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Trace elements in shells of common gastropods in the near vicinity of a natural CO_2 vent: no evidence of pH-dependent contamination

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Abstract

There is concern that the use of natural volcanic CO_2 vents as analogs for studies of the impacts of ocean acidification on marine organisms are biased due to physio-chemical influences other than seawater pH alone. One issue that has been raised is whether potentially harmful trace elements in sediments that are rendered more soluble and labile in low pH environments are made more bioavailable, and sequestered in the local flora and fauna at harmful levels. In order to evaluate this hypothesis, we analyzed the concentrations of trace elements in shells (an established proxy for tissues) of four species of gastropods (two limpets, a topshell and a whelk) collected from three sites in Levante Bay, Vulcano Island. Each sampling site increased in distance from the primary CO_2 vent and thus represented low, moderate, and ambient seawater pH conditions. Concentrations of As, Cd, Co, Cr, Hg, Mo, Ni, Pb, and V measured in shells using ICP-OES were below detection thresholds for all four gastropod species at all three sites. However, there were measurable concentrations of Sr, Mn, and U in the shells of the limpets *Patella caerulea*, *P. rustica*, and the snail *Osilinus turbinatus*, and similarly, Sr, Mn, U, and also Zn in the shells of the whelk *Hexaplex trunculus*. Levels of these elements were within the ranges measured in gastropod shells in non-polluted environments, and with the exception of U in the shells of *P. caerulea*, where the concentration was significantly lower at the collecting site closest to the vent (low pH site), there were no site-specific spatial differences in concentrations for any of the trace elements in shells. Thus trace element enhancement in sediments in low-pH environments was not reflected in greater bioaccumulations of potentially harmful elements in the shells of common gastropods.

1 Introduction

Marine ecosystems are increasingly subject to the influence of seawater pH reduction and alterations in carbonate chemistry resulting from oceanic absorption of anthropogenic CO_2 – a process known as ocean acidification (Feely et al., 2004; Orr et al., 2005). In efforts to understand the implications of these rapid changes on marine organisms, shallow submarine volcanic vents have become in situ models or analogues for studying the prospective impacts of ocean acidification on marine organisms (Hall-Spencer et al., 2008). The seawater released from submarine vents can vary in its chemical and physical properties (reviewed in Dekov and Savelli, 2004). Usually, the volcanic vent waters are acidic, reducing, and levels of metals are higher than those in the surrounding seawater (Dando et al., 1999). Moreover, the acidity of vent water increases the solubility, and thus, potentially, the bioavailability and toxicity of trace elements in seafloor in sediments surrounding the region of the vent (Kadar et al., 2007; Roberts et al., 2013).

In a recent related study, Vizzini et al. (2013) determined levels of trace elements in seafloor sediments and in the tissues of a local seagrass (*Cymodocea nodosa*) and its epiphytes across a spatial pH gradient in the near vicinity of a volcanic vent in Levante Bay on Vulcano Island in northeast Sicily, Italy. Their study was important because this particular vent system has become a popular analogue for numerous studies of ocean acidification (e.g., recently; Arnold et al., 2012; Lidbury et al., 2012; Kadar et al., 2012; Kerfahi et al., 2014; Milazzo et al., 2014). Employing several standard indices of trace element pollution, Vizzini et al. (2013) concluded that the bay is affected by a low contamination of trace elements in the sediments (and by extension the sediment–water interface) with the potential for adverse biological impacts – especially within a zone from approximately 150 to 350 m from the primary vent. Their analysis of levels of trace elements in a local common species of seagrass harboring epiphytes, and also in epiphytes separately, indicated that while levels of trace elements generally fell within the lower range of values detected in seagrasses in general, there was localized bioenrichment in three trace elements (Cd, Hg, and Zn) in both seagrass harboring epiphytes (300 m distant from the vent) and epiphytes alone (both at sites 227 and 500 m distant from the vent).

The purpose of the present study was to extend the analysis of the potential for bioaccumulation of trace elements in common ecologically-important benthic marine organisms that occur in the near vicinity of the vent system in Levante Bay on Vulcano Island. Among the most conspicuous macroinvertebrates represented in the rocky shore community are gastropods, and in particular, two species of limpets and one species of topshell and whelk. The limpets *Patella caerulea* (Linnaeus, 1758) and *P. rustica* (= textitlusitanica) (Linnaeus, 1758) and the topshell snail *Osilinus turbinatus* (Von Born, 1778) are abundant throughout the Mediterranean Sea on rocky shores in the intertidal and upper subtidal zones. They mainly graze on epilithic and epiphytic biofilms (i.e., bacteria, microalgae, macroalgal sporelings, protozoans, and detritus) (Della Santina et al., 1993; Bulleri et al., 2000; Crothers, 2001). A strong homing behaviour is well documented for the limpets, which often return to the same spot after eating, using sensitive chemoreceptors (Keasar and Safriel, 1994). The predatory muricid whelk *Hexaplex trunculus* (Linnaeus, 1758) is a more mobile gastropod species, generally found subtidally in shallow sheltered habitats or in deeper water on wave-exposed shores on rocky or sandy bottoms. *Hexaplex trunculus*, a commercially important delicacy, mainly feeds on barnacles, bivalves and gastropods (Sawyer et al., 2009), but is able to supplement its diet by scavenging and cannibalism (Rilov et al., 2004).

As the shells of gastropods have been validated as bioindicators of trace elements (Pearce and Mann, 2006; Bellotto and Miekeley, 2007; Pourang et al., 2014), we chose to evaluate levels and spatial patterns of trace elements in the shells of the four species of common gastropods. Our analysis facilitated an evaluation of whether there is evidence of elevated bioaccumulation of trace elements in common gastropods living in low-pH waters in close proximity to the primary vent.

2 Materials and methods

2.1 Gastropod collection and collecting sites

Collections took place in the near vicinity of the CO_2 vent system in Levante Bay, Vulcano Island, Sicily, Italy. Levante Bay is a shallow (2–3 m) microtidal region with well-defined carbonate chemistry (Johnson et al., 2013; Boatta et al., 2013; Milazzo et al., 2014). The volcanic activity of the vent acidifies the surrounding seawaters creating a pH gradient ranging from 6.8 to 8.2.

Adult individuals of four common gastropod species (two species of limpets and one species of topshell and whelk) were collected by hand in May 2013. Collections took place at three previously established sites (S1–S3) within the bay, each decreasing in distance from the primary vent. A map showing the collecting area and additional details about the three stations sampled in the present study is given in Johnson et al. (2013). Station 1 (S1) was located farthest from the vent (850 m distant; 38°25.184′ N; 14°57.696′ E) and represented an ambient or control pH (mean 8.18) and CO_2 level; Station 2 (S2) was closer to the vent (390 m distant; 38°25.193′ N; 14°57.763′ E) and represented an intermediate pH (mean 8.05) and CO_2 level which is forecast for approximately the middle of the century (Nakicenovic and Swart, 2000); Station 3 (S3) was located closest to the vent (300 m distant; 38°25.248′ N; 14°57.853′ E) and represented a low pH (mean 7.49) with a CO_2 level expected in the distant future. All three stations experience a stable, ambient temperature and salinity, but mean surface seawater pH variability increases with increasing proximity to the CO_2 seeps, likely varying due to changes in wind-driven currents (Boatta et al., 2013). These variability patterns in carbonate chemistry are typical of other CO_2 vent sites (Hofmann et al., 2011; Fabricius et al., 2011). Moreover, while the vent also releases some sulfur, it is localized and undetectable at the three sampling stations (Boatta et al., 2013).

Whole collected animals were placed in zip-lock bags and shipped to the University of Alabama at Birmingham, USA where they were frozen whole on arrival, then immediately dissected to remove all tissues and the shells stored frozen. Shells of five adult individuals of each of the four species of gastropod were haphazardly selected and soaked in a 10 % sodium hypochlorite (Chlorox bleach) solution for a period of several days to digest away all organic material (McClintock et al., 2011). The weak solution was exchanged daily as needed to promote digestion. Clean shells were generously rinsed with diH_2O and allowed to air dry to constant weight. Dried shells were wrapped in sterile gauze and then cracked with a hammer before being ground into a fine powder with an agate mortar and pestle.

2.2 Trace elemental analyses

Powdered gastropod shell material was analyzed by ACTLabs in Ontario, Canada (www.actlabs.com). 90–500 mg of each shell sample was digested with aqua regia (1 : 3 M nitric-hydrochloric acid freshly mixed) for 2 h at 95 °C. Samples were then cooled and diluted with diH_2O and levels of elements determined via inductively coupled plasma atomic emission spectroscopy using an PerkinElmer Optima ICP-OES. USGS-geochemical standards served as controls, and along with a blank, were analyzed every thirteen samples. The accuracy with ICP-OES for trace elements was consistently within ±0.01 % of the known value for the standards. Results of the trace element analysis were reported by ACTLabs in ppm and expressed in the present study as μg^{-1} dry wt.

2.3 Statistical analyses

Many of the trace elements in the shells were below detection limits and thus no data were available for analysis. However, for some elements there were measurable values for most, but not all, replicate shells (Table 1). Sophisticated techniques are available for providing estimates of censored data values (those below detection limits) such that parametric tests can be used on these data (e.g. Helsel, 2006). But, sample sizes $> n = 20$ per group are required in order to accurately characterize the distributions from which the data were sampled. Since our sample sizes ($n = 5$ per group) did not meet this requirement, we compared the levels of the trace elements between pH groups (sample sites) using nonparametric Kruskal–Wallis tests. Where a significant difference between groups was observed, pair-wise comparisons between pH groups were done using Dunn's tests (Dunn, 1964).

3 Results

Values of the trace elements As, Cd, Co, Cr, Hg, Mo, Ni, Pb and V in shells of all four gastropod species were less than detection limits of ICP-OES (see http://www.actlabs.com/page.aspx?page=501&app=226&cat1=549&tp=12&lk=no&menu=64&print=yes for list of detection limits for these elements) and thus could not be analyzed statistically. Median levels of Mn, Sr, and U in the shells of the limpets *Patella caerulea*, *P. rustica* and the topshell *Osilinus turbinatus* for the three sites are presented in Figs. 1–3. The levels of these trace elements in shells of all the two species of limpets and the topshell gastropod collectively across all three sites occurred in the order Sr > Mn > U. Median levels of Mn, Zn, Sr, and U in the shells of the whelk *Hexaplex trunculus* for the three samples sites are presented in Fig. 4. The levels of these trace elements collectively in shells of whelks across all three sites occurred in the order Sr > U > Zn > Mn. The only significant difference ($P = 0.027$) in the level of a trace element detected in shells sampled at the three sites occurred for U in the shells of the limpet *P. caerulea*. Here, a significantly higher concentration of U occurred in the shells of individuals collected at sites S1 and S2, the two sites farthest from the CO_2 vent. Levels of Mn in the shells of the whelk *H. trunculus* approached significance ($P = 0.073$) in terms of site-specific differences, yet, even if there had been a significant difference between sites, once again, the levels would have been highest at the site (S1) farthest from the vent and where seawater pH is ambient.

4 Discussion

A variety of trace elements are known, or based on their physical chemistry can be expected to be more soluble in low-pH environments in the proximity of underwater CO_2 vents. For example, Aiuppa et al. (2000) found that the solubility of Fe in the water column increased 40 % when pH decreased from 8.1 to 7.4 in thermal groundwater of Vulcano Island, Italy. Similarly, dissolved Fe was found to occur in higher concentrations in the seawater column near the primary CO_2 vent in Levante Bay, Vulcano Island (Boatta et al., 2013). Vizzini et al. (2013) hypothesize that in addition to Fe, the trace elements Cd, Co, Cr, Cu, Mn and V could be expected to occur in enhanced concentrations dissolved in the water column near CO_2 vents, and as a result, in lower concentrations in near-vent sediments. Other trace elements might be expected to display their highest concentrations in sediments near vents where acidity and reducing conditions are pronounced (Dando et al., 1999). In a detailed analysis of the distribution patterns of trace elements in the sediments of Levante Bay, Vizzini et al. (2013) found that concentrations of As, Ba, Hg, Mo, Ni, Pb, and Zn were generally highest close to the primary CO_2 vent, while Cd, Co, Cr, Cu, Fe, Mn, and V occurred in lower concentrations. Concentrations of several trace elements in the general region of the vent exceeded established known Sediment Quality Guidelines (SQG). For example, Threshold Effects Level (TEL) and Probably Effects Level (PEL) indices (MacDonald et al., 1996) were exceeded for As, Cr, Cu, and Hg, and in certain regions of the bay Ni exceeded the PEL index. Based on sediment quality indices of SQG and MSPI (Marine Sediment Pollution Index, Shin and Lam, 2001), Vizzini et al. (2013) concluded that Levante Bay has a generally low level of trace element contamination with a moderate potential for causing adverse biological impacts. According to the MSPI index, they found the sediments most enriched with trace elements in Levante Bay occurred at a distance approximately 150 to 350 m from the primary CO_2 vent, where the index values fell between average and adverse.

Despite potentially harmful levels of trace elements occurring in sediments within the region of the present study (our sampling site S3 was 300 m distant for the primary vent), we found no spatial pattern of trace element bioaccumulation in shells of any individuals that would suggest CO_2 vent-related contamination. Despite mollusc shells serving as well established proxies for trace element pollutants (Pearce and Shettler, 1994; Pearce and Mann, 2006; Bellotto and Miekeley, 2007; Pouranug et al., 2013), the majority (79 %) of the different trace elements in the shells of the limpets, snail and whelk (i.e., As, Cd, Co, Cr, Hg, Mo, Ni, Pb, V, and Zn), occurred at levels below those detectable using ICP-EOS. Moreover, trace elements that occurred in measurable concentrations in shells, Mn, Sr, and U in the limpets *Patella caerulea* and *P. rustica* and the topshell *Osilinus turbinatus*, and Mn, Sr, U, and Zn in the shells of the whelk *Hexaplex trunculus*, in large part showed no significant differences in concentrations between our three sampling sites. Moreover, in the one gastropod (the limpet *P. caerulea*) that did display a significant site-specific difference in the concentration of a trace element (Mn), the pattern was the opposite of what might have been expected. Here, Mn concentrations in the shells of *P. caerulea* were highest at S1, the sampling site that was most distant (850 m) from the primary vent, and well beyond the 150 to 350 m polluted sediment zone containing elevated trace elements (Vizzini et al., 2013). Finally, none of the trace elements that Vizzini et al. (2013) detected in sediments in Levante Bay at concentrations deemed potentially harmful to marine organisms (As, Cr, Cu, Hg, and especially Ni), were present in any of the shells of the four gastropod species at any of the sampling sites in detectable concentrations.

The shells of all four species of gastropods at all sites had significant concentrations of strontium that ranged from 846 to 1180 $\mu g\, g^{-1}$. Strontium is an alkaline earth metal known to be substituted for calcium in biological systems including shells. There were no site-specific differences between sampling sites and no apparent intraspecific differences in strontium concentrations measured in shells. Levels of strontium have been related to the two polymorphs of calcium carbonate that comprise gastropod shells, with higher strontium concentrations associated with species with shells of aragonitic

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rather than calcitic mineral composition (Cravo et al., 2002). The levels measured in shells in the present study were similar to strontium levels measured in the calcitic shells of the gastropod *Nerita albicilla* (1262 µg g^{-1}) (El Sorogy et al., 2013). Cravo et al. (2002) reported strontium concentrations in the shells of the conspecific limpet *Patella aspera* to be somewhat higher with a concentration of 1318 µg g^{-1}. The genus *Patella* is known to have shells constructed of layers of aragonite and calcite with either polymorph capable of occurring in greatest abundance (Cohen and Branch, 1992; Day et al., 2000), so the use of strontium as a simple correlate of aragonitic or calcitic shell construction is difficult. The shell of topshell *Osilinus turbinatus* is comprised of aragonite (Mannino et al., 2008); the composition the shell of the whelk *Hexaplex trunculus* is bimineralic comprised primarily of aragonite with lesser amounts of calcite (A. Duquette, unpublished data). There is little evidence that strontium bioaccumulates along with toxic metals in polluted marine environments (El-Sorogy et al., 2013).

Concentrations of manganese in the shells of the limpets *Patella caerulea* and *P. rustica* were highly variable (12 to 41 µg g^{-1}) and median values by site did not vary significantly. Levels of manganese were similar to those measured in the shells of the conspecific *P. aspera* (30 µg g^{-1}; Cravo et al., 2002). Median levels of manganese in the shells of the topshell *Osilinus turbinatus* and the whelk *Hexaplex trunculus* were lower (2.5 to 17 µg g^{-1}) than those measured in the shells of limpets. Inter-site levels of manganese in the shells of *H. trunculus* approached significance ($P = 0.073$), yet even assuming a significant difference and that manganese levels in the soft tissues of *H. trunculus* have been shown to increase with increased levels in sediments (María-Cervantes et al., 2009), the concentration of manganese was highest at the site furthest from the primary CO_2 vent. Levels of manganese in the gastropod shells in the present study generally fell within the maximum values measured in the shells of the gastropods *Nerita albicilla* (23 µg g^{-1}) and *Canarium gibbosus* (16 µg g^{-1}) on the coast of the Red Sea (El-Sorogy et al., 2013). Manganese is an essential trace element in biological systems that can be toxic at high concentrations. Ambient manganese concentrations in soil and in surface sediments in intertidal mud flats can range from 300 to

600 and from 100 to 1000 µg g^{-1}, respectively, and in seaweeds and shellfish from 130 to 755 and 3 to 660 µg g^{-1}, respectively (reviewed in Howe et al., 2004). Concentrations measured in the shells of the four gastropods in the present study across all three sites are well below manganese concentrations measured in the surrounding sediments of Levante Bay (89–945 µg g^{-1}; Vizzini et al., 2013). Vizzini et al. (2013) found that mean bioconcentrations of manganese in the leaves and the epiphytes of the seagrass *Cymodocea nodosa* in Levante Bay ranged from 99 to 474 and from 108 to 422 µg g^{-1}, respectively. These values are consistently higher than those measured in gastropod shells and this difference likely reflects the closer interaction that seagrasses and their associated epiphytes have with the water–sediment interface where dissolved metals occur in higher concentration.

The shells of all four species of gastropods at all three sites in Levante Bay contained uranium at median concentrations that ranged from 5 to 11 µg g^{-1} (all sites except one had median values of either 10 or 11 µg g^{-1}). There were no site-specific differences. The median value at the low end of the range of uranium concentrations measured occurred in shells of the limpet *Patella caerulea* at the site closest to the primary vent. The levels of uranium measured in the present study were four-fold to two orders of magnitude higher than levels detected in shells of the gastropods *Canarium gibbosus* (0.1–2.3 µg g^{-1}) and *Nerita albicilla* (0.1 µg g^{-1}) from various sites along the coast of the Red Sea (El-Sorogy et al., 2013). Vizzini et al. (2013) did not measure uranium in the sediments of Levante Bay so it is not possible to compare our shell values with the surrounding sediment concentrations. Elevated levels of uranium in sediments can be indicative of suboxic or anoxic bottom water (Jones and Manning, 1994) or high levels of organic matter (Spirakis, 1996).

Interestingly, Zn was present in the shells of the whelk *Hexaplex trunculus* at all three sampling sites (range = 8–12 µg g^{-1}) and yet undetectable in the shells of the other three gastropods. Levels of zinc in shells of *H. trunculus* were an order of magnitude lower than those in shells of bivalves from a polluted region of the coastal Red Sea (range = 20–174 µg g^{-1}; Ziko et al., 2001) and below the range of levels measured for sediments in Levante Bay (range = 18–48 µg g^{-1}; Vizzini et al., 2013). These levels were also at least two orders of magnitude lower than those measured in the soft tissues of individuals of *H. trunculus* living in a coastal lagoon in the Mediterranean heavily polluted by local mining operations (María-Cervantes et al., 2009). Levels of zinc in highly polluted sediments such as Deep Bay, Hong Kong can occur at concentrations as high as 240 µg g^{-1} (Tam and Wong, 2000). Toxic levels of zinc in marine sediments have been calculated using several common pollution metrics (CCME 1999) including the Interim Sediment Quality Guidelines (ISQG) (124 µg g^{-1}) and the PEL (271 µg g^{-1}). Both values are over one fold above those measured in gastropod shells in the present study. Zinc is a biologically essential metal that can accumulate either without excretion, or with excretion from a detoxified store or a pool of active metabolites (Rainbow, 2002). Zinc becomes more labile and therefore potentially more toxic to organisms when contaminated sediments are treated with elevated $p\mathrm{CO_2}$ simulating ocean acidification (Roberts et al., 2013). Accordingly, Vizzini et al. (2013) detected the highest concentrations of zinc in sediments sampled nearest the primary $\mathrm{CO_2}$ vent in Levante Bay. Populations of *H. trunculus* living in Levante Bay occur in association with sediments (McClintock and Amsler, personal communication, 2013), in contrast to the limpets and snail that are found in association with hard substrata. Other studies have suggested that differences in shell trace elements may reflect the habitat selection (living in association therein or consumption of sediment-associated prey) as it relates to the water–sediment interface where trace metal accumulation is greater (Campell and Tessier, 1996; see El-Sorogy et al., 2013). Living in direct contact with the sediment–water interface may explain why whelks in the present study bioaccumulate zinc in their shells. However, despite this accumulation, there was no pattern of increasing concentration of zinc in shells of individuals living closest to the primary vent site.

The present study does not allow an evaluation of whether soft tissue concentrations of a given trace element may have been higher than those levels bioaccumulated in the shells of gastropods (e.g. Belotto and Miekeley (2007) for mussels; Palpandi and Kesavan (2012) for gastropods). However, there are good reasons to use shells as proxies of pollutants other than ease of collection, processing, and information that can be gleaned from shell size and weight (Palpandi and Kesavan, 2012). The composition of mollusc shells is tightly correlated with the chemical minerals accumulated from the environment (Carel et al., 1987), and studies have shown that shells rather than soft tissues provide better measures of spatial patterns of trace metal pollutants (Pourang et al., 2014 and references therein). As the intent of the present study was to evaluate the hypothesis that common gastropods living in low-pH waters near the primary vent in Levante Bay bioaccumulate potentially harmful trace elements to a greater extent than those in conspecifics in neighboring ambient pH environments, our analysis of trace element contamination focused on shells. We found no evidence that any of fourteen common trace elements are bioaccumulated at higher concentrations in shells of a suite of the most common gastropods living in the vicinity of a CO_2 vent Levante Bay, Vulcano Island, in the Mediterranean Sea. Nonetheless, it is reasonable to expect that there may be negative impacts of trace element bioaccumulation on fauna that live within the sediments in Levante Bay and other marine CO_2 vent ecosystems (Widdicombe et al., 2011).

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Table 1. Numbers of each species at different pH levels that were analyzed for the metals shown. Given before the slash is the number of individuals $\geq$ the detection limit. Given after the slash is the number $<$ the detection limit.

	pH*	Mn	Zn	Sr	U
P. caerulea	H	5/0	1/4	5/0	5/0
P. caerulea	M	5/0	0/5	5/0	5/0
P. caerulea	L	5/0	0/5	5/0	2/3
P. rustica	H	5/0	0/5	5/0	5/0
P. rustica	M	5/0	0/5	5/0	3/2
P. rustica	L	5/0	0/5	5/0	5/0
H. truncatus	H	5/0	5/0	5/0	5/0
H. truncatus	M	4/1	5/0	5/0	5/0
H. truncatus	L	4/1	5/0	5/0	4/1
O. turbinatus	H	4/1	0/5	5/0	4/1
O. turbinatus	M	4/1	0/5	5/0	5/0
O. turbinatus	L	1/4	1/4	5/0	4/1

* H = high, M = medium, L = low.

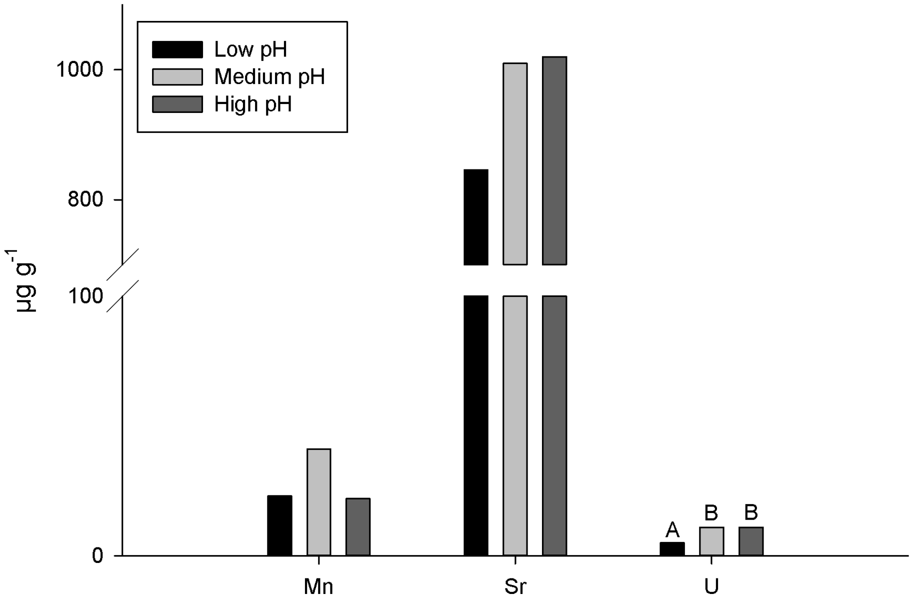

Fig. 1. Median levels of Mn, Sr, and U in the shells the limpet *Patella caerulea* at the three sample sites ($n = 5$ per site). Medians labelled with different letters differ significantly (Kruskal–Wallis test followed by Dunn's pairwise tests, $p < 0.05$).

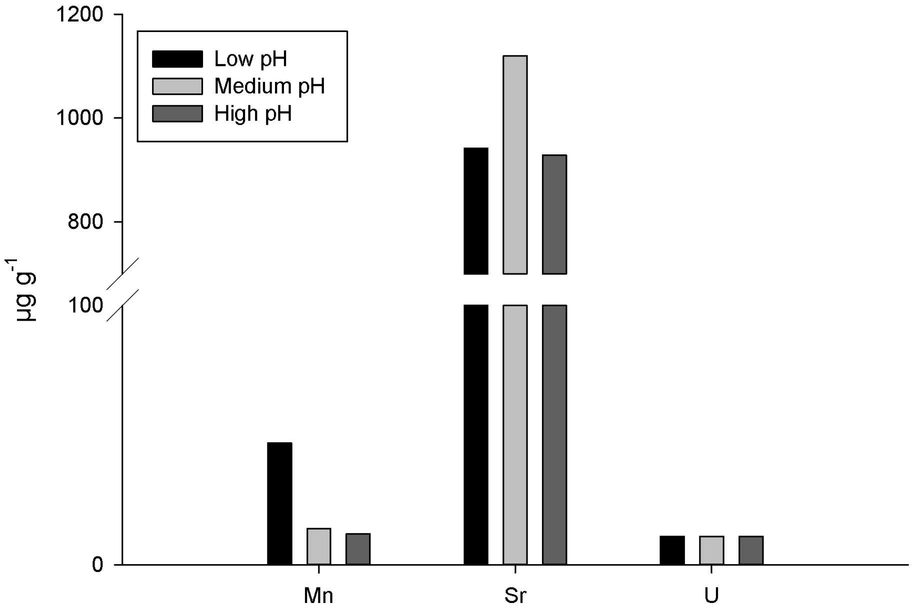

Fig. 2. Median levels of Mn, Sr, and U in the shells the limpet *Patella rustica* at the three sample sites ($n = 5$ per site).

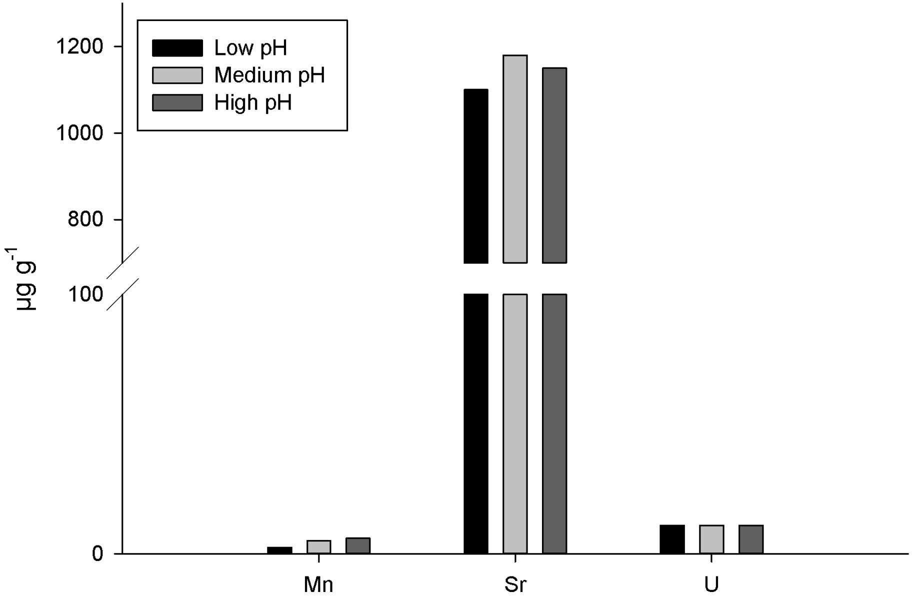

Fig. 3. Median levels of Mn, Sr, and U in the shells the topshell *Osilatus turbinatus* at the three sample sites ($n = 5$ per site).

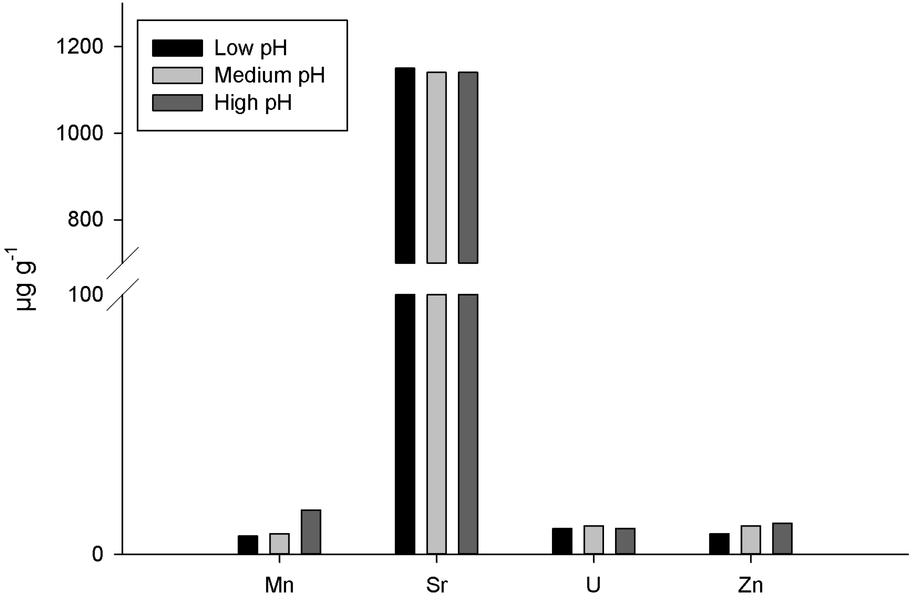

Fig. 4. Median levels of Mn, Sr, U, and Zn in the shells the whelk *Hexaplex trunculus* at the three sample sites ($n = 5$ per site).