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Density and patterns of orientation of *Patella caerulea* L. in the Bay of Koper (Gulf of Trieste, North Adriatic)

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ABSTRACT

This study deals with the density and patterns of head-orientation of the midlittoral limpet *Patella caerulea* L., along the Bay of Koper (Gulf of Trieste, North Adriatic). The aims were to determine the relationship in patterns of head-orientation and density of limpets on vertical, horizontal, wave exposed, and sheltered substrata. The results revealed that the density of limpets on vertical and exposed substrata was greater than those on horizontal and sheltered surfaces; the proportion of downward head-orientated limpets was higher in comparison with upward head-oriented limpets; also the proportion of downward-facing limpets was higher on vertical exposed surfaces than on the others.

Keywords: *Patella caerulea,* density, head-orientation, midlittoral, north Adriatic

INTRODUCTION

Among different species of midlittoral limpets, those belonging to the genus *Patella*, three species are very common along the Adriatic shores, namely: *Patella caerulea* L., *P. ulyssiponensis* Gmelin (= *Patella aspera* Lam), and *P. rustica* L. (= *P. lusitanica* Gmelin) (Grubelić, 1992; Šimunović, 1995; Zavodnik et al., 2005). Information on this genus along the Slovenian coast has mostly been occasional. Several authors mention only the species *P. caerulea* for the Slovenian shore (Matjašič & Štirn, 1975; Lipej et al., 2004; Pitacco et al., 2013; Battelli, 2016a, 2016c). De Min & Vio (1997) report the presence of *P. caerulea* all along the Slovenian coast as a very common species, while *P. ulyssiponensis* and *P. rustica*, as very rare.

Limpets are widely studied, because they are easily accessible and because–being sessile midlittoral organisms–they represent a very good study system for determining density and patterns of orientation. They play also an important role in the ecology of the midlittoral zone. Grazing, for example, exerts a very strong effect on midlittoral ecosystem (Poore et al., 2012), directly controlling the density and composition in algal assemblages and structuring rocky shore communities (Jenkins et al., 2005; Coleman et al., 2006; Moore et al., 2007; Battelli, 2016b).

Among different abiotic factors that influence patterns of limpet distribution and their density many authors consider many factors as: different types of wave exposure, substratum morphology and steepness, tidal dynamics, thermal and desiccation stress (Williams & Morritt, 1995; Harley, 2008; Miller et al., 2009; Fraser et al, 2010; Christofoletti et al., 2011; Prusina et al., 2014a). Limpet density depends also on biotic factors as intra and interspecific interactions (Coleman, 2010; Fraser, 2014b; Fraser et al., 2015a, 2015b).

Another characteristic of limpets that has aroused particular interest of many researchers is the orientation of their bodies. This characteristic can be defined as the fine-scale spatial position of an individual at a given point in time with respect to a given directional stimulus/factor (e.g. light, north/south, upwards/downwards) (Fraser et al., 2010, 2014a, 2014b). Orientation often differs between vertical and horizontal surfaces (e.g. Fraser et al., 2010; Fraser, 2014a, 2014b). In many species of limpet, including *P. caerulea*, individuals return to the same resting site, called 'homing' (Della Santina, 1994), after feeding.

Although a number of studies have been carried out on the biology, distribution and ecology of *Patella* species of the Adriatic Sea (e.g. Grubelić, 1992; Šimunović, 1995; Zavodnik et al., 2005; Prusina et al. 2014a, 2014b, 2015), very little is known about these species across the Slovenian coast, except the works of Battelli (2016a, 2016c) on the morphometric characteristics, vertical distribution and density of the limpet *Patella caerulea* L.

The present study was carried out along the south-western coast of the Bay of Koper and it deals with the density and patterns of head-orientation of the midlittoral limpet *P. caerulea* on differently sloped and differently wave-exposed surfaces. Our investigations aimed to determine (a) whether the density of individuals of the limpet *P. caerulea* differed between surfaces that are differently sloped (horizontal and vertical) and differently wave-exposed (exposed and sheltered); (b) whether a dominant head-orientation of this species of limpets existed and (c) whether the mean head-orientation of individuals of this species was different on horizontal, vertical, wave-exposed, and wave-sheltered surfaces.

MATERIAL AND METHODS

Study sites

The study was performed within the midlittoral zone at three locations interspersed along the southern part of Slovenian coast (Bay of Koper), total distance being approximately 5 km. Location Lo1 was situated on the coast between Koper and Izola (45º 32´ 49´´ N, 13º 41´11´´ E). Location Lo2 was situated in the northern part of Izola (45º 32´ 32´´ N, 13º 39´ 42´´ E), while location Lo3 was located in St. Simon Bay (45º 32´ 04´´ N, 13º 38´ 47´´ E) (Fig. 1). The locations Lo2 and Lo3 consisted of a rocky shore and had a horizontal extent of about 250 m. The location Lo1 was characterized by the presence of boulders of different size and slope. The substratum of the study locations consisted of limestone (Pavlovec, 1985). Each location contained a series of sites with differently sloped and exposure to wave-action.

The mean tidal amplitude in the midlittoral zone ranged from – 40 cm (MLLW) to + 49 cm (MHHW) and the mean sea level (MWL) was 218 cm (Fig. 2) (baseline measurements of the sea level are Mareographic zero at the tide gauge station in Koper) (Environ. Agency of the Republic of Slovenia (ARSO) (2016).

Experimental design

At each location two sites, one consisting of steeply sloped (vertical) rocky surfaces (>60°), and one of shallowly sloped (horizontal) (<30º), were chosen randomly. Each site was a

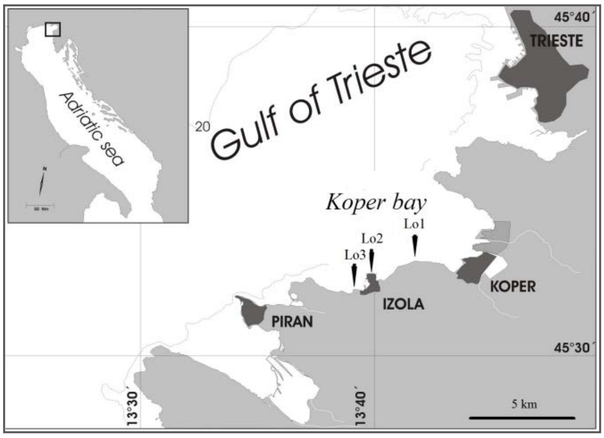


Figure 1. Map of the research area (Bay of Koper, Gulf of Trieste), indicating the sampling locations (Lo1, Lo2 and Lo3).

stretch of coastline of about 50 m. Within each site two transects, at least 10 m apart, were haphazardly selected: one on exposed and one on sheltered shore. Five 400 cm^2 (20 x 20 cm) replicate plots, 1 m apart, were randomly selected within each transect. In this experiment there were thus in total 30 plots from exposed and 30 from sheltered shore.

Sampling and statistical data analyses

Plots were sampled at low tide during August 2016 at shore levels of the midlittoral zone occupied by the limpet *P. caerulea*. On the basis of our observations, we found that the upper limit of the presence of *P. caerulea* individuals was principally the MHW, while the lower limit was the MLW (Fig. 2). It is important to note that *P. caerulea* individuals, after feeding, return to the same resting site (homing) and maintain the same direction (patterns of head-orientation).

The density of *P. caerulea* individuals within a plot was determined by counting the individuals present in the whole selected plot. The head-orientation of limpets was measured from field-derived photographs of limpets, and noting their head-orientations (posterior-anterior) *in situ*. In total 366 individuals of *P. caerulea* were examined.

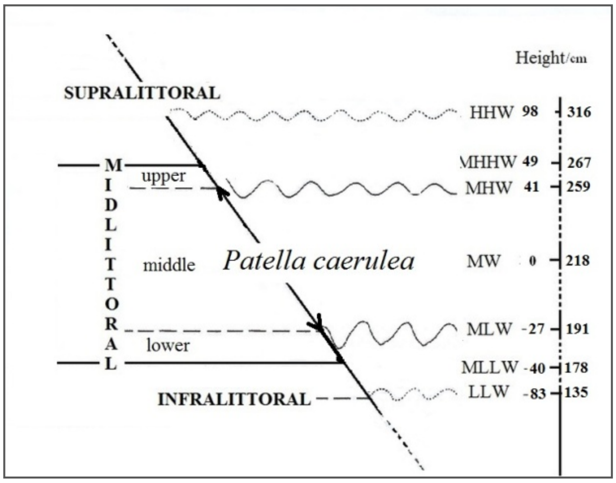


Figure 2. Schematic representation of the vertical zonation of the midlittoral zone indicating the horizons (upper, middle and lower) and the limits of distribution of *P. caerulea*. The relative average sea levels of the period 2005–2015 for the Bay of Koper are indicated (HHW – Higher High water; MHHW – Mean Higher High Water; MHW – Mean High Water; MW – Mean Water; MLW – Mean Low Water; MLLW – Mean Lower Low Water; LLW – Lower Low Water). Sources of data: ARSO 2016. (Redesigned after Battelli, 2016c).

The anterior of this species is easily identified as the profile of their shell is asymmetric, with the peak at the head (anterior) (Fig.3).

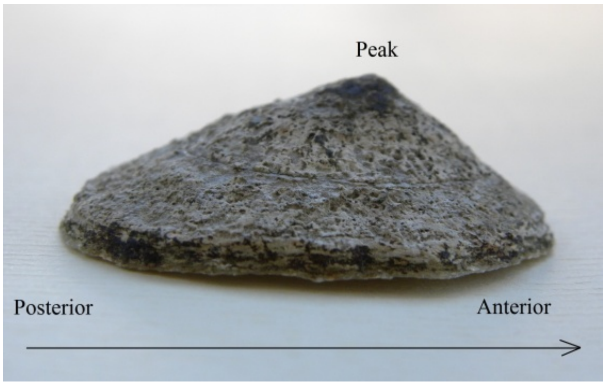


Figure 3. *P. caerulea* shell with annotated head-orientation (posterior-anterior).

On the basis of the head-orientation (posterior-anterior) on the shore (plot), the limpets were classified in six groups (where 0° is normal to gravity) (Fig.4), as follows:

1. Downwards-facing limpet: individuals with head-orientation between 135° and 225°;
2. Upwards-facing limpet: individuals with head-orientation between 315° and 45°;
3. Upwards-right-facing limpet: individuals with head-orientation between 45° and 90°;
4. Upwards-left-facing limpet: individuals with head-orientation between270° and 315°;
5. Downwards-right-facing limpet: individuals with head-orientation between 90° and 135°;
6. Downwards-left-facing limpet: individuals with head-orientation between 225° and 270°.

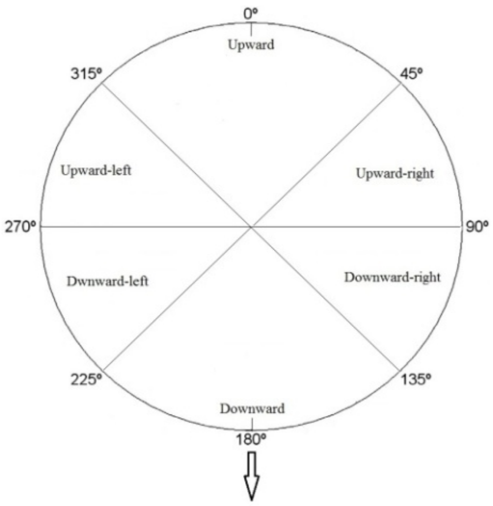


Figure 4. Schematic representation of limpet groups on the basis of their head-orientation. The degrees that delimit single groups (sectors) are indicated. Arrow indicates vertical downward direction (modified after Fraser et al., 2010).

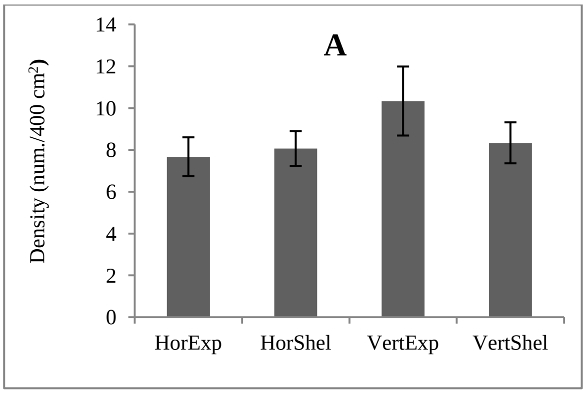
In order to establish whether there was a statistically significant difference between groups of samples, the raw data were subjected to statistical analysis using the non-parametric Kruskal-Wallis H-test (Kruskal & Wallis, 1952) and the Mann-Whitney U-test (Mann & Whitney, 1947). The data were expressed as the mean ± SE (standard error of mean) and in proportion (%).

The hypothesis that there was no difference between the density of the limpet *P. caerulea* individuals and differently sloped and differently wave-exposure surfaces, was tested using a 2-way analysis of variance (ANOVA), by IBM SPSS 23.0 program. The considered factors were: *steepness* (2 levels: steeply sloped and shallowly sloped surfaces) and *wave-exposure* (2 levels: exposed and sheltered surfaces). The assumption of homoscedasticity of variances was tested using Levene's test. Significance level was set at $p < 0.05$. If necessary, transformation was used to reduce the heterogeneity of variances, which, however, in some analyses could not be stabilized by the transformation. In this case we decided to perform the analyses anyway; but with p set at 0.01.

RESULTS

Density of limpets on differently sloped and differently wave-exposed surfaces

During our field sampling of the study on the density (individuals/400 cm^2) of *Patella caerulea*, 516 individuals were analysed. The mean density was 8.6 (individuals/400 cm^2) and it ranged between a minimum of 2 and maximum of 21 limpets, although groups of 5–10 limpets were more common. The results showed that the density of limpets on differently sloped and differently wave exposed surfaces was the highest (mean = 10.3, SE = 1.7, n = 15) on vertical-exposed surfaces and the lowest on horizontal-exposed (mean = 7.7, SE = 0.9, n = 15) surfaces (Fig. 5A).



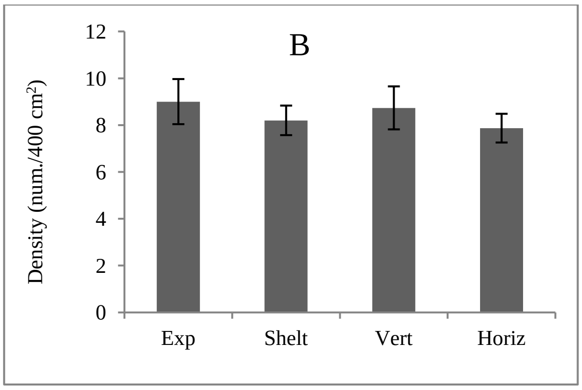


Figure 5. Comparison between density (mean ±SE) of *P. caerulea* populations on differently sloped and differently wave-exposed surfaces (combined-A, separated-B) across three locations (Bay of Koper). (HorExp = horizontal/exposed; HorShel = horizontal /sheltered; VertExp = vertical/exposed; VertShel = vertical/sheltered)

The comparison of the density of the populations of limpets between exposed, sheltered, vertical and horizontal surfaces showed slightly higher values on exposed (mean = 9.0, SE = 1.0, n = 30) and lower on vertical (mean = 8.7, SE = 0.9, n = 30) surfaces than on the others (Fig. 5B), but Kruskal–Wallis test showed that among these values there were no significant differences.

The results of 2-way ANOVA analysis showed that there were no significant differences in the density of *Patella caerulea* population among differently sloped and differently wave-exposed surfaces, as indicated in Table 1.

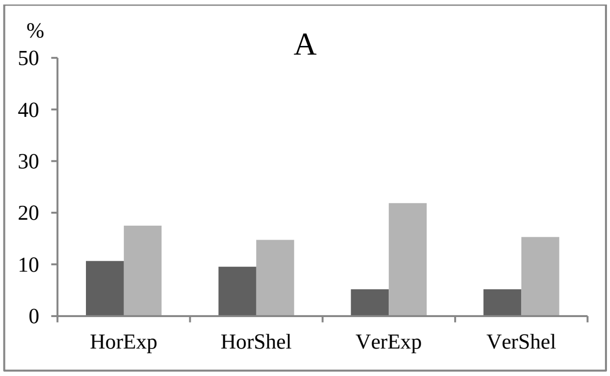
Table 1. 2-way ANOVA on the effect of differently sloped (horizontal and vertical) and differently wave-exposed (exposed and sheltered) surfaces on *P. caerulea* density, across three locations (Bay of Koper).

Dependent Variable: density, log transformation				
Source	df	MS	F	P
Slope	1	0.025	0.495	0.484
Exposure	1	3.938	0.001	0.978
Slope x Exposure	1	0.025	0.487	0.488
Error	56	0.051		
Total	60			
Corrected total	59			

Patterns of head-orientation of limpets on differently sloped and differently wave exposed surfaces

During our investigation from a total of 366 individuals of *P. caerulea*, on differently sloped and differently wave-exposed surfaces, 254 (69.40%) individuals were downward head-oriented (including downward-facing, downward-right-facing and downward-left-facing) and 112 (30.60%) were upward head-oriented (including upward-facing, upward-right-facing and upward-left-facing). The non-parametric Mann-Whitney U-test indicated that the difference between these values was statistically significant (p = 0.0286). Though the values of proportion of the single different head-oriented limpets varied between differently sloped and differently wave exposed surfaces, as illustrated in Figure 6A and Figure 6B, analysis, using Kruskal–Wallis test, showed that there were no significant differences between these surfaces.

From the Figure 6 it is evident that the total upward head-oriented limpets reached their highest values in terms of proportions on horizontal-exposed surfaces (10.7%) (Fig. 6A), on horizontal (20.2%) and on exposed (15.8%) surfaces (Fig. 6B). Conversely, the total downward head-oriented limpets showed consistently higher values on vertical-exposed surfaces (21.9%) (Fig. 6A), on vertical (37.2%) and on exposed (39.3%) surfaces (Fig. 6B).



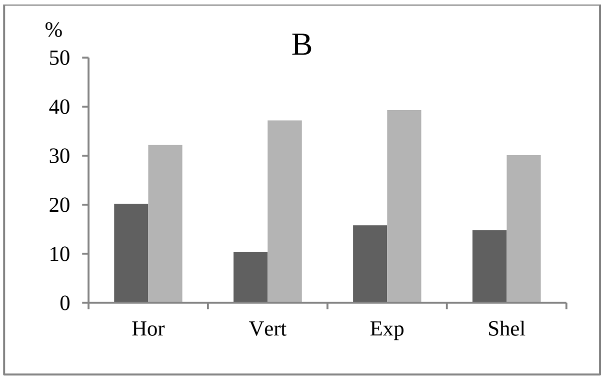


Figure 6. Comparison of proportions (%) of upward-facing (black bars) and downward-facing (grey bars) *P. caerulea* limpets on differently sloped and differently wave-exposed surfaces (combined-A, separated-B), across three locations (Bay of Koper).

The comparison of the proportions of differently head-oriented limpets showed some marked differences between differently sloped and differently wave exposed surfaces. Among upward head-oriented limpets the upward-left-facing ones reached the highest values on horizontal-sheltered surfaces (16.9%) (Fig. 7b) and on horizontal surfaces (16.7%) (Fig. 8a). On the contrary the upward-right-facing limpets reached the highest values on vertical-sheltered (16.0%) (Fig. 7d), and on sheltered surfaces (15.2%) (Fig. 8d).

Among downward-facing limpets, the highest values were found on vertical-sheltered surfaces (37.3%) (Fig. 7d), on vertical (33.3%) and on sheltered (32.9%) surfaces (Fig. 8d).

In the total research area the results of the analyses showed that the highest values reached the downward-facing limpets (29.8%) and the lowest the upward-facing limpets (5.7%) as illustrated in the Figure 8e.

DISCUSSION

The results of our investigation, on the distribution of populations of the species *Patella caerulea* across three locations of the bay of Koper, showed that the individuals of this species occupied mainly the middle horizon of the midlittoral zone. This is in accordance with Prusina et al. (2014b) and Battelli (2016a, 2016c), but in contrast with Della Santina et al. (1993) that reported this species as predominant in the lower midlittoral zone in the Mediterranean. This difference could be attributed to higher amplitude of tides in the north Adriatic (mean amplitude of about 90 cm) than in the central and southern part of the Adriatic and the Mediterranean Sea (mean amplitude 20-30 cm) as reported by Morri et al. (2003).

Many studies have documented that the distribution, abundance and behaviour of midlittoral organisms differ between differently sloped substrata (horizontal and vertical) (Della Santina, 1994; Williams *et al.*, 1999; Benedetti-Cecchi et al., 2000; Fraser, 2010) and differently wave-exposed substrata (Branch, 1981; Fraser et al., 2014a).

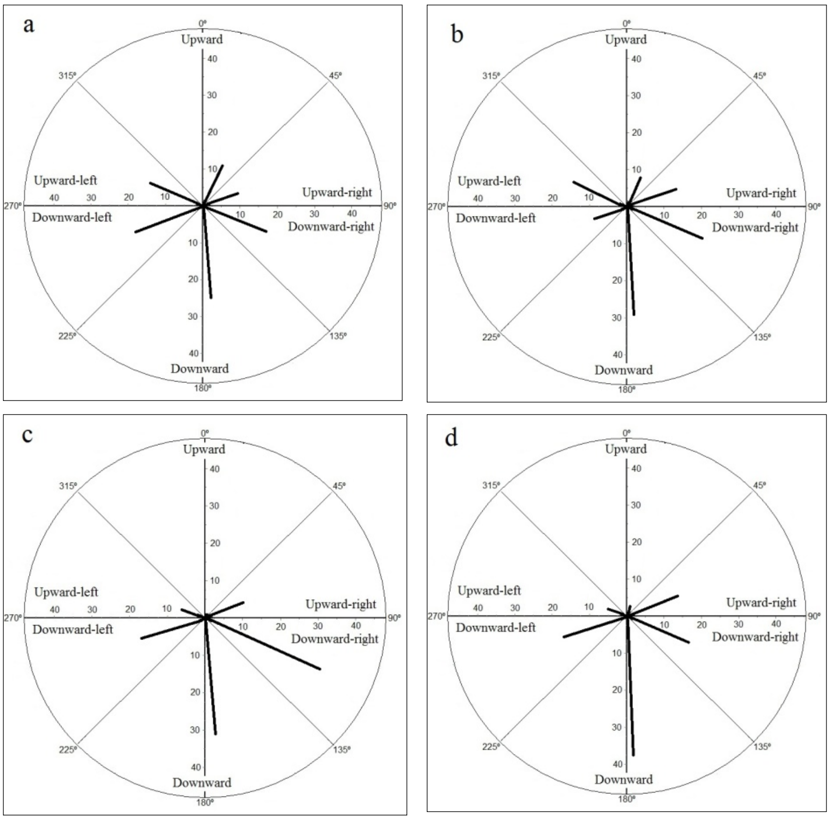
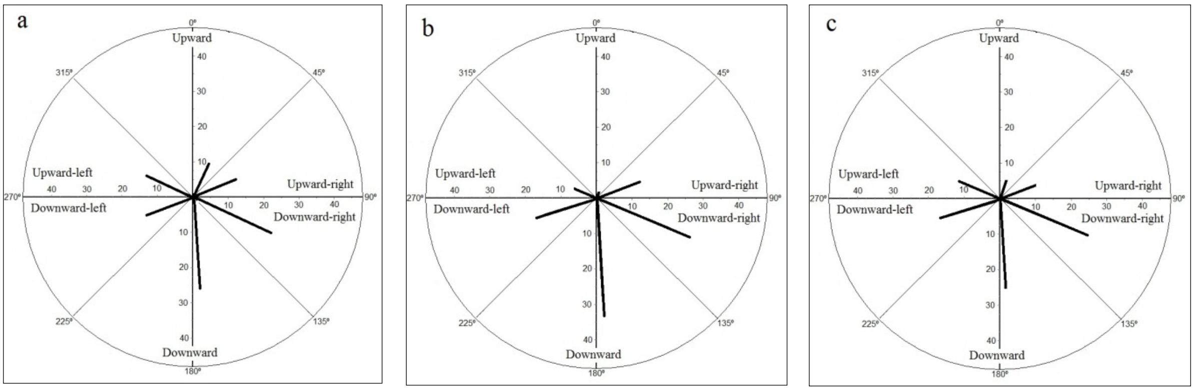


Figure 7. Patterns of head-orientation of *P. caerulea* at differently sloped and differently wave-exposed sites (a = horizontal-exposed; b = horizontal-sheltered; c = vertical-exposed; d = vertical-sheltered). Internal axes indicate the proportion (%) of *P. caerulea* populations in different head-orientation.



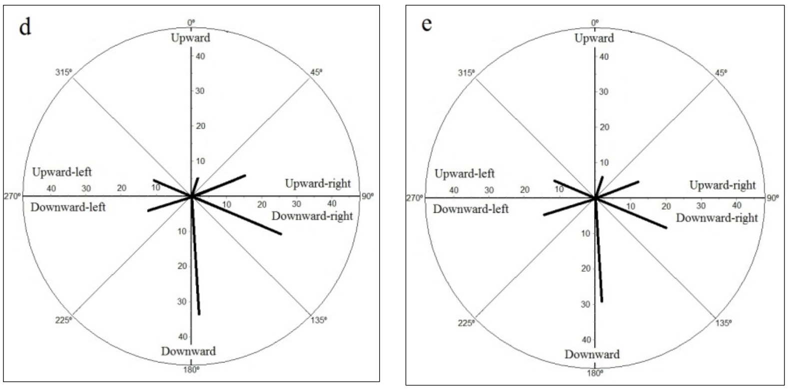


Figure 8. Patterns of orientation of *P. caerulea* at differently sloped sites (a = horizontal; b = vertical) and differently wave-exposed sites (c = exposed; d = sheltered); e = total research area. Internal axes indicate the proportion (percentage number) of *P. caerulea* in that orientation.

Their results are mainly in accordance with the results of our study, during which was observed that the density of investigated limpets on vertical and exposed substrata was greater than the density on horizontal and sheltered surfaces. Though, in future more studies are necessary to obtain a better understanding of these differences and to determine how limpets and other midlittoral organisms function.

Field sampling at the study sites showed that head-orientation of limpets varies among individuals: they are oriented in all directions, not only downwards. Our results showed that a proportion of the studied limpets consistently exhibit mainly a downward head-orientation (including the downward-facing, downward-right-facing and downward-left-facing). This result suggests that these limpets could be classified as “downwards-facing limpets” (i.e. limpets which mainly orientate downwards over the time). The number and the proportion of the downward-facing limpets were greater on vertical-exposed rocky surfaces compared to vertical-sheltered, horizontal-exposed and horizontal-sheltered surfaces. One of the explanations why limpets were mainly downward-orientated is primarily physiological (e.g. desiccation stress) (Garrity, 1984) and our study suggests that it is in this direction that we believe future works should be focused.

Differences in head-orientation may also depend on resting site, which differs among and within species and populations as documented by Branch (1981) and Iwasaki (1992). On the basis of our field observation we found out that after feeding individuals of *P. caerulea* return to the same resting site and–as reported by Fraser et al. (2010)–the limpets which return to the same resting site usually orientate in the same direction.

Several studies have found that by returning to the same resting site, limpets can achieve a closer fit to the rocky surface, reducing the risk of dislodgment by waves, predation and reducing desiccation stress (Garrity, 1984). Some authors (e.g. Williams et al., 1999; Fraser et al., 2010) documented that differences in desiccation and thermal stress may be a mechanism that may explain the head-orientation of limpets. On the other hand, a downward head-orientation may allow for greater flushing of the nuchal cavity to remove waste products, as observed by Williams et al. (1999).

Patterns of orientation of limpets may be influenced by chemical signals produced by several organisms (e.g. Hay, 2009). In gastropods, mucus is deposited during foraging and during low tide this mucus forms a pad between their foot and the substratum, which acts as information (Davies & Hawkins, 1998), signalling the resting site and the orientation for this site. This represents also good protection from desiccation (Wolcott, 1973), predation and dislodgment by waves (Smith, 1991; 1992).

CONCLUSIONS

In conclusion, the results of our investigations have shown that:

1. individuals of *Patella caerulea*, across three locations of the Bay of Koper, occupied mainly the middle horizon of the midlittoral zone and reached the highest density on vertical-exposed surfaces;
2. the head-orientation of the investigated limpets indicated a consistent downward-facing pattern on all selected surfaces (vertical, horizontal, wave-exposed and wave-sheltered).

Our results suggest that the challenge is now to explain why the observed differences occur and to determine what is the pattern of interaction strengths that characterize limpets populations of the midlittoral zone.

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Gustina i orijentisanost *Patella caerulea* L. u Koparskom zalivu (Tršćanski zaliv, sjeverni Jadran)

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SAŽETAK

Studija se bavi gustinom i orijentisanošću jedinke mediolitoralnog priljepka *Patella caerulea* L. duž obala Koparskog zaliva (Tršćanski zaliv, sjeverni Jadran). Cilj je bio odrediti povezanost u orijentisanosti i gustini priljepaka na vertikalnoj, horizontalnoj, izloženoj i zaklonjenoj podlozi. Rezultati pokazuju da je gustina priljepaka na vertikalnoj i izloženoj podlozi bila veća od one na horizontalnoj i zaklonjenoj podlozi; veći je bio udio priljepaka glavom orijentisanih na niže od onih koji su bili okrenuti ka više; takođe, najveći dio priljepaka na niže orijentisanih je bio na vertikalnoj izloženoj podlozi.

Ključne riječi: *Patella caerulea,* gustina, orijentisanost glave, mediolitoral, sjeverni Jadran