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Reproductive Biology of the Elegant Cuttlefish (*Sepia elegans*) in the Eastern Mediterranean

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Abstract

Reproductive biology of Mediterranean cuttlefish *Sepia elegans* has been studied in the Aegean Sea from total of 924 animals (432 males, 492 females) caught during monthly trawl surveys from May 2008 to April 2009. Cuttlefishes were measured, weighed, maturity assessed, and reproductive systems were removed and investigated in the lab. The size at maturity (ML_{50}) were 42 mm (males) ML and 41mm ML (females) respectively. Seasonal changes in a gonadosomatic index suggest extended reproductive period of the population with two peaks of spawning; once in July and in October. The ovulation pattern of S. *elegans* is asynchronous and spawning occurs continuously. The female potential fecundity was 513-1190. The spermatophore counts for mature males ranged 167-486. Size of adult individuals in the East Mediterranean was smaller than that in the Western Mediterranean probably due to lower productivity and high temperatures (hence expenses on metabolism).

Keywords: Reproductive biology, pink cuttlefish, *S. elegans*, Aegean Sea.

Doğu Akdeniz'de Küçük Mürekkep Balığının (*Sepia elegans*) Üreme Biyolojisi

Özet

Bu çalışmada *S.elegans*'a ait toplam 924 birey (432 erkek; 492 dişi) Ege Denizi'nden Mayıs 2008' den Nisan 2009 tarihleri arasında aylık olarak örneklenmiştir. Laboratuvara getirilen örneklerin manto boyları ölçülmüş, ağırlıkları tartılmış ve daha sonra üreme sistemleri çıkarılarak olgunluk safhaları belirlenmiş ve tartılmıştır. Elde edilen bireylerden populasyonun olgunlaşma boyları (ML_{50}) erkekler için 42 mm ML, dişiler için ise 41 mm ML olarak tespit edilmiştir. Mevsimsel gonasdosomatik indis değerlerinden bölgedeki populasyona ait bireylerin Temmuz ve Ekim ayları olmak üzere iki üreme piki oluşturdukları gözlenmiştir. Gonadlardaki yumurta ovulasyonunun ise asinkronik yapıda olduğu ve buna bağlı olarak yumurtlamanın sürekli olduğu tespit edilmiştir. Her iki cinsiyetteki bireylerin doğurganlıkları dişi bireylerde 513-1190 yumurta rasında iken erkek bireylerin ise 167-486 spermatofor arasında olduğu hesaplanmıştır. Ege Denizi'nde tespit edilen bireylerin manto boyları doğu Akdeniz'deki bireylerden daha küçük olduğu ve bunun başlıca sebebinin ise primer productivitenin batı Akdeniz'den daha az ve suların ise daha sıcak olmasından kaynaklandığından ileri gelebileceği düşünülmektedir.

Anahtar Kelimeler: Üreme biyolojisi, mürekkep balığı, *S.elegans*, Ege Denizi.

Introduction

Sepia elegans Blainville, 1829 is the smallest cuttlefish species among the Mediterranean Sepiidae. It occurs from the eastern Atlantic to the Mediterranean Sea and along the West African coast down to 15°S; from sublittoral zone to 500m depth (Jereb and Roper, 2005). This species is also tolerant to the brackish waters and penetrates into the Marmara Sea, where the salinity decreases to 22‰ (Katagan *et al*., 1993; Ünsal *et al*., 1999; Öztürk *et al*., 2014).

Researches on the Mediterranean cephalopods had shown that *S. elegans* is one of the most common species in the area (Belcari and Sartor, 1993; D'Onghia *et al*., 1996; Salman *et al*., 1997; Salman and Katagan, 2004). *S. elegans* plays an important role in the marine ecosystem. where also Kabasakal (2002) reported the species being a common occurrence in stomachs of sharks and rays.

Salman and Katagan (2004) reported this cuttlefish as an important by-catch species in trawl

fisheries of both Aegean Sea and the eastern Mediterranean Sea, from depths of 150 to 250 m. Even though this cuttlefish has no commercial value, so is discarded in Turkey, but in some European countries is sold both fresh and frozen (Jereb and Roper, 2005). Fate of discarded cuttlefish is unknown, but their survival rate might be low as in case of similar sized subadult common cuttlefish (Reville, 2012).

Though there are several studies on some biometrical features and distribution of *S. elegans* from the Mediterranean (Jereb and Ragonese, 1991; Ragonese and Jereb, 1991), and on some biological features from the Atlantic (Guerra and Castro, 1989) and from western Mediterranean (Mangold-Wirz, 1963), knowledge of its reproductive biology is superficial. The aim of this study is to investigate the reproductive biology of *S. elegans* in the Aegean Sea, where this species represent a potentially important but still not exploited commercial object.

Materials and Methods

Samples were collected onboard with commercial vessels operating during daytime by bottom trawl (44-mm mesh size in cod-end) on sandy and muddy bottoms at depth stratas of 150, 350, and 550 m in the Aegean Sea (Eastern Mediterranean Sea). Samplings were carried out monthly from May 2008 to April 2009 (Figure 1). The duration of each haul was 1 h, and towing speed varied between 2.2 and 2.5 knots. The entire cephalopod catch was preserved in 10% formalin solution onboard. A total of 924 *S. elegans* individuals (432 males and 492 females) were collected and studied in the lab.

The dorsal mantle lengths (ML) were measured within 1 mm, total body weights (BW) were taken within 0.01 g.

Subadult and adult female cuttlefishes were then dissected and the ovary and oviducts were excised; Gonad weights (GW) and oviduct weights (OV) were weighed within 0.0001 g. Reproductive systems were examined under binocular microscope (40X) after dissection. To estimate fecundity all oocytes from the ovary and the oviduct of twenty mature females (ML=41-53 mm) were separately counted and measured along the major axis to the nearest 0.1 mm. Potential fecundity (PF) of females was calculated as the sum of the oocytes in the ovary plus the eggs in the oviduct. Because in preserved stage V ovaries, the oviductal eggs often torn as a result of the separation during counting in the laboratory, we used Dursun *et al.* (2013) methodology, and counted fresh materials oviduct eggs on board.

Gonadosomatic indices (GSI) for females were calculated with GSI = (GW (including ovary weight and oviduct weight) / BW) x 100 for females and GSI = (GW (gonad weight) / BW) x 100) (except spermatophoric complex) for males (Gabr *et al.*, 1998). All spermatophores collected from 10 mature males (ML= 36–54 mm) were counted, and 40 spermatophores were measured from each specimen. The relative spermatophore length (SpL index) was calculated from SpL index = SpL x 100/ML). A maturity stage was assigned visually using a universal maturity scale adapted for cuttlefish from Arkhipkin (1992).

Length at maturity of population (ML_{50}) was defined as the length at which 50% of the population 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

$$L_{50} = \frac{-\ln(-\ln(0.5)) - a}{b} \cong \frac{0.3665 - a}{b}$$

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

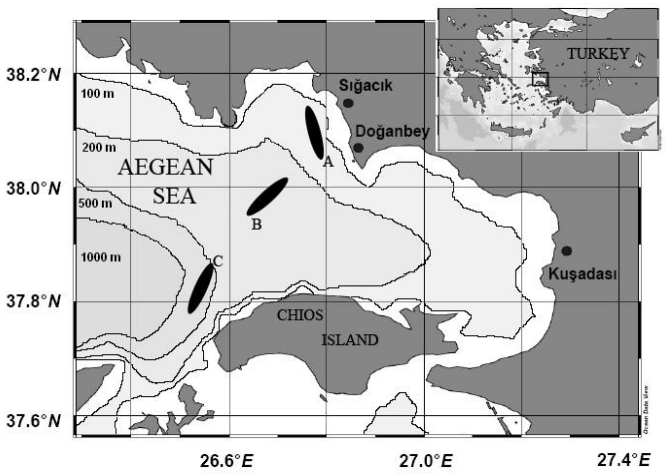

Figure 1. Sampling area (indicated by full dots).

Results

Size Distribution

The observed MLs of mature individuals ranged between 27-63 mm for females (mean 48.1±5.7 mm ML), and 28-54 mm for males (mean 42.5±4.2 mm ML). Cuttlefish size distribution varied seasonally with modal sizes increasing from spring to summer and then gradually decreasing throughout autumn into winter.

The size of mature animals were compared seasonally between sexes with t-test, mature females were always larger than mature males in all seasons (spring, t=6.826; summer t=9.173; autumn, t=4.315; winter, t=4.167) (Figure 2).

The length-weight relations were calculated with a power function as $TW♂=0.0026ML^{2.149}$ and $TW♀=0.0018ML^{2.284}$ for males and females, respectively. When the length-weight relationship was compared by ANCOVA (F=119.38; p=0.001), it showed that there was difference between the sexes as a result of females being larger and heavier than males (Figure 3).

Seasonality of Maturation and Spawning

Mature animals and small immature recruits were found in all seasons in numbers that allows the assumption that spawning occurs all year round (Table 1). Analysis of changes in size at maturity distribution with size and with season (Figure 2) and GSI (Figure 4) shows that there are likely several seasonal cohorts.

The largest spawning individuals occurred during summer catches. The mean size of mature animals decreased in autumn and was the smallest in winter and spring even when major recruitment occurs.

The reproductive system weight of the fully mature individuals with a total body weight between 0.7-3.6 g. was between 3.51–21.82 g. In fully mature animals the reproductive system consists from 61% (range 28-78%) ovary, 8% (range 1-28%) oviduct,

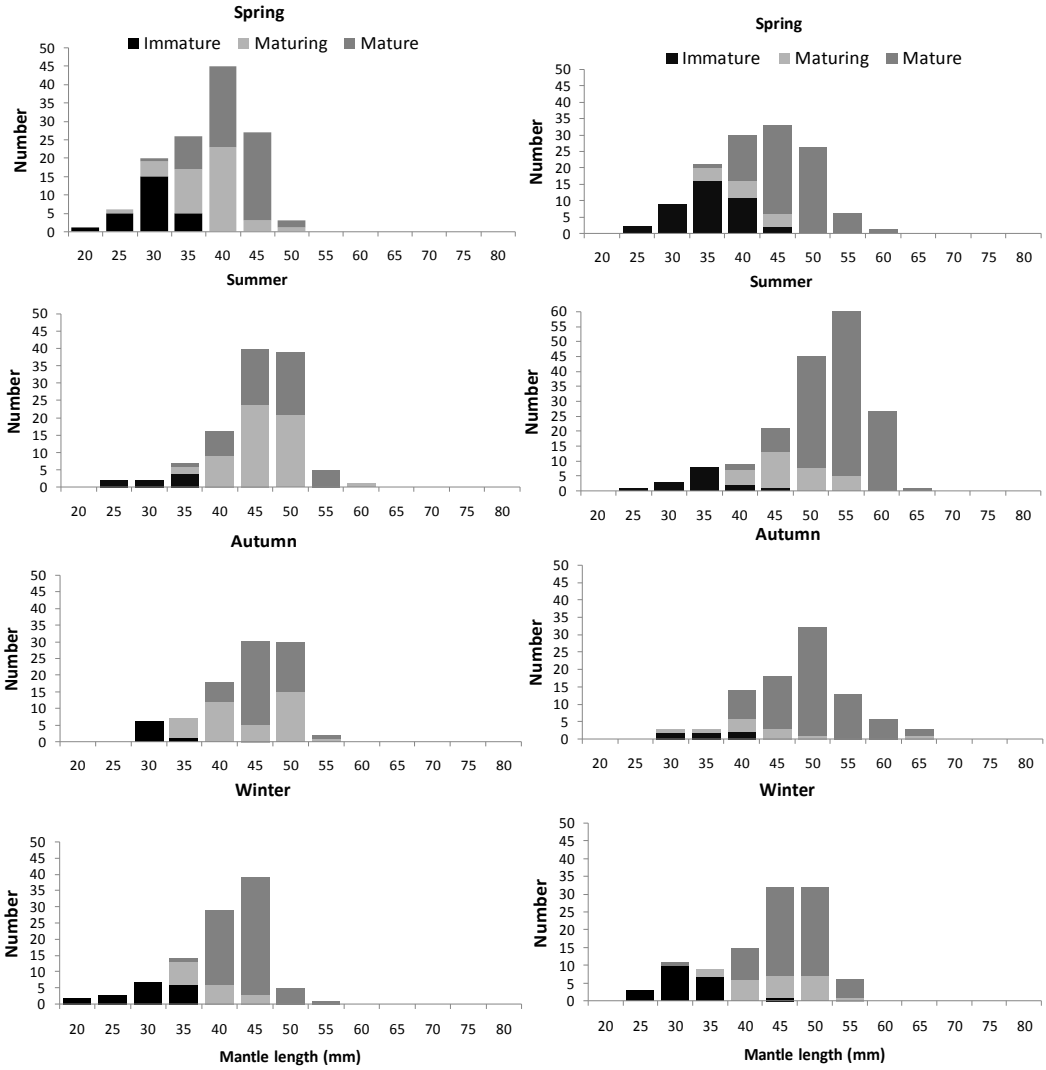

Figure 2. Seasonal length distribution of *S.elegans* in Aegean Sea.

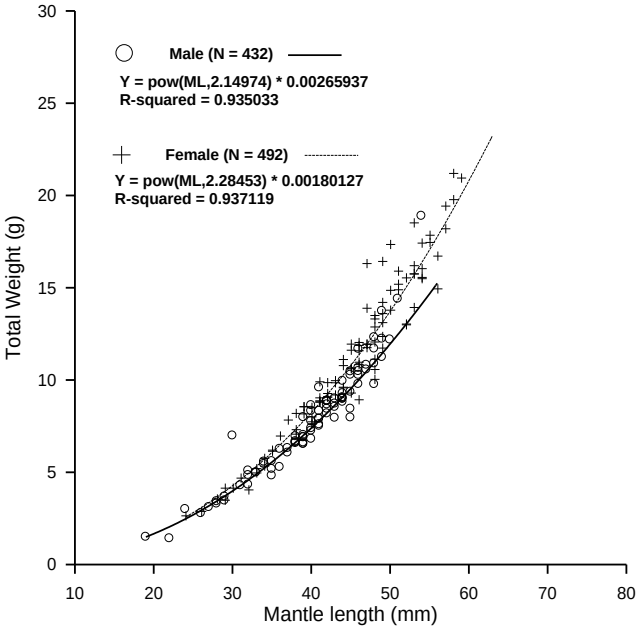

Figure 3. Mantle length-Total weight relationship of *S.elegans* from Aegean Sea.

Table 1. Monthly gonad stage distribution of *S.elegans*

Month	Male							Female						
	N	St-1	St-2	St-3	St-4	St-5	Total	N	St-1	St-2	St-3	St-4	St-5	Total
Jan	31		23	6	19	52	100	30		23	7	7	63	100
Feb	40	5	20	5	8	63	100	39		33	10	3	54	100
Mar	53	6	19	19	17	40	100	55	2	35	5	2	56	100
Apr	44	5	9	2	20	64	100	37	3	14	16	3	65	100
May	30	10	13	13	37	27	100	36	11	28	3	3	56	100
June	29			31	24	45	100	59	3	8	15	12	61	100
July	39		5	18	28	49	100	58		5	5	2	88	100
Aug	44	5	9	16	36	34	100	57	2	7	4	14	74	100
Sept	38		16	8	42	34	100	22		5	14	5	77	100
Oct	9			44	56		100	11		9		9	82	100
Nov	45		2	7	18	73	100	49	2	6	6	6	80	100
Dec	30		3		10	87	100	39		3		33	64	100
Total	432							492						

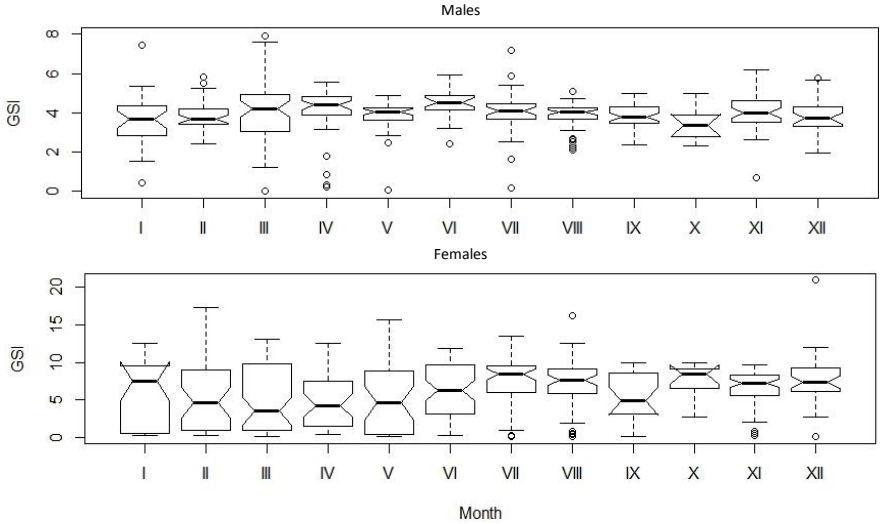

Figure 4. Monthly gonadosomatic index values of *S. elegans*.

31% (range 15-64%) nidamental glands in females and in males 60% (range 39-79%) gonad, 40% (range 20-60%) Needham's sac.

Seasonal dynamics of gonadal maturation was lead by males with females to follow. The male GSI values were increasing from January to April, whereas in females it occurred from March to June. Following decrease in male GSI occurs in June-October, and that of female in July–September. Then male GSI were stable between November and January, after what male GSI increased whereas female GSIs were decreasing in February–March (Figure 4). Generally such a complicated intra-annual dynamics of GSI mirrored relative abundance of mature animals in the population (Table 1).

Size at Maturity

The smallest mature animals caught in the Aegean Sea, were of 27 mm ML (females) and 28 mm (males). The size-at maturity ML_{50} was estimated at 41 mm ML for females and 42 mm ML for males. There was no difference in size at maturity (ML_{50}) between male and female individuals (Figure 5; Table 2).

Distribution of male and female size at stage of maturation is presented on Figure 6 and illustrates that cuttlefish go on growing while maturing between stages II and V.

Ovary Maturation and Fecundity

In the ovary of adult cuttlefish one might find oocytes at any stage of oogenesis; small protoplasmic oocytes of 0.5-0.9 mm in diameter predominated during most of the ontogeny. The oocyte stock could be divided into three groups of oocytes (small/protoplasmic oocyte, 0.1-0.9 mm; medium/vitellogenic large oocyte 1.0-2.7 mm, and large/ripe oocyte 2.8-4.2 mm). The length-frequency distribution of the oocytes at the different gonadal stages (Figure 7), demonstrate asynchronous ovulation with further accumulation of ripe oocytes in the oviduct.

Potential fecundity in (immature, maturing and mature) females of 33–59 mm ML (Stages II-V) varied between 513 and 1190 eggs (mean: 985). In the mature females the number of ripe eggs in the oviduct

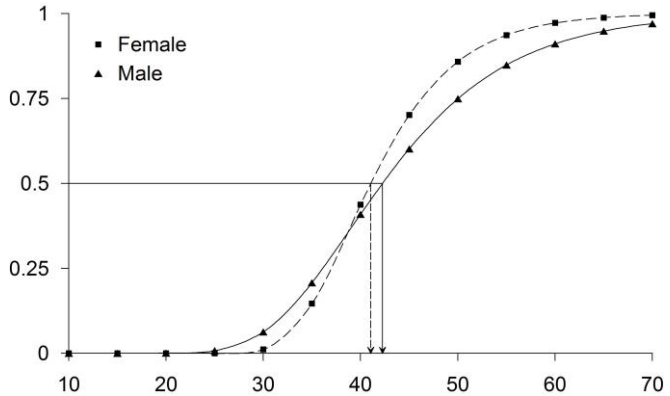

Figure 5. Male and female distribution of the 50% of the population (ML_{50}) at the size-at maturity.

Table 2. Percentage of *S. elegans* males and females in each maturity stage for each 5 mm size class

	Male							Female						
ML	N	St-1	St-2	St-3	St-4	St-5	Total	N	St-1	St-2	St-3	St-4	St-5	Total
0-14														
15-19	1	1					100							
20-24	11	36	55	9			100	5	20	80				100
25-29	35	6	77	11	3	3	100	24	17	75	4		4	100
30-34	54	4	26	22	28	20	100	37	11	68	16	3	3	100
35-39	109			14	32	54	100	64		17	23	8	52	100
40-44	136			11	15	74	100	103		4	9	16	72	100
45-49	77			8	40	52	100	136			4	8	88	100
50-54	8				13	88	100	85				7	93	100
55-59	1				1		100	34					100	100
60-64								4					100	100
65-69														
Total	432	9	47	53	104	219		492	9	62	36	39	346	

ML: Mantle length; ST: Maturity stage

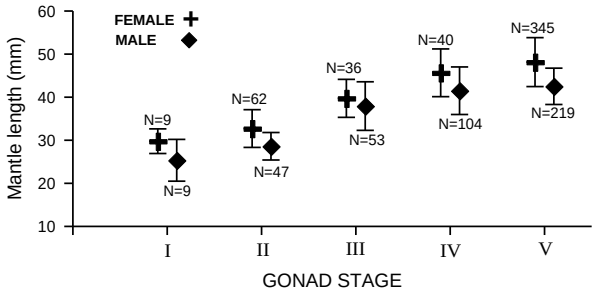

Figure 6. Male and female individuals GSI values according to gonad stage.

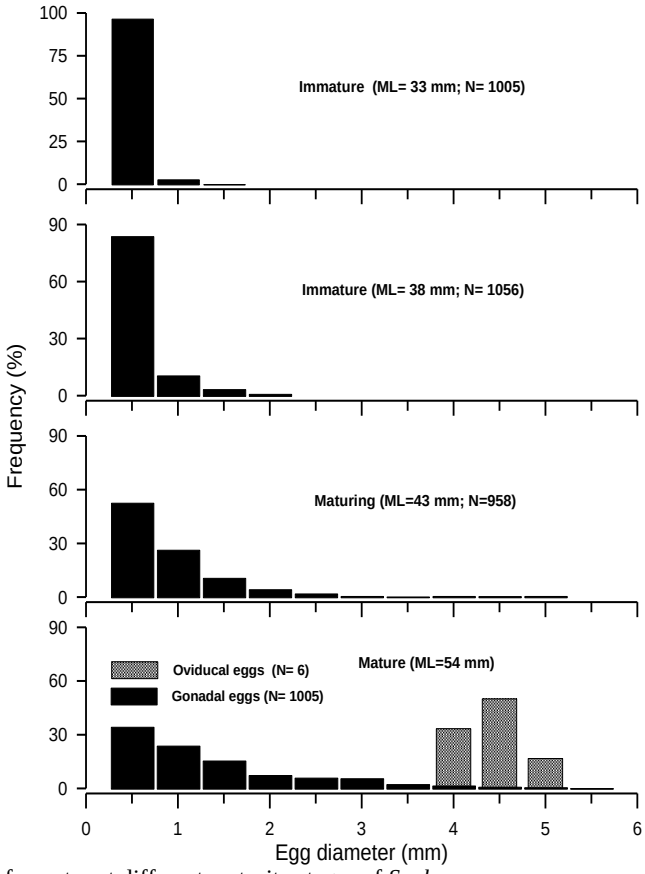

Figure 7. Size distribution of oocytes at different maturity stages of *S. elegans.*

was low, between 2-25 (mean 5 eggs). Their diameters were between 3.5–5.5 mm (mean: 4.9 mm) at the longest axis. The ML of the smallest individual with eggs in the oviduct was 27 mm. The calculated oviducal Egg diameter/ML index varied between 7.8% and 11.1% (mean 8.8%). Comparison between oviducal egg diameter and ML showed no correlation (r=0.1).

The number of spermatophores in the Needham sacs of ten mature males (36-54 mm ML) ranged from 167 to 486 (mean 370). Spermatophore lengths (SpL) varied between 3.9-5.5 mm (mean 4.7mm) and increased with male size (Figure 8). The calculated SpL/ML index varied between 9.1% and 11.4% (mean 10.5%).

Discussion

There was no *S. elegans* specimen collected from 550 m depth, during the sampling period, when trawls were carried at 150, 350 and 550 m depths over the study area which shows that depth limit of the species distribution is somewhere shallower.

Small amount of specimens (about 10% of 924) were collected from 350m depth in various months (May, September, October and January) during the sampling period.

It was supposed that pink cuttlefish can migrate to shallower waters for reproduction as it was found in Atlantic (Guerra and Castro 1989), and some parts of western and central Mediterranean (Volpi *et al*., 1990; Jereb and Ragonese, 1991; Tursi and D'Onghia,

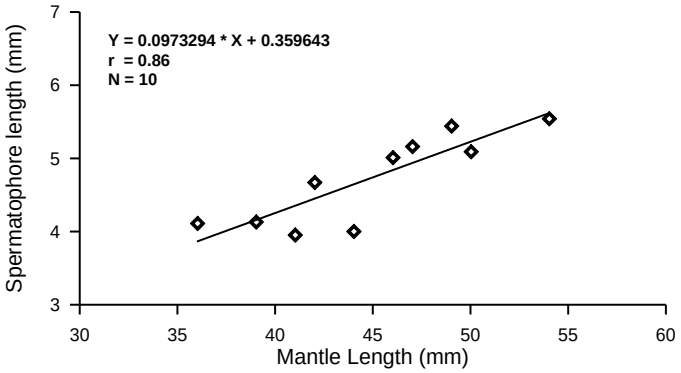

Figure 8. Mantle length-Spermatophore length relationship of *S. elegans.*

1992; Ciavaglia and Manfredi, 2009). Because fishery, in the area, was not allowed to shallower waters than 100 m we could not test this hypothesis for extremely warm east Mediterranean.

Across the species range, males of *S. elegans* reach maturation at smaller sizes than females (Mangold-Wirz, 1963; Guerra and Castro, 1989; Volpi *et al.*, 1990; Jereb and Ragonese, 1991; Belcari and Sartor, 1993; Ciavaglia and Manfredi, 2009). In the present study, males and females were at similar sizes (42 mm ML for males and 41 mm ML for females) both for size at maturity or for length at maturity of the population (ML_{50}). A similar phenomena was reported for *S. officinalis* - the species in which males are generally larger than females where no sexual dimorphism was found in the Aegean Sea (Önsoy and Salman, 2005), but such difference was found in *S. obignyana* (Dursun *et al.*, 2013). Adult animal size in the Aegean Sea was smaller than in the West Mediterranean (Mangold-Wirz, 1963), Ionian Sea (Tursi and D'Onghia, 1992) and Adriatic Sea (Ciavaglia and Manfredi, 2009).

There are few possible reasons for the decrease in size distribution of the samples from summer to winter; first may be the result of pressure caused by the start of the fishing season of the area, secondly may be the result post-spawning death of mature animals and also the overlapping effect of these two events (Figure 2).

The reproduction period of *S. elegans* occurs all year round with the peak between July and October. Respective peak in the recruitment of 20-35 mm ML occurs in winter and spring. Similar results were reported by Guerra and Castro (1989) from the coasts of Atlantic and, by Mangold-Wirz (1963) and Jereb and Roper (2005) from the western Mediterranean.

The spermatophore lengths of *S. elegans* from the western Mediterranean were between 3.5 and 5.5 mm (Mangold-Wirz, 1963), where the results are the same as in this study, between 3.6 and 5.4 mm. Therefore, there is no difference in spermatophore sizes between both sides of the Mediterranean. The calculated SpL/ML index of the Eastern Mediterranean varied between 9.1 and 11.4% (mean 10.5%) and western (Mangold-Wirz, 1963) varied between 9.2-10.9%, where the results were also similar. The small ratio difference is the result of the size difference between the samples of Mangold-Wirz (1963) and this study, where the mature males were between 32-60 mm ML and 28-54 mm ML, respectively.

However, the spermatophore counts were quite different between two habitats: maximum of 95 spermatophores was counted from the western Mediterranean and minimum 167 spermatophores was found in the eastern Mediterranean cuttlefish, which might be the result of either a sampling bias or rather caused by a difference in spawning patterns with western cuttlefish spending spermatophores more often than their eastern counterparts. However, spermatophore index values were found to be close in the present study than those reported by Mangold-Wirz (1963) from the western Mediterranean (9.1-11.4 versus 9.2-10.9). Oviducal egg diameters are similar between both parts of the sea: 3.7-4.2 mm in the western, and 3.5-4.2 mm in the eastern Mediterranean. However, oviducal egg indices were found to be higher in present study (7.8-11.1) than in the western Mediterranean 5.5-7.4 (Mangold 1963). This difference was caused by the difference in adult female size.

Unfortunately, there was no chance to compare fecundities between the western and the eastern Mediterranean because Mangold-Wirz (1963) reported only numbers of oocytes larger than 1 mm (about 400 eggs). It may be expected that the smaller animals have a longer spawning period as a result of having larger eggs with higher fecundity depending on the low oviduct capacity. Laptikhovsky *et al.* (2003) stated similar opinion for the correlation between the oviduct capacity and potential fecundity for an intermittent terminal spawner *Sepia officinalis* from the same family.

It has been observed that the oocytes of all stages are present in the ovary. According to Rocha *et al.* (2001), the ovulation pattern of S. *elegans* likely is asynchronous and that spawning is year-round. All year around spawning in *S. elegans* was also reported by Guerra and Castro (1989) from Atlantic Ocean. The egg count in oviducts were very low in contrast to numbers recorded in the ovary. It is possible because ovulated eggs are accumulated in oviducts,

which suggests asynchronous ovulation with continuous spawning (Figure 7). Actually these few eggs in oviducts might be leftovers of the previous egg laying rather than a new portion accumulating.

It might be concluded that *S. elegans* from the eastern Mediterranean has higher fecundity with larger eggs than the western Mediterranean as an adaptation of an oligotrophic environment of the eastern Mediterranean as also has been stated by Laptikhovsky *et al.* (2009; 2014).

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