A preliminary study of iron isotope fractionation in marine invertebrates (chiton, mollusca) in near shore environments

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1. INTRODUCTION

Iron plays a critical role in controlling biological productivity in the oceans (Martin et al., 1990; De Baar et al., 1995; Coale et al., 1996), and understanding the biogeochemical cycling of Fe is therefore key in reconstructing the history of life on Earth. One potentially rewarding way to reconstruct past marine conditions is to examine variations in the isotopic signature of iron. Changes to Fe isotope ratios occur due to shifts in redox state, chemical bonding environment, adsorption properties, and microbial and organic-ligand bonding processes (e.g., Matthews et al., 2001; Zhu et al., 2002; Beard et al., 2003a,b; Brantley et al., 2004; Croal et al., 2004; Welch et al., 2003; Johnson et al., 2005; Teutsch et al., 2005; Crosby et al., 2007; Matthews et al., 2008), and precise measurements of these isotopes could yield vital information about geochemical and ecological conditions in both present day and past environments.

While studies have examined isotopic variations of Fe in marine rocks (e.g., Matthews et al. 2004; Staubwasser et al., 2006; Severmann et al., 2006), marine organisms that accumulate significant amounts of Fe could also prove to be good environmental recorders. One group of marine molluscs that might fulfill this role is chitons (Figure 1a-b). Belonging to the class Polyplacophora, these molluscs graze on algae on the surface of rocks and other hard substrates in the near shore coastal environment using radula (or rasping tongue) made up of teeth impregnated with magnetite and other iron bearing minerals, such as ferrihydrite, goethite, and lepidocrocite (e.g., Lowenstam, 1962a; Towe and Lowenstam, 1967; Lowenstam and Kirschvink, 1996; Lowenstam and Weiner, 1989). Due to their high level of iron accumulation, the Fe isotopic signature of modern chiton radula might be expected to reflect ambient oceanic environments.

However, a number of factors may influence the isotopic composition of Fe accumulated in chiton teeth at any given location. Being primarily herbivorous, they extract nutrients from marine algae, which in turn absorb nutrients directly from seawater. As the

isotopic composition of Fe in seawater can vary spatially due to variations in the relative contributions of different sources, including continental runoff, aerosols, hydrothermal fluids, and oceanic crust alteration (Sharma et al., 2001; Anbar & Rouxel, 2007; Johnson et al., 2008; Homoky et al., 2012), the isotopic value recorded in invertebrate teeth could therefore change with geographical location. In addition, utilization by marine organisms and associated biological fractionation may also play an important role in determining Fe isotope compositions. Bacteria are known to form isotopically light magnetite during dissimilatory microbial reduction of Fe(III) oxyhydroxides (Johnson et al., 2005); other organisms, such as algae and even the chitons themselves, could also fractionate Fe isotopes as a result of biomineralization processes. Although Fe isotope signatures in higher organisms have been studied (e.g., Walczyk & von Blanckenburg, 2002; Hotz, 2011), little is currently known about the natural variation of metal isotopes in marine invertebrates or the influence that biological fractionation and environmental factors, such as geographical location and diet, may have on those signatures.

Here, in a preliminary study, we examine Fe isotopes in modern marine chitons collected from different locations in the Atlantic and Pacific oceans to determine the range of isotopic values that might be encountered and whether or not these isotopic signatures reflect seawater values. Furthermore, by comparing two different species that were collected from the same geographical location but have very different feeding habits, we make a *first* attempt to isolate the potential impact of diet on metal isotopic signatures. While our findings are not definitive, the small new dataset sheds light on the possible pathways of Fe biogeochemical cycling in near-shore environments, highlighting important new directions for future research.

2. METHODS

Ideally, chiton samples would have been obtained from a field campaign that collected specimens from different locations around the world. However, in this preliminary study such

an approach was not feasible, and instead samples were selected from the collections at the Peabody Museum of Natural History at Yale University. The samples were collected in the early 1900's and preserved in formalin, which primarily acts as an antimicrobial agent; although the effect of prolonged exposure of Fe oxides to formalin is not known, we assume no mineralogical or isotopic changes to have occurred in the samples. A total of 24 individual chiton specimens representing 5 different species from 4 different geographical locations were selected for analysis. A summary of the samples is given in Table 1. To represent high and low latitude sites from the Atlantic Ocean, chitons from Bermuda and New Brunswick, Canada, were sampled; from the Pacific Ocean, samples from Panama and Washington State, USA, were selected. In addition, from the Washington locality, two different species – *Tonicella lineata* and *Mopalia muscosa* that feed on predominantly green algae and red algae respectively – were selected for comparison. Of the 5 species investigated in this study, 3 inhabit the eulittoral (intertidal) zone, while 2 are found in the sublittoral (neritic zone). The eulittoral zone is characterised by tidal activity and extends from the low tide line to the high tide line leading to periodic dry and flood periods. The sublittoral zone starts immediately below the eulittoral zone and is permanently underwater. Sunlight penetrates to the seafloor in the eulittoral zone so that both the eulittoral and sublittoral zones are within the photic zone.

The protocol for sample preparation involved dissection of the chitons to extract the radula sac containing the magnetite-capped teeth; a magnetic separation technique was used to separate the radula from the organic matter. A single radula is made of two symmetric rows of teeth (Figure 1a). The total number and size of teeth of each radula can vary depending on the species. Here, each isotopic analysis (Table 1) represents a homogenised sample comprising all teeth of a complete radula for each individual specimen. Due to the small size of the radula for *Tonicella marmorea* from New Brunswick, the teeth from 8 individual

specimens were combined and homogenized to produce one isotopic measurement. One sample (YPM12739-16) was processed in duplicate, and a total of 18 values are reported here.

After separation, the radula were then processed in a clean room facility, where they were digested using ultrapure concentrated HCl; hydrogen peroxide was also added to remove any residual organic material. The digested sample solution was evaporated on a hot plate and re-dissolved in 6M HCl and then passed through chromatographic columns to isolate Fe (Zhu et al., 2002; Archer & Vance, 2004). Purity of samples and quantitative recovery of iron after the column separation procedure was verified by inductively coupled plasma - mass spectrometry (ICP-MS; Agilent 7500cx) analyses. Total Fe amounts ranged from 30 μg to 840 μg. Purity of Fe analyte solutions was found to be better than 99%, which is sufficient for accurate Fe isotope analyses using the method described below (Schoenberg and von Blanckenburg, 2005). Noteworthy, efficient separation of Cr and Ni from Fe was achieved, eliminating spectral interferences of ^{54}Cr on ^{54}Fe and ^{58}Ni on ^{58}Fe during mass spectrometric measurements of Fe isotope ratios. The procedure was also tested by processing the reference material IRMM-014 repeatedly through the same chromatographic separation protocol as the samples. This method yielded a δ^{56}Fe value for IRMM-014 of -0.03 ± 0.02 (2SE, n=16), which is identical with the unprocessed IRMM-014, within the external uncertainty of the method. Prior to isotope analysis, samples were dissolved in 0.3M HNO_3 and diluted to about 2 μg/ml Fe, matching the ion beam intensities (~20 V on ^{56}Fe; $10^{11}\Omega$ amplifier, H cones) of the bracketing standard (IRMM-014) within 10%. The Fe isotopic analyses were performed on a total set of 18 chiton samples using a *Thermo Scientific Neptune* multi collector inductively coupled plasma mass spectrometer (MC-ICP-MS) at GFZ Potsdam in Germany. The mass spectrometer is equipped with a *Neptune Plus Jet Interface Pump* and an *ESI Apex-Q* desolvating system (without membrane) with a ~ 50 μl/min PFA nebuliser for sample introduction. Iron isotope analyses were performed in ‘medium’ mass resolution mode (mass

resolving power m/Δm (5%, 95%) > 7600) to resolve all Fe isotopes from polyatomic interferences (mainly ArO, ArOH, and ArN, see Weyer and Schwieters, 2003, for details). Potential interferences from of ^{54}Cr on ^{54}Fe and ^{58}Ni on ^{58}Fe were monitored at masses ^{52}Cr and ^{60}Ni and corrections to Fe isotope ratios were made according to the method described in Schoenberg and von Blanckenburg (2005). In this study corrections made to the data are insignificant compared to the analytical uncertainty, due to the low impurity levels of Cr and Ni, i.e., ^{54}Cr/^{54}Fe < 0.005‰ and ^{58}Ni/^{58}Fe < 0.5%. The sample-standard bracketing method was used for mass bias correction (using IRMM-014 as bracketing standard), following the measurement procedure and data acceptance criteria of Schoenberg & von Blanckenburg (2005), and results are reported relative to the international reference material IRMM-014 using the delta notation:

$$\delta^{56}\mathrm{Fe} = \left(\frac{\left[{}^{56}\mathrm{Fe} / {}^{54}\mathrm{Fe} \right]_{\mathrm{sample}}}{\left[{}^{56}\mathrm{Fe} / {}^{54}\mathrm{Fe} \right]_{\mathrm{standard}}} - 1 \right) \times 1000 \quad .$$

Between 4 and 8 repeat measurements of each purified sample solution were performed in 2 or 3 independent analytical sessions; the mean δ-value of *n* replicates is reported in Table 1 together with the 95% confidence interval (2SE = $t \cdot \mathrm{SD}/\sqrt{n}$, with t = correction factor for small numbers of *n* from Student's t-distribution at 95% probability). For data quality control, measurement accuracy and precision was assessed by repeated analyses of an in-house working standard (HanFe: pure Fe solution used as control standard) in each analytical session, and four aliquots of the reference material IRMM-014 (δ^{56}Fe ≡ 0 ‰) were independently processed through the same chromatographic separation protocol as the samples. The uncertainty associated with the Fe separation and isotope analysis of IRMM-14 with δ^{56}Fe = -0.03 ± 0.05‰ (2σ = 2 standard deviation of the mean) and δ^{57}Fe = -0.04 ± 0.08‰ (2σ) agrees well with the mass spectrometric repeatability estimated over the course of this study from the HanFe standard with δ^{56}Fe = +0.27 ± 0.05‰ (2σ, *n*=59) and δ^{57}Fe of

+0.39 ± 0.08‰ (2σ), and from the dataset of the 18 investigated chiton teeth samples (Σn_i = 91 measured δ-values) according to $2 \cdot \sqrt{\{[\Sigma (x_i - x_{j\text{-}mean})^2] / [\Sigma (n_{i,j} - 1)]\}}$, for the jth chiton sample having a mean isotope composition $x_{j\text{-}mean}$ determined from i replicate analyses x_i, yielding ± 0.05 (2σ) and ± 0.08 (2σ) for δ^{56}Fe and δ^{57}Fe, respectively. Hence, the overall uncertainty estimate of the reported δ^{56}Fe and δ^{57}Fe values is ± 0.05‰ (2σ) and ± 0.08‰ (2σ), respectively.

3. RESULTS AND DISCUSSION

3.1 Fe isotopic measurements in chiton teeth

The δ^{56}Fe values measured in the samples cover a wide range, varying from -1.90‰ to 0.00‰ (Figure 2). Although the overall range is quite large, the chiton specimens from each of the different regions cluster reasonably close together, with each chiton group possessing a distinct isotopic composition: *Chiton tuberculatus* from the sub-tropical north Atlantic (Bermuda) has a mean Fe isotope signature of δ^{56}Fe = -0.23 ± 0.32‰ (2σ, 3 specimens), while the value for *Tonicella marmorea* from the North Atlantic (Grand Manan Island, New Brunswick, Canada) is -1.10‰. *Chiton stokessi* from the south Pacific (Panama) has a mean δ^{56}Fe value of -1.09 ± 0.44‰ (2σ, 5 specimens), while *Tonicella lineata* and *Mopalia muscosa* from the north Pacific (Puget Sound, Washington) possess mean δ^{56}Fe values of -0.65 ± 0.26‰ (2σ, 8 specimens) and -1.47 ± 0.98‰ (2σ, 5 specimens), respectively,

Such large variation in isotopic signatures between the chitons in the different locations might be expected given the widely varying δ^{56}Fe values reported for dissolved Fe (filtered <0.45 µm) in seawater in different oceans. Isotopically heavy values in δ^{56}Fe from +0.01‰ to +0.58‰ have been measured at different locations in the Pacific Ocean (Lacan et al., 2010; Radic et al., 2011). At different depths too within the water column, significant variations in Fe isotope compositions have been reported in the Pacific Ocean: in the San

Pedro Basin in the North Pacific, δ^{56}Fe values ranged from 0.00‰ at the surface to extremely negative values of -1.82‰ at a depth of 900 m. Large variations have also been reported in the Atlantic Ocean: δ^{56}Fe values in the range from -0.14‰ to +0.23‰ have been reported for the Atlantic Section of the Southern Ocean (Lacan et al., 2008, 2010), while values of -0.13‰ to 0.27‰ have been measured in the South East Atlantic (Lacan et al., 2010); in the North Atlantic δ^{56}Fe values varying between +0.30‰ to +0.71‰ have been reported in some studies (John and Adkins, 2010; John and Adkins, 2012; Lacan et al., 2010), while off the north-eastern coast of North America isotopic signatures in the range -0.90‰ to +0.10‰ have also been reported (Rouxel & Auro, 2010). Such geographical dependence of seawater isotopic signatures is generally thought to be due to changes in the balance of different inputs and the influence of utilisation of Fe as a nutrient by marine organisms (e.g, Radic et al., 2011). Negative seawater values could be due to dissimilatory iron reduction or high local flux from continental runoff flux (Anbar & Rouxel, 2007), while positive values have been interpreted as indicative of non reductive dissolution of sediments (Radic et al., 2011).

Seawater samples taken at the same site and time of chiton sampling were not available for Fe isotope analyses in this preliminary study. However, to allow a first-order assessment of biological fractionation during Fe uptake from seawater, we compare our data with published data for Fe isotopes of dissolved Fe from surface or shallow seawater measured at locations as close as possible to the chiton sampling sites (Figure 2). For the three regions for which seawater Fe isotope values are reported (the north Atlantic, the south Altlantic, and the north Pacific), δ^{56}Fe of dissolved Fe in surface seawater is more positive than the Fe in chiton teeth: the difference in δ^{56}Fe values between seawater and chiton theeth ($\Delta^{56}Fe_{sw\text{-}chiton} = \delta^{56}Fe_{seawater} - \delta^{56}Fe_{chiton\ theeth}$) at the different locations ranges from 0.28‰ to 1.14‰. Thus, overall, Fe in chiton teeth would seem to be isotopically lighter than Fe in seawater, suggesting that fractionation processes determine the Fe isotope signatures in chiton

teeth. In the following sections we discuss 3 possible fractionation mechanisms controlling Fe isotope fractionation in chiton teeth, and these pathways are summarized schematically in Figure 3.

3.2 Physiologically-controlled Fe isotope fractionation

One of the possible mechanisms leading to differences between isotopic values in bulk seawater and those in chiton teeth could be associated with the mode of biomineralization within the chitons. In addition to magnetite (Fe_3O_4), chiton radula contain other Fe minerals, including goethite (α-FeOOH), lepidocrocite (γ-FeOOH), and ferrihydrite (Fe_2O_3•$0.5H_2O$) (see Brooker and Shaw, 2012, and references therein). To form these minerals, iron originates as ferritin in the haemolymph and is delivered to the superior epithelial cells of the radula sac (Shaw et al., 2009). At a later stage, the ferritin is transferred to an organic matrix where it is deposited as ferrihydrite (Kim et al., 1989; Brooker et al., 2003). Despite the recent efforts in materials science to better understand Fe biomineralization (e.g, Weaver et al., 2010; Xiao and Yang, 2012), the precise mechanism by which the ferrihydrite precursor is transformed to magnetite remains undetermined. However, this transformation must involve a transition from an Fe(III) mineral (ferrihydrite) to a mineral that contains both Fe(II) and Fe(III) (magnetite). Moreover, changes in redox state can cause relatively large equilibrium Fe isotope fractionations (Wu et al, 2011; Frierdich et al., 2014); for example formation of magnetite from aqueous Fe(II) at 22°C leads to a mineral isotopic signature that is around ~1.6‰ heavier, while precipitation of ferrihydrite from aqueous Fe(II) leads to mineral isotopic values that are between 2.5‰ to 3.2‰ heavier. Mineralogical compositions in chiton teeth vary from species to species (Lowenstam and Weiner, 1989), and different proportions of the Fe bearing minerals could therefore result in distinct overall isotopic signatures. In future studies, precise measurements of Fe isotopes in the various mineralogical phases using new

techniques, such as laser ablation MC-ICP-MS, could be used to assess fractionation processes during biomineralization.

3.3 Diet-controlled fractionation

Another potential mechanism affecting the isotopic values in the chiton teeth is the direct ingestion of Fe that is isotopically distinct to iron in seawater. The main source of food for chitons is red and green algae; both types of algae are known to fractionate oxygen and carbon isotopes (Anderson and Arthur, 1983), and it is feasible that Fe isotopes might also be fractionated during uptake by algae. Algae contain high concentrations of Fe (e.g., García-Casal et al., 2007)**,** having developed a range of strategies for creating bioavailable Fe(II) from low solubility Fe(III) species, including the use of siderophores that facilitate photochemical redox cycling (e.g., Amin et al., 2009). Uptake mechanisms produce strong fractionations in terrestrial plants (von Blanckenburg et al., 2009; Guelke-Stelling and von Blanckenburg, 2012), and if enough Fe(II) is available, the light isotope may be preferentially absorbed, producing a light δ^{56}Fe signal which could depend on algal type.

Curiously, the isotopic values measured in the two chiton species from Puget Sound (Washington, USA) seem to have distinct values. The range in δ^{56}Fe values obtained from 5 individual specimens of *Tonicella lineata* is from -0.83‰ to -0.45‰ (mean δ^{56}Fe = -0.65 ± 0.26‰, 2σ); in contrast, more negative δ^{56}Fe values ranging from -1.90‰ to -0.94‰ (mean δ^{56}Fe = -1.47 ± 0.98‰, 2σ) were found for the 3 specimens of *Mopalia muscosa* (Figure 2). As one of the important differences between the two species is their contrasting diets (*T. lineata* predominantely feeds on red algae, while *M. muscosa* has a diet that includes both green and red algae (Boolootian, 1964; Demopulos, 1975), food sources could account for the different isotopic compositions. Furthermore, the variance associated with the δ^{56}Fe signature for *M. muscosa* is much higher than the variance for *T. lineata,* which would seem to be

consistent with the observation that chitons from the eulittoral zone (intertidal zone), such as *M. muscosa*, have less specific feeding habits, often ingesting both red and green algae and even animal matter (Boolootian, 1964; Morris et al., 1980).

While isotopic analyses of the different algal types would help evaluate the role of diet in determining the chitons' isotopic signatures, samples were not available for analysis in the current study. However, biological fractionation by algae is supported by an Fe isotope difference measured between phytoplankton and seawater, where an isotopic fractionation of about 0.25‰ favouring light isotopes was suggested to occur during uptake by phytoplankton (Bergquist and Boyle, 2006; Radic et al., 2011). Thus, the observed isotopic differences between seawater and chiton teeth could be at least be partially controlled by algal-mediated fractionation.

We note here that direct ingestion of Fe from rocky substrates with different isotopic signatures could also affect the chiton teeth (Lowenstam and Kirschvink, 1996). However, the Fe isotope composition of crustal igneous rocks is relatively restricted, ranging from about 0‰ to +0.4‰ in δ^{56}Fe (e.g, Beard et al., 2003, Poitrasson and Freydier, 2005) with an average igenous rock compostion of 0.1 ± 0.1‰ (2σ) (Beard et al., 2003). Modern marine sediments, such as terrigenous sediments, turbidite clays, and volcanoclastites, as well as altered oceanic cust, also have a restricted range of Fe isotope compositions clustered around the average igneous δ^{56}Fe value, with variations of less than 0.3‰ (e.g., Beard et al., 2003; Rouxel et al., 2003; Fantle and DePaolo, 2004), consistent with the homogeneous Fe-isotope composition found in loess and aerosols (Zhu et al. 2000). Thus, Fe derived from rocky subtrates is unlikely to account for the very light Fe isotope values we measured, although confirming this would have required *in situ* sampling that was beyond the constraints of this prelimary study.

3.4 Environmentally-controlled fractionation

Environmental conditions in the eulittoral and sublittoral zones are significantly different, and they could exert an additional control on the isotopic pools of bioavalaible Fe. In near-surface coastal seawater, dissolved bioavailable Fe(II) is thought to be produced by the photo-reduction of Fe(III) nanoparticles and complexes (e.g., Johnson et al., 1994; Barbeau et al., 2000; Barbeau, 2006; Fan, 2008). Measurements of seawater indicate that Fe(II) concentrations decrease significantly with depth in the top 10 m of the water column (e.g., Shaked, 2008), suggesting that photoreduction of Fe(III) to Fe(II) may be more effective in the shallower eulittoral zone than in the deeper sublittoral zone. Importantly, experiments have shown that the reductive dissolution of Fe-oxides produces isotopically light Fe(II) (e.g., Wiederhold et al., 2006; Beard et al., 2010), and photoreduction might be expected to produce bioavailable Fe(II) in seawater that possesses negative δ^{56}Fe values. Moreover, differences in levels of photoreduction could produce biovailable Fe(II) with light δ^{56}Fe values in the eulittoral zone and heavier isotopic values in the deeper sublittoral zone. Importantly, such a mechanism is consistent with the measurements of different chiton species from Puget Sound: Fe isotopes in *M. Muscosa* from the eulittoral zone are indeed lighter than those in *T. lineata* from the sublittoral zone. Thus, the different isotopic ratios measured in the two species may reflect different water depths and levels of photoreduction in the near shore environment.

CONCLUDING REMARKS

In this paper, we report on the Fe isotopic compositions of chiton radula from different marine locations in the Pacific and Atlantic oceans. We found a large variation in δ^{56}Fe values between the different locations, suggesting that the isotopic compositions may in part be controlled by variations in the local isotopic source signature due to changes in the relative balance of inputs from dissimilatory iron reduction, continental runoff, and non reductive dissolution of sediments. However, the distinct signatures recorded from two different species

analysed from Puget Sound, USA, suggest that Fe isotopes could be influenced by three main processes: (i) physiologically-controlled processes within the chitons that cause species dependent fractionation; (ii) diet-controlled variability resulting from different Fe isotope fractionation in the red and green algal food sources; (iii) environmentally-controlled fractionation that leads to variation in the isotopic signatures of bioavailable Fe in the different tidal zones.

Clearly the dataset presented in the current study possesses a number of limitations. Firstly, the number of chitons in our study is relatively small, a fact that complicates the interpretation of the results. In addition, although a dataset of published Fe isotope values for seawater exists, no Fe isotope data are available for algae and seawater from the exact locations from which the chiton specimens were collected; moreover, even if values were to be obtained for the present day, it is unclear how relevant such data would be for the samples in this study that were collected decades ago. In view of such constraints, our study must be regarded as a first attempt to tackle the complexities of Fe isotope fractionation in marine invertebrates, and our findings regarding the Fe isotope fractionation mechanisms are therefore preliminary. To determine the relative significance of the pathways controlling Fe isotopic signatures, a far more extensive sampling campaign – involving in-situ measurements of water, rock substrates, algae, and chitons – would be necessary.

Despite the limited dataset, the present study nevertheless yields a number of important conclusions. Although the results suggest that Fe-isotopes in bio-minerals do not necessarily record oceanic values, iron-concentrating organisms such as chitons (polyplacophora) and even limpets (archeogastropods) – which have teeth containing goethite (Lowenstam, 1962b) – could still record the signature of dissolved bioavailable Fe, and provide information concerning Fe biogeochemical cycling in near shore environments. Furthermore, in a similar way to oxygen and nitrogen isotopes, Fe isotopes could be used to distinguish between the primary sources of Fe in the diets of different organisms, serving as

an additional tool with which to probe ecological systems. Although the difficulties associated with identifying Fe-biominerals in the fossil record (Chang and Kirschvink, 1989) currently limit their potential usefulness in reconstructing past conditions, further documentation of Fe isotopes in seawater, algae, and higher organisms is expected to help track the present-day pathways and sources of Fe in marine environments.

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TABLE 1: SUMMARY OF ANALYSED CHITON SAMPLES.

Species	Individual specimens	Locality	Ecology	Specimen ID	δ^{56}Fe ‰	2SE ‰	δ^{57}Fe ‰	2SE ‰	*n*
Tonicella lineata	5	Puget Sound, WA, USA	Sublittoral, red algae	YPM-12716-01	-0.57	0.04	-0.84	0.07	4
				YPM-12716-02	-0.73	0.05	-1.07	0.07	4
				YPM-12716-03	-0.56	0.05	-0.83	0.05	5
				YPM-12720-04	-0.54	0.04	-0.82	0.07	5
				YPM-12720-05	-0.83	0.05	-1.22	0.06	4
Mopalia muscosa	3	Puget Sound, WA, USA	Eulittoral, green algae	YPM-12718-06	-1.58	0.04	-2.33	0.06	5
				YPM-12718-07	-1.90	0.02	-2.81	0.03	6
				YPM-12718-08	-0.94	0.04	-1.39	0.04	5
Tonicella marmorea	8	Grand Manan Island, New Brunswick, Canada	Sublittoral, red algae	YPM-12760-09	-1.10	0.05	-1.61	0.06	5
Chiton stokessi	5	Panama	Eulittoral, green algae	YPM-5176-10	-1.11	0.03	-1.63	0.05	6
				YPM-5176-11	-1.26	0.01	-1.86	0.03	6
				YPM-5176-12	-0.73	0.04	-1.03	0.07	5
				YPM-5176-13	-1.29	0.06	-1.89	0.06	4
				YPM-5176-14	-1.06	0.05	-1.54	0.09	4
Chiton tuberculatus	3	Bermuda	Eulittoral, green algae	YPM-12739-15	-0.38	0.01	-0.54	0.03	6
				YPM-12739-16a	0.00	0.05	0.00	0.09	4
				YPM-12739-16b	-0.28	0.02	-0.40	0.04	8
				YPM-12739-17	-0.28	0.05	-0.40	0.07	5

Iron isotope data are reported as permil deviation relative to the international reference material IRMM-014. Mean values of *n* replicate analyses of the same analyte solution are reported with their 95% confidence intervals (2SE = $t \cdot SD/\sqrt{n}$, with t = correction factor from Student's t-distribution at 95% probability). Uncertainties in δ^{56}Fe and δ^{57}Fe associated with the entire Fe isotope analytical procedure are estimated to be ±0.05 ‰ (2σ) and ±0.08 ‰ (2σ), respectively (see text).

Figure 1: (a) *Chiton tuberculatus* in the eulittoral zone, and (b) a radula sac containing the magnetite-capped teeth, indicated by the arrow. *C. tuberculatus* specimens are typically 14-80 mm in length (Glynn, 1970), and the one shown in (a) is approximately 50 mm long.

Figure 2: Fe isotope signatures of 18 chiton teeth analyses (grey circles). Analytical uncertainties in δ^{56}Fe are smaller than the symbols. Data points encircled by the red dashed lines indicate chitons with a red-algae rich diet, while green dotted lines indicate a predominantly green algae diet. Note that the values for the chitons with a red-algae diet from Washington cluster close together; by contrast, chitons from the same location with a diet of green algae have a larger variance and a lighter isotopic signature. The single value for *Tonicella marmorea* from Grand Manan Island represents the average of 8 homogenized specimens. For comparison, the average for igneous rocks (Beard et al., 2003) is indicated by the vertical grey line, while the range of Fe isotope values reported in the literature for dissolved Fe in surface and shallow (< 75 m depth) seawater (Lacan et al., 2008, 2010; John and Adkins 2010, 2012; Rouxel and Auro, 2010; Radic et al., 2011) is represented by the solid blue band at the bottom. Blue squares are published surface seawater isotope analyses of dissolved Fe from locations as close to the chiton sampling sites as available data permits. Data reported by Rouxel and Auro, (2010) for three sites located off the north-eastern Atlantic coast of North America are (a) Vineyard Sound on Cape Cod, Massachusetts, USA (−0.82 ‰); (b) Waquoit Bay on Cape Cod, Massachusetts, USA (−0.55 ‰); (c) Connecticut River estuary in Long Island Sound, Connecticut, USA (+0.04 ‰). These three sites are located within less than 150 km distance from each other, on average about 500 km south of the chiton sampling site at Grand Manan Island, New Brunswick, CA). Data for the North Atlantic (d) (+0.3 ‰; sampled about 100 km southeast from Bermuda, John and Adkins, 2010; John and Adkins, 2012) are compared with the Bermuda chiton sampling site. The closest available coastal seawater Fe isotope data to compare with the Puget Sound chiton sampling site (Washington, USA) is from the San Pedro Basin (e) (0 ‰; John and Adkins, 2010), which is located off the Atlantic coast near Los Angeles (California, USA), about 1500 km south from Puget Sound.

Figure 3: Schematic pathways for isotope fractionation of iron present in seawater and different chiton species. Fractionation could occur during (a) physiologically-controlled biomineralization processes within the chitons; (b) fractionation during uptake by algae and subsequent ingestion; and (c) environmentally-controlled photoreductive dissolution of Fe complexes and nanoparticles in seawater. The mechanisms are not mutually exclusive and the signatures in the chiton teeth could reflect a combination of different pathways.

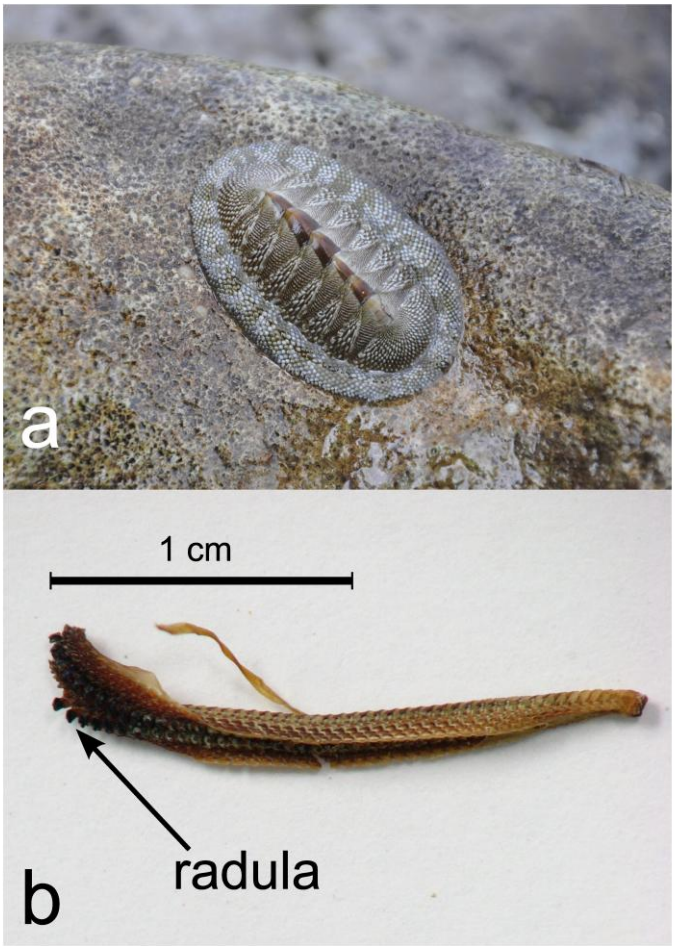

Figure 1

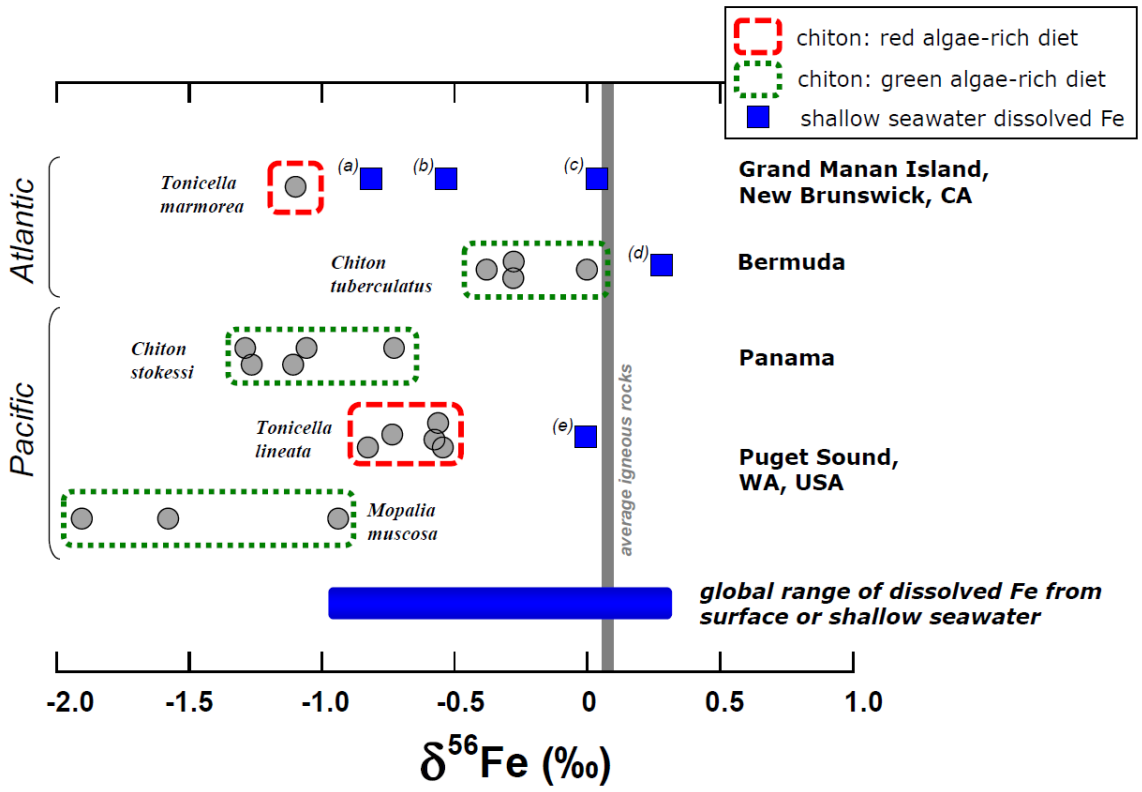
chiton: red algae-rich diet
chiton: green algae-rich diet
shallow seawater dissolved Fe
Atlantic
Pacific
Tonicella marmorea
(a)
(b)
(c)
Grand Manan Island, New Brunswick, CA
Chiton tuberculatus
(d)
Bermuda
Chiton stokessi
Panama
Tonicella lineata
(e)
Puget Sound, WA, USA
Mopalia muscosa
average igneous rocks
global range of dissolved Fe from surface or shallow seawater
-2.0
-1.5
-1.0
-0.5
0
0.5
1.0
δ56Fe (‰)

Figure 2

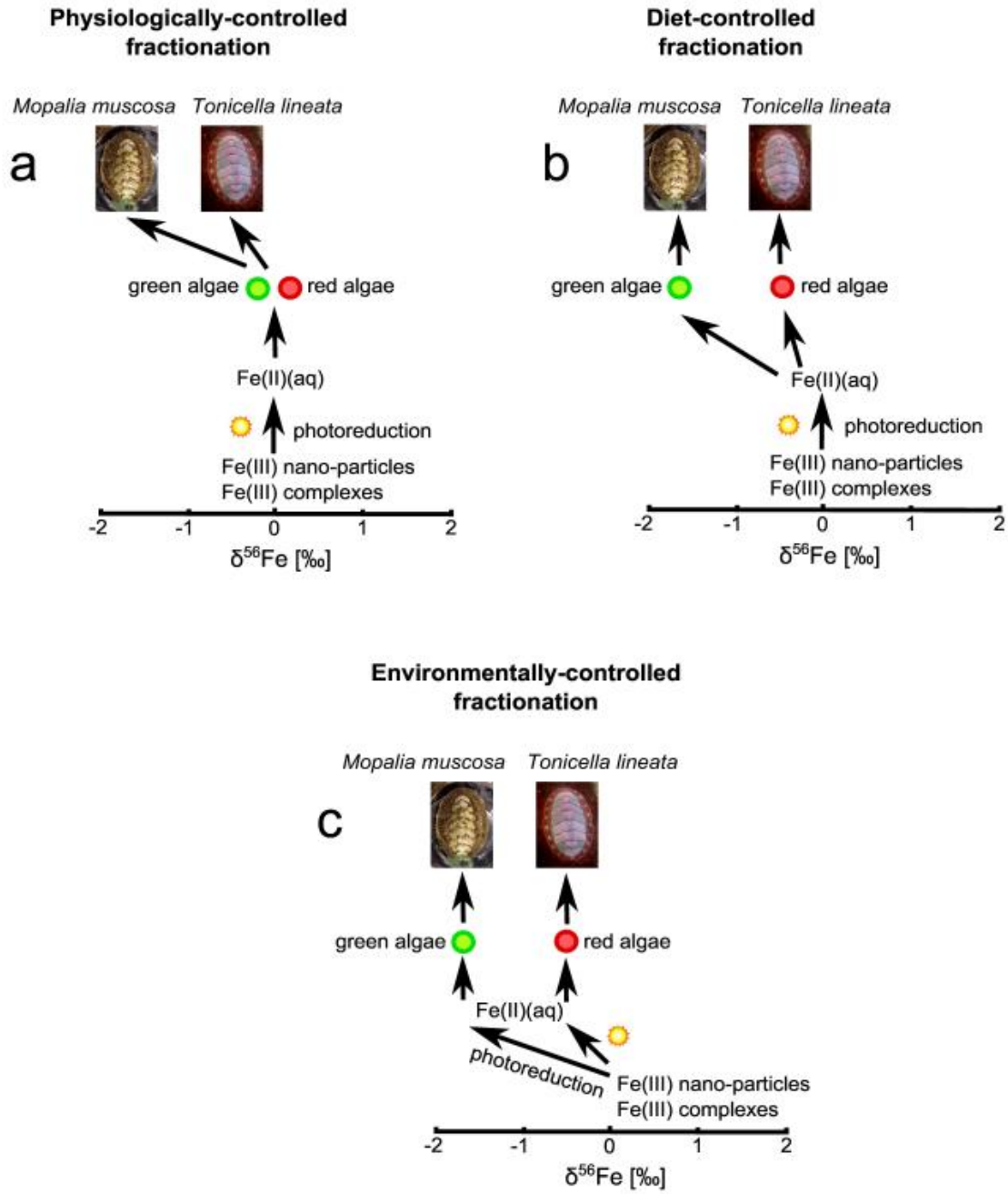
Physiologically-controlled fractionation
Mopalia muscosa
Tonicella lineata
a
green algae
red algae
Fe(II)(aq)
photoreduction
Fe(III) nano-particles
Fe(III) complexes
-2
-1
0
1
2
δ56Fe [‰]
Diet-controlled fractionation
Mopalia muscosa
Tonicella lineata
b
green algae
red algae
Fe(II)(aq)
photoreduction
Fe(III) nano-particles
Fe(III) complexes
-2
-1
0
1
2
δ56Fe [‰]
Environmentally-controlled fractionation
Mopalia muscosa
Tonicella lineata
c
green algae
red algae
Fe(II)(aq)
photoreduction
Fe(III) nano-particles
Fe(III) complexes
-2
-1
0
1
2
δ56Fe [‰]

Figure 3