Grazing under experimental hypercapnia and elevated temperature does not affect the radula of a chiton (Mollusca, Polyplacophora, Lepidopleurida)

Julia D. Sigwart[*], Nicholas Carey

Queen's University Belfast, Marine Laboratory, 12-13 The Strand, Portaferry, Co., Down, BT22 1PF, UK

ARTICLE INFO

ABSTRACT

Chitons (class Polyplacophora) are benthic grazing molluscs with an eight-part aragonitic shell armature. The radula, a serial tooth ribbon that extends internally more than half the length of the body, is mineralised on the active feeding teeth with iron magnetite apparently as an adaptation to constant grazing on rocky substrates. As the anterior feeding teeth are eroded they are shed and replaced with a new row. The efficient mineralisation and function of the radula could hypothetically be affected by changing oceans in two ways: changes in seawater chemistry (pH and pCO$_2$) may impact the biomineralisation pathway, potentially leading to a weaker or altered density of the feeding teeth; rising temperatures could increase activity levels in these ectothermic animals, and higher feeding rates could increase wear on the feeding teeth beyond the animals' ability to synthesise, mineralise, and replace radular rows. We therefore examined the effects of pH and temperature on growth and integrity in the radula of the chiton *Leptochiton asellus*. Our experiment implemented three temperature (~10, 15, 20 °C) and two pCO$_2$ treatments (~400 μatm, pH 8.0; ~2000 μatm, pH 7.5) for six treatment groups. Animals ($n = 50$) were acclimated to the treatment conditions for a period of 4 weeks. This is sufficient time for growth of ca. 7–9 new tooth rows or 20% turnover of the mineralised portion. There was no significant difference in the number of new (non-mineralised) teeth or total tooth row count in any treatment. Examination of the radulae via SEM revealed no differences in microwear or breakage on the feeding cusps correlating to treatment groups. The shell valves also showed no signs of dissolution. As a lineage, chitons have survived repeated shifts in Earth's climate through geological time, and at least their radulae may be robust to future perturbations.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Polyplacophoran molluscs (chitons) are marine grazers, found worldwide. The most distinctive feature of chitons is their dorsal shell formed of eight articulating plates; unusually for molluscs, these plates or valves are composed entirely of aragonite, rather than calcite (Runnegar, 1989). The valves are perforated by tissue-filled channels called aesthetes which terminate in sensory pores on the valve surface (Schwabe 2010). Chitons also possess a radula with enlarged scraping teeth mineralised with iron magnetite (Lowenstam, 1962). Similar iron-mineralised structures are found in patellogastropod limpet radulae and cephalopod beaks (Lowenstam and Weiner, 1989), although the large scraping teeth of chitons capped with magnetite have gained particular interest from materials science studies seeking to understand and replicate the biomineralisation process for industrial applications (Gordon and Joester, 2011; Wang et al., 2013).

The chiton radula is exceptionally long, and extends more than half the length of the body (Fig. 1), enclosed in a tissue sheath which contributes to growth and mineralisation of the radula in the posterior sections (Brooker et al., 2003). As in all molluscan radulae, when the anterior feeding teeth are eroded they are shed and replaced with a new row. Deposition of minerals within the teeth varies among species in sequence and relative contributions of iron, phosphorous and calcium (Macey and Brooker, 1996; Lee et al., 2000). One previous study reported damage to radulae in the limpet *Patella vulgata* under simulated ocean acidification (Marchant et al., 2010). The potential impacts of changing temperature and seawater chemistry on molluscs include altered calcification of shell material, increased metabolic costs, and

* Corresponding author. Tel.: +44 (0)780 5665 863.
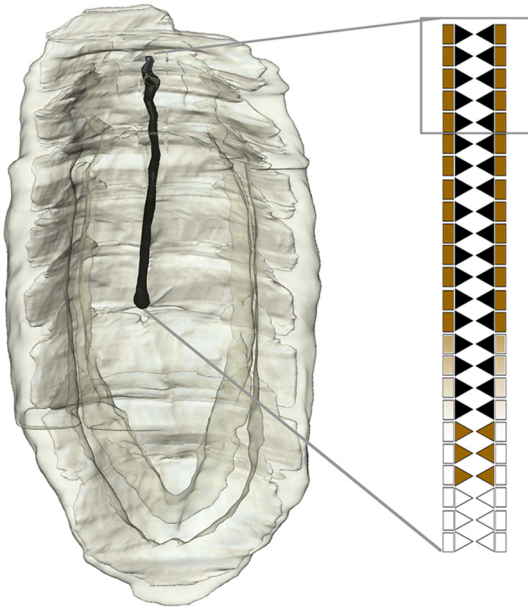 E-mail address: j.sigwart@qub.ac.uk (J.D. Sigwart).

Fig. 1. Schematic of the chiton radula; left, a 3D model of the radula (black) *in situ* in *Leptochiton* constructed from serial sections of *L. rugatus* (Sigwart, unpub. data), ventral view with the body and shells rendered transparent to illustrate a typical chiton radula within the body, extending from the mouth at top to the fifth shell valve. Right, proportional schematic representation of mineralisation fields in *L. asellus*, across the total radula length. Inner triangles represent the iron-mineralised major lateral teeth, boxes represent other adjacent teeth, in non-mineralised (white), partially mineralised (pale) and fully mineralised (black) regions. The box indicates the region illustrated in Fig. 2.

potential behavioural effects (Gazeau et al., 2013; Kroeker et al., 2013); radular damage represents another potential pathway that may threaten the survival of marine molluscs in future oceans.

Exposure to ocean acidification and warming conditions could affect the hard part anatomy of chitons (i.e. shells, and radula) in several ways, which we investigated morphometrically. Rising temperature should increase grazing rate due to increased metabolic demand (O'Connor, 2009), and therefore increased radular wear. Hypercapnia can also cause increased metabolic demand (Beniash et al., 2010), and has been shown to cause increased grazing in benthic marine herbivores (Burnell et al., 2013). Many critical biological processes are substantially affected by pH and this provides a mechanism under which calcification and other biomineralisation pathways, such as radula formation, could potentially be disrupted under ocean acidification conditions. So under climate change, a combination of increased grazing and a weaker radula could result in substantial damage to the radular teeth. If feeding was disrupted by damage to the radula, and poor nutrition leads to an inability to maintain shell integrity, antagonistic affects could seriously disable the animal's performance.

Shell dissolution under hypercapnia has been observed in many molluscs, but this apparently is mitigated by access to adequate nutrition in some species (Rodolfo-Metalpa et al., 2011; Thomsen et al., 2013). Shell calcification is not an environmentally-dependent process, and many molluscs can still maintain net calcification in conditions of under-saturated aragonite and calcite (Comeau et al., 2010; Gazeau et al., 2010). Aragonite, which makes up the shells of chitons, is ~50% more soluble in seawater than the calcite from which most other mollusc species form their shells (Mucci, 1983), making chiton valves potentially more physically vulnerable to direct effects of reduced seawater pH.

We tested several specific hypotheses for the effects of ocean acidification and warming on the subtidal chiton *Leptochiton asellus*, in particular that treatment conditions would: increase visible wear on radular teeth, decrease mineralisation efficiency of the growing radula, and promote dissolution of the shell surface around natural aesthete pores. Any effect on the rate of replacement or the biomineralisation pathway should be visually evident, in an increased number of non-mineralised (posterior) new teeth, or through damage to the anterior feeding tooth rows.

2. Material and methods

Specimens of *L. asellus* were obtained from stones collected by dredge (depth 30–40 m, T ≈ 10 °C) from Gullmar Fjord, Sweden and transferred to the Sven Loven Centre for Marine Sciences, Kristineberg and housed on stones and shell debris taken from the collection sites in flowthrough fjord-bottom seawater. These aquaria were set up following the approach previously used by the authors to successfully maintain healthy captive chitons for up to 18 months. Specimens ($n = 50$) were randomly assigned to one of six seawater treatments (Table 1): elevated pCO_2 (~2000 µatm, pH 7.5) or control pCO_2 (~400 µatm, pH 8.0), in one of three temperature treatments (10, 15, or 20 °C). Animals were maintained in treatment for 4 weeks, then dissected to examine radulae and valves.

Temperature was maintained through a controlled seawater supply (±0.2 °C). Carbonate chemistry was maintained using a computerised feedback system (AquaMedic) that regulates pH by addition of pure gaseous CO_2 directly into seawater (±0.02 pH units). Seawater samples for pH_T (total scale) and total alkalinity (A_T) were collected twice weekly. A_T was determined on filtered water samples with a titration system (Titroline Alpha Plus, SI Analytics). pH_T was measured using a Metrohm (827 pH lab) electrode, and adjusted for pH measurements on the total scale using Tris (Tris/HCl) and AMP (2-aminopyridine/HCl) buffer solutions with a salinity of 32 (provided by Unité d'Océanographie Chimique, Université de Liège, Belgium). Carbonate system speciation (Table 1) was calculated from pH_T and A_T with CO_2Calc (dissociation constants; Mehrbach et al., 1973, as refitted by Dickson and Millero, 1987).

We examined several *a priori* metrics to test our hypotheses: 1. radula mineralisation (tooth colour; number of fully-, partially- and un-mineralised teeth), 2a. cracks or breakage in major lateral teeth (presence or absence), 2b. "holes" in the radula, i.e. decoupling of central and 1st lateral teeth (presence or absence), and 3. megalaesthete pore diameter (µm) at the growing edge of a shell valve. Tooth mineralisation state in dissected radulae was first classified by colour under a dissecting microscope. Mineralisation efficiency of the growing radula can be quantified by scoring the number of tooth rows in discrete stages of mineralisation (Fig. 1): from posterior to anterior, the major lateral cuspids progress in mineralisation from colourless or white (unmineralised) to brown (partially mineralised) to black (fully mineralised). The lateral accessory teeth are mineralised much later (further forward), and appear brown (mineralised) only on the anterior-most portion of the radula. This creates three to four discrete zones of major/lateral tooth mineralisation: white/white (unmineralised), brown/white and black/white (two zones, partially mineralised, herein considered as one region), and black/brown (fully mineralised). These units correspond imprecisely to the developmental stages identified by Kirschvink and Lowenstam (1979).

Secondarily, the radular integrity and aesthetes were examined using a JEOL NeoScope SEM. Aesthete pore diameter was measured from captured images using ImageJ (NIH). In order to accurately measure aesthete pores, the proteinaceous surface layer of the dissected valve was removed by bathing for 60 s in sodium hypochlorite and then rinsing in distilled water. The metrics using SEM visualisation (radula and valves) were scored "blind", from images assigned numbers not associated with their treatment group.

Table 1
Carbonate system speciation ($\pm$SD) in experimental treatments. Total dissolved inorganic carbon (C_T), pCO_2 and calcium carbonate saturation state for calcite and aragonite (Ω_{Ca} Ω_{Ar}) as calculated from pH_T and total alkalinity (A_T) using CO_2Calc.

Treatment	T (°C)	pH_T	S	A_T	pCO_2 (µatm)	C_T (µmol/kg SW)	Ω_{Ca}	Ω_{Ar}
Low Temperature, Control pCO_2	9.4 ± 0.1	8.03 ± 0.07	32	2294 ± 27	428 ± 73	2137 ± 41	2.90 ± 0.37	1.83 ± 0.23
Low Temperature, Elevated pCO_2	9.4 ± 0.0	7.47 ± 0.09	32	2293 ± 52	1736 ± 368	2317 ± 55	0.88 ± 0.17	0.56 ± 0.11
Medium Temperature, Control pCO_2	15.6 ± 0.1	8.02 ± 0.10	32	2289 ± 31	446 ± 102	2093 ± 58	3.56 ± 0.69	2.27 ± 0.44
Medium Temperature, Elevated pCO_2	15.5 ± 0.2	7.42 ± 0.11	32	2291 ± 23	2024 ± 487	2306 ± 40	1.02 ± 0.25	0.65 ± 0.16
High Temperature, Control pCO_2	20.3 ± 0.1	7.99 ± 0.09	32	2289 ± 21	484 ± 119	2074 ± 49	3.92 ± 0.70	2.54 ± 0.45
High Temperature, Elevated pCO_2	20.3 ± 0.2	7.44 ± 0.12	32	2291 ± 35	1992 ± 503	2281 ± 51	1.27 ± 0.34	0.82 ± 0.22

3. Results and discussion

All control pCO_2 treatments were super-saturated and all elevated pCO_2 treatments under-saturated with respect to aragonite (Table 1). In under-saturated seawater conditions, aragonite should undergo dissolution rather than precipitation. Experimental conditions were stable; temperature, pH_T, pCO_2 and C_T differed between treatments with different setpoints (ANOVA, $p < 0.001$ in all four cases). Total Alkalinity (A_T) was stable and did not differ significantly between treatments (ANOVA, $F_{5,30} = 0.14$, $p = 0.98$).

After four weeks of constant grazing in treatment conditions all metrics showed no significant difference among treatment groups. Overall there is no evidence that incubation in hypercapnia or temperature produced related measurable changes in hard-part integrity (Fig. 2, Table 2).

All specimens included in the experiments ($n = 50$) were of small but adult size, approximately 1.5—2.0 cm in length, with an average of 50 tooth rows on the radula (minimum 41 to maximum 61 tooth rows recorded; no significant difference among treatment groups: Mood's test of medians, $\chi^2 = 3.933$,

df $= 5$, $p = 0.559$). Animals all showed the same proportion of mineralised and unmineralised teeth on the radular ribbon; there was no significant difference among the six treatments in number of unmineralised teeth (Mood's test of medians: $\chi^2 = 5.543$, df $= 5$, $p = 0.353$) or fully mineralised teeth (Mood's test of medians: $\chi^2 = 6.819$, df $= 5$, $p = 0.234$). The comparison of medians is the most appropriate test of central tendency in a nonparametric dataset. These data included a few outliers, but the median absolute deviation is 0 for all 18 median values reported here (6 treatment groups, unmineralised, fully mineralised, and total tooth counts).

In our experiments, visible wear on radular teeth was scored "blind". After determining the lack of mineralisation difference across all groups, we excluded specimens from the medium temperature (15 °C) treatments in investigating other metrics to better compare extremes of temperature and pH. The only other study so far published on the effects of hypercapnia on the radulae of grazing molluscs found extensive radular damage in a small sample size incubated for 5 days under similar conditions (pH 7.6; 2800 µatm pCO_2; 15 °C) (Marchant et al., 2010). In our experiments, the treatment group with least tooth damage, recorded as evidence
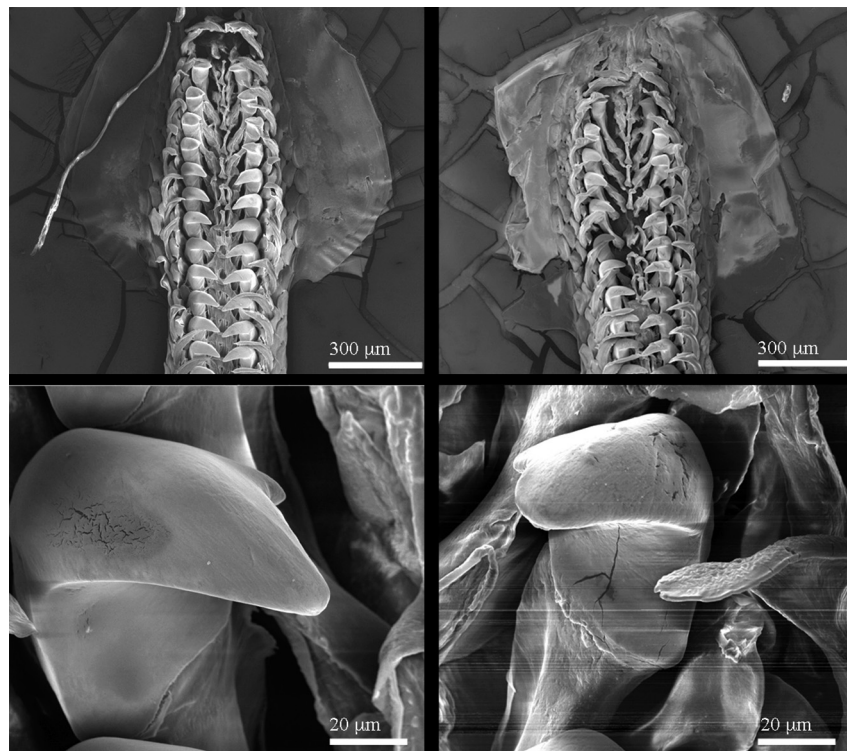

Fig. 2. Radulae of chitons in experimental (left) and control (right) treatments. The specimen on the left was in the most extreme experimental group, high temperature and elevated pCO_2 (20 °C; ~2000 µatm, pH 7.5); the right specimen was housed in control conditions or low (normal) temperature and pCO_2 (10 °C; ~400 µatm, pH 8.0). The damage visible in control specimens (right) is interpreted as attributed to handling damage rather than treatment.

Table 2
Morphometrics used to assess damage or alteration under treatment conditions for experimental chitons' radulae and shell pores (aesthetes): number of specimens, total number of radula rows per animal, number of radular rows with fully mineralised major laterals (anterior rows, cuspids visually black and opaque), number of rows with no evidence of mineralisation in any teeth (posterior rows, all transparent or white), evidence of cracks or holes in the radula (observed under SEM), and mean specimen diameter of megalaesthete pores on growing margin of valve II. Median values for tooth counts each represent data with median absolute deviation of 0.

	Control (pH 8.0)			Elevated pCO_2 (pH 7.5)			Total
	10 °C	15 °C	20 °C	10 °C	15 °C	20 °C	
Specimens per group	8	9	10	10	6	7	
Radular cracks (specimens)	4	–	5	4	–	2	
Radular holes (specimens)	2	–	0	0	–	2	
Radula tooth rows (median)	52	50	51	51	49	48	50
Mineralised tooth rows (median)	33	33	32	30	30	33	32
Unmineralised rows (median)	6	5	6	6	6	5	6
Aesthete diameter (μm), average ± σ	7.7 ± 1.2	–	8.7 ± 1.4	8.1 ± 0.7		8.1 ± 1.4	8.2 ± 1.2 μm

of cracks in the mineralised major lateral teeth in the feeding part of the radula, was the high temperature, high pCO_2 group (Fig. 2). Half of available specimens in the control pCO_2 groups (at 10 °C and 20 °C) had tooth cracks, compared to 40% in the low-temperature (10 °C), elevated pCO_2 group, and only 29% ($n = 2$) in the most "extreme" treatment (elevated pCO_2, 20 °C). These differences reveal interesting variability among individuals but do not represent any statistical difference among groups ($\chi^2 = 0.995$, df = 3, $p = 0.80$).

Only four specimens (low temperature, control pCO_2 $n = 2$; high temperature, elevated pCO_2 $n = 2$) showed "holes" in the radula. In fact, differences in the condition of the radula seem to be attributable to either natural variation in animal condition, or, more likely, handling in dissection (Fig. 2). Although robust, radulae are complex structures comprising an inordinate number of articulating parts. In life position the radula is longitudinally folded in the radular sheath, with the lateral teeth curled up around the central and mineralised major lateral cuspids. Mounting for SEM imaging requires that the width of the radula be flattened and adhered to the surface of the SEM stub. Even when a large sample of specimens are prepared simultaneously by an experienced anatomist, such as in this study, it is evidently possible to introduce variable levels of handling damage.

The seawater chemistry conditions associated with ocean acidification may not only alter structural integrity through affecting calcified hard parts, but may impact upon non-calcified organismal biomechanics. This includes weakened byssal attachment in mussels under high pCO_2 (O'Donnell et al., 2013), even when shell growth was unaffected. Therefore the biomechanical impacts upon organisms of altered seawater chemistry may be more complex than first thought, and not simply confined to impacts upon calcification.

Recent research has focussed interest on the unusual ability of chitons to biomineralise iron magnetite, which may be relevant to industry applications for nanotechnology (Gordon and Joester, 2011; Wang et al., 2013). The evidence here suggests that the radula biomineralisation pathway in L. asellus was not disrupted under treatment, or that the animals are able to compensate for environmental change and maintain regulated growth and mineralisation. This makes the chiton radula yet more interesting: these animals mineralise precise structures at nano scale, under a broad range of temperatures—from polar to tropical environments—and also under an apparently broad range of water chemistry outside the individual local environment.

There was no significant difference in size of natural shell pores (aesthetes) in any treatment (Kruskal–Wallis $\chi^2 = 2.994$, df = 3, $p = 0.393$) nor any visual sign of dissolution or pitting in any specimens. Chiton valves are composed of aragonite, which is naturally more liable to dissolution in low-pH conditions than its sister mineral calcite. However, most chitons have a proteinaceous layer on the shell dorsum, similar to the periostracum in other molluscan groups, which may provide a protective layer. Leptochiton asellus particularly is a member of the plesiomorphic order Lepidopleurida, which is characterised by thin and simple shells compared to the opposing order Chitonida. On that basis one might expect L. asellus to be particularly prone to shell damage under acidified conditions. Our experiment did not directly address shell mineralisation or strength, but the lack of apparent dissolution around the vulnerable aesthete pores is nevertheless an interesting negative result.

The order Lepidopleurida contains predominantly deep-sea species, found worldwide and ranging to depths as low as 7000 m (Kaas and van Belle 1985). Leptochiton asellus is broadly distributed across the NE Atlantic and can occur in the shallow subtidal where short term thermal excursions could reach 20 °C. However, the elevated temperature used here (20 °C) is potentially above the natural conditions experienced by the population sampled from a thermally stable Swedish fjord. The specimens in this study maintained normal shell and radular structures in altered temperature as well as pH. This suggests that any increase in metabolic activity and feeding with higher temperature was compensated by increased radular growth.

The experimental subjects here were housed in conditions with natural substrate and abundant available food (diatoms and biofilm presumed to be available on the aquarium substrate, taken from their native habitat). Other studies have also shown that animals' ability to compensate for abiotic stress and maintain normal physiological parameters is enhanced when the animals are well fed (Thomsen et al., 2013).

The results of this study are at odds with the one other published report of radular integrity in experimental ocean acidification (Marchant et al., 2010). Results from the radula of P. vulgata, were used to tentatively suggest the damage could indicate a trade-off of decreased radular replacement with increased general metabolic demand to maintain extracellular acid-base balance (Marchant et al., 2010). Evidence for similar trade-offs and compensatory mechanisms have been observed in several marine species (Stumpp et al., 2012; Collard et al., 2013; Timmins-Schiffman et al., 2013). Within chitons specifically there is good evidence that basic metabolism is variable among species (Carey et al. 2013). Clearly hypercapnia imposes additional or altered metabolic demands on animals even under laboratory acclimation irrespective of specific morphological changes tested herein. The physiological mechanisms underlying the compensation to abiotic change remain unknown. L. asellus belongs to a lineage that has persisted for over 300 million years and may be able to overcome even anthropogenic climate change. But results indicating robustness to particular stressors are important in informing predictions of the impacts of ongoing climate change.

Acknowledgements

References

Beniash, E., Ivanina, A.V., Lieb, N.S., et al., 2010. Elevated level of carbon dioxide affects metabolism and shell formation in oysters *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 419, 95–108 http://dx.doi.org/10.3354/meps08841.

Brooker, L.R., Lee, A.P., Macey, D.J., 2003. Multiple-front iron-mineralisation in chiton teeth (*Acanthopleura echinata*: Mollusca: Polyplacophora). Mar. Biol. 142, 447–454.

Burnell, O.W., Russell, B.D., Irving, A.D., Connell, S.D., 2013. Eutrophication offsets increased sea urchin grazing on seagrass caused by ocean warming and acidification. Mar. Ecol. Prog. Ser. 485, 37–46 http://dx.doi.org/10.3354/meps10323.

Collard, M., Laitat, K., Moulin, L., et al., 2013. Buffer capacity of the coelomic fluid in echinoderms. Comp. Biochem. Physiol. Part A 166, 199–206.

Comeau, S., Jeffree, R., Teyssié, J.-L., Gattuso, J.-P., 2010. Response of the Arctic pteropod *Limacina helicina* to projected future environmental conditions. PLoS One 5, e11362. http://dx.doi.org/10.1371/journal.pone.0011362.

Gazeau, F., Gattuso, J.-P., Dawber, C., et al., 2010. Effect of ocean acidification on the early life stages of the blue mussel (*Mytilus edulis*). Biogeosciences 7, 2051–2060.

Gazeau, F., Parker, L.M., Comeau, S., et al., 2013. Impacts of ocean acidification on marine shelled molluscs. Mar. Biol. 160, 2207–2245.

Gordon, L.M., Joester, D., 2011. Nanoscale chemical tomography of buried organic-inorganic interfaces in the chiton tooth. Nature 469, 194–197. http://dx.doi.org/10.1038/nature09686.

Kirschvink, J.L., Lowenstam, H.A., 1979. Mineralization and magnetization of chiton teeth: paleomagnetic, sedimentologic, and biologic implications of organic magnetite. Earth Planet. Sci. Lett. 44, 193–204.

Kroeker, K.J., Kordas, R.L., Crim, R., et al., 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Glob. Change Biol. 19, 1884–1896.

Lee, A.P., Brooker, L.R., Macey, D.J., et al., 2000. Apatite mineralization in teeth of the chiton *Acanthopleura echinata*. Calcif. Tissue Int. 67, 408–415.

Lowenstam, H.A., 1962. Magnetite in denticle capping in Recent chitons (Polyplacophora). Geol. Soc. Am. Bull. 73, 435–438.

Lowenstam, H.A., Weiner, S., 1989. On Biomineralisation. Oxford University Press, Oxford, UK.

Marchant, H.K., Calosi, P., Spicer, J.I., 2010. Short-term exposure to hypercapnia does not compromise feeding, acid-base balance or respiration of *Patella vulgata* but surprisingly is accompanied by radula damage. J. Mar. Biol. Assoc. U. K. 90, 1379–1384 http://dx.doi.org/10.1017/S0025315410000457.

Macey, D.J., Brooker, L.R., 1996. The Junction zone: initial site of mineralization in radula teeth of the chiton *Cryptoplax striata* (Mollusca: Polyplacophora). J. Morphol. 230, 33–42.

Mucci, A., 1983. The solubility of calcite and aragonite in seawater at various salinities, temperatures and 1 atmosphere total pressure. Am. J. Sci. 238, 780–799.

O'Connor, M.I., 2009. Warming strengthens an herbivore-plant interaction. Ecology 90, 388–398.

O'Donnell, M.J., George, M.N., Carrington, E., 2013. Mussel byssus attachment weakened by ocean acidification. Nat. Clim. Change 3, 587–590. http://dx.doi.org/10.1038/nclimate1846.

Rodolfo-Metalpa, R., Houlbrèque, F., Tambutté, É., et al., 2011. Coral and mollusc resistance to ocean acidification adversely affected by warming. Nat. Clim. Change 1, 308–312. http://dx.doi.org/10.1038/nclimate1200.

Runnegar, B., 1989. The evolution of mineral skeletons. In: Crick, R.E. (Ed.), Origin, Evolution, and Modern Aspects of Biomineralization in Plants and animals. Plenum Press, New York, pp. 75–94.

Stumpp, M., Trübenbacha, K., Brenneckea, D., et al., 2012. Resource allocation and extracellular acid–base status in the sea urchin *Strongylocentrotus droebachiensis* in response to CO_2 induced seawater acidification. Aquat. Toxicol. 110–111, 194–207.

Timmins-Schiffman, E., O'Donnell, M.J., Friedman, C.S., Roberts, S.B., 2013. Elevated pCO_2 causes developmental delay in early larval Pacific oysters, *Crassostrea gigas*. Mar. Biol. 160, 1973–1982.

Thomsen, J., Casties, I., Pansch, C., et al., 2013. Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field experiments. Glob. Change Biol. 19, 1017–1027 http://dx.doi.org/10.1111/gcb.12109.

Wang, Q., Nemoto, M., Li, D., et al., 2013. Phase transformations and structural developments in the radular teeth of *Cryptochiton stelleri*. Adv. Funct. Mater. http://dx.doi.org/10.1002/adfm.201202894.