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Reproductive biology of *Plesionika narval* in the SE Aegean Sea (Eastern Mediterranean)

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Abstract

Plesionika narval is a widespread species of the Pandalidae family, of particular high economic importance for small-scale shrimp trap fisheries in the Dodecanese Islands (SE Aegean Sea). Understanding its biology and reproduction are crucial for stock management. Reproductive biology aspects were studied through data collected during monthly experimental surveys with baited shrimps traps from November 2014 to October 2015 at a depth range of 10-150 m. A total of 3436 individuals within the size range of 6.46-20.20 mm carapace length (CL) was analyzed. Overall, mean female size was significantly higher than mean male size, while the mean size of ovigerous females was higher than that of non-ovigerous females. Mean carapace length of ovigerous and non-ovigerous females was significantly correlated to depth. Ovigerous females were observed throughout the study period; however, monthly proportions revealed April to October as the main reproductive period of the species in the area. The sex ratio showed a clear predominance of females in the shallow depth zone (10-25 m) and was found to be affected by sampling area and depth zone. Immature females were found from November to March. Mature females were found all year round, exhibiting higher percentages in March, June, July and September, coinciding with the main reproductive period. *Plesionika narval* seemed to spawn more than one time within the annual reproductive cycle. Gonadosomatic index obtained its highest mean values in May, June and September, thus revealing the main reproductive period. Size at first maturity for females (CL_{50}) was estimated at 11.7 mm.

Keywords: Narwal shrimp, reproduction, sex ratio, gonadosomatic index, gonads, size at first maturity, Mediterranean Sea.

Introduction

Plesionika narval is a widespread benthic species of the Pandalidae family, commonly found in the eastern central Atlantic and the western Indian Ocean, around Australia, the Red Sea, the western central Pacific Ocean and the Mediterranean Sea (Heldt & Heldt, 1954; Zariquiey-Alvarez, 1968; Holthuis, 1987; Thessalou-Legaki *et al.,* 1989; Koukouras *et al*., 1992; 1998; González *et al*., 1997; Carbonell *et al*., 2003; Kevrekidis & Galil, 2003; Li & Davie, 2006; Christodoulou *et al*., 2009). The species inhabits a large variety of habitats including muddy, sand-muddy, rocky bottoms and submarine caves and occurs at depths between 4m and 910m (Crosnier & Forest, 1973; Holthuis, 1987; Biscoito, 1993; Carbonell *et al.*, 2003).

Plesionika narval is of considerable economic importance for small scale fisheries, and therefore included in the FAO catalogue as a separate species (Holthuis, 1980). Specifically for the Dodecanese Islands (Southeastern Aegean Sea), *P. narval* is served as a delicacy and is of high economic importance for the local small-scale shrimp trap fisheries and the local economy. Knowledge of its biology and reproductive patterns, in space and time, are limited and of crucial importance for stock management.

Although *P. narval* is of particular interest to fisheries, few studies have been carried out on its reproduction, based only on ovigerous females and are those reported by Thessalou-Legaki *et al*. (1989) and Thessalou- Legaki (1992) for the Eastern Mediterranean Sea, González *et al*. (1997) for the eastern Central Atlantic, Arculeo & Lo Brutto (2011) for the Central Mediterranean Sea and Sousa *et al*. (2014) for the Northeastern Atlantic. However, none of the previous studies have studied its gonads or estimated its Gonadosomatic Index (GSI), probably because of the small size of the gonads (Kitsos *et al*., 2008). The present study provides detailed information on the reproduction of *P. narval* from two areas in the Southeastern Aegean (Eastern Mediterranean Sea) and studies the gonads of the species for the first time, presenting respective images.

Materials and Methods

Sampling - Data Collection

Individuals of *P. narval* were collected during monthly experimental surveys (November 2014 to October 2015) with baited shrimps traps (mesh size of 12 mm knot to knot), carried out in the Dodecanese Islands of Simi and Karpathos (Fig. 1) within the framework of the PLESIONIKA MANAGE project (Kalogirou *et al.*,

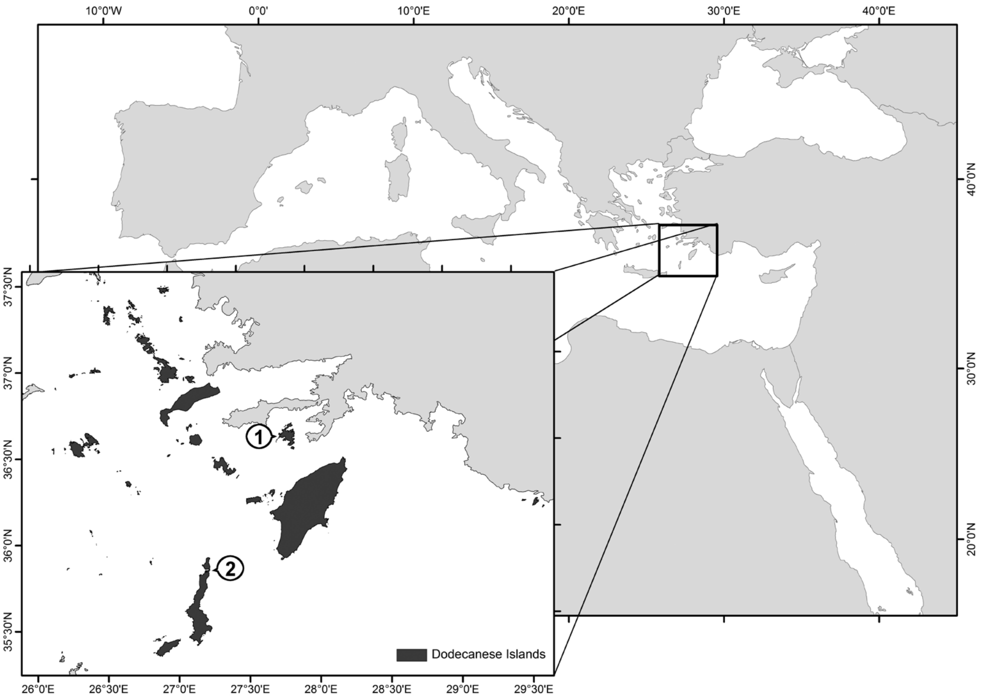

***Fig. 1*:** Map of the study area in the Dodecanese Islands showing Simi (1) and Karpathos (2) in the Eastern Mediterranean Sea.

2016). Specimens were collected from three depth zones: 10-25 m (A), 60-70 m (B) and 110-150 m (C). Samples were stored in 10% formalin immediately after capture and transferred to the laboratory for further analysis.

For each individual, carapace length (CL) was measured from the posterior margin of the eye orbit to the posterior dorsal margin of the carapace with electronic digital calipers (to the nearest 0.01 mm), body weight (BW) and gonad weight (GW) were measured using an electronic scale, with 0.001 g accuracy. Sex was determined under a stereoscope by observing the secondary sexual characteristics of the first and second pleopods (Thessalou-Legaki, 1989; Thessalou-Legaki *et al.*, 1989). Gonads were macroscopically examined under a stereoscope and classified to maturity stages based on their relative size, color and stage of development. In this study, the four –stage maturity scale described for *Plesionika martia* (Maiorano *et al.*, 2002) was adopted with some modifications to Stages I and II. Thus, Stage I refers to immature individuals, Stage II to developing and resting females, Stage III to early mature and Stage IV to fully mature individuals. Since post-spawning individuals never return to an immature phase (Stage I) resting individuals were classified as Stage II although their appearance was very similar to those of Stage I (Anastasopoulou *et al.*, 2015).

Females were examined for the presence of external eggs and classified as ovigerous or non-ovigerous. Three stages of egg development were estimated based on coloration and eye development in the eggs following Company& Sardá (1997) as:

Stage 1: eggs of recent spawning with embryos without eye pigmentation (intense blue in fresh samples, orange in the fixed samples),

Stage 2: slight eye pigmentation, visible embryos (green blue in fresh samples, orange in the fixed samples),

Stage 3: fully developed eyes and embryos (brown–transparent color in fresh and fixed samples).

Data Processing

The mean CL values between males and females were compared using the ANOVA test (Zar, 1984).

Sex ratio (Females * 100 / (Females + Males)) was estimated by month, area and depth zone. Multifactor analysis of variance (MANOVA) was performed using as factors, area (two levels: Simi and Karpathos), month (eleven levels: all months except February) and depth zone (three levels: depth A 10-25 m; depth B 60-70 m and depth C 110-150 m). Sex ratios were then calculated for those factors found to be statistically significant ($p < 0.05$) and the Chi-square test was used to evaluate the null hypothesis of equality of frequencies between sexes (*H*o: 1:1 ratio).

Mean female CL values (ovigerous and non-ovigerous) between the three examined depth zones were compared using the ANOVA test.

Monthly length frequency distributions by development stage of eggs for ovigerous females and non-ovigerous females, in 1mm intervals, were plotted for the whole period in order to ascertain if the presence of ovigerous females was continuous or showed any kind of seasonality.

Reproductive period was estimated taking into account the percentage females at various gonadal maturity stages and the percentage of ovigerous females. In order to determine the contribution of individual female size during the spawning period, two size classes were determined: Class 1: CL 9-14.99 mm and Class 2: CL $\geq$ 15 mm. The size of 9 mm was selected because all individuals with CL $\leq$ 9 mm were immature and non-ovigerous. Monthly changes in the percentage occurrence of ovigerous females were examined by sampling area (Simi and Karpathos), depth zone (A: 10-25 m; B: 60-70 m and C: 110-150 m) and size class.

Variations in relative frequency of the ovarian maturity stages were studied monthly and by size class. In addition, to provide evidence for the link between the gonad activity and spawning, the relative frequency of the various stages of ovarian maturity were computed for ovigerous females during early (recent) and late developmental stages.

The Gonadosomatic Index (GSI) was estimated according to the following formula from Strum (1978) and Grant & Tyler (1983):

$$GSI = (\text{Gonad weight (g)} / \text{Body Weight (g)}) * 100$$

A General Linear Model (GLM) analysis of ANOVA was used to investigate the effect of sampling area, month, depth zone and size class on GSI.

Size at sexual maturity (CL_{50}), i.e. size (in CL) at which 50% of females are ovigerous (King & Butler, 1985; Caldentey *et al.*, 1990), was estimated from the relationship between the proportion of ovigerous females and size class for the whole studied period because of the continuous reproduction of the species. The percentage of ovigerous females was fitted to a logistic equation, as follows:

$$P = 1 / (1 + \exp^{-(a + b * CL)})$$

where P is the balanced probability, a and b are equation parameters estimated by the linear least squares method using the logarithmic transformation.

Results

In total, 3432 individuals of *P. narval* were studied comprised of 1981 females and 1451 males during one annual cycle (from November 2014 to October 2015). The number of individuals analyzed for reproductive parameters are presented in Appendix 1.

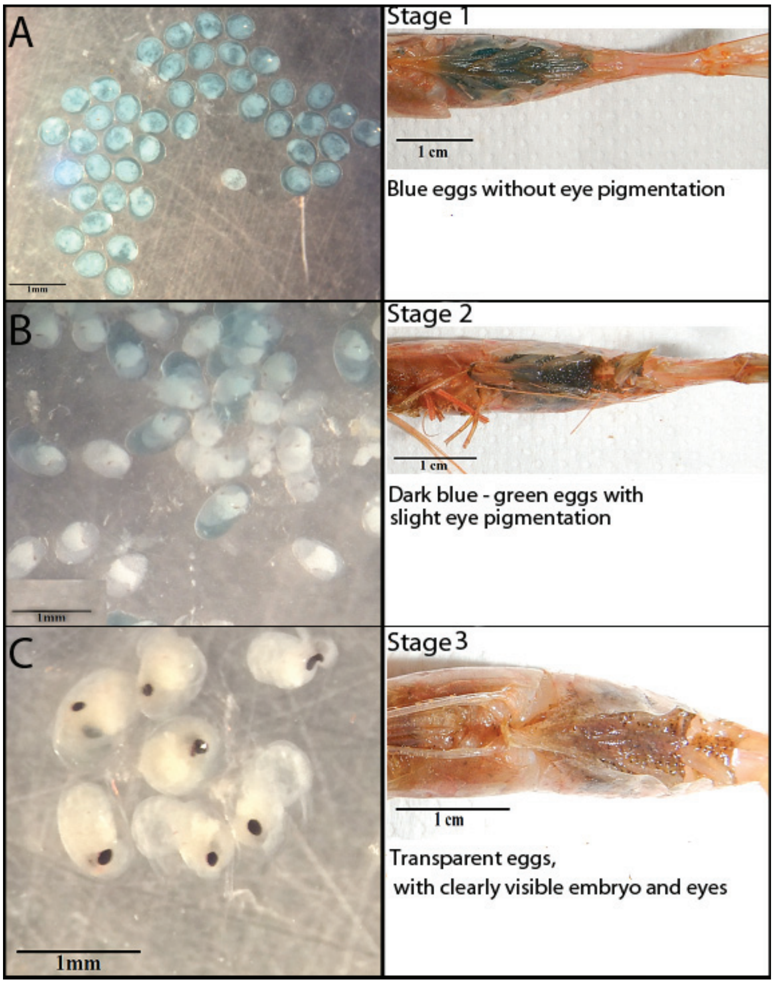

***Fig. 2*:** Egg developmental stages in ovigerous *Plesionika narval*.

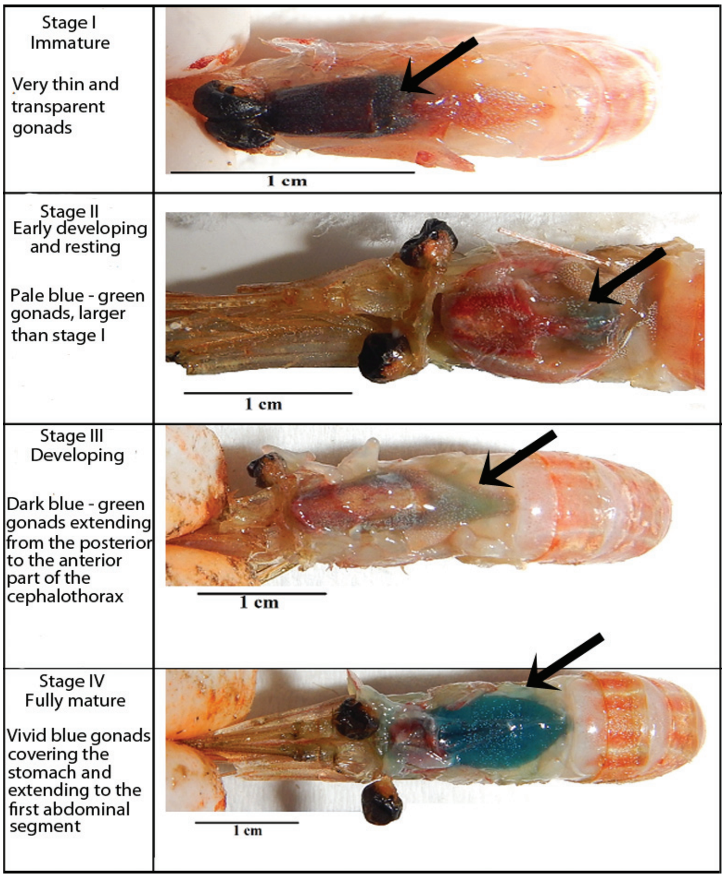

***Fig. 3*: Gonadal maturity stages of females *Plesionika narval*.

Stages of egg development and ovarian maturity

Images and descriptions for the three stages of egg development are shown in Figure 2. The four stages of ovarian maturity are shown in Figure 3.

Mean CL by month, depth zone and sampling area are given in Appendix 2. The sizes of males ranged from a minimum size of 6.46 mm to a maximum of 19.54 mm CL. The females varied from a minimum of 7.13 mm to a maximum of 20.20 mm CL. Mean female CL ± s.d ($CL_f = 13.83 \pm 2.2$) was found to be significantly higher than the mean male ($CL_m = 12.19 \pm 1.8$) (ANOVA, $F_{(1,3429)} = 555.67$, $p < 0.0001$).

Sex ratio

Sex ratio was found to be affected by sampling area (MANOVA, $F_{(1,17)} = 11.84$, $p = 0.0031$) and depth zone (MANOVA, $F_{(2,17)} = 19.31$, $p < 0.0001$), but not by month (MANOVA, $p > 0.05$). In Karpathos, females were significantly more abundant than males at the shallower depth zones of 10-25 m (χ^2-test, $\chi^2 = 240.41$ $p < 0.0001$) and 60-70 m (χ^2-test, $\chi^2 = 25.43$, $p < 0.0001$) whereas at the depth zone 110-150 m the female to male proportion was 1:1 (χ^2-test, $\chi^2 = 2.10$, $p > 0.05$). In Simi, the sex ratio was in favor of females in the shallow 10-25 m depth zone (χ^2-test, $\chi^2 = 58.90$, $p < 0.0001$). However, males were significantly dominant at depth zones 60-70 m (χ^2-test, $\chi^2 = 10.97$, $p = 0.0009$) and 110-150 m (χ^2-test, $\chi^2 = 14.03$, $p = 0.0002$).

Reproductive Parameters

The mean size (CL) of females was found to increase significantly with increased depth (ANOVA, $F_{(2,1978)} = 49.81$, $p < 0.0001$). Non-ovigerous female mean size was significantly higher only in the deeper depth zone (ANOVA, $F_{(2,844)} = 20.87$, $p < 0.0001$), whereas that of ovigerous females increased gradually as depth increased (ANOVA, $F_{(2,1131)} = 74.50$, $p < 0.0001$) (Fig. 4).

Monthly length frequency distributions by egg developmental stage for ovigerous and non-ovigerous females of *P. narval* are presented in Figure 5. Ovigerous females were present throughout the year and ranged from 13.84-97.73%. The presence of non-ovigerous females for all sizes was more evident during the period from November to March ranging 75.87-86.16%. A clear size separation between ovigerous and non-ovigerous females was evident from the length frequency distributions of November, January and March. The mean CL of ovigerous females ($CL_{ovig} = 14.30 \pm 0.06$ s.e) was statistically higher than that of non-ovigerous females ($CL_{non\text{-}ovig} = 13.19 \pm 0.07$ s.e) ANOVA, $F_{(1,1979)} = 138.81$, $p < 0.0001$). The smallest and largest ovigerous females of *P. narval* had

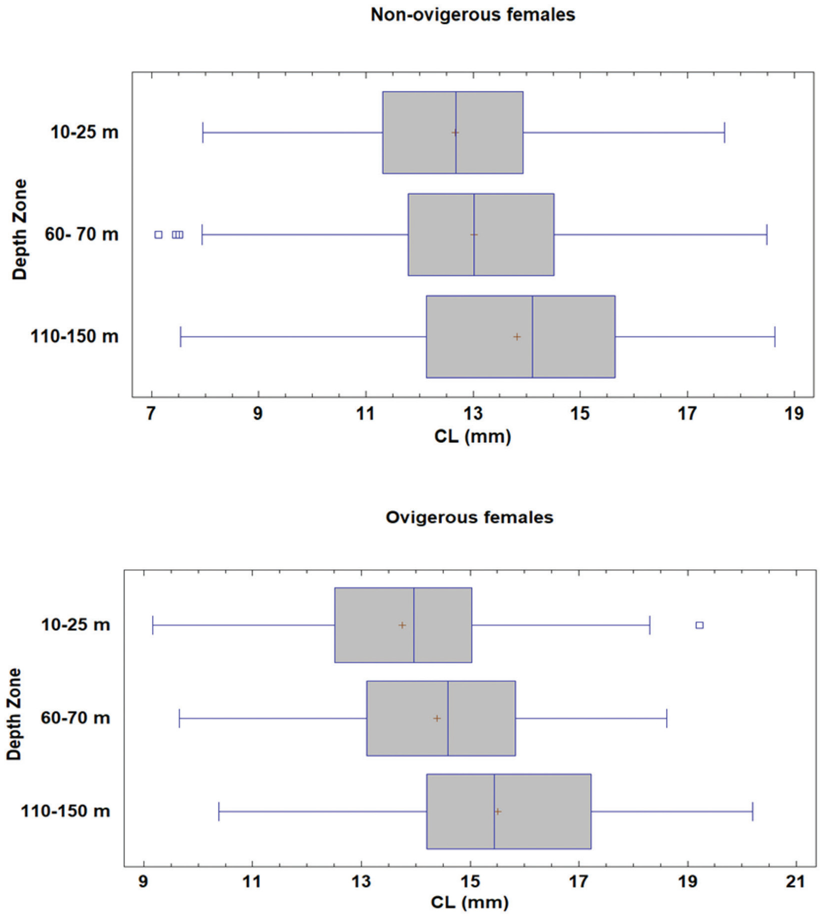

***Fig. 4*:** Box plots of the mean carapace length (CL) of non- ovigerous (up) and ovigerous (down) females by depth zone.

CLs of 9.16 mm and 20.20 mm and were found in July and October, respectively.

The monthly proportion of ovigerous females (Fig. 6a) showed higher values for the period between April (74.53%) and October (90.23%), indicating its main spawning period. Figure 6a also shows that the main reproductive period in Simi seems to end one month earlier (September) than in Karpathos. The monthly proportion of ovigerous females by depth (Fig. 6b) suggests the same pattern. The proportion of ovigerous females during the main reproductive period is higher for all depth zones. Regarding the size class, monthly percentages of ovigerous females (Fig. 6c) show that larger and therefore more experienced females (CL > 15 mm) contributed more actively from the onset of the reproductive period (April) than the smaller females which enter the spawning phase mainly in May.

Female gonad maturity stages by month are shown in Figure 7. Immature females (Stage I) were found during autumn and winter (November to March). However, fully mature females (Stage IV) were observed all year round (higher proportions in March: 37.95%; June: 38.04%; July: 37.71%; September: 44.23%), indicating a continuous reproduction of the species.

Female gonad maturity stages by size are presented in Figure 8. The smallest mature female was 9.32 mm CL and captured in September. Fully mature females of the first size group (9-14.9 mm CL) were more abundant in September (45.65%) and less in January (3.39%), whereas larger fully mature individuals of the second size group (CL ≥ 15 mm) were in high proportions in May (54.17%) and low in October (8.43%). Individuals smaller than 9 mm CL were all immature without external eggs on their pleopods.

A noteworthy fraction of ovigerous females of recent spawning (egg developmental Stage 1) was found to have gonads in Stage II while the majority of ovigerous females in the late egg stage (egg developmental Stage III) had gonads in advanced maturity (Stage IV) with eggs ready to be released for a new cycle of spawning (Fig. 9).

Gonadosomatic Index (GSI) was estimated from 1820 females. GLM analysis showed that GSI was affected by month, area, month within depth zone (nested) and month within size class (nested) (GLM ANOVA, $F_{(39,1814)} = 138.81$, $p < 0.0001$). The values of the GSI for each ovarian stage of *P. narval* are shown in Figure 10, while Figure 11 shows the monthly distribution of the GSI through an entire annual cycle by area, depth zone and size class. Maturity of females showed a slow but significant increase in GSI from ovarian Stage I to II, and from II to III, followed by a rapid increase into Stage IV

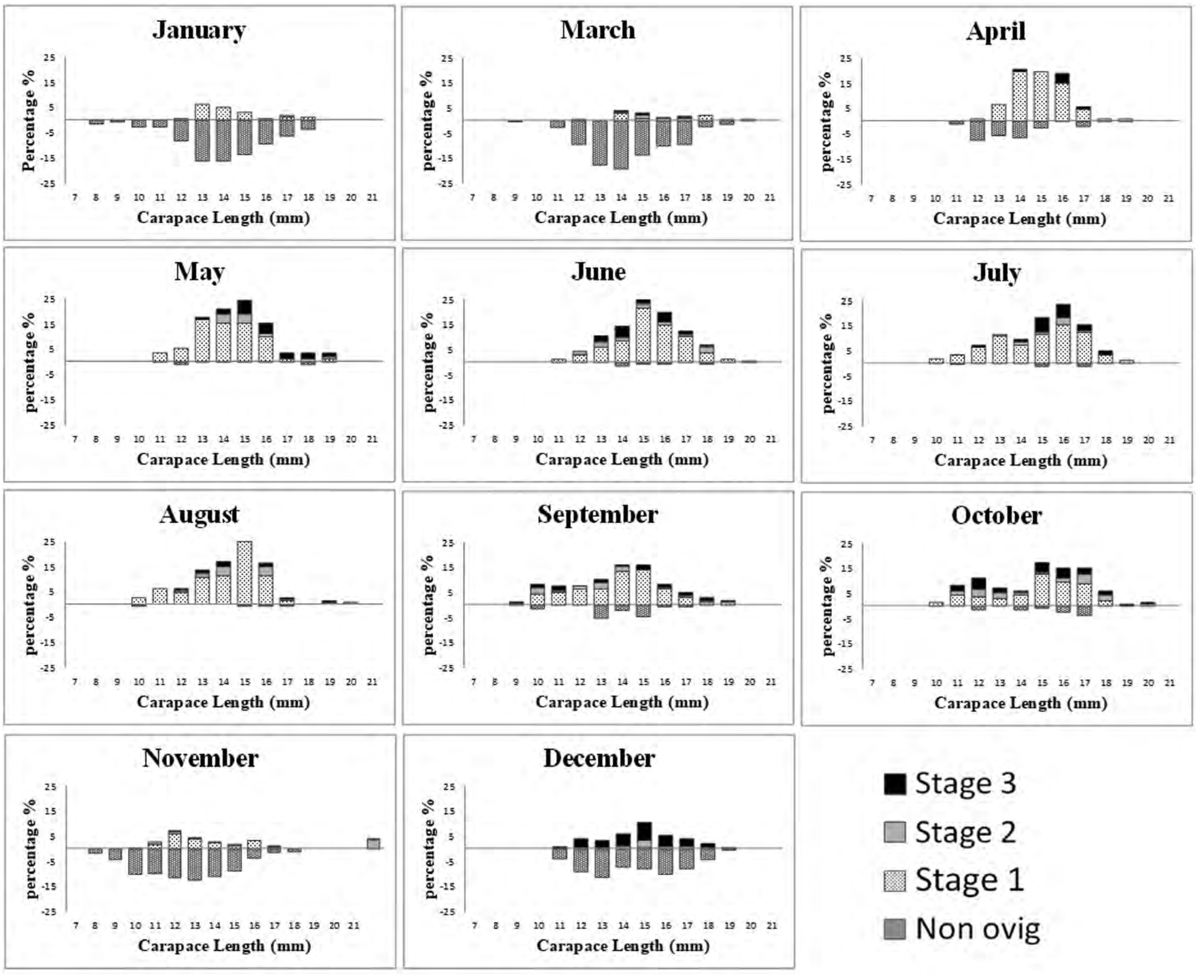

Fig. 5: Length frequency distribution by developmental stage of eggs for ovigerous females and non-ovigerous females of *Plesionika narval* caught in the Dodecanese islands (Eastern Mediterranean Sea). Upper the bar: ovigerous females by developmental stage and lower the bar: non-ovigerous females.

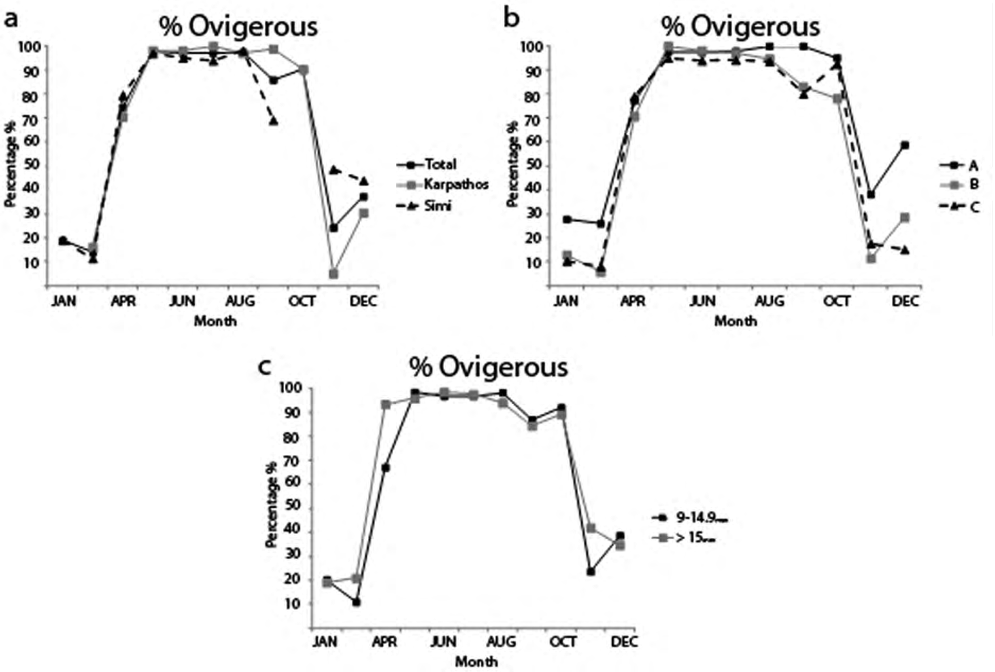

Fig. 6: Monthly changes in percentage occurrence of ovigerous females of *Plesionika narval* a) by area, b) by depth zone (A=10-25 m, B=60-70 m, C=110-150 m) and c) by size class.

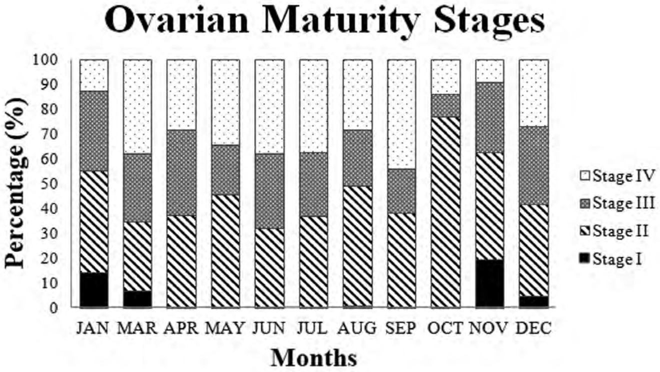

***Fig. 7*: Monthly percentages of gonadal maturity stages (I-IV) of female *Plesionika narval* caught in the Dodecanese Islands (Eastern Mediterranean Sea).

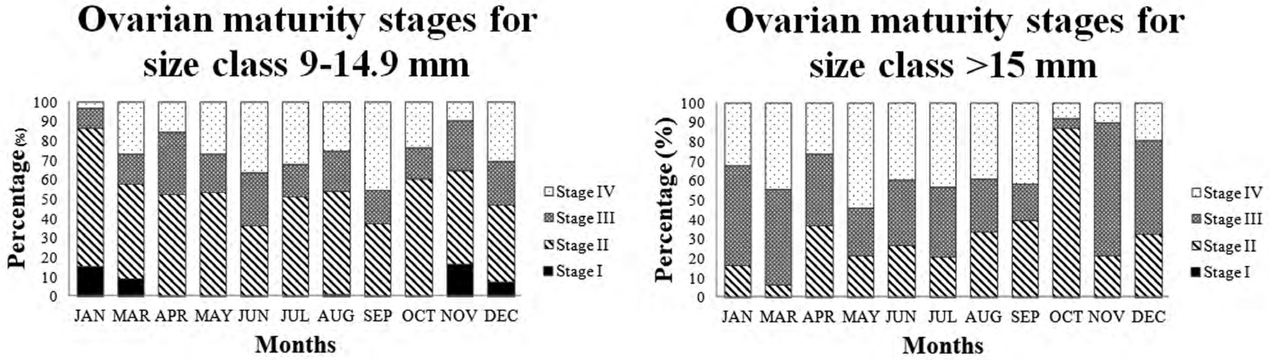

***Fig. 8*: Monthly percentages of gonadal maturity stages (I-IV) of female Plesionika *narval* by size class.

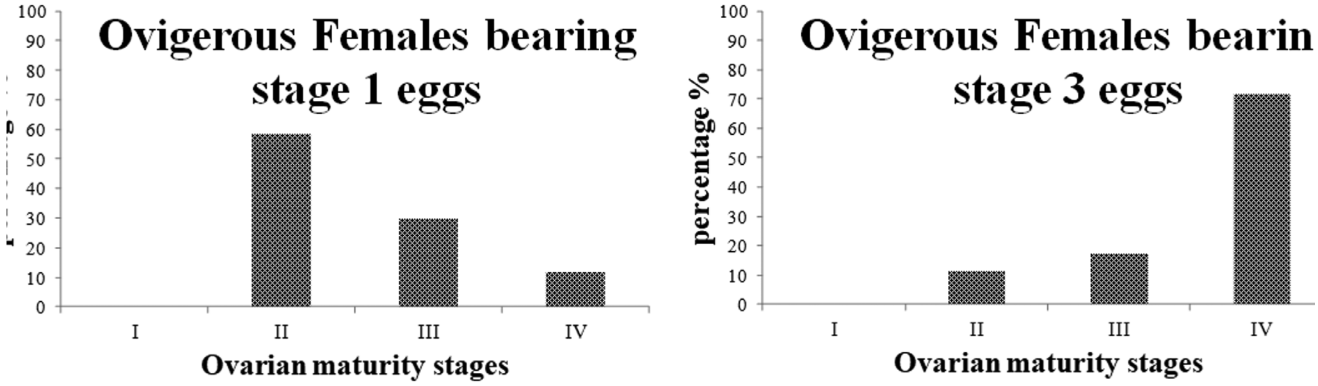

***Fig. 9*: Percentage occurrence of gonadal maturity stages of the ovigerous females Plesionika *narval* carrying eggs in the developmental Stage I (left) and III (right).

(Fig. 10). Mean peak values (± s.e) for the GSI occurred in September (2.39 ± 0.21), June (2.04 ± 0.20) and May (1.90 ± 0.24). In Karpathos, mean GSI was higher in May (2.49 ± 0.34) and September (1.98 ± 0.25), whereas in Simi the highest values of GSI were found in September (2.92 ± 0.34) and June (2.40 ± 0.40) (Fig. 11a). In the shallow zone (10 - 25 m) high GSI mean values occurred in June (2.02 ± 0.27) and December (1.88 ± 0.18). In the depth zone 60-70 m, the GSI peaks were observed in September (3.17 ± 0.38) and June (1.86 ± 0.38), while in the deep zone (110-150 m) peaks were observed in July (2.76 ± 0.60) and September (2.21 ± 0.32) (Fig. 11b).

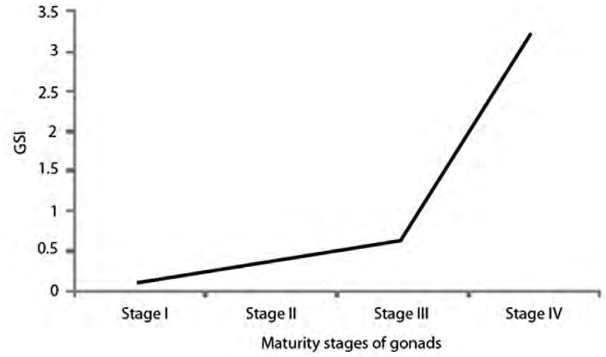

Fig. 10**:** Mean Gonadosomatic Index (GSI) by gonadal maturity stage for the females Plesionika *narval* caught in the Dodecanese Islands.

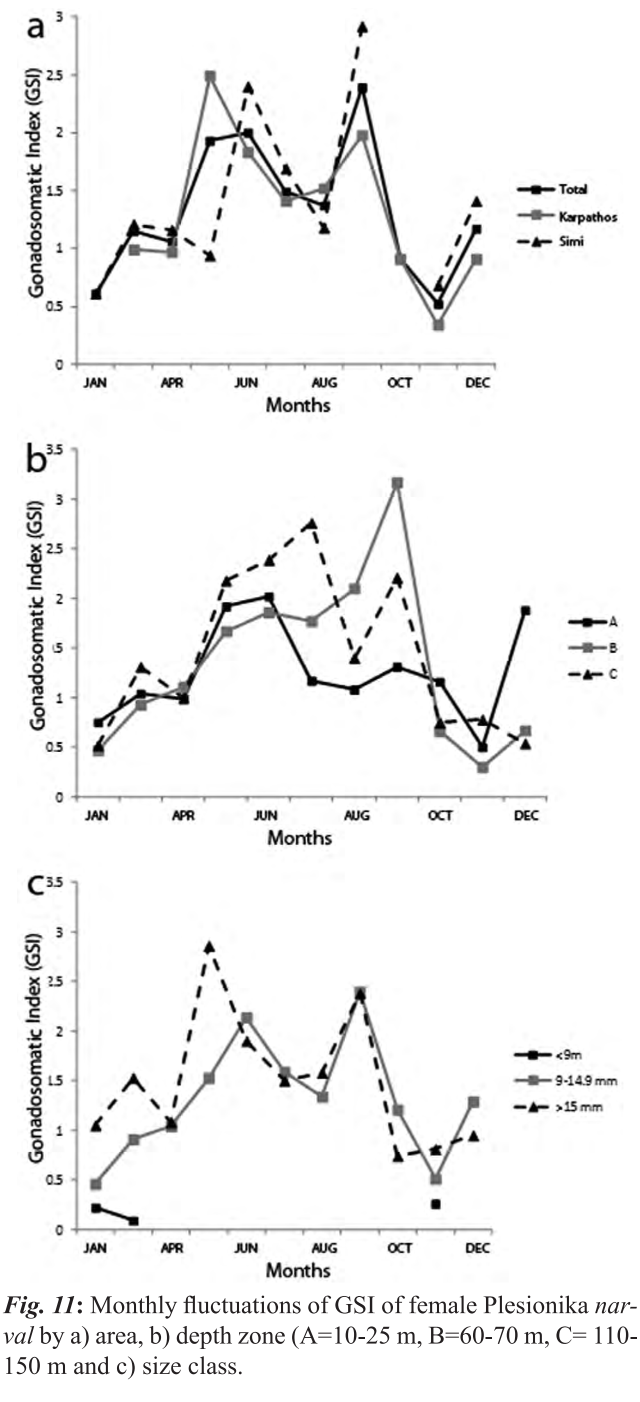

Fig. 11**:** Monthly fluctuations of GSI of female Plesionika *narval* by a) area, b) depth zone (A=10-25 m, B=60-70 m, C= 110-150 m and c) size class.

Smaller individuals (<9 mm CL) were immature and their mean GSI values were very low. Individuals at a size range of 9.0-5.0 mm CL, had GSI peaks in June (2.14 ± 0.28) and in September (2.40 ± 0.30), whereas larger individuals (>15 mm CL) had their peaks in May (2.86 ± 0.49) and September (2.38 ± 0.33) (Fig. 11c).

Size at sexual maturity

Size at first maturity (CL_{50}) was based on 1981 individuals and estimated at CL_{50} = 11.7 mm, (a = -2.515 ± 0.44, b = 0.215 ± 0.03) (Fig. 12).

Discussion

Stages of egg development and ovarian maturity

The images presented in this study with description on stages of egg development and staging of gonad maturity are the first of its kind for this species and constitute a useful tool for future studies.

Female mean size (13.83 mm CL) in the present study is in accordance with that reported for this species in previous studies from the Atlantic Ocean (Sousa *et al*., 2014), the Central Mediterranean Sea (Arculeo & Lo Brutto, 2011) and the Aegean Sea (Thessalou-Legaki *et al*., 1989). The higher mean female size (15.4 mm CL) reported by Gonzales *et al.* (1997) in the East Atlantic could be related to the greater maximum sizes of females (CL_f = 30.9 mm) caught in this area. The higher mean carapace length of females in comparison to males is in agreement with those reported by Thessalou-Legaki *et al.* (1989), Gonzales *et al*. (1997), Arculeo & Lo Brutto (2011), and Sousa *et al*. (2014) for the species. Moreover, female size predominance has been reported for most of the pandalid species (Company & Sarda, 1997; Maiorano *et al*., 2002; Koçak *et al*., 2012), possibly as a result of differential morphological development, reproductive behaviour and different growth rates.

Sex ratio

Sex ratios were significantly different between the two sampling areas and depth zones. In shallow waters the sex ratio was in favour of females in both sampling areas. Furthermore, in Karpathos, females were more abundant in the 60-70 m depth zone, while in Simi Island more males were collected from the 60-70 m and the 110-150 m depth zones. Similar results were obtained by Thessalou-Legaki *et al*. (1989) for Rhodes and Chalki islands of the Dodecanese archipelago and Gonzales *et al.* (1997) for the middle Eastern Atlantic Ocean. Specifically, Gonzales *et al*. (1997) suggest that the observed differences in sex ratios between sampling areas and depths could be related to different habitat preferences, which are strongly influenced by seasonal migrations and different growth rates in males and females. Moreover, the reproductive behaviour of the species, such as mating and coupling, could result in sex ratio differences in Karpathos and Simi islands.

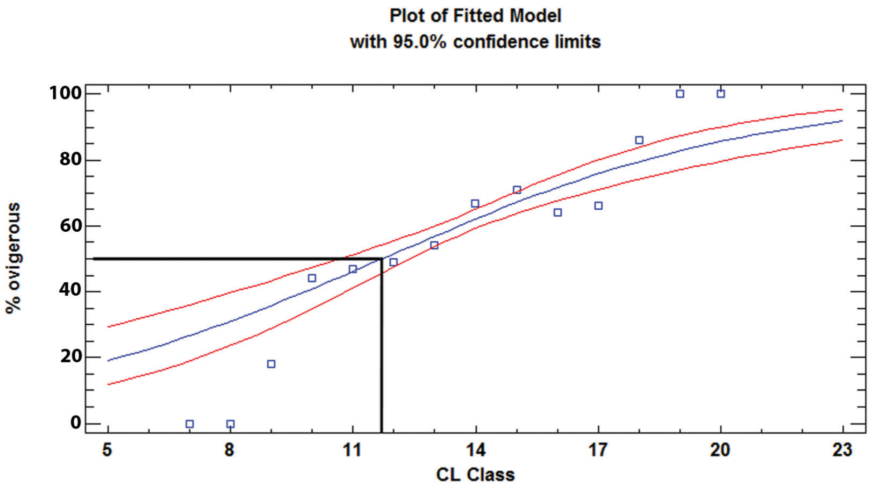

Fig. 12: Logistic curve of size at first maturity for Plesionika *narval*, based on the percentage of ovigerous females in each carapace length class. The line logistic curve corresponds to a proportion of 50% of ovigerous females; observed values (▫); external lines represent the confidence interval (CI) of estimate (95%).

Reproductive Parameters

Mean carapace length of ovigerous and non-ovigerous females was significantly correlated to depth. Similar results were found for *P. narval* in the Western and Central Mediterranean Sea (Carbonell & Abelló, 1998; Arculeo & Lo Brutto, 2011), in the Eastern Mediterranean Sea (Thessalou-Legaki *et al*., 1989; Thessalou-Legaki, 1992), and in the Eastern Central Atlantic (Gonzáles *et al*., 1997). Variation in other pandalid population structures in relation to depth have been recorded for a number of species such as for *Plesionika edwardsii* in the Western Mediterranean Sea (Company & Sardá, 1997) and the Eastern Central Atlantic (Santana *et al*., 1997), and for *Plesionika heterocarpus*, *Plesionika gigliolii* and *Plesionika martia* in the Western Mediterranean Sea (Company & Sardá, 1997). Company & Sardá (1997) suggested that this bigger-deeper phenomenon is related to the reproductive behaviour of the Pandalidae family as the recruitment of young shrimps occurs in specific areas. On the other hand the increase in female size in deeper waters leads to an increase in egg production per female at these depths (Thessalou-Legaki, 1992) which may balance the female predominance in the shallower waters. Company & Sarda (2000) found that some species belonging in the same family (e.g. *P. edwardsii*, *P. giglioli* and *P. martia*) with an intermediate depth range (e.g. 100-871 m) showed a shorter reproductive cycle particularly during spring and summer in comparison with species of the shallowest waters (e.g. *P. heterocarpus*) which have a predominant reproductive pattern and reproductive activity all year around. Based on these data of panadalid shrimps of the Mediterranean Sea, the above-mentioned authors supported their life-history adaptation with depth. A reason for this differentiation could be that the shallower waters are more productive throughout the year than the deeper waters, which allows the shallowest species to have a prolonged reproductive period in contrast to deepest species, which are distributed in a less productive zone and depend on maximum particulate sinking (Company & Sarda 2000 and references herein). A higher diversity of benthic communities inhibiting the Cretan continental shelf (Eastern Mediterranean Sea) in comparison to slope has also been well documented (e.g. Karakassis & Eleftheriou, 1997) and species that perform vertical migrations in the water column (e.g. decapod crustaceans) play a major role in downward benthopelagic coupling: the transfer processes from the pelagic domain to the benthic habitats and the responses of the latter (Tecchio *et al.*, 2013). *Plesionika narval* in the Southeastern Aegean Sea, is the main target species in the small-scale trap-fishery of the narrow shelf and steep slopes. According to classification of slopes by Company *et al.* (2003), *P. narval*, would be categorized in the upper shelf (<150 m). Unfortunately, there are not any comparable reproductive patterns except those of *P. heterocarpus*, in the Northwestern Mediterranean Sea upper slope. A possible explanation for the seasonality observed on the main reproductive period of *P. narval* might be seasonal mass fluxes, and that seasonal periods of organic input to the sea bottom would coincide with the main reproductive period, spring and summer months, as already found for other congeneric species in the Northwestern Mediterranean Sea (i.e. *Aristeus antennatus, P. acanthonotus*) (Company *et al.,* 2003). However, species reproductive strategies are based on a complexity of endogenous and exogenous factors such as environmental differences, energy investment, predation, food availability, light intensity, demographic aspects, phylogenetic constraints, phenotypic flexibility, etc. (Giangrande *et al.,* 1994; Company *et al.,* 2003), most of them not yet investigated, especially for the Eastern Mediterranean Sea, in order to make general conclusions and comparisons with other parts of the same basin.

The presence of ovigerous females carrying eggs of all developmental stages throughout the year confirms previous suggestions for a continuous reproductive strategy in *P. narval* (Gonzáles *et al.*, 1997; Arculeo & Lo Brutto, 2011; Sousa *et al.*, 2014) and other Pandalid species (*P. edwardsi*: Company & Sardà, 2000; *P. martia*: Maiorano *et al.*, 2002) in the Mediterranean Sea. However, the high increase in the percentage of ovigerous females from spring to autumn, suggests that the main spawning period for *P. narval* in the Eastern Mediterranean Sea could be defined as from April to October. The timing of this period is also supported by the high percentage of non-ovigerous females during the late autumn and the winter months (November to March). Spawning peaks seemed to vary between different geographical locations; for example, the spawning peak in the North East Atlantic was in late summer (Sousa *et al.*, 2014), in the Central East Atlantic and the Central Mediterranean Sea from April to June (Martins & Hargreaves, 1991; Gonzáles *et al.*, 1997; Arculeo & Lo Brutto, 2011) and in the Western Mediterranean Sea was found in March (Zariquiey Alvarez, 1968). In Greece (Eastern Mediterranean Sea), Thessalou-Legaki (1992) reported a high percentage of ovigerous females in August (the only sampling month) which is in agreement with our results. According to the results of the present study, the main spawning period of P. *narval* in Greece seemed to be more prolonged than in the other areas and it is extended into October. A prolonged reproductive period has also been suggested for other species of this family such as *P. heterocarpus* and *P. edwardsii* (Zariquiey Alvarez, 1968, Company & Sardà, 1997; González *et al.*, 1997; Colloca, 2002; Maiorano *et al.*, 2002; Chilari *et al.*, 2005; Koçak *et al.*, 2012).

The smallest ovigerous female was 9.16 mm CL, indicating that *P. narval* females > 9 mm CL can contribute to reproduction. According to the growth rate of the species, females become mature in the first year of their life (Kapiris, unpublished data). Arculeo & Lo Brutto (2011) found the smallest ovigerous female to be 7 mm in the Central Mediterranean Sea although the size range with the present work was similar. Monthly distributions for ovigerous females by size in relation to monthly GSI variations showed that smaller individuals become reproductively active a month later than larger ones (≥15 mm), probably because they invest more in growth.

No other study on the gonads of the species has been done so far. A reason for this could be their very small size, which makes the gonads of early stages hardly distinguishable. *P. narval* females with immature ovaries were captured in small percentages only during the November to March period. Individuals with fully mature ovaries (Stage IV) were observed throughout the research period and mainly in March, June, July and September. These results were confirmed by the presence of high percentages of ovigerous females found during the main reproductive period. The simultaneous occurrence of females at all stages of maturity and the presence of ovigerous females throughout the year indicate that *P. narval* is a continuous breeder. The increased percentage of females with mature ovaries observed in March coincides with the increased percentages of ovigerous females observed in April (the onset of the main spawning season). The lowest percentage of fully mature individuals was found in November, immediately after the main reproductive season. The maturation of the ovaries by size class also confirms that smaller females contribute later in the spawning.

The presence of ovigerous females carrying eggs in late maturity stage and mature ovaries, as well as the presence of ovigerous females carrying eggs in the first developmental stage and recovering or resting ovaries suggests that females could spawn more than once within their annual reproductive cycle.

GSI values ranged from 0.13 to 3.26 depending on the maturity stage of the gonads. GSI showed its highest values in May, June and September, during the main reproductive period, indicating that there are at least two spawning events each year for the females larger than 9 mm CL. In Karpathos, ovaries mature a month earlier (May) than in Simi (June) which could be related to several factors such as water temperature or food availability. In both islands, however, the main reproductive period ends in October. The absence of similar studies does not allow us to make any comparative analysis. Nevertheless, the GSI results are in accordance with the reproductive strategy of the species.

Size at sexual maturity

The size at first maturity of female *P. narval* of 11.7 mm CL is in agreement with that of 11.96 mm reported by González *et al.* (1997) in the Atlantic Ocean and smaller than that of 14.61 mm referred by Sousa *et al.* (2014) in the Northeastern Atlantic. The latter difference might be explained by different abiotic conditions (Sousa *et al.*, 2014) and trophic parameters that characterize various areas or the higher size range of specimens analysed by those authors. Since the size at first maturity has been estimated at 11.7 mm CL, the species is able to mature and spawn before they complete one year of life (Kapiris, unpublished data). The size at first maturity is of special importance in fisheries management and is widely used as an indicator for minimum permissible capture size (Lucifora *et al.*, 1999).

In conclusion, the monthly distribution of ovigerous females, GSI and ovary maturity of *P. narval* showed that the reproductive period of the species in the Eastern Mediterranean Sea is continuous with a higher predominance of ovigerous females during April to October, indicating this as the main reproductive period. The presence of ovigerous females throughout the year, allows the continuous entrance of new recruits to the adult population. The occurrence of ovigerous females in Stage I of egg development together with the occurrence of resting/recovery gonads and ovigerous females at Stage III of egg development together with mature gonads, indicates the existence of more than one spawning event during an annual cycle, in agreement with previous studies for decapod crustaceans (Company & Sardà, 1997; Maiorano *et al.*, 2002; Possenti *et al.*, 2007; Ahamed & Ohtomi, 2011). Bauer (1989) reported that this pattern of continuous reproduction is probably an adaptation for species with a short life span.

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Appendix 1 *Plesionika narval* individuals studied from each island, depth zone, and month

Sampling area	Depth zone (m)	*NOV*	*DEC*	*JAN*	*MAR*	*APR*	*MAY*	*JUN*	*JUL*	*AUG*	*SEP*	*OCT*
Karpathos	130-150	133	52	*	50	*	33	65	40	79	34	44
Karpathos	60-70	120	50	*	50	40	30	53	47	51	43	65
Karpathos	15-23	103	50	*	50	40	30	50	49	50	48	67
Simi	110-150	106	51	100	51	*	79	83	52	52	52	*
Simi	60-70	93	67	103	50	40	46	71	50	38	57	*
Simi	10-25	101	50	101	51	40	30	54	52	50	*	*

*no sampling

Appendix 2

Carapace length by sex, month, sampling area and depth zone
(CL: carapace length in mm; s.d.: standard deviation)

Sampling area	Month	Sex	Depth Zone	N	CL_{min}	CL_{max}	CL_{mean}	s.d.
Karpathos	November	♂	A	18	8.66	14.28	10.72	1.46
			B	41	7.75	15.40	10.47	1,61
			C	72	6.46	19.54	10.71	2.20
		♀	A	85	8.13	16.36	11.79	1.90
			B	79	7.13	15.33	11.77	2.10
			C	61	7.54	17.58	13.18	2.28
	December	♂	A	2	10.29	12.23	11.26	1.37
			B	25	7.80	15.03	12.26	1.75
			C	13	8.12	17.92	12.77	2.83
		♀	A	48	10.49	16.30	14.02	1.66
			B	25	10.42	17.87	14.86	2.09
			C	39	11.29	12.15	14.91	1.81
	March	♂	A	4	10.75	12.36	11.50	0.67
			B	22	10.97	14.88	12.73	1.14
			C	6	11.81	15.81	13.56	1.67
		♀	A	46	8.97	16.99	13.46	1.73
			B	28	12.34	18.48	14.97	1.62
			C	44	11.07	19.03	15.56	1.82
	April	♂	A	5	9.59	14.44	12.9	2.00
			B	13	9.98	12.95	11.92	0.88
			C					
		♀	A	35	11.02	15.59	13.82	1.22
			B	27	12.05	17.00	14.04	1.41
			C					
	May	♂	A	8	8.8	13,95	12.00	1.67
			B	7	10.78	13.88	12.25	1.24
			C	23	9.92	15.88	12.63	1.66
		♀	A	22	12.39	16.83	14.58	1.01
			B	23	12.10	16.63	13.77	1.22
			C	10	11.73	18.76	15.95	2.42
	June	♂	A	5	8.44	13.96	10.57	2.07
			B	21	7.78	13.97	11.94	1.59
			C	39	9.44	15.89	12.65	1.52
		♀	A	45	11.,67	19.22	14.47	1.54
			B	32	11.76	17.06	14.92	1.38
			C	26	12.67	18.47	15.68	1.55
	July	♂	A	1			11.22	
			B	14	9.13	14.38	12.67	1.31
			C	29	8.88	15.44	12.29	1.53
		♀	A	48	9.52	18.3	14.96	1.89
			B	33	11.44	17.40	15.66	1.16
			C	11	12.14	17.82	14.99	1.51
	August	♂	A	0				
			B	28	8.15	15.65	12.19	1.57
			C	81	7.79	17.09	12.36	2.22
		♀	A	86	9.32	16.64	13.55	1.81
			B	66	9.72	18.62	14.19	2.10
			C	32	9.17	19.86	15.88	2.57
	September	♂	A	12	9.07	15.83	11.47	1.81
			B	28	8.15	15.65	12.19	1.57
			C	81	7.79	17.09	12.36	2.22
		♀	A	86	9.32	16.64	13.55	1.81
			B	66	9.72	18.62	14.19	2.10
			C	33	9.17	19.86	15.88	2.57
	October	♂	A	6	10.71	16.73	14.26	2.53
			B	33	10.57	16.39	13.07	1.32
			C	4	12.48	15.55	14.16	1.33
		♀	A	61	10.40	17.53	13.88	1.88
			B	32	11.05	17.66	15.71	1.77
			C	40	12.01	20.20	17.10	1.55

(continued)

Appendix 2 *(continued)*

Sampling area	Month	Sex	Depth Zone	N	CL_{min}	CL_{max}	CL_{mean}	s.d.
Simi	November	♂	A	18	7.35	14.58	11.35	2.17
			B	65	6.69	15.91	10.91	2.01
			C	40	7.18	13.58	9.42	1.57
		♀	A	83	9.08	16.6	12.15	1.62
			B	28	8.33	16.96	13.14	2.36
			C	66	7.71	16.42	11.99	2.30
	December	♂	A	1			11.74	
			B	36	9.24	14.77	11.74	1.56
			C	17	10.14	15.68	13.08	1.69
		♀	A	49	10.26	16.28	13.47	1.54
			B	31	10.11	16.36	12.63	1.82
			C	34	11.27	18.48	14.86	2.23
	January	♂	A	29	10.25	15.48	12.32	1.41
			B	55	10.13	15.76	12.51	1.47
			C	61	11.13	16.06	13.61	1.20
		♀	A	72	7.95	17.69	13.45	1.50
			B	48	7.45	16.93	12.86	2.18
			C	39	10.97	17.67	15.04	1.72
	March	♂	A	16	9.13	14.03	12.61	1.23
			B	9	10.89	13.36	11.98	0.75
			C	21	7.79	14.21	12.36	1.61
		♀	A	35	10.02	17.27	13.86	1.58
			B	41	11.26	17.01	13.23	1.40
			C	30	10.32	16.32	13.19	1.66
	April	♂	A					
			B	15	9.17	14.76	12.10	1.56
			C	21	10.42	14.59	12.81	1.24
		♀	A					
			B	25	10.79	17.04	13.93	1.82
			C	19	11.82	18.35	14.72	1.68
	May	♂	A	11	10.50	13.89	12.00	0.99
			B	87	9.97	10.02	12.34	1.24
			C	69	9.86	16.41	12.68	1.63
		♀	A	19	10.24	13.33	13.59	1.81
			B	6	10.82	15.97	12.79	1.93
			C	10	10.75	18.48	14.11	2.05
	June	♂	A	24	9.01	13.84	11.60	1.18
			B	48	9.75	15.64	12.58	1.18
			C	76	9.59	16.68	13.14	1.50
		♀	A	30	10.61	17.02	13.94	1.55
			B	23	11.32	16.33	14.40	1.61
			C	7	11.82	18.66	14.87	2.29
	July	♂	A	7	8.74	12.49	11.43	1.31
			B	18	9.55	14.93	12.15	1.30
			C	46	8.08	15.47	13.04	1.39
		♀	A	45	9.16	16.01	12.88	1.69
			B	32	11.65	16.42	14.17	1.43
			C	6	15.00	18.68	16.50	1.45
	August	♂	A	22	9.23	13.99	11.35	1.22
			B	29	11.05	15.41	13.14	1.31
			C	38	7.88	15.79	12.50	1.34
		♀	A	28	9.62	16.69	12.78	1.77
			B	9	12.27	16.52	14.50	1.59
			C	14	10.89	15.79	13.92	1.53
	September	♂	A					
			B	29	9.19	15.44	11.62	1.53
			C	12	10.37	14.83	12.48	1.43
		♀	A					
			B	28	9.65	15.91	13.22	1.84
			C	40	12.26	18.01	14.92	1.29