RESEARCH PAPER

Fecundity and Spawning Strategy of Shortfin Squid *Illex coindetii* (Oegopsida: Ommastrephidae), In the Eastern Mediterranean

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Abstract

Samples of *Illex coindetii* were collected with bottom trawl net at 150, 350 and 550 m depths in the Aegean Sea (Eastern Mediterranean). Sampling was carried out monthly from May 2008 to April 2009. A total of 1415 *I. coindetii* individuals (551 males and 688 females and 176 unidentified sex) were collected. The length-weight relationship showed a negative allometry. There was a difference between the sexes in respect to length-weight relationships with females being larger and heavier than males. The smallest mature individual in the Aegean Sea, was observed to be 125 mm ML for females and 117 mm ML for males. The size-at maturity ML_{50} was calculated separately for males and females, and first maturation was reached at 164.8 mm ML for females and 139.3 mm ML for males. There were two peaks in GSI of females, one in spring and the other in autumn/early winter. Potential fecundities of mature females were between 46500 and 190000 and, there were 3000–7000 ripe eggs in both oviducts. Oocytes size groups distribution in ovary was bimodal at maturing, mature and advanced mature individuals so ovulation pattern was group-synchronous and reproductive strategy was supposed to be intermittent terminal spawning.

Keywords: *Illex coindetii*, Cephalopoda, reproductive biology, Eastern Mediterranean.

Introduction

Broadtail shortfin squid *Illex coindetii* (Verany, 1839), one of four species of *Illex* genus is a demersal, neritic species of the continental shelf and upper slope, occurring from the surface down to over 1000 m, with maximum concentrations depending on geographical location and distributed widely at both sides of Atlantic Ocean and in the Mediterranean Sea (Jereb & Roper, 2010).

I. coindetii is an opportunistic predator and plays "ecosystem accelerator" role in the marine ecosystem by supplying high quality protein for upper level predators (Jereb *et al*., 2010). In the eastern Mediterranean Sea, it was reported from stomach contents of predator teleost swordfish (Salman, 2004; Peristeraki, Tserpes & Lefkaditou, 2005), bluefin tuna (Karakulak, Salman & Oray, 2009), chondrichthyans (Kabasakal, 2002; Eronat & Özaydın, 2015), and marine mammal *Stenella coeruleoalba* (Salman, 2015).

I. coindetii is generally caught by bottom trawls as by-catch in *Nephrops norvegicus* and *Parapenaeus longirostris* targeted fishery in the Mediterranean. Its annual catch amount increased from 3,000 tons to 7,000 tons during the last decade (Jereb & Roper, 2010).

Despite many studies on the distribution of *I. coindetii* in the eastern Mediterranean (Degner, 1925; Adam, 1967; Ruby & Knudsen, 1972; Katagan, Salman & Benli, 1993; D'Onghia, Matarese, Tursi, Maiorano, & Panetta, 1995; Salman, Katagan & Benli, 1997, 1998; Lefkaditou, Siapatis & Somarakis, 2005), its biology (D'Onghia, Tursi, Papaconstantinou & Mataresse, 1991, Salman *et al*., 1997, 2003; Arvanitidis *et al*., 2002; Lefkaditou *et al*, 2005) there is little information about its fishery (Salman *et al*., 1997; Arvanitidis *et al*., 2002; Salman & Katagan, 2004) and no information on the reproductive biology of the species in the area.

The increasing fishing effort on *I. coindetii* in the entire Mediterranean resulted with a decrease in landing amounts of the area. The aim of this paper is to present the first detailed study on the reproductive biology of *I. coindetii* in the eastern Mediterranean, to fill the information gap on the reproductive biology of the species.

Materials and Methods

During sampling a 25 m commercial trawler was used. Samples were collected in day time by

commercial bottom trawl (44 mm mesh size in codend) from the depths where target species *Parapenaeus longirostris* and *Nephrops norvegicus* are caught, at 150, 350 and 550 m depths on sandy and muddy bottoms in the Aegean Sea (Eastern Mediterranean). Samplings were carried out monthly from May 2008 to April 2009 (Figure 1). Duration of each haul was an hour at 2.5 knots.

The total cephalopod catch were fixed in 10% formalin solution on board. A total of 1415 *Illex coindetii* individuals (551 males, 688 females and 176 undetermined) were collected. Dorsal mantle lengths (ML) were measured to the nearest 1 mm, and total body weights (BW) weighed with 0.01 g precision. The reproductive system and maturity stages were identified according to criteria by Lipinski (1979). Gonads and accessory glands were weighed within 0.0001 g precision. Potential fecundity (PF) was calculated by using gravimetric method both for ovary and oviduct. Three subsamples were taken from gonads of females at each maturity stage and from oviducts of mature females. All subsampled oocytes from the ovary and the oviducts were separately counted and measured along the major axis to the nearest 0.1 mm under binocular microscope. As the oocytes of females at early maturity stages couldn't be counted under the binocular microscope, histological slides were prepared to identify size distribution of oocytes from the gonads (Maturity stage III). Gonadosomatic indices (GSI) of both sexes were calculated for each sampling period (GSI=(Gonad weight (GW)/Body weight (BW)) x 100) and for male Spermatophore length index SpLI= (SpL (Spermatophore Length)x100/ML). The nonlinear relationship between BW and ML (BW=a*BW^b) was investigated by a linear regression analysis following logarithmic transformation of paired values i.e. log (BW)=log (a)+b*log (ML) (Tirasin, 1993). Here the constant b represents the rate of increase (i.e. slope) of BW against ML in log transformed relationship, whereas the constant log (a) represents the intersection point of the regression line on the BW axis. The linear transformation also allowed application of analysis of covariance (ANCOVA) to test whether BW-ML relationship varied by gender. A t-test was also used to test the hypothesis of isometric growth (i.e whether estimated b values differed from hypothetical value of 3 or not) both for females and males (Tirasin, 1993). Similarly, constants of the GW-BW relationship (GW=c*BW^d) were calculated by linear regression using the log transformed values. The ML at first maturity ML_{50} (the mantle length at which 50% of the shortfin squid matured) was determined for both females and males from the relationship between the percentages of mature shortfin squid and their corresponding ML. A logistic regression model relating the proportion (P) of sexually mature individuals to a given ML was chosen (P=e^{a+b*ML}/1+ e^{a+b*ML}) (Hosmer & Lemeshow, 2000). The parameters of this logistic regression, i.e. *a* and *b*, were estimated by means of fitting a generalized linear model using a binomial error to the observed maturity data. The values of ML_{50} were calculated subsequently. Linear and logistic regression analyses, t-tests and all other tests required for fulfilling the assumptions of linear regression i.e. normality, homoscedasticity etc. (Snedecor & Cochran, 1989) were carried out with R software (ver. 3.0.3).

For the histological observations ovaries were removed in lab and fixed in 10% formalin solution, dehydrated and then embedded in paraffin. Histological sections (5-8 μm thick) were cut and stained with Mallory's stain that distinguished the start of the yolk grain appearance in oocytes (blue color) in contrast to the pinkish cytoplasm. Stage of oocytes was determined according to Laptikhovsky and Arkhipkin (2001). Slides were measured and photographed under Olympus DP-20 digital camera attached to the CX-41 phase contrast microscope by using Olympus DP2-BSW application software.

Results

Length Frequency Distribution

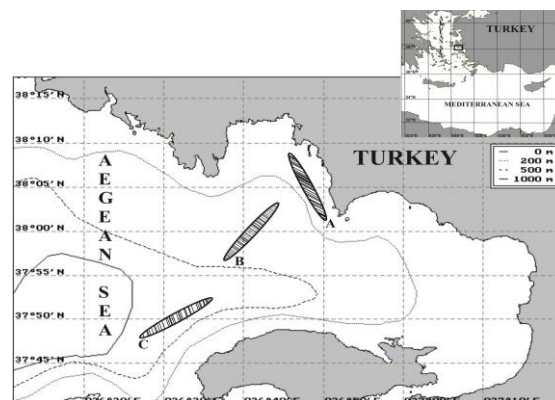Females mantle length ranged between 34–278 mm (avg±SD: 86.2±41.9 mm) and males between 35–

Figure 1. Sampling areas (A: 150 m; B: 350 m; C: 550m). (From GEBCO digital atlas).

188 mm ML (avg±SD: 88.4±37.1 mm). Small sized specimens (40–50 mm ML) were found in all months throughout the sampling period. Minimum sized individuals (30 mm ML) were sampled in December (Figure 2), and maximum sized ones were found in July and August.

The length-weight relations were calculated with a power function as TW♂=0.000004ML$^{2.952}$ and TW♀=0.00014ML$^{2.656}$ for males and females, respectively. Negative allometry was observed for both sexes. The relationship was found to be statistically different for two sexes with females being larger and heavier than males ANCOVA (F=133.22; P=0.001) (Figure 3).

Vertical Distribution

I. coindetii distributed in all depth strata, 150 m, 350 m and 550 m, but its abundance decreased with depth by the occurrence rates 64%, 29% and 7% respectively. Mantle length classes increased with depth. Average mantle lengths were 69.7±32.4 mm at 150 m, 91.8±39.2 mm at 350 m, and 127.5±54.1 mm at 550 m. Proportionally smaller specimens preferred 150 m depth which was the shallowest among the vertical sampling area. Vice versa, there were proportionally larger individuals mostly found at 550 m depth. Specimens found at 150 m depth had the smallest average mantle lengths and data showed that
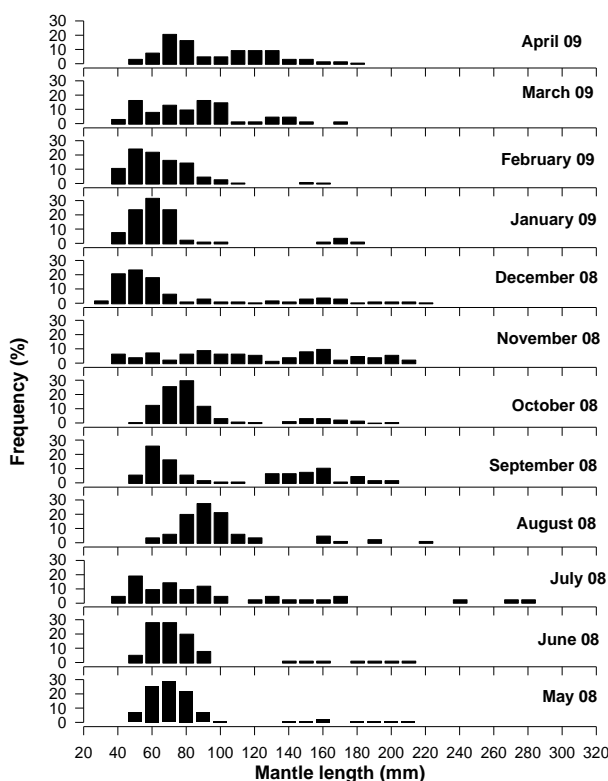

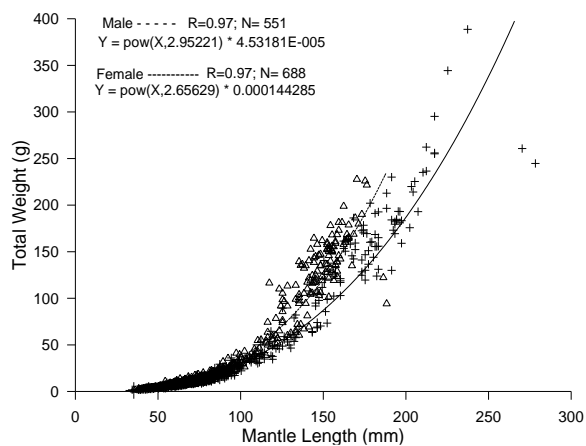**Figure 2.** Monthly mantle length distributions of *Illex coindetii*.

Figure 3. Mantle length-total weight relationship of *Illex coindetii* from the Aegean Sea.

the average mantle lengths increased with depth (F=146.67; P=0.000).

Specimens were caught in all months during the sampling period at 150 m depth, in respect of monthly vertical variance. At 350 m depth, samples were found in all months except late winter and early spring when the water temperature was at minimum (JICA, 1992). While the temperature raised in summer, *I. coindetii* was at 550 m depth, and disappeared during the cold winter conditions (Figure 4).

Maturity Stages

The smallest mature individual were 125 mm ML for females and 117 mm ML for males. The estimated logistic equations relating the proportion (P) of sexually mature individuals to ML were ($P=e^{-20.70153+0.13936*ML}/1+e^{-20.70153+0.13936*ML}$) for females, and ($P=e^{-16.82695+0.13887*ML}/1+e^{-16.82695+0.13887*ML}$) for males.

The size-at maturity ML_{50} of the population were defined as 164.8 mm ML for females and 139.3 mm ML for males. It was observed that the males could reach maturity at a smaller size than females (Table 1).

It was found that defined maturity stages were related to size, larger animals were at higher level of maturity. The average GSI values were low in the first stages, but in stages 4 and 5, a significant increase was observed (Table 2).

Mature females were found throughout the year, however the proportion of the mature females increased at the end of summer and early autumn then decreased at the end of winter (Table 3). Two peaks were observed in GSI values of females, the lower one at spring and early summer and the higher one at autumn and early winter (Figure 5; Table 3). The maximum GSI value of females were 14.2% at spring period, as the first reproduction period suggested, and 12.1% at autumn-winter period. The average GSI values were 1.51% for females and 1.16% for males. The linear regression analysis between the body weight and the weight of gonads (GW/TW) in females establish equation $R^2=0.82$ (GW=0.000371399 * $TW^{1.8613}$, N=688) (Figure 6).

Fecundity

Two maturing females (Stage IV) number of oocytes between 46500 to 185000 (PF) with 0.1 and 1.0 mm in diameter while in mature females (Stage V) oocytes diameter between 0.1–1.5 mm with number of oocytes between 164000 and 228000 (PF) were observed (Figure 7). There were 3000–7000 ripe eggs with 0.8 to 1.3 mm diameter found in both oviducts of mature females. The maximum potential
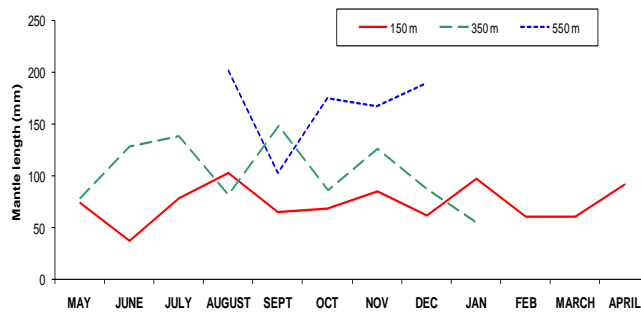

Figure 4. Monthly bathymetric mantle length distribution of *Illex coindetii* from the Aegean Sea.

Table 1. Percentage of *Illex coindetii* males and females in each maturity stage for 20 mm size classes (ML: Mantle length; ST: Maturity Stage)

Size Class (ML)	Male						Female					
	N	St-1	St-2	St-3	St-4	St-5	N	St-1	St-2	St-3	St-4	St-5
21-40	9	100					10	90	10			
41-60	126	68	21	1			172	73	27			
61-80	188	40	43	6			246	45	47	8		
81-100	68	9	53	38			117	24	60	16		
101-120	28		18	54	25	4	34	6	41	53		
121-140	41			24	39	37	17	12	18	59		12
141-160	71			3	11	86	25		8	40	12	40
161-180	18				6	94	27			4	19	78
181-200	2					100	25				16	84
201-220							11				9	91
221-240							2				50	50
241-260												
261-280							2					100
Total (N)	551	177	179	67	32	96	688	276	254	77	14	67

Table 2. Average GSI and size value for different maturity stages of *Illex coindetii*

Maturity Stages	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Average Size (♀)	62.7±15.5	75.7±17.9	104.3±26.6	181.6±25.1	182.5±25.8
Average GSI (♀)	0.31	0.41	0.66	5.45	9.11
Average Size (♂)	59.6±12.2	71.7±13.4.	97.6±21.7	133.6±14.6	151.3±12.9
Average GSI (♂)	0.10	0.19	0.89	2.49	2.35

Table 3. Monthly distribution of *Illex coindetii* females by maturity stages

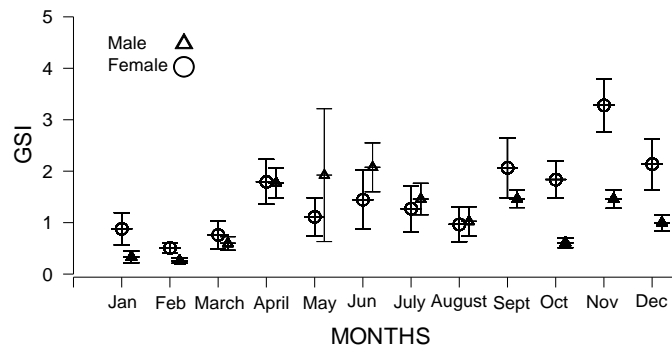|	Jan	Feb	March	April	May	Jun	July	Aug	Sept	Oct	Nov	Dec
St-1	31.8	38.5		10.2	90.8	88.5	14.3	32.3	13.5	38.4	13.0	59.2
St-2	61.4	49.5	73.1	55.9	1.5		74.3	38.5	51.4	39.0	28.3	14.3
St-3	2.3	10.1	23.1	22.0				21.5	13.5	11.6	15.2	8.2
St-4			3.8	3.4	1.5		5.7		2.7		10.9	4.1
St-5	4.5	1.8		8.5	6.2	11.5	5.7	7.7	18.9	11.0	32.6	14.3
N	64	25	34	64	36	141	45	48	43	105	25	58

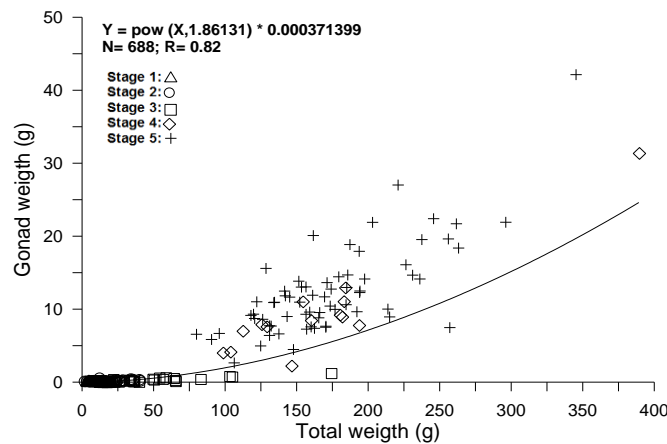**Figure 5.** Monthly gonadosomatic index values of *Illex coindetii.*

Figure 6. Total weight and gonad weight relationship by different gonad stages of *Illex coindetii*.

fecundity (MPF=ovarian eggs+ripe eggs in oviducts) of mature females (Stage V) were between nearly 50000 and 190000. Oocytes group and size distribution were bimodal at the maturing, mature and advanced mature individuals. Except advanced mature individuals, protoplasmic oocytes were predominating in maturing and mature individuals. They presented more than 80% of total oocytes stock in the ovary (Figure 7; Table 4). The photographs of

histological section of gonads from females at different stages of maturity and oviducts of mature females and oocyte distributions of related gonads were given at Table 3. There were 335-474 (mean 416±72) spermatophores found in Needham's sac of mature males of ML between 128 and 140 mm. Spermatophore lengths varied from 20 to 30 mm (mean 26.52±2.3 mm) was calculated.
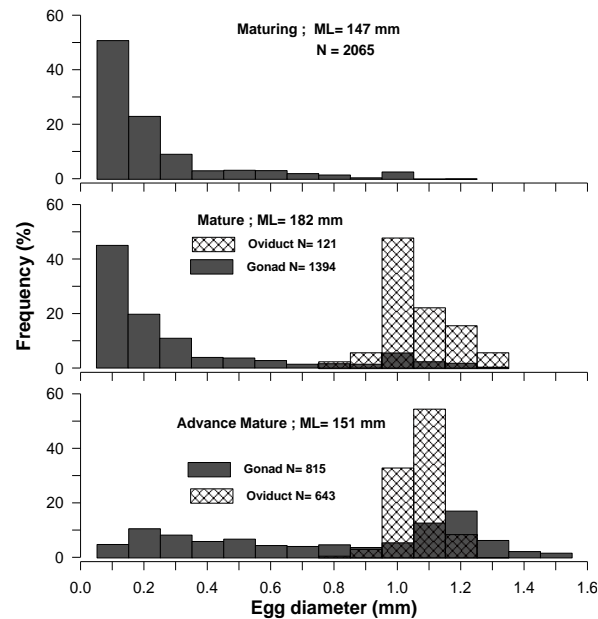
Figure 7. Egg diameter distribution percentages for different gonad stages of *Illex coindetii*.

Discussion

Length-Frequency Distribution

Samplings were carried out at three different depths (150, 350, and 550 m) where target species *Parapenaeus longirostris* fishery is conducted (220–400 m). *I. coindetii* had bi-modal length frequency distribution in the first group mantle lengths showed intensity between 40 and 90 mm and in the second group were ML between 140 and 180 mm ML (Figure 2). Also, bi-modal length distribution of *I. coindetii* was reported previously by Sanches *et al*. (1998) from the Catalan Sea and by Ceriola, Ungaro, and Toteda (2006) from the Adriatic Sea. Sanchez *et al*. (1998) reported that growth of summer spawners were faster than that of autumn spawners after hatching periods, following two distinct reproductive peaks within the year. According to Sanchez *et al*. (1998), *I. coindetii* could reach 80–90 mm ML after 180 days of age. In this study, it could be suggested that 35–50 mm ML small sized specimens sampled in December (~120-140 days old) were hatchings from summer reproduction period, and 40–50 mm ML small sized specimens sampled in July (~180-200 days old) were hatchings from autumn reproduction period.

Bathymetric Distribution

Juveniles and mature specimens of *I. coindetii* from Aegean Sea shared same depths, and large sized animals became abundant as the depth increased as also reported from the Catalan Sea (Sanchez *et al*., 1998) and the Sicilian Channel (Jereb & Ragonese, 1995). *I. coindetii* was the most abundant at 350 m depth according to the data of this study. Although D'Onghia *et al*. (1991) from Eastern coasts of Aegean
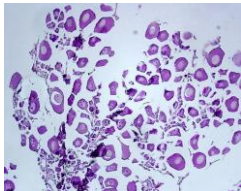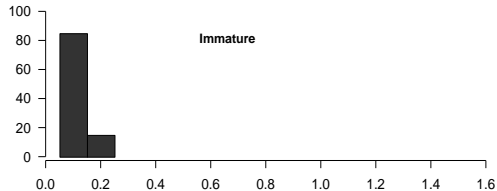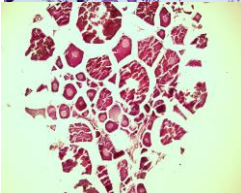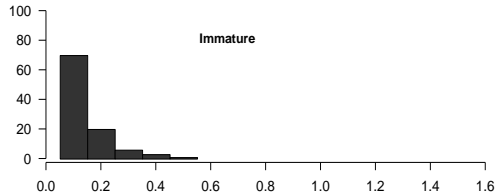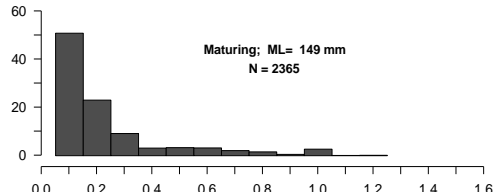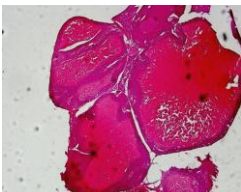
Sea, Salman *et al*. (1997) from Aegean Sea, and Salman and Katagan (2004) from North eastern Levant Sea reported that the fishing effort was higher at 100–200 m depths, it was also reported that fishing e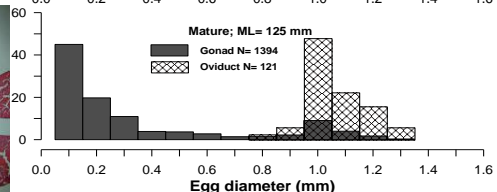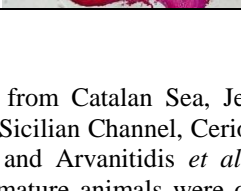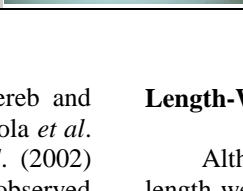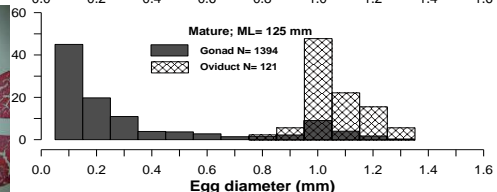ffort and abundance were higher at 100–200 m depths in other areas of the Mediterranean Sea such as Catalan Sea (Mangold-Wirz, 1963; Sanchez & Martin, 1993), Northern Tyrrhenian Sea (Belcari & Sartor, 1993), Southern Tyrrhenian (Perdichizzi *et al*., 2011), Sicilian Channel (Jereb & Ragonese, 1995), and Southern Adriatic (Ceriola *et al*., 2006). This vertical distribution differences between areas, and seasonal vertical difference on abundance observed in this study suggested that *I. coindetii* has vertical migrations depending on variations in bottom water temperature. Further studies on the distribution of this species relating to some oceanographic data, such as bottom water temperature and current systems, are required to gather more accurate information.

Maturity

The smallest males reached maturity at 117 mm ML and females at 125 mm ML. ML_{50} of males was calculated as 121.2 mm ML and that of females was 148.5 mm ML. The similar results observed in different parts of the Mediterranean Sea; Arvanitidis *et al*. (2002) from the neighboring Greek Seas ($ML_{50,}$ males 113 mm ML; females 179 mm ML), Ceriola *et al*. (2006) from Adriatic ($ML_{50,}$ males 137 mm ML; females 146 mm ML), Jereb and Ragonese (1995) from Sicilian Channel ($ML_{50,}$ males 120 mm ML; Females 150 mm ML) and Perdichizzi *et al*. (2011) from Southern Tyrrhenian Sea ($ML_{50,}$ males 105 mm ML; Females 150 mm ML).

Although there were reports from other areas of the Mediterranean such as Sanchez (1995) and

Table 4. Gonad development; Histological validation and oocytes distribution of *Illex coindetii*

Maturity Stages Modified from Lipinsky, (1979) and Nigmatullin and Markaida, (2009)	Histological view of gonads	Histological view of oviduct	Oocytes distribution of gonads
Stage 1- Sex is recognizable; all reproductive organs are tiny and translucent,			
Stage 2- Ovary is grayish, separate oocytes are hardly seen, Protoplasmic oocytes predominate.		Empty	Immature
Stage 3- Ovary is yellowish, separate oocytes are well seen but they are still small,		Empty	Immature
Stage 4- Oocytes attain maximum size but there is no ripe eggs in oviduct in squid and in genital coelome in females. Usually not copulated.		Empty	Maturing; ML= 149 mm N = 2365
Stage 5- There are some ovulated ripe eggs in oviduct. Usually copulated.			Mature; ML= 125 mm Gonad N= 1394 Oviduct N= 121

Sanchez *et al*. (1998) from Catalan Sea, Jereb and Ragonese (1995) from Sicilian Channel, Ceriola *et al*. (2006) from Adriatic, and Arvanitidis *et al*. (2002) from Greek Seas that mature animals were observed throughout the year, in this study, there were mature animals from all year round except March. This might be the result of the limited sampling scheme of this study. According to Perdichizzi *et al*. (2011), although no post-spawning specimen were found during the entire period of sampling, it can be said that spawning could occur throughout the year because of the findings of mature animals (even in low numbers) all year round. Besides that, there were definitely two reproduction peaks observed, one in early summer and the other in autumn. Similar reproduction period was reported by Sanchez (1995) from the Mediterranean coasts of Spain. Also since small sized animals were found throughout the year, it might be suggested that the reproduction occurs all year round with intensification in some seasons. Ceriola *et al*. (2006) also reported that the spawning of *I. coindetii* in the Adriatic Sea occurred throughout the year.

Length-Weight Relationship

Although it was reported by several studies on length-weight relationship (Ragonese & Jereb, 1992; Jereb & Ragonese,1995; Belcari, 1996; Hernandez-Garcia & Castro, 1998; Sanchez *et al*., 1998; Arkhipkin, Jereb & Ragonese, 2000; Arvanitidis *et al*., 2002) that males show positive and females show negative allometry, Sanchez *et al*. (1998) from Spanish coasts and Ceriola *et al*. (2006) from South Adriatic stated that both sexes show positive allometry. Besides, Belcari (1996) stated that mature animals show positive, and immature show negative allometry. However, in this study, it was found that both sexes showed negative allometry (♂2.95; ♀2.65). This might be explained by the lower sample number and higher ratio of immature animals in the sample.

Fecundity

Ripe eggs' diameters found in previous studies were 1.0–1.2 mm (Mangold-Wirz, 1963) and 0.7–1.2 mm (Sanchez *et al*., 1998). It was suggested that

variations of egg diameters might depend on geographic latitudes (Laptikhovsky, Pereira, Salman, Arkhipov, & Costa, 2009). Ripe egg diameters varied from 0.8 to 1.3 mm in this study and it is similar with the results of Gonzalez and Guerra (1996) from North-eastern Atlantic (egg diameters: 0.8–1.2 mm). Oocyte diameters of *I. coindetii* from the Eastern Mediterranean showed bimodal distribution and varied between 0.1 and 1.3 mm. Therefore, it refers to group-synchronous ovulation pattern according to Rocha, Guerra & Gonzales (2001). Diameters of previtellogenic oocytes ranged from 0.1 to 0.5 mm and the ratio of previtellogenic oocytes to total oocyte amount in gonad was 80% in maturing and mature females. Ovulation pattern defined for *I. coindetii* in this study proves the reproductive strategy hypothesis of intermittent terminal spawning also stated for the species by Gonzales and Guerra (1996).

Maximum spermatophore length values (spL 20–30 mm) observed in this study were found lower than those reported by Mangold-Wirz (1963) from the Western Mediterranean (spL 25–32 mm), and Gonzalez and Guerra (1996) from the North-eastern Atlantic (spL 11.1–37.7 mm). Gonzalez and Guerra (1996) stated that there was a positive correlation between spermatophore length and mantle length. The differences in maximum spermatophore lengths mentioned above may be caused by the differences in mantle lengths of male specimens, as males in this study were smaller than in other studies.

Acknowledgement

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