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Reproductive biology of the blue swimming crab, *Portunus segnis* (Forskål, 1775) in Yumurtalık Cove, North-eastern Mediterranean, Turkey

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Abstract

The blue swimming crab, *Portunus segnis* is a Lessepsian migrant into the Mediterranean and little is known about its biology in Iskenderun Bay, North-eastern Mediterranean, Turkey. In this study, we examined the sex ratio, gonadal development stages, gonadosomatic index, size at first sexual maturity, and fecundity of *P. segnis*. The specimens of *P. segnis* were collected monthly from July 2014 to June 2015 (except February) using a trawl net from Yumurtalık Cove. The crabs were found throughout the year and were abundant from July to October (74%). Carapace width (CW) range of all samples was 38.1-163.2 mm, and body weight ranged from 3.5 to 324.4 g. The population's sex ratio was Male:Female=0.7:1, which shows that the female population is more abundant. Fifty percent of the female crabs attained sexual maturity when they reached the size of 115-120 mm CW. The ovigerous crabs ranging between 101.4 and 154 mm CW, and 88.3-324.4 g in body weight can produce 139379 to 2745236 eggs. The mean fecundity of *P. segnis* was 1070425 ± 580978 eggs with mean carapace width of 130.0 ± 12.8 mm and mean body weight 191 ± 59 g. The crabs produced 5607.8 ± 2559.9 eggs (mean \pm sd) per gram of body weight. In Yumurtalık Cove, breeding of *P. segnis* was observed throughout the year, except in winter. Further studies are needed to assess the reproductive biology of blue crabs in other locations of Turkey.

Keywords: *Portunus segnis*, fecundity, size at sexual maturity, Iskenderun Bay, Lessepsian, Crustacea, Portunidae.

Introduction

The blue swimming crab, *Portunus segnis* inhabits a wide range of inshore and continental shelf areas, including sandy, muddy and sea grass habitats, from the intertidal zone to at least 50 m depth. This species is distributed in the western Indian Ocean, from the East coast of South Africa to Pakistan, the Persian Gulf and the Red Sea (Lai *et al.*, 2010).

In the North-eastern Mediterranean, the blue crab *Callinectes sapidus* is the most economically important decapods crustacean, with *Portunus segnis* is second place (Tureli *et al.*, 2000). The blue swimming crab, *Portunus segnis* (Forskål, 1775), previously synonymised with *P. pelagicus* (Linnaeus, 1758) (Lai *et al.*, 2010) is a Lessepsian migrant into the Mediterranean via the Suez Channel and has settled along the coasts of Egypt, Israel, Syria, Tunisia and Turkey. This species was reported for the first time in Turkey, at Iskenderun Bay (Gravel, 1928). According to FAO, in 2014, a total of 212.571 tonnes of *P. segnis* were caught in the world. According to the Turkish Statistical Institute, in 2015, a total of six tonnes of blue crab were caught in Turkey. Ozcan (2012) reported that *P. segnis* was commercially important for local fish markets in the bays of Mersin and Iskenderun where one crab specimen is sold for 0.25 to 0.42 Euro.

The swimming blue crab *P. segnis* has been studied throughout its distribution area, e.g. in the Persian Gulf,

Iran (Safaie *et al.*, 2013a,b; Hosseini *et al.*, 2012; Hosseini *et al.*, 2014 a,b). *P. segnis* is one of the dominant crab species found in Iskenderun Bay on the Turkish Mediterranean coast, although there are few studies about *P. segnis* in Turkey. Tureli and colleagues (2000) compared the meat composition and yield of *C. sapidus* and *P. segnis* caught in Iskenderun Bay. Gokoglu and Yerlikaya (2003) investigated the mineral contents of the blue crab and swimming crab, caught off the Gulf of Antalya. Ozcan (2003) researched the distribution of the blue crab (*C. sapidus*) and sand crab (*P. segnis*) in Iskenderun Bay. Ozcan and Akyurt (2006) have studied the population biology of *P. segnis*. Ayas (2010) and Ayas & Ozoğul (2011a), reported the chemical composition of *P. segnis* caught in Mersin Bay. Ayas & Ozoğul (2011b) also published information on the effect of vacuum packing on the quality of thermally processed crabmeat. Ozcan (2012) detected the barnacle *Chelonibia patula* on the carapace and chelipeds of some specimens of *P. segnis*. Ayas (2013) investigated the effects of gender and season on potentially toxic metal levels in two muscle types of adult *Portunus pelagicus*, caught in Mersin Bay. Inandi (2015) conducted research on the bio-ecological characteristics of the blue swimming crab in Iskenderun Bay. Ayas (2016) investigated the effects of season and sex on the nutritional quality of muscle types of *C. sapidus* and *P. segnis*. Thus, little is known about the biology of *P. segnis* in North-eastern Mediterranean of Turkey.



Fig. 1: Yumurtalik Cove (Iskenderun Bay, North-eastern Mediterranean, Turkey).

The reproductive biology of crustaceans has been widely studied, mainly of those species that have commercial value or ecological potential. Studies on the reproductive biology of *Portunus* species have been carried out in various regions: in Australia (Potter *et al.*, 1983; Sumpton 2001; de Lestang *et al.*, 2003; Kumar *et al.*, 2003; Johnson *et al.*, 2010); in India (Pillay & Nair, 1971; Sahoo *et al.*, 2011; Soundarapandian *et al.*, 2013); in the Philippines (Bato *et al.*, 1987); in Malaysia (Arshad *et al.*, 2006; Ikhwanuddin *et al.*, 2011; Ikhwanuddin *et al.*, 2012); in Iran (Kamrani *et al.*, 2010; Safaie *et al.*, 2013b; Hosseini *et al.*, 2012; Hosseini *et al.*, 2014 b); in Indonesia (Sara *et al.*, 2016; Zairion *et al.* 2015; Hamid *et al.*, 2016). In Turkey, however, no research has been conducted on the reproductive biology of Lesepsian *Portunus* species. The study of gonads, sizes at sexual maturity and fecundity are important aspects of the reproductive biology of crabs. The purpose of this study was to investigate the sex ratio, gonadal development stages, gonadosomatic index (GSI) and size at first sexual maturity of females, and estimate the fecundity of *P. segnis* in Yumurtalik Cove, Turkey. Information on reproductive biology plays an important role when formulating management decisions and policy advice for any fishery as spawning is the basis for recruitment. The results of this

study will be helpful to understand the breeding season of the species, determine its population dynamics and status of recruitment in Yumurtalik Cove. Also, the results will be useful for comparing the different stocks of *P. segnis* at different locations.

Material and Methods

Study Site and Collection of Samples

Samples of *P. segnis* were collected monthly from July 2014 to June 2015 (except February) from Yumurtalik Cove (Iskenderun Bay- North-eastern Mediterranean, Turkey) (Fig. 1). Specimens were sampled with a small bottom trawl net (15 m head rope with 14 mm-mesh cod end), towed for 45 minutes at 0-50m depths. Bottom water temperature, salinity and pH were measured by a CTD (YSI 6600 multiparameter probe). After sampling, the individuals were transferred to the laboratory in a cooler and stored in a deep freezer for further analysis. Water temperature fluctuated during the study period, with the highest average values occurring in August ($28.55 \pm 0.52^\circ\text{C}$) and the lowest average values occurring in March ($16.60 \pm 0.24^\circ\text{C}$). Salinity ranged from 37.40 ± 0.65 ‰ (the lowest in March, 2015) to 38.61 ± 0.78 ‰ (the highest in October, 2014).

Carapace Width and Body Weight Measurements and Sex Determination

A total of 140 females, 110 males and 70 juveniles of this species were collected. Each crab was measured and its carapace width (CW), carapace length (CL), body weight (BW) and sex were recorded. The CW and CL of each crab were measured to the nearest 0.01 mm using a digital calliper. The BW of the crab was measured to the nearest 0.1 g using a digital balance. An immature crab's abdomen is tightly sealed to the cephalothorax (Zairion & Fahrudin, 2015). Male blue swimming crabs have a V-shaped abdomen and female crabs have a broad and rounded abdomen (Hosseini *et al.*, 2014b)

Reproduction

Determination of the reproductive biology of *P. segnis* in Yumurtalık Cove was divided into 5 major parts described as follows:

Sex ratio

Total numbers of mature male and female crab data sets were used to calculate the sex ratio per month. The Chi square (X^2) statistic was used to test the difference between ratios in both sexes. The male to female sex ratio was determined every month as the proportion of females to males. To assess if the calculated monthly sex ratio differed significantly from the expected ratio (1:1), a chi-square goodness of fit test (χ^2) was used.

Gonad and Ovarian Development

The carapace of the crabs was opened to observe gonadal development. Each month, mature female ovaries were dissected and macroscopically examined to determine the maturity stages. Four stages were used to classify females based on the reproductive staging criteria developed by Kumar *et al.* (2000): stage I - gonad immature, ovary white coloured, stage II - gonad maturing or developing, ovary light yellow in colour, stage III - gonad mature, ovaries change colour to yellow/orange, stage IV - gonad ripe, ovaries turn dark, orangish or red.

Gonadosomatic index (GSI)

The weight of the ovaries was measured to the nearest 0.01 g. GSI was calculated by dividing the weight of the ovaries by the body weight and multiplying by 100 (Soundarapandian *et al.*, 2013).

Size at sexual maturity

The size at first sexual maturity of females was determined by using the minimum size class data of females. Gonad stages 3 and 4 and ovigerous crabs were assumed mature, while gonad stages 1 and 2 were assumed immature. The size at which 50% (L_{50}) of females were sexually mature was determined from the percentages of mature

female crabs in each of the five mm CW size classes and the logistic regression curve described by King (2007):

$$P = 1 / (1 + e^{-r(L_T - L_{50})})$$

where P: Percentage of mature individuals, r: slope of curve, L_T : Total size, L_{50} : size at which 50% of females were sexually mature.

Fecundity

A total of 44 ovigerous females were collected. Firstly, the egg batches were removed from the broad abdomen of the crab pleopod, and the wet weight of the total egg batch was measured. Three replicate subsamples were taken from each egg batch. Each of those subsamples was weighed to the nearest 0.001g, immersed in 30% glycerol solution and the number of eggs counted under a dissecting microscope. These data were then used to estimate the total number of eggs of each female. The relationship between batch fecundity (BF) and carapace width (CW) was calculated with the equation:

$$BF_j = a + bCW_j$$

where, a is a constant number, b is the slope of curve.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows (version 10). Differences in the regression slope for the relationships between fecundity and body size were analyzed statistically using a t-test. Statistical tests involved the use of simple regression analysis and one-way analysis of variance (ANOVA). ANOVA was used to test GSI differences per month. All statistical effects were considered significant at $\alpha = 0.05$.

Results

A total of 320 blue swimming crabs, 140 females (44 ovigerous females), 110 males and 70 juveniles were caught. The highest number individuals were caught in October (22.5% of total specimens). Mature crabs were found throughout the year (Fig. 2 and Fig. 3). The juveniles were found from July to October, and also in December and January but not in November (Fig. 2). Carapace width ranged from 55.3 to 163.2 mm in mature females and from 48.7 to 154 mm in mature males (Table 1). Carapace width of all samples ranged from 38.1 to 163.2 mm and body weight ranged from 3.5 to 324.4 g (Table 1). The females were wider than the males ($F_{1,2}=15.96$; $P=0.00$).

Sex ratio

The sex of crabs can be determined directly through visual observations by identifying the shape of the abdomen. During the study period, the expected sex ratio (1:1) was not recorded any month. Male crabs were predominant in July while the females were dominant in the

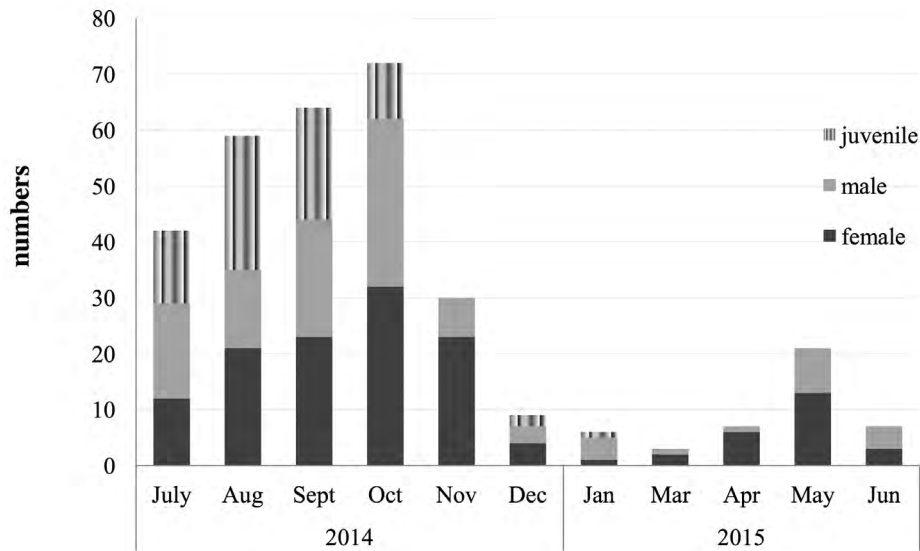


Fig. 2: Monthly density of all individuals.

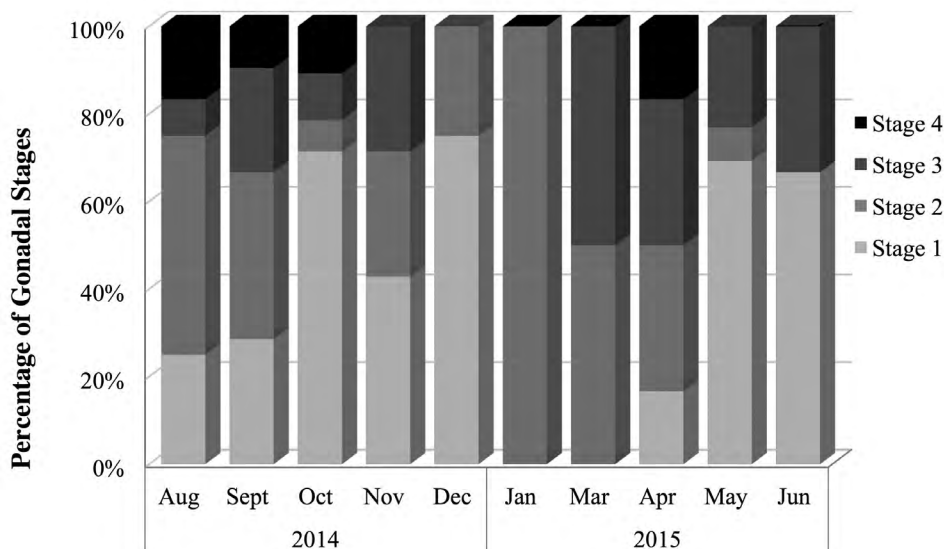


Fig. 3: The percentage of gonad maturity stages of female blue swimming crabs.

other months (Table 2). There were 250 samples of observed crabs consisting of 110 males and 140 females. The sex ratio was 0.7:1 or 44% male and 56% female. A chi-square (X^2) test indicated that this ratio was not significantly ($P > 0.05$) different from the expected 1:1 ratio. In other words, the ratio between males and females is not equal (one:one).

Gonad Development

The monthly variations in the different maturity stages of female ovaries are shown in Figure 3. In July, it was not possible to investigate the gonadal stages of the crabs.

Female crabs at maturity stage 1 occurred during all months, except in January and March, while October had

the highest percentages (71%) at that stage (Fig. 3). In January, all the examined specimens were at maturity stage 2. The highest percentage of crabs at maturity stage 3 was observed in March (50%). Maturity stage 4 specimens were found from August to October and in April, with the highest percentages in August and April.

Gonadosomatic Index (GSI)

The GSI values for the 113 female specimens ranged from 0.13 to 13.60% with a mean of 2.21 ± 2.67 (SD)%. In females, mean GSI values increased with a small peak from August to September and then declined in October. It increased again from December to March and the highest GSI value was observed in March (Fig. 4). It declined in April. It increased again from May to June. According

Table 1. The morphological characteristics of *P. segnis* in Yumurtalık Cove.

Sex	Number	CW (mm)	CL (mm)	W (g)
Male	110	48.7-154 (111.6±23.1)	10.7- 74.2 (51.2±11.9)	18.4-299.8 (124.3±76.9)
Female	140	55.3-163.2 (125.5±16.8)	24.5-74.6 (56.4±7.9)	10.9-324.4 (154.3±61.4)
Ovigerous female	44	101.4-154 (130.4±12.8)	45.9-72.9 (58.4±6.3)	88.3-324.4 (191±59)
Juvenile	70	38.1-117.4 (75.8±20.1)	17.2-51.5 (33.9±24.8)	3.5-109.3 (33.9±24.8)

Note: CW: carapace width, CL: carapace length, BW: body weight, numbers in parenthesis refers to mean ±SD.

to Figure 4, the peak of spawning and breeding seasons for females seems to occur three times during the year. Significant differences in females GSI were noted during the observation period. ($F_{1,10}=2.57; P=0.00$).

Size at first sexual maturity and $LM_{50}\%$

The smallest mature female was 80.63 mm CW, and the largest was 163.17 mm CW. The logistic curve fitting the estimation of carapace width at which 50 percent of female crabs are sexually mature is shown in Figure 5. Fifty percent of the crabs attained sexual maturity when they reached the size of 115-119.99 mm CW.

Fecundity

The ovigerous crabs in Yumurtalik Cove ranged from 101.4 to 154 mm in carapace width and 88.3-324.4 g in body weight (Table 1), with mean carapace

width 130.4±12.8 mm (mean±sd) and mean body weight 191±59 g (mean±sd). Monthly changes of ovigerous female frequency is shown in Figure 6. Three peaks were observed, one in August, one in September and the other one in May.

Determination of fecundity of *P. segnis* from Yumurtalik Cove showed that crabs can produce 139,379 (min) to 2,745,236 (max) eggs. During the study, the mean fecundity of *P. segnis* was 1.070.425±580.978 eggs (Fig. 7). Furthermore, the crabs produced 5607.8±2559.9 eggs (mean±sd) per gram of body weight. The estimated regression equation of CW on fecundity was $F=-2.10^6 + 25430.CW$ ($R^2=0,312$). Regression analysis showed that there was a significant weak positive relationship between carapace width and fecundity (Fig. 7). A linear regression of fecundity against carapace width for all crabs was significant ($F_{1,40}=18.1; P=0.00$).

Table 2. Monthly variation of sex ratio (male:female) and p-value (obtained from chi-square test) of *P. segnis* in Yumurtalık Cove.

Month	male	female	male:female	% of female	p value
Jul	17	12	1.4:1.0	41.3	0.112
Aug	14	21	0.6:1.0	60	0.633
Sep	21	23	0.9:1.0	52.22	0.618
Oct	30	32	0.9:1.0	51.6	0.486
Nov	7	23	0.3:1.0	76.6	0.022
Dec	3	4	0.7:1.0	57.1	0.951
Jan	4	1	4:1.0	20	0.104
Mar	1	2	0.5:1.0	66.6	0.709
Apr	1	6	0.1:1.0	85.7	0.113
May	8	13	0.6:1.0	61.9	0.585
Jun	4	3	1.3:1.0	42.8	0.483
Total	110	140	0.7:1.0	56	0.141

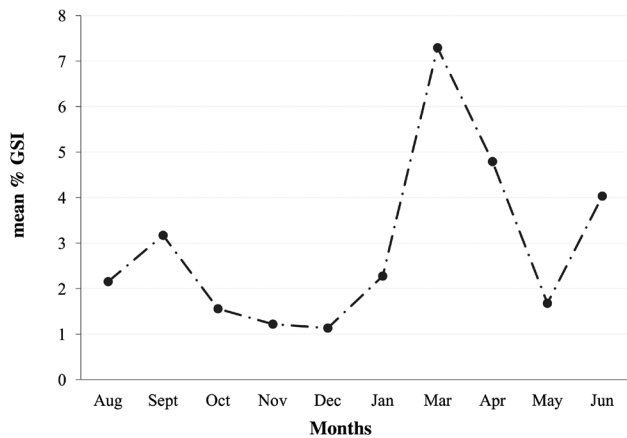


Fig. 4: Monthly variation of the mean values of the gonadosomatic index of female crabs.

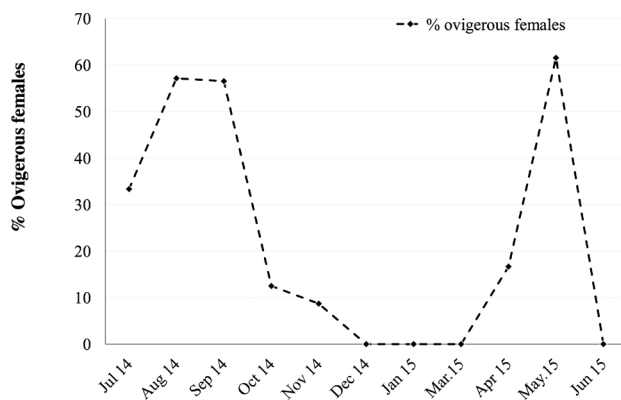


Fig. 6: The percentage of ovigerous crabs.

Discussion

During this study, carapace width (CW) of mature *P. segnis* was 48.7-154 mm in males and 55.3-163.2 mm in females (table 1). In Iskenderun Bay, Inandi (2015) reported a size of CW 47.2-169.8 mm in males and 51.3-171.5 mm in females. In the Persian Gulf, Hosseini and colleagues (2014a) caught *P. segnis* specimens with CW 75-175 mm in males and 70-165 mm in females, using a beam trawl. Furthermore, Hosseini and colleagues (2014b) also reported a CW in males of 60-175 mm and in females of 55-170 mm using a trawl net. When we compared our results with these studies, we found that the CW of crabs is similar. In Iskenderun Bay, Inandi (2015) reported that the CW of females is bigger than that of males but the difference was not significantly important. We also recorded CW of females wider than males and it was significantly important ($p=0.000$).

The crabs were found throughout the year and were abundant from July to October (74%) (Fig. 2). The reason for that is possibly due to the fact that from July to October mean water temperature (mean \pm SD, 27.3 \pm 1.5

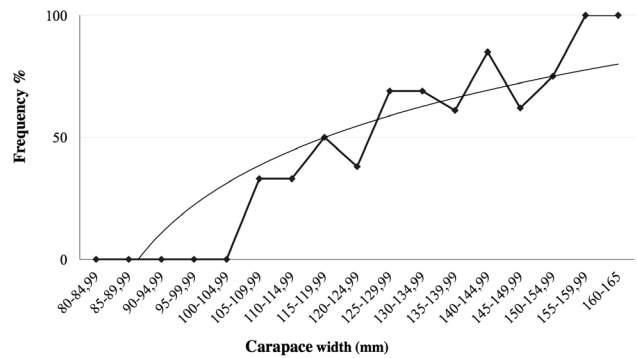


Fig. 5: A logistic curve for estimation LM₅₀%.

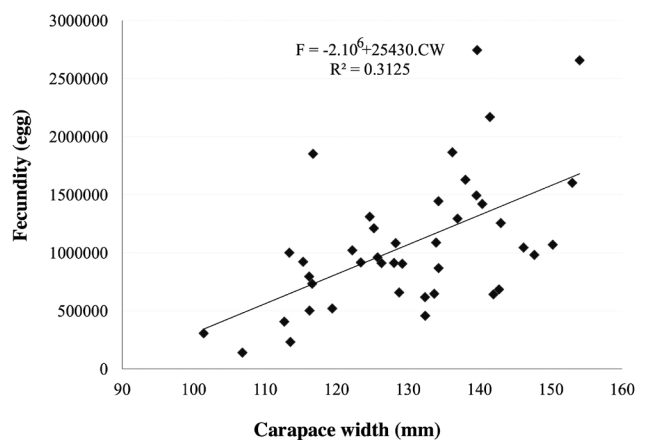


Fig. 7: Relationship between carapace width and fecundity of *P. segnis*.

$^{\circ}$ C) and salinities (mean \pm SD, 38.5 \pm 0.1 psu) were higher than during the other months.

In this study, the population sex ratio obtained was M:F=0.78:1, which showed a more abundant female population (table 2). Similar results were found for *P. segnis* in the Persian Gulf by Safaie *et al.* (2013b) and Hosseini *et al.* (2014a; b). However, in Iskenderun Bay, Inandi (2015); and in the Persian Gulf Kamrani *et al.* (2010) and Hosseini *et al.* (2012), found a sex ratio of M:F= 1:0.69, M:F=1.2:1.0 and M:F= 1:0.88 respectively. Some researchers have mentioned that crabs in bay ecosystems often do not migrate when spawning and, under certain conditions when there is a change in salinity, crabs spawn in the waters of the bay with high salinity (Sumpton *et al.*, 1994; de Lestang *et al.*, 2003). In our study, salinity ranged from 36.6 to 38.9 psu; it was quite high and our results show a more abundant female population in Yumurtalık Cove.

In the Persian Gulf, all stages of gonad development and ovigerous females of *P. segnis* were observed throughout the year (Kamrani *et al.*, 2010 and Safaie *et al.*, 2013). In this study, not all stages of gonad de-

velopment were observed throughout the year and no ovigerous females were noted from December to March. Spawning is correlated with seasonal changes in sea-surface temperature (Dhawan *et al.*, 1976). In our sampling area, water temperature is relatively low from December to March (mean 16.3 ± 0.3 (SD)).

Our research indicates that in females the highest percentage of mature and ripe gonads was observed from August to October and in April (Fig. 3). Furthermore, a higher proportion of ovigerous females occurred from August to September and in May (Fig. 6). According to Inandi (2015), in Iskenderun Bay, egg bearing individuals of *P. segnis* were obtained between April and November. In the Persian Gulf, Safaie *et al.* (2013) reported that the highest proportion of ovigerous *P. segnis* females was caught in October and September. Thus, in Iskenderun Bay and in the Persian Gulf, ovigerous *P. segnis* females were recorded in the same months. In Yumurtalik Bay, at the end of the spring to early autumn, water temperature is very high (mean 26°C) and can affect the reproduction of crabs in this area.

In this study, the size at which 50% of female *P. segnis* attain maturity was 115-120 mm CW (Fig. 5). It was similar to that of a corresponding study on *P. segnis* in the Persian Gulf (113 mm CW, Safaie *et al.*, 2013). However, Hosseini *et al.*, (2014b) found that 50% of female *P. segnis* reach sexual maturity in the Persian Gulf when CW reached 75 mm. Environmental conditions affect the growth and size of crabs at maturity. The results of Fisher's (1999) logistic regression model demonstrate the effects of salinity and temperature on percent maturity according to carapace width. The same author claimed that the size at maturity in female blue crabs decreases in line with increases in salinity and temperature; for example at low temperature and salinity (10°C and 0‰), 50% maturity was attained at 156 mm, whereas at high temperature and salinity (30°C and 30‰), 50% maturity is attained at 106 mm. In Yumurtalik Cove, during the study period, average water temperature was $16.6 \pm 0.2^{\circ}\text{C}$ (the lowest) and $28.5 \pm 0.5^{\circ}\text{C}$ (SD) (the highest); average salinity was $37.4 \pm 0.6\text{‰}$ (the lowest) and $38.6 \pm 0.8\text{‰}$ (SD) (the highest) (Figure 3). Thus, in Yumurtalik Cove, with high water temperature and salinity, 50% maturity was attained at 115-120 mm CW.

Fecundity refers to reproductive output, which indicates the number of eggs produced in every batch. Egg production is directly related to body weight and growth of individuals. In our research, the fecundity of *P. segnis* varied from 139,379 to 2,745,236 for CW ranging from 101.4 to 154mm and 88.3-324.4g body weight (Table 1). The mean number of eggs was $1,070,425 \pm 580,978$ (SD) for a female with a mean CW of 130.0 ± 12.8 (SD) mm and with a mean body weight 191 ± 59 g. In Iskenderun Bay, mean fecundity of *P. segnis* was $805,851 \pm 93,792$ eggs (range: 74,567- 1,929,117 eggs) (Inandi, 2015). In the Persian Gulf and the Sea of Oman, the fecundity of

P. segnis varied from 521,027 to 6,656,599 (103-155mm CW), with a mean of $2,397,967 \pm 1,326,721$ (SD) eggs. An analysis of the CW/ fecundity relationship found $F=0.063828 \text{ CW}+0.06$, indicating that fecundity increases with an increase of CW ($R^2=0.37$) (Safaie *et al.*, 2013). Our results also showed that fecundity increases with an increase of CW ($R^2=0.31$). High fecundity was found in larger crabs because of a longer intermolt period between population and egg extrusion than small crabs (Kamrani *et al.*, 2010), which indicates that they have more time to accumulate the energy reserves required to produce eggs. This difference accounts for the greater number of eggs produced by larger rather than small crabs (de Lestang *et al.*, 2003).

Conclusively, our findings show that *P. segnis* in Yumurtalik Cove -Iskenderun Bay (North-eastern Mediterranean) varied in their sex ratios, maturity size, fecundity, and size-fecundity relationships when compared to studies conducted in other water bodies. Some important aspects of the blue swimming crab's reproductive status have been documented in its distribution area (e.g. Safaie *et al.*, 2013; Hosseini *et al.*, 2014a). In contrast, despite extensive research on the reproductive biology of the blue swimming crab within its endemic range, there is little information on aspects of the reproductive status of the blue swimming crab outside of this range. The data obtained from the current study constitutes a useful source of information about the blue swimming crab outside its endemic ranges, the Mediterranean coast of Turkey in particular. Size at maturity for a single stock can differ by year and stock abundance (Fogarty & Lipcius, 2007). Along the North-eastern Mediterranean coast of Turkey, average-sized ovigerous crabs have a carapace width of 130.0 ± 12.8 mm and maturity size of 115-120 mm CW, which is larger than in endemic ranges. Furthermore, in Yumurtalik Cove, the breeding season of *P. segnis* extends throughout the year, except winter. However, further studies are required to assess the reproductive biology of blue crabs in other locations of Turkey.

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