Reproductive Biology of Sepiola intermedia (Sepiolidae: Cephalopoda) in the Aegean Sea, Eastern Mediterranean

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Abstract

A total of 111 Sepiola intermedia specimens were collected from the Aegean Sea at seasonally between 2007 and 2008. The maturity stages of both sexes and the gonadosomatic index values were estimated. It was observed that the ovulation pattern of S. intermedia is asynchronous and spawning occurs continuously. Potential fecundity ranges for females and males were determined to be between 148-406 eggs (mean 271) and 54-383 spermatophores (mean 197), respectively. The females with mantle lengths ML between 14-22 mm obtained 68 to 161 spermatangia (mean 96) ranging from 0.3-0.9 mm in length throughout the season inside their bursa copulatrix. The mantle lengths of mature females varied from 15 to 22 mm ML, those of mature males – between 15 and 23 mm ML. This study provides new records on the reproductive biology of S. intermedia.

Keywords: Sepiola intermedia, reproductive biology, histology, Aegean Sea, Mediterranean.

Sepiola intermedia (Sepiolidae: Cephalopoda)‘nın Ege Denizi’nde (Doğu Akdeniz) Üreme Biyolojisi

Özet


Anahtar Kelimeler: Sepiola intermedia, üreme biyolojisi, histoloji, Ege Denizi, Akdeniz.

Introduction

Sepiola intermedia Naef 1912, belonging to the subfamily of Sepiolineae, is a small bottom-living species that has a short life span and a maximum ML of 28 mm. It is found in the Aegean Sea as well as in other areas of the Mediterranean Sea and Northeastern Atlantic (Reid and Jereb, 2005). Although being captured in many Mediterranean areas, the species has no commercial value and there is no separate fisheries statistics available for it (Reid and Jereb, 2005).

According to Relini and Bertuletti (1989) S. intermedia is one of the Mediterranean’s patchy distributed sepiolid species, which has been reported from studies carried out in different parts of the Mediterranean. The species is the most abundant species among the genus Sepiola which have a vertical distribution between 8-200 m (Belcari et al., 1989; Relini and Bertuletti 1989; Sartor and Belcari 1995; Volpi et al., 1995; Würtz et al., 1995). Female Sepiolidae store spermatangia ejaculated spermatophores, either in a specialized seminal receptacle (Heteroteuthis) or on the bursa copulatrix (Sepiola, Sepietta) (Nesis, 1995; Hoving et al., 2008).

The shape of bursa copulatrix in the mantle cavity is used in the identification of the female species (Bello, 1995). Even though studies on S. intermedia’s spawning, fecundity and spermatophore...
numbers and length have been conducted (Gabel-Deickert, 1995; Salman and Önsöy, 2004), the reproductive biology of this species remains poorly known.

The present study contributes to our understanding of the biology of the species by providing new data on maturity, size, oocyte growth and ovary development and other reproductive parameters of both sexes.

Materials and Methods

Samples were collected within the scope of the “Analysis of Effect of Abiotic Environmental Factors on Economic Demersal Fish Population in the Gulf of Izmir” project on board R/V EGESUF. Daytime sampling was carried out seasonally from autumn 2007 to summer 2008 by a commercial bottom trawl (44 mm cod-end mesh size) from sandy and muddy bottoms within a 50-55 m depth of Izmir Bay, Aegean Sea (Eastern Mediterranean) (Figure 1). The entire catch of the cephalopods was fixed in 10% formalin solution on board. A total of 111 S. intermedia specimens (57 males + 54 females) were collected and identified following Reid and Jereb (2005) and Bello (1995). Dorsal mantle length (ML) and total body weight (BW) of the preserved individuals were measured to the nearest 1 mm and 0.01 g, respectively.

To assign maturity status of the studied specimens, Yau and Boyle (1996) and Laptikhovsky et al. (2008), sexual maturity criteria were used to create a new maturity scale (Table 1). Sexual maturity stages were assigned as “immature”, “maturing”, “mature” and “spent”.

All oocytes from the ovary and the oviduct were separately counted and measured along the major axis to the nearest 0.1 mm. Gonads (GW) and oviduct were weighed to the nearest 0.0001 g. Gonadosomatic indices (GSI) for females were calculated for each

![Figure 1. Sampling area (indicated by full dots)](image)

**Table 1.** Scale of the Sepiola intermedia’s maturity stages, which was modified from Yau and Boyle (1996) and Laptikhovsky et al. (2008)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juvenile</td>
<td>(I)</td>
<td></td>
</tr>
<tr>
<td>Immature</td>
<td>(II)</td>
<td>(I)</td>
</tr>
<tr>
<td>Maturing</td>
<td>(III)</td>
<td>(IV)</td>
</tr>
<tr>
<td>Mature</td>
<td>(V)</td>
<td>(V)</td>
</tr>
</tbody>
</table>

- Gonads are microscopic; sexes cannot be distinguished using a stereomicroscope.
- Testis is small and translucent. No spermatophores and no sperm in spermatophore.
- Nidamental glands are very small. Ovary is beneath and contains pre-vitellogenic oocytes that are transparent and <0.05 mm in length.
- Testis has enlarged. Usually a few spermatophores in the Needham’s sac.
- Nidamental glands are larger. Ovary is beneath the nidamental gland and contains medium size oocytes. No ripe eggs in the oviduct. Bursa copulatrix small and no spermatangia attached.
- Spermatangia rarely attach to the bursa copulatrix.
- Testis is large, opaque and white in color. Needham’s sac contains functional spermatophores.
- Ovary contains all stages oocytes. No ripe eggs in the oviduct. Spermatangia rarely attach to the bursa copulatrix.
- Ovary similar stage to IV but contains mature oocytes. A few ripe eggs in the oviduct. Spermatangia attach to the bursa copulatrix.
sampling period using equation (GSI = (GW (including ovary weight and oviduct weight) / BW) x 100). Males were calculated for each sampling period using (GSI = (GW (gonad weight) / BW) x 100) (except spermatophoric complex) (Gabr et al., 1998). Potential fecundities (PF) of mature females were computed as the sum of the oocytes in the ovary plus the oocytes in the oviduct. Relative fecundity (RF) was calculated as a ratio of potential fecundity (PF) to BW (RF = PF/BW). For mature male specimens, the relative spermatophore length (SpL index = SpL x 100/ML) was estimated. The structures of spermatophores were identified according to Weil (1927) and Voss (1969). Length at maturity of population (ML_{50}) was defined as the length at which 50% of the population investigated is mature (King, 1996). A log-log function was used to assess the proportion of the mature individuals by size class using nonlinear regression (Ilkyaz et al., 1998). The equations;

\[ r(l) = \exp(- \exp(-(a + bl))) \]

and

\[ L_{50} = \frac{-\ln(-\ln(0.5)) - a}{b} \approx \frac{0.3665 - a}{b} \]

were applied, where \( r(l) \) is the proportion of mature animals in each length class (%), \( l \) is the mantle length (mm), \( L_m \) is the mean mantle length at sexual maturity (50%, mm), \( a \) is intercept, and \( b \) is slope.

Both linear and nonlinear regression analyses of Snedecor and Cochran, (1989) were used. Gonads of 18 females (12 to 21 mm ML) at various maturity stages were used to study oogenesis. Ovaries were removed from the specimens that were fixed in 10% formalin solution on board as before. For preparations of histological sections of the reproductive system, paraffin blocks. Histological sections (5-8 μm thick) were cut and stained using hematoxylin-eosin (HE). The hematoxylin stains the membranes and proteinaceous structures blue and the eosin stains cytoplasmic structures pink. Histological sections were examined using a CX-41 phase contrast microscope and photographed with an Olympus DP-20 digital camera and a stereo binocular Olympus SZ-61.

**Results**

**Body Size**

The observed MLs ranged between 10-23 mm for males (average 17.8±3.1 mm ML), and 12-22 mm for females (average 17.1±2.4 mm ML). The t-test show no difference in the mantle length distributions (P>0.05).

Although mature specimens were observed in all seasons, the smallest individuals were found in spring and autumn. The mean MLs and their standard deviations were 18.1±2.9, 19.5±1.9, 16.6±2.5 and 18.1±2.9 for spring, summer, autumn and winter, respectively. Except in summer months, no statistical differences were observed between seasons (P<0.005).

**Maturity Stage**

The length-at maturity of the population ML_{50} was recorded for both sexes and average MLs at maturation were estimated as 17.4 mm females and as 17.3 mm males (Figure 2). Mean ML of immature female was 13±1.0 mm, maturing 15±1.52 mm and mature females 18±1.87 mm ML. In males, respective values were immature 12±1.70 mm, maturing 17±3.34 mm and mature 19±1.54 mm ML. For females, calculated average GSI values decrease starting from the autumn and reach a minimum in winter. Males had homogeneous GSI values throughout the year, and the maximum value of 2.4% was observed in spring (Figure 3).

**Gonad Development, Fecundity and Fertility**

In males GSI increase at maturation and maturity was much smaller than those of females (Figure 4).

Oocyte growth occurred asynchronously; small protoplasmic oocytes of 0.5-0.9 mm in diameter predominated during most of the ontogeny. The oocytes sizes varied from 0.1 to 4.2 mm. There were at least 3 groups of oocytes (small/protoplasmic oocyte, 0.1-0.9 mm; medium/vitellogenesis large oocyte 1.0-2.7 mm, and large/ripe oocyte 2.8-4.2 mm). The percentage of oocyte size distributions at different gonad stages are given in Figure 5.

The potential female fecundity varied between 148-406 oocytes, with a mean of 271. The number of ripe oocytes in the oviduct was 3-33, and their diameters were 2.6-4.2 mm, which corresponds to 11-26% (mean 17%) of ML (Figure 6A).

A linear relationship between the ML of the 15 mature females and 182 mature eggs from their oviducts shows that there is no correlation in between them (R=0.0031).

The estimated RF for pre-spawning females was 44.8-151.7 (mean 93.9) oocytes/g. The ML of the smallest individual with eggs in the oviduct was 15 mm. Mature male and female specimens were observed every season throughout the year.

Spermatangia attached in bursa copulatrix, which is a distal oviduct wrinkled area, were investigated. In all seasons, the specimens with ML between 14 and 22 mm had 68-161 spermatangia (mean 96) ranging from 0.3 to 0.9 mm inside their bursa copulatrix (Figure 6B).

In a transverse cut of the bursa copulatrix distal
section, a dense spermatangia group could be seen (Figure 7A).

In the longitudinal section of spermatangia, sperm mass at the aboral part and the cement body at the oral part of the spermatangia can be distinguished easily (Figure 7B). Implanted spermatangia have thin capillary-tubule with oral openings, which extend outwards and this may help spermatozoa transfer from the bursa copulatrix to the eggs to be fertilized (Figure 7B).

The number of spermatophores in the Needham sacs of seventeen mature males between 15 and 21 mm ML ranged between 54 and 383 (mean 195), their lengths varied from 5.0 to 10.0 mm (mean 7.7 mm). The calculated ML/SpL index was 32-52% (mean 40%). The relative size of sperm mass, cement body and the ejaculatory apparatus in respect to the total length of the spermatophore was estimated as 21.4±2.16, 15.2±1.54 and 63.4±1.05 respectively.

The spermatophores’ spiral coiled sperm mass, cement body and ejaculatory apparatus sections were distinctly recognized even inside the Needham sacs, which had been removed from examined males (Figure 8). The cement body, which is the middle part of a spermatophore connect to the ejaculatory apparatus consist of upper sac, which is connected by
a narrow bridge of the outer membrane to the lower sac (Figure 8).

**Discussion**

The bursa copulatrix of the subfamily Sepiolinae occur in different shapes and sizes. Spermatangia are implanted in bursa copulatrix, which is located in the genital opening of females, or into the wrinkled area (Jones and Richardson, 2011), which is called seminal receptacle. Implantation area of spermatangia is an enlarged area of distal oviduct (Rodrigues et al., 2012). According to Jones and Richardson (2011) species Sepiola atlantica contains 22 spermatangia in its bursa copulatrix, and by Rodrigues et al. (2011) it was reported that implanted spermatangia in the bursa copulatrix could survive up to 104 days.

Different numbers of spermatangia can be observed in subfamily Rossinae species, such as Rossia molleri 6-21 (Hoving et al., 2009); Semirossia patagonica 2-19 (Önsoy et al., 2008); Neorossia caroli 1-30 (Salman, 2011), which do not contain bursa copulatrix.

Yau and Boyle (1996) used egg diameter and bursa copulatrix length to estimate maturity indices in Sepiola atlantica but these indices are not applicable to other sepiolidae species because of species-specific length of bursa copulatrix. To determine the accurate sexual maturity stage of Sepiola intermedia, a new maturity scale was developed (Table 1).

The GSI values of male individuals, which remain stable throughout the year, indicate that males are equally actively reproduce all year round. Females being to mate while still maturing and mating and spawning continues throughout the year (Table 2). Also the occurrence of small immature individuals (between 10-14 mm ML) throughout the seasons supports this conclusion.

According to the results of this study, the size at maturity of both sexes of S. intermedia is 17 mm ML.
Successive cohorts of oocytes in different development stages can be distinguished in the ovaries of maturing and spawning females. These observations suggest that the ovulation pattern of this species is asynchronous which is supported by the classification of gonad developments proposed by Rocha et al. (2001).

It has been reported that in general, sepiolids spawn throughout the year whether their lifetime is shorter than a year like the Sepietta oweniana or more than a year such as Neorossia caroli (Boletzky 1975; Salman, 1998, 2011; Önsoy et al., 2013).

According to Gabel-Deickert (1995), Sepiolidae have low batch fecundity and an extended spawning

**Figure 7.** A view of the spermatangium in the bursa copulatrix of S. intermedia from histological sections A: General view, B: Horizontal section of a spermatangia (Sm: Sperm mass; Cb: Cement body; oo: Oral opening).

**Figure 8.** General view of the spermatophore, cement body and inner structure in the S. intermedia (Sm: Sperm mass; Cb: Cement body; EA: Ejaculatory apparatus).
period as a result of their short lifespan. From this, Boletzky (1983) reported that species are forced to spawn without any environmental stimuli, as it is also the case for *S. intermedia*. Spawning without any environmental signal and low batch fecundity indicate that this species is a continuous spawner. Similar spawning strategies were reported for subfamily Rossinae (Laptikhovsky et al., 2008), *Heteroteuthis dispar* (Hoving et al., 2008), and *Rondeletiola minor* (Onsoy et al., 2013). In this study, the maximum batch fecundity was estimated to be 33 eggs.

As emphasized by Gabel-Deickert, (1995) to know the reproductive biology of members of a subfamily Sepiolinae is better by investigating and comparing with other sepolid members, which gives knowledge on the reproductive characteristics of the species separated from each other under taxonomic differences in the evolutionary process.

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**References**


<table>
<thead>
<tr>
<th>ML (mm)</th>
<th>Stage</th>
<th>SptN</th>
<th>SptL (mm)</th>
<th>Season</th>
</tr>
</thead>
<tbody>
<tr>
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<td>68</td>
<td>0.3-0.7</td>
<td>Spring</td>
</tr>
<tr>
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<td>Mature</td>
<td>68</td>
<td>0.3-0.8</td>
<td>Summer</td>
</tr>
<tr>
<td>19</td>
<td>Mature</td>
<td>78</td>
<td>0.4-0.9</td>
<td>Summer</td>
</tr>
<tr>
<td>14</td>
<td>Maturing</td>
<td>76</td>
<td>0.3-0.9</td>
<td>Autumn</td>
</tr>
<tr>
<td>19</td>
<td>Maturing</td>
<td>111</td>
<td>0.5-0.9</td>
<td>Autumn</td>
</tr>
<tr>
<td>16</td>
<td>Maturing</td>
<td>146</td>
<td>0.3-0.8</td>
<td>Autumn</td>
</tr>
<tr>
<td>20</td>
<td>Mature</td>
<td>77</td>
<td>0.4-0.7</td>
<td>Winter</td>
</tr>
<tr>
<td>22</td>
<td>Mature</td>
<td>161</td>
<td>0.4-0.8</td>
<td>Winter</td>
</tr>
<tr>
<td>15</td>
<td>Mature</td>
<td>80</td>
<td>0.3-0.8</td>
<td>Winter</td>
</tr>
</tbody>
</table>

(SptN: Number of Spermatangia; SptL: Spermatangium length)