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ORIGINAL PAPER

Shorter but thicker: analysis of internal growth bands in shells of intertidal vs. subtidal Antarctic limpets, *Nacella concinna*, reflects their environmental adaptation

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Abstract

The limpet *Nacella concinna* is a dominant macroinvertebrate along the coastal Antarctic Peninsula with two ecotypes inhabiting intertidal and subtidal areas, respectively. The ecological aim of the study was to understand whether higher stress competence and migratory energy expenses in intertidal Antarctic limpets shorten their lifetime and limit the shell growth rate compared to their sublittoral conspecific. We evaluated shell morphometry, age and internal shell growth bands in a large number of intertidal and subtidal *N. concinna* shells in Potter Cove, South Shetland Islands. Comparisons of their morphometrics showed that intertidal limpets are relatively shorter and less wide, and have higher shell mass, i.e. at common shell height, intertidal shells are relatively thicker and heavier than those of subtidal specimens. Internal shell growth bands showed alternating wide opaque (faster growth in summer) and thin translucent bands (slow growth in winter). The maximum age read was close to 20 years for both groups. Comparisons of von Bertalanffy growth curves showed for shell length and shell width lower growth rate *k* in intertidal animals than in subtidal ones associated to a great variability, with no differences in other growth parameters. However, when shell height vs. age is considered, no differences were observed for any growth parameter. Curtailed variability of growth rates in the intertidal population reflects either a limitation of the food reserves or feeding time, or an energy gap for shell growth due to the costs for migratory movements and stress defense.

Keywords Gastropoda · Age · Morphotypes · Tidal zones · Shell growth pattern

Introduction

Benthic organisms of limited mobility, but with vertical distribution along intertidal and subtidal gradients, develop functional traits, adaptive to different microhabitats (rock surfaces, tidal pools, mud flats, etc.) at different tidal ranges (high and low intertidal, shallow subtidal; e.g., Underwood 1979; Abele et al. 1998; Garcia 2014; Gleason et al. 2018). Aside from environmental drivers, species interactions e.g., predation and competition for space or food, determine species distribution and, therewith, community structure across tidal ranges and microhabitats (Barnes 2002; Barnes and Kuklinski 2003; Molis et al. 2011; Valdivia et al. 2011; Clark et al. 2017). Shell growth rates, morphology and thickness are major functional traits of gastropod and bivalve molluscs, and are strongly influenced by seawater temperature, ion composition, pH, oxygenation, as well as by species interactions and competition for space (Moss et al. 2016; Lomovasky et al. 2018). Because only a small number of species is able to tolerate the extreme conditions

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at high-intertidal levels, environmental effects are the major determinants of shell growth in the upper intertidal (Reise 1998; Gosling 2003; Cognie et al. 2006), whereas species interactions dominate in the low-intertidal and -subtidal zones (Kochmann et al. 2008; Valdivia et al. 2011).

The limpet *Nacella concinna* is one of the most abundant macroinvertebrates in shallow coastal ecosystems of the Antarctic Peninsula where it colonizes intertidal and subtidal habitats down to around 100-m (Powell 1951; Walker 1972). Highest densities of *N. concinna* at 25 de Mayo/King George Island (KGI) are observed at low-intertidal- and shallow-subtidal levels (Valdivia et al. 2014: for Fildes Peninsula). Recruitment occurs in the majority in subtidal areas from where some individuals over ~ 20 mm shell length migrate towards the intertidal (Brêthes et al. 1994) as a mechanism for relaxing intraspecific competition.

Two distinct morphotypes have been described throughout different biogeographic populations of the Antarctic limpet: a seasonal migratory intertidal type with relatively taller and more voluminous shells (inner shell volume, see Weihe and Abele 2008) than the subtidal ecotype which occurs below 4 m water depth (Walker 1972; Nolan 1991; Kim 2001; de Aranzamendi et al. 2008, 2010). These studies used size frequency analysis, to document depth zonation of the two shell morphs, which is backed by their phenotypic adaptation to intertidal (tall shells) and subtidal (flat shells) habitats (Waller et al. 2006; Weihe and Abele 2008). No evidence has so far been obtained for genetic depth zonation, i.e. two sympatric species co-existing at different depth levels (Hoffman et al. 2010; but see de Aranzamendi et al. 2008). The high top intertidal *N. concinna* ecomorph is one of the few macroinvertebrates that survives on rocky surfaces in the splash zone of the upper littoral. This presumes enhanced tenacity (Davenport 1988), pronounced resistance to desiccation (Weihe and Abele 2008), and enhanced thermal tolerance and righting ability of high intertidal limpets with taller and more massive shells (Morley et al. 2010). Nevertheless, predation by gulls could also contribute to the differentiation of limpets since individuals with flatter shells (compared to higher shells in the littoral zone) and/or with the apex displaced towards the anterior side are more likely to be predated (Castillo et al. 2019).

Furthermore, several studies have assessed seasonal and site-dependent growth rates of *N. concinna*, using mark/recapture experiments in combination or not (Picken 1980; Nolan 1991) with calcein labelling (Clarke et al. 2004) and size frequency analyses (Brêthes et al. 1994) for different locations along the Antarctic Peninsula and the islands of the Scotia Arc. To better understand the adaptive response to intertidal and subtidal conditions, it is, however, necessary to analyze and compare shell growth rates of both ecotypes. Hence, in the present study we analyzed internal growth band patterns of 179 shells of intertidal and subtidal limpets from Potter Cove (PC), KGI. We determined the individual growth and age of both ecotypes and derived growth constants for different shell morphometric measures (length, width, height) from the respective growth models. The ecological aim of the study is to understand whether higher stress competence and migratory energy expenses in intertidal Antarctic limpets shorten their lifetime and limit shell growth rate and maximum lifespan compared to their sublittoral congeners.

Material and methods

Study area and sampling procedure

The samples were collected during summer seasons of 2006, 2009, 2013 and 2014 in PC, KGI, South Shetland Islands. Two sites were sampled in PC: Peñón 0 (P0; 58° 40′ 51.7″ S, 62° 14′ 22.5″ W) and Peñón 1 (P1; 58° 40′ 52.5″ S, 62° 14′ 50.9″ W) on Potter Peninsula (southern PC shore, Fig. 1). Both P0 and P1 represent coastal rock heads on the outer southern shore of PC, with extended shallow intertidal offshore areas composed of boulders (volcanic rock and glacial moraines, Lindhorst and Schutter 2014; Heredia Barión et al. 2019). These boulders are surrounded by a sand and gravel covered intertidal, in which rock pools form during low tides (Fig. 2). The adjacent sublittoral zone is composed of sandy and rocky bottom (Heredia Barión et al. 2019).

At both sites, animals were hand collected in the intertidal at 0-m water depth whereas subtidal animals were collected by SCUBA divers at 30 m. In both samplings, we aimed to cover the entirety of available size range. Animals below 20 mm shell length were, however, underrepresented especially in the intertidal population. Small individuals of < 15-mm shell length were specifically "targeted" by our samplings, and a small number of these small-sized individuals could be included in the analysis.

Shell morphology of *N. concinna*

Limpets were collected from intertidal (P0, $n = 74$; P1 $n = 182$) and subtidal zones (P0, $n = 153$; P1, $n = 141$). Whereas the soft tissues were used for other studies, the calcitic shells were preserved for morphometric and growth analysis. Shell length (L; the anterior–posterior axis), height (H; vertical distance from the apex to the ventral margin), width (W; the maximum distance between the lateral shell margins; all using calipers with precision ± 0.10 mm), and shell mass (SM) were determined (precision ± 0.01 g). The mean morphometric variables were compared between intertidal and subtidal ecotypes by t-test analyses. The relationships among H, L, W and SM were fitted to the best model (linear or exponential). To compare the morphometric

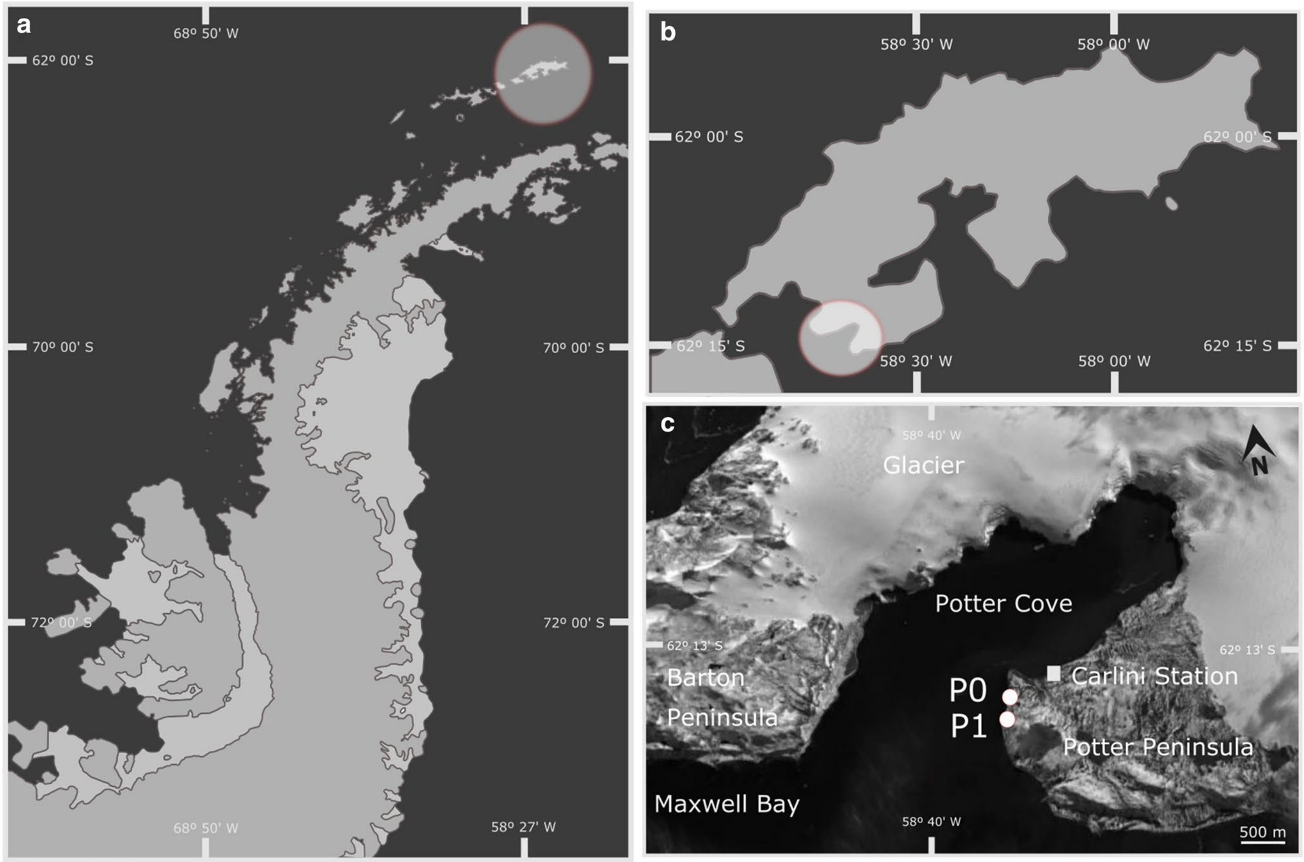

Fig. 1 Location of sampling sites of *Nacella concinna*, **a** Antarctic Peninsula, **b** 25 de Mayo/King George Island, South Shetland Islands and **c** Potter Cove (62°14′ S, 58°38′ W); extracted from Google earth. *P0* Peñón Cero, *P1* Peñón 1

relationships between morphotypes, a full interaction ANCOVA model with subsequent Tukey multiple comparison tests was applied when assumptions of normality, homoscedasticity and parallelism were fulfilled (Zar 1999) using STATISTICA 10.0 software. To enhance overall data homogeneity, we restricted the statistical analysis to the size range $\geq$ 1.60 mm to $<$ 19.70 mm shell height ($\geq$ 7 mm to $<$ 47 mm length) in both samples.

Internal shell growth patterns and growth rate of *N. concinna*

Shells for growth analysis were taken from the two areas and combined: intertidal $n = 63$ (P0, P1; length (L) range 6.30 mm and 37.93 mm) subtidal $n = 116$ (P0, P1, length (L) range 7.53 mm and 56.09 mm), omitting individuals with abraded shells owing to ice scour or unsuccessful attacks of seagulls (see Cadée 1999). Individual age was inferred from internal shell growth bands that were interpreted to represent growth during one calendar year (see below), following Richardson (2001) and Colonese et al. (2011). The shells were individually embedded in polyester resin and sectioned along the axis of maximum growth from the apex to the ventral margin using a Bosch GDC 34 saw with a diamond blade. Cross-sections were polished on lapidary wheels using grits of 400, 600, 1200, 2400 and 4000 grade and diamond solutions of 0.10 µm and 0.05 µm. The polished cross-sections were stained with Mutvei's solution (see Schöne et al. 2005) to facilitate optical growth band readings with a stereomicroscope (Stereo Microscope System SZX16 with a Digital Camera DP73, Olympus, and a cellSens Standard 1.15 program from Olympus), using reflected light. In stereomicroscopy, unstained shell cuts show internal shell growth bands that appear as translucent and opaque growth bands (see Jones et al. 1990). Additional Mutvei´s technique makes the growth bands appear in different shades of blue, staining sugars that are generally more concentrated at major growth lines and etching the shell increments between growth lines that contain fewer organics, improving the visibility of growth structures within the shell (see Schöne et al. 2005). Description of the shell layers (a sheet-like composition consisting of single microstructure) was based on previous studies, and layer definitions were taken

Fig. 2 **a** Picture from intertidal rocky shore sampling site in summer at Potter Cove (photograph D. Abele), **b** *Nacella concinna* limpets inside a rocky tidal pool from Peñón Cero site (photo M.C. de Aranzamendi) and **c** *Nacella concinna* at subtidal zones (photo C. Lagger)

from Fuchigami and Sasaki (2005). Patellidae shells have between four and six different structural layers defined by their position relative to the muscle attachment scar (the myostracum). Layers above the myostracum (outer layers) are designated $M+1$, $M+2$ and so on. Layers below the myostracum (inner layers) are designated $M-1$, $M-2$ and so on. The number, arrangement, and crystal structure of these layers varies by genera (see Fuchigami and Sasaki 2005; Prendergast and Schöne 2017). Under the premise that the major growth bands are formed annually, following previous age validation by O^{18} isotopic measurements after Colonese et al. (2011) and calcein-labelled internal growth band analysis by Clarke et al. (2004), each distance between two subsequent winter growth lines (translucent band) marks the shell growth during one calendar year (opaque growth band; see Prendergast and Schöne 2017).

Additionally, to confirm the detected shell growth patterns, acetate peel replicates of the cross-sectioned surfaces were produced for some exemplary shells following Rhoads and Lutz (1980). The polished unstained cross-sections were etched 17 min using 0.5% DECAL agent. Acetate peels were microscopically examined to analyze the calcium carbonate shell structure. The results were compared with the stereomicroscopy images obtained with the Mutvei's technique. The number of internal translucent bands and the corresponding length (L), height (H), and width (W) were used to generate age–length, age–height or age–width data for each individual. Based on these individual measurements, a von Bertalanffy growth model was fitted to the shell length or height or width-at-age data using:

$$L_t = L_\infty\left(1-\mathrm{e}^{-k(t-t_0)}\right)\ (\text{mm},\ \text{year}),$$

where L_∞ or H_∞ or W_∞ is the asymptotic length or height or width, k is the growth constant, t the age, and t_0 the age at zero length or height or width. To fit the model to the data, the maximum likelihood method was used following Edwards (1992) and Hilborn and Mangel (1997).

To compare growth measurements between intertidal and subtidal ecotypes, the von Bertalanffy k, t_0 and L_∞ or H_∞ or W_∞ parameters from each tidal habitat were pairwise compared, using the likelihood ratio test (Cerrato 1990; Aubone and Wohler 2000). Bivariate confidence limits (Cls) for k, t_0, L_∞, H_∞ and W_∞ were determined using profile likelihood (see Hilborn and Mangel 1997).

Results

Nacella concinna shell morphology

Limpets collected from intertidal and subtidal zones ranged from 6.30 to 46.83 mm L and 7.53 to 58.07 mm L, respectively. Mean length and width of intertidal limpets were significantly smaller than in the subtidal limpets (Table 1).

The ratios between L vs. H, L vs. W, L vs. SM, and H vs. all variables were fitted best by exponential models for both intertidal and subtidal limpets ($p < 0.05$; Table 2). At shell length ≥ 20 mm, morphological differences between both groups become apparent (Fig. 3a–d). The ANCOVA analyses of ln-transformed data sets indicate that slopes of the linear regressions for all analysed relationships differ significantly between both groups ($p < 0.0001$, Table 2). This reflects higher H and SM, and lower W for a mean co-variable "shell length (L)" in intertidal compared to subtidal limpets. Using "ln H" as co-variable results in higher values

Table 1 *Nacella concinna* shell morphometrics of intertidal ($n = 255$) and subtidal ($n = 294$) individuals from Potter Cove, King George Island, South Shetlands

	Length (mm)	Height (mm)	Width (mm)	Shell mass (g)
Intertidal	31.32 ± 7.05	11.26 ± 3.21	21.99 ± 5.01	1.57 ± 1.01
Minimum	6.30	1.25	4.46	0.01
Maximum	46.83	21	33.84	7.31
Subtidal	38.60 ± 8.60	11.54 ± 3.22	26.64 ± 6.01	1.52 ± 0.77
Minimum	7.53	1.61	5.16	0.01
Maximum	58.07	19.63	42.99	4.58
t-test	$t_{549} = -14.45$	$t_{549} = -3.55$	$t_{549} = -13.09$	$t_{541} = -3.35$
	$*p < 0.0001$	$p = 0.0536$	$*p < 0.0001$	$p = 0.0853$

All values are means ± SD, with minimum and maximum values found

Asterisks represent statistically significant values ($p < 0.05$)

Table 2 Morphometric relationships in *Nacella concinna* from intertidal and subtidal zones as described by exponential function $y = ax^b$ (all significant relationships, $p < 0.05$; best fit); and Tukey test to compare the morphometric relationships between intertidal and subtidal ecomorphs

Variable *x*	Variable *y*	Site	*a*	*b*	*r2*	*n*	Tukey test
L	*H*	Intertidal	0.11	1.34	0.95	274	a
		Subtidal	0.16	1.17	0.73	262	b
L	*W*	Intertidal	0.69	1.001	0.99	274	a
		Subtidal	0.74	0.98	0.97	262	b
L	SM	Intertidal	0.00002	3.25	0.95	274	b
		Subtidal	0.00004	2.85	0.76	262	a
H	*L*	Intertidal	5.63	0.71	0.95	273	b
		Subtidal	7.77	0.66	0.77	305	a
H	*W*	Intertidal	3.91	0.72	0.95	273	b
		Subtidal	5.25	0.67	0.78	305	a
H	SM	Intertidal	0.004	2.364	0.95	273	b
		Subtidal	0.006	2.207	0.89	305	a

Equal letters indicate no significant differences at $p = 0.05$. The average ratio of dependent variable to covariate decreases in alphabetical sequence

H shell height, *L* shell length, *W* width, *SM* shell mass

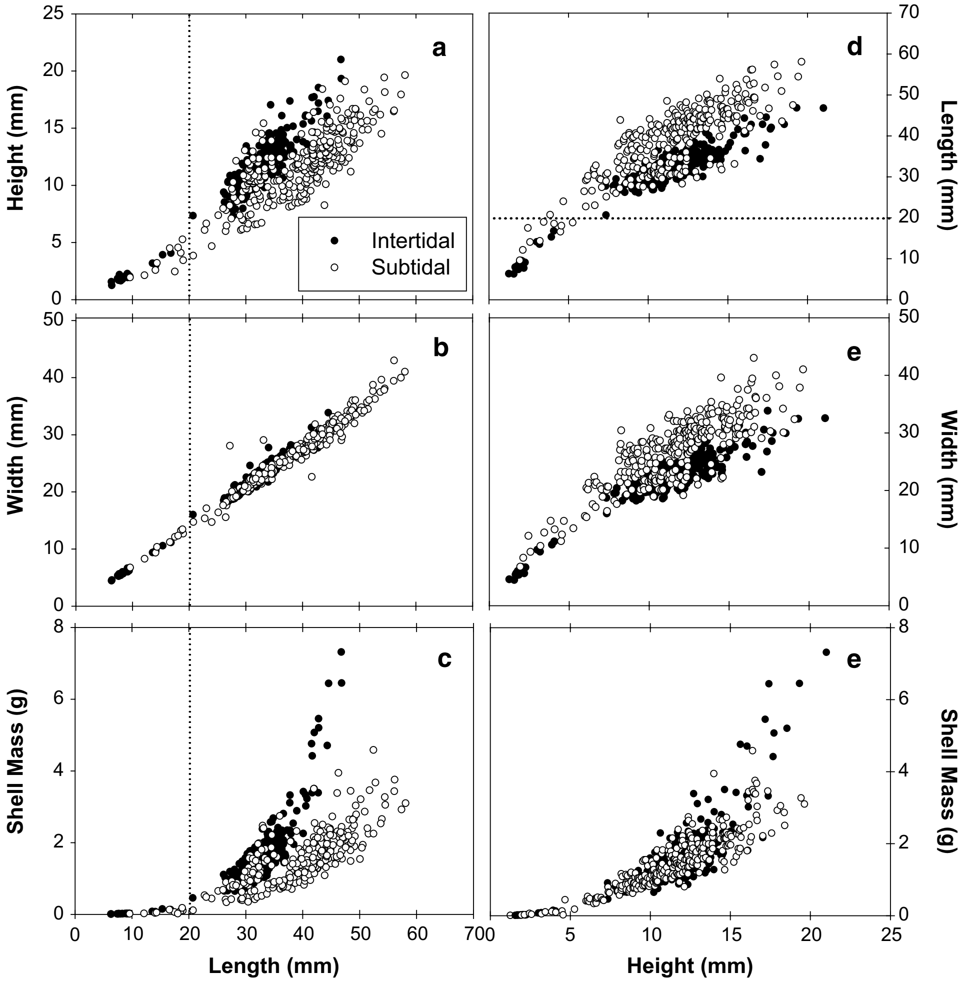

Fig. 3 Morphological relationships in *Nacella concinna* from intertidal and subtidal sites from Potter Cove, Antarctic Peninsula

of *L* and *W* for a mean standardized *H* in subtidal limpets (Table 2). The relationship between *H* and SM showed different slopes too ($p < 0.0001$, Fig. 3f), with higher SM in intertidal limpets for bigger animals and lower SM for smaller ones. Hence, plasticity of allometric shell growth distinguishes the Antarctic limpets from both tidal levels.

Nacella concinna individual shell growth

Nacella concinna shells are composed of four different microstructural layers (Fig. 4). The myostracum (*M*) separates the outer layers ($M+1$ and $M+2$) from the inner layer ($M-1$). In the apex of the shell, the myostracum occupies the area close to the top. The internal shell cuts under stereomicroscopy (Fig. 4a, b) and acetate peel technique for both morphotypes revealed a pattern of alternating broad opaque (brown colour) and narrow translucent bands inside each layer (Fig. 4b: subtidal specimen). The internal growth bands showed a parallel growth layering in the apex area (Fig. 4b, c). Staining cuts with Mutvei's solution visualized each internal translucent growth band (indicative of slow growth) as layers appearing in different shades of blue. Growth bands run from the apex area (Fig. 4c) along the entire inner shell section (Fig. 4d) to the point where they cross the outer shell layer in a cluster of narrow translucent growth lines, forming an exterior growth ring (Fig. 4e).

We used the internal translucent growth bands for the analysis of individual age to infer maximum population lifespan (see Fig. 5). Maximum age reads were 19 and 20 years for individuals from both shore levels, indicating animals to live to age max at both depth levels. In spite of the fact that most studies report (or repeat) a minimal shell length of 20 mm for limpets migrating towards the intertidal, when looking specifically for small individuals, they are indeed present intertidally. Thus, the smallest individuals we encountered in the PC intertidal were between 5 and 10 mm in length and had a shell height around 2 mm (Fig. 5). These animals were aged to 1–2 years.

The parameters of the von Bertalanffy growth curves (Fig. 5) for length (*L*), height (*H*) and width (*W*) annual growth

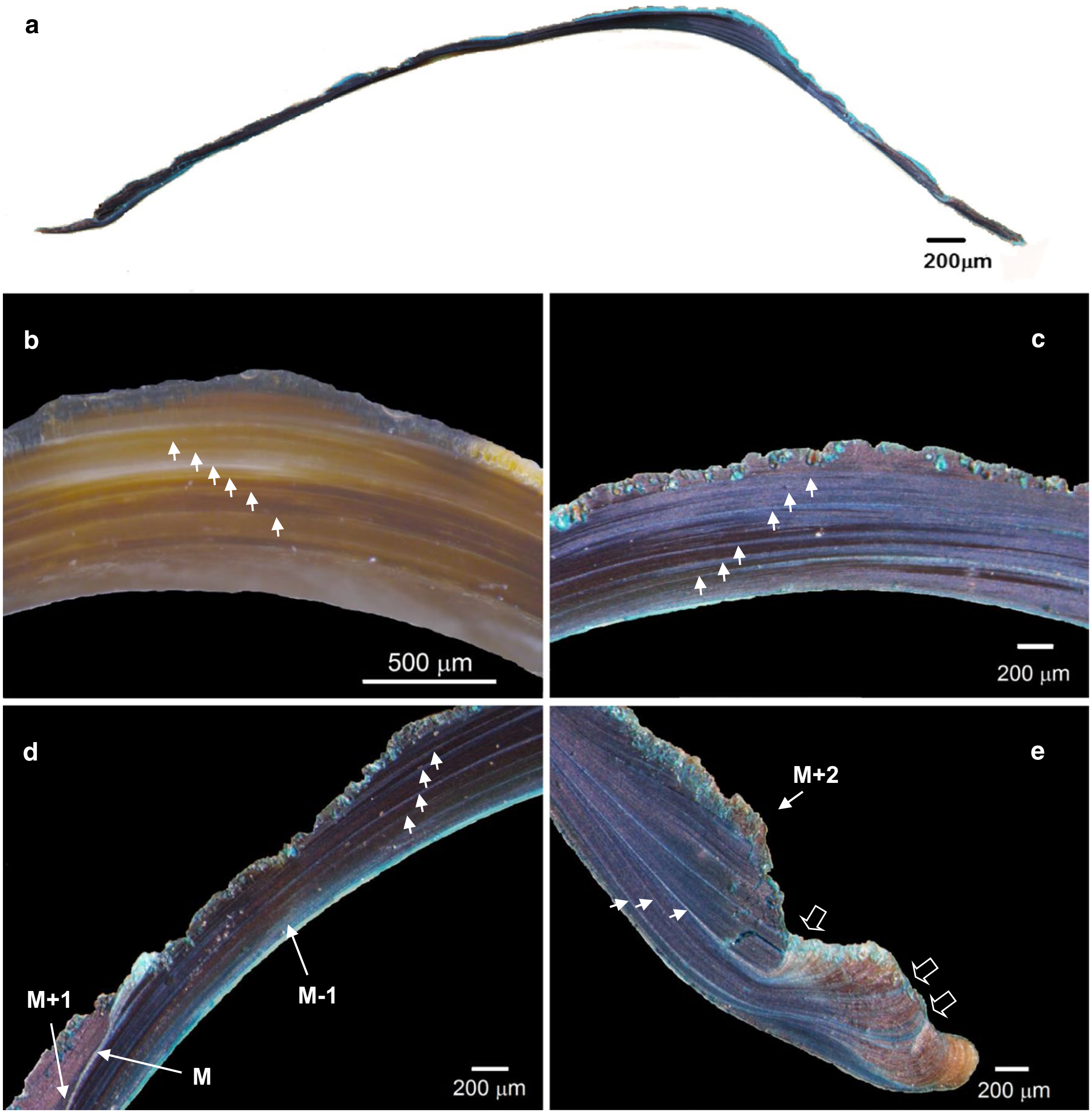

Fig. 4 Internal shell cuts of *Nacella concinna*, **a** shell cross-section of *N. concinna* along the axis of maximum growth in length, **b** internal shell growth bands in the apex section observed under stereoscopy showing a pattern of alternating broad opaque (brown colour) which represent high growth rate and narrow translucent bands, which represent slow growth, **c**–**e** shell sections stained with Mutvei's solution, **c** apex area, **d** body shell, and **e** shell margin showing the internal growth bands all the way along a cross shell section. White arrows: translucent growth bands (low growth), open white arrows (exterior rings): point where translucent growth bands cross the outer shell layer to form an exterior ring on the shell surface. The myostracum (M) separates the outer layers ($M+1$ and $M+2$) from the inner layer ($M-1$)

are summarized in Table 3. Comparison of the growth curves by the likelihood ratio test indicates lower shell length and width growth rate k (χ^2_{179} = 13.22, $p=0.0003$; χ^2_{179} = 12.62, $p=0.0004$, respectively) of intertidal than subtidal limpets especially at young ages < 10 years (Fig. 5). No significant differences in L_∞ and t_0 between the two tidal groups were detectable (χ^2_{179} = 0.78, $p=0.3762$ and χ^2_{179} = 1.55, $p=0.2136$, respectively). Likewise, W_∞ and t_0 did not differ (χ^2_{179} = 0.44, $p=0.5091$ and χ^2_{179} = 1.99, $p=0.1581$, respectively). Furthermore, no significant difference in shell height vs. age was observed for any growth parameter ($H_\infty \chi^2_{179}$ = 0.001, $p=0.9717$; k, χ^2_{179} = 2.05, $p=0.1524$; t_0, χ^2_{179} = 0.17, $p=0.6747$). For all three-growth parameters, CIs (Table 3; Fig. 5) for the subtidal population were wider than for the intertidal limpet population, reflecting higher plasticity of subtidal animal shell growth rates.

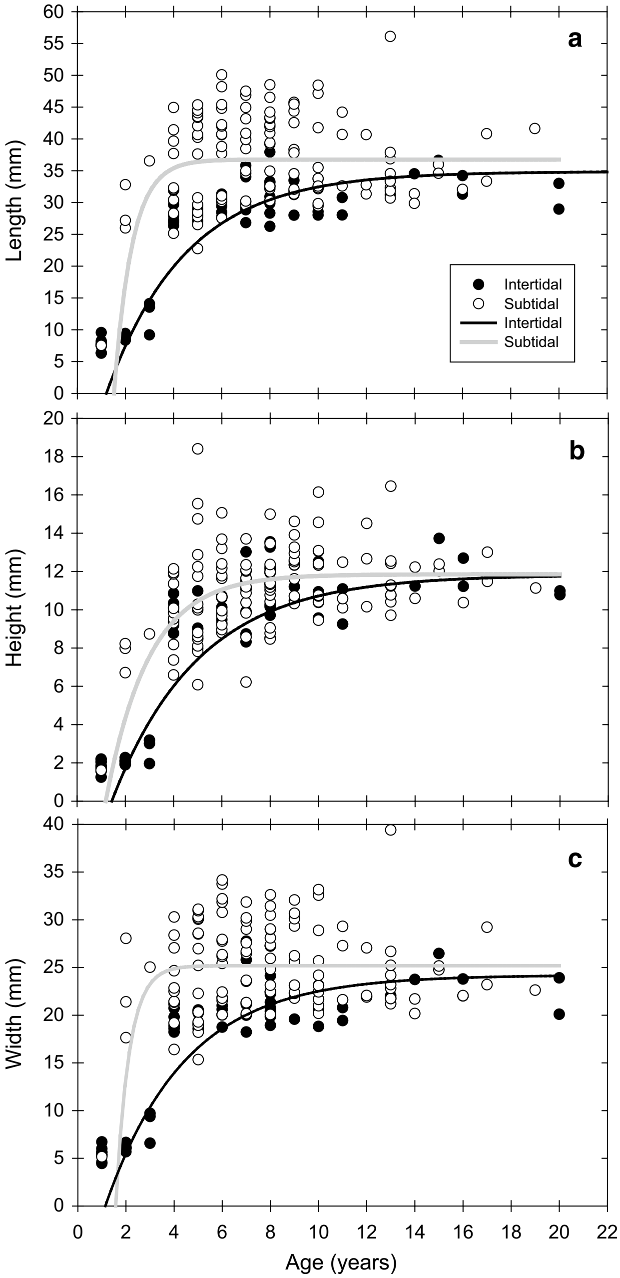

Fig. 5 Size-at-age data and corresponding von Bertalanffy growth curve in *Nacella concinna* from intertidal and subtidal zones from Potter Cove, Antarctica, **a** length vs. age data, **b** height vs. age data and **c** width vs. age data

Discussion

The ecological background of the growth band patterns in Potter Cove

Alternating patterns of shell growth bands (fast and slow growth) in Antarctic shallow water *N. concinna* has mainly been attributed to the strong seasonality in both pelagic and benthic primary production (Picken 1980; Clarke et al. 2004; Fraser and Peck 2007). Limpets are grazers with a highly seasonal diet of bacterial biofilms, epiphytic microalgae, and macroalgal propagules, with availability of at least the last two components restricted to the short Antarctic spring and summer season (Brêthes et al. 1994; Iken et al. 1998; Choy et al. 2011; Suda et al. 2015; Campana et al. 2018). Contrary to the seasonality of diet, temperature differences in Antarctic surface waters are less pronounced. Seasonal sea surface temperatures in PC range between 0.5 and 2 °C in summer (January to March) and vary from − 2.5 to 0 °C in winter (June to September, see Schloss et al. 2012 for the inner PC, Barnes et al. 2006 for KGI upper 10–20 m water depth). The maximal rates of protein synthesis and incorporation into the soft tissue in *N. concinna* was found around 1 °C, stimulating at least soft tissue growth in summer (Fraser and Peck 2007). As the same biological processes also support the accrual of the organic shell matrix, we assume that these temperatures are also supporting faster summer shell growth rates. In addition, higher temperatures support calcium carbonate accretion and thus facilitate formation and maintenance of the inorganic shell matrix (see Abele et al. 2017). A similar effect on *N. concinna* shell growth rates was seen by Clarke et al. (2004) in years with higher water temperatures at Rothera Point. Most studies suggest food availability and water temperature to control annual shell growth rates of Antarctic limpets, with likely interaction between both factors (i.e., higher water temperatures also affording faster growth of macroalgae and benthic diatoms in coastal areas), and this seems to also hold true for the PC population.

Shell growth in intertidal and subtidal limpets from King George Island

Our analysis of the internal shell growth patterns of *N. concinna* from the South Shetland Islands revealed typical annual growth increments: opaque bands that form during the austral summer season (see Clarke et al. 2004) alternating with translucent winter growth bands. The same seasonal pattern of growth band alterations has already been confirmed for Patellidae gastropods from different habitats around the world (Richardson 2001; Colonese et al. 2011; Prendergast and Schöne 2017). We used the existing information on patellid growth structures to derive individual ages and growth rates for Antarctic limpets from KGI and approximated a maximum lifespan of 20 years at both tidal levels based on our larger sample batch. Additionally, thorough searches in the intertidal we could collect a small number of animals < 20 mm shell length, hence showing that recruitment could happens in the intertidal at KGI as suggested by Brêthes et al. (1994) but we cannot completely rule out the possibility that these small limpets have migrated from the subtidal. The models

Table 3 Parameters of the general von Bertalanffy growth functions for length (*L*) vs. age, height (*H*) vs. age and width (*W*) vs. age with 95% confidence limits (Cls) in *Nacella concinna* from intertidal and subtidal zones at Potter Cave

	L_∞ (mm) (Cl)	k (year^{-1}) (Cl)	$t0$ (year) (Cl)	n	Lmax (mm)	Age$_{max}$
Intertidal	34.86 (28.91; 40.82)	0.31 (0.14; 0.47)	1.20 (0.64; 1.77)	63	37.93	20
Subtidal	36.75 (35.13; 38.37)	1.33 (0.38; 2.29)	1.52 (1.28; 1.78)	116	56.09	19
	H_∞ (mm) (Cl)	k (year^{-1}) (Cl)	$t0$ (year) (Cl)	n	Hmax (mm)	Age$_{max}$
Intertidal	11.82 (9.70; 13.94)	0.28 (0.12; 0.44)	1.44 (0.90; 1.98)	63	13.72	20
Subtidal	11.85 (11.43; 12.26)	0.56 (0.36; 0.76)	1.19 (0.63; 1.74)	116	18.40	19
	W_∞ (mm) (Cl)	k (year^{-1}) (Cl)	$t0$ (year) (Cl)	n	Wmax (mm)	Age$_{max}$
Intertidal	24.20 (19.81; 28.59)	0.29 (0.12; 0.47)	1.14 (0.52; 1.75)	63	27.74	20
Subtidal	25.18 (24.28; 26.09)	1.80 (−0.22; 3.83)	1.57 (1.35; 1.79)	116	39.41	19

for length and width indicate faster growth and more variable growth rates in the subtidal population in very young limpets between 2 and 5 years of age (Fig. 5). Curtailed variability of growth rates in the intertidal population reflects either a limitation of the food reserves or feeding time, or an energy gap for shell growth due to the costs for migration and stress defense.

A lifespan of 20 years is considerably longer than that observed in the sister genus *Cellana* from warm climates (*C. eucosmia*: 5 years, *C. karachiensis*: 6 years, *C. radiata*: 4 years; Saad 1997). This agrees with the general perception of life in the slow lane (for growth and reproduction) in polar ectotherms, which eventually attain longer life spans than temperate and tropical sibling species (Clarke 1996; Philipp and Abele 2010; see also Moss et al. 2016). Based on von Bertalanffy growth models, several studies report a variety of growth constants (k) and asymptotic shell length (L_∞) for different latitudes, seasons, and limpet size classes, compiled by Clarke et al. (2004, see Appendix Table). Most studies did not differentiate between intertidal and subtidal limpets and Clarke and colleagues concluded growth performance in different studies to be strongly biased by the size range sampled (smaller limpets growing faster than medium and bigger) and by the applied methods. Length growth rate k for *N. concinna* shells in Clarke's table differ between 0.06 and 0.32 and are thus in a range somewhat lower (subtidal) than our findings (Table 3). Interestingly, these authors reported fivefold higher growth constant k for limpets on Signy Island than Rothera shores that supports the idea that in areas with more food availability, as in PC or Signy, growth rates would be faster. This suggests that faster and more variable growth of young specimens in PC subtidal areas is likely due to better food availability (quantity) and extended feeding in permanently submerged habitats. Besides, limpets need a wet surface for feeding excursions and breathing, reducing feeding possibilities of intertidal limpets to high tides or tidal pools. However, it is important to highlight that Clarke et al. (2004) used different methods for growth model calculations, including size frequency analysis, external ring readings, field experiments of capture and recapture with increment marking; so, these k values are hard to compare and they should be taken as "indicative" rather than definite numbers.

The ecological causes of taller and more massive but relatively shorter shells in the intertidal

The morphometric analyses in this study showed that intertidal limpets are relatively shorter, but have higher SM, i.e. at common shell height, they have relatively thicker and heavier shells than those of subtidal specimens. As shell growth and repair are energetically costly (Day et al. 2000), this raises questions as to the environmental factors limiting the energy available for growth of shell circumference (relation between length and width), whereas at the same time a process must be at work that supports higher SM of intertidal limpets. Table 3 indicates 4- and sixfold higher shell length and width growth in subtidal than intertidal individuals, with no difference in the k for height, supporting the idea of a compensation effect of higher erosive stress in the intertidal (see below).

It is interesting to note in this context, that slower growing limpets in intertidal pools on KGI can episodically experience temperatures of up to 10 °C on sunny days during the austral summer (Abele et al. 1998; and see Waller et al. 2006 for Adelaide Island). High temperatures and water evaporation, even in the Antarctic, might increase calcium carbonate saturation in small water bodies such as shallow intertidal pools, which would support the higher SM of intertidal specimens through enhanced calcium carbonate precipitation on the shell. This effect is currently unexplored for limpets, but has been demonstrated for calcium carbonate precipitation in cyanobacterial mats on intertidal flats by Kremer et al. (2008) and warrants further investigation.

Heavier shells of intertidal than subtidal limpets have already been reported by Shabica (1971, 1976), who attributed this phenomenon to intense wave action and ice impact. Cadée (1999) reported a great proportion of abraded shells in the intertidal zone of KGI that he related to mechanical damage from non-lethal predation by different bird species. Day et al. (2000) observed that higher erosive pressure is compensated for by continual shell growth. These authors experimentally applied protective coating to South African limpets and observed that the "coated limpets" grew thicker shells than the unprotected ones. Hence, mechanical damage and subsequent repair raise additional energetic costs for shell formation and maintenance in the shallows (see Day et al. 2000; Lomovasky et al. 2005, 2006), but also lead to a permanent renewal and thickening of the shell tops.

Functionally the taller shells support righting activity of animals dislodged by wave action (Morley et al. 2010). Furthermore, a sex bias could also underly the morphometrics of intertidal vs subtidal shells. Kim (2001) analyzed mean shell size and the sex ratio in both sub-populations of KGI limpets in the neighboring Marian Cove. He found males (M) to have significantly larger mean shell size (length measurements) than females (F) across all water depths. The sex ratio (M:F) was 1:1.36 ($n = 3316$) at intertidal level and dropped to 1:0.98 ($n = 211$) in the subtidal. This means that smaller females outnumber the bigger males in intertidal environments at least on KGI, presumably altering growth rates as a side effect. Kim's interesting data (never fully published except in the thesis report) open new research questions with respect to similar phenomena in other areas of the maritime Antarctic, and the gender ecology and sex-based migration behavior in this interesting species. The method we have applied to analyze the internal shell growth bands in the Antarctic limpet could be a valuable tool to be included into such investigations in the future.

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Compliance with ethical standards

Conflict of interest We declare that we do not have any conflicts of interest.

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