Population Characteristics of the Limpet *Patella caerulea* (Linnaeus, 1758) in Eastern Mediterranean (Central Greece)

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Abstract: Limpets are pivotal for structuring and regulating the ecological balance of littoral communities and are widely collected for human consumption and as fishing bait. Limpets of the species *Patella caerulea* were collected between April 2016 and April 2017 from two sites, and two samplings per each site with varying degree of exposure to wave action and anthropogenic pressure, in Eastern Mediterranean (Pagasitikos Gulf, Central Greece). This study addresses a knowledge gap on population characteristics of *P. caerulea* populations in Eastern Mediterranean, assesses population structure, allometric relationships, and reproductive status. Morphometric characteristics exhibited spatio-temporal variation. Population density was significantly higher at the exposed site. Spatial relationship between members of the population exhibited clumped pattern of dispersion during spring. Broadcast spawning of the population occurred during summer. Seven dominant age groups were identified, with the dominant cohort in the third-year class. Significant negative allometric relationships were exhibited between morphometric characteristics. Differences in growth patterns among populations were indicated.

Keywords: *Patella caerulea*; Eastern Mediterranean; population structure; spawning season; age groups; allometric relationships

1. Introduction

Limpets of the genus *Patella* are grazing gastropods, common inhabitants of the hard substrate communities in the midlittoral and upper infralittoral zones of the East Atlantic and Mediterranean coasts in temperate latitudes [1,2]. They play an important role in controlling algal coverage and consequently, the ecological succession and biological communities established in coastal zones [3–7]. They are considered the “keystone” species of the midlittoral zone [8–12] and are widely collected for human consumption and as fishing bait. Limpets are pivotal for structuring and regulating the ecological balance of intertidal communities, directly through the key process of grazing that determines macroalgal abundance, and indirectly by enhancing or inhibiting the establishment of other organisms [3,6,12,13].

In the Mediterranean Sea the genus *Patella* is represented by four species: *P. caerulea*, *P. ulysseponensis*, *P. rustica*, and *P. ferruginea* [14]. The native Mediterranean limpet *P. caerulea* (Linnaeus, 1758), the common or rayed Mediterranean limpet, is among the most common of rocky shores in the whole infralittoral and midlittoral Mediterranean basin [15], also present in a few places in the eastern Atlantic (Bay of Biscay, Canary Islands, Moroccan coast) [16–18] and considered endemic to the Mediterranean Sea [19,20].

Abiotic factors, mainly environmental, have been suggested to effect morphological changes on the shell shape in several species of Atlantic and Mediterranean limpets [21–23]. Limpet distribution and density can be affected by both abiotic and biotic factors. Abiotic factors include wave exposure, substratum morphology, and inclination [18,24–26] whereas biotic factors include intra and interspecific competition [27–29].
Although a number of studies have been carried out on the biology, distribution, and ecology of *Patella* species in the Mediterranean Sea [7,14,15,25,30–36], little is known on the occurrence and population characteristics of *P. caerulea* in eastern Mediterranean, apart from fragmented information (morphometry, distribution, ecology, reproduction) occasionally collected in the framework of several ecological studies [26,37–41].

Our aim is to address a knowledge gap on population characteristics of *P. caerulea* populations in Eastern Mediterranean with an extensive assessment of *P. caerulea* population structure and characteristics, allometric relationships and reproductive status on the upper infralittoral zone in eastern Mediterranean. The present work can be used as a baseline in an ongoing study on the effects of climate change to this very sensitive part of the coastal zone, especially in climate sensitive Eastern Mediterranean.

2. Materials and Methods

2.1. Study Area

Pagasitikos, a semi-enclosed landlocked gulf, is located in the north western part of the Aegean Sea (Figure 1). The mean depth is 69 m, with its deepest area (108 m) located at the eastern part where larger depth gradients are observed. Total gulf area is 520 km$^2$ and total volume 36 km$^3$ [42], connected with the Aegean Sea and north Evoikos through the narrow (5.5 km) and relatively deep (80 m) Trikeri channel [43]. Pagasitikos is a rather sensitive ecosystem due to its semi-enclosed nature and shallow depths. The predominant weak winds of the area result in small to moderate water currents while renewal occurs mainly through the deep-water layer of the Trikeri channel [44]. In contrast with other enclosed gulfs in Greece, Pagasitikos is considerably less eutrophic with a unique and stable circulation pattern which includes a cyclone in the central-west and an anticyclone in the eastern part [45]. Water renewal of the gulf takes approximately 105 days according to Petihakis et al. [46]. The only major city is Volos located at the north part of the gulf, with a population of 120,000 inhabitants and a well-developed industrial sector.

2.2. Field Sampling

Four study stations were selected (Figure 1) based on the degree of exposure and anthropogenic pressure. Two stations were selected at each site in order to obtain disparate samples. The first two stations were located at the first study site (Site 1 Agios Stefanos, Latitude: 39.298531 and Longitude: 22.941732) characterized by low wave exposure (approximately 7 degree slope) and low anthropogenic pressure only in the summer months whereas the third and fourth stations were located at the second study site (Site 2 Plakes, Latitude: 39.348283 and Longitude: 22.969542) characterized by mid to high wave exposure (approximately 10 degree slope) and high anthropogenic pressure (trampling by swimmers) year round. Both study sites experience similar wave-wash because of maritime traffic.

Monthly samples of *P. caerulea* were recorded and collected at each sampling station (Figure 1) from April 2016 to April 2017. Population density and distribution patterns were estimated using randomly placed quadrant plots (20 × 20 cm metal frame). The frame was placed randomly ten times on the substrate covering a sampling area of 0.04 m$^2$ recording *P. caerulea* numbers at each quadrant for every placement [47]. From each sampling station and period, 20 *P. caerulea* individuals, randomly chosen from the quadrat counts were transferred to the laboratory. Four biometric characteristics were determined for each individual (shell length—greatest distance between the anterior and posterior, shell width—greatest distance perpendicular to the anterior posterior axis, shell height—greatest vertical distance from the apex to the shell base, posterior shell length—distance between apex and posterior shell base) were recorded using a digital Vernier caliper to the nearest 0.01 mm (Figure 2). Limpet shells were individually weighed (Wt) on a digital balance to the nearest 0.01 g. Soft tissues were carefully separated from the shells and washed in distilled water to remove dirt. Soft entire bodies were dried at 80 °C for 48 h and then weighted to determine dry tissue mass (Wf). The main abiotic factors, i.e., temperature, salinity, and dissolved oxygen were measured on the water surface with
the portable instrument HACK HQ 40d multi (HACK, Colorado, CO, USA) pH was measured with the portable instrument WTW PH 340i/SET (WTW, Weilheim, Germany). Based on sea temperature, monthly means of these data were processed through seasons.

![Map of Pagasitikos Gulf](image)

**Figure 1.** Pagasitikos Gulf depth profile and sample locations, Site 1 (sheltered), Site 2 (exposed).

### 2.3. Data Analysis

The null hypothesis of no significant spatial and temporal differences in the abundance and the mean values of the biometric characters of the limpet were tested with one way ANOVA [48] and hierarchical structure using Nested ANOVA. Normal distribution was assessed using the Anderson-Darling normality test. Bartlett’s and Levene’s tests were used to assess homogeneity of variance. Statistical analyses were performed using Minitab 19 software (Minitab, Pennsylvania, USA) with significance level at $p < 0.05$. The spatial distribution of *P. caerulea* was estimated calculating Morisita index of aggregation (I8) [49,50].
To test the null hypothesis of randomness (significance of departure from random distribution), \( (I \delta = 1) \) the chi-square test was used (d.f. = \( n - 1 \)) [50–52]. Spatial and temporal abundance variation was assessed using one-way ANOVA, Tukey’s pairwise comparisons were used to identify possible differences between sample means. Condition index (the general physiological condition of the mollusk) was calculated for each individual according to Nakhlé et al. [53] as the ratio: (dry mass of soft tissues/shell weight) \( \times \) 100.

The non-parametric Kruskal–Wallis test was used to investigate the possible temporal differences. The equation describing the best fit (highest correlation coefficient) was identified with the use of Curve Expert 1.4 software (Hyams Development, Alabama, USA). All identified relationships were curvilinear power regression equations (\( Y = aX^b \)). Allometric relationships were assessed for each population and were compared using the standard student t-test. Regression equations for each population were compared between sampling sites, using the two-sample t-test. Length frequency distribution calculated per 1 cm size classes, were divided into age groups using Bhattacharya’s method [54] in order to obtain initial guesses, using the software FiSAT II (FAO, Rome, Italy) (version 1.2.2.) [55]. The results were refined using the maximum likelihood concept (NORMSEP) [56] separating normally distributed components of size-frequency samples (modal progression analysis).
3. Results

3.1. Physio-Chemical Measurements

The physical parameters measured in the water surface showed similar spatio-temporal values. Temperature ranged seasonally from 13.17 °C to 27.12 °C. Minimum surface sea water temperature was recorded in February and maximum in July. Salinity ranged seasonally from 36.12 psu to 38.46 psu. Minimum salinity was recorded in August and maximum in March. Monthly surface temperature and salinity exhibited minor variations between sites, indicating homogeneity (Table 1).

Table 1. Spatial variation of the physical (T, temperature; S, salinity) and chemical (pH; O\textsubscript{2}, dissolved oxygen) parameters recorded in Pagasitikos gulf surface layer.

| Sampling Period | Site 1 | Site 2 |
|-----------------|--------|--------|
|                 | T (°C) | S (psu) | pH | O\textsubscript{2} (mg/L) | T (°C) | S (psu) | pH | O\textsubscript{2} (mg/L) |
| April           | 16.06  | 38.54  | 8.32 | 6.79 | 16.95  | 38.16  | 8.32 | 6.86 |
| May             | 21.21  | 37.54  | 8.29 | 5.88 | 19.92  | 37.54  | 8.26 | 2.48 |
| June            | 25.04  | 37.47  | 8.26 | 6.61 | 26.45  | 36.64  | 8.24 | 5.71 |
| July            | 27.08  | 36.46  | 8.26 | 5.44 | 27.12  | 36.18  | 8.23 | 2.45 |
| August          | 26.67  | 36.12  | 8.26 | 2.06 | 26.78  | 36.76  | 8.31 | 5.02 |
| September       | 24.28  | 36.84  | 8.29 | 4.92 | 25.03  | 36.49  | 8.29 | 5.92 |
| October         | 21.79  | 36.42  | 8.31 | 5.22 | 22.04  | 36.84  | 8.35 | 5.54 |
| November        | 18.31  | 36.71  | 8.34 | 2.48 | 17.95  | 36.99  | 8.39 | 3.67 |
| December        | 16.01  | 36.87  | 8.31 | 2.99 | 15.64  | 36.21  | 8.24 | 4.97 |
| January         | 13.96  | 36.99  | 8.27 | 5.21 | 13.17  | 38.21  | 8.33 | 4.01 |
| February        | 13.28  | 37.67  | 8.28 | 3.04 | 13.46  | 38.03  | 8.27 | 5.11 |
| March           | 13.31  | 37.78  | 8.23 | 4.76 | 13.53  | 38.46  | 8.29 | 6.49 |
| April           | 14.62  | 38.12  | 8.24 | 6.45 | 14.93  | 37.78  | 8.24 | 6.12 |

3.2. Biometric Relationships

Total number of \textit{P. caerulea} specimens collected was 1040. Shell length ranged from 9.3 to 45.1 mm, shell width from 5.7 to 40.9 mm, shell height from 2.8 to 12.8 mm, posterior shell length from 0.8 to 21.9 mm, total weight from 0.2 to 8.4 gr, and foot weight from 0.04 to 2.4 gr. Temporal variation of biometric characters was exhibited, with highest values in spring and lowest values in autumn. No spatial differences were exhibited in the shell length and shell width between sites. However spatial differences were exhibited, with significantly higher shell height, posterior shell length, total weight, and foot weight at the sheltered site (Site 1). The full set of biometric measurements at each sampling site are presented in Table 2. Limpet shell length, shell width, shell height, and total weight frequency distributions (Figures 3–6) indicated greater variability of the morphometric characteristics of \textit{P. caerulea} population at the sheltered site (Site 1).

3.3. Population Density

Population density at the sheltered site (Site 1) was higher in winter (126.7 ind/m\textsuperscript{2} ± 55.4) followed by summer (120.0 ind/m\textsuperscript{2} ± 52.7), autumn (115.8 ind/m\textsuperscript{2} ± 37.4), and spring (107.5 ind/m\textsuperscript{2} ± 35.0). Analysis of variance did not indicate significant differences in temporal population density at the sheltered site. Population density at the exposed site (Site 2) was higher in spring (195.0 ind/m\textsuperscript{2} ± 34.4) followed by summer (165.83 ind/m\textsuperscript{2} ± 13.77), winter (128.3 ind/m\textsuperscript{2} ± 29.3), and autumn (125.8 ind/m\textsuperscript{2} ± 20.1). Overall \textit{P. caerulea} population density was 135.63 ind/m\textsuperscript{2} ± 41.95. Population density at Site 1 (sheltered) was 117.6 ind/m\textsuperscript{2} ± 39.9 and at Site 2 (exposed) 153.75 ind/m\textsuperscript{2} ± 37.0. Highest temporal abundance of the total population was recorded in spring (151.3 ind/m\textsuperscript{2} ± 23.3) followed by summer (142.9 ind/m\textsuperscript{2} ± 17.4), winter (127.5 ind/m\textsuperscript{2} ± 16.2), and autumn (120.8 ind/m\textsuperscript{2} ± 11.2). Results of Nested ANOVA did not indicate temporal differences in the density of the total population. Spatial differences in the population density between sampling sites were indicated with higher abundance observed at the exposed site (Site 2), however not statistically significant (Table 3).
Table 2. Spatial and temporal variation of the biometric characters, mean value + standard error (SE) (L shell length, W shell width, H shell height, PL posterior shell length, Wt total weight, Wf foot weight) measured of the *Patella caerulea* population in Pagasitikos Gulf and one way ANOVA results (F, p).

| Sampling Period | No. of Individuals | L ± SE | W ± SE | H ± SE | PL ± SE | Wt ± SE | Wf ± SE |
|-----------------|--------------------|--------|--------|--------|---------|---------|--------|
| Site            | (n)                |        |        |        |         |         |        |
| Site 1          | 520                | 23.47 ± 0.23 | 19.09 ± 0.21 | 6.22 ± 0.07 | 11.21 ± 0.13 | 1.43 ± 0.05 | 0.51 ± 0.06 |
| Site 2          | 520                | 23.24 ± 0.15 | 18.83 ± 0.13 | 5.97 ± 0.05 | 10.89 ± 0.09 | 1.24 ± 0.02 | 0.37 ± 0.01 |
|                 | F = 0.66, p = 0.42 | F = 1.13, p < 0.05 | F = 8.51, p < 0.05 | F = 4.12, p < 0.05 | F = 12.23, p < 0.001 | F = 20.58, p < 0.001 |
| Season          | (n)                |        |        |        |         |         |        |
| Winter          | 242                | 22.78 ± 0.27 | 18.71 ± 0.23 | 5.97 ± 0.08 | 10.57 ± 0.14 | 1.15 ± 0.04 | 0.32 ± 0.01 |
| Spring          | 318                | 25.14 ± 0.25 | 20.46 ± 0.24 | 6.56 ± 0.08 | 12.50 ± 0.15 | 1.66 ± 0.06 | 0.52 ± 0.02 |
| Summer          | 240                | 23.60 ± 0.28 | 19.13 ± 0.25 | 6.39 ± 0.09 | 10.91 ± 0.16 | 1.48 ± 0.06 | 0.46 ± 0.02 |
| Autumn          | 240                | 21.32 ± 0.25 | 17.04 ± 0.23 | 5.28 ± 0.07 | 9.73 ± 0.13 | 0.94 ± 0.03 | 0.27 ± 0.01 |
|                 | F = 39.02, p < 0.001 | F = 37.07, p < 0.001 | F = 50.82, p < 0.001 | F = 68.59, p < 0.001 | F = 39.80, p < 0.001 | F = 51.18, p < 0.001 |
| Total           | 1040               | 23.56 ± 0.14 | 18.96 ± 0.12 | 6.09 ± 0.04 | 11.05 ± 0.08 | 1.34 ± 0.03 | 0.41 ± 0.01 |

Figure 3. Shell length-frequency distribution of *Patella caerulea* population with overlaid fitted normal distribution at both sites.

Figure 4. Shell width-frequency distribution of *Patella caerulea* population with overlaid fitted normal distribution at both sites.
Figure 5. Total shell height-frequency distribution of *Patella caerulea* population with overlaid fitted normal distribution at both sites.

Figure 6. Total weight-frequency distribution of *Patella caerulea* population with overlaid fitted lognormal distribution at both sites.

Table 3. Nested ANOVA results of spatial and temporal effects on the surveyed *Patella caerulea* population abundance.

| Source of Variation | DF | SS      | MS      | F      | p       |
|---------------------|----|---------|---------|--------|---------|
| Site                | 1  | 7884.38 | 7884.38 | 4.45   | 0.077   |
| Season              | 6  | 10,397.92 | 1732.99 | 1.25   | 0.334   |
| Error               | 16 | 22,195.83 | 1387.24 |        |         |
| Total               | 23 | 40,478.13 |        |        |         |
No spatiotemporal differences were recorded among sampling stations within each sampling site. Spatial abundance is shown in Figure 7. Comparative densities and morphometric characteristics of *P. caerulea* from various Mediterranean areas are displayed in Table 4.

Table 4. Densities and morphometric characteristics of *Patella caerulea* reported from various Mediterranean areas.

| Area                        | Density (ind/m²) | Shell Length (mm) | Shell Width (mm) | Shell Height (mm) | Total Weight (gr) | References |
|-----------------------------|------------------|-------------------|------------------|-------------------|-------------------|------------|
| Strait of Gibraltar         | 125              | 28.87 ± 3.66      |                  |                   | 2.87 ± 1.09       | [37]       |
| Strait of Gibraltar         | 100              | 28.87 ± 3.66      |                  |                   | 2.87 ± 1.09       | [39]       |
| French Mediterranean coast   | 16.5 ± 9.9       |                   |                  |                   |                   | [38]       |
| Northern Adriatic            | 3–38             | 13.5–56.1         | 19.91–25.52      | 3.5–17.5          |                   | [25]       |
| Tunisia                      | 21.03–35.57      | 17.93–30.15       | 4.99–9.4         |                   |                   | [40]       |
| Turkey (Izmir Bay)           | 24.36–30.75      | 10.5–46.9         | 6.26–8.9         | 2.59–4.21         |                   | [41]       |
| Pagasitikos Gulf (Central Greece) | 135.63 ± 41.95  | 23.36 ± 4.46      | 18.96 ± 4.01     | 6.09 ± 1.38       | 1.34 ± 0.89       | Present study |

3.4. Distribution Pattern

Spatial point pattern analysis used to examine the spatial distribution of *P. caerulea*, exhibited significant clustered spatial dispersion pattern in spring at the sheltered site (Site 1), and in winter and spring at the exposed site (Site 2) (Table 5).

3.5. Condition Index (C.I.)

Analysis of variance indicated temporal differences in the C.I. (Table 6). Tukey pairwise comparisons between sample means revealed significant temporal differences in the C.I. of the total population between spring (31.36 ± 5.04) and summer (31.45 ± 4.39), in comparison with winter (28.03 ± 4.65) and autumn (28.90 ± 4.78).

Maximal C.I. occurred in April 2017 (32.762 ± 7.38) followed by August (32.143 ± 4.78) and September (32.043 ± 4.54), whereas minimal C.I. occurred in February (26.58 ± 4.47) followed by November (27.425 ± 4.75). Spawning season of *P. caerulea* occurs at the end of summer (Figure 8), indicated by an increase of the C.I. from winter to summer (February to July) and a sudden decrease at the end of summer (September).
Table 5. Population density (ind/m²) and dispersion pattern of *Patella caerulea* per sampling site and period (SD standard deviation, Iδ Morisita index of aggregation, DF degrees of freedom, X² chi-square values corresponding to the statistic obtained with indication of their significance at 95% confidence level).

| Sampling Period | Sites | Population Density (ind/m² ± SD) | Iδ  | X²    | Dispersion Pattern | Significance Level | DF |
|-----------------|-------|----------------------------------|------|-------|--------------------|--------------------|-----|
| April           | Site 1| 55 ± 30.73                       | 1.29 | 6.18  | clustered          | ns                 | 9   |
|                 | Site 2| 177.5 ± 2.56                     | 1.13 | 8.29  | random             | ns                 | 9   |
| May             | Site 1| 122.5 ± 91.6                     | 1.51 | 24.74 | clustered          | <0.05              | 9   |
|                 | Site 2| 225 ± 55.3                       | 1.05 | 4.89  | random             | ns                 | 9   |
| June            | Site 1| 132.5 ± 85.8                     | 1.38 | 19.98 | clustered          | <0.05              | 9   |
|                 | Site 2| 202.5 ± 113.3                    | 1.29 | 22.8  | clustered          | <0.05              | 9   |
| July            | Site 1| 115 ± 69.9                       | 1.34 | 15.31 | clustered          | ns                 | 9   |
|                 | Site 2| 152.5 ± 27.5                     | 1.03 | 1.79  | random             | ns                 | 9   |
| August          | Site 1| 175 ± 76.3                       | 1.11 | 7.71  | random             | ns                 | 9   |
|                 | Site 2| 165 ± 71.9                       | 1.17 | 11.27 | random             | ns                 | 9   |
| September       | Site 1| 70 ± 30.7                        | 1.18 | 4.86  | random             | ns                 | 9   |
|                 | Site 2| 180 ± 85.6                       | 1.21 | 14.71 | random             | ns                 | 9   |
| October         | Site 1| 105 ± 28.4                       | 1.07 | 2.76  | random             | ns                 | 9   |
|                 | Site 2| 105 ± 61.0                       | 1.31 | 12.76 | clustered          | ns                 | 9   |
| November        | Site 1| 85 ± 44.4                        | 1.25 | 8.35  | clustered          | ns                 | 9   |
|                 | Site 2| 145 ± 35.0                       | 1.05 | 3.03  | random             | ns                 | 9   |
| December        | Site 1| 157.5 ± 80.0                     | 1.24 | 14.63 | clustered          | ns                 | 9   |
|                 | Site 2| 127.5 ± 71.2                     | 1.29 | 14.59 | clustered          | ns                 | 9   |
| January         | Site 1| 102.5 ± 60.6                     | 1.32 | 12.89 | clustered          | ns                 | 9   |
|                 | Site 2| 150 ± 60.1                       | 1.15 | 8.67  | random             | ns                 | 9   |
| February        | Site 1| 190 ± 62.6                       | 1.1  | 7.42  | random             | ns                 | 9   |
|                 | Site 2| 95 ± 38.7                        | 1.15 | 5.68  | random             | ns                 | 9   |
| March           | Site 1| 87.5 ± 51.7                      | 1.32 | 10.99 | clustered          | ns                 | 9   |
|                 | Site 2| 140 ± 96.9                       | 1.44 | 23.95 | clustered          | <0.05              | 9   |
| April           | Site 1| 77.5 ± 36.2                      | 1.20 | 6.09  | random             | ns                 | 9   |
|                 | Site 2| 137.5 ± 71.9                     | 1.25 | 13.54 | random             | ns                 | 9   |

Figure 8. Temporal variability (95% confidence interval for the mean of each group) in the condition index of *Patella caerulea*.
Table 6. ANOVA results of reproductive status of the surveyed *Patella caerulea* population.

| Source of Variation | DF | MS   | F     | p    |
|---------------------|----|------|-------|------|
| Abundance           | 3  | 773.98 | 34.35 | <0.001 |
| Error               | 1036 | 22.53 |       |      |
| Total               | 1039 |      |       |      |

3.6. Age Composition

Seven dominant age groups were identified (Figure 9, Table 7). The dominant cohort was the third-year class, with 42% of the total population.

![Figure 9. Patella caerulea cohorts identified with modal progression analysis.](image)

Table 7. Population characteristics of the age groups identified for the entire population (mean length, standard deviation, population size, separation index, and population percentage at each age class.

| Age Group | Mean Length (mm) | Standard Deviation | Population Size | Separation Index (SI) | Population % |
|-----------|------------------|--------------------|-----------------|-----------------------|--------------|
| 1         | 11.83            | 2.220              | 8               | 3.370                 | 0.82         |
| 2         | 18.78            | 1.900              | 228             | 2.620                 | 23.41        |
| 3         | 23.13            | 1.420              | 408             | 2.400                 | 41.89        |
| 4         | 26.17            | 1.110              | 213             | 3.930                 | 21.87        |
| 5         | 30.96            | 1.330              | 78              | 3.010                 | 8.01         |
| 6         | 34.62            | 1.100              | 28              | 3.890                 | 2.87         |
| 7         | 40.53            | 1.940              | 11              | 3.370                 | 1.13         |

3.7. Allometric Relationships

Equations describing the best fit (highest correlation coefficient) between different morphometric characteristics of *P. caerulea* populations throughout the sampling period and their allometric relationships are displayed in Table 8. Comparison of regression lines (Table 8) indicated no significant difference in regression lines of shell length vs. shell width among sampling sites. Significant differences among sampling sites were observed in the slopes of the regression lines of total weight vs. shell length and total weight vs. shell width. Highly significant differences among sampling sites were observed in the slopes and intercepts of the regression lines of shell weight vs. shell height, shell length vs. shell height, and shell width vs. shell height. Significant differences exhibited in the regression lines are indicative of differences in the growth patterns among studied populations.
Table 8. Allometric equations between shell length (L), shell width (W), shell height (H), and total weight (Wt) of *Patella caerulea* for sampling sites, Site 1 (sheltered) and Site 2 (exposed), during the study. N: number of individuals, $R^2$: coefficient of determination, t-test: statistical significance of the allometric relationship, allometry: allometric relationship between the two variables, slopes (b): statistical comparison between the slopes of the equations, intercepts (a): statistical comparison between the intercepts of the equations, significance level (ns: non-significant, *: $p < 0.05$, **: $p < 0.001$).

| Sampling Site | Equation | N  | $R^2$ | t-test | Allometry | Slopes | Intercepts |
|---------------|----------|----|-------|--------|-----------|--------|------------|
| Site 1 Wt     | $Wt = 0.000083234 \times L^{3.03929}$ | 520 | 96.2  | ns     | Isometry  | *      | ns         |
| Site 2 Wt     | $Wt = 0.000133015 \times L^{2.80733}$ | 520 | 90.8  | ns     | Isometry  |         |            |
| Site 1 W      | $W = 0.000496301 \times W^{2.65566}$ | 520 | 94.6  | **     | -ve Allometry | *      | ns         |
| Site 2 W      | $W = 0.000750993 \times W^{2.50773}$ | 520 | 89.8  | **     | -ve Allometry |         |            |
| Site 1 Wt     | $Wt = 0.0135219 \times W^{2.48436}$ | 520 | 91.5  | **     | -ve Allometry | **     | **         |
| Site 2 Wt     | $Wt = 0.0291897 \times W^{2.07913}$ | 520 | 82.7  | **     | -ve Allometry | **     | **         |
| Site 1 L      | $L = 5.72765 \times H^{0.774654}$ | 520 | 88.4  | **     | -ve Allometry | **     | **         |
| Site 2 L      | $L = 8.04943 \times H^{0.595556}$ | 520 | 76.2  | **     | -ve Allometry | ns     | ns         |
| Site 1 W      | $W = 1.88689 \times H^{0.855741}$ | 520 | 93.2  | **     | -ve Allometry | ns     | ns         |
| Site 2 W      | $W = 2.14392 \times H^{0.812053}$ | 520 | 90.3  | **     | -ve Allometry |         |            |
| Site 1 Wt     | $Wt = 4.12051 \times H^{0.841331}$ | 520 | 87.4  | **     | -ve Allometry | **     | **         |
| Site 2 Wt     | $Wt = 6.32293 \times H^{0.612731}$ | 520 | 70.6  | **     | -ve Allometry | **     | **         |

4. Discussion

Several studies of rocky coastal communities at temperate latitudes have identified limpets as “keystone” grazers [57] and important structuring agents [58] with numerous functions, including roles as herbivores, as prey for higher trophic-level consumers, and as space occupiers that limit the recruitment of other invertebrates [8,10,59]. Limpets are highly vulnerable because of their restricted habitat and easy access to humans [60], threatened by a number of stressors [61] including habitat degradation [62], overfishing [63], pollution and sedimentation [64,65], eutrophication [66], invasive species [67], and climate change [68], among others. These stressors often act simultaneously [61,69].

4.1. Biometric Relationships

Significant differences were also exhibited on a temporal scale, with smallest values occurring during autumn and largest during spring, possibly attributed to the reproductive cycle of *P. caerulea* in the study area with spawning occurring at the end of summer. In Turkey (Izmir Bay) [41] minimum shell length was reported in autumn ($25.13 \pm 0.77$ mm) and maximum in summer ($29.88 \pm 0.87$ mm), maximum total weight in summer ($3.92 \pm 0.29$ mm) and minimum in fall ($2.85 \pm 0.26$ mm). Long-term studies on rocky shores have shown that communities are continually changing, with both rapid inter-annual fluctuations and longer-term trends [70].

*P. caerulea* biometric relationships exhibited significant differences on a spatial scale, with significantly higher shell height, posterior shell length, total weight and foot weight at the sheltered site, possibly attributed to the variable degree of wave exposure and anthropogenic pressure among sites and density differences.

Several studies support that greater growth in sheltered areas is due to lower densities per unit of grazeable area, meaning higher food availability [71–73]. Shell alterations in gastropods can be induced by environmental and biological factors, such as wave action, salinity, and predatory pressures [74]. Limpets from very exposed sites often appear to be different from specimens living in more sheltered situations [75]. Limpets tend to vary greatly in size, shape, sculpture, and color pattern, because of ecological factors (particularly the relative exposure to wave stress, and the nature of the substratum) [76]. Limpet growth rate and biomass are highly dependent on rates of algal production [77] and the microalgal film coating the rocky shore, consisting predominantly of organic material, diatoms, and cyanobacteria that provide a food supply for microphagous grazers [71].
4.2. Population Density

Population density (135.63 ind/m² ± 41.95) displayed seasonal variation, with highest numbers observed in spring (151.3 ind/m² ± 23.3) followed by summer (142.9 ind/m² ± 17.4), winter (127.5 ind/m² ± 16.2), and autumn (120.8 ind/m² ± 11.2). Higher abundance was exhibited at the exposed site (Site 2) (153.75 ind/m² ± 37.0) compared to the sheltered (Site 1) (117.6 ind/m² ± 39.9), however, it was not statistically significant. In agreement with [71], who concluded that limpet density declines with increasing shelter and [78] who suggested that the overall density of *P. vulgata* declines as shelter increases, a possible direct result of predatory activity, since heavy wave action can limit the activity of predators and grazers at exposed sites [79,80]. Patellid limpets are consumed by a wide variety of predators including fish [81], octopi [82], crabs [83], other gastropods [84], starfish [85], birds [86], and humans [87]. It has been suggested [88] that predation is an important factor controlling limpet abundance on the lower shore. Taking into consideration that limpets have annual recruitment, yearly effects of predation could be quite substantial. Predators have been shown to regulate the abundance of juvenile mussels on rocky shores in New Zealand [28] and predation by crabs in the U.K. can influence the diversity of intertidal prey assemblages [29]. Predators can be significantly more abundant on sheltered than on exposed shores [89] resulting in greater direct and indirect effects of predation on sheltered shores.

Several studies reported higher densities of *Patella* sp. in exposed areas, inversely related to body size and reproductive investment [71,78,90]. It has been suggested [90] that the density of *Patella* populations is determined by the balance between recruitment and survival. According to the intraspecific variability among populations of *P. vulgata*, abundance differences might be related to the aggregation of specimens in relation to trophic resources or use of habitat, being indifferent whether they are on rocky shores or artificial seawalls [91].

Variations in the density of *Patella* spp. have been observed in areas with different degrees of wave exposure [71,90]. According to Casal et al. [2] the estimated average densities of *P. vulgata* and *P. depressa* were lower in sheltered than in exposed areas. In contrast no differences were observed in the density of limpets between sheltered and exposed sites in Scotland [92].

4.3. Distribution Pattern

Clustered spatial distribution pattern was indicated for *P. caerulea* in spring at the sheltered site and in winter and spring at the exposed site, possibly a consequence of recruitment. Recruitment of *Patella* is lower in sheltered areas [72,73], which is attributed to lower connectivity or poorer conditions for settlement [93]. Habitat physical characteristics may have a great influence in processes such as recruitment, competition, and predation, which, in turn, affect the structure of populations [94,95]. Changes in the spatial distribution of organisms can have implications for all parts of an ecosystem, biotic and abiotic. Clustering, dispersion, or spatially random patterns of individuals may be evidence of either an endogenous biological or ecological process or a response to environmental variation or environmental change [96]. The importance of several abiotic factors affecting the settlement and recruitment of benthic organisms (orientation of the substratum, texture, current regime, sediment load, light attenuation/depth, and water quality and nutrient level) has been pointed out [97]. Differences in substratum preferences in *P. caerulea* and *P. ulysseiponensis*, such that larvae of both species settled preferably on sedimentary rocks rather than on igneous ones have been identified [98].

4.4. Condition Index

Spawning of *P. caerulea* occurs during the end of summer, which is indicated by an increase of the C.I. from winter to summer (February to July) and a sudden decrease at the end of summer (September). The highest C.I. can signify the period before spawning in gastropods, water temperature can also affect both the index and reproduction of *Patella* species [99]. *P. caerulea* is a hermaphrodite protandric species and along the southwestern coast of Italy spawning occurs from autumn to spring [100].
In Turkey (Izmir) maximum C.I. of *P. caerulea* occurs during winter (41.20 ± 1.18) and minimum in fall (30.46 ± 1.54) [41]. Significant temporal differences of the condition index were exhibited, with summer displaying the largest difference. In Tunisia maturation began in August/September in both sexes for *P. caerulea*. Male spawning took place between March and August 2006 and between April and June. Female spawning took place between April and July. A secondary spawning was observed in January for both sexes [101]. It has been reported [102] that limpets spawn in winter (November, January, February, March) in south eastern Australia, whereas in Adriatic Sea the reproductive cycle of *P. rustica* is between November and December [36].

4.5. Age Composition

Seven cohorts were identified with the dominant cohort in the third-year class, with roughly 42% of the total population. Growth and longevity of *P. vulgata* are directly related to habitat with limpets under the shelter of fucoids growing rapidly but may live only 2–3 years, whereas those on bare rock grow slowly but live up to 15–17 years [78,90,99]. Such differences in life history pattern due to local environmental conditions may drive spatial variability in the duration of such cycles.

4.6. Allometric Relationships

Significant negative allometric relationships exhibited in the morphometric characteristics of the populations, indicated differences in growth patterns, with the tendency of *P. caerulea* to grow in weight, faster than in width and height and in length and width, faster than in height. Significant differences in growth patterns among the studied populations were indicated possibly because of variable degree of exposure of the sampling sites. *Patella* species show substantial morphological variability possibly attributed to environmental variation [16,18,22,103].

A curvilinear function of shell length vs. shell height was reported by [75] for *Patella* sp. In Portugal, by [16] for *P. ulyssiponensis* and *P. vulgata* in the UK, and by [104] for *P. ulyssiponensis* and *P. caerulea* in southern Spain. Power regression equations (curvilinear) in three sites in Tunisia with significant negative allometric relationships for *P. caerulea* describing the relationships of shell length vs. shell height, shell length vs. shell width, and shell width vs. shell height were reported [40]. In western Algeria [105] *P. rustica* exhibited similar curvilinear negative allometric regression equations, describing the relationships between shell length, shell height, and total weight.

Rocky intertidal communities are sensitive to the effects of anthropogenic disturbance. Common forms of disturbance range from indirect (often chronic) agents, such as sewage and industrial effluent [106], to more direct agents, such as harvesting and development [107–110]. These impacts have led to decreased biomass, decreased species richness, and shifts in community composition worldwide [106,107,111,112]. Identification of the simultaneous effects of multiple human-induced threats is one of the major challenges in modern ecology [69,113] directly affecting biodiversity. All stressors have the potential to cause important changes in population and/or assemblage dynamics and their effects need to be reduced [114,115].

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