Mediterranean Marine Science

Vol 16, No 2 (2015)

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

I. PRUSINA, M. PEHARDA, D. EZGETA-BALIC, S. PULJAS, B. GLAMUZINA, S. GOLUBIC

doi: 10.12681/mms.1121

To cite this article:

PRUSINA, I., PEHARDA, M., EZGETA-BALIC, D., PULJAS, S., GLAMUZINA, B., & GOLUBIC, S. (2015). Life-history trait of the Mediterranean keystone species Patella rustica: growth and microbial bioerosion. *Mediterranean Marine Science*, *16*(2), 393–401. https://doi.org/10.12681/mms.1121

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.1121

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

I. PRUSINA[1], M. PEHARDA[2], D. EZGETA-BALIĆ[2], S. PULJAS[3], B. GLAMUZINA[1] and S. GOLUBIĆ[4]

[1] Department of Aquaculture, University of Dubrovnik, Ćira Carića 4, 20000 Dubrovnik
[2] Institute of Oceanography and Fisheries, Šetalište Ivana Meštrovića 63, 21000 Split
[3] Faculty of Science, University of Split, Teslina 12, 21000 Split, Croatia
[4] Biological Science Center, Boston University, 5 Cummington Street, Boston, MA 02215-2406, USA

Corresponding author: ivana.prusina@unidu.hr

Handling Editor: Marco Oliverio

Received: 3 November 2014; Accepted: 13 February 2015; Published on line: 22 May 2015.

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_∞) was 38.22 mm and the growth constant (K) was 0.30 year^{-1}. Growth performance index value of *P. rustica* (Ø') 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), *Cellana toreuma* (Richardson & Liu, 1994), *Scutellastra granularis* (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 microboring organisms (Richardson & Liu, 1994). Microboring 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 mesospatial 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

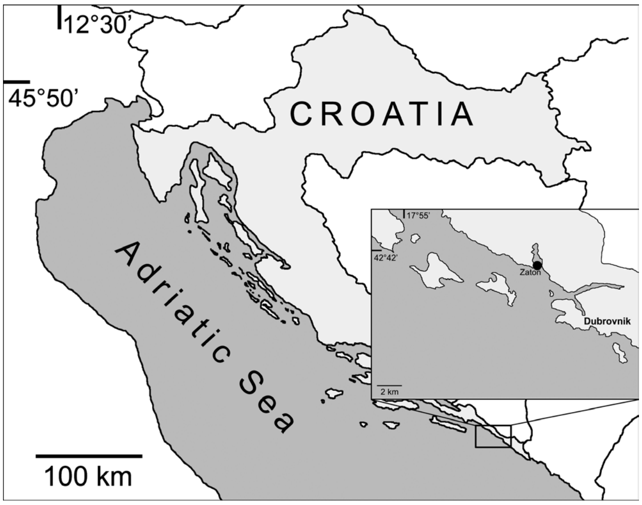

Fig. 1: Map of the Adriatic Sea showing the study area in Zaton Bay near the city of Dubrovnik in Croatia.

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

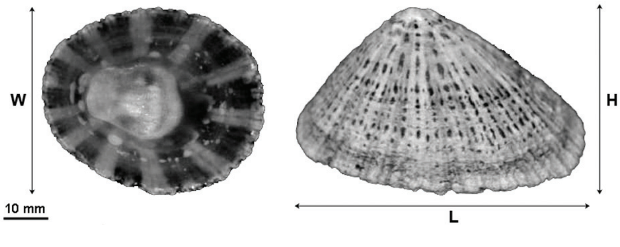

Fig. 2: *Patella rustica* shell with annotated shell dimensions: shell length (L), shell width (W) and shell height (H).

Obtained age, length, width and height data for each of the 120 individuals, were fitted to the generalized von Bertalanffy growth function ($L_t = L_\infty\ (1\text{-}e^{-K\ (t\text{-}to)})^D$, where L_t is the shell length at the time t; L_∞ 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_∞) and height (H_∞).

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 = uninfected 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×, 200× and 400× 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 ± SD = 20.2 ± 6.2 mm, n = 120), width from 6.2 to 27.8 mm (16.2 ± 5.2 mm) and height from 2.8 to 11.8 mm (6.6 ± 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 α (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.

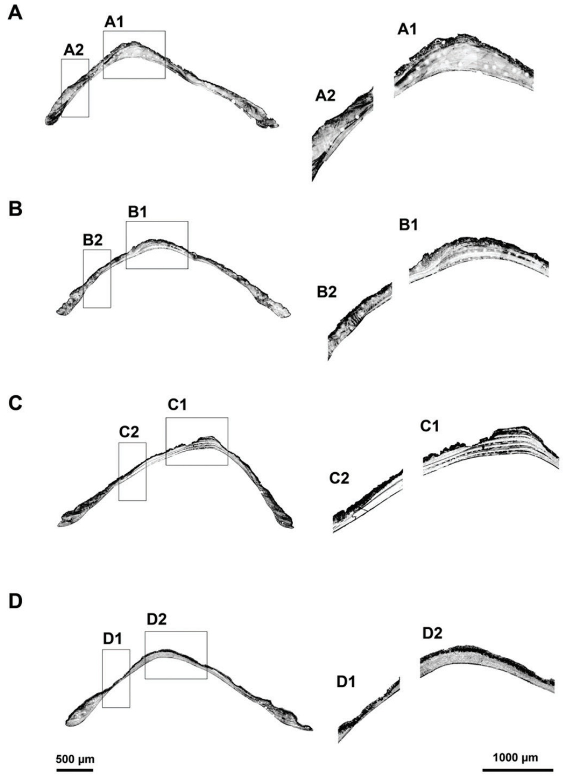

Fig. 3: Acetate peel replicas of shells at varying stages of endolith infestation: **A.** category 0 = uninfected shells with intact periostracum, apex (A1), periostracum (A2). **B.** category 1 = shells with eroded apex (B1) while outer striation on periostracum (B2) become indistinct. **C.** category 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.

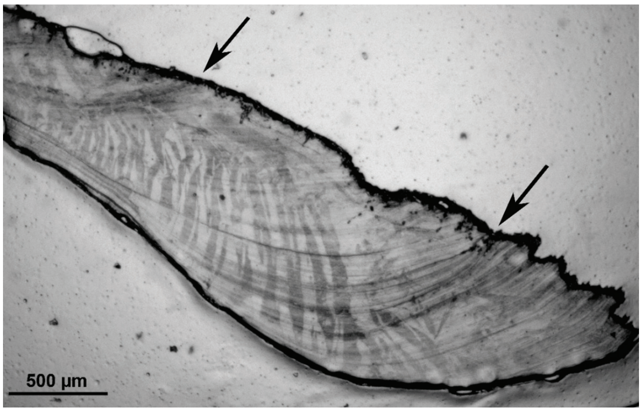

***Fig. 4*:** Photomicrograph of acetate peel replica of an individual *Patella rustica* shell section collected in November and used for growth line validation. Black arrows indicate the position (the beginning) of inner growth lines.

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 from 11.4 to 16.9 mm (X ± SD = 14.4 ± 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 ± SD = 2.9 ± 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 structure. 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 ± SD = 19.67 ± 5.8 mm) i.e. younger (X ± SD = 2.67 ± 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 ± SD = 25.18 ± 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.

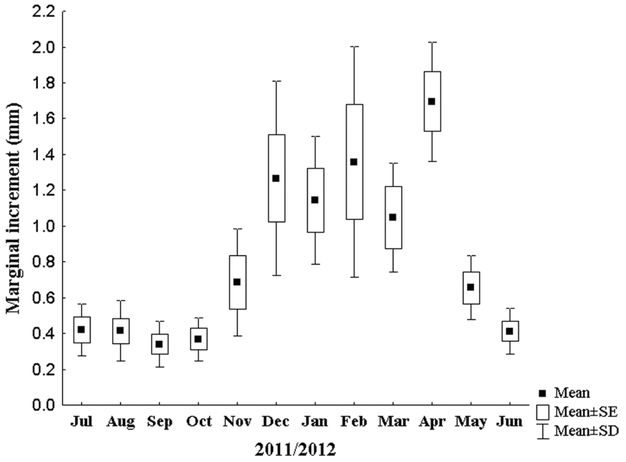

***Fig. 5*:** Seasonal variations in the distance from the last growth line and the shell margin (marginal increment width) of *Patella rustica* collected monthly from July 2011 to June 2012. SE–standard error, SD–standard deviation.

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

	L_∞ / W_∞ / H_∞ (mm)	K (year^{-1})	t_0 (year)	D
Length	38.22	0.30	-0.70	1.44
Width	30.99	0.30	-0.51	1.38
Height	11.97	0.41	-2.97	5.87

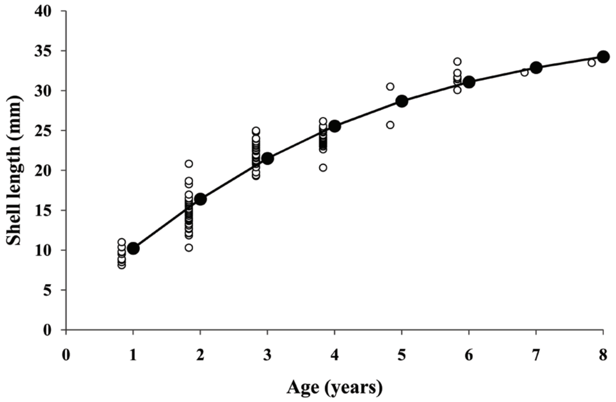

***Fig. 6*:** Age to shell length relations obtained from the annually resolved growth lines of *Patella rustica*. Obtained 120 age at length data pairs were fitted to the generalized von Bertalanffy growth function for length: $L_t = 38.22\ (1-e^{-0.30\ (t+0.70)})^{1.44}$.

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

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*

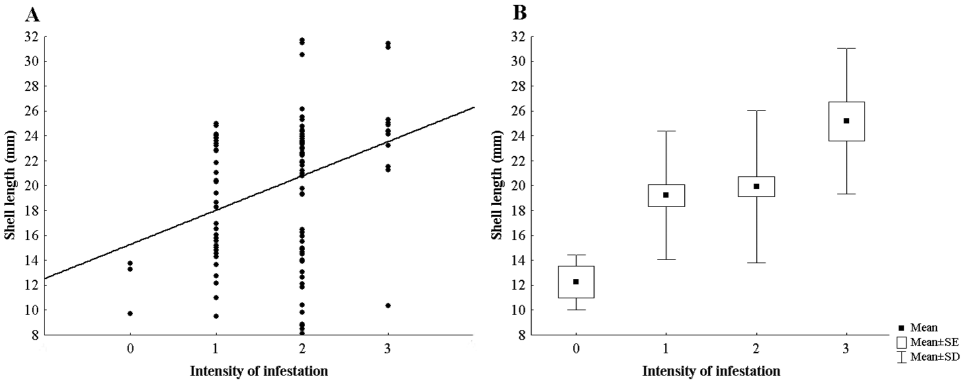

Fig. 8: Relationships between intensity of endolith infestation and shell length of *Patella rustica*: linear regression showing a significant relationship between intensity of infestation and shell length (**A**); infestation severity increased with shell length (**B**). SE–standard error, SD–standard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

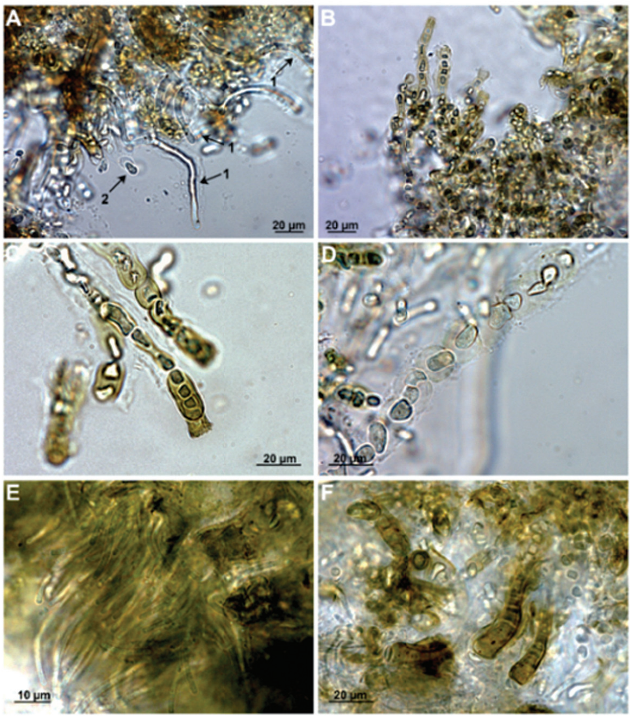

Fig. 9: Cyanobacteria infesting *Patella rustica* shell. A. *Mastigocoleus testarum* (1) and *Hormathonema paulocellulare* (2). B. *Hyella caespitosa* with scytonemin-stained envelopes. C. *Hyella caespitosa* from illuminated surface of the bored shell with stained envelopes. D. *Hyella caespitosa* with 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 branching. *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 then 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 ± SD = 18.7 ± 2.2 °C and 18.0 ± 0.8 °C, respectively), while the maximum growth seems to occur in colder months, i.e. from October to February (X ± SD = 12.1 ± 5.0 °C and 17.7 ± 3.8 °C, air and sea temperatures respectively).

Some of the possible reasons for annual growth line formation are food supply, thermal tolerance and reproductive 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 ($Ø' = \log K + 2\log L_\infty$) were calculated according to Pauly and Munro (1984) for the majority of published studies of limpets where estimates of K and L_∞ were available. Comparisons based simply on K parameter are not considered reliable because of the negative correlation of K and a L_∞ (Sparre & Venema, 1998; Clarke *et al*., 2004). Growth performance index (Ø') 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 year^{-1}) falls in the middle of the values reported for different limpet species, ranging from 0.06 to 1.40 year^{-1}. Growth performance of *P. rustica* (Ø'=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_∞ were available from the literature. Data are arranged by latitude to ease comparison (N = northern hemisphere, S = southern hemisphere). L_∞ = asymptotic maximum length, K = growth constant, Ø' = growth coefficient.

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

Acknowledgements

This project was conducted as part of a doctoral thesis (I.P.), and supported by the Croatian Ministry of Science, Education and Sports. The authors would like to thank the two anonymous reviewers for their helpful and constructive comments that greatly improved the final version of the manuscript.

References

Bowman, R.S., 1981. The morphology of *Patella* spp juveniles in Britain and some phylogenetic inferences. *Journal of the Marine Biological Association of the UK*, 61, 647-666.

Branch, G.M., 1974b. The Ecology of *Patella* Linnaeus from the Cape peninsula, South Africa. III. Growth rates. *Transactions of the Royal Society of South Africa*, 41 (2), 161-193.

Branch, G.M., 1981. The biology of limpets: physical factors, energy flow, and ecological interactions. *Oceanography and Marine Biology - An Annual Review*, 19, 235-379.

Brêthes, J.C., Ferreyra, G., de la Vega, S., 1994. Distribution, growth and reproduction of the limpet *Nacella* (Patinigera) *concinna* (Strebel 1908) in relation to potential food availability, in Esperanza Bay (Antarctic Peninsula). *Polar Biology*, 14, 161-170.

Bretos, M., 1978. Growth in the keyhole limpet *Fissurella crassa* Lamarck (Mollusca: Archaeogastropoda) in Northern Chile. *The Veliger*, 21, 268-273.

Bretos, M., 1980. Age determination in the keyhole limpet *Fissurella crassa* Lamarck (Archaeogastropoda, Fissurellidae), based on shell growth rings. *The Biological Bulletin*, 159, 606-612.

Brey, T. 2001. Population dynamics in benthic invertebrates. A virtual handbook: http://www.awi-bremerhaven.de/Benthic/Ecosystem/FoodWeb/Handbook/main.html. Alfred Wegener Institute for Polar and Marine Research, Germany.

Chelazzi, G., Della Santina, P., Santini, G., 1994. Rhythmical timing and spatial scattering of foraging in a homer limpet (*Patella rustica*). *Behavioral Ecology*, 5, 288-292.

Clarke, A., Prothero-Thomas, E., Beaumont, J.C., Chapman, A.L., Brey, T., 2004. Growth in the limpet *Nacella concinna* from contrasting sites in Antarctica. *Polar Biology*, 28, 62-71.

Coleman, R.A., Underwood, A.J., Benedetti-Cecchi, L., Åberg, P., Arensa, F. *et al*., 2006. A continental scale evaluation of the role of limpet grazing on rocky shores. *Oecologia*, 147, 556-564.

Creese, R.G., 1981. Patterns of growth, longevity and recruitment of intertidal limpets in New South Wales. *Journal of Experimental Marine Biology and Ecology*, 51, 145-171.

Crisp, D.J., Wieghelland, J.G., Richardson, C.A., 1990. Tidal microgrowth patterns in *Siphonaria gigas* (Gastropoda, Pulmonata) from the coast of Costa Rica. *Malacologia*, 31, 235-242.

Ćurin, M., Peharda, M., Calcinai, B., Golubić, S., 2014. Incidence of damaging endolith infestation of the edible mytilid bivalve *Modiolus barbatus*. *Marine Biology Research*, 10 (2), 179-189.

Day, E.G., Branch, G.M., Viljoen, C., 2000. How costly is molluscan shell erosion? A comparison of twopatellid limpets with contrasting shell structures. *Journal of Experimental Marine Biology and Ecology*, 243, 185-208.

Della Santina, P., Sonni, C., Sartoni, G., Chelazzi, G., 1993. Food availability and diet composition of three coexisting Mediterranean limpets (*Patella* spp.). *Marine Biology*, 116, 87-95.

Ekaratne, S.U.K., Crisp, D.J., 1982. Tidal micro-growth bands in intertidal gastropod shells, with an evaluation of band-dating techniques. *Proceedings of the Royal Society B*, 214, 305-323.

Ercegović, A., 1932. Études écologiques et sociologiques des Cyanophycées lithophytes de la côte Yougoslave de l'Adriatique. *Bulletin International de l'Académie Yougoslave de la Sciences des Arts*, 26, 33-56.

Espinosa, F., Gonzáles, A.R., Maestre, M.J., Fa, D., Guerra-García, J.M. *et al*., 2008. Responses of the endangered limpet *Patella ferruginea* to reintroduction under different environmental conditions: survival, growth rates and life history. *Italian Journal of Zoology*, 75, 371-384.

Fenger, T., Surge, D., Schöne, B., Milner, N., 2007. Sclerochronology and geochemical variation in limpet shells (*Patella vulgata*): A new archive to reconstruct coastal sea surface temperature. *Geochemistry, Geophysics, Geosystems*, 8 (7), Q07001.

Fischer-Piètte, E., 1948. Sur les éléments de prospérité des Patelles et sur leur spécificité. *Journal de Conchyliologie*, 88, 45-96.

Gilman, S.E., 2007. Shell Microstructure of the Patellid Gastropod *Collisella scabra* (Gould): Ecological and Phylogenetic Implications. *The Veliger*, 48 (4), 235-242.

Golubić, S., Friendmann, I., Schneider, J., 1981. The lithobiontic ecological niche, with special reference to microorganisms. *Sedimentary Geology*, 51, 475-478.

Golubić, S., Schneider, J., 2003. Microbial endoliths as internal biofilms. p. 249-263. In: *Fossil and Recent Biofilms*. Krumbein, W.E., Dornieden, T., Volkmann, M. (Eds). Kluwer Academic Publishers, Dordtrecht.

Gray, D.R., Hodgson, A.N., 2003. Growth and reproduction in the high-shore South African limpet *Helcion pectunculus* (Mollusca: Patellogastropoda). *Journal of African Zoology*, 38 (2), 371-386.

Guerra, M.T., Gaudêncio M.J., 1986. Aspects of the ecology of *Patella* spp. on the Portuguese coast. *Hydrobiologia*, 142, 57-69.

Gulland, J.A., 1983. *Fish stock assessment: A manual of basic methods*. Vol. 1. Chichester, John Wiley and Sons, FAO/ Wiley series on food and agriculture. Vol. 1, 223 pp.

Hawkins, S.J., Hartnoll, R.G., 1983. Grazing of intertidal algae by marine invertebrates. *Oceanography and marine biology: an annual review*, 21, 195-282.

Henninger, T.O., 1998. Aspects of the ecology and reproductive biology of the limpet, *Helcion pruinosus* (Gastropoda: Prosobranchia). MSc Thesis, Rhodes University, South Africa, 118 pp.

Jenkins, S.R., Coleman, R.A., Burrows, M.T., Hartnoll, R.G., Hawkins, S.J., 2005. Regional scale differences in determinism of limpet grazing effects. *Marine Ecology-Progress Series*, 287, 77-86.

Kaehler, S., McQuaid, C.D., 1999. Lethal and sub-lethal effects of phototrophic endoliths attacking the shell of the intertidal mussel *Perna perna*. *Marine Biology*, 135, 497-503.

Kenny, R., 1969. Growth characteristics of *Acmaea persona*. *The Veliger*, 11, 336-339.

Kenny, R., 1977. Growth studies of the tropical intertidal limpet *Acmaea antillarum*. *Marine Biology*, 39, 161-170.

Khow, A.S., 2007. Growth determination of tropical limpet *Cellana testudinaria* (Linnaeus, 1758) living on the rocky shore of Ohoiwait, Southeast Moluccas, Indonesia. *The Journal of Coastal Development*, 10 (2), 89-103.

Kido, J.S., Murray, S.N., 2003. Variation in owl limpet *Lottia gigantea* population structures, growth rates, and gonadal production on southern California rocky shores. *Marine Ecology-Progress Series*, 257, 111-124.

Lima, F.P., Queiroz, N., Ribeiro, P.A., Hawkins, S.J., Santos, A.M., 2006. Recent changes in the distribution of a marine gastropod, *Patella rustica* Linnaeus, 1758, and their relationship to unusual climatic events. *Journal of Biogeography*, 33, 812-822.

MacClintock, C., 1967. *Shell structure of patelloid and bellephontoid gastropods (Mollusca).* Peabody Museum of Natural History, Yale University, Bulletin 22, pp. 128.

Marquet, N., Nicastro, K.R., Gektidis, M., McQuaid, C.D., Pearson, G.A. *et al*., 2013. Comparison of phototrophic shell degrading endoliths in invasive and native populations of the intertidal mussel *Mytilus galloprovincialis*. *Biological Invasions*, 15, 1253-1272.

Mauro, A., Arculeo, M., Parrinello, N., 2003. Morphological and molecular tools in identifying the Mediterranean limpets *Patella caerulea*, *Patella aspera* and *Patella rustica*. *Journal of Experimental Marine Biology and Ecology*, 295, 131-143.

Menge, B.A., Freidenburg, T.L., 2011. Keystone species. p. 613-631. In: *Encyclopedia of Biodiversity,* Volume 4, Levin, S.A. (Ed), Academic Press.

Pantazidou, A., Louvrou, I., Economou-Amilli, A., 2006. Euendolithic shell-boring cyanobacteria and chlorophytes from the saline lagoon Ahivadolimni on Milos Island, Greece. *European Journal of Phycology*, 41 (2), 189-200.

Pauly, D., Munro, J.L., 1984. A simple method for comparing the growth of fishes and invertebrates. *Fishbyte,* 1, 5-6.

Power, M.E., Tilman, D., Estes, J.A., Menge, B.A., Bond, W.J. *et al*., 1996. Challenges in the quest for keystones. *BioScience,* 46, 609-620.

Prusina, I., Sarà, G., De Pirro, M., Dong, Y.W., Han, G.D. *et al*., 2014a. Variations in physiological responses to thermal stress in congeneric limpets in the Mediterranean Sea. *Journal of Experimental Marine Biology and Ecology*, 456, 34-40.

Prusina, I., Ezgeta-Balić, D., LJubimir, S., Dobroslavić, T., Glamuzina, B., 2014b. On the reproduction of the Mediterranean keystone limpet *Patella rustica*: histological overview. *Journal of the Marine Biological Association of the UK*, 94 (8), 1651-1660.

Radtke, G., Golubić, S., 2011. Microbial euendolithic assemblages and microborings in intertidal and shallow marine habitats: Insight in cyanobacterial speciation. In: *Advances in Stromatolite Geobiology*. Reitner, J., Queric, N.V., Arp, G. (Eds). Lecture Notes in Earth Sciences, 131, 213-244.

Riascos, J.M., Heilmayeer, O., Oliva, M.E., Laudien, J., Arntz, W.E., 2008. Infestation of the surf clam *Mesodesma donacium* by the spionid polychaete *Polydora bioccipitalis*. *Journal of Sea Research*, 59, 217-227.

Ribeiro, P.A. 2008. Dispersal and connectivity of northeastern Atlantic patellid limpets: a multidisciplinary approach. PhD Thesis. University of Southampton, United Kingdom, 279 pp.

Ribeiro, P.A., Xavier, R., Santos, A.M., Hawkins, S.J., 2009. Reproductive cycles of four species of *Patella* (Mollusca: Gastropoda) on the northern and central Portuguese coast. *Journal of the Marine Biological Association of the UK*, 89 (6), 1215-1221.

Richardson, C.A., 2001. Molluscs as archives of environmental change. *Oceanography and marine biology: an annual review*, 39, 103-164.

Richardson, C.A., Liu, J.H., 1994. Tidal microgrowth bands in the shell of the intertidal limpet *Cellana toreuma* (Reeve 1855) from the shores of Cape d'Aquilar, Hong Kong. p. 445-465. In: *Proceedings of the 3rd International Workshop on the Malacofauna of Hong Kong and southern China 1993.* Hong Kong University Press, Hong Kong.

Santini, G., Chelazzi, G., 1995. Gyclogen content and rate of depletion in two limpets with different foraging regimes. *Comparative Biochemistry and Physiology - Part A*, 2 (3), 271-277.

Šimunović, A., 1995. Ecological study of Prosobranchiata (Gastropoda) in the eastern part of the Adriatic Sea and their relationship to benthic biocoenoses. *Acta Adriatica*, 36 (1-2), 3-162.

Sokolova, I.M., Pörtner, H.O., 2003. Metabolic plasticity and critical temperatures for aerobic scope in a eurythermal marine invertebrate (*Littorina saxatilis*, Gastropoda: Littorinidae) from different latitudes. *Journal of Experimental Biology*, 206, 195-207.

Southward, A.J., 1964. Limpet grazing and the control of vegetation on rocky shores. p 265-273. In: *Grazing in terrestrial and marine environments.* Crisp, D.J. (Ed). Blackwell, Oxford.

Sparre, P., Venema, S.C., 1998. *Introduction to tropical fish stock assessment, Part 1*, *Manual.* FAO Fisheries Technical Paper*,* No 306/1, Rev.2, 407 pp.

Stefaniak, L.M., McAtee, J., Shulman, M.J., 2005. The costs of being bored: Effects of a clionid sponge on the gastropod *Littorina littorea* (L). *Journal of Experimental Marine Biology and Ecology*, 327, 103-114.

Surge, D., Wang, T., Gutiérrez-Zugasti, I., Kelley, P., 2013. Isotope sclerochronology and season of annual growth line formation in limpet shells (*Patella vulgata*) from warm- and cold-temperate zones in the eastern north Atlantic. *Palaios,* 28 (6), 386-393.

Tatarenkov, A., Johannesson, K., 1994. Habitat related allozyme variation on a microgeographic scale in the marine snail *Littorina mariae* (Prosobranchia: Littorinacea). *The Biological Journal of the Linnean Society*, 53, 105-125.

Taylor, C.C., 1958. Cod growth and temperature. *Journal du Conseil Permanent International pour l'Exploration de la Mer*, 23, 366-370.

Vahl, O., 1971. Growth and density of *Patina pellucida* (L.) (Gastropoda: Prosobranchia) on *Laminaria hyperborea* (Gunnerus) from western Norway. *Ophelia*, 9, 31-50.

Vat, L.S., 2000. The growth and reproduction of *Patella granularis* (Mollusca: Patellogastropoda) on the southeast coast of South Africa. PhD Thesis. Rhodes University, South Africa, 247 pp.

Vidal, M., Fornós, J.J., Gómez-Pujol, L., Palmer, M., Pons, G.X. *et al*., 2013. Exploring rock coast bioerosion: rock fragment intestine transit time and erosion rates computation of the gastropod *Monodonta articulata* (Lamarck, 1822). *Journal of Coastal Research*, Special Issue No. 65.

Ward, J., 1967. Distribution and growth of the keyhole limpet *Fissurella barbadensis* Gmelin. *Bulletin of Marine Science*, 17 (2), 299-318.