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RESEARCH ARTICLE

Some biological parameters of *Patella caerulea* from the Black Sea

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ABSTRACT

Limpets are common inhabitants of the midlittoral and upper infralittoral zones and play an important ecological role in the coastal ecosystem. The study aimed to assess growth, meat yield, morphological aspects, condition and reproduction features of *Patella caerulea*. About 58% of collected Mediterranean limpets were concentrated in the 25.0-34.9 mm shell length group. The average meat yield ratio was calculated as 39.34%. Mean growth increments for shell length (SL) and total weight (TW) were 23.99% and 97.99%, respectively. Results exhibited relatively high correlation coefficients among variables. Mean condition factor value was calculated as 14.2. Spawning occurs over a short period with ovigerous females observed on two month periods. Mean fecundity was calculated as 90,983±28,675 eggs/g whereas mean egg diameter was estimated as 160.6 µm. This study presents first baseline information about biological and morphological of Mediterranean Limpets population in Black Sea.

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Introduction

Marine gastropods are situated at several levels in the food chain, which increases their determining role in the functioning of marine ecosystems (Hakenkamp & Morin, 2000). Limpets of the genus *Patella* are grazing gastropods, common inhabitants of the hard substrate communities in the mid-littoral and upper infralittoral zones of the East Atlantic and Mediterranean coasts in temperate latitudes (Vafidis et al., 2020). Limpets are considered the "keystone" species of the mid-littoral zone and are widely collected for human consumption and as fishing bait (Menge, 2000). They play an essential role in controlling algal coverage and consequently, the ecological succession and biological communities established in coastal zones (Prusina et al., 2015; Vafidis et al., 2020).

The Mediterranean limpet *Patella caerulea* Linnaeus, 1758 is among the most common species of rocky shores in the infralittoral and midlittoral, especially Mediterranean (Küçükdermenci et al., 2017). *P. caerulea* is considered endemic to the Mediterranean Sea (Christiaens, 1973). It is very

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prevalent in Turkish seawater (Öztürk & Ergen, 1996). This species primarily feed by scraping algae off rocks and plants such as Cyanophyceae, *Ulva lactuca*, *Corallina elongate* (Silva et al., 2008; Ayas, 2010). *P. caerulea* is a hermaphrodite protandric species and may reach a length of 70 mm (Kastanevakis et al., 2008). Mediterranean limpet fulfills the criterion an ideal bioindicator for heavy metal contamination is extensively used in marine monitoring programs (Storelli & Marcotrigiano, 2005).

Various studies have been conducted on the morphology, biology, ecology, and distribution of *Patella* species, especially in the Mediterranean Sea (Navarro et al., 2005; Espinosa et al., 2006; Prusina et al., 2014; Prusina et al., 2015; Bouzaza & Mezali, 2018). In Turkey, generally, morphometric, taxonomic, distribution, and heavy metals content studies on *P. caerulea* have been conducted (Bat et al., 1999; Akşit & Falakalı Mutaf, 2007; Ayas, 2010; Çulha & Bat, 2010). Although *P. caerulea* is the most dominant intertidal gastropod on the rocky shore of the Black Sea in Turkey, there is no information about these species in the region yet. The present study aims to make an extensive assessment of population structure and characteristics, growth, meat yield and reproductive status of *P. caerulea* in the Black Sea. To our knowledge, this is the first study in Turkey of the species, on the Black Sea.

Material and Methods

Study Area and Sampling

This study was conducted along the Turkish coast of the Black Sea (between 41.149538° N, 37.273525° E–40.971953° N, 38.059734° E). In this study, *P. caerulea* were collected from the intertidal zone of the coastline monthly from April 2018 to March 2019. Limpets were collected by hand with a penknife, and injured limpets (broken, shattered, severed) were not included in the sampling. Collected specimens were put into drums filled with seawater and then transferred to the laboratory. Collected samples were identified on the basis of form and external sculpture of the shell, the color of the pallial tentacles and the color of the foot (Christiaens, 1973).

Growth and Morphological Analyses

Shell length (SL – the longest distance between the anterior and posterior of limpet), shell width (SWi – the longest distance perpendicular to the anterior-posterior axis), and shell height (SH – the longest vertical distance from the apex to the shell base) of samples were measured using a digital caliper to the nearest 0.01 mm. Total wet weight (TW), meat weight (MW), and shell weight (SWe) was measured with an electronic balance of 0.001 g accuracy. The growth performance of samples according to *SL* and *TW* was determined with the formula below:

$$\text{SL increment (\%)} = [(SL_n - SL_{n-1})/SL_{n-1}] \times 100 \quad (1)$$

$$\text{TW increment (\%)} = [(TW_n - TW_{n-1})/TW_{n-1}] \times 100 \quad (2)$$

where n is the number of size classes (Ricker, 1975). The *SL*, *SWi* and *SH* relationships with each one were determined using the equation (3).

$$W = aL^b \quad (3)$$

Linear relationships between *SL* and *TW*, *SWe*, and *MW* were also analyzed (Le Cren, 1951; Pauly, 1980; Erkoyuncu, 1995). The condition factor (*K*) was calculated using the formula (Equation 4):

$$K = 100 \times \frac{TW}{SL^3} \quad (4)$$

where *TW* was total weight (g), and SL was shell length (mm) of collected samples (Bagenal, 1978).

Reproduction, Fecundity, and Egg Diameter

Sex was determined using macroscopic methods. The spawning period was determined by analyzing the monthly variation in the gonad maturity as well as the gonadosomatic index (GSI). In this study, 191 females were used to calculate the gonadosomatic index as described by (Bagenal, 1978) (Equation 5):

$$GSI = \left[\left(\frac{Gonad\ weight}{Meat\ weight}\right) \times 100\right] \quad (5)$$

The limpet eggs were removed from 26 ovigerous females in the spawning period (in October and November), and the total weight of eggs was measured using a balance with a sensitivity of 0.0005 g. Fifty eggs were taken from different regions of an ovary, and egg diameters were measured with an calibrated ocular micrometer. Eggs were counted with an ocular micrometer as (Equation 6):

$$F = n\left(\frac{W_0}{X}\right) \quad (6)$$

where W_0 denotes the weight of gonad and n represents the number of eggs in the subsample, X stands for subsample weight (g), F represents the number of eggs (Bagenal, 1978; Kwei, 1978; Jones et al., 1990).

Data Analysis

Statistics and data analyses were performed by statistical software SPSS v26.0. The normality of the data was checked

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using the Kolmogorov-Smirnov test, depending on the sample size. In the former analysis, homogeneity of variance was tested using Levene's test. Since the data in this study were not normally distributed, monthly morphological values were compared using the Mann-Whitney U-test. Monthly and combined variables were analyzed with the Pearson correlation and regression analysis to investigate the relationships among morphological characters (Sokal & Rohlf, 1969; Düzgüneş et al., 1983). Hierarchical Ward cluster analysis after Z score correction was used to determine closely related variables (Lopez et al., 2004). The sex ratio of samples, the expected 1:1 male to female ratio was assessed using the chi-square (χ^2) test (Düzgüneş et al., 1983).

Results and Discussion

Size Distribution and Meat Yield

In the present study, 1830 *P. caerulea* individuals were sampled during the study period. Shell length distribution of collected samples showed that 57.98% of Mediterranean limpets were in a range of 25 to 34.9 mm. It was observed that 77.40% of the samples collected were smaller than 5 g, according to the TW (Figure 1). Ayas et al. (2008) reported the SL, SWi, and SH in a range of 19–39 mm, 15–33 mm and 5–11 mm from samples caught in the Mersin Bay (Mediterranean). Küçükdermenci et al. (2017) reported the minimum and maximum SL as 24.4 and 30.7 mm in Izmir Bay (Aegean Sea), respectively. Vafidis et al. (2020), who conducted a study in the Pagasitikos Bay (Aegean Sea), determined the average SL, SWi, and SH as 23.36, 18.96, and 6.35 mm, respectively. In our study, biometric parameters were more variable compared to similar studies, possibly attributed to variation in the number of samples or sampling regions. The environmental and ecological factors (food availability, water parameters, and meteorological factors) may lead to morphological variations in limpets of the same species that live in different ecosystems (Crothers, 1983). Monthly biometric measurements and statistical differences of *P. caerulea* are presented in Table 1. A closer look at the monthly SL reveals that the maximum value was 31.70 mm in December and the minimum value of 25.80 mm in May. Minimum SL, SWi, SH, TW, and MW were determined in May and July. High SL, SWe, TW, and SW values were obtained in April and December.

Average MW of *P. caerulea* was calculated as 1.49±0.02 g with a rate of 39.34% of TW. In other words, 1 kg of edible meat can be obtained from approximate the 2.5 kg live weight of *P. caerulea*. It was determined that the minimum and maximum meat yield ratio was 35.32% in November and 43.68% in August, respectively. A weak relationship was found between the SL and MW. However, the maximum ratio of meat weight (41.56%) was observed in the 25.0-29.9 mm shell length classes (Figure 2). The mean meat weight of samples was significantly different by month (χ^2= 99.851; df= 11; .001). Similarly, Küçükdermenci et al. (2017) determined the percentage of meat yield as 37% in Izmir (Turkey) for *P. caerulea*. Hamad et al. (2019) who conducted a study in Libya, determined the average meat yield as 1.09 g. The present study's findings concerning meat yield are in agreement with the findings reported by Küçükdermenci et al. (2017) and Hamad et al. (2019). Water conditions, especially temperature, may also affect the meat yield of *Patella* species (Fretter & Graham, 1976). Thus, it is usual to observe differences in meat yield.

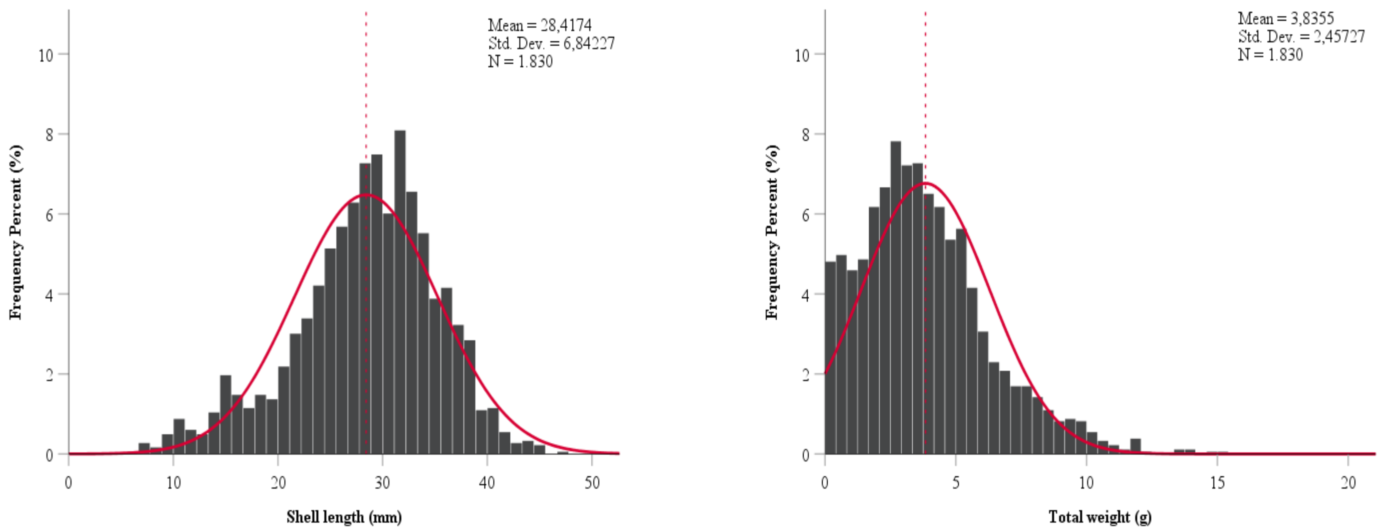

Figure 1. Density histogram of shell length (SL) and total weight (TW) of population and dashed red lines indicating the means and red lines representing the normal curve of the population for the *P. caerulea*

Table 1. Monthly and totally morphometric measurement values of *Patella caerulea* (N = 1830)

		April A	May B	June C	July D	Aug E	Sep F	Oct G	Nov H	Dec I	Jan J	Feb K	Mar L	Mean
SL (mm)	Mean	31.04	25.80	28.46	26.20	28.84	28.65	28.51	28.53	31.70	28.54	26.74	27.66	28.42
	±SE	0.54	0.71	0.51	0.41	0.38	0.44	0.68	0.56	0.49	0.57	0.54	0.61	0.16
	Min	7.26	6.31	9.84	10.13	15.99	9.27	6.72	7.75	10.09	10.33	9.32	12.10	6.31
	Max	44.29	43.42	40.43	43.35	38.72	47.05	44.75	45.00	43.02	45.26	38.86	40.40	47.05
	Dif.	a,f,g	b,c	b,d,e	c	a,d,h,i	a,e,h,i	a,b,c	a,b,c	f	b,c,d,e,g	b,c,h	b,c,i	**
SWi (mm)	Mean	25.14	20.62	22.92	20.91	23.17	23.22	23.12	23.15	26.03	23.22	21.51	22.51	22.99
	±SE	0.46	0.61	0.46	0.36	0.33	0.39	0.61	0.50	0.44	0.50	0.47	0.55	0.14
	Min	4.82	4.09	6.61	7.23	12.13	6.53	5.03	5.38	7.47	7.15	6.30	8.55	4.09
	Max	35.98	35.78	36.26	36.85	31.18	38.12	39.02	36.49	36.49	38.04	32.94	35.53	39.02
	Dif.	a,h,i	b,d,g,j	b,c,e	d	e	a,e,f	a,b,d,e	a,c,e,g	h	c,e,i,j	b,d,e	b,d,e	**
SH (mm)	Mean	10.46	9.10	10.05	9.09	10.10	10.31	10.84	10.49	10.85	9.75	9.49	9.72	10.04
	±SE	0.23	0.31	0.25	0.19	0.17	0.21	0.34	0.25	0.20	0.23	0.22	0.27	0.07
	Min	2.29	1.54	2.94	2.64	4.83	2.35	1.14	1.71	3.22	2.61	2.67	3.15	1.14
	Max	15.64	17.10	18.85	15.57	14.88	19.15	22.81	17.69	15.60	16.91	15.67	18.23	22.81
	Dif.	a,e,f,g	b,c,d	a,b	b	a,c,e,f,g	a,d,e,f,g	a,e,f,g	a,e,f,g	a	b,e	b,f	b,g	**
TW (g)	Mean	4.93	3.30	4.08	3.00	3.76	4.08	3.93	3.91	4.51	3.75	3.16	3.55	3.84
	±SE	0.21	0.23	0.21	0.13	0.14	0.17	0.26	0.21	0.17	0.20	0.17	0.21	0.06
	Min	0.06	0.02	0.10	0.10	0.51	0.12	0.04	0.04	0.14	0.15	0.08	0.24	0.02
	Max	14.62	11.87	10.75	10.41	7.88	12.47	15.05	13.97	10.39	12.02	8.90	13.44	15.05
	Dif.	a	b,c,d	a,b,d	c	d,g,i,j	a,d,e,j	a,b,c,d	a,d,f,i,j	a,g,h	b,c,d,h	c,i	b,c,j	**
SWe (g)	Mean	2.23	1.56	1.78	1.31	1.66	1.78	1.79	1.85	2.22	1.75	1.48	1.68	1.76
	±SE	0.10	0.12	0.09	0.06	0.06	0.07	0.12	0.10	0.09	0.10	0.08	0.10	0.03
	Min	0.02	0.01	0.04	0.04	0.25	0.05	0.02	0.02	0.08	0.06	0.05	0.11	0.01
	Max	6.71	6.67	5.71	5.10	4.24	5.20	6.49	7.08	5.55	5.57	4.87	7.58	7.58
	Dif.	a,i,j	b,d,e	a,b,c,e	d	e	e,f	a,e,g,i	a,e,h,i	i	c,e,j	b,d,e	b,d,e	**
MW (g)	Mean	1.85	1.24	1.68	1.20	1.64	1.69	1.61	1.37	1.60	1.40	1.20	1.39	1.49
	±SE	0.08	0.09	0.09	0.06	0.06	0.07	0.10	0.07	0.06	0.07	0.06	0.08	0.02
	Min	0.02	0.01	0.05	0.04	0.20	0.04	0.01	0.01	0.05	0.05	0.03	0.10	0.01
	Max	5.19	4.52	4.38	4.38	3.44	5.90	5.37	4.05	3.46	4.23	3.87	4.57	5.90
	Dif.	a	b,d,h	a,e,g,i	b,c	a,e,g,i	a,e,g,i	a,d,e,g,i	b,e,f,h	a,f,g,i	b,g,h	c,h	b,h,i	**

Note: Superscript and capital letters denote comparisons between two months (a capital and a superscript). There is no statistical difference between a capital letter and each superscript letter ($P < 0.05$). A – November, B – December, C – January, D – February, E – March, F – April, G – May, H – June, I – July, J – August, K – September, L – October.

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Table 2. Proximity matrix of six morphometric variables for *P. caerulea*

Proximity Matrix						
	SL	SWi	SH	TW	SWe	MW
SL	0.00					
SWi	435.36	0.00				
SH	4582.08	2252.68	0.00			
TW	8306.20	5012.97	578.12	0.00		
SWe	9614.60	6047.51	946.03	62.59*	0.00	
MW	9788.57	6188.20	1001.81	79.88*	3.39*	0.00

Note: Values with * indicates that the morphometric characters are very close.

Table 3. Mean (±SE) and size increment rates (%) of SL (mm) and TW (g) for sampled *P. caerulea* during the study

Size classes	N	%	Mean SL	Mean TW	SL increment (%)	TW increment (%)
5.00-9.99	18	0.98	8.52±0.26	0.08±0.01	-	-
10.00-14.99	72	3.93	12.89±0.19	0.30±0.01	51.18	264.85
15.00-19.99	119	6.50	17.36±0.14	0.70±0.02	34.73	136.81
20.00-24.99	275	15.03	22.86±0.08	1.69±0.03	31.69	141.99
25.00-29.99	539	29.45	27.65±0.06	3.09±0.03	20.95	82.43
30.00-34.99	522	28.52	32.34±0.06	4.88±0.05	16.95	58.10
35.00-39.99	238	13.01	37.01±0.08	7.39±0.11	14.43	51.39
40.00-44.99	43	2.35	41.62±0.20	9.93±0.30	12.46	34.35
45.00-49.99	4	0.22	45.59±0.49	11.32±1.05	9.54	13.92
Combined	1830	100	28.42±0.16	3.84±0.06	23.99	97.98

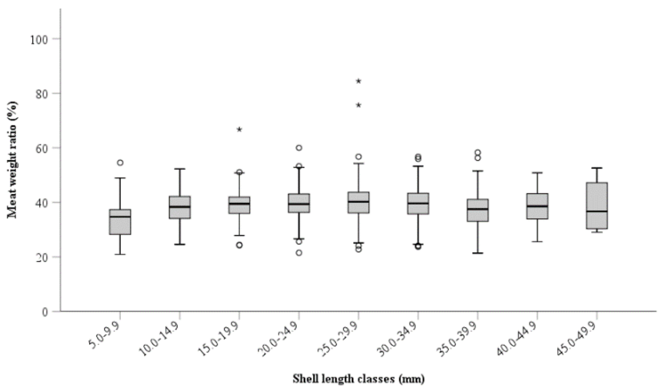

Figure 2. Boxplot of the percentage of meat weight (%) for the Mediterranean limpets according to shell length classes. The whiskers represent the ranges for the bottom 25% and the top 25% of the data values while bold horizontal lines represent the mean values. Circles and stars outside the whisker represent outliers (95%).

Biometric Relationships and Growth

According to the proximity matrix, close relationships were exhibited between SWe and MW (3.39), TW and SWe (62.59), and TW and MW (79.88). For instance, the relationships of SL with SWe and MW were highly distant. However, there appeared to be weak relationships of SL and SWi with other variables, a tendency similar to that presented by the correlation analysis (Table 2, Figure 3).

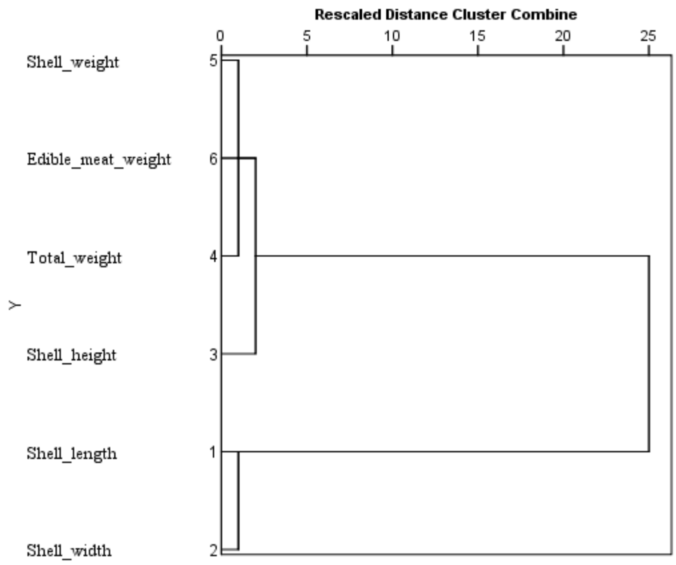

Figure 3. Dendrogram topology of the morphometric variables of *P. caerulea*.

The mean growth increment value was calculated as 23.99% and 97.99% according to the SL and TW, respectively.

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According to SL and TW growth increments, the highest growth (51.18% and 264.85%, respectively) occurred in 10.0–14.9 mm size class individuals (Table 3). Both growth types inversely correlated with the size classes but seemed to be close relations. Furthermore, the growth of individuals decreased with the increase of SL size classes and almost plateaued after 50 mm. The most remarkable difference between SL and TW increments concerning the models is that the TW growth increment increases after 40 g weight classes (Figure 4).

In this study, the finding showed that the correlation coefficients among variables were quite high (Table 4). According to Pearson correlation values, the strongest and weakest correlations were determined between SL and SWi (R^2= 0.982) and between ST and MW (R^2= 0.868), respectively. A linear relationship and strong interaction (R^2= 0.95) were reported between SL and SWi of *P. caerulea* in the Mediterranean was found by Bouzaza and Mezali (2018). These findings are consistent with our results. Unlike these findings, Belkhodja and Romdhane (2012) reported that curvilinear regression equations between morphometric measures for Mediterranean limpets in Tunisia. Compared with our findings, weaker correlation coefficients (R^2= 0.962 and R^2= 0.908 for sheltered and exposed, respectively) were obtained of morphometric measurements of *P. caerulea* by Vafidis et al. (2020). *Patella* species may show significant morphological variability depending on environmental variation (Mauro et al., 2003). Correlations among the morphometric parameters are of particular significance in terms of understanding of smoothness of organism shell structure. It should be underlined, however, that big sample sizes could make a slight difference among the correlations that are significant (Aydın et al., 2014). The relationship of SL with TW and the linear relationship of SL with SWi and SH are presented in Figure 5. However, the b value of SL and TW of individuals was found as 3.09 (positive allometry). Considering the growth value (b > 3), it can be said that the sampled limpets have well enough environmental conditions, and the total growth of the individuals is provided as required. The b value was reported as 2.89 between SL and TW in the exposed site in Pagasitikos Gulf. Isometric growth type for SL and TW was reported by Vafidis et al. (2020). This value may vary depending on many conditions such as the number of samples, catch season, characteristics of the aquatic ecosystem, gonadosomatic index, and nutrition (Bagenal, 1978).

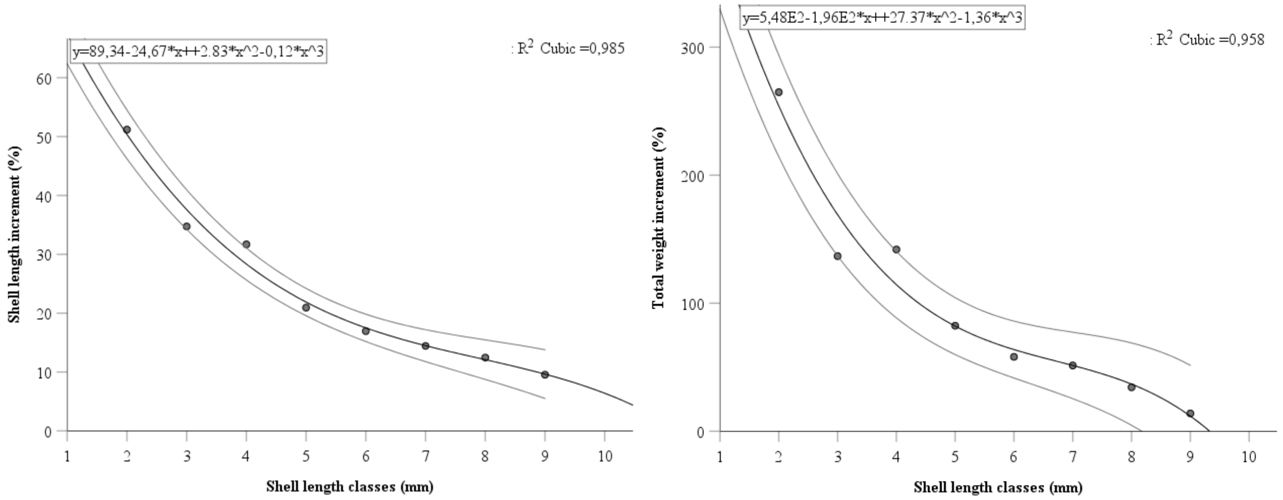

Figure 4. Growth increments (%) indicating the size classes for the Mediterranean limpets, where shell length from 5.0 to 49.9 mm are represented as 1 to 10. Grey lines in the graph represent the mean confidence intervals.

Table 4. Pearson correlation coefficients among morphometric variables for *P. caerulea*

Variables	SL	SW_i	SH	TW	SW_e	MW
SL	1					
SW_i	0.982	1				
SH	0.910	0.913	1			
TW	0.899	0.904	0.896	1		
SW_e	0.882	0.891	0.878	0.967	1	
MW	0.880	0.880	0.868	0.955	0.890	1

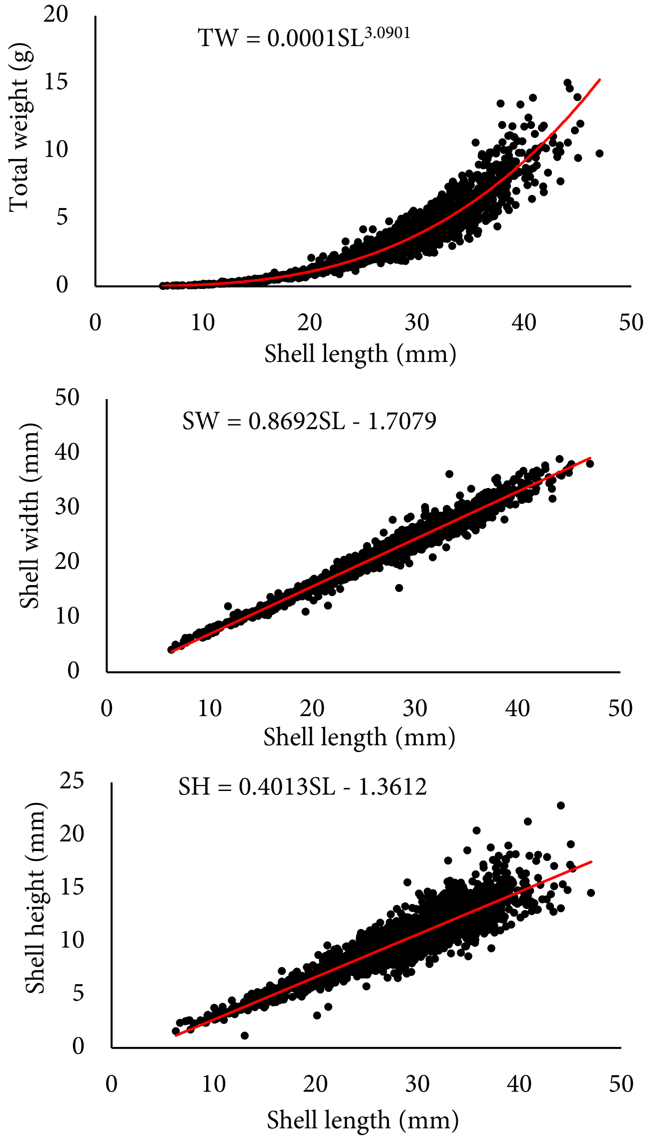

Figure 5. Nonlinear regressions SL-TW, linear regression SL-SWi and SL-SH for *P. caerulea* from the study area (N = 1380). The red lines represent the regression curve.

Condition Factor (K)

The mean condition factor (K) value was calculated as 14.2 for sampled individuals from the study area. The maximum K value occurred in June with 15.47, whereas the minimum K value occurred in December with 12.79. A significant increase in the condition of the individuals was observed in the summer with 15.03 (Figure 6). The mean K value of individuals was significantly different by monthly (χ^2= 164.21; df= 11; .001). The highest condition factor can signify the period before spawning in gastropods; water temperature may also affect both the index and reproduction of *Patella* species (Fretter and Graham, 1976; Vafidis et al., 2020). Based on this statement, it was seen that the increase in the K value before the ovulation period was significant. Thus, it was determined that the spawning season started in October and ended in November. The maximum and minimum K value of *P. caerulea* was reported as 41.20±1.18 in winter and as 30.46±1.54 in autumn, respectively, by Küçükdermenci et al. (2017). Vafidis et al. (2020) conducted that the maximum K value occurred in April (32.762±7.38), whereas the minimum K value occurred in February (26.58±4.47). The findings of our study were lower than the findings in other studies. Factors, such as different areas, spawning periods, and water parameters, may cause monthly changes in the condition of the limpets (Fretter & Graham, 1976; Belkhodja et al., 2011).

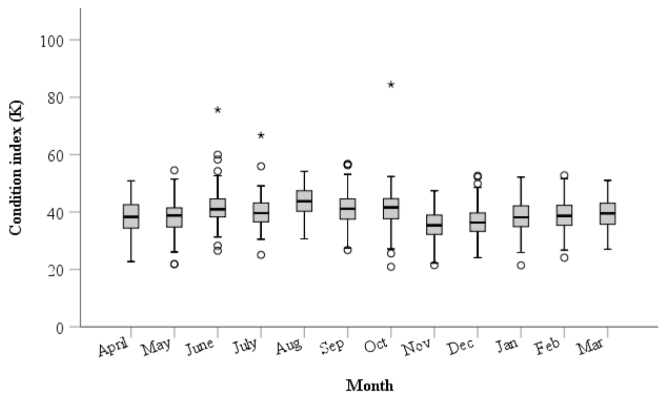

Figure 6. Boxplot of the monthly condition factor (K) for the *P. caerulea*. Horizontal lines in the boxes represent the mean values, and whiskers represent the standard deviations. Error bars represent 95% confidence intervals.

Reproductive Characteristics

Ovigerous females were observed for only two months (October and November), implying that spawning takes place for quite a short period for *P. caerulea*. The overall sex ratio of all individuals was calculated as 1:1.93 (M/F), with a significant deviation from the expected 1:1 ratio (χ^2= 29.19; df= 1; .05) in the spawning period. The GSI values of *P. caerulea* ranged from 1.41 to 12.32%, with a mean value of 5.69%. It has been reported that the Mediterranean limpets spawn period is in winter in south Eastern Australia (Parry, 1982), whereas in Eastern Tunisian coasts was between February and May (Gharred et al., 2019). These values are different from those obtained in the present study, suggesting that the regional differences may play a significant role in the spawning period. The fecundity of 26 ovigerous females ranged from 39,773 to 127,273 with an average value of 90,983±28,675 eggs/g. The egg diameter of samples ranged between 115.0 µm and 242.5 µm (mean 160.6 µm). Gharred et al. (2019) reported the mean egg diameters for three sites on the eastern Tunisian coast as 56.93, 41.22 and 40.71 µm, respectively. The egg diameters of Mediterranean limpets are unequivocally higher than those obtained in the

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present study. The present monitoring study suggests that reproduction parameters of Mediterranean limpets may exhibit variations depending on the regions.

Conclusion

In conclusion, due to its ever-changing spatiotemporal structure, the Black Sea should be closely monitored in terms of biodiversity, food chains and ecosystems. Thus, it is essential and valuable to know the current status, growth, and reproductive performance of available populations. This study presents baseline information on the Mediterranean Limpets population in this area, where no studies have been carried out. This study assessed growth, morphometric parameters and population aspects of the Mediterranean limpet, contributing valuable new data on *P. caerulea* along the Black Sea coastal zones.

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Compliance with Ethical Standards

Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by MA, AES and UK. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

Ethics committee approval is not required. All authors declare that this study does not include any experiments with human or animal subjects. All applicable international, national, and institutional guidelines for the care and use of animals were followed by the author.

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