insularis* females oocyte diameter in the different maturity stages

Oocyte diameter (μm)					
Stage	Nov	Noc	Range	Mean	SD
I	13	1,560	16.16–145.83	62.91 **a**	20.60
II	9	1,080	40.00–440.00	109.37 **b**	29.80
III	7	840	66.92–774.68	235.30 **c**	108.01
IV	6	720	120.26–1211.02	444.52 **d**	177.87

Different letters indicate significant differences between the maturity stages

Nov number of ovaries, *Noc* number of oocytes

($F = 63.66$, gl $= 3$, $P < 0.05$), showing itself to be a good parameter to determine the reproductive status in females of *O. insularis*.

Discussion

The general gonadal development process observed in *O. insularis* was similar to other species, such as *O. vulgaris* (Jiménez-Badillo et al., 2008), *O. hubbsorum* (Pliego-Cárdenas, 2011), and *O. maya* (Avila-Poveda et al., 2009). However, some differences were detected, mainly in females. The longitudinal lines

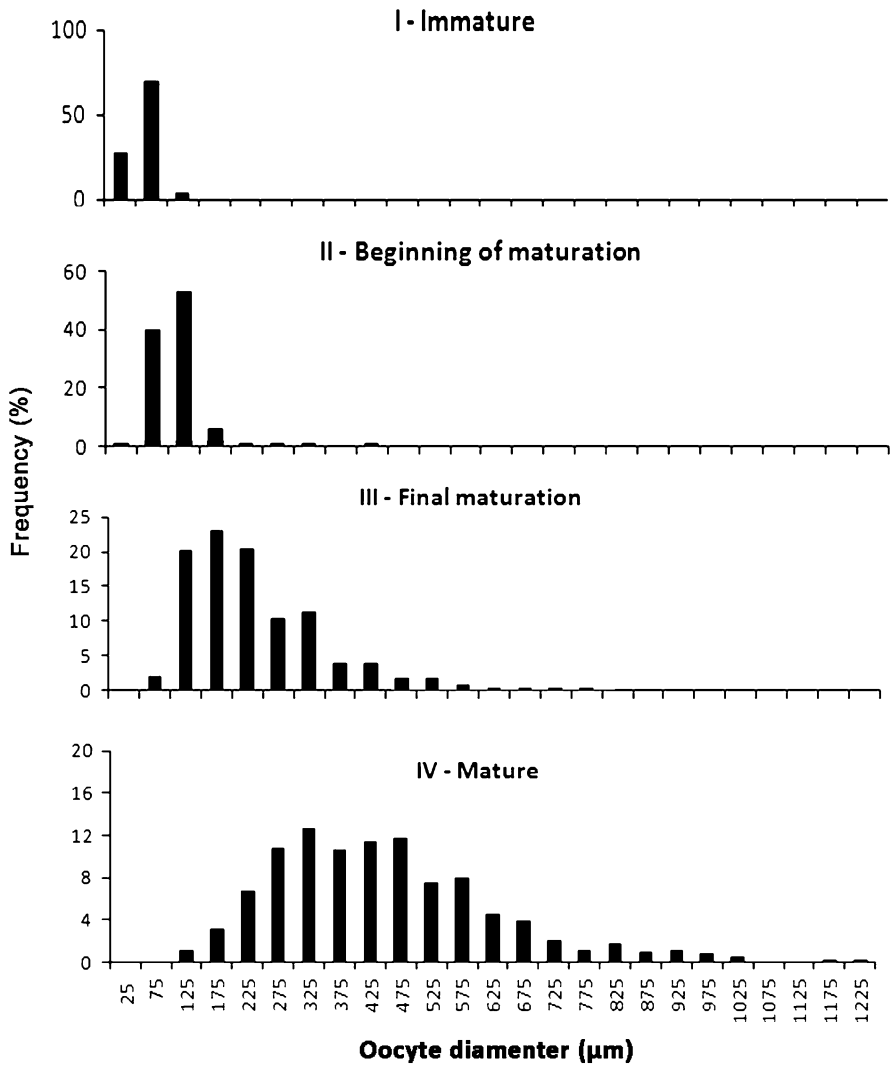

Fig. 6 Relative frequency of the oocytes by size classes (diameter in μm) in the different maturity stages of *O. insularis*

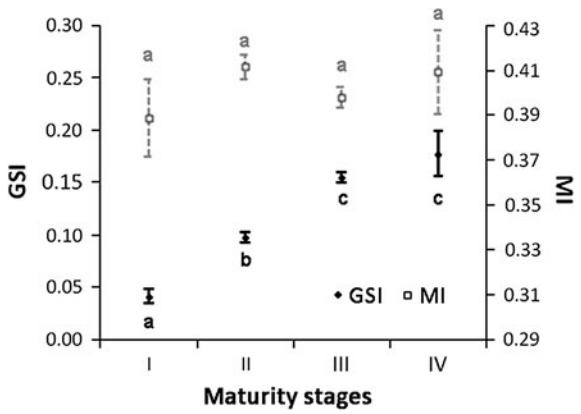

Fig. 7 Variation of the reproductive index between the maturation stages of *O. insularis* males. *GSI* gonadosomatic index, *MI* maturation index. The *vertical bars* indicate the mean standard error. *Different letters* indicate significant differences between the maturation stages

found in all extensions of the oviducal glands of the *O. insularis* females differed from the denticulate pattern described in the apical region of *O. vulgaris*, *Eledone cirrhosa*, and *E. moschata* glands (Guerra, 1975; Caparro, 2010). It was also noted that the average sizes of the ovaries, oviducal glands, and oocytes in each maturation stage registered for *O. insularis* were inferior to the values obtained for *O. vulgaris* and *O. mimus* (Guerra, 1975; Olivares-Paz et al., 2001; Gonçalves et al., 2002).

The number of stages in macroscopic maturity scales attributed to octopus varies between authors: for *O. vulgaris* Guerra (1975) described three stages for males and four for females, Quetglas et al. (1998) described three for both sexes and Arkhipkin (1992) described up to seven stages for males and females; Perez & Haimovici (1991) recorded four stages for both sex of *Eledone massyae*. The wide variation of maturity stages reflect the different criteria adopted for the authors during the gonad analyses (Gonçalves et al., 2002). The four stage scale proposed for both sexes of *O. insularis* in this study has the advantage of being equivalent microscopically and macroscopically, due to the strong correlation between both and with gonadal maturity indices.

Preliminary estimates of the fecundity of *O. insularis* females are at most around 120,000 compared with nearly 800,000 observed for *O. vulgaris* by Oosthuizen & Smale (2003) or 700,000 for *O. tetricus* and *O. cyanea* (Mangold, 1987; Carvalho & Reis, 2003; Boyle & Rodhouse, 2005). *Octopus insularis* is a smaller species than *O. vulgaris*, therefore its fecundity is expected to be lower, once that the amount of eggs produced by an individual is closely related to its size and body weight (Mangold, 1983; Boyle & Rodhouse, 2005). However, relative fecundity was 121 oocytes/g compared with 465 oocytes/g recorded for *O. vulgaris* (Hernández-García et al., 2002) from the Canary Islands and the relative size of the ovaries was also smaller than other species (Guerra, 1975; Olivares-Paz et al., 2001; Gonçalves et al., 2002) suggesting a lower reproductive investment in this tropical octopus. Larger samples are necessary to confirm these preliminary observations regarding the fecundity of *O. insularis* female.

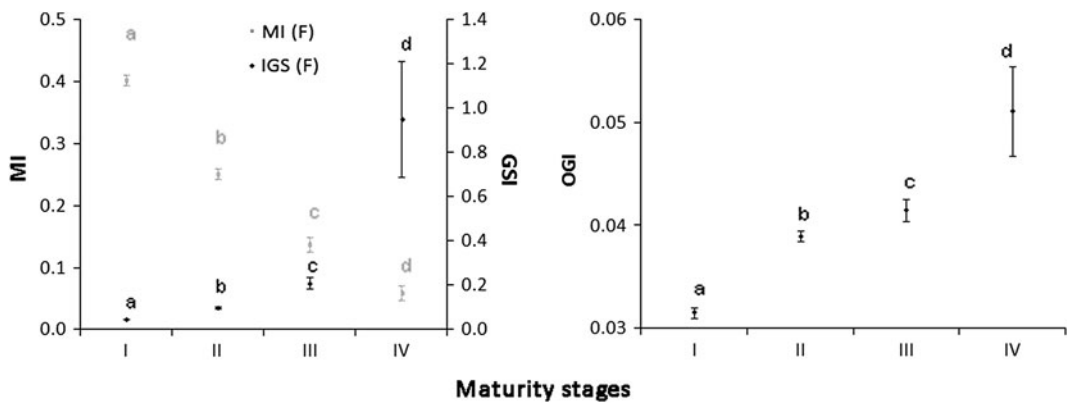

Fig. 8 Variation of the reproductive indexes between the maturity stages of *O. insularis* females. *GSI* gonadosomatic index, *MI* maturation index, *OGI* oviducal gland index. The *vertical bars* indicate the mean standard error. *Different letters* indicate significant differences between the maturation stages

The size and weight of ovaries were not different in the stages I and II, while in the final stages was observed a substantial increase in the female gonad. This is a pattern observed in many cephalopod species, in which the growth of the ovary is mediated by gonadotropin hormone produced by the optic gland. Gonadotropin promotes the formation and multiplication of a complex of follicular cells that accelerate the vitellogenesis process (O'Dor & Wells, 1978; Boyle & Rodhouse, 2005). Several studies have shown that intense proliferation of germinal cells in early stages does not imply immediate growth ovarian, which occurs significantly only during vitellogenesis (Idrissi et al., 2006; Jiménez-Badillo et al., 2008). In this stage, the yolk accumulates in the lumen of the ovary, with considerable increases in oocyte size, which affects the mass and volume of the gonad. This growth is clearly reflected in the macroscopic scales and indices of sexual maturation (Olivares-Paz et al., 2001; Boyle & Rodhouse, 2005).

The presence of oocytes in more than one development stage in the ovaries in stages III and IV shows that vitellogenesis is not synchronous in all oocytes. This ovulation pattern is known as group-synchronous, in which at least two populations of developing oocytes can be recognized simultaneously (Rocha et al., 2001). Spawning of octopuses can last between 25 and 125 days (Mangold, 1987) and it is likely that part of the oocytes continue to mature after spawning initiation. Thus, *O. insularis* spawns repeatedly during a single reproductive event. This strategy can expand the reproductive success of the species, once the total and immediate spawning would leave the offspring most vulnerable to the biotic and abiotics events as predation or environmental disturbances (Jennings et al., 2001). The same pattern was observed for *O. vulgaris* in the southern Moroccan Atlantic (Idrissi et al., 2006) and *O. mimus* in Chile (Olivares-Paz et al., 2001). On the other hand, Boyle & Rodhouse (2005) affirm that oocytes grow in different rates and it is possible that not all of them reach the maturation due to competition between them for resources (blood supply) and space.

The spermatogenesis process in *O. insularis* was very similar to that described for *O. vulgaris* (Gonçalves et al., 2002; Rodríguez-Rúa et al., 2005; Idrissi et al., 2006). This process is initiated by the transformation of spermatogonia, after successive mitotic divisions in primary spermatocytes, which give rise to secondary spermatocytes through the first meiotic division. A second meiotic division transforms these into spermatidia, which differentiate into spermatozoa (or spermatozoids) in a process called spermatogenesis, in which the flagellum formation occurs (Mangold, 1987; Gonçalves et al., 2002; Boyle & Rodhouse, 2005).

For *O. insularis* males, the maximum number of spermatophores found in the Needham's sac was 66, which was several times lower than those observed for *O. vulgaris:* 374 in Galicia (Otero et al., 2007), 276 in the Gulf of Cadiz (Silva et al., 2002), and 109 in the Portuguese coast (Carvalho & Reis, 2003). Also, the production of spermatophore by individual body weight registered in this study (0.02–0.15 spermatophore/g) was lower than the values obtained for *O. vulgaris* (0.03–0.55 spermatophore/g) (Hernández-García et al., 2002). As in females, males of this species have a lower fecundity than *O. vulgaris*, probably because of their smaller size (Boyle & Rodhouse, 2005). However, this could also be related to a higher investment in growth rather than reproduction, eventually explained by the faster growth and shorter life span, as expected for organisms of warm water (Rosa et al., 2012) and preliminarily observed by Batista (2011).

As observed in other *Octopus* species, males of *O. insularis* mature at smaller sizes than females (Hernández-García et al., 2002; Rodríguez-Rúa et al., 2005; Avila-Poveda et al., 2009; Batista, 2011). The presence of spermatozoa in the spermathecae of immature females indicates that males compete to copulate with females even before they reach gonadal maturation, as the sperm can be stored for long periods before fertilization (Froesch & Marthy, 1975). Given the possible earlier maturation in males compared to females, the occurrence of mature males for most of the year (Hernández-García et al., 2002; Rodríguez-Rúa et al., 2005; Avila-Poveda et al., 2009) and the females' capacity to store sperm for prolonged periods (Mangold, 1987; Rodríguez-Rúa et al., 2005; Jiménez-Badillo et al., 2008), it can be deduced that copulations occurs opportunistically, as octopuses are described as solitary animals (Wells & Wells, 1956; Hanlon & Messenger, 1996). This characteristic allows the possibility of copulations during longer periods, and not only at the time and space where mature females are found.

According to Quinteiro et al. (2011) a female may copulate with several males throughout its life and the

male that perform the last copulation will have more chance to be the genitor of the offspring. If this is the case, this study suggests that adult males might be able to form couples with subadults and adults females to ensure higher chances of paternity of the offspring, while the younger females have a solitary behavior with opportunistic copulations. In fact, Huffard et al. (2008) and TS Leite (pers. obser.) had observed the formation of couples, in which the males protected the females of others of the same species, in adult individuals of *Abdopus aculeatus* and *O. insularis*, respectively. Further research is necessary to better understand this behavior of *O. insularis*.

In relation to the reproductive indices analyses, the GSI, OGI and, mainly, the MI for females and the GSI for males presented better efficiency to represent the reproductive stages of *O. insularis*. The MI registered for females decreased during the reproductive stages, which, according to Guerra (1975), occurs due to strong relation between the ovary and oviducts weight and the oviducal glands. According to the author, during the initial stages of gonadal development, the ovary weighs about five times more than the oviducal complex, while in advanced stages it can reach a weight of 50 times higher.

Among the *O. insularis* males, the relation of testis weight and Needham's complex weight (MI) with the maturation process was not evident; this is different to that observed by Guerra (1975) for *O. vulgaris* in the Mediterranean, which showed a clear relation between the weights of the different parts of the masculine gonad. *Octopus vulgaris* is found in temperate and subtropical waters, while *O. insularis* is the dominant species in tropical waters, and this environment, with lower seasonal variability, could provide the ideal conditions for it to permanently produce and liberate spermatophores, with frequent weight oscillation in the different parts of the gonad.

This study is the first to detail the process of gonadal development and identified the best reproductive indices that reflected the sexual maturation stage of *O. insularis*. Moreover, the results obtained highlighted important features of the reproductive strategies and biology of this tropical octopus, such as the lower fecundity (in relation to *O. vulgaris*), the presence of mature males in the population year round and the sperm storage capacity in females.

Conclusions

Despite the fact that *O. insularis* has a general gonadal development similar to *O. vulgaris*, some differences were observed: *O insularis* has longitudinal lines found in all extensions of the oviducal glands, relatively smaller gonads, lower absolute and relative fecundity, year round production and release of spermatophores and group-synchronous ovulation. The distinct reproductive features of *O. insularis* in Rio Grande do Norte seems to be related to less variable conditions in the tropical environments, in which the benefits of a longer life cycle and higher fecundity are offset for a shorter life cycle and lower fecundity, typical of tropical organisms with high metabolism associated to permanent clear and warmer water. Management should take into account the differences and establish specific rules for the Northern Brazil octopus fisheries.

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