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ORIGINAL ARTICLE

On gonadic maturation and reproductive strategy in deep-sea benthic octopus *Graneledone macrotyla*

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Abstract The new information reported in this paper is based on five maturing and mature females of the large-tuberculate octopus *Graneledone macrotyla*. These specimens were caught in bottom trawl surveys ATLANTIS 2009 (February 24 to April 1, 2009) and ATLANTIS 2010 (March 9 to April 5, 2010) carried out off the Argentinean Economic Exclusive Zone. Capture depth ranged from 475 to 921 m and sea bottom temperature between 2.8 and 3.1 °C. Development of the complex ovary, oviducts, and oviducal glands during gonadic maturation is described. The absence of spermathecae in the oviducal glands and the presence of fertilized eggs inside the ovary suggested that fertilization took place within the ovary. Histological techniques showed the presence of four types of oocytes. Maturing oocyte size–frequency distribution was polymodal. Fluorescence reaction showed that atresia occurred in both early and later oocyte maturation stages. Atresia affected 48–55 % of the initial number of oocytes. The maximum observed potential fecundity was estimated at 250–300 eggs. *G. macrotyla* showed a group-synchronous ovulation pattern, regulative atresia, and a batching spawning pattern with a few egg batches spawned intermittently over an extended period of spawning.

Keywords Reproduction · Atresia · Deep-sea octopods · *Graneledone macrotyla* · Patagonian slope · Southeast Atlantic

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Introduction

Cold-water deep-sea octopods, both in Arctic and Antarctic waters, have large eggs (15–35 mm length) and low fecundity, ranging from a dozen to a few hundred oocytes, and hatchlings which are among the largest and most advanced known (Kuehl 1988; Laptikhovsky 1999, 2001; Laptikhovsky et al. 2007; Voight and Drazen 2004).

Bello (2006) suggested that the large-egged *Graneledone pacific* is a "multiple spawner," based on the simultaneous occurrence of oocyte cohorts at different oogenic stages in five females collected in the north-east Pacific Ocean, as deep as 2765 m. This reproductive strategy requires a group-synchronous ovulation and somatic growth between separate spawning events (Rocha et al. 2001). Nevertheless, the scattered information available on the reproduction of deep-sea octopods suggests that oocyte growth and maturation seem to be synchronous, although in maturing females, the oocyte size distribution appears to be bimodal or, rarely, polymodal (Kuehl 1988; Laptikhovsky 1999, 2001; Barrat et al. 2008). The existence of synchronous or group-synchronous ovulation in these species is, therefore, controversial.

Observations recorded from ROVs (Drazen et al. 2003) and submersibles (Voight and Grehan 2000) have shown that these deep-sea octopods attach their eggs in shelters on rocky ground, on outcrops and ledges, and that females

brood their egg mass. Whether a brooded egg mass is spawned simultaneously or in several successive batches during an extended spawning period is also a matter of discussion. Laptikhovsky (pers. comm.) suggested that this question can only be solved by studying postovulatory follicles (POFs), which provide information on how many eggs and batches were actually laid. At present, however, only few specimens in such a reproductive stage have been studied extensively.

Another question relates on whether females continue to grow between the successive release of egg batches, as seems to occur in many cirrate and some incirrate octopods considered as "continuous spawners" (Rocha et al. 2001).

On the Patagonian slope (475–921 m), the large-tuberculate octopus *Graneledone macrotyla* Voss, 1976 inhabits shallower waters than in the sub-Antarctic area (1,647–2,044 m). The species inhabits the plume of cold sub-Antarctic waters, which is pushed far north into the southwestern Atlantic by the Falkland-Malvinas Current (Guerra et al. 2012). These authors described, for the first time, the female genitalia of *G. macrotyla*. Owing to the presence of a bimodal size distribution of oocytes, a reproductive strategy with multiple spawning was suggested. Besides, the presence of shrivelled oocytes within the ovary was considered as a symptom of atresia. Cases of atresia, or degeneration of some oocytes as a result of intragonad competition for nutrient supply, have been reported in *Eledone cirrhosa* (Boyle and Chevis 1991), the squids *Loligo vulgaris reynaudii* and *Dorytheuthis gahi* (Sauer et al. 1993; Melo and Sauer 1998; Laptikhovsky and Arkipkin 2001), and large-egged deep-sea squids (Laptikhovsky et al. 2007). Although recent observations have shown that massive atresia is relatively common in fish (Rideout and Tomkiewicz 2011), and cases of massive atresia have been recorded in deep-sea octopods (Kuehl 1988; Laptikhovsky 1999), the later has not received special attention to date. Accordingly, the aim of this paper is to study atresia in females of *G. macrotyla* during gonadic maturation and how it affects the poorly known reproductive strategy of this species.

Materials and methods

Eleven specimens of *G. macrotyla* (Table 1) were caught in two multidisciplinary research cruises conducted by the Instituto Español de Oceanografía (IEO) to assess the biomass of the main commercial fish stocks in the High Seas of the Southwest Atlantic by using the swept area method through a bottom trawl Lofoten type gear (Portela et al. 2010). The surveys ATLANTIS 2009 and 2010 were carried out between February 24 and April 1, 2009 (Guerra et al. 2011) and from March 9 to April 5, 2010, respectively, on board the R/V *Miguel Oliver*. Depth of captures ranged from 475 to 921 m. Sea bottom temperature varied from 2.8 to 3.1 °C.

On board, catches were sorted immediately after capture. Cephalopods were stored in labelled plastic bags and frozen at −20 °C on board. Specimens were subsequently transported to the Instituto de Investigaciones Marinas (IIM, CSIC) in Vigo, Spain, for detailed examination.

After thawing at room temperature, the specimens were identified and sexed. Five females were identified and preserved in ethanol 70 %. All measurements and histological sections were taken on preserved specimens. All specimens were stored in the collection of the MDMG: Museo do Mar de Galicia (Vigo, Spain).

Two indices were calculated: the proximal oviduct length index (POLI: proximal oviduct length/dorsal mantle length 100×), and the distal oviduct length index (DOLI: distal oviduct length/dorsal mantle length 100×).

The histological study of the oviducal gland and oviducts was carried out on three specimens MDMG552011, MDMG68A2011, and MDMG68B2011 (Table 2), whose glands had a maximum diameter of 12.1, 16.0, and 27.0 mm, respectively.

Table 1 Reproductive traits of *G. macrotyla* females

Sp. No.	ML (mm)	TW (g)	Mat	Type of oocytes and eggs						OGD (mm)	POLI	DOLI
				S1	S2	S3	S4	S5	Total			
MDMG552011	78.0	318	Sm	230	2				232	12.1	6.4	61.0
MDMG68C2011	80.0	666	Sm	258	9				267	9.2	7.5	69.3
MDMG472011	107.0	760	M	–	–	3	103	3	109	19.0	8.7	50.0
MDMG68A2011	98.2	1,064	M	–	–	4	95	1	100	16.0	9.1	52.0
MDMG68B2011	110.0	1,076	M	–	–	108	–		108	27.0	9.0	53.3

Sp No Specimen catalogue number, *MDMG* Museo do Mar de Galicia, Vigo, Spain; *ML* mantle length, *TW* total weight, *Mat* maturity stage (*Sm* maturing, *M* mature), *OGD* oviducal gland diameter, *POLI* proximal length oviduct index, *DOLI* distal length oviduct index. For S1–S5 see explanation in Table 2

Table 2 *Graneledone macrotyla* oocyte and egg characteristics

Type of oocytes/eggs	S1	S2	S3	S4	S5
Length (mm)	1.0–3.0	3.1–9.0	18.8–25.5	12.8–17.2	18.8–20.5
Width (mm)	–	5.4–1.0	11.7–9.5	4.3–3.0	12.2–11.4
OW ± SD (mg)	–	–	733.86 ± 71.84 (*n*:7)	355.67 ± 120.5 (*n*:7)	1,399 ± 139.31 (*n*:7)
Outer case	Rigid, opaque	Rigid, opaque	Rigid, translucent	Rigid, translucent	Hard, opaque
Longitudinal striae	No	No	Yes	Yes	No. Smooth surface
Attached in bunch	Yes	Yes	Yes	Yes	No. Free within ovary
Distal micropyle	Yes, narrow	Yes, narrow	Yes, narrow	Outer case widely open	No
Peduncle	Long	Long	Long, 17–19 mm	Long	Short, 4–5 mm
Fertilized	No	No	No	No	Yes
Observations	Swollen AO	Swollen	Swollen	Flabby, empty AO	Hen's egg shape. Color: beige

OW oocyte weight in mg, *AO* atretic oocytes

Histological studies of oocytes in different stages of development were carried out on the specimens MDMG552011, MDMG68A2011, MDMG68B2011, and MDMG472011, which represent the four maturation stages observed (Table 2). Oocytes were fixed with a solution of 10 % buffered formaldehyde in a ratio volume formaldehyde/tissue approximately equal to 50. After fixative action, profuse cleaning with water was done. Then, each sample was dehydrated in ethylic alcohol in increasing concentrations from 70 to 100 % and embedded individually in paraffin blocks (58 °C) according to standard histological techniques (Gabe 1968). Longitudinal sections at different levels were produced (6–8 μm thick) and stained with Harry's hematoxylin–eosin. In order to distinguish between postovulatory follicles (POFs) and atretic oocytes (AO), oocyte preparations were evaluated using a fluorescence microscope with a B-2A filter set with a 450–490 nm excitation filter, a 505-nm dichroic mirror and a 520-nm barrier filter (Saborido-Rey et al. 2007). Observations were carried out, and photographs taken, with a binocular (63× magnification) and a light microscope (100–1,000× magnification) coupled with a Nikon DXM 1200F digital video camera. The software used was NIS-Elements D 3.00 SP6 (Build 539) (© 1991–2008 Nikon Laboratory Imaging).

Results

Reproductive system maturation

Measurements of reproductive traits of maturing and mature female *G. macrotyla* (Fig. 1a) are shown in Table 1. In the mid-portion of each oviduct, there is an oviducal gland, which changes in size and color from white-beige (Table 1; Fig. 1b) to blue–blackish during the maturation process (Table 1; Fig. 1c, d). The proximal oviducts are short (POLI 6.4–9.1) and wide. The distal oviducts are long (DOLI 50.0–69.3) and wider in the proximal than in the distal part. Their walls and shape experience changes during growth and maturation. While being thin in maturing females, with the apex practically closed and adopting a hook-like structure, the walls in mature females are thick and completely open (Fig. 1b, c). Conversely, the maturation process involves lengthening of oviducts and widening of oviducal glands. The ovary is almost spherical and does not experience any substantial changes during growth and maturation, except an increase in volume from 8.2 cm^3 (ovary: 2.5 cm diameter in maturing female MDMG552011) to 143.7 cm^3 (ovary: 6.5 cm diameter in mature female MDMG68A2011).

The oviducal gland is structurally formed by one huge concentric peripheral gland around the oviduct, separated by a thin sheet of connective tissue (Fig. 2a). The peripheral gland is formed by groups of concentric cells with basal nuclei and a central lumen; in females close to maturity, their cytoplasm is densely packed with eosin granulations (Fig. 2b). Spermathecae were absent. No spermatangia or free sperm were observed within the ovaries or attached to the egg filaments.

Oocytes

Four types of oocytes (S1–S4) and fertilized eggs (S5) were observed inside the ovaries. Their characteristics are shown in Table 2 and Figs. 3, 4, 5, 6, 7. During oogenesis, in addition to a progressive increase of oocytes in size, the outer case of oocytes transforms from rigid and opaque in the earlier stages (S1 and S2, Fig. 3a–c) to translucent and with longitudinal striae in the more advanced stages of maturation (S3, Fig. 3d). During these stages (S1–S4), all oocytes were attached to the ovary by long peduncles forming a bunch (Fig. 3b). Oocytes at stages S1 and S2 showed no longitudinal striae and had yellow–brown spots on the surface (Fig. 3b). A very narrow micropyle was

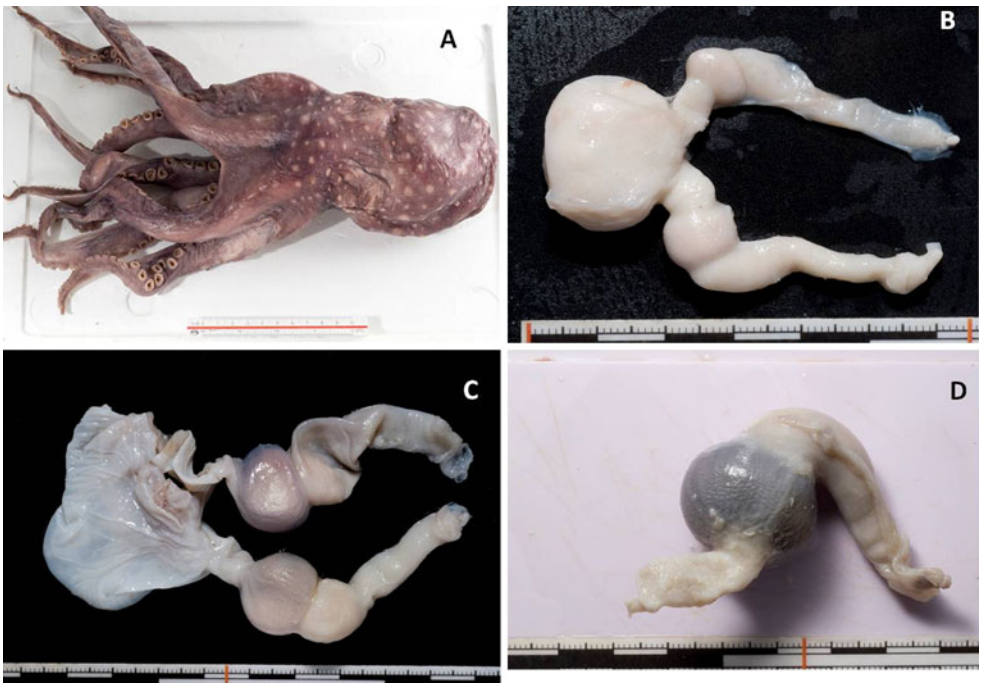

Fig. 1 *Graneledone macrotyla.* **a** Dorsal view of the specimen MDMG68B2011; **b** reproductive system of the maturing female MDMG552011; **c** reproductive system of the mature female MDMG472011; **d** detail of the oviducal gland of specimen MDMG68B2011

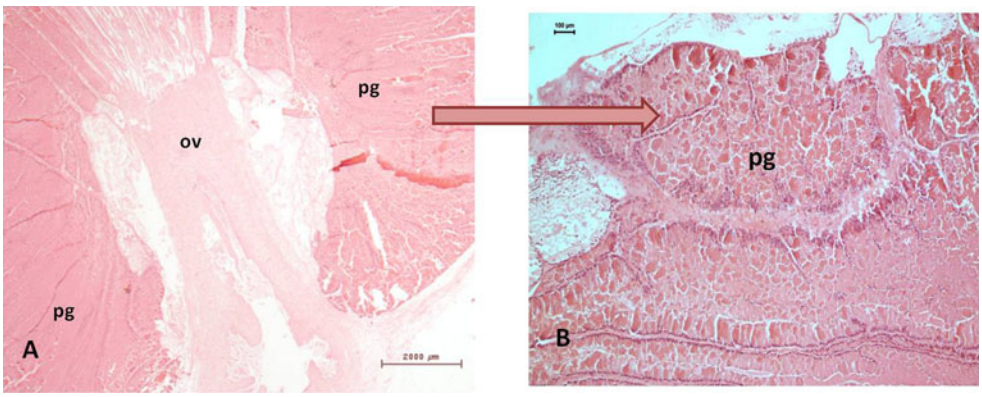

Fig. 2 *Graneledone macrotyla*. Oviducal gland of mature females MDMG68A2011 and MDMG68B2011. **a** *Longitudinal section* in the *middle portion* stained with hematoxylin–eosine (h–e); *ov* oviduct, *pg* peripheral gland, **b** detail of the *pg* showing eosinophil granulations

present at the distal pole of all oocytes of stages S1–S3 (Fig. 3e).

In the maturing female MDMG552011, 90.5 and 9.5 % of the oocytes present within the ovary were in stages S1 and S2, respectively. In the other maturing female (MDMG68C2011), the values amounted to 92.9 and 7.1 %, respectively (Table 1). The total number of oocytes in these cases did not exceed 267, which suggests that the potential fecundity (PF) of *G. macrotyla* is low (approximately between 250 and 300 oocytes). Moreover, the presence of oocytes differing in size and stage of maturation indicates that oogenesis of this species is group-synchronous.

All oocytes (108) found within the ovary of the mature female MDMG68B2011 were in stage S3 (Figs. 3d, 4a, b; Table 1). No POFs were found in the histological sections examined.

An oocyte in stage S4 is shown is Fig. 4c. Its outer case was rigid and showed longitudinal striae. All oocytes at that stage showed long peduncles and were attached to the ovary forming a bunch (Fig. 4a). Their most conspicuous characteristics were an outer case, widely open in the distal zone, with a flaccid consistency. Mature females MDMG472011 and MDMG68A2011 showed 94.5 and 95 % of their oocytes in this stage.

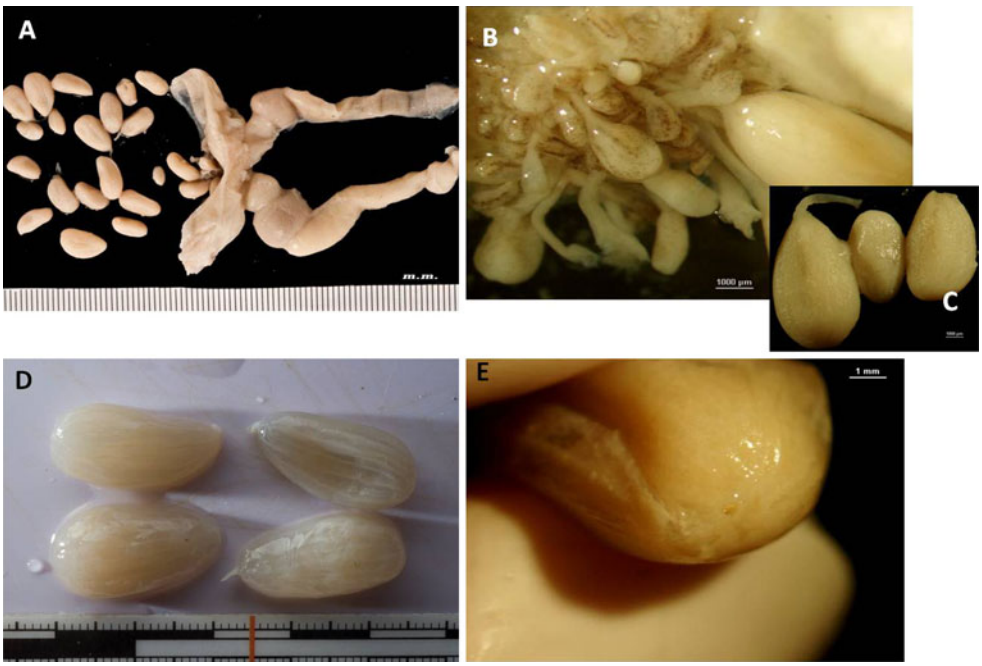

Fig. 3 *Graneledone macrotyla.* **a** Oocytes in stage S2 from maturing female MDMG552011 (see Table 2 for details); **b** oocytes S1 and S2 within the ovary forming a bunch; **c**, details of oocytes S1 and S2; **d** Oocytes S3 from mature female MDMG68B2011 showing *longitudinal* striae; **e** micropyle was present in S1–S3 oocytes

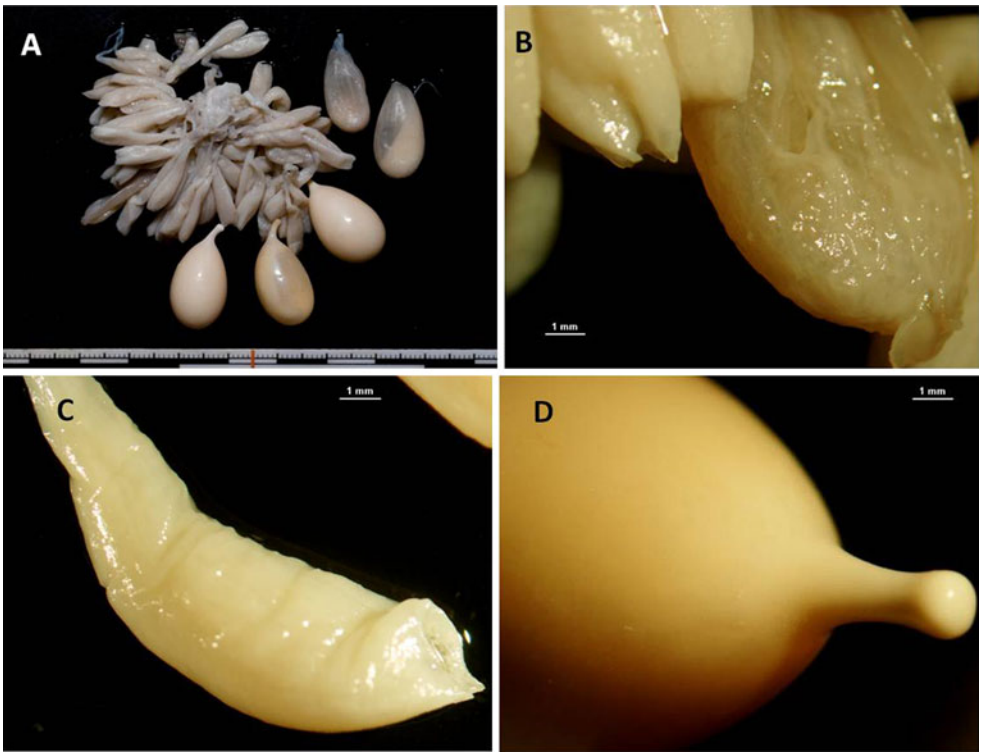

Fig. 4 *Graneledone macrotyla.* Mature female MDMG472011. **a** Oocytes and fertilized eggs within the ovary; **b** detail of oocytes S3 and S4; **b** oocyte S4; **d** fertilized egg

Within the ovaries of the mature females MDMG472011 and MDMG68B2011, three and one fertilized eggs (S5) were found, respectively (Table 1; Fig. 4a, d). These eggs were free within the ovary and oval in form. Their external capsule was hardened, lacking longitudinal striae, and beige in color. The micropyle had disappeared and their peduncles

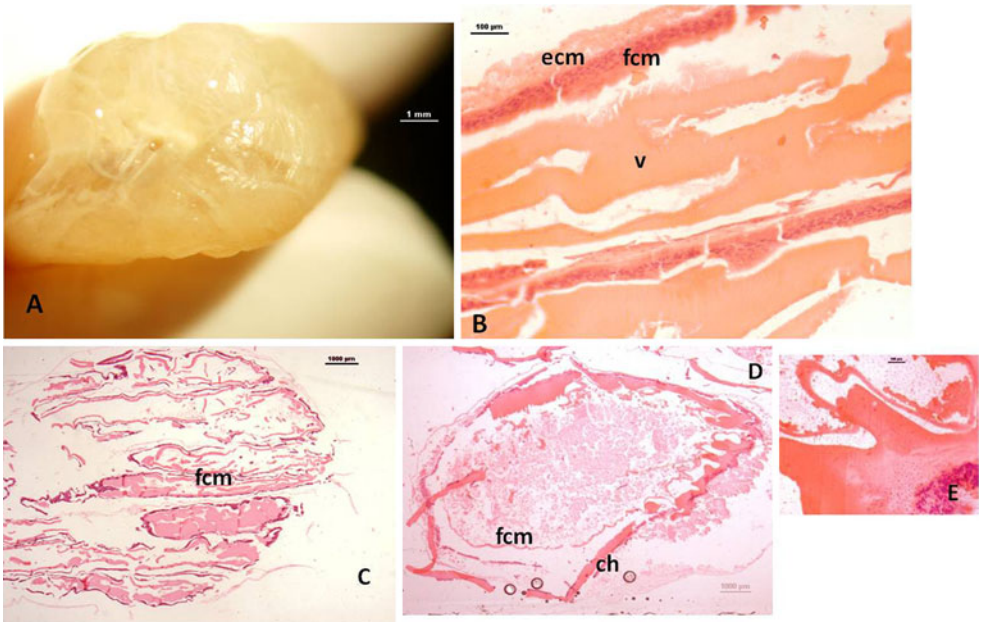

Fig. 5 *Graneledone macrotyla.* Mature female No MDMG68B2011. **a** External aspect of an oocyte type S3 showing *longitudinal* striae and micropyle; **b** longitudinal section of that oocyte, *Ecm* epithelial cell membrane, *fmc* follicular cell membrane, *v* vitellus, **c** oocyte S3; **d**, longitudinal section of oocyte S3 showing *fmc* and chorion (*ch*); **e** detail of the chorion

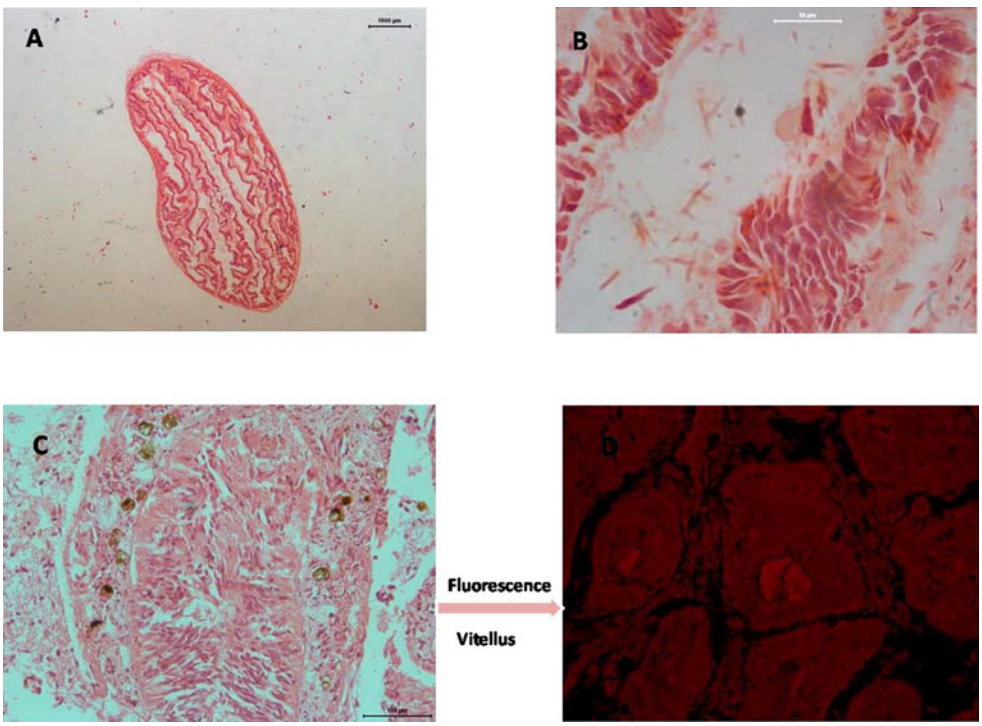

Fig. 6 *Graneledone macrotyla.* Maturing female MDMG552011. **a** *Longitudinal section* of an oocyte in stage S1 stained with h–e; **b** detail of the follicular cells of that oocyte; **c** longitudinal section of another oocyte S1 stained with h–e; **d** vitellus, atresia

considerably shortened (Table 2; Fig. 4d). No POFs were found in these females.

Figure 5 illustrates the external aspect of an oocyte of the mature female MDMG68B2011 in stage S3 showing longitudinal striae and a micropyle (Fig. 5a). A longitudinal section of that oocyte stained with h–e (Fig. 5b) indicated the existence of a typical epithelial cell membrane, a follicular cell membrane, a vitellus at an

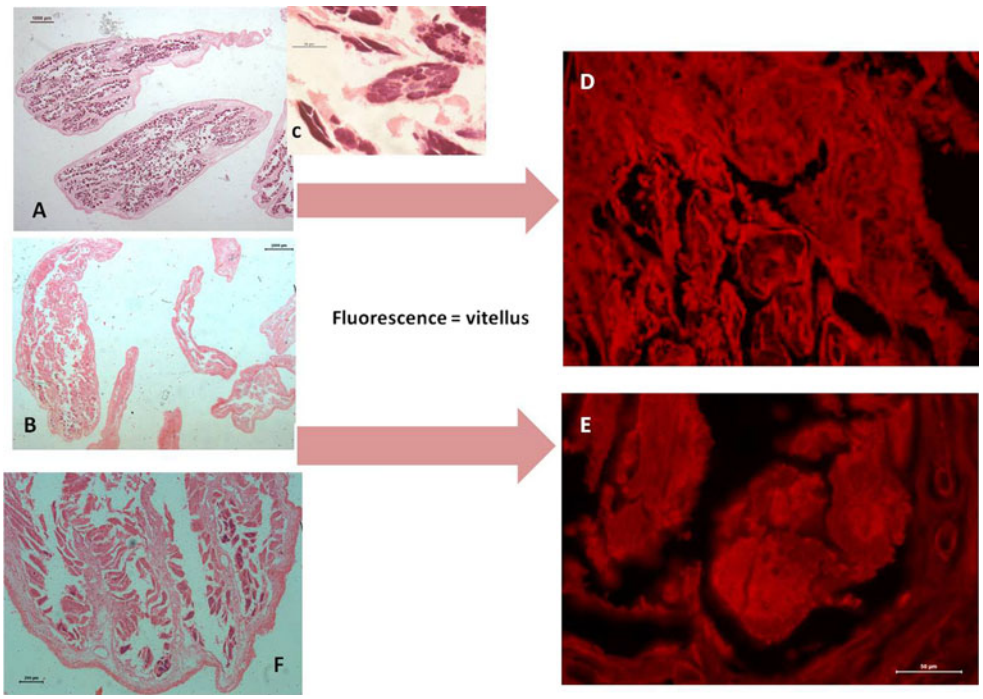

Fig. 7 *Graneledone macrotyla.* **a** *Longitudinal section* of an oocyte type S4 stained with h–e from mature female MDMG472011; **b** longitudinal section of an oocyte type S4 stained with h–e from mature female MDMG68A2011; **c** detail of granulated cells; **d**, **e,** and **f** fluorescence reaction of oocytes from MDMG472011 and MDMG68A2011, vitellus present, follicular cell membrane (*fcm*) in regression, atresia

advanced stage of maturation, and chorion already formed (Fig. 5c–e).

Size and depth of the longitudinal striae suggest that S4 oocytes are at a more advanced stage than S2 oocytes, but at a less advanced stage than S3 oocytes.

Atresia

Histological images of an oocyte in stage S1 show jumbled structures and a swelled connective tissue, while follicular cells are difficult to detect (Fig. 6a–d). Fluorescence reaction revealed that vitellus was present at this stage, which indicated that these were actually oocytes and not POFs. Therefore, these oocytes were considered atretics (AO). Initial oocytes manifesting signs of degeneration represented high rates (48–55 %) of oocytes present inside the ovaries of the maturing females MDMG552011 and MDM68C2011. On the other hand, oocytes S2 showed follicular cells arranged in longitudinal stripes and an organization of maturing cells with follicular folding.

Figure 7 shows a longitudinal section of type S4 oocyte from the mature females MDMG472011 and MDMG68A2011 stained with h–e. Taking into account that these oocytes lost their form (Fig. 7a, b), that the fluorescence reaction was positive, showing the presence of vitellus (Fig. 7d, e), and that their follicular cells were located around and overlapping the ooplasma (Fig. 7f), these oocytes should also be considered as atretics (AO) and not as POFs.

Therefore, as suggested by the presence of AO in stages S1 and S4, oocyte degeneration may occur in both early and late stages of maturation.

Discussion

Maturation of the reproductive system and oocyte fertilization

The ovary, oviducts, and oviducal glands complex of *G. macrotyla* is similar to that described by Bello (2006) for *G. pacifica,* although the swelling of the distal oviduct just behind the oviducal glands present in *G. pacifica* (Bello 2006) was not observed in *G. macrotyla*. However, it should be considered that some deformations may have occurred in the preserved specimens (Voight 2001). The proximal oviduct of *G. pacifica* is slightly longer than in *G. macrotyla*. The POLI estimated from Bello (2006), excluding the probably erroneous measurement of 23 mm, ranged from 7.0 to 11.3, whereas in *G. macrotyla* that index ranged from 6.4 to 9.1. However, the contrary applies to the distal oviduct, with a DOLI ranging from 17.3 to 26.0 in *G. pacifica* (Bello 2006), and from 52.0 to 69.3 in *G. macrotyla* (Table 1).

As in *G. pacifica* (Bello 2006), the overall maturation process in *G. macrotyla* involves lengthening of oviducts and widening of oviducal glands. There is no possible comparison between the changes in color observed in the oviducal glands of *macrotyla* during the maturation process and those of *pacificus*, because Bello (2006) did not indicate anything in this regard.

It remains uncertain whether fertilization in *G. macrotyla* takes place inside the ovary, as observed in two species of the *Eledoninae* (Perez et al. 1990), or whether the oocytes pass along the oviducts, as in *Octopodinae* and *Bathypolypodinae* (Mangold 1989. Nonetheless, the absence of spermathecae in the oviducal glands and the presence of fertilized eggs inside the ovary of *G. macrotyla* suggest that fertilization takes place within the ovary. On the other hand, considering the relationship between sperm morphology and the mode of fertilization (Franzén 1955; Healy 1988), the type of spermatozoid found in the Graneledoninae (Roura et al. 2009) suggests a mode of fertilization in this subfamily which is more similar to that in the Octopodinae (Longo and Anderson, 1970) and the Bathypolypodinae (Roura et al. 2010) than to that displayed by the Eledoninae (Selmi 1996).

Oocytes, reproductive strategy, and fecundity

All oocytes (108) within the ovary of the mature female MDMG68B2011 were in an advanced stage (S3). There were no oocytes left in earlier stages of maturation (Table 1), and no POFs were found in the histological sections examined. Such a scenario has never been observed in any deep-sea octopus before. Two alternative interpretations are possible: (1) Synchronous maturation of oocytes which are spawned in one or several batches. This assumption is consistent with the oocyte size frequency in the female MDMG472011, but it is inconsistent with the scenario shown by the maturing females MDMG552011 and MDMG68C2011. (2) Group-synchronous production of oocytes, many of which are reabsorbed (as observed) while the others undergo maturation and are eventually spawned in a single or several batches. Atresia of very early oocytes is called "regulative atresia" and seems to be relatively frequent in other species (Laptikhovsky et al. 2007). This second interpretation neither contradicts the scenario shown by the mature females MDMG472011 and MDMG68A2011 nor that of the maturing females MDMG552011 and MDMG68C2011, and this could also be the case with *G. pacifica*, since Bello's (1990) conclusions were based on subadult specimens.

Consequently, *G. macrotyla* seems to have a group-synchronous pattern of oocyte production, regulative atresia, and maturation of remanent oocytes, which are spawned in a single or several (few) batches.

The presence of oocytes at different stages of maturation and, particularly, the occurrence of a distinct group of oocytes in an advanced maturation stage were considered to be evidence of multiple spawning in *G. pacifica* and in *G. macrotyla* (Bello 2006; Guerra et al. 2012). However, the term "multiple spawning" should only be applied to species with group-synchronous oocyte production, monocyclic spawning pattern, and somatic growth between separate egg batches, such as the lesser Pacific strip octopus *Octopus chierchiae* from low inter-tidal tropical areas (Rodaniche 1984; Rocha et al. 2001). Although this reproductive strategy would be the most suitable for deep cold-water octopods, the existence of somatic growth between spawning events in *Graneledone* species is controversial. *Graneledone* females have to brood an egg mass and protect it against predators, as recorded by submarines (Voight and Grehan 2000) and ROVs (Drazen et al. 2003). In this situation, feeding would be fairly casual. It is hard to believe that after a few months of brooding under a condition of half starvation, a female would be able to lay a new egg mass in a different place, and even to grow between these spawning events (Laptikhovsky pers. comm.). Thus, *G. macrotyla* should not be considered a multiple spawner. On the other hand, since growth does not take place between egg batches, *G. macrotyla* should neither be considered a continuous spawner.

The reproductive traits and the characteristics of the developing oocytes found in the five *G. macrotyla* females (Tables 1 and 2) suggest that the species' maximum observed potential fecundity (PF) or total number of all oocytes or initial oocyte reserve (Laptikhovsky 2001) ranged between 250 and 300 eggs, similar to that of *G. pacifica* (Bello 2006). Two octopod species from the Bering Sea caught at depths of 200–600 m, *Bathypolypus salebrosus* and *Benthoctopus* sp. aff. *sibiricus,* showed maximum PF of 204 and 195, respectively (Laptikhovsky 1999). PF in *Benthoctopus eureka*, an octopod of cold waters from 80 to 2500 m depth around the Falkland (Malvinas) islands, ranged from 250 to 535 (Laptikhovsky 2001), which are scenarios comparable to that of *G. macrotyla*.

Atresia

Atretic oocytes were found along with those in stages S1 and S4 (Table 2). Atresia has been observed in many marine organisms, including cephalopods (e.g., Guraya 1986; Melo and Sauer 1998; Boyle and Chevis 1991; Laptikhovsky 1999, 2001; Laptikhovsky and Arkipkin 2001). In the case of *G. pacifica*, a set of oocytes develop to maturity and are spawned, whereas the remaining oocytes fail to grow beyond about 3 mm length and degenerate (Bello 2006). The percentage of atretic oocytes found in

the squid *Doryteuthis gahi* inhabiting the shelf and continental slope was about 1 % of the total number of oocytes (Laptikhovsky and Arkipkin 2001). However, atresia can affect a much higher percentage of initial oocytes; for instance, in the Antarctic Eledoninae, *Pareledone charcoti*, *P. polymorpha* and *P. turqueti* 42, 29, and 54 %, respectively, of the initial number of oocytes were resorbed (Kuehl 1988). Moreover, residual degenerated oocytes amounted to 60–80 % in *Bathypolypus salebrosus,* and 50–60 % in *Benthoctopus* sp. aff. *sibiricus* from Artic waters (Laptikhovsky 1999). Thus, atresia percentages found in *G. macrotyla* coincide with those observed in other cold-water octopods.

Several factors have been advanced as possible reasons for oocyte atresia, mainly in fish, as summarized by Melo and Sauer (1998). These include overcrowding, environmental conditions such as temperature and day length, food restriction, and body size. The possible explanation for atresia in *L. vulgaris reynaudii* was that atresia plays a role in the removal of surplus oocytes, given that oogenesis is energetically costly, particularly in short-living species (Melo and Sauer 1998). The scanty number of female *G. macrotyla* examined, as well as the lack of knowledge on many aspects of the species' life cycle, does not allow us to formulate any well-founded hypothesis. However, given the stable oceanographic conditions of the bottom deep waters along most of the Argentine continental slope, the most plausible explanation for atresia in *G. macrotyla* might be food restriction. Atlantic cod females initiate gonad development up to seven months prior to spawning (Burton et al. 1997). During that period, impaired or non-improving feeding conditions may render reproduction less attractive; accordingly, energy temporarily invested in gonads can later be reabsorbed through atresia (Kjesbu et al. 1991).

Although no POFs were observed in female *G. macrotyla*, the existence of POFs in the ovaries examined cannot be excluded. This is because of the inadequate process of conservation of the specimens, the difficulty of obtaining good histological sections from a material unsuitably preserved, and finally, because POFs would be attached forming a bunch inside the ovary together with the other types of oocytes, making their identification difficult.

Degeneration of oocytes within the ovaries could be an artifact produced by cell damage due to preservation, since the animals were frozen during 12 and 5 months on the ATLANTIS 2009 and 2010 cruises, respectively. It has been observed that conservation hampers the identification of yolk granules with visible or fluorescent light (Saborido-Rey 2012). Furthermore, the high salt content of marine invertebrate cells leads to extensive cellular damage during freezing (Dixon et al. 2002), and freezing induced artificial cleavage of apoptosis-related proteins in human bone marrow (Schmidt-Mende et al. 2000). Nevertheless, if the preservation was the reason, why do specimens caught at the same time, in the same place and preserved in the same conditions (MDMG 68A2011 and MDMG68B2011) show such different oocyte stages (S3 and S4, see Table 2) within their ovaries? Although similar levels of atresia were observed in ovaries of *Muusoctopus* sp that were fixed in formalin straight after capture (Laptikhovsky pers. comm.), further analyses using fresh material are needed to elucidate whether the scenario shown in some *G. macrotyla* females is a matter of apoptosis or atresia.

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