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Validation of macroscopic maturity stages of the Patagonian red octopus *Enteroctopus megalocyathus*

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Testes and ovaries of Enteroctopus megalocyathus *collected along the Patagonian Atlantic coast were analysed histologically to validate the macroscopic maturity scales adopted for this species. Changes through the course of development of the seminiferous tubules and of the oocyte/follicular cell complexes were characterized and these were classified into five and six microscopic categories of development respectively. A histological maturity index, based on the frequencies of microscopic categories, was used to assess the correspondence between macroscopic maturation stages and the microscopic level of development of the gonadal tissue. Seminiferous tubules showed a regular and progressive pattern of microscopic development within each macroscopic stage and between consecutive macroscopic stages. However, a minority of males exhibiting seminiferous tubule with sperm did not display macroscopic characteristics of the mature-spawning stage. In females, an overlapping of microscopic categories was observed in maturing macroscopic stages. Previtellogenic oocytes were not present at mature-spawning or spent stages. Significant changes in the histological maturity index were observed between consecutive macroscopic stages, confirming the validity of macroscopic maturity scales of both sexes. In addition, by considering both macroscopic and microscopic criteria, it was possible to determine the overall state of development and functioning of the reproductive system during sexual maturation of this species.*

Keywords: *Enteroctopus megalocyathus*, gametogenesis, histology, maturation scales, Patagonian Atlantic coast, spawning mode

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INTRODUCTION

Information about the reproductive biology of an exploited cephalopod population is necessary both for further assessment and for setting management regulations. The macroscopic determination of the maturation stage by visual examination of gonads is a relatively rapid and inexpensive manner of determining the reproductive status (Sauer & Lipinski, 1990; Boyle & Rodhouse, 2005). However, it is often difficult to set discrete categories and define discrete reference points based on macroscopic criteria because maturation progresses continuously. Besides, macroscopic analysis alone may lead to a misclassification of the maturity state of animals (Mangold, 1987). On the other hand, histological analysis could provide more precise data about the changes occurring in gonads. It serves as a tool to validate the adopted macroscopic stages by linking categories of microscopic development (i.e. stages describing the degree of development of spermatogenesis or oogenesis) to macroscopic maturity stages, although it might be a time consuming and expensive task. In addition, the knowledge about the reproductive strategy of the cephalopod species would improve through histological observations (Sauer & Lipinski, 1990; Melo & Sauer, 1999; Gonçalves *et al.*, 2002; Díaz-Uribe *et al.*, 2006).

The Patagonian red octopus, *Enteroctopus megalocyathus* (Gould, 1852), is a relatively large octopus receiving growing attention regarding its fishing and its potential of culture in the southern tip of South America (Argentine and Chilean coasts) (Ortiz *et al.*, 2006, 2011; Perez *et al.*, 2006; Farías *et al.*, 2011). Geographical distribution of this species, ranges from 41°S to 56°S in the south-west Atlantic Ocean, and from 42°S to 56°S in the south-eastern Pacific Ocean, with a bathymetric range of distribution from intertidal areas up 240 m depth (Ré, 1998; Osorio *et al.*, 2006; Ibáñez *et al.*, 2009) (Figure 1). Along the Patagonian Atlantic coast, the species is harvested by artisanal fisheries and by sporting fishermen in intertidal and subtidal areas. Although the fisheries in this region have been operating at least since the 1960s, they are unregulated and official catches are not recorded (Cinti *et al.*, 2003; Ortiz *et al.*, 2011). On the southern Chilean coast, captures by diving approximate 500 tons per year, although at present in main fishing areas there exist regulations that forbid the fishing of this species (Ibáñez & Chong, 2008; Sernapesca, 2011).

Sexual maturation of *E. megalocyathus* has recently been assessed on the northern Atlantic Patagonian coast (San José and Nuevo Gulfs) (Figure 1) (Ortiz *et al.*, 2011). In that work, maturity stages were assigned taking into account the macroscopic (i.e. morphochromatic) characteristics of

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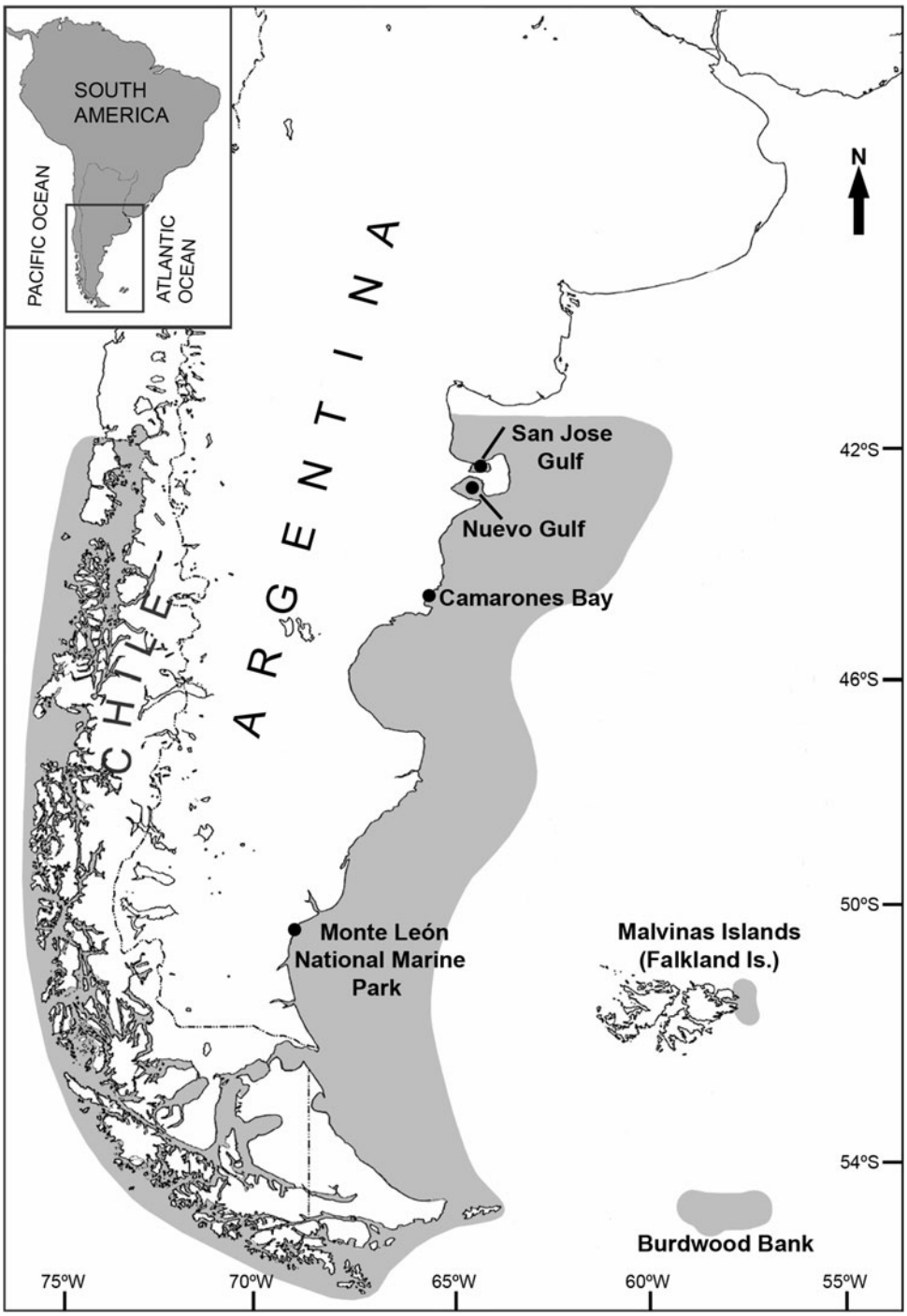

Fig. 1. Geographical distribution of *Enteroctopus megalocyathus* (in grey) according to Ré, (1998, 2008) and Ibáñez *et al.* (2009). Dots indicate sampling areas along the Patagonian Atlantic coast.

the reproductive system defined by scales developed for *E. megalocyathus* (Tables 1 & 2). Nevertheless, these macroscopic maturity scales remain to be validated. In addition, little is known about the gametogenesis of *E. megalocyathus* as well as many other aspects of its reproductive biology. The aims of this work were to define microscopic categories of development by histological analysis of the gonads of both sexes, and to validate the macroscopic scales of *E. megalocyathus*, in order to establish these scales as a tool that will allow for further maturity assessments.

MATERIALS AND METHODS

Samples were obtained from January 2006 to June 2007 by monthly diving surveys up to 25 m depth in San José Gulf and Nuevo Gulf. In the same period additional animals were collected in the intertidal areas of Camarones Bay and Monte Leon National Park (Figure 1). In all cases, animals were captured with a hook.

Total weight (TW), and dorsal mantle length (ML) were registered to the nearest 1 g and 1 mm respectively in 40

Table 1. Macroscopic maturity stages, frequency of seminiferous tubule categories and developmental phases resulting from the overall state of development and functioning of the reproductive system of *Enteroctopus megalocyathus* males.

Macroscopic maturity stages*	Macroscopic characteristics*	Seminiferous tubule categories (%)					Developmental phases of the reproductive system
		I	II	III	IV	V	
I. Juvenile	Difficult to determine sex and recognize terminal organ with the naked eye. Hard to differentiate genital bag from the rest of the internal organs	99	1				Physiologically maturing and functionally immature
II. Immature	White testicle bigger than spermatophoric complex. Terminal organ easy to recognize. Well defined reproductive system. Genital bag of smaller-size appearance than the rest of visceral mass	17.1	82.9				
III. Advanced maturity	Large, yellowish testicle, of bigger size appearance than spermatophoric complex. Ducts of the spermatophoric complex slightly swollen with shreds of spermatophores in formation inside. Eventually, small fragments of an outer tunic without inner content can be found in the spermatophoric sac. The reproductive system has a similar size to the rest of the viscera		27.9	66.2	5.9		Physiologically mature** and functionally immature
IV. Mature-Spawning	Turgescent spermatophoric complex of a size appearance similar or bigger than testicle with wide duct lumen. Mature spermatophores stored in the spermatophoric sac and/or the terminal organ. Maximum-sized reproductive system (often bigger than the rest of visceral mass)			4.1	93.6	2.3	Physiologically and functionally mature

*, from Ortiz *et al.* (2011); **, some animals which presented spermatozoa in seminiferous tubules.

males and 43 females, which were assigned to a maturity stage (Tables 1 & 2). Besides, the weight of the ovary (OV), and the testis (TE) were recorded to the nearest 0.01 g (Table 3). Fresh gonads were fixed in Bouin's solution for 7 days and stored in ethanol 70% for histological examination. Tissue samples taken from the mid-area of testis and ovaries were then dehydrated through an alcohol series, cleared in xylol, and embedded in paraffin wax. Afterwards, sections 6 μm thick were cut with a rotary microtome and stained with eosin and haematoxylin.

Changes through the course of the seminiferous tubules development in males and of oocyte/follicular cell complexes development in females were characterized and classified into categories of microscopic development. Categories were counted along two random perpendicular 10 mm lines, running from the periphery towards the middle of the gonads. In addition, a fraction of oocytes in each category was measured under microscope using their largest diameter.

To assess the correspondence between macroscopic maturation stages and the level of seminiferous tubules and oocyte development, a histological maturity index (HMI) was calculated for each individual according to Sauer & Lipinski's (1990) equation:

$$\mathrm{HMI} = \frac{\sum_{i=1}^{s} (n_i \; x \; i)}{\sum_{i=1}^{s} n_i}$$

where i is a discrete number which represents each microscopic category, s is the maximum number of categories recognized for each sex, and n_i the absolute frequency of seminiferous tubules or oocytes in the category i resulting from the sum of the counts made on each gonad. Kruskal–Wallis (KW) and t planned multiple comparison tests were performed to compare HMI between consecutive macroscopic maturity stages (Conover, 1999). Statistical analyses were performed with GraphPad InStat and Excel packages.

RESULTS

Categories of microscopic development

Five and seven microscopic developmental categories were recognized in gonads of males (Figure 2) and females (Figures 3 & 4) respectively.

MALES

Seminiferous tubule category I: the tubule is poorly developed, has a low cell density and the central lumen is difficult to recognize. Cytologically, there are only rounded spermatogonia attached to the inner tubule wall (germinal epithelium) (Figure 2A) and occasionally a few early spermatocytes are present.

Seminiferous tubule category II: the tubule in this category is well defined and wider than that in category I. Central lumen is now recognized but there are no cells inside. At this stage, there is evidence of the cellular multiplication process. The spermatogonias predominate, and early and secondary spermatocytes appear, increasing cellular density. Smaller than spermatogonias and distinguished by their large nuclei, spermatocytes appear in groups that extend from the germinal epithelium towards the lumen (Figure 2B). No spermatids are present.

Seminiferous tubule category III: the tubule shows a well defined central lumen and a high cell density. In this stage the maturation process stands out and a decrease in cell size

Table 2. Macroscopic maturity stages, frequency of oocyte categories and developmental phases resulting from the overall state of development and functioning of the reproductive system of *Enteroctopus megalocyathus* females.

Macroscopic maturity stages*	Macroscopic characteristics*	Oocyte categories (%)							Developmental phases of the reproductive system
		Previtellogenic oocytes				Vitellogenic oocytes			
		I	II	III	IV	V	VI	VII	
I. Juvenile	Difficult to determine sex with the naked eye. Hard to differentiate reproductive system from the rest of the internal organs	66.9	33.1						Physiologically maturing and functionally immature
II. Immature	Small-sized, white ovary filled with liquid. The reproductive system differentiates itself from the rest of the internal organs, but its size is still small compared to them. Distal oviducts narrow with respect to oviducal glands. White oviducal glands. Proximal oviducts are distended and easily visualized	0.6	9	44.9	45.5				
III. Beginning of maturation	Medium-sized ovary and from white to ivory in colour. Ivory oviducal glands. Distal oviducts slightly swollen. Proximal oviducts not easily visualized. A few, small and longitudinally striped oocytes become evident through the ovary wall. Slightly distinguishable rings in the proximal oviducts		0.5	8.4	65.5	25.6			
IV. Advance maturity	Maximum-sized, ivory ovary of a firm consistency. Reproductive system of similar size to the rest of the visceral mass. Oviducal glands with two distinct bands: one narrow and ivory band and one broad and dark band. Distal oviducts very swollen, widened and filled with liquid. Medium-sized oocytes, with longitudinally striped appearance recognizable through ovary wall. Ring-like structures in the proximal oviducts clearly distinguishable					19.9	79.6	0.5	
V. Mature-spawning	Pink ovary lacking in firmness. Oviducal glands with ivory and dark bands. Unexpanded distal oviducts. Mature (smooth) oocytes in the proximal oviducts and free in the ovary lumen						42.5	57.5	Physiologically and functionally mature
VI. Spent	Pink and flaccid ovary, with a few or no eggs inside. Post-ovulatory follicles distinguishable through the ovary wall. Thick and unexpanded distal oviducts. Widened proximal oviducts							100**	Spent

*, from Ortiz *et al.* (2011); **, corresponding to only two oocytes found in one ovary.

Table 3. Macroscopic maturity stages and morphometric and gravimetric characteristics of *Enteroctopus megalocyathus* histologically analysed.

Macroscopic stages	N	Total weight (g) Mean range	Mantle length (mm) Mean range	Gonad weight (g) Mean range
Males				
I	10	126	65	0.08
		31–215	45–81	0.01–0.2
II	10	642	107	2.7
		404–1331	94–138	0.4–9.3
III	10	1064	151.7	25.6
		667–1553	134–189	10.3–44.3
IV	10	1409	161	67.4
		831–2295	140–190	42–116
Females				
I	5	70	46.7	0.13
		6–255	22–84	0.01–0.33
II	12	677	111	1.76
		215–1148	80–144	0.32–4.27
III	8	1191	140.6	11.6
		769–1613	110–176	1.84–34.3
IV	10	1646	171.8	153
		1157–2448	145.5–200	24–335.5
V	5	1538	171.3	213.6
		1029–2792	142–220	22.3–647
VI	3	948.5	136.2	7.3
		822–1075	131.5–141	4.7–9.9

is evident from the germinal epithelium towards the central lumen (Figure 2C). There are spermatogonias, many early and secondary spermatocytes and small spermatids. A few spermatozoa are present in some of the samples partially occupying the central lumen, in which case they are noticeable by their acidophilic tails.

Seminiferous tubule category IV: the lumen is slightly wider and it is densely covered by the tails of spermatozoa. A great abundance of spermatids and numerous heads of spermatozoa arranged in groups are located in the periphery of the lumen. There are also many spermatocytes and very few spermatogonias (Figure 2D). Along with the discharge of spermatozoa from the tubule, cell density decreases and the central lumen increases in size. In advanced stages of this process, there seem to be no cells associated with the germinal epithelium (Figure 2E).

Seminiferous tubule category V: tubules are either empty or have very few sperm tails. Tubular limits defined in earlier stages by germinal epithelium are not easily recognized (Figure 2F).

FEMALES

Oocyte category I: the oocyte is spherical, it has a small average width of 35 μm (SD = 11.2, N = 60) (Figure 3A). The nucleus presents a prominent nucleoli and heterochromatic clumps. Follicle cells are scarce or absent. When present, they begin to differentiate themselves from the connective tissue overlaying the oocyte.

Oocyte category II: the oocyte has an ovoid shape and it is attached to the ovarian connective tissue by a short stalk. Ovoid follicle cells form a single-layer which spreads from the stalk side, and surround the oocyte, except in the area that will become the animal pole of the egg (opposite the side of the stalk). The mean width of the oocyte is 75 μm (SD = 17.7, N = 60) (Figure 3B).

Oocyte category III: follicle cells are distributed in two layers, except in the side opposite the stalk, where follicle flat cells have formed multiple layers. These cells become incorporated to the inner layer of follicle ovoid cells (Figure 3C). Follicular cells of the outer layer surrounding the oocyte appear dispersed (Figure 3D). In the nucleus of the oocyte a prominent nucleoli persists and the oocyte average width is 135 μm (SD = 21.1, N = 60).

Oocyte category IV: at the beginning of this stage, the inner layer of follicle cells starts to invade the oocyte as folds and they become cuboidal. Blood capillaries develop at the bases of the folds (Figure 3E). Proliferation of follicular epithelium continues. In the second phase, the folds extend deeper into the oocyte increasing their number. The outer layer is formed by scattered flat cells surrounding the oocyte. The inner layer of follicle cells become columnar but keep the separation between them (Figure 3F). The oocyte has an average width of 240 μm (SD = 68.8, N = 60).

Oocyte category V: the onset of vitellogenesis takes place (Figure 4A). The outer layer of follicle cells is not recognizable. Follicle cells of the inner layer have prominent nuclei and the separations between these cells disappear forming a follicular syncytium (Figure 4B). Oocytes width increases on average three times (mean = 607 μm, SD = 172, N = 60).

Oocyte category VI: droplets of chorionic material are secreted by follicle cells and fuse along the inner surface of the epithelium, beginning to form the chorion. The large nuclei of follicle cell epithelium are oriented to cytoplasm of the oocyte (Figure 4C). Folds of follicular syncytium begin to regress, their number decrease, and the oocyte width continues to increase (mean = 1550 μm, SD = 0.39, N = 60) by the active secretion of yolk droplets inside (Figure 4D).

Oocyte category VII: the follicle cell epithelium becomes thin (Figure 4E). The whole oocyte is packaged with yolk and the chorion is fully formed (Figure 4F). Detached from the egg string, the mature oocyte will be ready for ovulation.

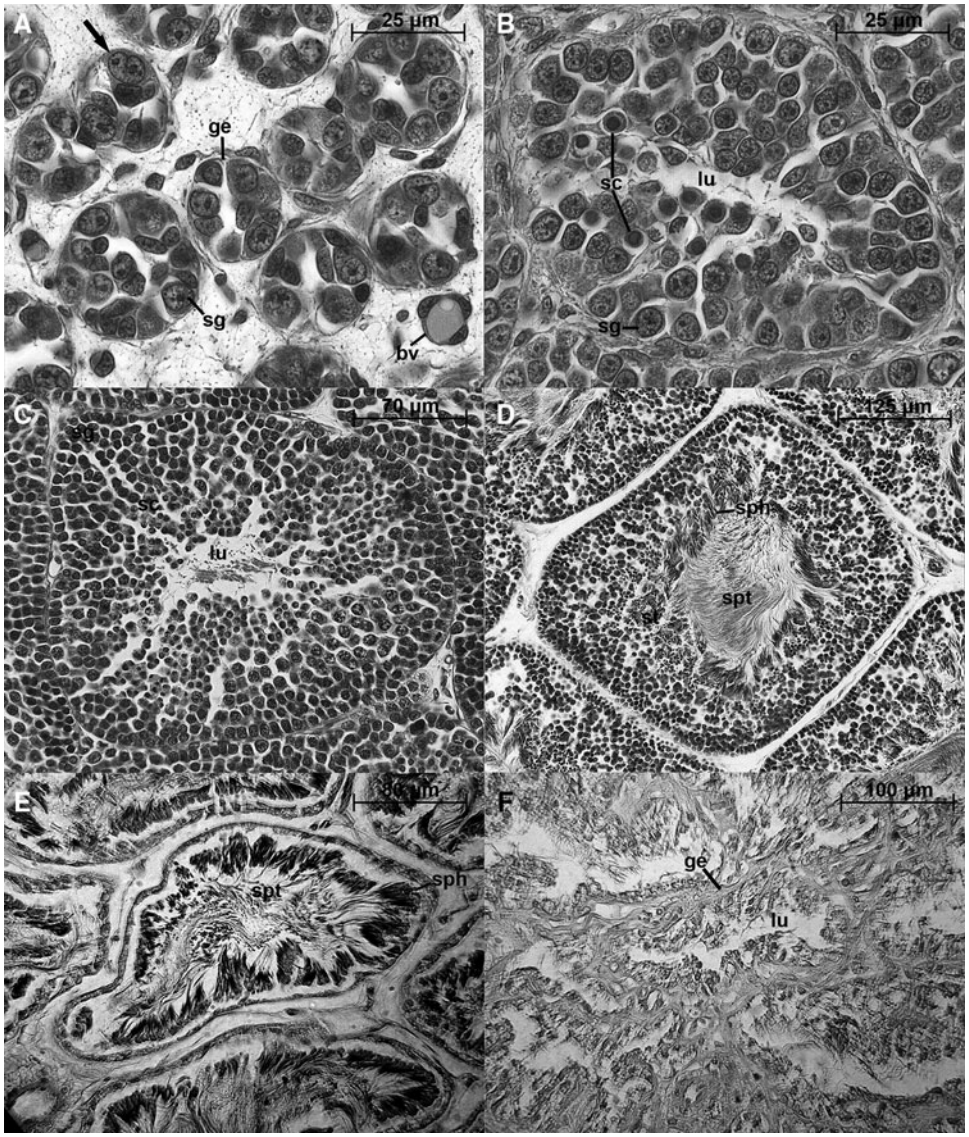

Fig. 2. *Enteroctopus megalocyathus*. Seminiferous tubule categories: (A) category I; (B) category II; (C) category III; (D) (early) and (E) (late), category IV; (F) category V. bv, blood vessel; ge, germinal epithelium; lu, central lumen; sc, spermatocytes; sg, spermatogonia; sph, spermatozoa heads; spt, spermatozoa tails; st, spermatids. Arrow head, an espermatogonia undergoing mitotic division.

Oocyte width reaches the maximum (mean = 1980 µm, SD = 0.54, N = 28).

MICROSCOPIC CATEGORIES ALONG MACROSCOPIC MATURITY STAGES

In males, within each macroscopic stage of maturation and between consecutive stages, seminiferous tubules showed a regular and progressive pattern of development (Table 1). Spermatozoa were recorded from macroscopic stage III in seminiferous tubules categories III and IV. At the stage IV, almost all testes exhibited a central lumen of tubules filled with spermatozoa, except for one testis which presented partially spent tubules. Spent tubules (category V) were only observed in one male which exhibited a small testis (TE weight = 42 g) but still had spermatophores stored in the spermatophoric sac and terminal organ (Tables 1 & 3).

In females, the greatest overlap of oocyte categories was observed in macroscopic stages II and III. Previtellogenic oocytes were registered up to stage III, from which the first early vitellogenic oocytes (oocyte category V) appeared. Nearly mature and mature oocytes (categories VI and VII respectively) were observed from stage IV on, but mature oocytes reached their maximum frequency at macroscopic stage V (Table 2). In addition, at the latter stage, distinct post-ovulatory follicles begin to be observed indicating recent spawning. An increased number of postovulatory follicles, and a very few (only two mature oocytes in one ovary) or no oocytes inside ovaries were registered at macroscopic stage VI (Figure 5; Table 2).

Validation of macroscopic maturity stages

The HMI values of males and females increased and showed highly significant differences through macroscopic stages (KW = 36.1, $P < 0.001$ and KW = 30.6, $P < 0.001$ respectively) (Figure 6). In males, highly significant differences were found between all consecutive stages (I–II: $t = 10$, $P < 0.01$; II–III: $t = 10$, $P < 0.01$; III–IV: $t = 9.5$, $P < 0.01$).

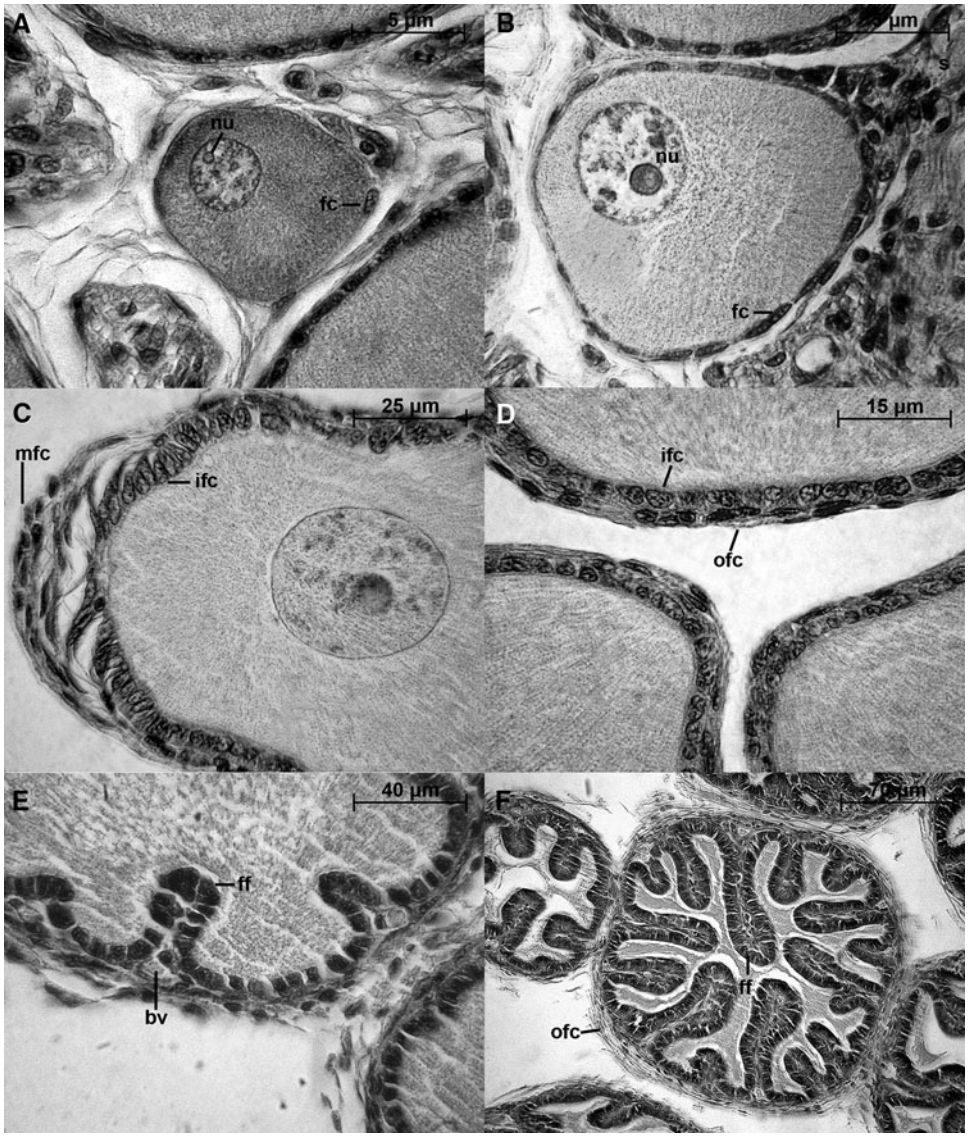

Fig. 3. *Enteroctopus megalocyathus*. Previtellogenic oocyte categories: (A) category I; (B) category II; (C & D) category III; (E & F) category IV. bv, blood vessel; fc, follicular cells; ff, follicular folds; ifc, inner layer of follicular cells; mfc, multiple layer of follicular cells; nu, nucleolus; ofc, outer layer of follicular cells; s, stalk side.

In females, significant differences were found between macroscopic maturity stages I–II ($t = 8.8$, $P < 0.01$), II–III ($t = 9.2$, $P < 0.01$), III–IV ($t = 9.5$, $P < 0.01$), and IV–V ($t = 7$, $P < 0.05$). Due to the fact that in macroscopic stage VI almost all oocytes have been expelled this stage was not used for the statistical comparison.

DISCUSSION

As for other cephalopod species, a comparison between spermatogenesis and oogenesis showed that the maturity process in males can be described in a lower number of categories than females. Besides, the development of seminiferous tubules and the oocyte/follicular cell complexes in *Enteroctopus megalocyathus* proceeds along schemes broadly similar to those described for other octopod species (Pujals, 1986; Coelho, 1990; Boyle & Chevis, 1992; Gonçalves *et al.*, 2002; Rodríguez-Rúa *et al.*, 2005). In the case of females, the rapid increase of the area of the single and the double layers of follicular cells is often attributed to mitosis (Cowden, 1968; Boyle & Chevis, 1992). However, it is remarkable that at oocyte category III, an active proliferation of follicular cells occurs at the opposite stalk side of the oocyte. These follicular cells migrate and eventually enclose the oocyte (Figure 3C). In addition, they also seem to contribute to increase the area of the follicular cell layer during the infolding process (i.e. at oocyte category IV) (Figure 3E, F). A similar pattern of follicular cells proliferation has been described for *Eledone cirrhosa* (Lamarck, 1798) (Boyle & Chevis, 1992).

Histological analysis of gonads coupled with macroscopic observations would permit the definition of developmental phases during sexual maturation on the basis of the degree of development of sexual cells, and once they become mature, their transport and accumulation in different parts of the reproductive system (i.e. the overall state of development and functioning of the reproductive system) (Arkhipkin, 1992; Sauer & Lipinski, 1990; Nigmatullin *et al.*, 2003). In males at stages I and II, germinal cells were observed in early stages of differentiation (i.e. up to seminiferous tubule

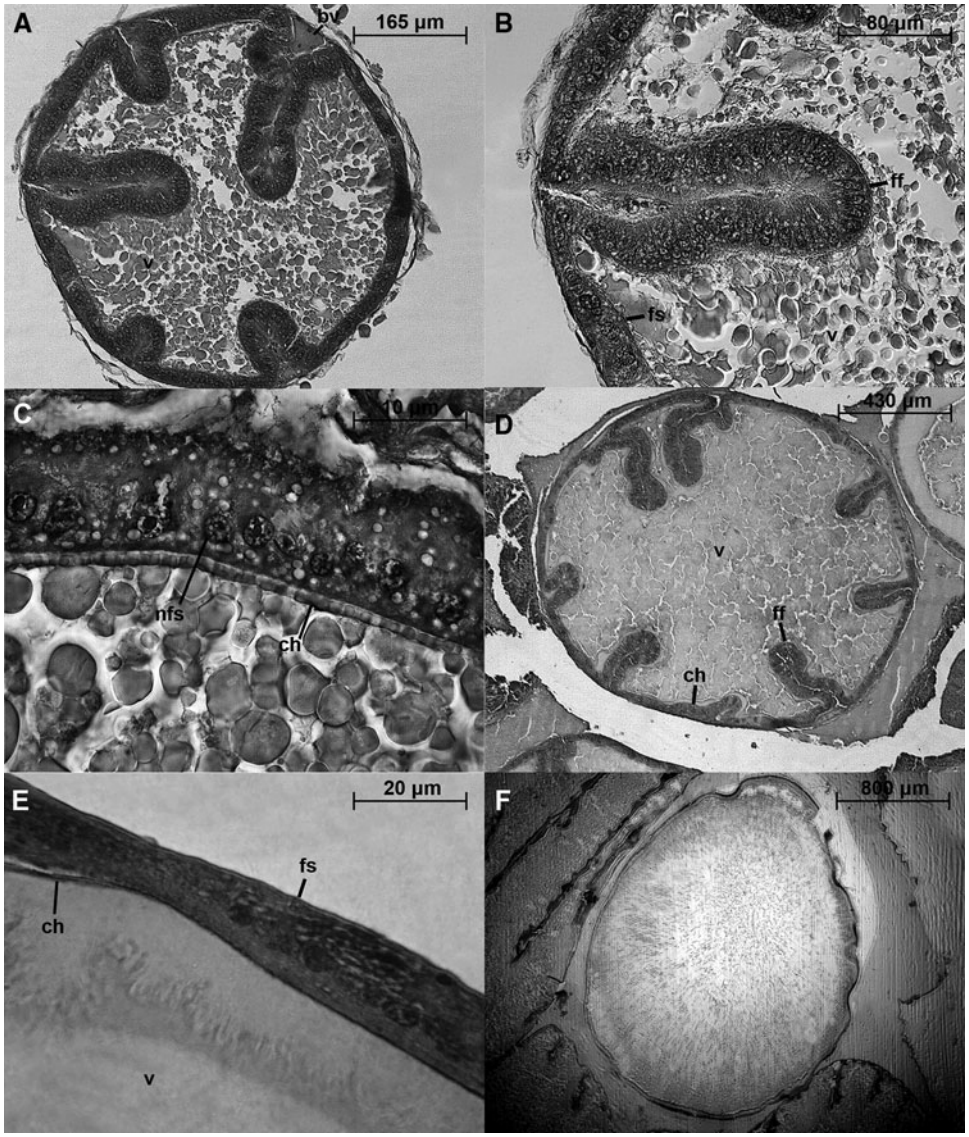

Fig. 4. *Enteroctopus megalocyathus*. Vitellogenic oocyte categories: (A & B) category V; (C & D) category VI; (E & F) category VII. bv, blood vessel; ch, chorion; ff, follicular folds; fs, follicular syncytium; nfc, nucleus of the follicular syncytium; v, vitellus.

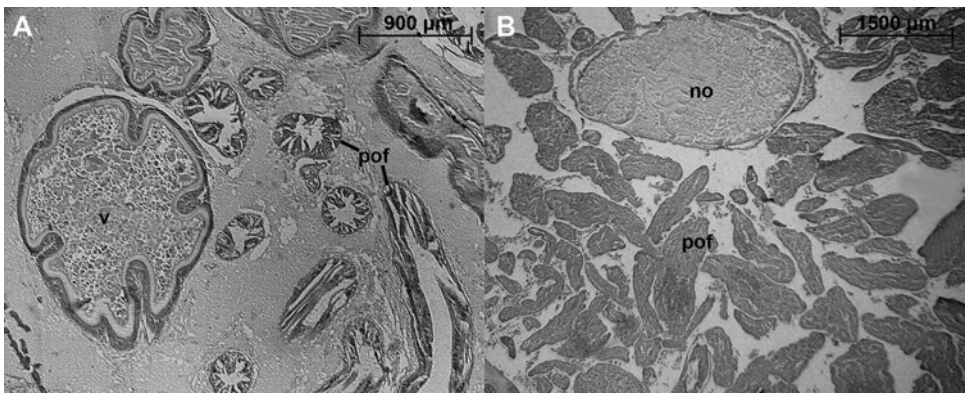

Fig. 5. *Enteroctopus megalocyathus*. Cross-section of an ovary at mature-spawning (A) and at spent stage (B). no, mature oocyte not evacuated; pof, postovulatory follicles; v, vitellus.

category II) and no spermatophores. These stages can be categorized into histologically (or physiologically) maturing and functionally immature phases. At stage III, TE weight increased sharply as a consequence of a continuous increase of the density and the diversity of the germinal cells. In addition, some animals at this stage exhibited a few spermatozoa in their testis and fragments of an outer tunic without inner content in the spermatophoric sac (Tables 1 & 3).

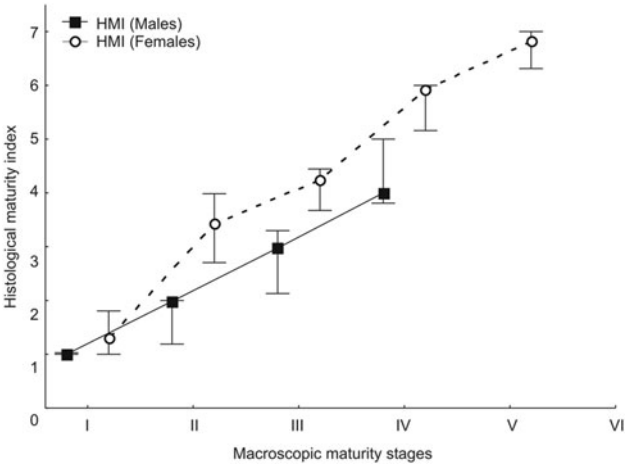

Fig. 6. Median (± minimum–maximum) values of the histological maturity index (HMI) of males and females of *Enteroctopus megalocyathus* by macroscopic stages.

The latter would be a process occurring before the animals reach functional maturation, prior to the formation of mature spermatophores (Lipinski & Underhill, 1995; Nigmatullin *et al.*, 2003). Thus, at least a fraction of stage III males could be considered to be in a physiological maturity phase but still functionally immature. In contrast, stage IV males which presented the lumen of seminiferous tubules covered by spermatozoa (i.e. seminiferous tubule category IV) and mature spermatophores storage in the spermatophoric sac and/or the terminal organ could fall into a physiologically and functionally mature phase (Table 1). On the other hand, an analysis was conducted in stage IV males which exhibited signs of gonad exhaustion (i.e. seminiferous tubules partially spent or without sperm and reduced testis weight) (Table 1). Since these octopuses still had storage spermatophores, they could be in an active spawning condition, a fact that makes it difficult to have clear criteria to determine the spent stage in this sex. In females, the sharply OV weight increase at stage IV is primarily due to a size increase of the oocytes as a consequence of the vitellogenesis (Tables 2 & 3). As a proof that mating can occur in stage IV females, evaginated spermatophores were observed attached to their distal oviducts (Ortiz *et al.*, 2011). Nevertheless, up to that stage, they should be considered to be in a functionally immature and physiologically maturing phase because most oocytes are still in a maturing developmental state (99.5% of oocyte categories V and VI). Conversely, stage V of females should be considered in a functionally and physiologically mature phase because mature oocytes are found inside ovary and distal oviducts (i.e. in a spawning condition) (Table 2). Thereafter, stage VI would not depend on the oocyte/follicular cells complexes development. At this stage, the morphological characteristics of the gonad define a clear set of criteria of the spent stage of this sex (Table 2) that is also used to recognize the spent stage in other octopus species (Pujals, 1986; Gonçalves, *et al.*, 2002; Leporati *et al.*, 2008).

In both sexes of *E. megalocyathus* at the most advanced states of maturation, histological analysis reveals that animals do not have a stock of germinal cells that would permit a second spawning (Figures 2F & 5B). In addition, at macroscopic stage VI females undergo a decrease of TW, and ML (Table 3) (Ortiz *et al.*, 2011). At spent stage, these microscopic and macroscopic features are conditions associated with senescence (Arnold & Williams-Arnold, 1977; Anderson *et al.*, 2002; Gonçalves *et al.*, 2002) expected to occur when *E. megalocyathus* approaches the end of its life span. On the other hand, although an overlapping of oocyte categories was observed through maturation, group-synchronous ovulation occurs in the ovary at mature-spawning stage. At that stage postovulatory follicles were seen alongside nearly mature and mature oocytes (Table 2; Figure 5A). These findings provide indirect evidence that *E. megalocyathus* has an egg lying period confined to a few days or weeks during which nearly mature oocytes become ready to be spawned. In addition, somatic growth does not seem to take place between spawning events (Table 3). Therefore, *E. megalocyathus* could be considered an intermittent terminal spawner (*sensu* Rocha *et al.*, 2001). Observations during spawning on captive *E. megalocyathus* could confirm this hypothesis.

The agreement between macroscopic and histological criteria confirms the validity of macroscopic maturity scales of both sexes. However, as in ichthyology, maturity staging by macroscopic examination could contain a subjective judgment (Gerritsen & McGrath, 2006). This would be particularly relevant in the intermediate ('maturing') stages of the *E. megalocyathus*'s scales that are defined by the relative size and colour of the different parts of the reproductive organs (Tables 1 & 2). These stages have been the most abundant within catches in fishing seasons and areas of the northern Atlantic Patagonian coast (Ortiz *et al.*, 2011). Thus, assays investigating the variability among assessors in the assignment of macroscopic maturity stages of *E. megalocyathus* could be performed in order to check the accuracy of the data gathered in this process.

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