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Reproductive biology of *Neorossia caroli* (Cephalopoda: Sepiolidae) in the Aegean Sea

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SUMMARY: A total of 171 specimens of *Neorossia caroli* were collected by monthly sampling using a bottom trawl with 44-mm mesh size. The depth range of trawls varied between 150 and 550 m. The maximum size (dorsal mantle length, ML) was 41 mm for males and 50 mm for females. Seasonal gonadal stages of both sexes were examined using a gonadosomatic index. It has been observed that the pattern of ovulation of *N. caroli* is asynchronous and that spawning is continuous. The potential fecundity of females ranged between 317 and 685 oocytes (mean 548). As for males, the number of spermatophores per individual varied between 50 and 71 (mean 61). The smallest female with mature oocytes in the oviduct was 36 mm ML, while the smallest mature male with spermatophores was 20 mm ML. A peak in maturity was observed between July and September. Females appeared to reach larger sizes than males.

Keywords: *Neorossia caroli*, fecundity, reproductive biology, Aegean Sea.

RESUMEN: BIOLOGÍA REPRODUCTIVA DE *NEOROSSIA CAROLI* (CEPHALOPODA: SEPIOLIDAE) EN EL MAR EGEO. – Se realizó un muestreo mensual con red de arrastre de fondo, cuyo tamaño de mallas en el copo fue de 44 mm, en el mar Egeo en el cual se recolectaron 171 ejemplares de *Neorossia caroli*. El rango de profundidad de los arrastres varió entre 150 y 550 m. El tamaño máximo (longitud dorsal del manto, LM) fue de 41 mm para los machos y de 50 mm para las hembras. Se examinó la variación estacional de los estadios gonadales de ambos sexos mediante un índice gonadosomático. Se ha observado que el patrón de ovulación de *N. caroli* es asincrónico y que su freza es continua. La fecundidad potencial de las hembras varió entre 317 y 685 ovocitos (media 548), variando entre 50 y 71 espermatóforos (61 en promedio) en el caso de los machos. La hembra más pequeña con ovocitos maduros en el ovario midió 36 mm de LM, mientras que el macho maduro más pequeño con espermatóforos fue de 20 mm de LM. Se observó la existencia de un pico de puesta entre julio y septiembre. La distribución de tallas de ambos sexos mostró que las hembras alcanzan mayores tamaños que los machos.

Palabras clave: *Neorossia caroli*, fecundidad, biología reproductiva, mar Egeo.

INTRODUCTION

The carol bobtail squid (*Neorossia caroli*, Joubin, 1902) is distributed from southwestern Iceland to southern Africa in the Atlantic and the Mediterranean. The species is considered to have one of the longest life spans and to be the most bathyal squid species (Reid and Jereb, 2005).

The vertical distribution of *N. caroli* in different geographical regions has been investigated by Mangold-Wirz (1963a), Villanueva (1992), Salman *et al*. (1997), Jereb *et al*. (1998), Collins *et al.* (2001), Lefkaditou *et al.* (2003) and Sifner *et al.* (2007). Although the most shallow distribution (81 m) was reported in the Sicily strait (Jereb *et al., 1*998) and the deepest (1744 m) in the western Mediterranean (Villanueva, 1992), the species is mostly abundant between 400 and 700 m throughout the Mediterranean. Mangold-Wirz (1963a) redescribed the species and discussed its systematic position, distribution and biology. However, very little is known regarding the reproductive biology of the species in the Mediterranean Sea (D'Onghia *et al*., 1993; Cuccu *et al.*, 2007).

The incubation period of cephalopods living in cold waters can last from 3 months to one year depending on the temperature (Boletzky, 1994). Cold-water ce-

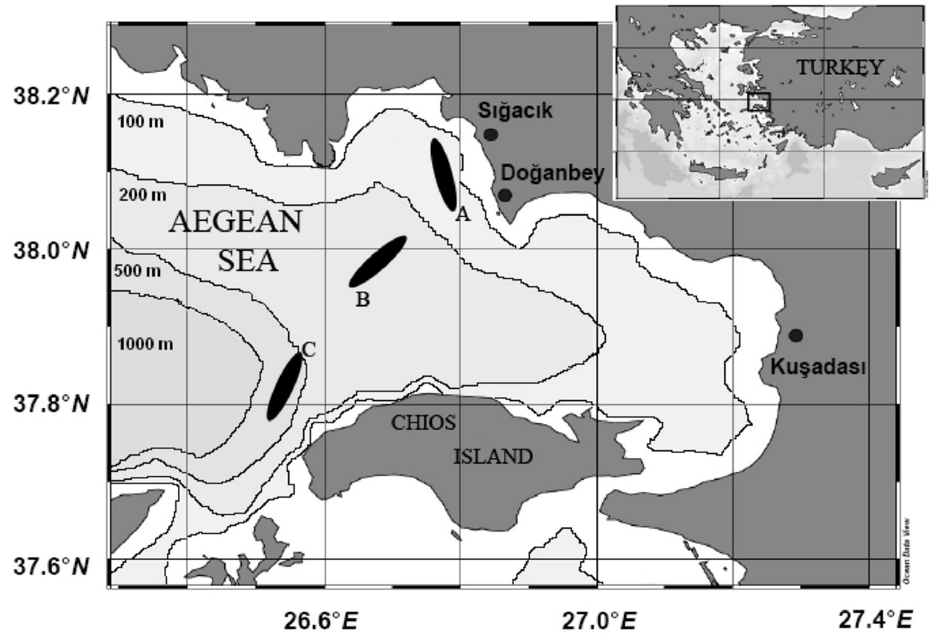

FIG. 1. – Sampling area (indicated by full trimmings; A150 m; B 350 m; C 550 m sampling area).

phalopods have evolved different reproductive strategies compared to their warm-water relatives (Rocha *et al.*, 2001).

Members of the Sepiolidae family are intermittent spawners (Rocha *et al.*, 2001) that produce relatively large eggs, generally >10% of mantle length (ML) (Laptikhovsky *et al.*, 2008). *N. caroli* is larger than other sepiolids of the Mediterranean. They deposit their eggs as small clusters on bivalve shells and other solid substrates (Mangold-Wirz, 1963b; Cuccu *et al.*, 2007). Members of the Sepiolidae family have short lifespans and generally tend to live for a year on average. However, *N. caroli*, which prefers to live in deep and cold waters, may live for up to 24 months (Reid and Jereb, 2005). Of minor commercial importance, it is taken usually as trawl fishery bycatch. Separate statistics are not reported for this species. It is sold fresh and frozen in fish markets with other bobtail squids (Reid and Jereb, 2005).

The aim of the present study was to provide information on the reproductive biology of *N. caroli* in the Aegean Sea.

MATERIALS AND METHODS

Samples were collected during daytime by commercial bottom trawl (44 mm mesh size in codend) on sandy and muddy bottoms within 150, 350 and 550 m depth in the Aegean Sea. Sampling was carried out monthly from May 2008 to April 2009 (Fig. 1).

Total catches of cephalopods were fixed in 4% formalin solution on board. Cephalopod species were separated into each taxon in the laboratory according to Reid and Jereb (2005). A total of 171 *N. caroli* individuals (81 males + 90 females) were collected. Of the preserved squid, dorsal mantle length (ML, mm) and total body weight (BW, 0.001g) were recorded. Sexual maturity stages were assigned as immature, maturing, mature, and spent according to Laptikhovsky *et al.* (2008) and Önsoy *et al.* (2008). Gonads and accessory glands were weighed to the nearest 0.0001 g. All oocytes from the ovary and the oviduct were separately counted and measured along the major axis to the nearest 0.1 mm. Gonadosomatic indices (GSI) of both sexes were calculated for each sampling period (GSI = [GW/BW] x 100) (Gabr *et al.*, 1998), and reproductive system indices (RSI), as a ratio between reproductive system mass (RSM) and body mass (BM) (RSI = [RSM/BM] x 100) Önsoy *et al.* (2008). Potential fecundity (PF) of mature females was computed as the sum of the oocytes in the ovary plus the eggs in the oviduct. Relative fecundity (RF) was calculated as a ratio of fecundity (F) to BW (RF = F/BW). Both linear and nonlinear regression analyses (Snedecor and Cochran, 1989) were carried out to investigate the existence of any functional relationship between the spermatophore length and male ML, between GW and BW, and between ML and BW.

RESULTS

Length-frequency relationship

The frequency distribution indicated that the 35-40 mm ML length class contained the highest number of individuals (Fig. 2). The ranges of observed MLs were 13-41 mm for males, and 16-50 mm for females. Although small-sized individuals were encountered in all seasons, they appeared to be more frequent in

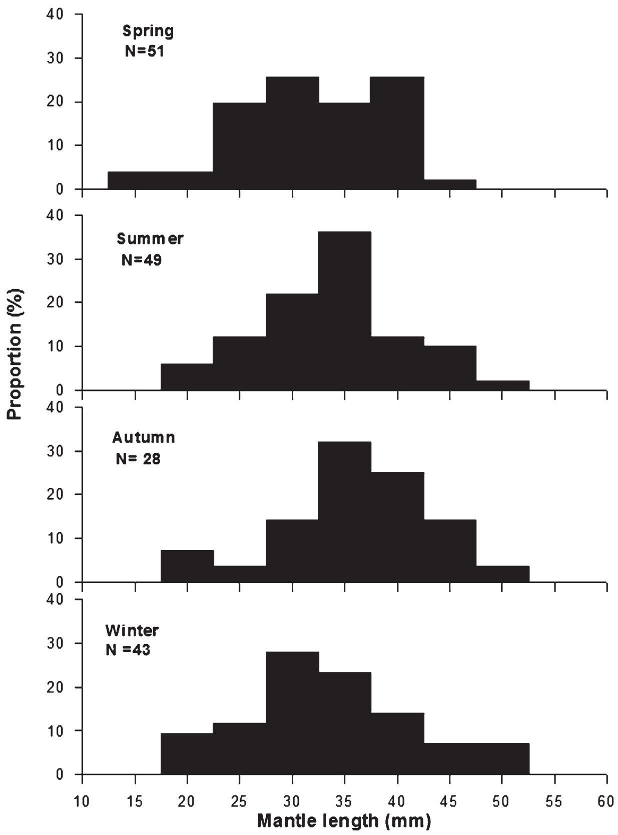

Fig. 2. – Seasonal distribution of *Neorossia caroli* in the Aegean Sea.

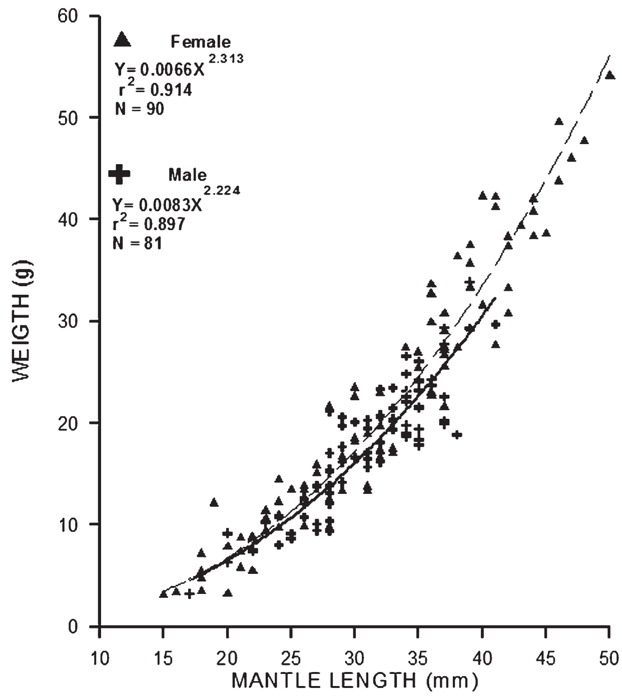

Fig. 3. – Comparison of ML and BW relationship of male and female *N. caroli*

spring (Fig. 2). The smallest individual was also found in spring. Mean MLs and their standard deviations were 30±7 mm, 31±7 mm, 34±7 mm and 32±8 mm for spring, summer, autumn and winter, respectively (Fig. 2). Although the relationship between ML and BW did not significantly vary according to gender, because the size ranges of male and females were different, a separate power curve was estimated for each sex (Fig. 3).

Maturity stage

Maturity stages were recorded for both sexes. Mantle lengths of females ranged from 15 to 50 mm. Sexual maturity in females were observed at 40-44 mm ML in 50% of the total maturing population. The smallest mature individual was measured in the 25-29 mm size class. The distributions of gonad stages according to size were: immature 15-25 mm, maturing 22-40 mm and mature females 29-50 mm ML (Table 1). In males, first sexual maturity was observed in the 30-34 mm size class in 50% of the mature population while the mantle length of males ranged from 20 to 41 mm. Sexual maturing of males ranged from 20 to 30 mm and mature males were observed between 20 and 41 mm (Table 1).

Due to the very small sample sizes, GSI values calculated for October and November may be disregarded (Table 2). The results of the remaining sampling period indicate that *N. caroli* females show one very pronounced reproductive peak annually. This peak occurs between July and September, with an average GSI value of 3.5%. The calculated GSI values decreased,

Table 1. – Percentage of *Neorossia caroli* males and females in each maturity stage for 5 mm size class.

Size Class (ML) mm	Male				Female			
	N	Immature (Stage 1)	Maturing Stage (2)	Mature (Stage 3)	N	Immature (Stage 1)	Maturing Stage (2)	Mature (Stage 3)
10-14					1	100		
15-19					2	100		
20-24	3		66.7	33.3	7	57.1	42.9	
25-29	9		100		13	30.8	61.5	7.7
30-34	24		25.0	75.0	16		93.8	6.3
35-39	34			100	13		84.6	15.4
40-44	10			100	21		23.8	76.2
45-49	1			100	12			100
50-54					5			100
Total	81				90			

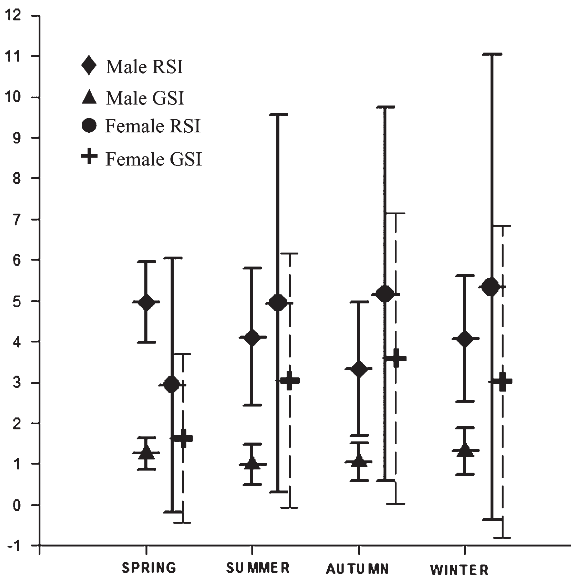

FIG. 4. – Seasonal variation of the Gonadosomatic index for male and female *N. caroli* from the Aegean Sea; bars ± standard deviations.

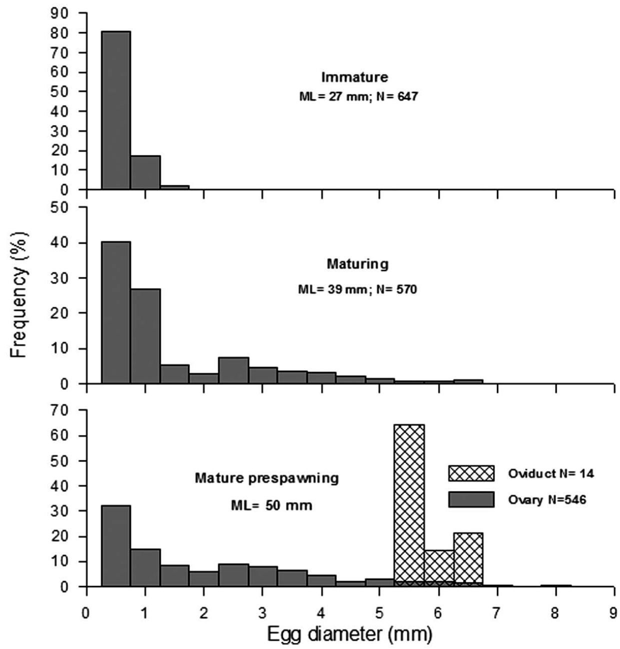

FIG. 5. – Size distribution of oocytes at different maturity stages of *N. caroli*

starting from winter and reaching the minimum value in spring (Fig. 4). On an individual basis, however, extreme GSI values of 10% were observed in autumn and winter. Males had homogeneous GSI values throughout the year, and the maximum value of 3% was noted in winter. The relative weight of the reproductive system (RSI) in mature males ranged from 2.9% to 6.9% (mean 4.8%). In mature females it ranged from 3.3% to 16.7% (mean 8.9%).

Gonad development, fecundity and fertility

Six mature males were randomly sampled to determine the size and reproductive output. The numbers of spermatophores observed ranged from 50 to 71 per individual, with a spermatophore size range of 9.5-18.4 mm. The average number was 61 spermatophores per individual, and the average spermatophore length was 14.3 mm. Average spermatophore length increased linearly with male mantle length.

Oocyte growth occurred asynchronously; small protoplasmic oocytes of 0.5-1.0 mm predominated during most of the ontogeny (Fig. 5). Egg size varied from 0.1 to 8.1 mm in diameter. There were at least 3 groups of eggs (small/protoplasmic oocyte, 0.1-3.0 mm; medium/vitellogenic large oocyte 3.0-5.5 mm, and large/ripe oocyte 5.5-8.0 mm). The modal frequency length of these small oocytes increased slightly in mature animals. The percentage egg size distributions at different gonad stages are given in Figure 5. There were mature male and female animals in every month throughout the year (Table 2). The potential fecundity in females changed between 317 and 685 eggs, with a mean of 548. The number of ripe eggs in the oviduct varied between 3 and 23, and their diameter was between 5.0 and 7.2 mm, which corresponds to 11-18% of ML. Usually, larger mature females had larger eggs

TABLE 2. – Percentage of distribution of male and female *Neorossia caroli* according to maturity stages in months.

Months	Males				Females			
	N	Immature (Stage 1)	Maturing Stage (2)	Mature (Stage 3)	N	Immature (Stage 1)	Maturing Stage (2)	Mature (Stage 3)
January	7			100	3		100	
February	9		11.11	88.89	6		33.33	66.67
March	2			100	5		80.00	20.00
April	5		20.00	80.00	9	22.22	44.44	33.33
May	11		9.09	90.91	18	11.11	55.56	33.33
June	14		14.29	85.71	5	80.00		20.00
July	11		36.36	63.64	11		54.55	45.45
August	2		50.00	50.00	7		42.86	57.14
September	9		44.44	55.56	13	7.69	46.15	46.15
October	1			100	1		100	
November	2			100	2			100
December	8		37.50	62.50	10	50.00	10.00	40.00
Total	81				90			

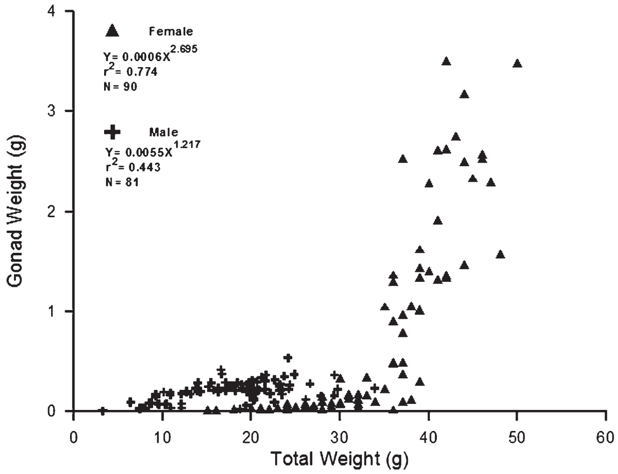

FIG. 6. – Relation between *N. caroli* male and female gonad weight and total body weight.

in their oviducts. The RF calculated for pre-spawning females was between 12.4 and 26.7 (mean 17.9) egg/g. The smallest individual having eggs in the oviduct had an ML of 36 mm. Power regression analysis between total female body weight and weight of gonads showed a determination coefficient value of 0.77. In males, gonad development with respect to body weight had a lower determination coefficient value of 0.44 (Fig. 6).

Mated females, carrying spermatophore reservoirs were observed in most months of the year, except for January, March and October (Table 3). These were all mature individuals containing mature eggs in the oviduct. The spermatophore reservoirs were located generally on the left ventro-lateral area of the body and rarely around the left eye. The number of spermatophore reservoirs in a mated female was between 1 and 30, with an average of about 10 (Table 3).

DISCUSSION

According to the results of this study, the maturation size for female *N. caroli* was 40-44 mm ML_{50}. Based on the gonad maturation phase (stage 3), the smallest female individual was 29 mm ML (Table 2). The smallest individual containing eggs in the oviduct was 36 mm ML. These findings were smaller than those recorded at the same development stages by Cuccu *et al*. (2007) for *N. caroli* in the western Mediterranean. A difference in the size of maturation due to the geographic differences was reported by Salman (1998) for a different sepiolid species, *Sepietta oweniana.*

When the geographic distribution of MLs of both sexes is considered, the maximum in the western Mediterranean is between 50 and 70 mm ML (Cuccu *et al*., 2007); in the Strait of Sicily in the central Mediterranean, the maximum size range is between 46 and 60 mm ML (Jereb *et al.,* 1998); and in the present study, the maximum size range was found to occur between 41 and 50 mm ML for males and females, respectively. Accordingly, results of this study agree with those of Boyle *et al.* (1988), who reported that there is a decrease in the size of the species as you move from the west to the east of the Mediterranean. Geographic size differences have also been observed for *S. oweniana* by Salman (1998). These changes can be attributed to the spatial differences in environmental variables such as temperature, light and food availability.

The size difference observed in female and male *N. caroli* specimens in the present paper is common for most Rossiinae species. Mangold (1987) recorded this fact and also that males mature earlier than females. On the other hand, Rossiinae females grow larger than males. A similar situation was reported for *Rossia macrosoma* by Salman and Önsoy (2010).

TABLE 3. – Location and number of spermatophore reservoirs in copulated *Neorossia caroli* females caught in different months

Months	Mantle length	Spermatophore Number	Spermatophores localities
January		No Spermatophore found	
February	30	6	Around the left eye
	39	10	Left ventro-lateral mantle
	46	8	Left ventro-lateral mantle
	47	24	Left ventro-lateral mantle, around the left eye
	48	12	Left ventro-lateral mantle, around the left eye
March		No Spermatophore found	
April	36	8	Ventro-lateral left eye
	40	10	Left ventro-lateral mantle
May	34	7	Left ventro-lateral mantle
	38	4	Ventro-lateral left eye
	44	15	Left ventro-lateral mantle
June	50	30	Left ventro-lateral mantle
July	34	15	Left ventro-lateral mantle
	40	10	Left ventro-lateral mantle
August	42	4	Left ventro-lateral mantle
	44	10	Left ventro-lateral mantle
September	39	10	Left ventro-lateral mantle
	45	10	Left ventro-lateral mantle
October		No Spermatophore found	
November	41	4	Left ventro-lateral mantle
	44	1	Left ventro-lateral mantle
December	40	10	Left ventro-lateral mantle
	44	8	Left ventro-lateral mantle

Small oocytes were predominant (0.1-3.0 mm diameter) in gonads of *N. caroli* at all maturity stages (Fig. 5). Successive cohorts of oocytes in different development stages can be distinguished in the ovaries of ripening and spawning females. These observations suggested that the development of oocytes in the ovaries was asynchronous (Laptikhovsky *et al.,* 2008). Therefore, *N. caroli* can be described as a species with an asynchronous ovary maturation with very large eggs (~8 mm), a fecundity with several oocytes and a high reproductive output, together with continuous intermittent spawning with low batch fecundity. Similar spawning strategies have been reported for 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).

Small individuals (<20 mm ML) were rarely captured in this study, probably due to the selectivity of the trawl net used in sampling. The occurrence of individuals of 20 mm ML during all seasons may indicate that this species spawns throughout the year. *N. caroli,* similar to other sepiolid species living in deep and stable waters (Boletzky, 1986), also have continous spawning strategies.

Although mature *N. caroli* females in this study showed a peak between July and September, as Mangold (1963a) reported, Boletzky (1975) indicated that this species could also spawn in other months. The fact that mature and mated *N caroli* females examined in this study carry spermatophore reservoirs in almost all months of the year is consistent with Boletzky (1975).

Boletzky and Boletzky (1973) reported that, depending on the ambient temperature, the incubation of eggs in *R. macrosoma* can last for a few months. Boletzky (1994) suggested that the incubation period can sometimes last for about a year for large cephalopod eggs undergoing slow development at low temperature. Members of Rossiinae deposit large eggs sized between 8 and 12 mm. Large eggs with a large yolk are necessary for a holobenthic life strategy so that new born individuals can start life directly in the benthic zone without a pelagic phase. According to Laptikhovsky *et al.* (2008) this fact may be the reason why the female individuals of *N. caroli* and *R. macrosoma* (members of the Rossiinae family, which have eggs larger than 10% ML) are larger than males. Boletzky (1986) pointed out the crucial question of whether these small hatchlings can actually be considered less competitive than the larger bottom-living hatchlings.

In order to understand how variation in ecological factors causes the adoption of different spawning strategies in cephalopods during the course of evolution, future targeted studies on the spawning of as many species as possible living in different ecosystems are needed.

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