

# Growth and longevity of *Lithophaga lithophaga*: what can we learn from shell structure and stable isotope composition?

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**Abstract** Longevity of bivalves has been an intriguing issue, especially for those species of interest for human consumption. Reliable age and growth estimates often require the combination of several methods. In this study, we analyzed changes in shell structure including ridges on the external shell surface and growth lines observed in acetate peel replicas of shell sections of the European date mussel *Lithophaga lithophaga*, as well as the oxygen and carbon isotope values ( $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ ) of the shell. High variations in growth rates between individuals were noted. Ontogenetic ages of analyzed shells varied from 10 to 54 years (30.6–93.6 mm). According to results of generalized von Bertalanffy growth function,  $L_{\infty}$  was 107.6 mm, and  $k$  was  $0.03 \text{ year}^{-1}$ .  $\delta^{18}\text{O}$  results strongly suggest that growth ridges visible on the external shell surface of *L. lithophaga* are formed annually. The  $\delta^{18}\text{O}$  values ranged from  $-0.2$  to  $2.7 \text{ ‰}$  ( $\bar{x} = 1.15 \pm 0.72 \text{ ‰}$ ), which equates

to a temperature range of  $13 \text{ °C}$ . The reconstructed seawater temperatures ( $T_{\delta^{18}\text{Oshell}}$ ) ranged between  $12$  and  $25 \text{ °C}$ , a range in good agreement with measured temperature. Stable carbon isotope values decreased through ontogeny and ranged between  $-2.05$  and  $2.32 \text{ ‰}$  ( $\bar{x} = 0.01 \pm 0.89 \text{ ‰}$ ). Results of this study provide the first stable isotope data for *L. lithophaga* shells and show the potential of this species as a geochemical sclerochronological archive.

## Introduction

Bivalves are one of the most ecologically diverse marine taxa. Members of this class live from the intertidal zone all the way to the deep sea hydrothermal vents at depths  $>3000 \text{ m}$ , from sheltered to exposed habitats, and from brackish to saline environments (Gosling 2003). Bivalves range in size from  $<1 \text{ mm}$  (e.g., *Bentho-cardiella striatula*) to more than  $120 \text{ cm}$  (*Tridacna gigas*) (Abele et al. 2009; Haszprunar and Wanninger 2012). They exhibit different life styles including being permanently attached to the hard substrate, and burrowing in soft substrates, as well as free swimming (Gosling 2003). Diversity in Class Bivalvia is also evident in extreme variations in longevity between species, from small warm water *Donax* clams that live  $\sim 1$  year (for review see Powell and Cummins 1985) to *Neopycnodonte zibrowii* (Wisshak et al. 2009) and *Arctica islandica* (e.g., Butler et al. 2013; Schöne 2013) that have been documented to live for over 500 years.

Longevity of bivalves has been an intriguing issue, especially for those species of interest for human consumption. Many commercially interesting bivalves have been studied in great detail, from the ecological stand point, as well as from the stand point of stock management and their potential for aquaculture production. The European date

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mussel *Lithophaga lithophaga* (Mytilidae) has been considered a delicacy in many countries and—because its habit of boring into hard substrates leads to destructive fishing methods—has been protected in many parts of the Mediterranean. This endolithic species can attain sizes of up to 90 mm, mostly inhabits shallow waters along the entire Mediterranean coast (Fisher et al. 1987; Poppe and Goto 2000) and lives inside galleries bored in calcareous rock by glandular secretions (Morton and Scott 1980).

Most of the studies conducted on *L. lithophaga* addressed the anthropogenic impact on *L. lithophaga* habitat (e.g., Fanelli et al. 1994; Devescovi et al. 2005; Parravicini et al. 2010; Guidetti 2011), different aspects of tissue contamination (Dujmov and Sučević 1990; Deudero et al. 2007; Jaafar Kefi et al. 2012a, b), or reproduction (Valli et al. 1986; Šimunović et al., 1990; Galinou-Mitsoudi and Sinis 1994, 1997; Jaafar Kefi et al. 2014). *Lithophaga lithophaga* reproduces once a year following a decline in sea water temperature and has an unequal sex ratio, with males being dominant at smaller size categories (Galinou-Mitsoudi and Sinis 1994; Jaafar Kefi et al. 2014). Growth and longevity of *L. lithophaga* have been studied by Kleeman (1973a, b) and Valli et al. (1986) in the north Adriatic and Galinou-Mitsoudi and Sinis (1994, 1995) in the Aegean Sea and according to the latter its longevity was estimated at >50 years.

Due to its longevity (Galinou-Mitsoudi and Sinis 1994; 1995), *L. lithophaga* is an interesting subject for bivalve ecology and sclerochronology study. One of the key issues in these studies is the establishment of the periodicity of growth line formation, for which geochemical analysis of the shells is a well-established method (Cardoso et al. 2013a, b). Therefore, the principal objective of this study was to assess the periodicity of growth line formation in *L. lithophaga* using oxygen and carbon stable isotope analysis. Further objectives were to estimate the maximal longevity and determine shell growth parameters, and to assess the potential of *L. lithophaga* as a sclerochronological archive.

## Materials and methods

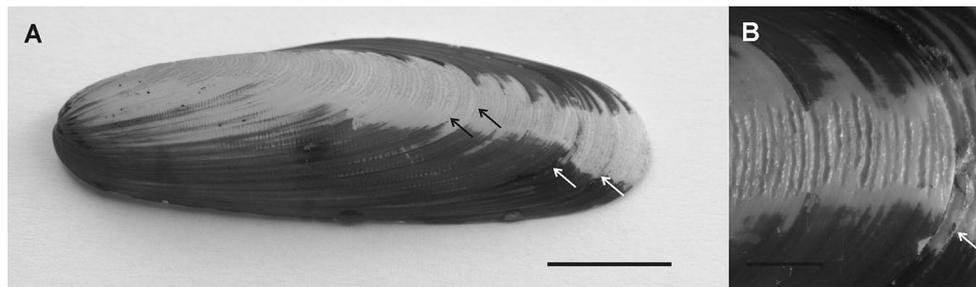
*Lithophaga lithophaga* specimens were collected from south side of the island of Čiovo, Croatia (43°29'01"N, 16°21'56"E), on July 2, 2013, by SCUBA divers from 2 to 3 m depth. During sampling, a Cera-Diver® temperature probe (Schlumberger water services) was anchored at 2 m depth and was kept in the boundary layer for a period of 1 year with periodic downloading of data. Measurements were taken once an hour, and mean daily variations of temperature for each sampling date are presented.

In the laboratory, the length, width and height of each shell ( $N = 84$ ) were measured with digital vernier calipers.

Due to breaking of shells during removal of individuals from the hard substrate, it was not possible to measure the height and width of six individuals. Two methods were applied for studying age and growth of *L. lithophaga*: (a) analysis of growth lines in shell cross sections and (b) analysis of growth ridges on the external shell surface. In order to study internal growth lines, shells ( $N = 20$ ) ranging in size from 37.0 to 79.3 mm ( $\bar{x} = 58.8 \pm 13.2$ ) were embedded in epoxy resin and cut along the length or height axis using a Struers Labotom 3 saw. Shell sections were ground on a series of wet silicone carbide papers (220–1200 grit), polished using 3  $\mu\text{m}$  diamond paste and etched in 0.1 M HCl for 2–4 min. Acetate peels were prepared according to the method of Richardson (2001), observed under a Zeiss Axio Lab.A1 microscope and photographed with a Zeiss AxioCam ERc 5 s.

Age estimate based on external growth ridges was performed by two experienced readers. Out of 84 collected shells, a total of 13 were considered to be unreadable by both readers as a result of shell breakage during removal from the substrate, disturbances in the shell, or because the readers did not agree on a single interpretation. Agreement was reached for the remaining 71 individuals, and these were further processed. The distance from the umbo to each growth ridge on the external shell surface was measured with a digital vernier caliper with a precision of 0.01 mm. The resulting 1882 age-at-length data pairs were fitted to the generalized von Bertalanffy growth function for length ( $L_t = L_\infty \times (1 - e^{-K \times (t - t_0)^D})$ ) where  $L_t$  is the shell length at time  $t$ ,  $L_\infty$  is the asymptotic length,  $K$  is the growth coefficient ( $\text{year}^{-1}$ ),  $t_0$  is the length at time zero, 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). A growth performance index ( $\phi = \log k + 2\log L_\infty$ ) was applied for comparison of growth with previous studies (see Pauly and Munro 1984).

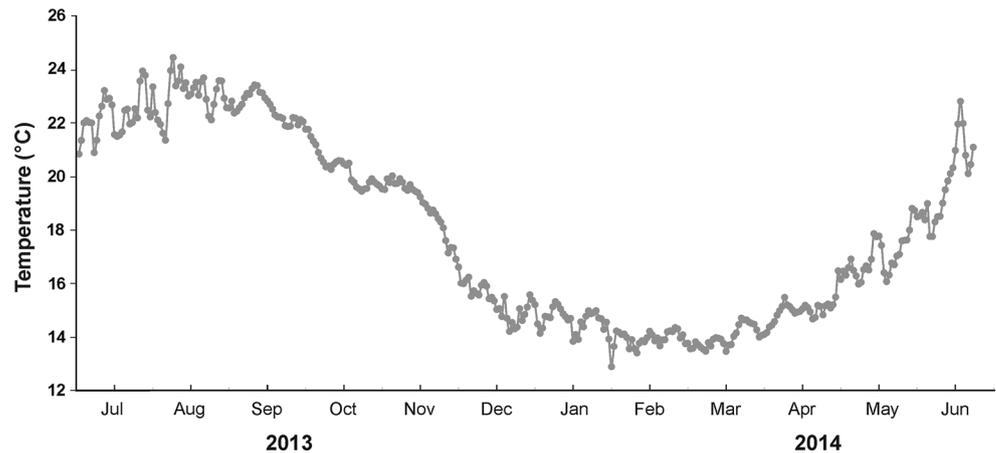
To establish the periodicity of growth line formation of *L. lithophaga*, the oxygen and carbon isotope values ( $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ , respectively) of the calcium carbonate were determined at sub-annual resolution. Due to the relatively thin shell, it was not possible to collect samples for geochemical analysis (>50  $\mu\text{g}$ ) from the shell cross sections. Therefore, the inner portion of the valves was first filled with epoxy resin to stabilize the shell and prevent breakage during drilling of samples. Subsequently, epibionts as well as the periostracum were physically removed. Samples were obtained by drilling shallow swathes in the external shell surface parallel to the growth lines. Drilling was conducted by hand under a Zeiss Stemi DV4 stereo zoom magnification using a DREMEL Fortiflex drill equipped with 200- $\mu\text{m}$  tungsten carbide dental drill bits. Collection



**Fig. 1** Photograph of external shell surface of shell LL73I sampled for stable isotope analysis. **a** Black arrows indicate position of two growth ridges on the external shell surface. White arrows indicate

position of disturbance lines. Scale bar 1 cm. **b** Close up of drilling tracks. White arrow indicates position of disturbance line. Scale bar 2 mm

**Fig. 2** Sea water temperature variation at Čiovo sampling site recorded in a period from July 2, 2013, to June 20, 2014. Values presented as daily means



of calcium carbonate powder from the external shell surface after reinforcing the shell with epoxy resin was previously successfully done for *Macoma balthica* (Cardoso et al. 2013a). Samples were collected from 6.1 mm from the umbo to the ventral margin of shell ID LL73I [total shell length 55.0 mm (Fig. 1)]. This shell was selected randomly from medium-sized shells. Due to shell erosion in the umbo region, it was not possible to collect samples closer to the umbo. Between 2 and 8 samples were drilled per growth increment. In total 147 discrete samples of shell material were collected.

All samples were analyzed on a Thermo Finnigan MAT 253 continuous flow—isotope ratio mass spectrometer coupled to a GasBench II at the Institute of Geosciences of the University of Mainz (Germany). They were measured against an in-house Carrara marble calibrated against the international isotopic reference standard NBS-19. Shell isotopic ratios are reported in conventional delta ( $\delta$ ) notation relative to the VPDB standard. Internal precision, based on replicated measurements of the Carrara standard, was 0.04 ‰ for  $\delta^{18}\text{O}$  and 0.02 ‰ for  $\delta^{13}\text{C}$ .

The *L. lithophaga* shell consists of both calcite and aragonite. Calcite is present just below the periostracum, while the inner shell layer is composed of aragonite

(Harper unpublished data). Since samples for stable isotope analysis were drilled from the outer shell surface inward, temperature reconstruction was done using the equation of Epstein et al. (1953), calibrated for biogenic calcite. This equation was rewritten by Sharp (2006) in a form appropriate for calcite and water oxygen isotope data expressed relative to VPDB and VSMOW, respectively:

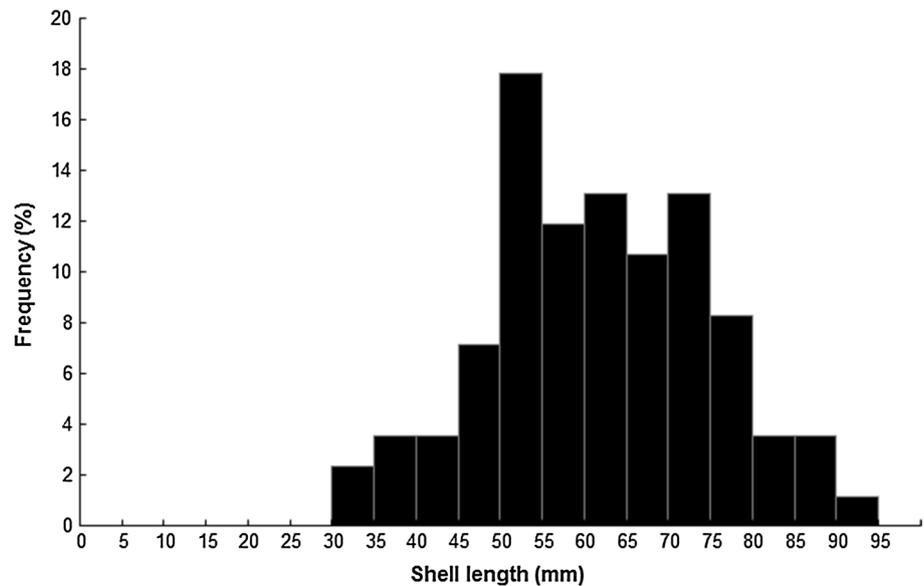
$$t(^{\circ}\text{C}) = 15.75 - 4.3(\delta^{18}\text{O}_{\text{c-PDB}} - \delta^{18}\text{O}_{\text{w-SMOW}}) + 0.14(\delta^{18}\text{O}_{\text{c-PDB}} - \delta^{18}\text{O}_{\text{w-SMOW}})^2 \quad (1)$$

As no measurement of the oxygen isotope composition of seawater has been carried out close to the sampling site, we used a  $\delta^{18}\text{O}_{\text{w-SMOW}}$  value of 1.30 ‰, measured by Stenni et al. (1995) at their station D4 (surface water) located ca. 60 km west-southwest from our sampling site.

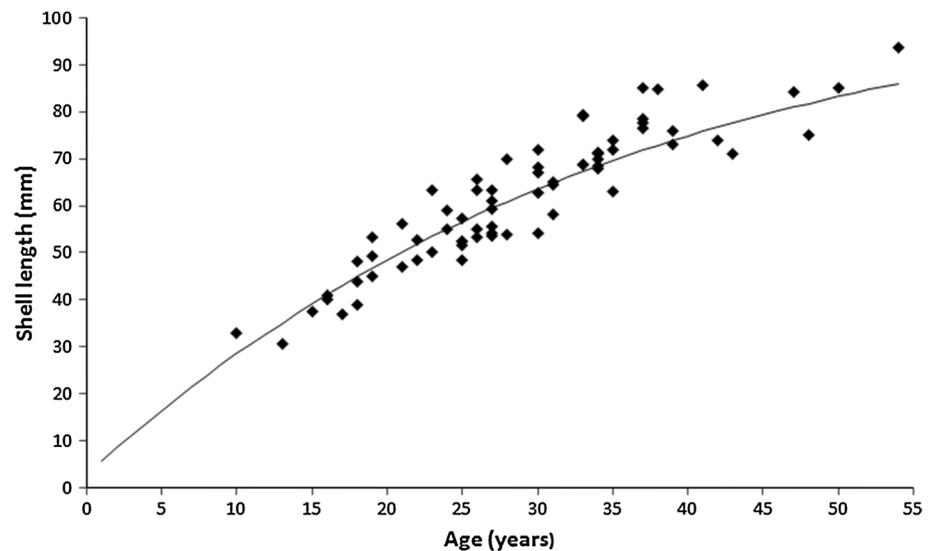
## Results

Temperature values recorded between July 2, 2013, and June 20, 2014, revealed pronounced seasonal patterns (Fig. 2) with lowest temperatures of 12.6 °C (January

**Fig. 3** Length frequency structure of sampled *Lithophaga lithophaga* ( $N = 84$ )



**Fig. 4** Age-at-length data of *Lithophaga lithophaga* collected at Čiovo in 2013 ( $N = 84$ ). General von Bertalanffy growth curve has been fitted to the data  $L_t = 107.6 \times (1 - e^{-0.03(t + 1.24)})^{1.056}$



28, 2014). Temperatures lower than 15 °C were recorded between mid-December 2013 and mid-April 2014, while temperatures above 20 °C were recorded from July to mid-October 2013, and in mid-June 2014. Highest recorded temperatures equaled 25.8 °C (August 9, 2014).

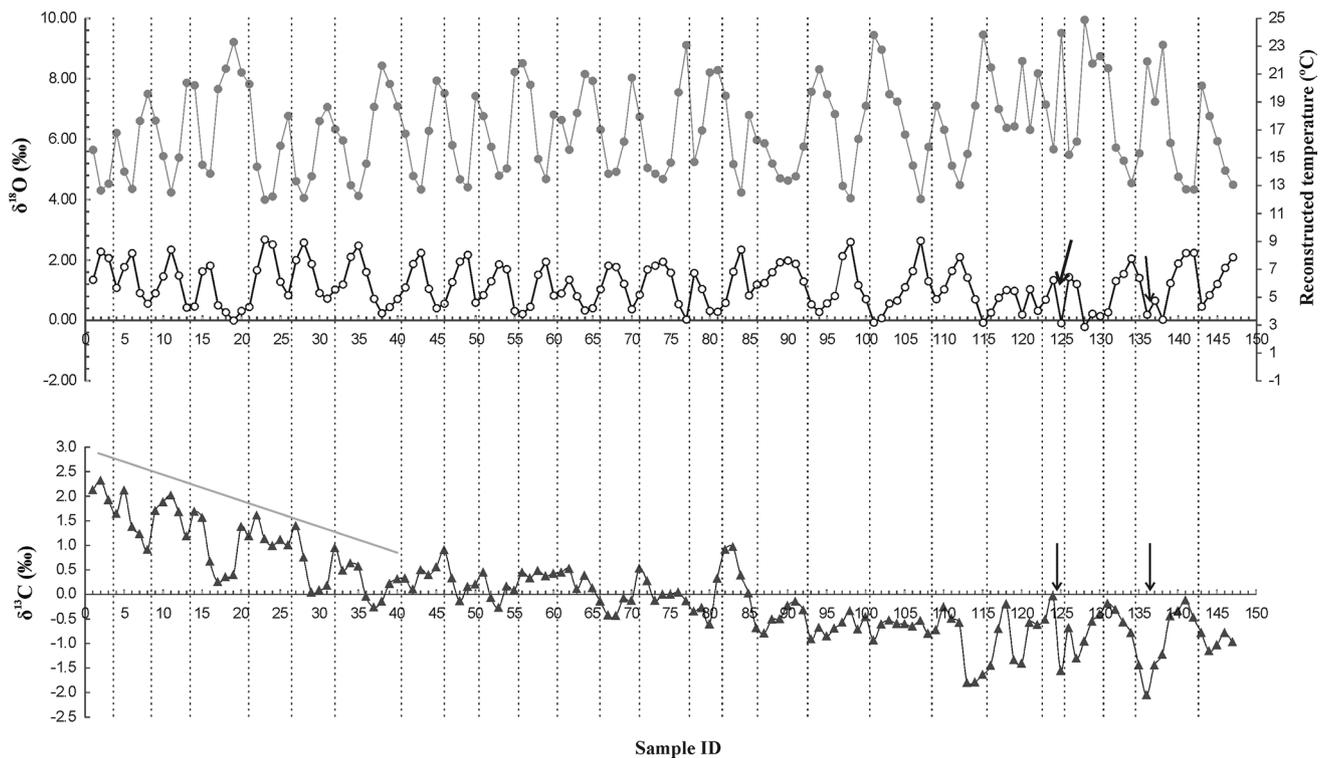
The 84 *L. lithophaga* shells ranged in length from 30.6 to 93.6 mm ( $\bar{x} = 61.7 \pm 13.3$  mm, Fig. 3). The relationship between length and height was described with the following equation

$$L = 3.20 \times H + 0.82 \quad (r^2 = 0.9031, p < 0.001, N = 78), \quad (2)$$

and that between length and width was described with this equation

$$L = 3.16 \times W + 8.66 \quad (r^2 = 0.863, p < 0.001, N = 78). \quad (3)$$

Age and growth analysis was performed by counting and measuring distances between the umbo and the beginning of each external ridge on the external surface of the 71 shells that were judged to be readable by two experienced observers. High variations among growth rates between individuals were noted. Under the assumption of an annual periodicity of external ridge formation, the age of the analyzed shells varied from 10 to 54 years ( $\bar{x} = 29.0 \pm 8.9$  years). According to the results of the generalized von Bertalanffy growth function conducted on 1882 age-at-length data pairs,  $L_\infty$  was 107.6 mm,  $k$  was 0.03 year<sup>-1</sup>,  $D$  was 1.056, while  $t_0$  was -1.243 (Fig. 4).



**Fig. 5** Comparison of  $\delta^{18}\text{O}$  values (white circles), reconstructed temperature (gray circles) and  $\delta^{13}\text{C}$  values (black triangles) for *Lithophaga lithophaga* shell (LL 731) sampled from umbo toward the shell margin. Perpendicular lines indicate position of external growth

ridges. Black arrows indicate position of two disturbance lines on external shell surface. Gray line is placed about first measured seven growth increments characterized with a rapid decrease in  $\delta^{13}\text{C}$  values

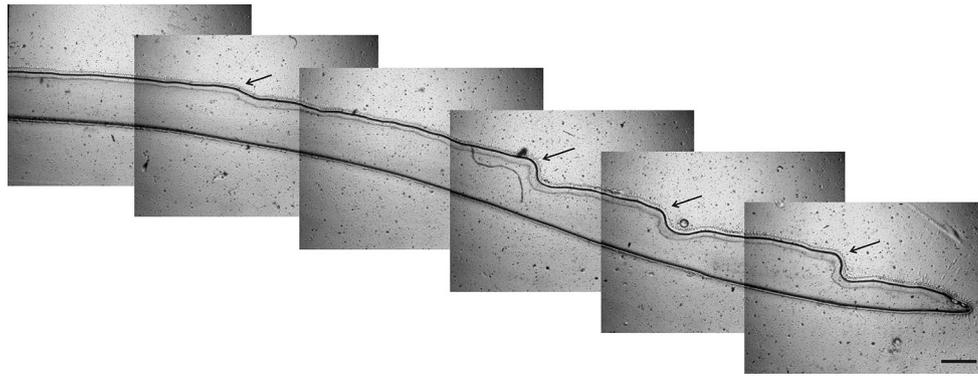
The  $\delta^{18}\text{O}$  values ranged from  $-0.2$  to  $2.7$  ‰, which corresponds to a temperature range of  $13$  °C (Fig. 5). The reconstructed seawater temperatures ( $T_{\delta^{18}\text{Oshell}}$ ) ranged between  $12$  and  $25$  °C. The observed cyclicity in the  $\delta^{18}\text{O}$  values corresponds to the number of growth rings ( $N = 26$ ) observed on the external shell surface of the analyzed sample (*L. lithophaga* ID = LL 731). Due to the relatively small number of calcite samples analyzed per growth increment, it was not possible to precisely determine the period when the growth line was formed. In the ontogenetically youngest part of the shell (i.e., first 16 growth increments), it appears that the annual growth line likely formed in a period when the water temperature decreased (September–November). This pattern is not clear in ontogenetically older increments.

Stable carbon isotope values decreased through ontogeny and ranged between  $-2.05$  and  $2.32$  ‰. There appear to be two distinct patterns in the  $\delta^{13}\text{C}_{\text{shell}}$  time series. The ontogenetically youngest one is characterized by a relatively rapid decrease in stable carbon isotope values that was noted for the first seven growth increments sampled (those closest to the umbo), which generally had positive stable carbon isotope values (Fig. 5). The second growth pattern was observed from the 7th sampled increment to the

shell margin and was characterized by a slower decrease in stable carbon isotope values. Negative stable carbon isotope values were generally observed after the 13th sampling increment, with the exception of several negative values observed between the 7th and 13th increment. Seasonal periodicity, with variance in minimal and maximal values, was observed for all sampled increments.

## Discussion

Acetate peels have been successfully used for the study of the growth dynamics of many bivalve species in different types of habitats. Furthermore, it has been demonstrated that they can be used for the interpretation of growth at different temporal scales from days to years (Richardson 2001). In this study, we attempted to apply this method for the analysis of *L. lithophaga* growth and longevity. Problems encountered included variations in the appearance of lines along the shell section, absence of shell material due to erosion especially around the hinge, and changes in the shell structure associate with the presence of endoliths. Due to the characteristic elongated shape of the shell, the appearance of growth lines in prepared samples seems to



**Fig. 6** Composite photomicrographs of acetate peels of *Lithophaga lithophaga* shell section (LL 711). Black arrows indicate position of four growth ridges. Scale bar 500  $\mu$ m

be highly dependent on sectioning angles, and therefore not reliable for interpretation. The same was true for growth ridges present on the external shell surface. In certain parts of the shells (for example close to the shell margin), they were more prominent, while in other parts they were very hard to identify (Fig. 6). Difficulties in interpreting growth lines in acetate peels of shell sections were also encountered in a recent study conducted by Bagur et al. (2013) on *L. patagonica* from Argentina. These authors observed discrepancies in the number of growth lines present in the hinge region and along the shell margin. According to their study, only clefts (ridges) present in the outer shell layer and representing changes in the direction and rate of shell deposition were reliable for estimating age of this species. This is in accordance with our findings for *L. lithophaga*. Results of our study indicate that ridges on the external shell surface of *L. lithophaga* can be reliably counted, distances between them measured and the obtained data used for growth and age analysis. The hypothesis that these ridges are deposited on an annual cycle was successfully validated using stable oxygen isotope analysis, and it was clearly demonstrated that one ridge is formed annually (see more elaborated discussion below). Exceptions are disturbance ridges that periodically appear on the external shell surface and that, we hypothesize, can in most cases be easily distinguished from the annual ridges by their appearance (see Fig. 1). Disturbance ridges are characterized by change in angle of shell deposition and are more pronounced than annual ridges. They most likely form due to stress caused to the animals due to illegal date shell harvesting and destruction of habitat that still occurs in the area. According to Galinou-Mitsoudi and Sinis (1994), *L. lithophaga* deposits two ridges per year, with a ridge representing reproduction being deposited before, and, very close to, an annual ridge, thus giving the appearance of a double ridge on the external shell surface. In shells from Čiovo analyzed in this study, we did not observe two ridges close to each other on the

shell surface and it is possible that, at this site, reproduction coincides with the period of annual ridge formation. Temporal and spatial variations in reproduction cycle have been noted previously for many bivalve species. In a recent study, Hernández-Otero et al. (2014) presented data on differences in the reproductive cycle of the commercial sword razor clam *Ensis magnus* (Schumacher, 1817) at six shellfish beds in the Ilia de Pontevedra (NW Spain) and have observed a 15-day to 1 month delay in advanced stages of gametogenesis and maturation between the inner and the outermost site of the Ria. Although both our sampling site and that of Galinou-Mitsoudi and Sinis (1994) are located in Mediterranean, it is possible that they are variations in *L. lithophaga* reproductive cycle between these sites.

Our data on growth and age of *L. lithophaga* are comparable to research conducted by Galinou-Mitsoudi and Sinis (1994, 1995) in Greece. Galinou-Mitsoudi and Sinis (1995) analyzed shells ranging in length from 0.65 to 9 cm and noted high variation in growth rates between individuals ( $N = 106$ ). Individual variations were noted in previous studies for other bivalve species. According to Bayne (2004), faster growth of individual is a result of combination of increased rates of feeding, reduced metabolic rates and lower metabolic costs of growth. Due to a boring lifestyle of *L. lithophaga*, it is to be expected that access to food (due to penetration angle and depth) varies between the individuals and that this can at least partially be responsible for individual variations.

The oldest shell had 54 annual ridges and its length was 81.6 mm, while the largest analyzed shell (90 mm) had 40 annual ridges. Similar longevity was recorded in our study. We found 54 annual ridges in a specimen with a shell length of 93.6 mm. Data obtained for growth parameters by Galinou-Mitsoudi and Sinis (1995) and in our study are not directly comparable since we used different growth functions, Ford-Walford plot and generalized von Bertalanffy growth function, respectively. Asymptotic length

of *L. lithophaga* obtained by Galinou-Mitsoudi and Sinis (1995) was 81.4 mm, while in our study it was 107.6 mm. The corresponding growth coefficient values were 0.04 and 0.03 year<sup>-1</sup>, while values for length at time zero were -0.64 and -1.24. However, growth performance index ( $\phi$ ) values obtained in our study (2.54) and studies conducted on *L. lithophaga* from Greece (2.42) are rather similar.

Validation of growth line formation is one of the key issues in the study of growth and age of marine bivalves and several methods have been successfully applied in previous studies. Methods depend on species characteristics as well as available logistic support in terms of field or laboratory access and include mark-recapture (e.g., Sejr et al. 2002; van der Geest et al. 2011), marginal increment analysis (e.g., Peharda et al. 2012; Bagur et al. 2013), geochemical variations in shell composition (e.g., Thébault et al. 2009; Cardoso et al. 2013a, b) and cross-matching (e.g., Brocas et al. 2013, Bušelić et al. 2015). Due to slow growth rates and boring behavior, the first two methods are not suitable for validation of growth lines in *L. lithophaga*. A pre-requisite of the cross-matching method is that growth increments can be measured with high precision and that the studied species shows inter-annual variations in growth that are consistent between individuals in a population (see Butler et al. 2010). In this study, we analyzed the periodicity of growth line formation in *L. lithophaga* shells by analyzing variations in oxygen ( $\delta^{18}\text{O}$ ) and carbon ( $\delta^{13}\text{C}$ ) isotope values of shell calcite, and have evaluated them as potential proxies for environmental variability.

Analysis of stable oxygen isotopes has been successfully used to validate seasonality in growth line deposition for many marine bivalve species including *Pinna nobilis* (Richardson et al. 2004), *Arctica islandica* (Schöne et al. 2004; Foster et al. 2009), *Comptopallium radula* (Thébault et al. 2007), *Panopea abrupta* (Goman et al. 2008), *Scrobicularia plana* (Santos et al. 2012), *Eurhomalea exalbida* (Yan et al. 2012), *Ensis directus* (Cardoso et al. 2013b), *Macoma balthica* (Cardoso et al. 2013a), *Glycymeris bimaculata* (Bušelić et al. 2015) etc. In shell samples collected from *L. lithophaga*, oxygen stable isotope profiles showed seasonal cycles corresponding to the annual growth ridges.

The results strongly suggest that growth ridges visible on the external shell surface of *L. lithophaga* are formed annually. This is because (1) the number of  $\delta^{18}\text{O}$  cycles found on specimen LL73I was exactly the same as the number of external growth ridges, and (2) the position of growth ridges usually occurred shortly after the minimum  $\delta^{18}\text{O}$  values. This would suggest that growth ridges are formed in a period when temperatures start to decrease. This is in agreement with Galinou-Mitsoudi and Sinis (1995) who observed formation of annual growth ridges during fall after reproduction. Nevertheless, the spatial (and therefore temporal) resolution of our  $\delta^{18}\text{O}$  values is too low

to assess the period when growth ridges are formed with a high degree of certainty. Indeed, because of the drilling angle, our samples represented a certain degree of time averaging, in some cases most likely several months.

The reconstructed range of seawater temperatures recorded by *L. lithophaga* shells over the period 1987–2012 (between 12 and 25 °C) is consistent with temperature records obtained from the temperature logger in 2013–2014. This indicates that shell calcite can capture both the lowest and highest temperatures. According to Galinou-Mitsoudi and Sinis (1995), over 95 % of the yearly growth of *L. lithophaga* shells occurs during the warm part of the year, i.e., from May to October. Further analysis is needed on shells collected from different temperature regimes to gain a better insight into the relationship between seawater temperature and bivalve growth rate.

Since stable carbon isotope ratios in shells are affected by metabolic factors and environmental conditions, their profiles in shells are less clear than those of  $\delta^{18}\text{O}$  (Wefer and Berger 1991; McConnaughey and Gillikin 2008). Isotopic fluctuations including shell  $\delta^{13}\text{C}$  trends toward lower values with increasing ontogenetic age are common in both marine and freshwater mollusks (for review see McConnaughey and Gillikin 2008; see also Schöne et al. 2011). Possible explanations are changes in respiration with increasing size (Lorrain et al. 2004), as well as changes in calcification physiology or hydrology (McConnaughey and Gillikin 2008). An ontogenetic decrease in shell  $\delta^{13}\text{C}$  values with increasing ontogenetic age has been noted for *L. lithophaga* analyzed in this study. It is interesting to note that there appears to be inter-annual differences in the decrease, with  $\delta^{13}\text{C}$  values decreasing more rapidly during the first years (~7) of life, with a slowdown in decrease after that time. It is hypothesized that this might reflect ontogenetic changes in boring depth and associated changes in the pore water chemistry. A more prominent decrease in  $\delta^{13}\text{C}$  during the first few years of life was previously noted for the deep sea oyster *Neopycnodonte zibrowii* (Wisshak et al. 2009). By comparison, no age-related trends were found in  $\delta^{13}\text{C}$  in shell samples of *Arctica islandica* with biological ages between 42 and 391 years collected at four distinct sites (Butler et al. 2011; see also Schöne et al. 2011).

Range and standard deviation of 4.37 and 0.89 ‰ for  $\delta^{13}\text{C}$  values, respectively, are rather higher than in previous studies conducted on some other species. Butler et al. (2011) and Schöne et al. (2011) determined the range of  $\delta^{13}\text{C}$  in shell samples of mature *Arctica islandica* to be lower than 1 ‰. In a recent study conducted by Bušelić et al. (2015) on *Glycymeris bimaculata* from the eastern Adriatic, seasonal variability of  $\delta^{13}\text{C}$  was observed with a range of 1.2 ‰ (0.8–2.0 ‰). Range in  $\delta^{13}\text{C}$  values observed for *Macoma balthica* (aragonite shell) was up to 3.5 ‰ (Cardoso et al. 2013a).

Results of this study provide the first stable isotope data for *L. lithophaga* shells and show the potential of this species as a geochemical sclerochronological archive. Although the shell of this species is thin and rather challenging to work with, and due to fragility is not easily preserved in fossil records, its longevity still makes it an interesting archive for changes occurring over its life span. Future studies should attempt higher-resolution sampling in order to obtain more data points per growth increment. Further, more data are needed on  $\delta^{13}\text{C}$  DIC of the Adriatic waters as well as on local hydrographic and ecological conditions (specifically salinity, primary production and ecosystem respiration) in order to interpret the potential of stable isotope data as environmental proxies, especially for carbon. According to previous studies,  $\delta^{13}\text{C}$  records depends on the context and can be a record of salinity,  $\text{CO}_2/\text{O}_2$  ratio, DIC content, productivity or the physiology of the animal (e.g., McConnaughey and Gillikin 2008; Schöne et al. 2011; Goodwin et al. 2013).

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#### Compliance with Ethical Standards

Sampling permits were obtained from all relevant Institutions in Croatia. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. All authors approved the final version of the manuscript and consent to submit has been received from all co-authors and institutions.

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