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Silicon isotopes in Antarctic sponges: an interlaboratory comparison

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Abstract: Cycling of deepwater silicon (Si) within the Southern Ocean, and its transport into other ocean basins, may be an important player in the uptake of atmospheric carbon, and global climate. Recent work has shown that the Si isotope (denoted by δ^{29}Si or δ^{30}Si) composition of deep sea sponges reflects the availability of dissolved Si during growth, and is a potential proxy for past deep and intermediate water silicic acid concentrations. As with any geochemical tool, it is essential to ensure analytical precision and accuracy, and consistency between methodologies and laboratories. Analytical bias may exist between laboratories, and sponge material may have matrix effects leading to offsets between samples and standards. Here, we report an interlaboratory evaluation of Si isotopes in Antarctic and sub-Antarctic sponges. We review independent methods for measuring Si isotopes in sponge spicules. Our results show that separate subsamples of non-homogenized sponges measured by three methods yield isotopic values within analytical error for over 80% of specimens. The relationship between δ^{29}Si and δ^{30}Si in sponges is consistent with kinetic fractionation during biomineralization. Sponge Si isotope analyses show potential as palaeoceaongraphic archives, and we suggest Southern Ocean sponge material would form a useful additional reference standard for future spicule analyses.

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Key words: biogeochemistry, calibration, nutrient, porifera, silicic acid, Southern Ocean

Introduction

Silicon isotopes and previous interlaboratory calibrations

The use of silicon (Si) isotopes in geosciences has expanded in the past decade to include cosmochemistry (e.g. Georg *et al*. 2007), earth surface processes (e.g. Opfergelt *et al*. 2009), palaeoceanography (e.g. de la Rocha *et al*. 1998, Brzezinksi *et al*. 2002, Beucher *et al*. 2007, 2008), palaeolimnology (Street-Perrott *et al*. 2008, Leng *et al*. 2009, Swann *et al*. 2010) and biological systems, including Si uptake by diatoms (de la Rocha *et al*. 1997), plants (Opfergelt *et al*. 2006, Hodson *et al*. 2008) and sponges (de la Rocha 2003, Hendry *et al*. 2009, 2010, Wille *et al*. 2010).

Si is present in three stable isotopes: ^{28}Si (92.22%), ^{29}Si (4.68%) and ^{30}Si (3.08%). The fractionation factor during a reaction from A to B, α, is defined by Eq. (1):

$$ {}^{x}\alpha = \frac{\left(\frac{{}^{x}Si}{{}^{28}Si}\right)_{A}}{\left(\frac{{}^{x}Si}{{}^{28}Si}\right)_{B}}, \qquad (1) $$

where x is either of the two minor isotopes, ^{29}Si or ^{30}Si. The per mil (‰) Si isotopic composition is expressed relative to the NIST standard, NBS 28, according to Eq. (2):

$$ \delta^{x}Si = \left\{ \frac{\left(\frac{{}^{x}Si}{{}^{28}Si}\right)_{sample}}{\left(\frac{{}^{x}Si}{{}^{28}Si}\right)_{NBS28}} - 1 \right\} \times 1000. \qquad (2) $$

The relationship between measured δ^{29}Si and δ^{30}Si can be used to demonstrate mass dependent fractionation, according to Eq. (3):

$$ {}^{29}\alpha = {}^{30}\alpha^{z}, \qquad (3) $$

where z~0.52 and depends on the ratio of the isotope masses and on whether the fractionation reaction is kinetic or in equilibrium (reviewed by Reynolds *et al*. 2007).

A previous interlaboratory comparison has been published using three isotopic standards: a rock standard IRMM-018, a "Diatomite" standard, and a highly fractionated SiO_2 material, "Big Batch", originally prepared at the University of California, Santa Barbara (UCSB) by Mark Brzezinksi and Christina de la Rocha (Reynolds *et al*. 2007). However, IRMM-018 is no longer produced commercially and did not show a good level of reproducibility between research groups,

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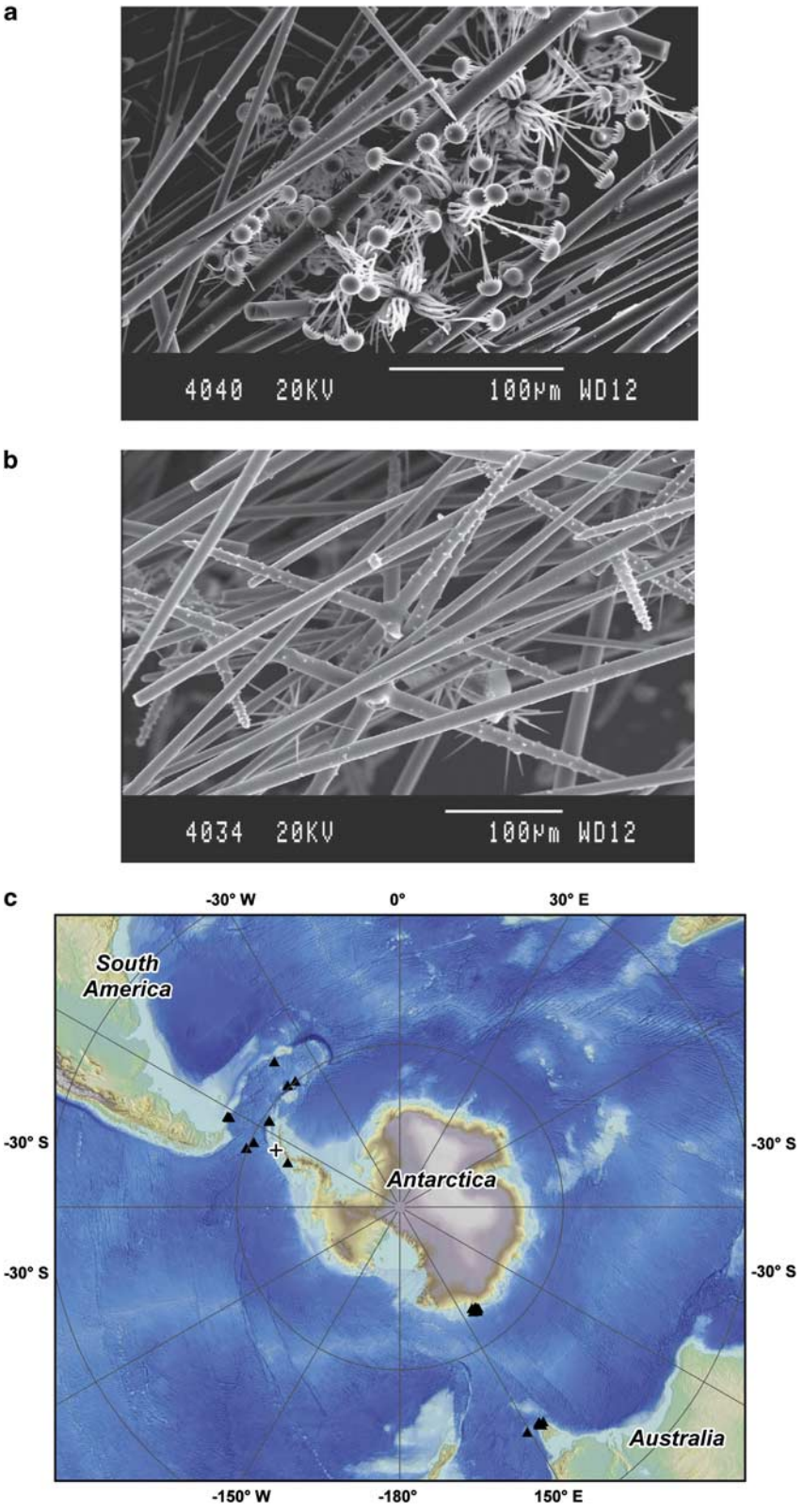


Fig. 1. Scanning electron micrographs of sponge spicules: **a.** *Acoelocalyx* sp., and **b.** *Rosella* sp. **c.** Map of sample collection area. Black triangles show location of modern sponges from the Scotia Sea/Drake Passage (Hendry *et al*. 2010, this study) and the Pacific Sector (Wille *et al*. 2010). The cross shows the location off Anvers Island of sponge LMG08, analysed by Hendry *et al*. (2010) and NIGL/WHOI (this study). Map produced by K. Scanlon, USGS.

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either as a result of isotopic heterogeneity or contamination, so is not a useful reference standard.

Sponge silicon isotopes and deepwater silicic acid

Reconstructing Southern Ocean intermediate and deep $Si(OH)_4$ through time is essential to our understanding of silica cycling, and its potential impact on the carbon cycle. Firstly, diatom blooms rely on upwelling sources of $Si(OH)_4$ because efficient utilization removes almost all of the Si from surface waters (Ragueneau *et al*. 2000, Falkowski *et al*. 2004). Secondly, heat transport by the oceans is influenced strongly by high-latitude deepwater formation processes and meridional distribution of deepwater masses. Ocean circulation and biogeographical variations in algal populations results in enriched $Si(OH)_4$ in Antarctic Bottom Water (AABW) compared to North Atlantic Deep Water (NADW) (Garcia *et al*. 2006). This difference allows dissolved Si to be used as a tracer of southern component water masses in the Atlantic over 10^3–10^4 year timescales. Thirdly, whole ocean changes in Si cycling over long timescales ($> 10^4$–10^5 years) will be reflected in deep water $Si(OH)_4$ concentrations to a greater extent than surface values, which are probably influenced by local processes such as productivity (de la Rocha & Bickle 2005). The Southern Ocean has been the major sink of opal for the past 2–3 million years, and is a key source area for such palaeoceanographic records (Cortese *et al*. 2004).

The Si isotopic composition of sponges has been recognized as a potential archive of deepwater Si chemistry (de la Rocha 2003, Hendry *et al*. 2010, Wille *et al*. 2010). Siliceous sponges (Phylum Porifera, Classes Demospongea and Hexactinella) produce needle-like skeletal elements, called spicules, from hydrated amorphous silica. Spicules can be further subdivided into larger megascleres and smaller microscleres (Fig. 1a & b). Uptake of ambient $Si(OH)_4$ occurs via a sodium transporter, which resembles active transporters isolated from other metazoans. The silica deposition occurs about a central organic filament in the axial canal of the filament. Biosilicification is carried out by sclerocyte cells both intra- and extra-cellularly and is controlled by the enzymes silicatein, which promotes condensation reactions, and silicase, which dissolves silica (Uriz *et al*. 2000, 2003, Foo *et al*. 2004, Müller *et al*. 2007). Silicatein is the predominant component of the axial filament and is found on the surface of spicules and in the extracellular space, resulting in lamellar growth (Müller *et al*. 2007). During these reactions, sponges fractionate Si isotopes with respect to the ambient $Si(OH)_4$, such that spicules have some of the lightest Si isotopic signatures known in natural systems (de la Rocha 2003).

With the development of Si isotopes in sponges as a geochemical proxy, it is essential to determine analytical precision and accuracy, and ensure there are no systematic differences between methodologies. Here, we present an interlaboratory comparison of the Si isotopic composition of sponges from the Southern Ocean and the Antarctic Peninsula. We review two independent methods for sample preparation, using three different instruments for Si isotope analysis. We show that sponge Si isotope ratios are homogeneous and influenced strongly by environmental parameters. Further work on the sponge Si isotope uptake and systematics would benefit greatly from a suitable interlaboratory reference standard. Although "Diatomite", which contains non-opal impurities, and "Big Batch" reproduced well ($\delta^{30}Si \sim +1.27$ and -10.48‰ respectively), neither standard has an isotope composition or microstructure similar to sponges (Schroeder *et al*. 2008).

Methods

Sample collection and initial preparation

We collected and analysed modern specimens of sponges from a north–south transect across the Southern Ocean, encompassing a range of $Si(OH)_4$ concentrations (12–120 μM) and depths (300–2500 m). Sponges were collected aboard the RV *Nathaniel B. Palmer* from sites in the Drake Passage and Scotia Sea (April–May 2008; Fig. 1c). Additional samples were collected from Anvers and Adelaide islands off the West Antarctic Peninsula. Spicules were isolated from organic matter by heating three times in concentrated HNO_3 and H_2O_2 (reagent grade), rinsing each time with 18 MΩ Milli-Q water. Detrital lithogenic grains were then physically removed until visual inspection showed the sample to comprise pure sponge spicules. The spicules were then further chemically cleaned by heating in 50% HNO_3 and 10% HCl (in house Teflon distilled) for two hours, followed by five Milli-Q rinses. Subsamples were analysed by three different laboratories using different methods: stepwise fluorination followed by gas-sourced Isotope Ratio Mass Spectrometry (IRMS) at the NERC Isotope Geosciences Laboratory (NIGL; Leng & Sloane 2008), wet alkaline extraction followed by analysis by NuPlasma Multi-Collector Inductively Coupled Plasma Mass Spectrometer (MC-ICP-MS) at Oxford University (Georg *et al*. 2006, Hendry *et al*. 2010) and wet alkaline extraction followed by analysis by Neptune MC-ICP-MS (Van den Boorn *et al*. 2006, Wille *et al*. 2010, this study) at Woods Hole Oceanographic Institution (WHOI).

Stepwise fluorination/IRMS (NIGL)

The subsamples were processed using stepwise fluorination, designed for both $\delta^{30}Si/\delta^{29}Si$ and $\delta^{18}O$ analysis of silica, to convert the Si (and O) to a gaseous phase (Leng & Sloane 2008). First, the samples were dehydrated at 250°C to remove surface and loosely bound water. Secondly, the samples went through a pre-fluorination reaction with a stoichiometric deficient quantity of bromine pentafluoride at low temperature to remove hydroxyl groups and any remaining loosely bound water. Thirdly, the samples were fully fluorinated at 450°C for

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Table I. Operating conditions of the Neptune MC-ICP-MS at WHOI. Note that the senstitivies reported are for high resolution settings on the Neptune, and medium resolution settings on the NuPlasma.

Parameter	Operating conditions and comments	
	Neptune (High resolution)	NuPlasma (Medium resolution)
Cones	Nickel X-cones	Nu instrument experimental WA cones
Nebulizer uptake rate	50 μl min^{-1}	100 μl min^{-1}
Cup configuration	Centre cup - left "shoulder" of ^{28}Si	L5 - ^{28}Si
	H1 - ^{29}Si	Ax - ^{29}Si
	H2 - ^{30}Si	H6 - ^{30}Si
Cycles/block	30	20
Integration time/cycle	4.2 sec	8 sec
Sensitivity	10–15 V for 1.5 ppm Si (^{28}Si)	~10 V for 0.6 ppm Si (^{28}Si)
	300–600 mV for 1.5 ppm Si (^{29}Si & ^{30}Si)	300–500 mV for 0.6 ppm Si (^{29}Si & ^{30}Si)
Blank	~50 mV on ^{28}Si	~30 mV on ^{28}Si
Blank/signal	< 1% (typically ~ 0.5–0.8%)	< 1% (typically < 0.5%)

12 hours, during which the Si is converted to silicon tetrafluoride (SiF_4), which is then measured off-line for ^{28}Si, ^{29}Si and ^{30}Si simultaneously using a Finnegan MAT253 IRMS. Repeat measurements of standards indicate a reproducibility of < 0.24‰ (2 SD) for $\delta^{29}Si$ and $\delta^{30}Si$.

Wet alkaline extraction/NuPlasma MC-ICP-MS (*Oxford*)

The subsamples were dissolved by wet alkaline extraction (Cardinal *et al*. 2007). Briefly, the spicules were dissolved in 4 ml 0.2 M NaOH per mg silica, left at 100°C for three days, and sonicated on a daily basis to aid dissolution. The solutions were acidified with 0.2 M HCl (in house Teflon distilled) to a pH > 2.

The Si concentrations of the dissolved samples and standards were determined using a heteropoly blue photospectrometric method (Ultra Low Range solutions, Hach). Quantitative separation of Si from major ions was achieved using a cation exchange resin (BioRad AG50W-X12; Georg *et al*. 2006). The standard and samples (< 0.4 ml) were introduced to the column, which contained a volume of wet resin at neutral pH suitable for the amount of Na added (0.8 to 1.8 ml). $Si(OH)_4$ is in equilibrium with the anionic silicate species $H_3SiO_4^-$ for the pH range 2–8 (Georg *et al*. 2006), and can simply be eluted with Milli-Q water. Recent studies have shown pH does not affect Si yield or fractionation on the column (Savage *et al*. 2010). Our previous tests have shown this method results in 100% yields for both commercially available Si solutions and dissolved biogenic opal samples (Hendry *et al*. 2010, Hendry unpublished data). There are traces of organic matter bound within biogenic opal, including sponge spicules, which may form small silico-organic complexes that cannot be removed from the Si and may cause instabilities with plasma source mass spectrometry. However, our measurements of the organic content of sponge spicules show the organic content is ~ 0.1% or lower, and any problems arising from this material will be minimal.

The Si isotope measurements of the Si isotopes (^{28}Si, ^{29}Si and ^{30}Si) use the NuPlasma MC-ICP-MS (University of Oxford), again allowing simultaneous measurement of the different isotopes. The mass spectrometer was operated in medium resolution mode to resolve interferences on masses 28 (e.g. $^{14}N^{14}N^+$, $^{12}C^{16}O^+$), 29 ($^{14}N^{15}N^+$, $^{13}C^{16}O^+$, $^{12}C^{17}O^+$) and 30 ($^{15}N^{15}N^+$, $^{13}C^{17}O^+$, $^{12}C^{18}O^+$). Samples were introduced via a self-aspirating PFA micro concentric nebuliser (ESI) plugged into a Cetac ARIDUS-2 desolvator unit, supplied only with Ar and not N_2. Uptake rates varied slightly, but were typically around 100 μl min^{-1} (Table I). The samples were bracketed with a concentration-matched NBS28 standard (~600 ppb Si) to correct for mass bias and drift (Georg *et al*. 2006, Reynolds *et al*. 2007), and isotope ratios calculated according to Eq. (2). Standards were analysed before every batch run to ensure accuracy. Repeat dissolutions and repeat aliquots of the same dissolution indicate a good

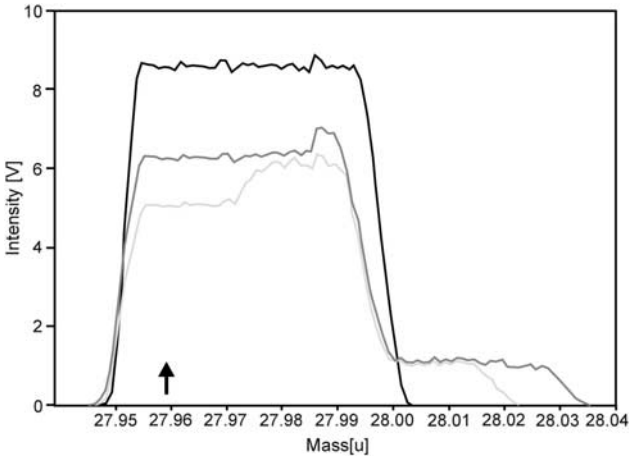


Fig. 2. Peak scans for silicon isotopes ^{28}Si (black), ^{29}Si (dark grey) and ^{30}Si (pale grey) in high-resolution and dry plasma mode, obtained with a 1.5 ppm solution of Si in ~0.05N Teflon-distilled HCl. Note the vertical exaggeration for ^{29}Si (x 13.88) and ^{30}Si (x 16.35). The measurements were carried out on the left side of the peak plateau to avoid the interferences, clearly visible in high-resolution mode (the black arrow shows the position of the magnet).

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Table II. δ^{30}Si measurements of standards, comparing a previous interlaboratory comparison (Reynolds *et al.* 2007), and measurements made at WHOI (this study). Numbers in parentheses show 1SD.

Standard	δ^{30}Si (‰) (Reynolds *et al.* 2007)	δ^{30}Si (‰) WHOI All measurements	δ^{30}Si (‰) WHOI Mean values for each aliquot
Diatomite	+1.26 (0.22)	+1.25 (0.12) $n = 15$	+1.28 (0.07) $n = 5$
Big Batch	-10.48 (0.22)	-10.61 (0.14) $n = 9$	-10.61 (0.15) $n = 3$

level of reproducibility around 0.1‰ (2 SD) for δ^{30}Si and 0.2‰ (2 SD) for δ^{30}Si (Hendry *et al.* 2010). Full details of experimental set-up and quality control criteria are published elsewhere (Georg *et al.* 2007, Savage *et al.* 2010).

Wet alkaline extraction/Neptune MC-ICP-MS (WHOI)

Aliquots of a solution prepared from a sponge collected from Anvers Island off the West Antarctic Peninsula (LMG08), prepared by wet alkaline extraction and column separation as outlined above, were introduced into the Thermo Neptune MC-ICP-MS instrument. The instrument was operated in a dry plasma mode, with the sample introduced via a self-aspirating PFA micro concentric nebuliser, plugged into an ARIDUS-1 (not connected to N_2) with an uptake rate of 50 μl min^{-1}, in high-resolution mode to resolve potential interferences as outlined above. The instrument was left to stabilize after plasma ignition for approximately an hour before tuning on a daily basis, during which gas flow and ion optics are optimized for maximum sensitivity and peak shape on ^{28}Si (Fig. 2). Peak centring was carried out on ^{28}Si prior to measurement. Operating conditions are outlined in Table I.

Mass bias and drift were accounted for by standard-sample bracketing matching samples and bracketing standards. Our initial tests showed intensity matching of bracketing standards and samples of within 20% resulted in acceptable levels of reproducibility for the known reference standards (± 0.15‰ for δ^{30}Si). To provide a conservative limit, the bracketing standards and samples were intensity matched within 15%. The blank is monitored by analysing a 0.05N HCl solution run prior to and after each standard-sample bracket, and is < 1% of the signal (Table I). Blank corrections do not make significant differences to the calculated δ^{29}Si or δ^{30}Si for standards or samples.

Values of δ^{29}Si and δ^{30}Si were calculated offline using Eq. (2), taking an average of the two bracketing standards for each sample. Data that did not meet strict quality control criteria were rejected, meeting guidelines based on Van den Boorn *et al.* (2006):

a) Initial tests showed that for a standard deviation of greater than 1 x 10^{-5} on either ^{29}Si /^{28}Si or ^{30}Si /^{28}Si ratios resulted in an error greater than ~ 0.2‰ on the resulting δ^{29}Si and δ^{30}Si values, which were generally

Table III. Sponge silicon isotopes measured by IRMS at NIGL. For more information about the specimens, see Hendry *et al.* 2010.

Specimen code	δ^{29}Si (‰)	δ^{30}Si (‰)
NBP0805-TB1-3	-0.56	-1.05
NBP0805-TO1-57	-1.69	-3.27
	-1.68	-3.28
NBP0805-TB4-24	-1.21	-2.36
NBP0805-DR29-47	-1.80	-3.63
NBP0805-TO1-27	-1.91	-3.71
NBP0805-DR7-47	-1.69	-3.29
NBP0805-TB4-3	-0.87	-1.72
NBP0805-DR34-47	-1.15	-2.22
NBP0805-DR35-111	-1.23	-2.39
NBP0805-TB1-6	-0.54	-1.02
	-0.48	-0.90
NBP0805-DR13-47	-1.55	-3.00
NBP0805-TO3-111	-1.36	-2.62
	-1.26	-2.43
NBP0805-TO3-100	-1.55	-2.99
	-1.49	-2.94
NBP0805-TB4-27	-1.34	-2.59
NBP0805-DR16-27	-2.04	-3.94
LMG08	-1.71	-3.29

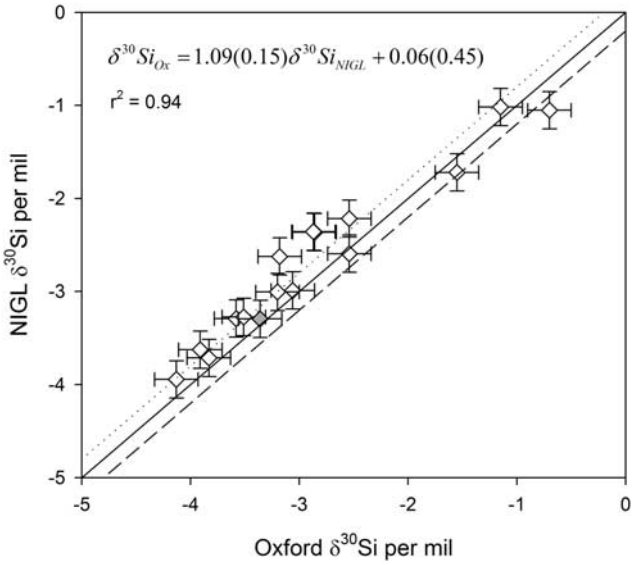


Fig. 3. Interlaboratory comparison of sponge silicon isotopes, measured by *NuPlasma* MC-ICP-MS at Oxford (Hendry *et al.* 2010) and Finnegan MAT253 at NIGL (this study). Error bars show long-term reproducibility (2 SD). Note that measurements were made on non-homogenized subsamples of sponge specimens, explaining the small variations between laboratories. The grey diamond shows sponge LMG08. Solid line shows 1:1, dashed lines show ± 0.2‰.

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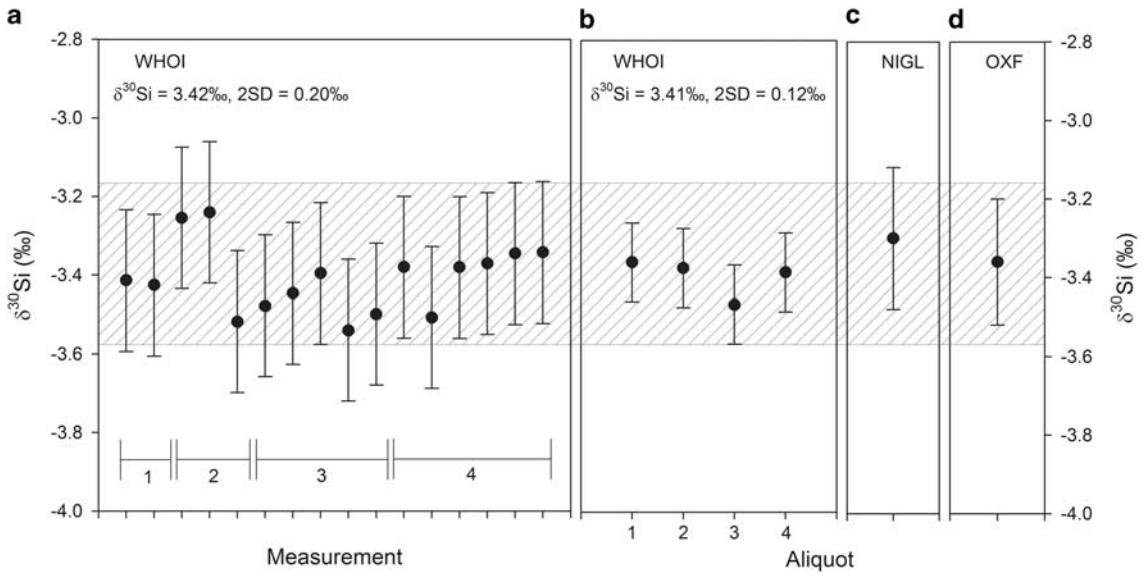


Fig. 4. Repeat measurements of different subsamples of sponge LMG08, collected near Anvers Island off the West Antarctic Peninsula. Different aliquots of a subsample were measured at WHOI on different occasions over four months. **a.** All measurements made at WHOI (error bars show 2 SD), **b.** the mean value for each aliquot measured at WHOI (error bars show 2 SD), **c.** subsample measure at NIGL (error bars show long-term reproducibility 2 SD), **d.** mean value for the subsample measured in Oxford (mean of eight repeat measurements, error bars show 2 SD). The grey hatched area shows the mean value for all measurements (± 0.2‰).

non-mass dependent. Any samples or bracketing standards run with a standard deviation above this threshold were rejected.

b) If the difference between two successive bracketing standards exceeded 0.4‰ for δ^{30}Si (double the approximate long-term reproducibility 2 SD), the data were rejected.

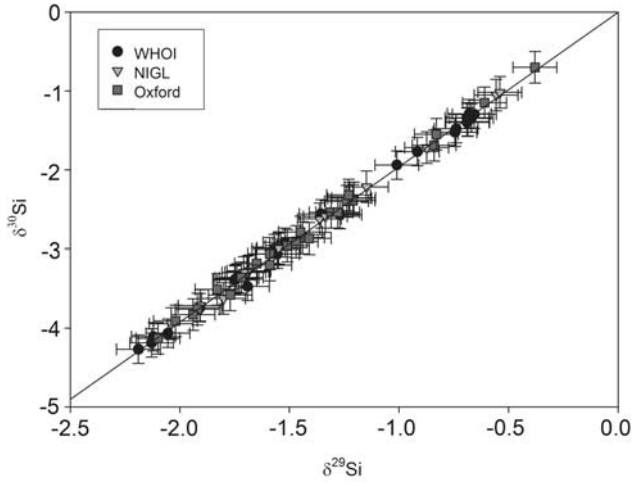


Fig. 5. Three-isotope plot of all the silicon isotope measurements of all sponge samples analysed at the three laboratories. The slope of the plot is consistent with mass dependent fractionation, such that $\delta^{29}Si \sim 0.51\delta^{30}Si$. Error bars show long-term reproducibility (2 SD). The line shows mass dependent fractionation with slope 0.51.

The mean of all replicates that meet the criteria above (typically $n = 3$) for each aliquot was calculated and reported relative to NBS28.

Measurement precision was assessed using previously calibrated standards ("Diatomite" and "Big Batch"; Reynolds *et al*. 2007). Diatomite showed a good level of reproducibility, with $\delta^{29}Si \sim 0.66$‰ and $\delta^{30}Si \sim 1.26$‰ depending on whether all measurements are included, or mean values of each aliquot undergoing independent chemical separation (Table II). Big Batch showed greater variability (Table II), appearing to be more sensitive to drift due to larger fractionation, perhaps as a result of matrix effects from its high molybdenum content (Reynolds *et al*. 2007). Repeat measurements of sample aliquots, within and between runs, show typical variability < 0.1‰ for δ^{29}Si and δ^{30}Si. However, the sample size of measurements for each aliquot is small ($n \sim 3$), such that calculating standard deviations is not valid, and so the long-term reproducibility for diatomite is used as a more conservative estimate of total error (Table II).

All three methods show a very similar long-term reproducibility (2 SD ~ 0.2‰ for δ^{30}Si), in agreement with the finding of the previous interlaboratory calibration of standards that differences between reported results should be limited to 0.2‰ for δ^{30}Si (Reynolds *et al*. 2007).

Results

The δ^{30}Si values of sixteen modern sponges measured at NIGL varied from approximately -0.5 to -4‰ (Table III), all

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Table IV. A comparison of the linear regressions of $\delta^{29}Si$ on $\delta^{30}Si$ at the three laboratories (model II linear regression, calculated using R, showing 95% confidence intervals). Note that all laboratories show results consistent with kinetic fractionation (gradient = 0.505 or 0.509); only one laboratory shows results consistent also with equilibrium fractionation (gradient = 0.518).

Laboratory	Gradient $\delta^{29}Si$ vs $\delta^{30}Si$			Intercept $\delta^{29}Si$ vs $\delta^{30}Si$		
		Max	Min		Max	Min
Oxford	0.504	0.516	0.492	-0.021	+0.016	-0.057
NIGL	0.507	0.519	0.500	-0.021	+0.012	-0.054
WHOI	0.509	0.515	0.502	-0.007	+0.014	-0.027

in range of other published values (de la Rocha 2003, Hendry *et al*. 2010, Wille *et al*. 2010). Subsamples from these specimens had been previously analysed at Oxford (previously reported in Hendry *et al*. 2010). Note that although the spicules were not homogenized prior to analysis, subsample measurements of any particular specimen are in good agreement (Fig. 3; regressing Oxford data on NIGL data yields $r = 0.97$, slope = 1.09, intercept = 0.06‰). Over 80% of the specimens yielded $\delta^{30}Si$ values that agreed within ± 0.2‰, (i.e. agreement within analytical error), and all agreed within ± 0.3‰ (including OT3-111 and DR-111, two specimens of the same species of demosponge). Repeat subsamples analysed at NIGL agreed within ± 0.1‰ for $\delta^{30}Si$ (Table III).

Subsamples from a sponge collected near the Antarctic Peninsula, LMG08, were measured by Oxford (Hendry *et al*. 2010), NIGL and WHOI (both this study), and also showed a high level of reproducibility (Fig. 4; Oxford $\delta^{30}Si$ = -3.36 ± 0.16‰ (one aliquot with repeat measurements; $n = 8$), NIGL (one aliquot) $\delta^{30}Si$ = -3.3‰, WHOI (all measurements, $n = 16$) = -3.41 ± 0.18‰; WHOI (mean values for independent aliquots, $n = 4$) $\delta^{30}Si$ = -3.40 ± 0.10‰, for 2 SD).

All three laboratories show mass dependent measurements for sponge samples, with the slope of the three isotope plot of $\delta^{29}Si$ vs $\delta^{30}Si \sim 0.51$ (Fig. 5, Table IV, see discussion below).

Discussion

Isotopic homogeneity in sponges

The agreement between the different laboratories, utilizing different preparation and measurement techniques, indicates that $\delta^{30}Si$ measurements obtained for Southern Ocean sponges are robust and there is no method dependent fractionation of Si isotopes. Furthermore, given that the subsamples were not taken from a homogenized mass of spicules, our data also indicate there is a high level of homogeneity within Southern Ocean sponges. There are other lines of evidence for isotopic homogeneity within specimens of Southern Ocean sponge:

a) Spicules taken from external layers (dermal) and internal layers (perenchymal) from a sponge collected in the Drake Passage have, within error, the same isotopic composition (dermal $\delta^{30}Si$ = -2.87‰ and perenchymal $\delta^{30}Si$ = -2.96‰; Hendry *et al*. 2010).

b) Core top measurements of megaspciules picked from Scotia Sea sediments ($\delta^{30}Si \sim$ -3.9‰) are within error of modern sponges living near to the core site ($\delta^{30}Si \sim$ -4.1‰; Hendry *et al*. 2010), comprising both mega and microspicules (Fig. 1a).

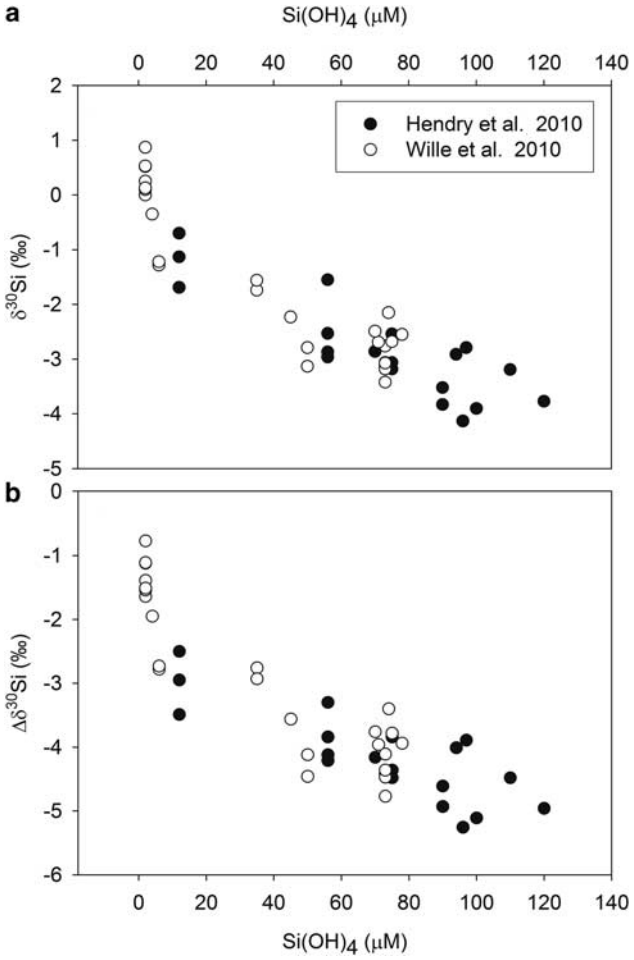


Fig. 6. Two independent studies of Southern Ocean sponge silicon isotopes from the Drake Passage/Scotia Sea (Fig. 1c; Hendry *et al*. 2010) and the Pacific Sector (Wille *et al*. 2010). **a.** The relationship between the sponge silicon isotope composition and ambient silicic acid, **b.** the relationship between the fractionation factor, $\Delta\delta^{30}Si$, defined as the difference between the isotopic composition of the sponge and ambient seawater: $\Delta\delta^{30}Si \approx \delta^{30}Si_{sponge} - \delta^{30}Si_{seawater}$

Kinetic uptake of Si by sponges

The relationship between ambient $Si(OH)_4$ and isotopic composition of sponges suggests a growth rate effect (Hendry *et al*. 2010). Kinetic uptake of Si has been observed during sponge growth (Frøhlich & Barthel 1997) and supported by the highly fractionated nature of sponge Si isotopes, and

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by theoretical isotopic fractionation models (Wille *et al.* 2010). The relationship between $\delta^{29}Si$ and $\delta^{30}Si$ can be used to determine whether kinetic or equilibrium fractionation has occurred: equilibrium fractionation yields a slope on a three-isotope plot (Fig. 5) of 0.518 whereas kinetic fractionation results in slopes of either 0.509 or 0.505 for Si atoms and SiO_2 respectively (Reynolds *et al.* 2007). Data from all three laboratories are consistent with kinetic fractionation (either Si or SiO_2, Reynolds *et al.* 2007) within 95% confidence intervals calculated by model II linear regressions (Table IV), whereas only data from one laboratory is within range of equilibrium fractionation. This supports the notion of kinetic uptake of Si by deep sea sponges in the Southern Ocean.

Use of sponge spicules as geochemical archives

Understanding the impact of surface biological production on carbon export in the past relies on the reconstruction of the nutrient supply from upwelling deep waters, both within the Southern Ocean and further afield. Sponges are an ideal marine geochemical archive of deepwater nutrients, given their ubiquitous distribution on the oceans. We confirm that Antarctic sponge Si isotopic composition show a robust relationship with ambient $Si(OH)_4$, are internally homogeneous, and measurements are reproducible irrespective of preparation and analytical method. Two independent calibrations, produced by different laboratories, show ambient $Si(OH)_4$ is the dominant control over both the Si isotopic composition of, and fractionation by, sponges collected in different sectors of the Southern Ocean (Hendry *et al.* 2010, Wille *et al.* 2010; Figs 1c & 6). There is no consistent species specific offset in isotopic composition or fractionation: different species from the same site show similar isotopic composition and two specimens of the same species from different $Si(OH)_4$ environments show different isotopic compositions (Hendry *et al.* 2010). There is no evidence for a significant influence of temperature, pH, or salinity on isotopic fractionation (Hendry *et al.* 2010, Wille *et al.* 2010). The scatter in the $Si(OH)_4$–$\delta^{30}Si$ calibration, which may reflect secondary vital effects, results in an uncertainty of any reconstructing $Si(OH)_4$ concentration of approximately ± 20 μM (Hendry *et al.* 2010).

The relationship between $Si(OH)_4$ and $\delta^{30}Si$ from the two calibrations agree well between $Si(OH)_4$ concentrations of ~5 and 80 μM. However, at concentrations ~2 μM, only sampled by one calibration, sponge $\delta^{30}Si$ become isotopically heavier than expected, suggesting either an exponential relationship with $Si(OH)_4$ concentration (Wille *et al.* 2010) or a non-linearity arising from physiological stress under low Si. Given the scatter in the calibration, a simple linear fit between $\delta^{30}Si$ and $Si(OH)_4$ is likely adequate for most applications; for shallow water environments experiencing low $Si(OH)_4$, an exponential fit may be appropriate. Further work is required to validate this calibration in other ocean basins, experiencing different nutrient regimes and physical environmental parameters. Culturing studies growing sponges under extreme concentrations of $Si(OH)_4$ (less than 5 μM and greater than 120 μM) may also elucidate the linear vs exponential nature of the relationship between $Si(OH)_4$ and $\delta^{30}Si$, and resolve the cause of scatter in the calibrations.

Sponge $\delta^{29}Si$ and $\delta^{30}Si$ standard

The isotopic homogeneity in sponges makes them an ideal target for a reference standard, which would be particularly valuable should sponge spicules become a more commonly used geochemical archive. The sponge LMG08 has now been analysed by three laboratories, using two different preparation methods and three different instruments, and yielded (within error) the same value (mean $\delta^{29}Si$ value from the three laboratories = -1.72 ± 0.01‰, mean $\delta^{29}Si$ value of all measurements = -1.72‰, 2 SD = 0.08‰; mean $\delta^{30}Si$ value from the three independent laboratories = -3.35 ± 0.06‰, mean $\delta^{30}Si$ value of all measurements = -3.37‰, 2 SD = 0.17‰; Fig. 4). Additional subsamples are available from the authors for future interlaboratory tests.

Conclusion

The Southern Ocean is an important location for studying deepwater Si cycling because of the regional importance of global biological productivity and its sensitivity to well documented proximal climatic changes. Sponge spicule $\delta^{29}Si$ and $\delta^{30}Si$ are promising new proxies for past deepwater silicic acid concentrations, and Southern Ocean downcore records of spicules could be applied to a wide range of climatic and palaeoceanographic questions. We show here that Si isotopic measurements of Antarctic sponge spicules are robust and reproducible, confirming that the overriding control over silicon isotopes is the ambient $Si(OH)_4$ concentrations in which the sponges grow. The relationship between $\delta^{29}Si$ and $\delta^{30}Si$ is consistent with kinetic fractionation during sponge growth. Lastly, we suggest Southern Ocean sponges would act as an ideal new standard for future spicule analyses.

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