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Reproductive biology of *Charybdis* (*Goniohellenus*) *longicollis* Leene, 1938 (Brachyura: Portunidae), in the north-eastern Mediterranean Sea, Turkey

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ABSTRACT

The swimming crab, *Charybdis (Goniohellenus) longicollis* Leene, 1938, is a Lessepsian migrant into the Mediterranean and little is known about its biology in Iskenderun Bay, north-eastern Mediterranean, Turkey. The size of crabs, sex-ratio and spawning period of this species was defined from 951 specimens in order to understand their reproduction and development in the North-Eastern Mediterranean Sea. The crabs were found to occur throughout the year. Carapace width (CW) ranged between 7.22 to 100.60 mm and total weight ranged from 1.34 to 164.09 g. Male-female sex ratio was 0.6:1. Highest gonado-somatic index (GSI) was seen in March and September for females and in May for males. Ovigerous females were present throughout the year, with peak spawning activity in July and September. The CW of the ovigerous female crabs ranged between 23.71 and 95.93 mm. In winter months, GSI of males and females decreased. However, spawning season of *C. (G.) longicolis* in Yumurtalık Cove was observed throughout the year.

Keywords: *Charybdis (Goniohellenus) longicollis*, Crustacea, Iskenderun Bay, Lessepsian, Portunidae, Reproductive biology

Introduction

The swimming crab, *Charybdis (Goniohellenus) longicolis* Leene, 1938*,* is known to occur in the East African coast, the Red Sea, the Gulf of Aden, the Gulf of Oman and the Arabian Gulf. *C. (G.) longicollis* was first recorded in the Mediterranean in 1954 from Mersin Bay, Turkey (Holthuis, 1961). Since then, it has invaded and established a large population throughout the north-eastern Mediterranean coast of Turkey (Ozcan *et al.,* 2005; Marun, 2016) and has been recorded all along the Levantine coast, from Egypt to the Aegean coast of Turkey. Portunidae is the most successful family in colonisation of Mediterranean coasts. In fact, among the 54 Brachyura listed by Galil *et al.* (2015), 14 species belong to this family with 6 species in the genus *Charybdis* (Kondylatos *et al*., 2017).

Previous studies on *C. (G.) longicollis* have reported the species' distribution (Kocatas,1981; Kocatas and Katagan,1994; 2003; Enzenross and Enzenross, 1995; Zenetos *et al.,* 2005; Naderloo and Sari, 2007), parasites (Oksnebjerg *et al.,* 1997; Galil and Innocenti, 1999; Innocenti *et al.,* 2010; Ozcan and Katagan, 2016; Innocenti *et al.,* 2017), width/length-weight and width-length relationships from the north-eastern Mediterranean coast of Turkey (Sangün *et al.,* 2009) and sex distribution, morphometric aspects, condition factor and fecundity in Iskenderun Bay (Marun, 2016).

However, very little is known about the biology of *C. (G.) longicollis* in any area of its distribution, probably because it does not reach a commercial size and value. In Turkey, there have been no studies on the reproductive biology of the species. The purpose of this study was to investigate on the reproductive biology of female *C. (G.) longicollis* in Iskenderun Bay, Yumurtalık Cove, Turkey.

Materials and methods

C. (G.) longicollis were sampled monthly in the Yumurtalık Cove 36° 42' N; 35° E - 35° 49' E between July 2014 and June 2015 using a small shrimp trawl (15 m head rope with 14 mm-mesh cod end) towed for 45 min at three different depths; 0-10, 10-20 and 20-50 m. Bottom water salinity, temperature, dissolved oxygen (DO) and depth were measured at each station using a CTD probe (YSI 6600 multiparameter probe) and all individuals were held in ice until laboratory analyses.

In the laboratory, crabs were sorted sex-wise and individual records of carapace widths (CW, mm) and total weight (TW, g) were taken. Missing legs were noted and the opposing leg, if present, was removed, weighed and its weight was added to the total (Gelpi *et al.,* 2009).

The total number of male and female crabs was used to calculate the sex ratio per month. Chi-square (χ^2) statistic was used to test the difference between the sexes.

Juveniles and adults (including ovigerous females) were described by the form of the abdomen and the presence of egg masses (Williams, 1974). In females, ovaries were dissected out and examined to classify the maturity stages (gonad development stages) (Wenner, 1989). Four stages were categorised as follows: stage 1 - immature ovary, white; stage 2 - maturing or developing ovary, light yellow; stage 3 - mature ovary, yellow/orange and stage 4 - ripe ovary, dark orange or red.

Weight of the ovaries of crabs was measured to the nearest 0.001 g. GSI was calculated using the following formula (Soundarapandian *et al.,* 2013):

GSI = (Wet weight of the gonad/Wet weight of the body) x 100

Fecundity was estimated by analysing 118 female crabs. Egg mass colours were classified as bright orange=1, dark orange=2, brown=3, dark brown=4 and black=5 (Jivoff *et al.,* 2007) and then, the egg masses were removed from the pleopods for measurement of weight. From each egg mass, three 0.01 g subsamples of eggs were randomly separated, weighed, spread on the microscope slide with one side striped with glycerol (30%) and the eggs were counted under a stereo microscope **(**Prager *et al.,* 1990). The number of eggs in the sample was taken as the average numbers of eggs from the three subsamples. The total number of eggs for each female was extrapolated from the whole egg mass. Linear and logarithmic regression analyses and exponential functions were used to establish the relationships between fecundity and body size (CW, TW) and egg mass weight (EW). Differences in the regression slope for the relationships between fecundity and body size were analysed by t-test. Spawning peak was determined as the months with highest number of ovigerous females.

The size at first sexual maturity of females was determined using the minimum size class data of the ovigerous females.

Statistical tests involved the use of simple regression analysis and ANOVA (Freund and Wilson, 2003). SPSS was used for all statistical analyses. All statistical effects were considered significant at p = 0.05.

Results

Water temperature ranged from 15.93 to 30.13°C and the lowest and highest values were measured at 0-10 m during all the sampling cruises. The average salinity ranged between 37.40±0.65‰ (March 2015) and 38.61±0.78‰ (October 2014). Temperature and salinity were seasonally variable. The mean dissolved oxygen (DO) rate ranged from 5.99 to 8.43 mg l^{-1}. There was also a seasonal trend for dissolved oxygen. Hypoxic (<2 mg l^{-1}) bottom water was not found in any station in the Yumurtalik Cove.

During the study period, a total of 951 crabs, which consisted of 523 females (55%), 213 of which were ovigerous), 350 males (36.8%) and 78 juveniles (8.2%) were caught. The crabs were abundant between June (17.45%) and July to December (61.30%).

Male crabs were larger than females (F_{1-3}=65.06; P=0.00), CW in male ranged between 14.73-71.88 mm (42.29±0.43) and in females varied from 7.12 to 100.6 mm (Table 1).

The overall male to female ratio showed female dominance during the study period (0.6:1). Chi-square (χ^2) test indicated that the difference between the numbers of males and females was significant ($p<0.05$) (Table 2).

The monthly distribution of maturity stages of females are shown in Fig. 1. Maturity stage 1 occurred during all months. In December, all the specimens were in this stage. Fully mature ovaries were seen throughout the sampling period except in December and January, with the highest percentages in September and July.

GSI values for the 170 females ranged from 1.63 to 5.37% with a mean value of 3.38±1.17%. Those of 172 males ranged from 0.17 to 0.58% with a mean of

Table 1. Morphological characteristics of *C. (G.) longicollis* in Yumurtalık Cove

Sex	Number	CW (mm)	CL (mm)	W (g)
Male	350	14.73-71.88 (42.29±0.43)	4.49-57.92 (25.98±0.28)	1.34-129.53 (17.05±0.57)
Female	310	17.22-100.60 (38.75±0.49)	16.33-61.68 (23.20±0.21)	1.80-164.09 (11.74±0.76)
Ovigerous female	213	23.71-95.93 (34.57 ±0.42)	14.58-33.36 (21.18±0.17)	2.97-88.84 (9.54±0.43)
Juvenile	78	23.64-48.42 (33.19±0.66)	13.17-32.10 (20.45±0.43)	2.51-31.99 (8.09±0.66)

Values are : Minimum-Maximum; Values in parantheses refers to Mean ±SE
CW: Carapace width, CL: Carapace length, W: Body weight,

Table 2. Sex ratio of *C. (G.) longicollis* in Yumurtalık Cove

Month	Male	Female	Male:Female	% of Female	χ^2
Jul	39	82	0.5:1	67.7	0.00*
Aug	71	79	0.9:1	52.6	0.51
Sep	43	49	0.9:1	53.2	0.53
Oct	33	49	0.7:1	59.7	0.07
Nov	29	69	0.3:1	70.4	0.00*
Dec	19	7	2.7: 1	26.9	0.01*
Jan	7	9	0.8:1	56.2	0.61
Mar	17	26	0.6:1	60.4	0.17
Apr	10	21	0.5:1	67.7	0.04*
May	22	36	0.6:1	62.0	0.06
Jun	60	96	0.6:1	61.5	0.00*
Total	350	523	0.6:1	59.9	0.00*

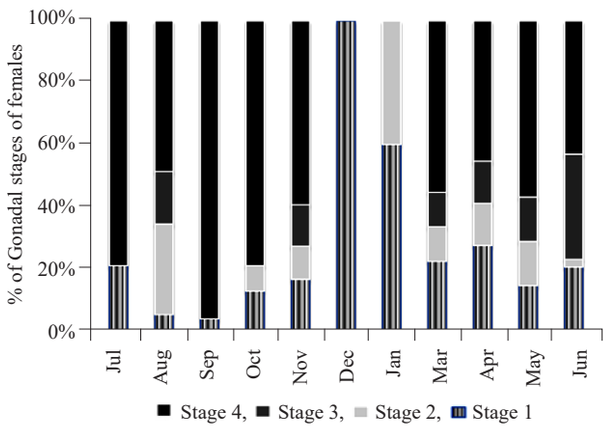

Fig. 1. Monthly distribution (%) of *C. (G.) longicollis* female maturity stages

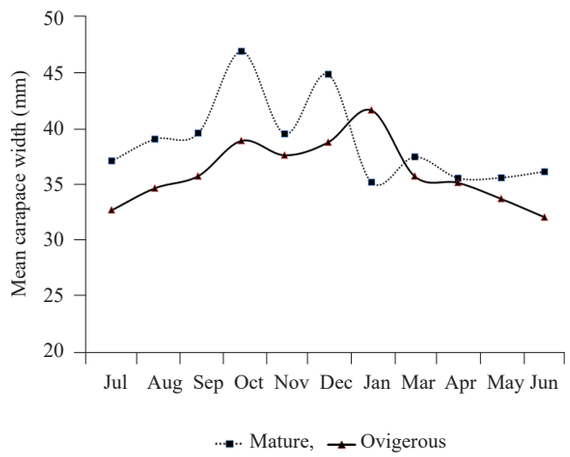

Fig. 2. Monthly mean size (CW) of mature and ovigerous *C. (G.) longicollis* females in Yumurtalık Cove

0.35±0.14%. In females, the mean GSI values increased with two peaks in September and March. They declined in December and had a slight oscillation in June. In males, the highest GSI values were estimated for November and May. In winter months, the GSI values of males and females decreased. The spawning season for females was observed two times in spring and fall months.

Ovigerous females were collected during the study period. Higher proportion of ovigerous females occurred from June to November. The highest numbers of ovigerous individuals were caught in July (Fig. 2). Ovigerous female crabs ranged between 23.71 and 95.93 mm in CW and 2.97 and 88.84 g in body weight (Table 1).

Fecundity of *C. (G.) longicollis* was 99176±51899 eggs, ranging between 22200±8879 (CW=22.33 mm) and 292608±38728 eggs (CW=45.21 mm). The mean fecundity increased with increasing total weight ($F_{9\text{-}108}$=7.96; $p<0.05$) and mean fecundity differences among size classes were significant ($F_{4\text{-}113}$=11.42; $p<0.05$). Mean fecundity very weakly correlated with carapace width ($r=0.17$, $p<0.01$); however, there was a high and positive correlation of weight ($r=0.57$, $p<0.01$) with egg weight ($r=0.67$, $p<0.01$) and mean fecundity. The regression slopes for fecundity-weight ($t=7.5$; $p=0.00$) and fecundity-egg weight ($t=12.2$; $p=0.00$) were significant. The regression between fecundity and weight was $F=26973.9+7158.6^{*}W$ ($R^2=0.330$; $F_{1\text{-}117}=57.1$; $p=0.00$). The estimated regression equation of egg weight on fecundity was $LnF=11.29+0.94^{*}Ln\ EW$ ($R^2=0.563$; $F_{1\text{-}117}=149.6$; $p=0.00$).

Discussion

In this study, females were found to be dominant in the population. Similar observation was reported by Sangun *et al.* (2009) and by Ozcan and Katagan (2016) in Iskenderun Bay. In contrast, Marun (2016) reported that male crabs were dominant in the population (M:F=1:0.88). Pereira *et al.* (2009) stated that predominance of adult females is seen mainly in areas of higher salinity. The

overall male to female ratio through the year was 0.6:1 in this study. which could be attributed to high salinity and temperature.

Sangün *et al.* (2009) reported the mean carapace width of *C. (G.) longicollis* population as 38.13±0.54 mm (22.46-59.06 mm) and the population's mean carapace width as 23.66±0.32 mm (14.18-35.71 mm). They stated that males had mean carapace width of 25.44±0.58 mm (14.18-35.71 mm) while this value for the females was 22.59±0.34 mm (14.19-31.85 mm) in Iskenderun Bay. Ozcan and Katagan (2016) reported that largest number of crabs were between 30 and 45 mm CW. The crabs captured ranged from 0.6 to 50.75 g in weight. Marun (2016) stated that the distribution of carapace width of the Lessepsian crabs ranged between 19.2 mm and 67.3 mm, with a mean value of 39.97±0.23 mm, while the total weight values varied between a minimum of 0.8 g and a maximum of 50.75 g. In the present study, the mean CW of the crabs were similar, while the minimum values were lower and the maximum values were higher (Table 1). The reproductive season for the females was observed with two peaks in spring and fall months in the Iskenderun Bay, with ripe females (maturity stage 4) occurring from March to November, with the highest percentages in September and July and immature females (maturity stage 1) and ovigerous females occurring in all the months. Spawning is correlated with seasonal changes in sea temperature (Dhawan *et al.,* 1976). Spawning usually occurs in waters with temperature and salinity values favourable for hatching of eggs and growth of larvae. The salinity and temperature range inside the Yumurtalık Cove seemed to characterise it as a spawning area. The prevalence of mature females as well as the high percentage of ovigerous females indicates that the invasion of *C. (G.) longicollis* is complete and the species is well established within the area, as suggested by Rabaoui *et al.* (2015).

Marun (2016) caught ovigerous females from April to November. Eggs of 31 females were counted, and the mean fecundity of *C. (G.) longicollis* was estimated as 4058.88±640.01 eggs per female (137-15047 eggs per female). The mean fecundity in the presesnt study was lower. Variations in fecundity may have been caused by several factors such as the technique used for counting eggs, time and stage of embryonic development of eggs at the time of counting and genetic characteristics of the populations (Severino-Rodrigues *et al.*, 2013).

Fecundity in brachyuran crabs is variable and highly dependent on the size of females (Perry and Vanderkooy, 2015). In the present study, the mean fecundity increased as total body weight increased and there was significant difference between the size classes. Marun (2016) estimated the regression between fecundity and weight relationship as $F=976.72^{*}W-832.45$ $72^{*}W-832.45$ ($R^2=0$, 4205), which is similar to the results obtained in the presesnt study.

This study provides useful information on the aspects of the reproductive status of *C. longicolis* in the Mediterranean Sea. The population, characterised by densities of adult and ovigerous female specimens, confirms that *C. (G.) longicollis* is well established in the Mediterranean coast of Turkey. Further studies are needed for assessing the potential relationships with other brachyuran species in order to foresee possible negative impacts due to the invasion.

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