



# The zinc isotopic composition of siliceous marine sponges: Investigating nature's sediment traps<sup>☆</sup>



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## ABSTRACT

The zinc (Zn) content and isotopic composition of marine biogenic opal have the potential to yield information about the nutrient availability, utilization and export of particulate organic matter from surface to deep waters. Here, we report the first measurements of the Zn isotopic composition of deep-sea marine sponge skeletal elements – spicules – collected in the Southern Ocean. Our results highlight different Zn uptake and isotopic fractionation behavior between the two major siliceous sponge clades (hexactinellids and demosponges), which is most likely linked to sponge feeding strategy. Hexactinellid spicule Zn isotopic compositions are not fractionated with respect to seawater, most likely due to Zn transport via the open internal structure of the sponges. In contrast, demosponge spicules exhibit a wide range of Zn isotopic compositions that are related to the opal Zn concentration, most likely reflecting variable Zn isotope compositions in the organic matter particles on which they feed, and internal fractionation processes.

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## 1. Introduction

Dissolved zinc (Zn) is an essential metallic co-factor in enzymes such as carbonic anhydrase (Lane and Morel, 2000), and may potentially play a role in phytoplankton growth under certain conditions (Morel et al., 1994; de la Rocha et al., 2000; Franck et al., 2003; Sunda and Huntsman, 2005). Zinc generally exhibits a seawater depth profile similar to that of major nutrients, with concentrations ranging over several orders of magnitude (from pmol to nmol kg<sup>-1</sup> levels), consistent with uptake by biological utilization, followed by remineralization at depth (Lohan et al., 2002; Croot et al., 2011). Furthermore in seawater, the dissolved Zn pool is dominated (>95%) by organic ligand bound Zn, particularly in the surface ocean, leaving the free bioavailable Zn<sup>2+</sup> pool even smaller (Bruland, 1989; Bruland and Lohan, 2003).

Despite the evidence linking Zn with biological cycling and ocean productivity, there is a need for a greater understanding of the exact role that Zn plays as a micronutrient for phytoplankton (Sinoir et al., 2012). This limitation on the understanding of Zn ocean cycling has been hindered by analytical challenges, which are being addressed through general improvements in sampling and measurement

techniques, and through large-scale efforts such as the GEOTRACES program (e.g. Boyle et al., 2012). Furthermore, the advent of techniques for measuring Zn isotopes (Marechal et al., 1999) has added an extra tool for studying Zn oceanic cycling (e.g. Bermin et al., 2006).

The major Zn input flux to the oceans is likely to be rivers, with aerosols (and hydrothermal) potentially contributing some part as well. The Zn isotopic composition of crustal material appears to be homogeneous centered around a  $\delta^{66}\text{Zn}$  (Eq. (1)) of +0.3‰ (Marechal et al., 2000; Archer and Vance, 2004; Cloquet et al., 2008):

$$\delta^{66}\text{Zn} = \left[ \left\{ \frac{\left( \frac{^{66}\text{Zn}}{^{64}\text{Zn}} \right)_{\text{sample}}}{\left( \frac{^{66}\text{Zn}}{^{64}\text{Zn}} \right)_{\text{JMC-Lyons}}} \right\} - 1 \right] \times 1000. \quad (1)$$

The major Zn input flux to the oceans (rivers and aerosols) may be assumed to be in that range. Marine derived materials (e.g. diatom opal, manganese nodules, carbonate sediments, algae) show both lighter and heavier Zn isotope compositions (Marechal et al., 2000; Pichat et al., 2003; Andersen et al., 2011). Uptake of Zn into organic matter results in preferential incorporation of light Zn isotopes (Marechal et al., 2000; John et al., 2007; Cloquet et al., 2008; Peel et al., 2009), whereas adsorption processes may lead to heavier Zn isotopic compositions (e.g. Gelabert et al., 2006; John et al., 2007; Juillot et al., 2008). As already mentioned, due to the low abundance of Zn in seawater, measuring Zn is an analytical challenge and

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available seawater Zn isotope data are presently sparse (Bermin et al., 2006; Boyle et al., 2012). These limited data suggest that the Zn isotopic compositions of deep waters with high dissolved Zn concentrations (nM levels) from remineralization of organic matter at depth have a  $\delta^{66}\text{Zn}$  close to the crustal material. For deep seawater samples (below 500 m depth) limited  $\delta^{66}\text{Zn}$  data have been reported in Andersen et al. (2011) and Boyle et al. (2012). A mean  $\delta^{66}\text{Zn}$  of  $0.45 \pm 0.09\text{‰}$  was obtained for samples from the Indian and Pacific Oceans (Andersen et al., 2011), and a mean  $\delta^{66}\text{Zn}$  of  $0.42 \pm 0.12\text{‰}$ , from GEOTRACES IC1 BATS station in the Atlantic Ocean (Boyle et al., 2012). Furthermore, recently a larger compilation of Zn isotope data of deeper seawater profiles (below 1000 m), with high dissolved Zn concentrations (nM levels) from the Atlantic, Pacific and Southern Oceans, exhibits relative uniform Zn isotope compositions with a mean  $\delta^{66}\text{Zn}$  of  $+0.53 \pm 0.14\text{‰}$  (Zhao et al., in review).

The nutrient like behavior of Zn suggests that the biological cycling of Zn and associated Zn isotope fractionation are the dominating processes controlling the marine Zn budget. Zn isotopes in carbonate sedimentary records over time have been interpreted in the context of changes in these biological processes: heavier isotopic compositions of carbonates are thought to reflect larger degrees of biological utilization, with the incorporation of lighter Zn leaving the residual seawater (and carbonate sediments) isotopically heavy (Pichat et al., 2003; Kunzmann et al., 2013). Furthermore, it has been shown, that the Zn/Si ratio of biogenic opal produced by photosynthetic diatoms relates to the availability of free  $\text{Zn}^{2+}$  ions in surface seawater, and is a potential proxy for environmental conditions (Ellwood and Hunter, 1999; Ellwood and Hunter, 2000; Hendry and Rickaby, 2008; Jaccard et al., 2009). A study of Zn incorporation and Zn isotope ratios in diatom opal across a transect in the Southern Ocean suggests that diatom opal chemistry may record the bioavailable Zn abundance and isotopic shifts in surface seawater (Andersen et al., 2011). The  $\delta^{66}\text{Zn}$  in diatom opal appears to record  $\delta^{66}\text{Zn}$  in surface seawater, which is driven isotopically heavier as a consequence of the biological utilization of Zn, taking up isotopically lighter Zn (Andersen et al., 2011). Conversely, the Zn/Si ratio of opaline skeletal elements (spicules) made by deep-sea sponges (Porifera) – belonging to the Class Hexactinellida – has been shown in one study to relate to the rain of Particulate Organic Carbon (POC; Ellwood et al., 2004). This raises the potential for the Zn/Si ratios of spicules produced by

sponges, which are simple, filter-feeding animals without tissue grade organization of cells, to be used as a measure of organic export flux (Ellwood et al., 2004; Ellwood et al., 2005). An archive of the flux of particulate organic matter to the seafloor would allow the quantification of past changes in the supply of nutrients to the benthos, an important parameter for the reconstruction of past carbon cycling and benthic ecology. To date, however, to the best of our knowledge there have been no further studies of sponge Zn/Si since Ellwood et al. (2005), or any studies investigating the Zn isotopic composition of spicules.

Here, we present new Zn concentration and isotope data from deep-sea sponges collected from the Southern Ocean. Our results highlight some specific differences in Zn uptake between the major classes of siliceous sponges, the hexactinellids and demosponges, and their potential uses as paleoenvironmental indicators.

## 2. Methods and materials

Sponges were collected by trawl and dredge from the Drake Passage and Scotia Sea (Fig. 1) from the R/V Nathaniel B. Palmer in April–May 2008. The specimens were dried for transport (see Hendry et al., 2010 for full details).

Subsamples were initially cleaned to remove external organic matter by heating three times in  $\text{H}_2\text{O}_2$  (30%, reagent grade) at  $80\text{ °C}$ , rinsing three times in  $18\Omega\text{-cm}$  Milli-Q water, heating three times in concentrated in-house Teflon-distilled  $\text{HNO}_3$  at  $100\text{ °C}$  and rinsing five times in Milli-Q water. Remaining lithic particles were removed physically from the samples. For some samples, generally those with smaller spicules, heavy liquid separation was used to remove remaining lithic particles (Hendry and Rickaby, 2008). The samples were then 1) reductively cleaned by heating twice in 1 M hydroxylamine chloride–acetic acid at  $100\text{ °C}$  for 1 h (Ellwood and Hunter, 1999), and were rinsed three times in Milli-Q water each time; 2) oxidatively cleaned by heating in strong acid solution (double Teflon-distilled  $\sim 7\text{ N HNO}_3$ ,  $\sim 1\text{ N HCl}$ , University of Bristol) for at least 2 h on a hotplate at  $120\text{ °C}$ , and were rinsed five times in Milli-Q. The spicules were dissolved in  $\sim 3\text{ N HF}$  (Romil ultra-pure), and dried down prior to repeat dissolution in  $\sim 3\text{ N HF}$  to maximize the removal of Si, which can result in a polyatomic interference on Zn isotopes in plasma mass spectrometry (Andersen et al., 2011).

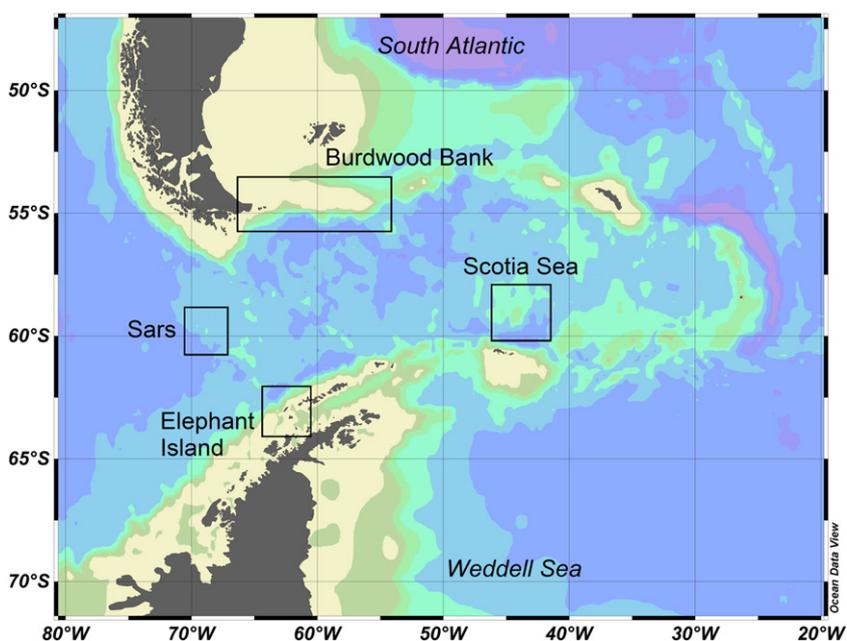


Fig. 1. Sample location map with key areas highlighted in boxes. Map drawn using Ocean Data View.

The residue was taken up in 1 ml ~0.3 N HNO<sub>3</sub> and fluxed on a hotplate for 1 h. 100 µl of this solution was diluted with 900 µl ~0.3 N HNO<sub>3</sub> for trace metal analysis using a Thermo Element II (University of Bristol; e.g. Fig. 2).

The remaining sample was dried down, spiked with a <sup>67</sup>Zn–<sup>64</sup>Zn double spike (aiming for a total Zn spike-sample ratio of ~1) and allowed to equilibrate (Bermin et al., 2006; Andersen et al., 2011). 0.5 ml splits were taken from samples containing more than ~200 ng total Zn, such that all samples contained a total of 10–200 ng Zn. The spiked samples were dried down and taken up in 1 ml 1 N HCl and fluxed on a hotplate for several hours. The samples were then purified using anion column exchange resin (AG MP-1). The pre-cleaned resin was pretreated with 5 ml Milli-Q water, 5 ml 0.3 N HNO<sub>3</sub>, 2 ml Milli-Q water, and 1 + 3 ml 1 N HCl before loading the samples in 5 ml 1 N HCl, and the resin was then washed with 3 × 5 ml 1 N HCl before Zn elution with 1 + 3 ml ~0.3 N HNO<sub>3</sub>. The samples were dried down, before being taken up in 1 ml 1 N HCl, fluxed for at least an hour on a hotplate, and the column chemistry repeated to ensure quantitative separation of Zn from other matrix elements. Previous studies on samples with a similar matrix have shown that matrix elements in the Zn fraction are at trace levels after chemical separation (Andersen et al., 2011). The samples were dried down a final time, before being taken up in 0.3 N HNO<sub>3</sub> and fluxed on hotplate for several hours.

The samples were analyzed for Zn isotopes using a Thermo Neptune MultiCollector Inductively Coupled Plasma Mass Spectrometer (University of Bristol) using methods outlined in Bermin et al. (2006) and Andersen et al. (2011). In Andersen et al. (2011) the external reproducibility (2SE) is estimated at ±0.1‰ for measured

samples with lower internal errors (2SE). For a few smaller samples, a higher uncertainty (±0.15‰ 2SE) is associated (Table 1). Total procedural blanks range from 0.8 to 1.9 ng Zn, contributing 1–15% of the measured samples. Laboratory blanks measured in Bermin et al. (2006), in the same laboratory used in this study, yielded δ<sup>66</sup>Zn of 0.17 ± 0.18‰. Thus, blank adjustments for most samples result in a shift of <0.02‰, and are within the external reproducibility estimate for all samples.

### 3. Results

Sixteen sponge specimens were analyzed (eight hexactinellids and eight demosponges) from six different Southern Ocean localities (Fig. 1). For three of the sponges, subsamples from different parts of the specimen were analyzed.

#### 3.1. Elemental concentrations

The Zn concentration in the sponge samples ranged from 0.03 to 4.4 ppm (Zn/Si approximately 0.03 to 4 µmol/mol; Table 1; Fig. 3A), which is a similar range found by previous studies (Ellwood et al., 2004) and generally lower, or within the lower range, observed for diatom opal (Hendry and Rickaby, 2008; Andersen et al., 2011).

Plotting the sponge Zn concentration versus water depth (Fig. 3A) or the water's silicic acid concentration (Fig. 4A), the two major classes of sponge appear to have different behaviors; although both classes show a weak negative correlation between Zn concentration and silicic acid (hex:  $r = -0.80$ ,  $p = 0.006$ ,  $n = 10$ ; demo:  $r = -0.59$ ,  $p = 0.09$ ,  $n = 9$ ; Fig. 4A), for a particular ambient

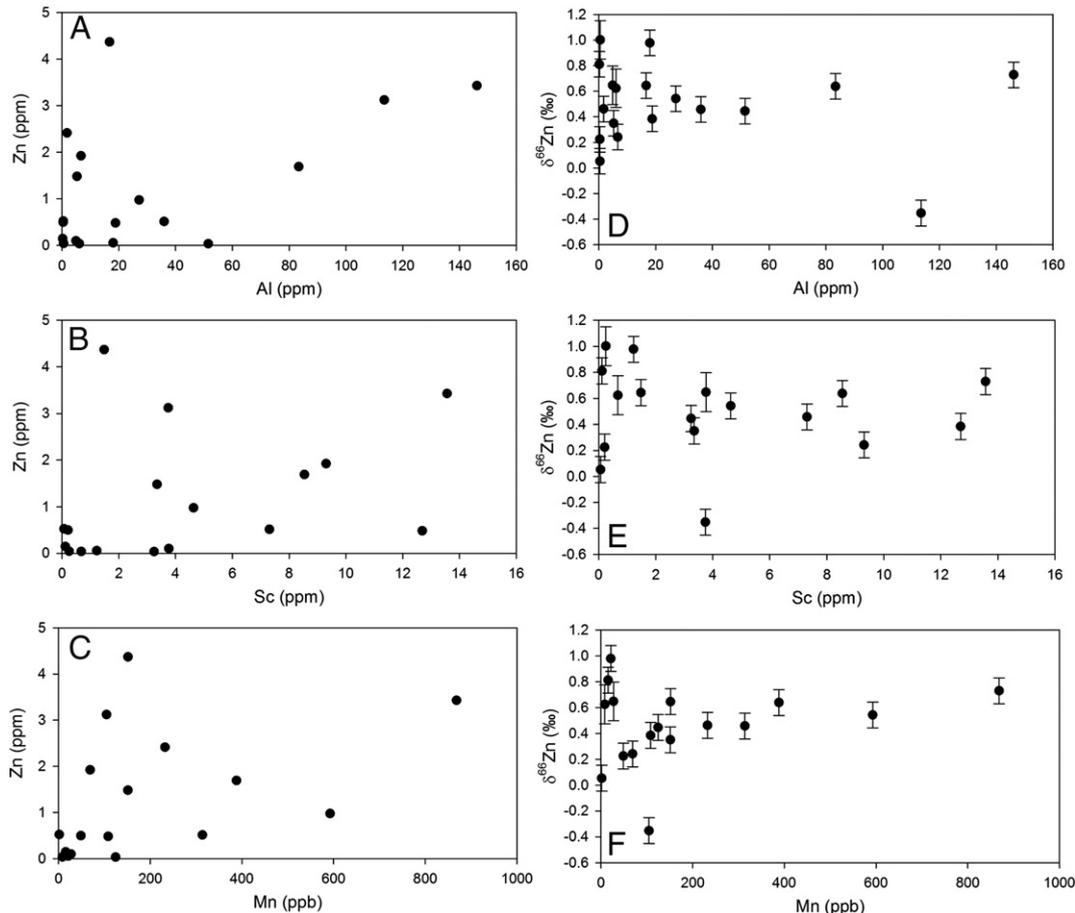


Fig. 2. All marine sponge spicule Zn concentration (A, B, C) and Zn isotope (D, E, F) results plotted against trace metal content aluminium, scandium and manganese. Error bars show the external reproducibility (2SE).

**Table 1**  
Zn concentration and isotope composition of deep-sea sponges from the Southern Ocean (for full details of sample locations, see Hendry et al., 2010; H and D refer to Hexactinellids and Demosponges respectively). Depths are from the official station log of cruise NBP0805.

Sample code	Depth (m)	Location	Si(OH) <sub>4</sub> (μM)	Zn (ppm)	δ <sup>66</sup> Zn (‰)	2SD
NBP0805-D7-47 (H)	2109–2422	Scotia Sea	120	0.03	0.45	0.04
NBP0805-D7-48 (H)	2109–2422	Scotia Sea	120	1.69	0.64	0.04
NBP0805-D13-47 (H)	2097–2477	Scotia Sea	110	0.51	0.46	0.06
NBP0805-TB1-3 (D)	306–333	Burdwood Bank	12	0.06	0.98	0.10
NBP0805-TB1-6 (D)	306–333	Burdwood Bank	12	3.12	−0.35	0.03
NBP0805-TB3-23 (D)	2215–2343	Burdwood Bank	97	0.10	0.65	0.12
NBP0805-TB4-24 (D)	804–828	Burdwood Bank	56			
Parenchymal				0.50	0.22	0.04
Dermal				0.52	0.05	0.04
NBP0805-TB1-11 (H)	306–333	Burdwood Bank	12	3.43	0.73	0.06
Full repeat				4.37	0.64	0.03
Analytical repeat <sup>a</sup>					0.67	0.07
NBP0805-TB4-3 (D)	804–828	Burdwood Bank	56	0.04	0.62	0.17
NBP0805-D16-47 (H)	930–1070	Elephant Island	96	2.41	0.46	0.07
NBP0805-OT1-27 (H)	407–428	Elephant Island	90	0.98	0.54	0.03
NBP0805-TO3-108 (H)	689–914	SARS Seamount	75	1.48	0.35	0.07
NBP0805-TO3-111 (D)	689–914	SARS Seamount	75	0.04	1.00	0.16
Full repeat				0.03	1.04	0.05
Analytical repeat <sup>a</sup>					0.96	0.17
NBP0805-D34-47 (H)	838–899	SARS Seamount	75	0.48	0.38	0.04
Analytical repeat <sup>a</sup>					0.41	0.08
NBP0805-D35-111 (D)	672–718	SARS Seamount	70	0.14	0.81	0.06
NBP0805-D40-47 (H)	1294–1351	SARS Seamount	94	1.92	0.24	0.05

<sup>a</sup> Analytical repeats do not include a full repeat of subsampling, cleaning or dissolution.

inorganic nutrient concentration (i.e. Si(OH)<sub>4</sub>), hexactinellids have a higher, and more variable, Zn content than demosponges.

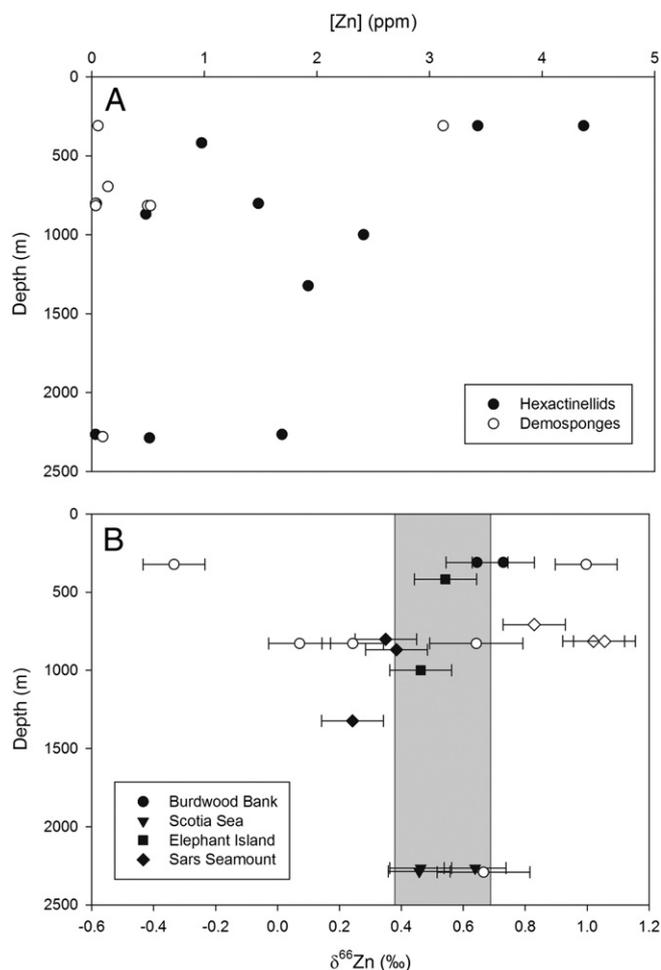
### 3.2. Zinc isotopes

The sponge Zn isotopic compositions, expressed as δ<sup>66</sup>Zn, ranged from −0.35 to +1.04‰ for the demosponges whereas the hexactinellids show a more narrow range of δ<sup>66</sup>Zn (+0.24 to +0.73‰, Table 1; Figs. 3B, 4B). This is a larger range than diatom opal (Andersen et al., 2011). At greater water depths (>1000 m) and high silicic acid conditions (>80 μM [Si(OH)<sub>4</sub>]), all sponge Zn isotopes are similar to the deep sea-water Zn isotopic composition (+0.53 ± 0.14‰, Figs. 3B, 4B, Zhao et al., in review); at shallower depths/lower silicic acid conditions the range of demosponge Zn isotopes is greater, even at the same locality (Fig. 3B), whereas the hexactinellids record the signature of the deep seawater. Thus, hexactinellids show roughly constant δ<sup>66</sup>Zn even with varying Zn content, whereas the demosponge spicules are isotopically light with high Zn contents and isotopically heavier when the Zn content is lower (Fig. 5A).

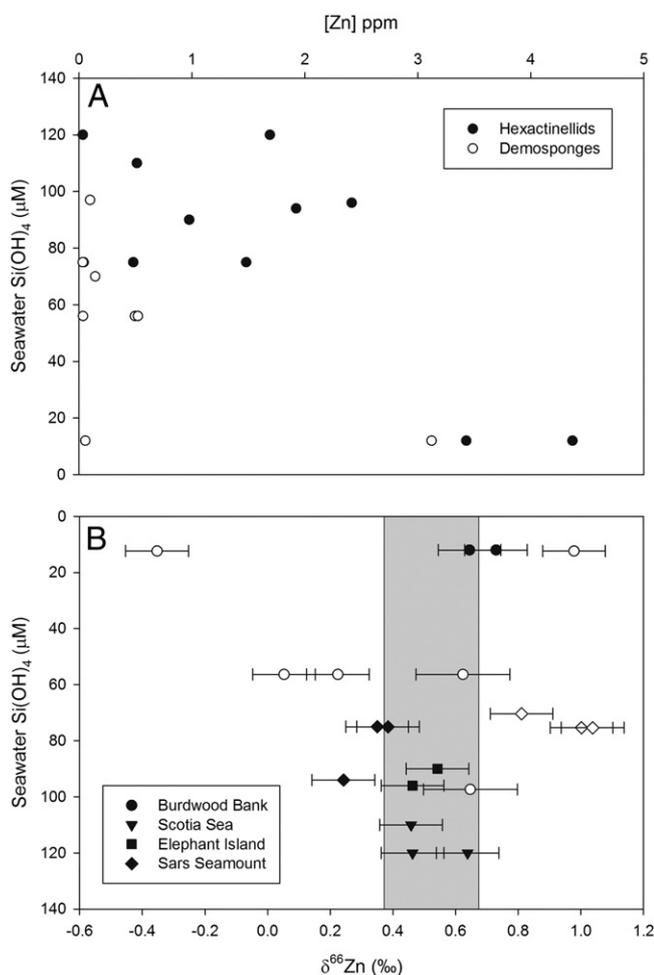
## 4. Discussion

### 4.1. Zinc in sponges versus contamination from lithogenic particles

Our results indicate that there is variability in marine sponge opal Zn isotopic composition, which represents a primary signal recorded within the spicules, as opposed to secondary contamination. There are several reasons why it is possible to rule out significant Zn contamination from either lithogenic particles, secondary coatings on the spicules, or contaminants during sample preparation. Firstly, the sponge spicule samples showed very low concentrations of elements associated with lithogenic material, such as aluminium (Al), scandium (Sc) and manganese (Mn). The Al concentrations in the spicules were maximum at ~160 ppm, compared to a maximum of ~1500 ppm in clean diatom samples (Hendry and Rickaby, 2008); similarly, Sc concentrations in the sponge spicules reached a maximum of 14 ppm compared to ~250 ppm in cleaned diatom opal (Andersen et al., 2011). The Mn concentrations in the cleaned spicules reached up to 0.9 ppm compared to concentrations of 0.2–1.8 ppm for cleaned diatoms (Andersen et al., 2011). Our spicule Mn concentrations approximate to a Mn/Si ratio of



**Fig. 3.** (A) Zn concentrations and (B) Zn isotopic composition of marine sponge spicules plotted against depth for the different classes (solid symbols hexactinellids; hollow symbols demosponges), and coded via shape for the different key sampling regions (see Fig. 1). The gray bar shows deep seawater Zn isotope composition. Error bars show the external reproducibility (2SE).

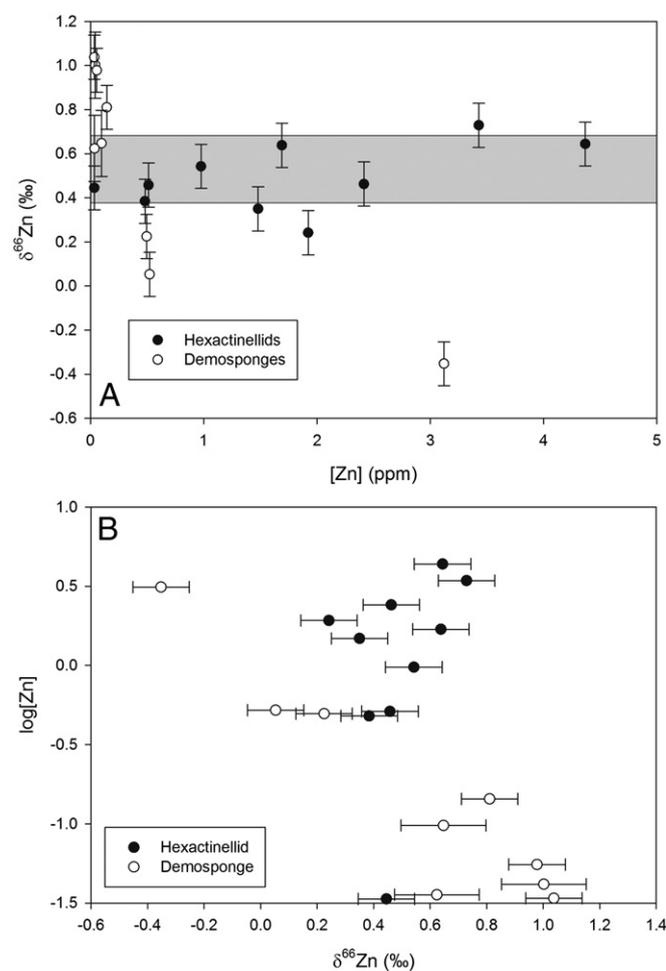


**Fig. 4.** (A) Zn concentration and (B)  $\delta^{66}\text{Zn}$  in marine sponge spicules plotted against silicic acid concentration. Error bars show the external reproducibility (2SE).

1  $\mu\text{mol/mol}$ , comparable to that found previously from sedimentary spicules (Ellwood et al., 2005). Furthermore, there is no consistent relationship between Al, Sc or Mn concentrations and either the Zn concentration (Fig. 2A–C) or  $\delta^{66}\text{Zn}$  of the spicules (Fig. 2D–F). Secondly, the spicule samples showed a very good degree of reproducibility with respect to the Zn isotope composition. Repeat measurements of samples from the same sponge, which had undergone the full chemical processing separately, yielded the same  $\delta^{66}\text{Zn}$  within analytical uncertainty (Table 1). This was even the case when spicules of differing morphologies, for example dermal and parenchymal spicules from the demosponge specimen NBPO805-TB4-24, were sampled from different parts of the sponge. This not only indicates that sponge spicules are homogeneous with respect to Zn isotopes, but also provides evidence that contamination that influenced  $\delta^{66}\text{Zn}$  is unlikely to have occurred, and that the cleaning procedure did not result in fractionation. Thirdly, the range of spicule  $\delta^{66}\text{Zn}$  exceeds the range of  $\delta^{66}\text{Zn}$  observed in lithogenic marine sediments (+0.2 to +0.8‰, Marechal et al., 2000).

#### 4.2. Zinc incorporation into sponge spicules

Our results show that the two clades, hexactinellids and demosponges, exhibit different trends between environmental parameters and both Zn concentrations and isotopic compositions. Hexactinellids generally have a higher Zn content than demosponges for a given water depth or  $\text{Si}(\text{OH})_4$  content (Figs. 3A, 4A) but have a near uniform  $\delta^{66}\text{Zn}$  signature. Demosponges also show a greater range in  $\delta^{66}\text{Zn}$ , with greater variability in shallower/lower  $\text{Si}(\text{OH})_4$  waters (Figs. 3B, 4B), and systematic changes in  $\delta^{66}\text{Zn}$  with spicule



**Fig. 5.** A) Zn content vs. Zn isotopic composition and B)  $\log[\text{Zn}]$  vs. Zn isotopic composition for marine sponge spicules plotted for the different classes. The gray bar shows deep seawater Zn isotope composition. Error bars show the external reproducibility (2SE).

Zn content (Fig. 5). Demosponges, unlike hexactinellids, show a linear trend in a plot of  $\delta^{66}\text{Zn}$  versus the logarithm of the Zn concentration ( $\log[\text{Zn}]$ ), indicating that the isotopic variation observed is not a result of mixing between two sources of Zn with different isotopic compositions but is consistent with a Rayleigh fractionation type process (Fig. 5B). We propose a theoretical model to explain the contrasting trends between Zn concentration and  $\delta^{66}\text{Zn}$  observed based on different Zn uptake mechanisms, which reflect fundamental differences in nutrient acquisition in hexactinellids and demosponges.

#### 4.3. Particle uptake: sponges as nature's sediment traps

The two sponge classes have different feeding strategies and internal physiologies (e.g. Reuswig, 1990), which we suggest results in two different Zn fractionation mechanisms (Fig. 3B). Demosponges feed by phagocytosis, carried out by flagellated collar bodies (choanocytes) on nearly all surfaces, which are able to take up a wide range of particle sizes, including engulfing particles  $>50 \mu\text{m}$  straight into the cell (Reuswig, 1971). Some evidence points towards selective feeding of nanoplankton in the 2–20  $\mu\text{m}$  range (Eda Topcu et al., 2010). Differentiated cells then move the food particles in vesicles through a canal system to other cells for utilization. In contrast, hexactinellids have an open “trabecular” network (syncytium) for communication between cells (Hooper and Van Soest, 2002). Feeding in hexactinellids is less well understood: some studies suggest that they rely only on dissolved organic matter (Reuswig, 1990), but other evidence points towards an ability to

take in selected small particles (colloids and “ultraplankton” e.g. bacteria  $<5 \mu\text{m}$ ) via choanocytes; nutrients are then distributed via cytoplasmic streams within the syncytium (Pile et al., 1996; Wyeth et al., 1996; Pile and Young, 2006; Yahel et al., 2006; 2007).

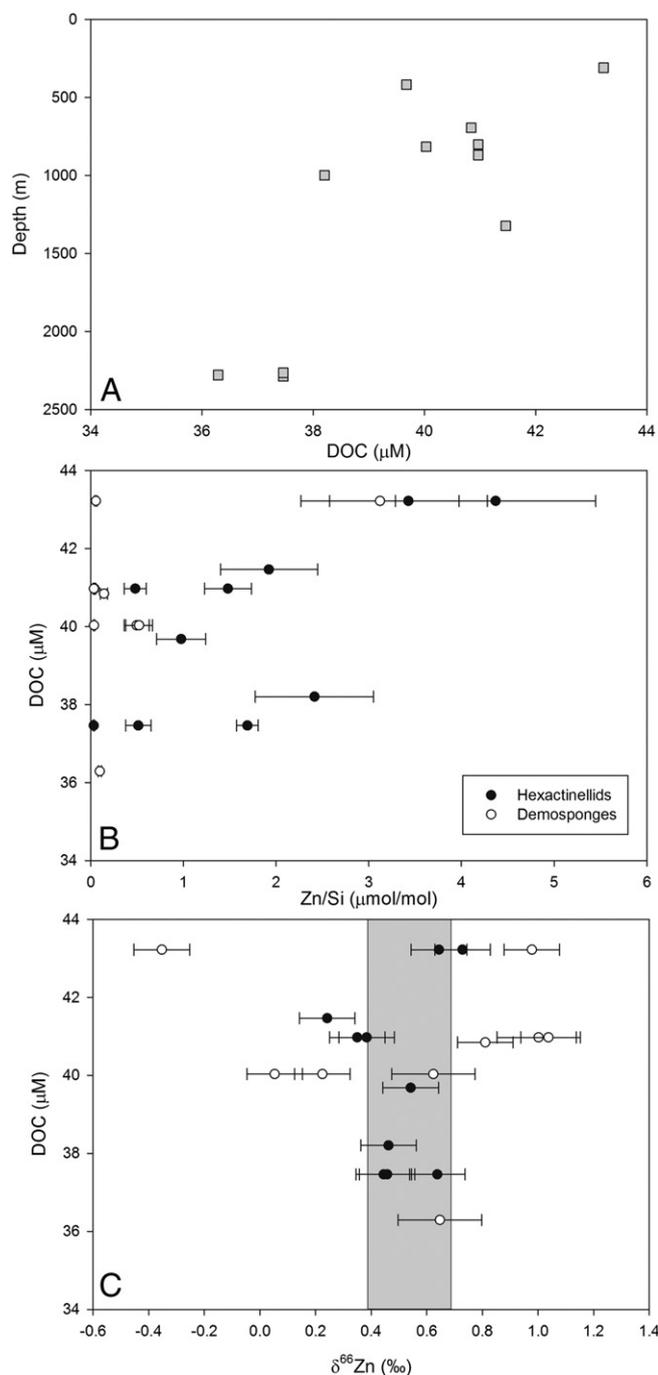
In order to understand the impact that dietary Zn has on sponge spicule Zn isotopic composition, it is essential to understand the isotopic fractionation that occurs during the formation and sinking of particulate organic matter. Existing evidence points towards isotopic fractionation of Zn during uptake into and adsorption onto settling particulate organic matter (e.g. John et al., 2007). A lacustrine sediment trap study highlighted seasonal variation in the  $\delta^{66}\text{Zn}$  of sinking particulate matter, due to preferential uptake of lighter Zn isotopes during phytoplankton growth (Peel et al., 2009). In the marine realm,  $\delta^{66}\text{Zn}$  of sinking particulate matter from the Atlantic Ocean from 250 to 2500 m water depth showed isotope compositions either similar or lighter compared to typical lithogenic values, ranging from  $+0.18\%$  to  $+0.34\%$  (Marechal et al., 2000). However, firstly – in both studies – the measured particulate matter consisted of lithogenic, particulate inorganic, and organic fractions (e.g. Bory et al., 2001), such that a greater percentage of marine-POM could have driven the flux to a lighter isotopic composition in the Atlantic samples, and, secondly, it is unlikely that either study will represent a steady-state system typical of the deeper ocean.

#### 4.3.1. Hexactinellids

The weakly significant inverse relationship between Zn content and seawater  $[\text{Si}(\text{OH})_4]$  observed for hexactinellids (Fig. 4A) suggests that Zn incorporation into their spicules does not simply reflect the concentration of the total dissolved Zn pool, given the general positive correlation between seawater  $[\text{Si}(\text{OH})_4]$  and dissolved Zn (e.g. Bruland, 1980; Bruland and Franks, 1983), a trend also observed in seawater profiles in the vicinity of the sponge sampling sites (Zhao et al., in review). Instead, hexactinellid Zn concentration is likely to be controlled by factors that relate to the growth of the sponge, including inorganic nutrient availability, surface productivity and the rain of organic matter to the seafloor. Our results show a weak positive relationship between ambient Dissolved Organic Carbon (DOC measurements from A. Burke, pers. com.) and hexactinellid spicule Zn concentration, or Zn/Si in  $\mu\text{mol/mol}$  calculated from the measured Zn concentrations, the spicule weights and assuming a molecular mass of biogenic opal of 66 g/mol (Mortlock and Froelich, 1989) (hex:  $r = 0.68$ ,  $p = 0.03$ ,  $n = 10$ ; cf demo:  $r = 0.44$ ,  $p = 0.23$ ,  $n = 9$ ; Fig. 6B). Although Particulate Organic Carbon (POC) measurements were not made at the sampling localities, the weak relationship between hexactinellid Zn content and ambient DOC would most likely result in a weak positive trend between spicule Zn/Si and ambient POC because of the coupled POC–DOC profiles in seawater (Carlson et al., 2000). The fit between Zn/Si and DOC would probably be improved through measurement of Si concentrations in the samples, rather than relying on calculating Zn/Si for weighed samples assuming a molecular mass of opal; we estimate 20% error on our Zn/Si values due to uncertainties on molecular mass of hydrated sponge opal.

Although our data support the positive relationship found between POC and hexactinellid spicule Zn/Si found previously (Ellwood et al., 2004), our data show larger variability and we suggest that other factors that co-vary with POC may influence hexactinellid Zn incorporation, e.g. DOC, growth rate, and the physiological condition of the individual. In particular, spicule growth rate has a strong dependence on ambient  $[\text{Si}(\text{OH})_4]$  (Fröhlich and Barthel, 1997; Reincke and Barthel, 1997; Maldonado et al., 2011), and spicule Zn concentration may, in turn, depend on growth rate: the faster the spicules grow, the higher the Si:Zn ratio and the lower the spicule Zn concentration.

The tight coupling of inorganic nutrients, growth rate, POC and DOC means that it is challenging to deconvolve the influence of the different parameters on spicule Zn content in hexactinellids. However, the hexactinellid  $\delta^{66}\text{Zn}$  values are similar to the average Zn



**Fig. 6.** (A) The Dissolved Organic Carbon (DOC) content of seawater plotted vs. depth; (B) the estimated Zn/Si and (C) Zn isotopic composition (error bars show the external reproducibility (2SE)) of marine sponge spicules plotted for the different classes vs. DOC. The gray bar shows deep seawater Zn isotope composition.

isotopic composition of the deep-ocean ( $<200 \text{ m}$ , Zhao et al., in review) and show no clear relationship with DOC (Fig. 6C). Hence, we suggest that the Zn incorporated into hexactinellid spicules largely derives from the inorganic dissolved pool, and that the weak inverse Zn concentration relationship with water depth derives from a growth rate effect, or from Zn loosely bound with dissolved organic ligands or small ( $<5 \mu\text{m}$ ) particulates, having a similar  $\delta^{66}\text{Zn}$  composition as the seawater dissolved Zn. Uptake of Zn from this internal pool results in very little isotopic fractionation, causing no net change from seawater values. Some minor deviation towards lighter values observed in one of the hexactinellid samples ( $+0.25 \pm 0.10\%$ ), which is barely outside analytical uncertainty of the deep seawater isotopic value, may be a

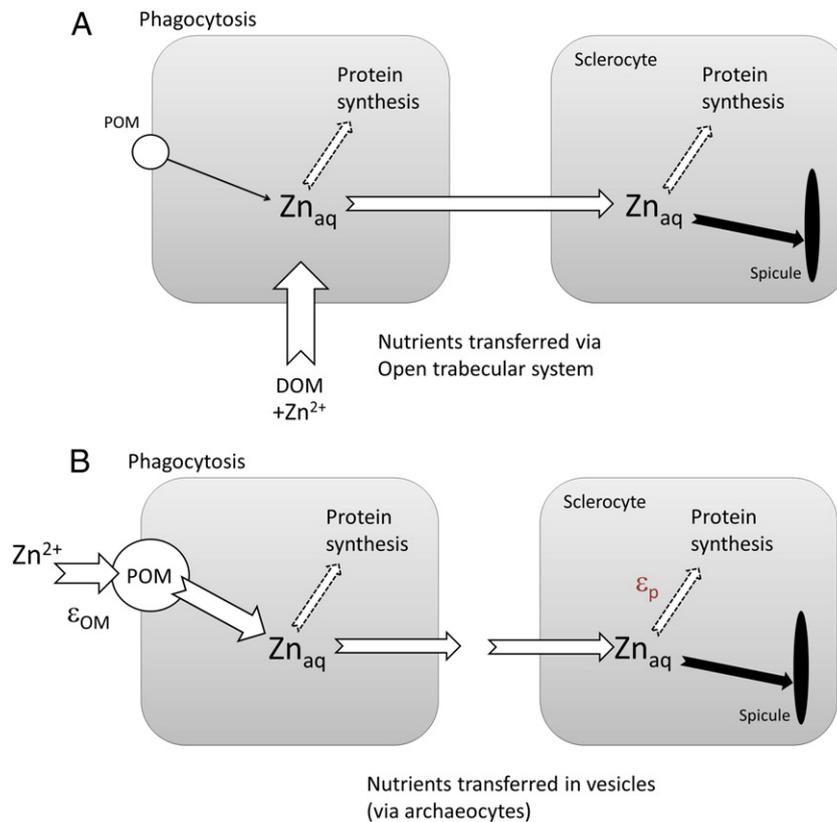
result of some uptake of Zn incorporated within POM, with a lighter Zn isotope composition. Due to the open trabecular system, there is no depletion or distillation of isotopes in the internal pool of nutrients (Fig. 7A). Hence it is most likely that external processes and environmental parameters control the Zn content and isotopic composition of hexactinellid spicules, possibly via the sponge growth rate.

#### 4.3.2. Demosponges

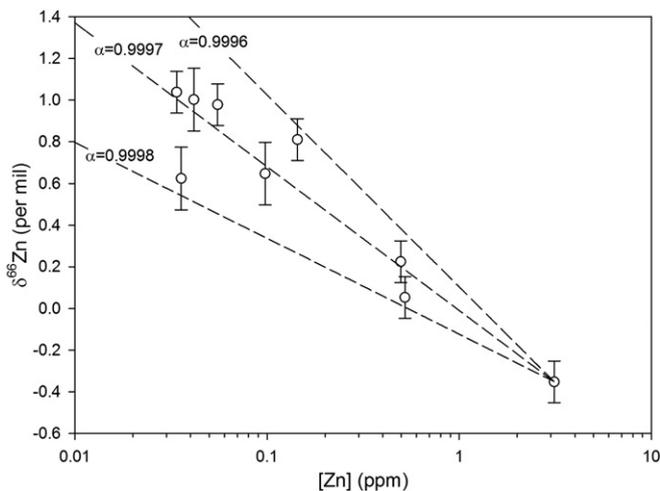
Demosponges, conversely, can utilize a larger range of particle sizes, and may take up a greater amount of their nutrients from particulate matter compared to hexactinellids (Reiswig, 1971; Reiswig, 1990; Bory et al., 2001). Hence, the variable Zn isotopic compositions of demosponge spicules could be explained by different Zn isotopic compositions of particulate material, for example isotopically light Zn from organic matter (e.g. John et al., 2007; Peel et al., 2009). Additionally, the Zn may then be internally fractionated during uptake of Zn into cellular material (e.g. metalloenzymes), which may explain the heavier Zn isotope values obtained in some spicule samples. Internally, the nutrient pools in demosponges are not connected by an open trabecular system, but rely on transportation by specialized cells, which may result in isotopic distillation. Progressive depletion of the internal pool of Zn may result in isotopic fractionation such that as more of the Zn available internally is taken up into “soft” cellular material, less is available for incorporation into the spicules. Assuming that the spicule Zn content reflects the ambient Zn concentration of the internal pool, as the cellular Zn progressively decreases, the spicule Zn content will also decrease. Similarly, assuming  $\delta^{66}\text{Zn}$  reflects the isotopic composition of the intracellular fluids,

where the soft cellular material fractionates the Zn isotopes towards lighter values, the spicules become isotopically heavier as the Zn availability decreases (Fig. 7B).

Based on the above assumptions, Zn uptake and utilization efficiency can be modeled using a simple “Rayleigh” fractionation style model. We assume that the initial Zn available within the sponge cells originates from the POM on which the organism feeds. The internalized Zn will then evolve with an isotopic fractionation during the progressive depletion of the Zn pool ( $\epsilon_p$  in Fig. 7). The composition of POM is unknown, but our best estimate comes from sample NBP805-TB1-6, with the lightest Zn isotopic composition of  $\delta^{66}\text{Zn} -0.35\%$  and highest demosponge spicule Zn concentration of 3.12 ppm (Fig. 8). This Zn isotopic composition is  $\sim 0.85\%$  lighter than the deep seawater value, which is similar to the largest Zn isotope fractionation observed for diatom culturing experiments (0.2 to 0.8‰, John et al., 2007) and  $\sim 0.8\%$  lighter  $\delta^{66}\text{Zn}$  values observed in settling organic particulates during algal blooms in Lake Greifen, Switzerland (Peel et al., 2009). Given this assumption for Zn isotope and concentration of the POM the sponges feed on, the evolution of the Zn may then be modeled using different internal fractionation factors ( $\epsilon_p$ ) in relation to the data, where the best fit suggest a  $\epsilon_p$  of  $\sim -0.3\%$  (Fig. 8). There are of course other additional factors that may influence the Zn concentration and isotopic composition of the spicules, for instance differences in the utilization efficiency or fractionation factor of individual sponges and variations in POM Zn isotope compositions (e.g. due to seasonal changes in surface productivity and Zn uptake) and Zn concentrations could influence spicule composition. However, the data observed so far are entirely consistent with our model, such that the Zn content of demosponges most



**Fig. 7.** Schematic to illustrate our model of Zn isotopic fractionation in hexactinellids and demosponges. (A) Hexactinellids feed on ultraplankton/dissolved organic matter, and have an open “trabecular” network for communication between cells (see main text for references). The majority of dietary Zn is associated with dissolved organic matter or bound loosely with small particulates; there is little internal fractionation such that hexactinellid spicules record seawater isotopic compositions. (B) Demosponges feed on larger particles by phagocytosis, carried out by cells on nearly all surfaces. Zn incorporation into the organic particles results in isotopic fractionation ( $\epsilon_{OM}$ ). Differentiated cells (archaeocytes) then move the nutrients via vesicles through a canal system e.g. to sclerocyte cells that produce spicules. The nature of the nutrient distribution system in demosponges may be responsible for the fractionation of Zn isotopes: as more of the dietary Zn is incorporated into cellular material (with fractionation factor  $\epsilon_p$ ), the remaining Zn available for incorporation into spicules becomes progressively isotopically heavier.



**Fig. 8.** Fractionation model of Zn isotope uptake by demosponges, assuming a constant Zn isotopic composition of organic matter, but varying  $\epsilon_p$ , the isotopic fractionation ( $\alpha$  shown by dashed lines) associated with cellular uptake of Zn (maximum [Zn] ~3.1 ppm). Error bars show the external reproducibility (2SE).

likely reflects internal processes relating to feeding strategy and physiology that influence either fractionation or Zn utilization efficiency. The dominant internal control on demosponge Zn isotope fractionation results in a poor relationship with external, environmental parameters such as organic matter concentrations (e.g. Fig. 6).

## 5. Zinc isotopes in opal: a paleoproductivity proxy?

Our investigations into Zn isotopic fractionation during spicule formation have provided insight into important physiological differences between the two major clades of marine sponge: the demosponges and the hexactinellids. Furthermore, the results highlight the potential use of spicules as geochemical archives.

Paleoproductivity estimates drawn from Zn isotopes have been carried out previously using carbonate material (Pichat et al., 2003; Kunzmann et al., 2013). Pichat et al. (2003) investigated Zn isotope composition in coccolith-dominated leached sediment fractions from the equatorial Pacific over the past 175 ky. The Zn isotope compositions of leached carbonate sediments show cyclic behavior, which corresponds to glacial–interglacial timescales, and heavier Zn isotope compositions are tentatively correlated with high surface ocean biological productivity, where light Zn is progressively utilized by biological matter and exported (Pichat et al., 2003). Similar interpretations have been made of Zn isotope compositions in dolostone from the Ediacaran period (~635 ka) just prior to the Marinoan “Snowball Earth” period (Kunzmann et al., 2013). Here, again, deviations to heavier Zn isotopic compositions in the carbonates were interpreted to reflect increased primary productivity in the surface ocean. However, there are still significant uncertainties in regard of what carbonates actually record in their Zn isotope compositions due to the current lack of core-top calibrations and laboratory experiments for Zn isotopes in carbonates. In contrast, the systematic behavior of Zn isotope and concentration systematics in diatom opal may provide a more robust estimate of Zn utilization in the surface ocean (Andersen et al., 2011). However, using sediment archives of surface productivity may be spatially limited and reveal information on local – rather than regional or global – processes, requiring an exact knowledge of the paleoceanographic setting.

Hexactinellids appear to closely track the Zn isotopic composition of deep seawater and thus offer an established archive for recording the deep seawater Zn isotopic composition and changes in global Zn utilization back through time. The recent compilation of deep seawater Zn

isotope composition suggests that the major modern ocean basins are close to uniform (Zhao et al., *in review*), thus any change in this deep seawater Zn isotopic composition implies a major change in ocean chemistry. For instance, a more vigorous biological pump may lead to an increased burial of organic carbon and a change in the total Zn inventory in the deep ocean. If the ocean Zn input is constant and the organic carbon draws down isotopically light Zn, it could be expected that the ocean Zn inventory would decrease in size and that the seawater  $\delta^{66}\text{Zn}$  would be driven towards heavier values. Any such shift in the deep ocean  $\delta^{66}\text{Zn}$  would be detectable in the Zn isotopic composition of hexactinellid spicules, although the robust interpretation of such a proxy would require a greater understanding of the global cycling of Zn and its isotopes (e.g. Zhao et al., *in review*).

The long fossil record of sponges, including hexactinellids, means that they are ideal target archives of marine Zn cycling over a wide range of timescales. Sponges appear in the fossil record as far back as the Ediacaran (Brasier et al., 1997), and so may provide a well-constrained record of Zn cycling across the late Precambrian–Cambrian boundary. During the Phanerozoic, sponge spicules could be used to investigate Zn cycling during periods of widespread changes in ocean productivity over major climate intervals, such as the Ocean Anoxic Events of the Mesozoic (e.g. Monteiro et al., 2011), and the Cenozoic glaciations (e.g. Palike, 2012). For the latter, spicule-rich sediments from the Southern Ocean could be targeted, to complement existing records of spicule silicon isotopes, in order to investigate global productivity changes across the Eocene–Oligocene boundary as a result of Antarctic glaciation (de la Rocha, 2003; Egan et al., 2013).

## 6. Conclusions

We have produced the first zinc (Zn) isotope measurements from modern sponge spicules collected in the Southern Ocean. Our results show that sponge opal has a large range in Zn isotopic compositions, which reflect differences in the feeding behavior and physiology of the two major classes of sponges, hexactinellids and demosponges. Hexactinellids show a weak negative relationship between spicule Zn concentration and silicic acid concentration and a weak positive relationship with DOC. While it is difficult to deconvolve the possible influencing factors, we suggest that a combination of environmental factors can influence Zn uptake into hexactinellids via growth rate, including inorganic nutrients and dissolved or particulate organic matter supply. Hexactinellids record the average deep ocean  $\delta^{66}\text{Zn}$  composition, and may prove useful as archives of past whole-ocean changes in Zn cycling. Conversely, demosponges show weaker links between Zn incorporation and environmental conditions, and their Zn isotopic compositions reflect a combination of particulate organic matter composition and internal fractionation processes. Our hypotheses can be tested through further investigation of  $\delta^{66}\text{Zn}$  compositions of water column particulate matter, and the isotopic fractionation processes that occur during speciation of dissolved Zn.

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