www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 10: 81-86 (2010) DOI: 10.4194/trjfas.2010.0112

Reproductive Biology of the Bobtail Squid *Rossia macrosoma* (Cephalopoda: Sepiolidea) from the Eastern Mediterranean

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Received 30 December 2008
Accepted 20 October 2009

Abstract

Rossia macrosoma was investigated based on material collected from the eastern Mediterranean Sea, to determine potential fecundity (PF), reproductive strategies and size at maturity. The female potential fecundity ranged between 126 and 931, while the value was 15 to 103 for male spermatophores. Egg sizes varied from 0.2 to 10.0 mm, and spermatophore lengths ranged from 12.3 to 18.8 mm. Animals became mature at smaller sizes than those from the western Mediterranean, thus, their maximum sizes were smaller either. According to egg diameter distributions in gonads, *R. macrosoma* has asynchronous ovary maturation, spawning more than once. Therefore, *R. macrosoma* is an intermittent spawner similar to other sepiolid species.

Keywords: *Rossia macrosoma*, fecundity, reproductive biology, Aegean Sea.

Doğu Akdeniz'de *Rossia macrosoma*'nın (Cephalopoda: Sepiolidea) Üreme Biyolojisi

Özet

Bu çalışmada doğu Akdeniz'de *Rossia macrosoma*'nın yumurtlama stratejisi, yumurtlama verimliliği (PF) ve ilk olgunlaşma boyları incelenmiştir. İncelenen dişi bireylerin yumurta verimliliği 126-931 arasında iken erkek bireylerde spermatofor verimliliği ise 15-103 arasında tespit edilmiştir. Dişi bireylerdeki yumurta çapları 0,2-10,0 mm arasında, erkek bireylerin spermatofor boyları ise 12,3-18,8 mm arasında ölçülmüştür. Çalışmamızda incelenen bireylerin batı Akdeniz'deki akrabalarına göre daha erken boyda olgunlaştıkları ve bununla birlikte maksimum boyunda batıdaki bireylere göre daha küçük olduğu tespit edilmiştir. *R. macrosoma*'nın gonadlarındaki yumurta çaplarının dağılımına bakarak asenkronik ovaryum olgunlaşması olduğu tespit edilmiştir. Bu da *R. macrosoma*'nın diğer sepiolidler gibi aralıklı yumurtlayan bir tür olduğunu göstermektedir.

Anahtar Kelimeler: *Rossia macrosoma*, yumurta verimliliği, üreme biyolojisi, Ege Denizi.

Introduction

Sepiolid cuttlefish, commonly known as bobtail squids, are benthic neritic cephalopod molluscs distributed in tropical, temperate and polar waters of all oceans. The family includes three subfamilies, i.e., Heteroteuthinae, Rossiinae and Sepiolinae, the latter two exhibiting benthic lifestyle, contrary to the oceanic and pelagic Heteroteuthinae. There are two different reproductive strategies – benthic and pelagic, in the family Sepiolidae like the other cephalopods. Pelagic cephalopod species have high potential fecundities and smaller egg diameters, while low fecundities and larger egg diameters are observed in benthic forms (Mangold, 1987). This fact is also similar for all sepiolids (Önsoy, 2007). The small, benthic reproductive strategist subfamily Sepiolinae members have 2-5 mm ripe egg diameters, whereas larger Rossiinae members have 4-9 mm. One of the Rossiinae members having larger ripe eggs is *Rossia macrosoma* (Delle Chiaje, 1829).

Species of Rossiinae generally prefer cold waters, occurring in polar waters and deep seas between 200-2000 m, usually deeper than 500 m (Jereb and Roper, 2005). The deepest recorded member of Rossiinae was collected from 1744 m depth in the Western Mediterranean (Villanueva, 1992). Members of family Sepiolidae are intermittent spawners (Rocha *et al*., 2001). They produce

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in cooperation with Japan International Cooperation Agency (JICA), Japan

relatively large eggs. *R. macrosoma* (Delle Chiaje, 1829) is much larger than other sepiolids of the Mediterranean, and distributed over sandy and muddy bottoms at depths ranging from 32 to 899 m in the Northeastern Atlantic and the Mediterranean (Jereb and Roper, 2005). They deposit their eggs as small clusters on bivalve shells and other solid substrates (Mangold-Wirz, 1963; Boletzky, 1983; Cuccu *et al.*, 2007). Volpi *et al.* (1995) stated that this species was one of the most abundant sepiolids in the bathyal muddy biocenosis of northern Tuscany. Thus, it is important in the diet of humans and some chondrichthyans (Roper *et al.*, 1984; Bello, 1997).

Although there were some investigations about other sepiolids' reproductive biology, biochemical features and systematic positions (D'Onghia *et al.*, 1994; Gabel-Dickert, 1995; Volpi *et al.*, 1995; Boucher-Rodini *et al.*, 1995; Boletzky, 1995; Lefkaditou and Kaspiris, 1998; Salman, 1998; Bello and Dickert, 2003; Salman and Önsoy, 2004), there was a limited number of studies to clarify ovulation type and spawning strategies of *R. macrosoma* (Laptikhovsky *et al.*, 2008).

Materials and Methods

A total of 189 specimens (91 females, 98 males) were investigated from different localities of the eastern Mediterranean (37 specimens) and the Aegean Sea (152 specimens). Samples were collected between 1991 and 1993 using a bottom trawl (20 mm mesh size, knot-to-knot) on board the R/V K. Piri Reis, in depths ranging from 100 to 500 m (Figure 1).

Body weight (to the nearest 0.01 g) and dorsal mantle length (ML, to the nearest 1 mm) measurements were taken from the samples, which were fixed with 4% formalin. Maturity stages were determined according to Önsoy *et al.* (2008) by using a three-stage scale (immature, maturing and mature).

In dissected females, development of eggs in the ovary and oviduct was considered to determine maturity stage (Figure 2).

Figure 1. Sampling area (indicated by full dots).

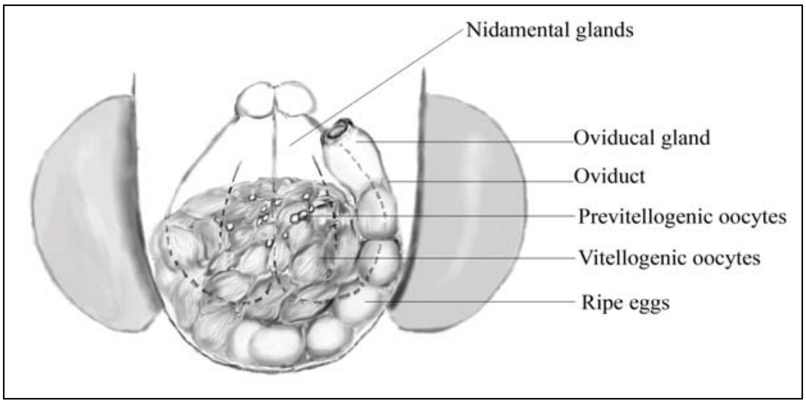

Figure 2. Schematic illustration of the reproductive system of *R. macrosoma* (Original).

All oocytes and the ripe eggs were counted and measured separately along the major axis to the nearest 0.1 mm. Then, the potential fecundities (PF: total oocyte stock in the ovary and oviduct) were determined. The relative fecundities (RF) were calculated by dividing the potential fecundity body weight (BW), (RF=PF/BW). Gonads were weighed to the nearest 0.0001 g. In males, Needham's sac was weighed and spermatophores counted and a sampled.

The ovulation pattern of *R. macrosoma* was identified by considering the size variations of oocytes in each ovary. To determine a reproductive cycle, gonadosomatic index (GSI= (GWx100)/BW) values were calculated. Both linear and nonlinear regression analyses (Snedecor and Cochran, 1989) were used to investigate existence of any functional relationship between the female mantle length and egg diameter.

Results

Male Reproductive system

Mantle lengths of males were between 16 and 47 mm (mean: 32.8 mm) and of these mature males were between 26 mm and 47 mm (mean: 37.1 mm). Males started to mature at the 25 mm size class, and 18% of the males were matured at the 30 mm size class (Table 1).

There were 15 to 103 (mean: 46) spermatophores in Needham's sacs in males between 26 and 47 mm ML. Spermatophore lengths varied from 12.3 to 18.8 mm (mean: 16.2 mm) and increasing with male size. The relationship between mantle length and average spermatophore length was linear (Figure 3). The relative fecundities ranged from 1.19 to 65.93 number/g (mean: 22.96 number/g).

Table 1. Size classes and percentages of maturation of *R. macrosoma* specimens

Size Class (mm)	Female (♀)				Male (♂)			
	N	Immature	Maturing	Mature	N	Immature	Maturing	Mature
10-14	6	100						
15-19	8	100			7	100		
20-24	7	100			13	46	54	
25-29	11	100			11	55	27	18
30-34	10	60	40		19		5	95
35-39	16	44	31	25	25			100
40-44	15		7	93	21			100
45-49	5			100	2			100
50-54	2			100				
55-59	9			100				
60-64	2			100				
Total	91				98			

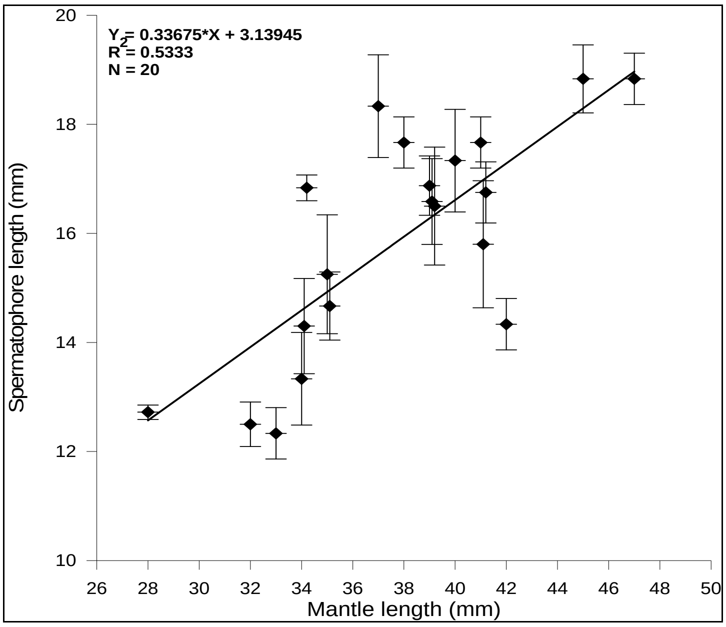

Figure 3. Relationship between mantle length and average spermatophore length in *R. macrosoma*. Bars denote ± sample standard deviations separately estimated for each male.

Female Reproductive System

Mature females first appeared in the population at the 35 mm size class and 50% of females were mature at the 40 mm size class (Table 1).

Seasonal average GSI values determined for females were between 2.4% (Spring) and 9.2% (Summer). For males, they were between 2.2% (Winter) and 4.2% (Autumn). Although mature females and males were found all around the year, the maximum GSI values were observed in summer and autumn (Figure 4).

Female potential fecundities were between 126 and 931 oocytes. There were some empty follicular sheaths (post ovulatory follicles) (N= 15–200) in 6 mature female ovaries. Maximum potential fecundities (MPF = PF + Empty Follicles) in those females ranged from 202 to 1035. No significant relationship, either linear or nonlinear form, was determined between the calculated potential fecundity and female mantle length. The number of eggs did not increase with advancing mantle lengths. In immature females, the relative fecundities varied from 21.05 to 65.93 oocyte/g (mean: 43.95 oocyte/g), whereas in maturing females, it was between 6.25 to 15.11 oocyte/g (mean: 9.21 oocyte/g). The relative fecundities in mature females ranged from 1.19 to 22.14 oocyte/g (mean: 10.42 oocyte/g).

The egg sizes varied from 0.2 to 10.0 mm in diameter. There were at least 3 groups of eggs (protoplasmic oocyte, vitellogenic large oocyte, ripe oocyte) in mature females. Eggs (protoplasmic oocytes) with 0.2 – 3.0 mm diameter were predominant in all maturity stages. The modal length of these small oocytes slightly increased in mature animals. Percentages of egg length distribution in different gonad stages were given in Figure 5. The number of ripe eggs in the oviduct varied between 6 and 26, and their diameter were between 5.0 and 10.0 mm. usually, larger mature females had larger eggs in their oviducts (Figure 5).

Females were between 11 and 62 mm (mean: 35.1 mm ML). Mature females ranged from 37 to 62 mm ML (mean: 46.5 mm ML), whereas other females at previous maturation stages varied from 11 to 59 mm ML (mean: 29.7 mm ML). Females were larger than males in investigated animals (Table 1).

Discussion

The first sexual maturation was found at 25–29 mm ML for males and 35–39 mm ML for females in the eastern Mediterranean, whereas *R. macrosoma* from the western Mediterranean achieves the mature stage at larger ML sizes (males: 35 mm, females: 50 mm ML; Mangold-Wirz, 1963). In addition, there are larger maximum mantle length values reported from the western Mediterranean (Males: 57 mm, females: 84 mm ML) probably depending on the temperature. Boyle *et al.* (1988) report a general marked decrease in size of cephalopods from the Western Mediterranean to the Eastern Mediterranean. This fact has also been observed in this study. The geographic size differences are also reported for other sepiolid species (Salman, 1998; Salman and Önsoy, 2004). The geographic variation in size may result from different environmental and biological factors such as heat, light and food (Salman, 1998).

In this investigation, *R. macrosoma* females from the eastern Mediterranean were 931 oocytes. Mangold-Wirz (1963) previously reported the potential fecundity of this species as 150 oocytes at maximum. It is probable that the differences were resulted from the exclusion of the perivitellogenic stage in the other study.

Spermatophore numbers of males were between 15 and 103. This is similar to the mean number of 85 spermatophores given by Mangold-Wirz (1963). It was observed that large sized mature specimens produce larger spermatophores, when compared to smaller specimens (Figure 3). Mangold-Wirz (1963)

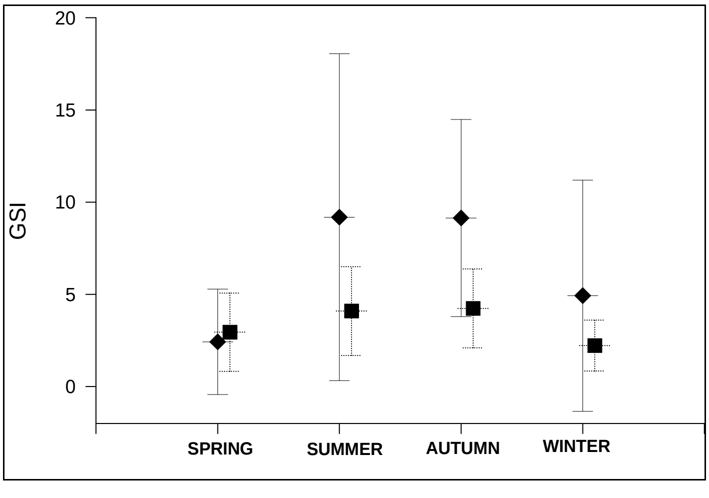

Figure 4. Seasonal average gonadosomatic index value of *R. macrosoma* in the Aegean Sea and Eastern Mediterranean (♦Female GSI; ■ Male GSI, bars denote ± sample standard deviations).

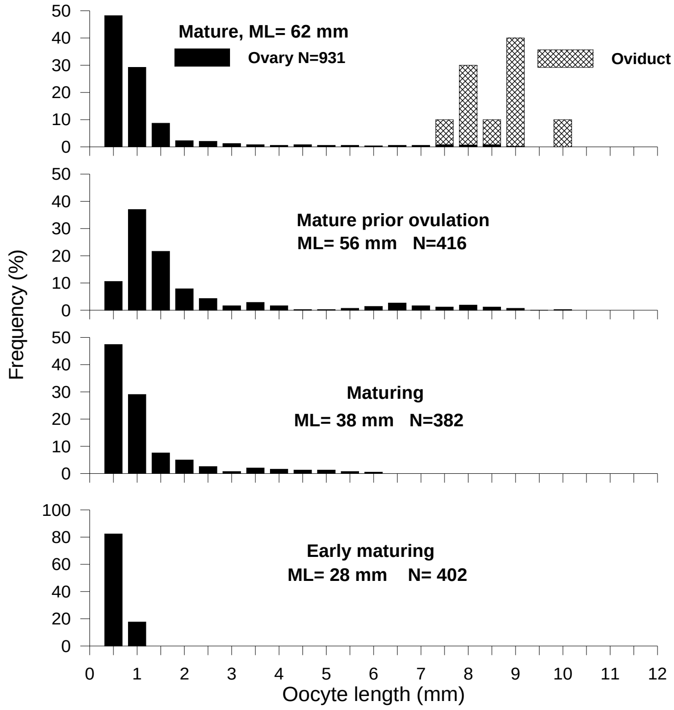

Figure 5. Oocyte length frequencies in *R. macrosoma.*

reported the spermatophore lengths between 23.0 and 26.5 mm and the spermatophore index between 46.5 and 63.8 based on results obtained from the western Mediterranean Sea. Spermatophore lengths were between 12.3 and 18.8 mm and the spermatophore index between 34.0 and 50.3 in this study. These results show that *R. macrosoma* from the eastern Mediterranean has shorter spermatophores than those from the western Mediterranean. Furthermore, this shows that the specimens in the eastern Mediterranean become mature at a small size than in the western Mediterranean. Similar observations are available for *Sepietta oweniana* (Salman, 1998) and *Eledone moschata* (Önsoy and Salman, 2004).

According to GSI values, gonad development peaked during the summer season. However, there were mature specimens throughout the year, indicating that *R. macrosoma* probably spawns anytime of the year. Similarly, Mangold-Wirz (1963) stated that this species had a long annual spawning period. In addition, it was reported that mature females of *R. macrosoma* were present all over the year in the Sicilian waters. However, the frequency of occurrence of mature females increased markedly in summer and fall (Jereb *et al.,* 1998).

There were different size groups of eggs in ovaries of *R. macrosoma*. The eggs were small in the previtellogenic stage. Large eggs (3.0-10.0 mm in diameter) were observed in the vitellogenic stage, and ripe eggs were released from follicular sheaths and were pushed to oviduct. Ripe eggs in the oviduct were 5.0 and 10.0 mm in diameter and they were smaller than large eggs in the ovary because of their lack of follicular sheath. Lefkaditou and Kaspris (1998) reported similar results. There were different size groups of oocytes in the ovary at the same time, so the ovulation pattern could be considered as group-synchronous ovulation. It was predicted that the species spawns more than once, because the percentages of the ripe eggs were fewer than that of other eggs in the ovary. This indicates that *R. macrosoma* has an asynchronous ovary maturation with very large eggs (~10 mm), fecundity with several oocytes and high reproductive output, continuous intermittent spawning with low batch fecundity. There were similar spawning strategies observed in other sepiolid species (Gabel-Deickert, 1995; Lefkaditou and Kaspiris, 1998; Rocha *et al.*, 2001; Bello and Deickert, 2003; Salman and Önsoy, 2004; Önsoy *et al.*, 2008; Laptikhovsky *et al.*, 2008).

Acknowledgements

We sincerely thank Dr.Vladimir Laptikhovsky for his valuable comments on preparation of this manuscript and also Dr.Murat Bilecenoğlu for improving the English text. We would furthermore like to acknowledge the contributions from the two anonymous referees and the assistance provided by the crew of R/V K. Piri Reis.

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