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RESEARCH PAPER

Residual Eggs in Post-Mortem *Sepia officinalis* (Mollusca: Cephalopoda)

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Abstract

Pre-spawning cuttlefish were collected between March-June 2008 and April 2012 from spawning grounds in Izmir Bay (Aegean Sea). A total of ten females (ML 99-148 mm) were captured alive and were kept in laboratory tanks where they spawned later. After spawning and consequent death the females were dissected. The residual eggs were counted and measured. Their numbers ranged between 1022 and 3036 with asynchronous size distribution. Histological sections demonstrated incipient atresia in most of oocytes that means that in spite of high residual fecundity these females hardly could reproduce further.

Keywords: *Sepia officinalis*, Cephalopoda, residual eggs, histology.

Introduction

Traditionally the term and concept of fecundity provides the most important criterion to describe the reproductive potential of a species (Voss 1983). Nearly all coleoid cephalopods spawn only once in their lifetime releasing one or many egg masses (Rocha *et al.* 2001), and because of this "dying after spawning" cliché is used (Nesis 1995) though in many species (like pelagic octopod *Argonauta*) spawning period might occupy most of the life. Fecundity and spawning patterns are keystones of cephalopod reproduction and were studied across different taxa. There are numerous data on number of oocyte in pre-spawning females (Arkhipkin 1996, Boletzky 1987; Forsythe *et al.* 1994, Laptikhovsky & Arkhipkin 2001, Laptikhovsky & Nigmatullin 2005, Laptikhovsky *et al.* 2003, Mangold-Wirz 1963, Melo & Sauer 1998, Nigmatullin & Markaida 2009) but very little is known about how many of them remain after spawning, so how efficiently the potential fecundity is used. The Common cuttlefish, *Sepia officinalis* (Linnaeus, 1758), was dubbed as a "white mouse" among cephalopods since it has been used for many laboratory studies (Boletzky 1983). It is known that *S. officinalis* survives well under captivity and may spawn over numerous laboratory generations (Forsythe *et al*. 1994). Laptikhovsky *et al.* (2003) reported *S. officinalis* to have a high potential fecundity in nature, with egg production between 6000 and 7000 per individual female.

Numbers of residual egg in a cuttlefish gonad have been reported by Boletzky (1987) as a single observation, and there is no study of the corresponding histology of the ovaries. The aim of this study was to investigate a maximum available number of deceased *S. officinalis* including histological studies, thus obtaining more detailed information on the reproductive biology and spawning efficiency in this species.

Materials and Methods

The female cuttlefish were collected in two different periods, between March-June 2008 and April 2012 using fishing poles and gillnets. The animals were first maintained in sea water tanks measuring 2×2×0.5 m (Şen 2009, 2013) containing 2000 l of circulating sea water (flow rate 400 l/h). Thereafter they were kept in smaller cylindrical polyester tanks measuring 90 x 70 cm, holding each 430 l (90x70 cm), sea water circulating at a rate of 100 l per hour. Spawning cuttlefish were fed *ad libitum* with anchovy (*Engraulis encrasicolus*), bogue (*Boops boops*), and Tompot blenny (*Parablennius gattorugine*), Black goby (*Gobius niger*), crab (*Goneplax rhomboides*) and with shrimp (*Palaemon* sp.), all either alive or just died. The spawning females eagerly consumed

this food and stop feeding only the day before the death. Since all the individuals were spawning in the same tank, it was impossible to allocate an egg mass to an individual cuttlefish. In 2008's five female individuals (one individual' head was eaten by other individuals after death) a total of 1567 egg were recorded. In 2012 the eggs were not counted. Spent individuals were collected as soon as it was found dead during daily inspection, than were preserved whole in 10% formalin solution right after collection. Five individuals collected in 2008 were all dissected; out of five dead females of the year 2012 where one was preserved and the other four were dissected (Table 1). Dorsal mantle length (DML, mm) was measured from all of them within 1 mm, and body weight (BW) – within 1 g.

Gonads of dissected animals were removed and all gonads were weighted to the nearest 0.01 g and oocytes were counted and measured under a microscope to the nearest 0.01 mm. Stages of oogenesis were assigned according to Laptikhovsky and Arkhipkin (2001), and atretic eggs were staged according to Melo and Sauer (1998). Two ovaries were studied histologically. The ovary sections were cut at a thickness of 6 µm, staining of histological slides was done using the Mallory Method; and photographed using an Olympus CX-41 microscope with DP-20 digital camera attachment.

Results

Spent females: Ten females that were analyzed in this study had DML ranging from 99 to 148 mm and BW of 80-380 g. Gonad weights from 9 dissected animals ranged between 2.2-11.8 g, (% BW). No correlation was found between gonad weight and total body weight (r = 0.14; P=0.704).

In 50 day period where five captive individuals from 2008, a total of 1567 eggs were counted (average 313 per sample). Individuals spawning period, total spawned egg amount, and time of death are given in Table 2.

Ovarian Oocytes

The total oocyte count in spent females resulted in 1,022-3,036 oocytes (Figure 1). These oocytes ranged between 0.1 mm to 7.5 mm. Small pre-vitellogenic oocytes of ca 1 mm strictly predominated representing around 70% of the total gonad (Figure 1). No correlation was found between BW and residual oocyte counts (r = 0.10; P= 0.759), and between DML and residual oocyte counts (r = 0.18; P=0.184).

Histological observations; two types of oocytes were observed in studied gonad samples: normal and atretic, both either pre-vitellogenic or vitellogenic. No empty follicular sheath was observed on histological sections.

1-Pre-vitellogenic normal oocytes; nucleus is clearly seen; cytoplasm is homogeneous without yolk droplets. Follicle cells are cubic or cylindrical beginning to form epithelial folders into the oocyte (Figure 2A-B).

2- Vitellogenic normal oocytes; follicular folds are conspicuous, deeply penetrate inside the oocyte;

Table 1. Features of the analyzed female *S .officinalis* (ML: Manle length; TW Total weight; GW: Gonad weight; N= Resudial egg number)

Date	ML(mm)	TW(g)	GW(g)	N	Remarks
April 2008	99	-	2,8	1022	No head
	108	80	2,2	2236	
	120	204	9,2	2246	Histology
	148	380	2,6	2336	
	121	215	6,9	3036	
April 2012	110	153	5,8	2631	
	112	163	6,8	2143	
	142	369	2,4	-	Histology
	122	222	10,6	2424	
	135	262	11,8	2580	Histology

Table 2. Spawning period, total spawned egg amount, and time of death of March-June 2008 samples (+ : Time of Death)

	Days after Capture											
♀	1	5	10	15	20	25	30	35	40	45	50	
1	----------+											
2	----------+											
3	----------+											
4	----------+											
5	----------+											
∑	Spawned eggs amount		322		483		432		209		121	1567

yolk droplets are already present in oocyte cytoplasm as blue spots (Figure 3A-C) (Figure 2A).

3-Pre-vitellogenic atretic oocytes; follicular cell are mis-shaped but still forming a complete follicular envelope. Follicular cells often have pyknotic nuclei (Figure 2A). No pyknotic nucleus was observed in the eggs themselves.

4-Vitellogenic atretic oocytes; Follicular epithelium sheath disintegrates; follicle cell nuclei are pyknotic (Figure 3D-F). Oocyte cytoplasm becomes vacuolised, and egg nuclei become pyknotic also indicating that phagocytosis began. (Figure 4).

Discussion

Size range of residual oocyte (0.1-7.5 mm) corresponds to that of viable oocytes in mature spawning individuals (Laptikhovsky *et al.* 2003) and fit definition of the intermittent terminal spawning as assigned by Boletzky (1987). Even though the size of the animal gets larger there is no correlation with the amount of leftover residual oocytes.

Cuttlefish can spawn at least 4-50 eggs in a batch (Boletzky 1987). Although it is reported that in the laboratory *S. officinalis* total fecundity can be up to 3000 eggs (Boletzky 1975, 1987; Domingues *et al.* 2001, 2002; Forsythe *et al.* 1994; Hanley *et al.* 1998; Şen, 2009; Villa 1998) but in the wild the fecundity might be different from that of the captive individuals.

Melo and Sauer (1998) reported for *Loligo vulgaris reynaudii* that more than 80% of the residual oocytes are atretic in spent females, which is in agreement with our observations.

According to Young *et al.* (2000) when epithelial cells enter to apoptosis stage, their nuclei transforms into pyknotic nuclei and cell membranes disintegrate.

The residual fecundity observed in this study

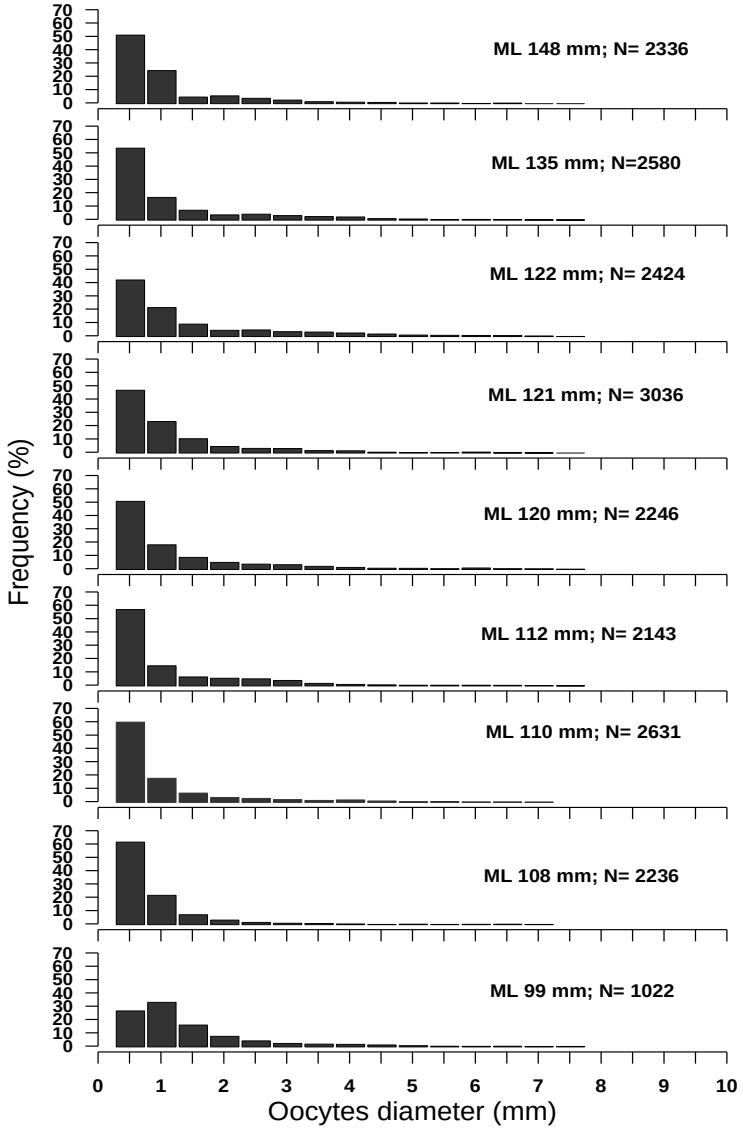

Figure 1. All the *Sepia officinalis* gonad' residual oocytes diameter distribution**.**

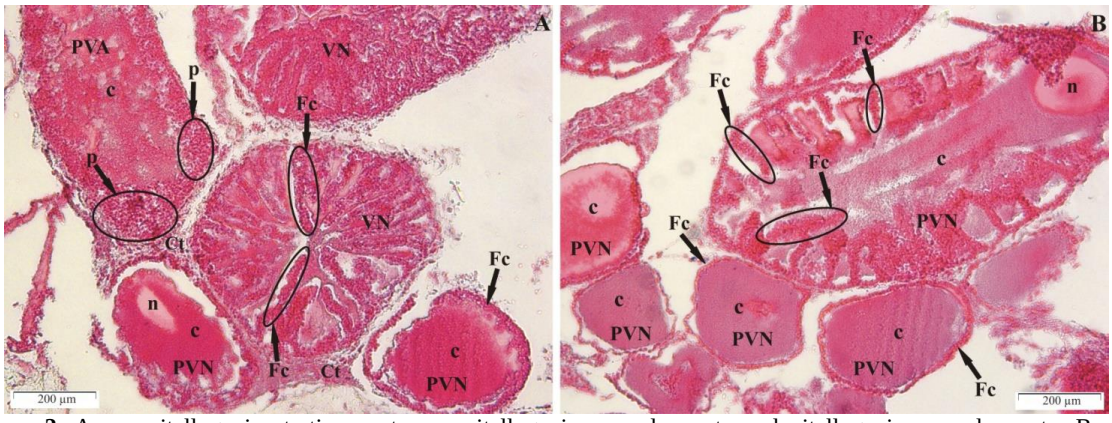

Figure 2. A; pre-vitellogenic atretic oocyte, pre-vitellogenic normal oocyte and vitellogenic normal oocyte. B: pre-vitellogenic normal oocyte (c; cytoplasm Fc; follicle cells, n; nucleus, P; pyknotic nuclei, PVA; pre-vitellogenic atretic oocyte, PVN; pre-vitellogenic oocyte, VN; normal vitellogenic oocyte,)

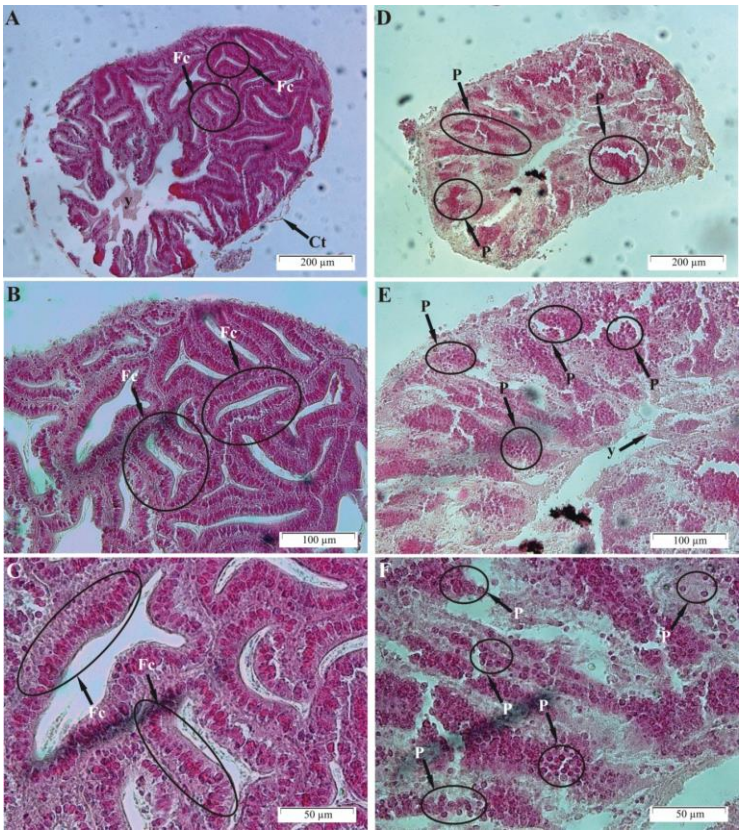

Figure 3. Comparison normal and atretic oocytes in the residual gonad of *S. officinalis*. A-C vitellogenic normal oocyte. D-F vitellogenic atretic oocyte. (Fc; follicle cells, Ct; connective tissue, P; pyknotic nuclei, Y; yolk,).

was found to be around 50 % possible potential fecundity (a total of 1567 eggs were spawn and total of 10876 residual oocytes in the ovaries were counted). In this case, it is claimed to be that the real fecundity is way lower than potential fecundity. This indicates that, estimation of potential fecundity might be only a very crude estimation of reproductive output, as well as there is a room for individual variation in efficiency of the use of this oocyte stock. It is generally in agreement with estimation of post-spawning ovary degeneration and residual fecundity in other cephalopod species like *S. officinalis* (Boletzky 1987), *Illex agrentinus* (Laptikhovsky & Nigmatullin, 1993), and upper bathyal Octopods such as *Graneledone*, *Adelieledone* and *Muusoctopus* (Laptikhovsky, 2013).

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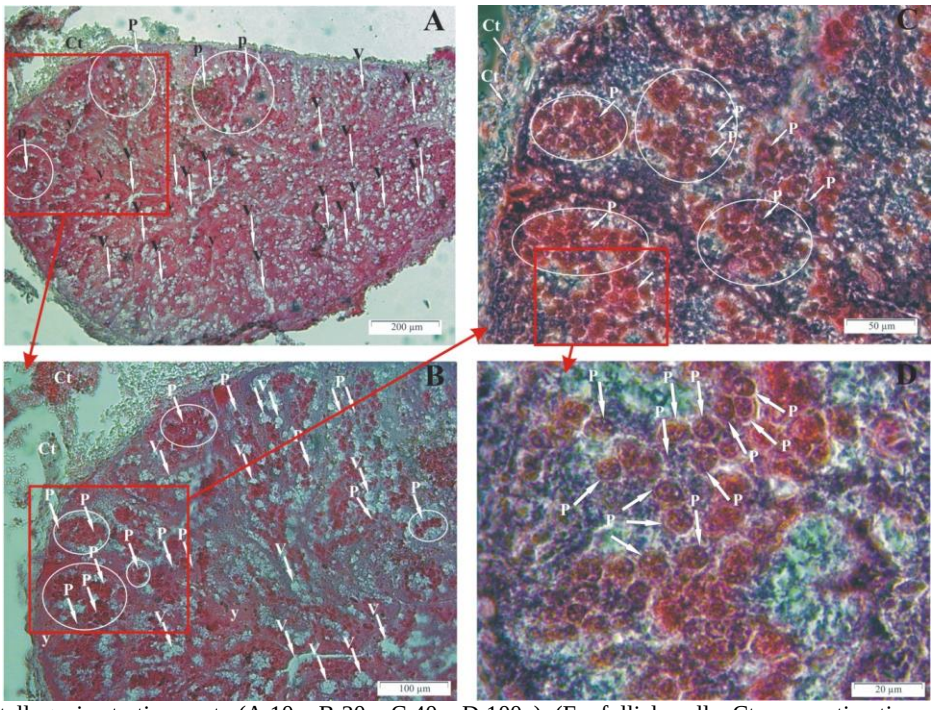

Figure 4. Vitellogenic atretic oocyte (A;10x, B;20x, C;40x, D;100x). (Fc; follicle cells, Ct; connective tissue, P; pyknotic nucleus, V; vacuol, Y; yolk,).

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