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## Life-history trait of the Mediterranean keystone species *Patella rustica*: growth and microbial bioerosion

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### Abstract

The age and shell growth patterns in populations of *Patella rustica* in the Adriatic Sea were determined by analyzing the inner growth lines visible in shell sections. Marginal increment analysis showed annual periodicity with the annual growth line being deposited in May. The growth analysis of 120 individual shells showed that 90.8 % of collected shells were less than 4 years of age and only two shells (1.6 %) were older than 6 years. Population structure was described and generalized von Bertalanffy growth parameters were calculated: asymptotic length ( $L_{\infty}$ ) was 38.22 mm and the growth constant (K) was 0.30 year<sup>-1</sup>. Growth performance index value of *P. rustica* ( $\Phi'$ ) was 2.64, among the lowest ranges reported for limpet species. *Patella rustica* shells were degraded to different degrees by microbial bioerosion. The damage was most often restricted to the oldest parts of the shell, i.e. apex of the shell, posing difficulties in determining the exact position of the first growth line. The microboring organisms identified were pseudofilamentous and filamentous cyanobacteria *Hormathonema (Solentia) paulocellulare*, *Hyella caespitosa*, *Mastigocoleus testarum* and *Leptolyngbya* sp. The overall intensity of infestation was relatively low, but increased in severity with shell age and length. The present study is first to introduce the use of inner growth lines in *Patella rustica* shell sections as a reliable method for age determination. It provides the first insight into the growth patterns of this keystone species, while taking the interference by microbial shell bioerosion in consideration.

**Keywords:** *Patella rustica*, shell growth, acetate peel replicas, euendoliths, bioerosion.

### Introduction

Patellogastropod limpets are among the best known marine herbivores on rocky shores worldwide (Branch, 1981; Jenkins *et al.*, 2005). They feed on microbial biofilms, which are primarily composed of cyanobacteria and microalgae, including diatoms, spores and other propagules of macroalgae and invertebrates (Jenkins *et al.*, 2005; Coleman *et al.*, 2006). Intertidal communities are fundamentally structured by limpet grazing (Southward, 1964; Hawkins & Hartnoll, 1983; Jenkins *et al.*, 2005; Coleman *et al.*, 2006): being generalist grazers they can indirectly enhance or inhibit the establishment of other organisms and are therefore rightfully considered to be keystone species in intertidal communities (*sensu* Power *et al.*, 1996, redefined in Menge & Freidenburg, 2011). Their sharp radulas are able to scrape away thin layers of limestone together with microboring cyanobacteria (Golubić & Schneider, 2003). Because limpets are able to mechanically browse the rock, they are considered to be the most effective contributors to bioerosion of limestone coasts (Vidal *et al.*, 2013). Despite their diversity

and ecological significance, which prompted intensified research over the past decades, the knowledge about limpets' life-history, their age and growth pattern is limited. While growth patterns in bivalve shells have been extensively studied, less attention has been paid to the growth of gastropod shells since configuration of their shell is often considered challenging for growth studies (Richardson & Liu, 1994; Richardson, 2001).

To determine limpets' growth and longevity, most studies used capture-mark-recapture technique (Kenny, 1977; Bretos, 1978, 1980; Kido & Murray, 2003; Clark *et al.*, 2004; Gray & Hodgson, 2003; Espinosa *et al.*, 2008) or length-frequency distribution analysis (Guerra & Gaudêncio, 1986; Brêthes *et al.*, 1994; Khow, 2007). In contrast, shell sectioning method has the advantage to enable analysis of microgrowth patterns in shells (MacClintock, 1967). Tidal periodicity has been described in different limpet species including *Patella vulgata* (Ekaratne & Crisp, 1982), *Fissurella crassa* (Bretos, 1978), *Cellanotoreuma* (Richardson & Liu, 1994), *Scutellastra granulata* (Vat, 2000) and *Helcion pectunculus* (Gray & Hodgson, 2003), and also in the pulmonate limpet *Siphonaria gigas*

(Crisp *et al.*, 1990). Recent studies used a combination of shell growth patterns and stable isotope ratios of oxygen and carbon to understand the seasonal periodicity of shell growth in the European limpet *P. vulgata* from the eastern North Atlantic (Fenger *et al.*, 2007; Surge *et al.*, 2013).

One of the challenges in the study of molluscan growth is the identification of individual growth lines, especially in the oldest parts of the shell, due to erosion and change in structure of the shell infested by micro-boring organisms (Richardson & Liu, 1994). Micro-boring organisms or euendoliths (Golubić *et al.*, 1981) actively penetrate hard minerals causing severe bioerosion of shells with sub-lethal or even lethal effects, usually resulting in reduced shell growth (Marquet *et al.*, 2013; Ćurin *et al.*, 2014). Both, predominance and intensity of endolithic infestations can vary over micro- and meso-spatial scales. Since the identification of growth lines is in general use for determination of bivalve growth and age, we address here the microboring as a potential limitation and source of error.

The focus of the present study was the Lusitanian limpet *Patella rustica* Linnaeus, 1758, one of the four limpets of the genus *Patella* distributed along the Mediterranean shores. Being present only in the upper intertidal zone (Šimunović, 1995; Mauro *et al.*, 2003), this species is adapted to life in harsh environment where food supply is limited and desiccation stress occurs daily. The objective of this study was to investigate the age and shell growth patterns in populations of *P. rustica* in the Adriatic Sea by analyzing the inner growth lines visible in shell sections and acetate peel replicas. The study describes the incidence of euendolith infestation and its effect on shell growth (i.e. shell length) with the principal microboring organisms identified.

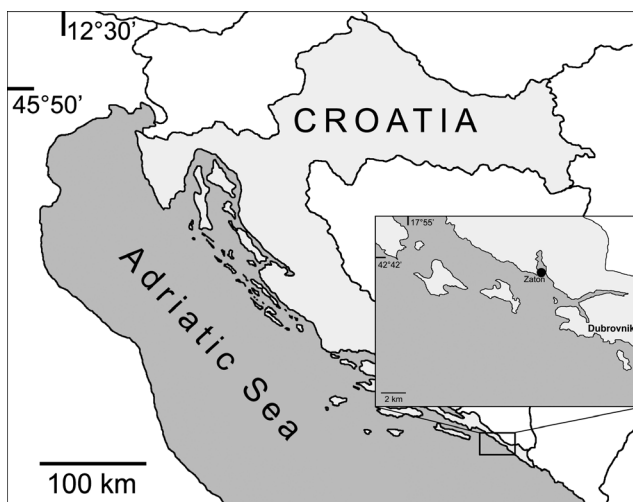
## Materials and Methods

### Study site and sampling

Specimens of *Patella rustica* were collected by hand on rocky shores of Zaton Bay (Fig. 1), situated in the southeastern Adriatic Sea, near the city of Dubrovnik in Croatia (42°41'40.77"N, 18°02'32.19"E). Seawater temperature varied during the time of collection from 12.5 °C in February to 25.8 °C in September, and air temperatures from 7.5 °C in February to 27.1 °C in August (see Prusina *et al.*, 2014b). To validate growth line formation, 3 to 6 small individuals (shell length < 17 mm) were collected monthly from July 2011 to June 2012. For age and growth determination, a total of 120 limpets were collected at random in September 2011.

### Shell growth measurements

Shell length (L, greatest distance between the anterior and posterior ends of the shell), shell width (W, greatest distance between margins perpendicular to the

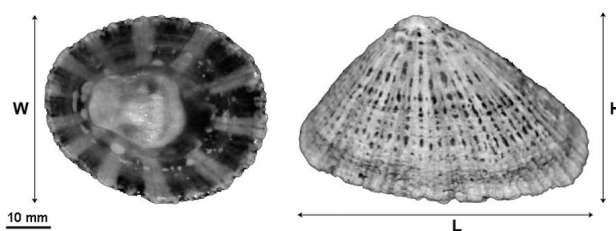


**Fig. 1:** Map of the Adriatic Sea showing the study area in Zaton Bay near the city of Dubrovnik in Croatia.

anterior/posterior axis) and shell height (H, greatest vertical distance from the apex to the base of the shell) were measured with Vernier calliper to the nearest 0.1 mm (Fig. 2). The ratio of limpet height (H) to length (L) was log-plotted by the function  $H = c \times L^\alpha$ , to determine whether the shell growth is isometric ( $\alpha = 1$ ) or allometric (e.g.  $\alpha > 1$ ).

Shells were embedded in epofix resin (Struers) and sectioned through the apex along the maximum growth axis using Struers Labotom 15 cut-off saw. The cut surface was ground on trimite (80-1200 grit) and polished with 3- $\mu$ m diamond paste. The polished sections were washed with detergent, air-dried and etched in 0.1 M-hydrochloric acid solution (HCl) for 1 min, washed in water and air-dried. Acetate peel replicas of the polished and etched sections were prepared according to the method by Richardson (2001). Photomicrographs of acetate peel replicas were made with Zeiss AxioCamERc 5s at 2.5 $\times$  magnification. Timing of growth line formation was determined by marginal increment analysis, i.e. by measuring the distances from the last visible growth line to the shell margin on small individuals (shell length < 17 mm) collected monthly over a period of one year using Zeiss Axio Vision Rel 4.8 software.

Following the validation of annual periodicity of growth-line formation, inner growth lines were identified from 120 individuals collected in September 2011.



**Fig. 2:** *Patella rustica* shell with annotated shell dimensions: shell length (L), shell width (W) and shell height (H).

Obtained age, length, width and height data for each of the 120 individuals, were fitted to the generalized von Bertalanffy growth function ( $L_t = L_\infty (1 - e^{-K(t-t_0)})^D$ , where  $L_t$  is the shell length at the time  $t$ ;  $L_\infty$  is the asymptotic maximum length i.e. the length at which further growth is stopped;  $K$  is growth constant,  $t_0$  is the length at time zero (Gulland, 1983) and  $D$  determines the shape of the curve (inflection point if  $D > 1$ ). Calculations were performed by an iterative nonlinear least square method (Newton algorithm; see Brey, 2001 for details). The same was applied for width and height by changing  $L_t$  for  $W_t$  and  $H_t$  respectively to obtain asymptotic maximum width ( $W_\infty$ ) and height ( $H_\infty$ ).

### Endolith analysis

Acetate peel replicas of 120 individuals used in age analysis were examined for endolith infestation rates. Shells were classified into five categories ranging from 0 to 4, based on subjective scale of infestation intensity (Ćurin *et al.*, 2013; Marquet *et al.*, 2013): 0 = uninfested shells with intact periostracum; 1 = shells with eroded apex while outer striation on periostracum become indistinct; 2 = shells with completely eroded apex and visible damage on the outer shell surface; 3 = heavily infested shells, periostracum completely absent; category; 4 = deformed shells, extremely damaged (Fig. 3).

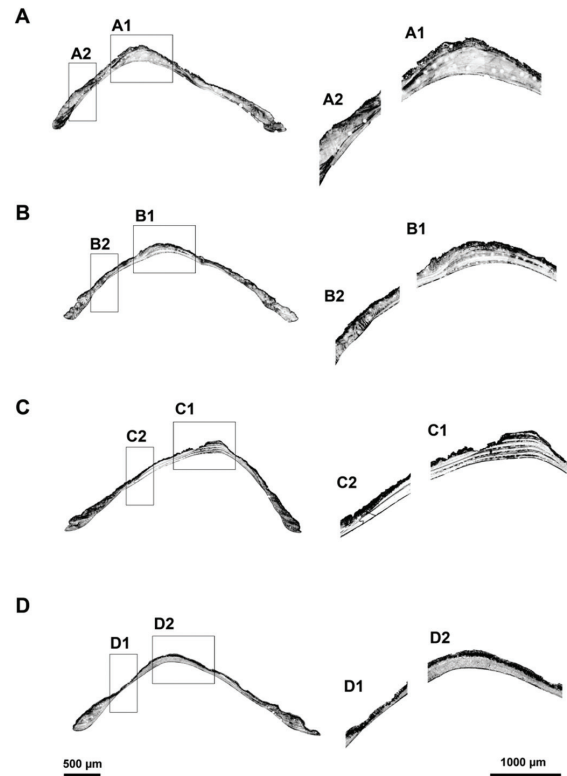
The identification of the euendolithic species was determined on shells decalcified for 3 h in 8 % HCl. The organic residue was mounted on microscopic slides and analyzed with Zeiss Axio Lab.A1 microscope at 100 $\times$ , 200 $\times$  and 400 $\times$  and photo documented with a Zeiss AxioCamERc. The species were identified and compared with the published record (Ercegović, 1932; Riascos *et al.*, 2008; Radtke & Golubić, 2011). The degree of infestation of shells in relation to shell length were analysed using the non parametric Kruskal-Wallis test. The critical probability value was set at 0.05.

### Results

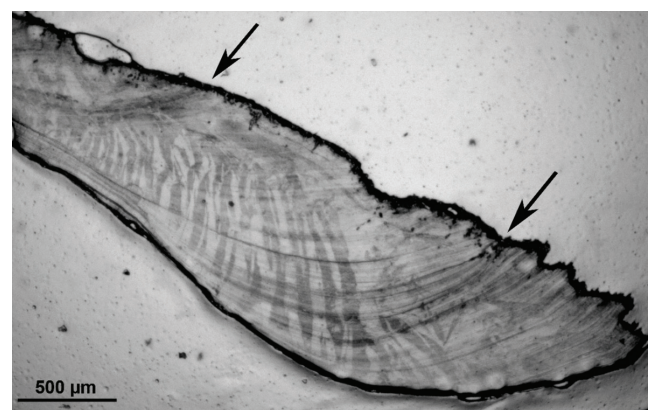
#### Age and growth analysis

The shell length in studied populations ranged from 8.1 to 33.6 mm ( $X \pm SD = 20.2 \pm 6.2$  mm,  $n = 120$ ), width from 6.2 to 27.8 mm ( $16.2 \pm 5.2$  mm) and height from 2.8 to 11.8 mm ( $6.6 \pm 2.1$  mm). All data were log-transformed before analysis in order to linearize the allometric effect of size. Regression analysis ( $H = 0.52 \times L^{1.66}$ ) performed on a plot of log shell length against log shell height yielded the values of  $\alpha$  (1.66),  $c$  (0.52) and  $r^2$  (0.967) and showed that the growth of *P. rustica* population was allometric, i.e. increasing in height faster than length.

Annual growth lines, separated by microgrowth increments, were visible microscopically in acetate peel replicas of shell sections (Fig. 4). Growth line validation was performed on smaller individuals ranging in length



**Fig. 3:** Acetate peel replicas of shells at varying stages of endolith infestation: **A.** category 0 = uninfested shells with intact periostracum, apex (A1), periostracum (A2). **B.** category 1 = shells with eroded apex (B1) while outer striation on periostracum (B2) become indistinct. **C.** category 2 = shells with completely eroded apex (C1) and visible damage on the outer shell surface (C2). **D.** category 3 = heavily infested shells, apex eroded (D1), periostracum completely absent (D2). Category 4 (deformed shells, extremely damaged) was not recorded in the examined limpets.



**Fig. 4:** Photomicrograph of acetate peel replica of an individual *Patella rustica* shell section collected in November and used for growth line validation. Black arrows indicate the position (the beginning) of inner growth lines.

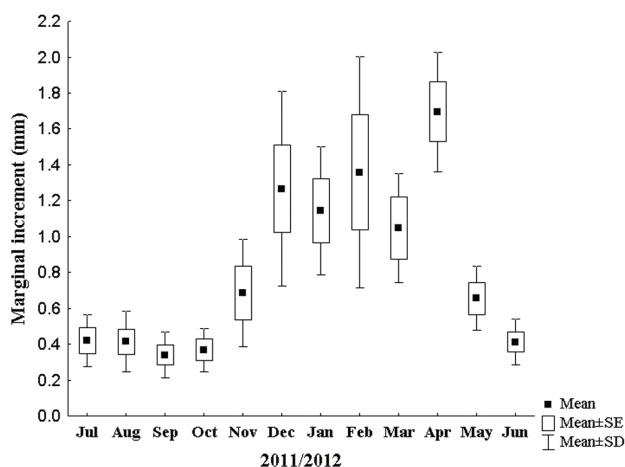
from 11.4 to 16.9 mm ( $X \pm SD = 14.4 \pm 1.6$  mm,  $n = 60$ ). Nine individuals, i.e. 15 % were omitted from the analysis due to poor growth line visibility. The results showed that one dark growth line representing slow shell

deposition (Richardson, 2001) is formed annually and it was visible at or near the margin of shells collected from May to October (Fig. 5). Based on these data, growth line formation was set for May.

Since the reproductive peak of *P. rustica* was in November (see Prusina *et al.*, 2014b), the birth date was set as December 1st. Consequently, a first growth line represents six months instead of twelve and this calculation was taken into account when estimating the age and growth of *P. rustica*. Growth was analysed on 120 individuals collected in September 2011. The estimated age of analyzed individuals ranged from 0.83 to 7.83 years ( $X \pm SD = 2.9 \pm 1.4$  years,  $n = 120$ ). The population growth curve data obtained from the age-at-length data are shown in Table 1 and Figure 6. Overall, 90.8 % of collected individuals were < 4 years old with 34 individuals (28.3 %) belonging to second (1.83 year) and fourth (3.83 years) age class. Only two (1.6 %) individuals were more than 6 years old (6.83 and 7.83 years).

### Endolith analysis

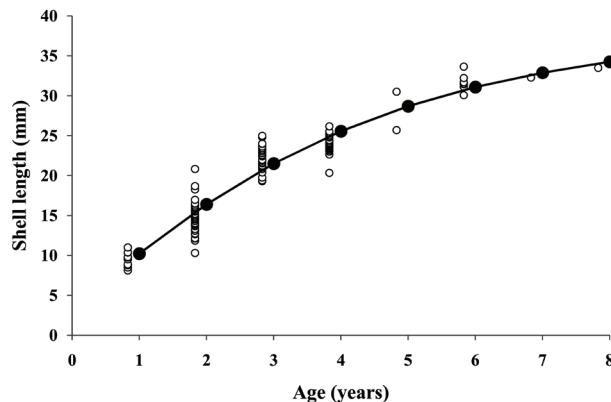
*Patella rustica* shells were exposed to different degrees of degradation by microbial bioerosion (see Fig. 3). Intensity of endolith infestation was determined by macroscopic examination of shell acetate peel replicas and represent only major interferences affecting shell struc-



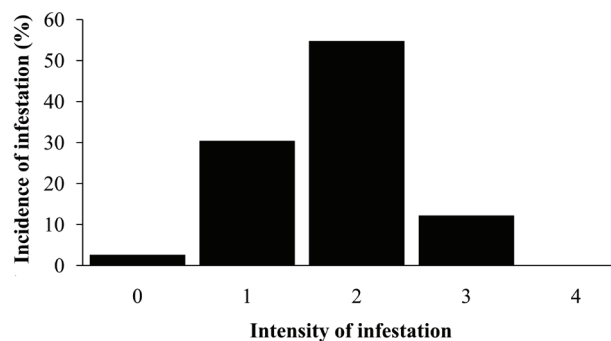
**Fig. 5:** Seasonal variations in the distance from the last growth line and the shell margin (marginal increment width) of *Patella rustica* collected monthly from July 2011 to June 2012. SE—standard error, SD—standard deviation.

**Table 1.** Generalized Von Bertalanffy growth parameters for each measured dimension ( $L_{\infty}$  - asymptotic maximum length,  $W_{\infty}$  - asymptotic maximum width,  $H_{\infty}$  - asymptotic maximum height), K (growth constant),  $t_0$  (initial condition parameter) and D (shape of the curve).

	$L_{\infty} / W_{\infty} / H_{\infty}$ (mm)	K (year <sup>-1</sup> )	$t_0$ (year)	D
<b>Length</b>	38.22	0.30	-0.70	1.44
<b>Width</b>	30.99	0.30	-0.51	1.38
<b>Height</b>	11.97	0.41	-2.97	5.87



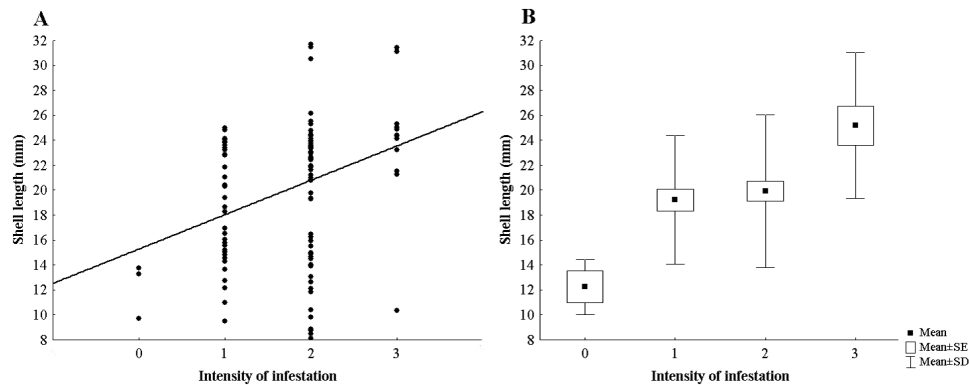
**Fig. 6:** Age to shell length relations obtained from the annually resolved growth lines of *Patella rustica*. Obtained 120 age at length data pairs were fitted to the generalized von Bertalanffy growth function for length:  $L_t = 38.22 (1 - e^{-0.30(t+0.70)})^{1.44}$ .



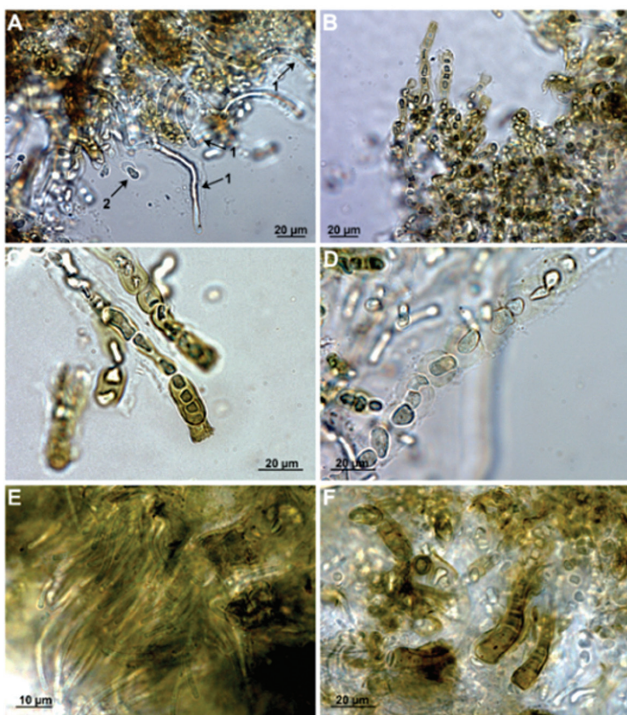
**Fig. 7:** Incidence of endolith infestation in *Patella rustica* shells by infestation categories (as described in Fig. 3).

ture. Looking at the entire sample, intensity of infestation (Fig. 7) was relatively low as 85.2 % of all analysed limpets were classified under category 1 and 2. No limpets exhibited endolith-induced shell fractures (category 4). Linear regression showed a significant relationship between intensity of infestation and limpets' length/age ( $r^2 = 0.10$ ,  $p < 0.0001$ , Fig. 8A). Infestation severity increased with shell length (Fig. 8B). Statistically significant difference was noted in the length of shells in relation to intensity of endolithic infestation: smaller limpets had lower infestation intensities (Kruskal-Wallis test,  $H_3 = 16.35$ ,  $p < 0.001$ ). Initial infestation (category 1 and 2) occurred in smaller ( $X \pm SD = 19.67 \pm 5.8$  mm) i.e. younger ( $X \pm SD = 2.67 \pm 1.2$ ) limpets. Heavily damaged shells (category 3) occurred in 12.2 % of all analyzed limpets, and all of them belonged to larger length classes ( $X \pm SD = 25.18 \pm 5.9$  mm). However, incidence of category 3 damage was also recorded in one limpet with shell length of 10.35 mm that was < 1 year old.

Microboring organisms present in *P. rustica* shells were observed microscopically upon decalcification. The identified species were cyanobacteria that penetrate inside the shell when the periostracum is damaged, including some that colonize as epiliths on the shell surface. Five taxa were identified (Fig. 9): *Mastigocoleus*



**Fig. 8:** Relationships between intensity of endolith infestation and shell length of *Patella rustica*: linear regression showing a significant relationship between intensity of infestation and shell length (A); infestation severity increased with shell length (B). SE—standard error, SD—standard deviation.



**Fig. 9:** Cyanobacteria infesting *Patella rustica* shell. A. *Mastigocoleus testarum* (1) and *Hormathonema paulocellulare* (2). B. *Hyella caespitosa* with scytonemin-stained envelopes. C. *Hyella caespitosa* from illuminated surface of the bored shell with stained envelopes. D. *Hyella caespitosa* with colourless envelopes from light-protected deeper layers of the shell. E. *Leptolyngbya* sp., forming dense mat-like patches on the shell surface. F. *Calothrix* sp., an epilith that dominates the surface of *P. rustica*.

*testarum* Lagerheim, *Hormathonema paulocellulare* Ercegović and *Hyella caespitosa* Bornet & Flahault were endolithic, while *Leptolyngbya* sp. and *Calothrix* sp. were recognized as epilithic. *Mastigocoleus testarum* (Fig. 9A) was identified by its characteristic branching pattern and the presence of terminal heterocysts. *Hormathonema paulocellulare* (Fig. 9A) is characterised by blue-grey gelatinous envelope forming parallel tunnels perpendicular to the surface with few lateral branch-

ing. *Hyella caespitosa* (Fig. 9B-D) has larger, scarcely branched pseudofilaments that penetrate perpendicular to the shell surface. Pseudofilaments are with one or more rows of cells while mucilaginous sheaths that are firm, thick, usually layered, rarely gelatinized and commonly observed to separate the cells. *Leptolyngbya* sp. (Fig. 9E) has long sheathed filaments, solitary or coiled into clusters and fine mats, usually with colourless facultative sheaths that opened at the apical end. *Calothrix* sp. (Fig. 9F) is an epilithic cyanobacterium with basal heterocysts and tapered trichomes apically attenuated into a cellular whip, surrounded by divergent sheath coloured by brown, UV-protecting scytonemin pigment.

## Discussion

Limpets, like bivalves, deposit annual growth lines and tidal microgrowth lines within their shell structure tracing environmental conditions in which they were growing, and providing important life history information (Richardson & Liu, 1994; Richardson, 2001). Age determination based on shell sections has proven to be more reliable than the outer surface lines that, for the most species, are not visible or clear enough (see Richardson, 2001). This study represents the first use of inner growth lines in *Patella rustica* shell sections as a reliable method for analyzing its growth and age. A dark and narrow line representing slow shell growth is deposited annually in *P. rustica* collected from May to October and a wider, light line representing periods of intensive shell growth is deposited from October to February. Based on the present data, the growth line formation set in May concurred with increase of air and sea temperatures in that month ( $X \pm SD = 18.7 \pm 2.2$  °C and  $18.0 \pm 0.8$  °C, respectively), while the maximum growth seems to occur in colder months, i.e. from October to February ( $X \pm SD = 12.1 \pm 5.0$  °C and  $17.7 \pm 3.8$  °C, air and sea temperatures respectively).

Some of the possible reasons for annual growth line formation are food supply, thermal tolerance and repro-

ductive processes such as gonad development and spawning. According to the reproductive study from the same location (Prusina *et al.*, 2014b), *P. rustica* has a prolonged period of gamete development starting from February and extending to September when majority of limpets (70 % of females and 100 % of males) contained gonads in late developmental stages. The spawning peak was in November and December for both sexes, followed by planktotrophic larval stage in December (Prusina *et al.*, 2014b). The assumption is that limpet's larvae can spend up to 4 weeks in plankton before settlement, however, due to their weak swimming activity they act as passive particles. Thus their distribution is limited by various environmental factors, such as the sea surface temperature (SST), hydrodynamic patterns and habitat availability (Ribeiro *et al.*, 2008). The onset of sexual maturity has often been linked with a decrease in growth rate (Vahl, 1971). This is not always the rule, because strong positive correlation between growth and reproduction has been documented for seven South African *Patella* species (Branch, 1981), implying adequate food supply during the reproductive season. Environmental stress and limited food supply supports the strategy of diverting the energy from growth to reproduction and other way around according to needs (Branch, 1981).

Generally accepted rule is that temperate limpets grow more rapidly in summer due to more food abundance (Brêthes *et al.*, 1994; Vat, 2000). This however, may not be the case for *P. rustica*. High shore species such as *P. rustica* are considered to be employing 'energy conservation' strategy because of their unstable environment and limited food resources due to prolonged emersion periods (Sokolova & Pörtner, 2003; Prusina *et al.*, 2014a). In addition, according to the r-K theory, existence of species occupying such environments depends greatly on recolonisation and will therefore benefit most from a larger investment in reproduction (Branch, 1981). The growth of biofilm microorganisms on which *P. rustica* feeds is not restricted during the mild Mediterranean winters, but rather by excessive insolation and heat impact during the summer months. The frequent wave splashing during winter months facilitates the foraging of limpets, whereas the desiccation stress reduces it to a minimum (Santini & Chelazzi, 1995). Accordingly, the limpets in winter carry out longer and irregularly timed feeding excursions (Chelazzi *et al.*, 1994) and obtain sufficient energy for both, faster growth and gonad maturation.

Growth variability and the estimated maximum size depend greatly on environmental conditions or substratum morphology within a locality (Branch, 1974b; Vat, 2000). A substantial body of literature dealing with growth patterns of different vetigastropod limpet species from different latitudes were summarised in Table 2 so as to obtain an insight into relationships between growth rate and latitude. Overall growth performances ( $\phi' = \log K + 2\log L_{\infty}$ ) were calculated according to Pauly and Munro (1984) for the majority of published studies of limpets where estimates

of K and  $L_{\infty}$  were available. Comparisons based simply on K parameter are not considered reliable because of the negative correlation of K and a  $L_{\infty}$  (Sparre & Venema, 1998; Clarke *et al.*, 2004). Growth performance index ( $\phi'$ ) is considered to be species-specific and to represent the physiological capacity of the organism with genetically predetermined factors. The growth constant (K) calculated for *P. rustica* in this study ( $0.30 \text{ year}^{-1}$ ) falls in the middle of the values reported for different limpet species, ranging from 0.06 to  $1.40 \text{ year}^{-1}$ . Growth performance of *P. rustica* ( $\phi'=2.64$ ) is however, among the lowest of the ranges reported, ranging from 1.98 to 3.65 (see Table 2). Since data obtained in this study are the first to describe growth patterns of *Patella rustica*, intraspecific variability and comparisons with Von Bertalanffy growth parameters in dependence of different locations and latitudes were not included in the present study. A broad relationship between observed growth rate and latitude does exist at the macro-ecological scale. The general consideration is that limpets in tropics grow more rapidly than temperate or polar limpets, i.e. species from higher latitudes vs. species from lower latitudes (Clarke *et al.*, 2004; Khow, 2007). It is not yet clarified whether physiological constraints, a reduced or prolonged growing season or combination of both might be the cause of dissimilar growth rates at differing latitudes (Clarke *et al.*, 2004). Environmental factors that are changing with latitude and are most likely to influence growth rate of limpets are temperature, insolation and/or photoperiod (Clarke *et al.*, 2004).

Additional factor can be the bioerosional damage by euendoliths, which may pose energy drainage and growth slow-down. *Patella rustica* population from Zaton Bay showed a high incidence of endolith infested shells with infestation severity increasing with shell length. The damage was low to moderate and restricted mostly to the oldest parts of the shell, i.e. apex of the shell. This is also the reason why shell infestation caused difficulties in defining the exact position of the first growth line in the majority of the examined limpets. The endolith community observed in decalcified shell fragments consisted of coccoid and filamentous cyanobacteria corresponding to the microbial composition prevalent in the rocks of the upper intertidal zone (Ercegović, 1932; Della Santina *et al.*, 1993).

Occurrence of shell-penetrating marine cyanobacteria has been widely reported since the end of the 19th century (Pantazidou *et al.*, 2006; Radtke & Golubić, 2011). In limpets, the high degrees of shell erosion have been reported for *Scutellastra granularis* and *Cellana toreuma* (Richardson & Liu, 1994; Vat, 2000). When settling on shells, endoliths can cause substantial damage disrupting carbonate substrates and forming boreholes and tunnels (Ćurin *et al.*, 2014). Successful colonisation of shells by endoliths mostly depends on prior damage or removal of the periostracum. In most cases, these activities do not affect the limpet, although occasional thinning due to bioerosion may lead to shells fragility and pose a

**Table 2.** Growth performances data calculated for different vetigastropod limpets where estimates of K and  $L_{\infty}$  were available from the literature. Data are arranged by latitude to ease comparison (N = northern hemisphere, S = southern hemisphere).  $L_{\infty}$  = asymptotic maximum length, K = growth constant,  $\emptyset'$  = growth coefficient.

species [family]	location	latitude	$L_{\infty}$ (mm)	K year <sup>-1</sup> (mm)	$\emptyset'$	reference
<i>Acmea persona</i> [Acmeidae]	Newport, USA	44°N	41.6	0.26	2.65	Kenny, 1969
<b><i>Patella rustica</i> [Patellidae]</b>	<b>Zaton Bay, SE Adriatic</b>	<b>42°N</b>	<b>38.2</b>	<b>0.30</b>	<b>2.64</b>	<b>present study</b>
<i>Patella ferruginea</i> [Patellidae]	Ceuta, North Africa (inside port)	35°N	119.6	0.14	3.30	Espinosa <i>et al.</i> , 2008
<i>Patella ferruginea</i> [Patellidae]	Ceuta, North Africa (outside port)	35°N	113.6	0.06	2.89	Espinosa <i>et al.</i> , 2008
<i>Lottia gigantea</i> [Lottiidae]	Orange County, California	33°N	67.7	0.18	2.92	Kiddo & Murray, 2003
<i>Fissurella barbadensis</i> [Fissurellidae]	Barbados	13°N	33.9	0.13	2.17	Ward, 1967
<i>Cellana testudinaria</i> [Nacellidae]	Ohoiwait, Indonesia	5°S	33.1	1.4	3.19	Khow, 2007
<i>Fissurella crassa</i> [Fissurellidae]	Huayquique, North Chile	20°S	94.5	0.16	3.15	Bretos, 1980
<i>Helcion pruinosus</i> [Patellidae]	SE South Africa	32°S	28.8	0.67	2.74	Henniger, 1998
<i>Scutellastra granularis</i> [Patellidae]	SE South Africa (mussel beds)	33°S	27.1	0.25	2.26	Vat, 2000
<i>Scutellastra granularis</i> [Patellidae]	SE South Africa (aeolianite)	33°S	31.9	0.32	2.51	Vat, 2000
<i>Scutellastra granularis</i> [Patellidae]	SE South Africa (quartzite)	33°S	33.0	0.33	2.56	Vat, 2000
<i>Helcion penitunculus</i> [Patellidae]	SW South Africa	33°S	30.9	0.61	2.77	Gray & Hodgson, 2003
<i>Cymbula granatina</i> [Patellidae]	SW South Africa	34°S	94.0	0.51	3.65	Branch, 1974b
<i>Cymbula oculus</i> [Patellidae]	SW South Africa	34°S	79.0	0.58	3.56	Branch, 1974b
<i>Helcion pruinosus</i> [Patellidae]	SW South Africa	34°S	29.6	0.69	2.78	Henniger, 1998
<i>Scutellastra cochlear</i> [Patellidae]	SW South Africa	34°S	49.0	0.33	2.90	Branch, 1974b
<i>Scutellastra granularis</i> [Patellidae]	SW South Africa	34°S	40.0	0.56	2.95	Branch, 1974b
<i>Scutellastra longicosta</i> [Patellidae]	SW South Africa	34°S	68.0	0.30	3.14	Branch, 1974b
<i>Nacella polaris</i> [Nacellidae]	Signy Island, Antarctic	61°S	34.4	0.32	2.58	Clarke <i>et al.</i> , 2004
<i>Nacella polaris</i> [Nacellidae]	Esperanza Bay, Antarctic	63°S	71.4	0.08	2.61	Brêthes <i>et al.</i> , 1994
<i>Nacella polaris</i> [Nacellidae]	Rothera Point, Antarctic, 1997	67°S	34.6	0.08	1.98	Clarke <i>et al.</i> , 2004
<i>Nacella polaris</i> [Nacellidae]	Rothera Point, Antarctic, 1998	67°S	61.4	0.11	2.62	Clarke <i>et al.</i> , 2004
<i>Nacella polaris</i> [Nacellidae]	Rothera Point, Antarctic, 1999	67°S	49.7	0.07	2.24	Clarke <i>et al.</i> , 2004

treat to limpet's survival (Richardson & Liu, 1994; Day *et al.*, 2007). This condition is aggravated in harsh intertidal environments, characterised with greater wave impact that may cause shell erosion, including the damage to the periostracum. It was noted that endolith-infested individuals of the gastropod *Littorina littorea* continually added new inner layers of the shell, and in that way reduced the total shell volume that consequently affected their body mass (Stefaniak *et al.*, 2005). The same was concluded for endolith-infested mussels which deposited significantly more shell material in order to overlay damaged parts, but at the expense of gonad development (Kaehler & McQuaid, 1999). Limpets can also lay down internal shell layers continuously in order to counteract the erosion of outer shell layers (Branch, 1974b; Day *et al.*, 2000). When studying the energetic cost of total shell reproduction, Day *et al.* (2000) concluded that erosion is energetically costly and that the compensation for it occurs at the expense of growth and reproduction, meaning that as the erosion increases with age, greater amount of energy is attributed to shell repair.

The present study provides the first insight into the growth patterns of the high shore limpet *Patella rustica* in the Adriatic Sea, using the analysis of inner growth lines for age determination. Changes in the marine environment and thermal tolerance of an individual may influence its seasonal growth patterns. Given the present trends in environmental changes, it is essential to estab-

lish baseline growth patterns for this species in order to study intraspecific differences across latitudinal gradient throughout the Mediterranean Sea. Environmental and isotopic analysis would be a useful follow-up to substantiate the results from this study.

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