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RESEARCH ARTICLE

Revisiting the silicon isotopic signal of sponge skeletons and its implications

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Abstract

This study investigates the relationship between the silicon (Si) isotopic composition of sponge skeletal silica ($\delta^{30}Si_{bSi}$) and seawater characteristics in sponge habitats, specifically the concentration of dissolved silicic acid and its Si isotopic signature ($\delta^{30}Si_{dSi}$). Initially, these correlations were considered a promising calibration proxies for paleoceanographic reconstructions, but the incorporation of subsequent data points into the dataset over the past decade has highlighted complexities in how sponges fractionate silicon isotopes during silicification processes. We revisit the historical dataset, including a detailed examination of each datapoint to identify biases related to environmental, biological, and taxonomic factors. We also contribute new isotopic data obtained by multi-collector inductively coupled plasma mass spectrometer analysis, specifically targeting underrepresented low-silicic-acid environments. This revised dataset highlights that anomalies in the calibration, in particular species with fused skeletal frameworks, remain incongruous. We found that part of the problem is that the relationship between silicic acid concentration and $\delta^{30}Si_{bSi}$ in the revised dataset of only Demospongiae follows a distinct, statistically robust, non-linear trend different from the weak, linear fit in Hexactinellida. Consequently, isotopic data from these two sponge classes should not be combined for calibration analysis, if possible. Yet, while the robust non-linear regression for only Demospongiae revitalizes the proxy, the relationship becomes asymptotic at silicic acid values above 200 μM, limiting its applicability to Cenozoic and Mesozoic conditions and excluding early Paleozoic scenarios with high concentrations of silicic acid. Practical recommendations for using and improving the proxy are discussed.

Silicon (Si) is an abundant chemical element on earth, forming part of minerals, rocks and soils (i.e., lithogenic silica). It is also incorporated by some unicellular and multicellular organisms in the form of soluble silicic acid, which, through biological processes, is polymerized to produce siliceous skeletal components (i.e., biogenic silica). Silicon has three natural stable isotopes, ^{28}Si, ^{29}Si, and ^{30}Si. The Si isotopic signature of a biological or lithological siliceous material is usually described through its $\delta^{30}Si$ value, which indicates the per mil (‰) deviation between the $^{30}Si/^{28}Si$ ratio in the material and a standard (*see* Materials and methods). It has long been recognized that the $\delta^{30}Si$ value of biogenic silica (hereafter, $\delta^{30}Si_{bSi}$) varies over a broader range than that of the lithogenic materials sourcing the silicic acid used to produce biogenic silica. This results from isotopic fractionation during biological silicification, which preferentially incorporates the lighter isotope (Douthitt 1982; De La Rocha et al. 1997).

In the ocean, diatoms produce the bulk of biogenic silica in the form of their frustules (Tréguer et al. 2021), but there are also important contributions by siliceous radiolarians (Llopis Monferrer et al. 2020) and siliceous sponges (Maldonado et al. 2012, 2019). When all these silicifiers die, their siliceous

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skeletons sink to the seafloor, with a part of that biogenic silica being dissolved into silicic acid and the rest being progressively buried by subsequent sediment deposition. Despite limited understanding of the mechanisms that fractionate Si isotopes during biological silicification, numerous studies have identified correlations between the $\delta^{30}Si_{bSi}$ of the biogenic silica produced by the silicifying organisms and the concentration or the isotopic composition of the silicic acid dissolved in the seawater where the organisms live (i.e., $\delta^{30}Si_{dSi}$). These relationships, which may vary across different groups of silicifying organisms, have been recognized as a potential reference system to infer past changes in silicic acid concentration and $\delta^{30}Si_{dSi}$ values, as well as in cycling of Si associated nutrients, across various paleoceanographic time scales, based on the $\delta^{30}Si_{bSi}$ values of biogenic silica preserved in sediments (De La Rocha 2006; Wille et al. 2010; Hendry and Robinson 2012). However, although this framework theoretically provides a tool to investigate changes in the silicon cycle of past oceans and the dynamics of silicifying organisms through time, the interpretation of the information contained in the $\delta^{30}Si_{bSi}$ values of biogenic silica buried in sediments is not a straightforward process (Sutton et al. 2013; Frings et al. 2024).

Initial isotopic studies of diatom silica in laboratory cultures revealed that these unicellular organisms preferentially take up the lighter Si isotope during their growth, so that the silicic acid in the remaining solution becomes progressively isotopically heavier, with a fractionation of approximately −1.1‰ (De La Rocha et al. 1997). This observation led to the proposition that downcore diatom $\delta^{30}Si_{bSi}$ values could archive changes in silicic acid utilization in surface waters through time, making them a suitable tool for understanding the role that diatom production plays in past carbon cycling (De La Rocha et al. 1998). Later laboratory culture studies, however, revealed potential species-specific fractionation (Sutton et al. 2013). Furthermore, subsequent field studies brought to light additional challenges in assessing isotopic fractionation factors in diatom assemblages, including bloom dynamics, dissolution, and the impact of other environmental parameters (Hendry and Brzezinski 2014; Sutton et al. 2018; Frings et al. 2024).

An additional limitation of using diatom Si isotopes to reconstruct past oceanic changes is that, as photoautotrophic organisms, they inherently capture only the conditions of the photic zone, rather than recording conditions in the vast aphotic zone of the ocean, which ultimately gives rise to deep and bottom water masses that upwell to supply primary production. This gap led to an increased research interest in exploring the potential of sponge silica as an archive of past ocean change. A priori, a number of factors suggest that the sponge silica preserved in marine sediments may serve as a valuable tool for paleoceanographic inference: (i) On average, sponges produce isotopically lighter silica ($\delta^{30}Si$: −6.74‰ to +0.9‰) than that of diatoms ($\delta^{30}Si$: −0.8‰ to +3.1‰), with a kinetic fractionation about 3 times that of diatoms, creating a broader signal range for analysis (Frings et al. 2016; Cassarino et al. 2018; Pack et al. 2023); (ii) Sponges produce silica at substantially slower rates compared to diatoms (revised in Maldonado et al. 2010, 2020), minimizing the influence of significant isotope distillation, such as Rayleigh-type fractionation, which prominently affects diatom silica during population blooms (De La Rocha et al. 1997, 1998); (iii) Unlike diatoms, whose lifetime does not typically exceed a week, sponge individuals can live for decades to millennia, depending on the species, creating a continuous archive of environmental conditions (Jochum et al. 2012, 2017); (iv) Sponge silica dissolves more slowly than diatom silica (Kamatani and Oku 2000; Maldonado et al. 2022), being therefore better preserved in sediments (Maldonado et al. 2019); (v) the isotopic signal of siliceous spicules in the superficial sediments has been shown to be minimally affected by post-depositional alterations (Hendry and Robinson 2012).

Initial exploration of the potential of sponge $\delta^{30}Si_{bSi}$ value sparked a variety of applied studies on paleoceanography, involving different time scales (Ellwood et al. 2010; Griffiths et al. 2013; Fontorbe et al. 2016; Rousseau et al. 2016; Conley et al. 2017; Ding et al. 2017; Jochum et al. 2017; Chen et al. 2020). In practical terms, most of those studies decoded the isotopic information of the sponge silica throughout what can be defined as a "multispecies calibration" approach. It consists of establishing mathematical relationships between the silicic acid concentration of the seawater in the sponge habitat and the $\delta^{30}Si_{bSi}$ of the sponge silica, as well as between silicic acid concentration and the "apparent fractionation" of the sponge silica ($\Delta^{30}Si_{bSi}$). The latter is understood as the difference between the $\delta^{30}Si_{bSi}$ of the silica and that of the seawater, that is, $\Delta^{30}Si_{bSi} = \delta^{30}Si_{bSi} - \delta^{30}Si_{dSi}$ (Hendry and Robinson 2012). These empirical relationships, initially based on Southern Ocean sponge studies (Hendry et al. 2010; Wille et al. 2010) and then expanded to a more global dataset (Hendry and Robinson 2012), indicated that both sponge $\delta^{30}Si_{bSi}$ and $\Delta^{30}Si_{bSi}$ were predictable functions of silicic acid concentration, as shown by their respective robust hyperbolic-decay regression models ($\delta^{30}Si_{bSi}$: $R^2 = 0.85$, $p < 0.01$, $n = 62$; $\Delta^{30}Si_{bSi}$: $R^2 = 0.83$, $p < 0.01$, $n = 62$). These mathematical relationships were supported by mechanistic biological models that fitted fractionation factors to uptake, efflux and polymerization processes (Milligan et al. 2004; Wille et al. 2010; Hendry and Robinson 2012). A subsequent study by Cassarino et al. (2018) added 103 sponge silica samples from the tropical Atlantic to the pre-existing sponge dataset and found that what was an initially robust, hyperbolic-decay regression became weaker, strongly disrupted by biogenic silica samples of some species in the Class Hexactinellida that appeared to have a differential fractionation. This effect was attributed to the species having part or all their siliceous skeletal components fused to each other into a massive "dictyonal" framework. This is a skeletal condition typical of dictyonine hexactinellids, which results from a process of hypersilicification (Supporting Text Note 1).

Subsequent studies have reported additional variability in the Si isotopic fractionation of sponges, including differences between different types of sponge skeletal components, even when they are not hypersilicified (Hendry et al. 2019, 2024). The ultimate mechanisms leading to such a variability remain poorly understood. As a result, the incremental addition of species data points to the global sponge dataset over the last decade has weakened the relationship, reducing the R^2 value to 0.46, which accounts for approximately half of the association strength between the variables initially presented in the proxy ($R^2 = 0.85$) by Hendry and Robinson (2012). Therefore, the expansion of the isotopic sponge dataset has gradually tempered initial expectations of applicability in paleoceanographic reconstructions. To revitalize the proxy, it is essential to identify potential issues within the dataset and constrain sources of variability.

Upon initially reviewing the historical sponge dataset from which the relationship between concentration of silicic acid and $\delta^{30}Si_{bSi}$ was derived, two key aspects were identified that may require reconsideration and revision, thereby motivating this study. First, the relationship between silicic acid concentration and $\delta^{30}Si_{bSi}$ had been established from a global dataset that could potentially be influenced by certain biases resulting from logistic constraints. Sampling was primarily conducted during opportunistic actions framed within large-scale projects focused predominantly on the oceanography of high-latitude regions, which typically have relatively high silicic acid concentrations. Therefore, the current dataset underrepresents ocean regions with low silicic acid concentrations. Second, the regional bias also extends to the sponge component, which, despite currently amalgamating data from multiple species, provides a biased representation both taxonomically and skeletally of the various siliceous lineages within the phylum Porifera. In this regard, the effects into the pre-existing model of incorporating data from tropical and temperate shallow-water species, whose siliceous skeletons would have developed under low silicic acid concentrations (i.e., $< 5\ \mu M$), remain unexplored. The spicules utilized for palaeoceanographic reconstructions have, to date, originated largely from deeper environments. Overlooking shallow-water environments is not conceptually trivial. Shallow-water ecosystems host not only iconic environments such as coral reefs, seagrass meadows, kelp forests (and others), but they also sustain the majority of the demosponge fauna, along with important reservoirs of sponge silica in their sediments (Maldonado et al. 2005, 2019; Bertolino et al. 2012; Costa et al. 2021).

In this context, our study revises the practical and conceptual framework of the sponge silica-based proxy, with the aim of revitalizing its applicability in paleoceanographic reconstructions.

Materials and methods

Sponge datapoints from previous literature

A dataset was compiled to contain the extant sponge species for which published information on the $\delta^{30}Si_{bSi}$ of their silica is available in association with information on the silicic acid concentration and $\delta^{30}Si_{dSi}$ of the seawater. Data were extracted from Hendry et al. 2010, 2024; Wille et al. 2010; Hendry and Robinson 2012; Jochum et al. 2017; Cassarino et al. 2018; and Riesgo et al. 2020. These datasets were subsequently examined for potentially problematic data points, as indicated in the sections of Results.

New sponge datapoints

Sample collection

The global dataset compiled from the literature was complemented with new information from 48 sponge silica samples that belonged to 23 species, representing all 3 classes of siliceous sponges. In Supporting Data file S1 and S2, it is summarized that the newly added species were collected from different oceans (Mediterranean Sea, Eastern North Atlantic, Caribbean, Central and Eastern Pacific, and Yellow Sea), spanning a wide range of depths (0.3–830 m) and habitats (intertidal and subtidal rocky bottoms, coral reefs, mangroves, seamount tops, and other deep-water rocky and soft bottoms). The sampling effort extended over two decades of fieldwork (1994–2013), with collections being accomplished by a variety of methods, which included snorkeling and scuba diving at sublittoral depths, along with trawling nets, ROVs, and manned submersibles for deep-water sampling (Supporting Data File S1 and S2). Data on silicic acid concentration and stable silicon isotopic compositions of seawater ($\delta^{30}Si_{dSi}$) in the sponge habitats are also provided in Supporting Data file S1 and S2.

Silica cleaning

The collected sponge specimens were initially dried at 60°C for several days. Subsequently, $1 \times 1 \times 2$ cm portions of dry tissue were dissected to obtain the siliceous skeleton, a process that involves digesting the organic constituents of the sponge to finally obtain clean biogenic silica. Initially, we used two different cleaning procedures and examined whether one or another method could somehow alter the original Si isotopic values of the silica. This precautionary test was deemed necessary, as recent studies have shown rapid post-mortem exchange of stable oxygen isotopes occurring between ^{18}O-enriched seawater and the silica of diatoms and, to a lesser degree, sponges (Akse et al. 2020). This alteration causes the $\delta^{18}O$ values of the silica to no longer accurately reflect the isotopic composition of the ambient seawater at the time of silicification, thereby compromising their applicability in paleoceanographic reconstructions.

To examine whether our process of silica cleaning could also alter the original Si isotopic ratios in the sponge biogenic silica, two cleaning methods were tested and the results compared. For that test, 17 out of 23 newly studied species were each represented by two tissue subsamples from the same specimen (Supporting Data File S1). One subsample was immersed in 25 mL Pyrex test tubes filled with concentrated

nitric acid and boiled over an open flame for several hours in continuous manual agitation to eliminate all the organic sponge elements and until the sponge silica was cleaned (i.e., it became bright white in color). The other subsample was immersed for 24 to 48 h in a cold mix (1:4) of concentrated nitric and sulfuric acid. Under both protocols, once the organic components disappeared and the silica was clean, test tubes were centrifuged for 5 min at 1500 rpm, the acid supernatant eliminated by pipetting, and the tubes refilled with Milli-Q water. Rinsing and refilling with Milli-Q water was repeated 5 to 10 times, depending on the samples. After two final rinses in 100% ethanol, test tubes were dried at 60°C for 24 h.

Significant differences in mean (± SD) stable silicon isotopic compositions ($\delta^{30}Si_{bSi}$) between cleaning methods were not found, according to a paired *t*-test ($\delta^{30}Si_{hot\ cleaning} = -0.57‰ \pm 1.35‰$, $\delta^{30}Si_{cold\ cleaning} = -0.56‰ \pm 1.39‰$, $n = 17$, paired-$t = 0.20$, $p = 0.843$; Supporting Data File S3). Consequently, the $\delta^{30}Si_{bSi}$ values of the various subsamples from a single individual were averaged (Supporting Data File S4) and the resulting mean added as a new species data point to the global dataset (Supporting Data File S5).

Si isotopic analysis of silica from collected sponges

Full details of the methodology of isotopic analyses can be found in Hendry et al. (2015). Briefly, the samples were analyzed for silicon isotope ratios ($^{29}Si/^{28}Si$, $^{30}Si/^{28}Si$) using a Thermo Neptune multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS) at the University of Bristol (Bristol Isotope Group). Machine blanks were monitored, being < 1% of the signal on ^{28}Si. Silicon isotope ratios were double-normalized using standard-sample bracketing and magnesium isotope correction, and are reported in delta notation (Eq. 1) relative to NBS28 (RM8546). During the study, two reference standards were analyzed to monitor external accuracy and precision: "Diatomite," which yielded a mean value of +1.26‰ (± 0.09 2SD, $n = 5$); and sponge standard "LMG08," which yielded a mean value of −3.45‰ (± 0.12 2SD, $n = 10$), both in agreement with published values (Reynolds et al. 2007; Hendry and Robinson 2012). For all samples and standards, the gradient of the linear regression line between $\delta^{29}Si$ and $\delta^{30}Si$ was 0.516, which is consistent with mass-dependent fractionation.

$$\delta^{30}Si = \left\{ \left[\frac{\left(^{30}Si/^{28}Si\right)_{sample}}{\left(^{30}Si/^{28}Si\right)_{NBS28}} \right] - 1 \right\} \times 1000 \quad (1)$$

Statistical analyses

The relationship between environmental concentration of silicic acid and either $\delta^{30}Si_{bSi}$ or $\Delta^{30}Si_{bSi}$ values of the sponges was examined by goodness-of-fit analysis using SigmaPlot 15 Software. In addition to the elemental statistics for the goodness of fit analyses, which are presented in the main text and corresponding figures, extended statistical outputs are provided in the form of Supporting Information, as specified throughout the main text.

To investigate whether differences in the stable silicon isotope composition of biogenic silica ($\delta^{30}Si_{bSi}$) between sponges from the classes Demospongiae and Hexactinellida are primarily driven by variations in environmental parameters specific to their respective habitats, we used non-parametric Mann–Whitney *U* tests. These tests assessed between-class differences in median depth, silicic acid concentration, and the $\delta^{30}Si_{dSi}$ of the seawater associated with the silica samples.

Results

The calibration proxy tested with new datapoints

The dataset initially compiled from the literature included 183 data points, to which our 23 new data points were subsequently added. All new data points were taxonomically identified to the species level and represented the three lineages of siliceous sponges: Class Demospongiae (18 spp.), Class Hexactinellida (4 spp.), and Class Homoscleromorpha (1 sp.). After including these new data points, the resulting dataset contained information on the Si isotopic signature (i.e., $\delta^{30}Si_{bSi}$ and $\Delta^{30}Si_{bSi}$) of the skeletal silica of 206 sponge species, along with information on silicic acid concentrations and $\delta^{30}Si_{dSi}$ of the seawater in which the sponges lived (Supporting Data File S5). The analysis of this global data set indicated that the relationship between silicic acid concentration and $\delta^{30}Si_{bSi}$ was equally well-fitted either by (i) a hyperbolic 3-parameter decline ($n = 206$, $R^2 = 0.512$, $p < 0.001$; Akaike information criterion corrected for sample size AICc = −24.9: predicted residual error sum of squares PRESS = 180.2; Fig. 1a) or (ii) an exponential 3-parameter decay model ($n = 206$, $R^2 = 0.513$, $p < 0.001$, AICc = −25.3, PRESS = 179.9). *See* Supporting Information (Statistics S1) containing extended statistics for comparison of models. Likewise, the relationships between silicic acid concentration and $\Delta^{30}Si_{bSi}$ could be equally well-fitted through a hyperbolic 3-parameter decline model ($n = 206$, $R^2 = 0.317$, $p < 0.008$; AICc = −14.9; PRESS = 190.1; Fig. 1b) or an exponential 3-parameter decay model ($n = 206$, $R^2 = 0.315$, $p < 0.001$, AICc = −14.5, PRESS = 190.4). *See* Supporting Information (Statistics S2) containing extended statistics for comparison of models. Comparison of the regression coefficients (R^2) of this new global dataset with those of previous datasets in the literature reveals that the addition of our new samples from shallow waters further weakened the relationship between environmental silicic acid concentration and either sponge $\delta^{30}Si_{bSi}$ or $\Delta^{30}Si$. As noted by Cassarino et al. (2018), the correlation is markedly disrupted by the skeletal frameworks of dictyonine hexactinellids, the silica of which often reaches $\delta^{30}Si_{bSi}$ values below −3.5‰ (Fig. 1a) even at moderate silicic acid concentrations (15–30 μM). Our analyses also encompassed samples of lithistid demosponges,

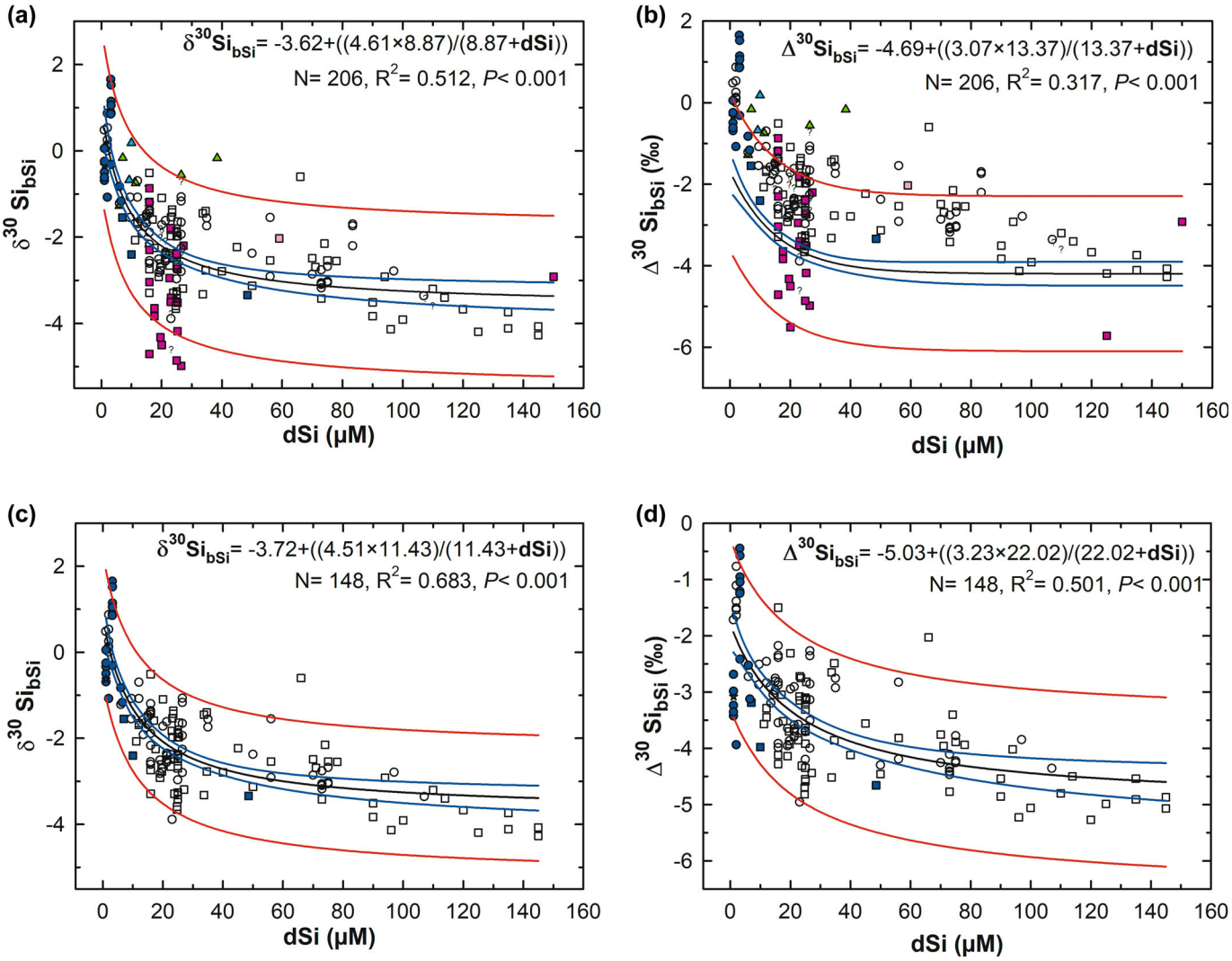

Fig. 1. Correlations between sponge stable silicon isotope compositions and dissolved silicon concentrations in seawater based on a global dataset. (**a**, **b**) Best-fitting, 3-parameter hyperbolic decay regression "$y = y_0 + (a \times b)/(b + x)$" between silicic acid concentration values of seawater (dSi) and stable isotopic composition ($\delta^{30}Si_{bSi}$) values of the sponge silica (a), as well as between silicic acid and apparent fractionation $\Delta^{30}Si_{bSi}$ (b), as resulting after pooling together for analysis all data available in the literature for demosponges and hexactinellids. White squares, white circles, and light-green triangles respectively refer to data on hexactinellids, non-lithistid demosponges, and lithistid demosponges compiled from the literature. Dark blue squares and circles are newly added hexactinellids and non-lithistid demosponges, respectively, while light blue triangles are newly added lithistids. A star symbol indicates a newly added member of the class Homoscleromorpha. Purple and light pink squares indicate respectively data on hexactinellids with dictyonal fused skeletons published previously in the literature and newly added in this study. A question mark symbol (?) refers to data from sponges in previously published studies lacking assignation to taxonomic class. The red lines indicate the 95% prediction interval and the blue lines the 95% confidence interval of the regression model (black line). (**c**, **d**) Best-fitting, 3-parameter hyperbolic decay regression between silicic acid and $\delta^{30}Si_{bSi}$ in sponge silica (c), as well as between silicic acid and $\Delta^{30}Si_{bSi}$ (d), as resulting from revising the combined data set of demosponges and hexactinellids to remove dictyonine hexactinellids, lithistid demosponges, sponges of unknown class assignation, and other problematic data points. *See* Supporting Information (Statistics S1–S4) for extended statistics of each of the four regression analyses.

commonly alluded to as "rock-like" sponges, because their skeletal components (known as desmas) fuse or interlock to form rigid skeletal frameworks, which are hard like rocks and analogous to the skeletal frameworks of dictyonine hexactinellids. In contrast to dictyonine hexactinellids, the seven lithistid demosponges in the dataset had silica with relatively high $\delta^{30}Si_{bSi}$ values compared to their non-lithistid demosponge counterparts, which also disrupted the regression (Fig. 1a).

Identifying problems in the calibration dataset

Based on the analyses in Fig. 1a,b, it was evident that "lithistid" demosponges and "dictyonine" hexactinellids, distinct types of outliers characterized by hypersilicified skeletal

components (Supporting Test Note 1) needed to be eliminated from the dataset prior to further analysis. In this regard, the initial global data set (i.e., Supporting Data File S5) was also revised for additional problematic or low-quality data points that could impact the proxy calibration, as follows: (1) In the study by Hendry and Robinson (2012), the sponge collected from Woods Hole at 10 m depth and reported as of unknown class allocation is highly likely to be a demosponge, given its habitat. (2) In the study by Wille et al. (2010), two samples originally defined as Hexactinellida *Farrea* sp. are herein allocated into the group of dictyonine fused skeletons, since this is the skeletal condition of the genus *Farrea*. (3) The $\delta^{30}Si$ values given by Wille et al. (2010) for a sponge identified as Dictyoceratida Dysideidae and another identified as Dictyoceratida Irciniidae cannot be used, since all the members of those sponge families lack a siliceous skeleton. The values reported in the study may likely derive from the fact that these sponges collect from the bottom both sand grains and spicules shed to the sediments by other sponges. Such foreign materials are incorporated into their epithelia and into the organic spongin fibers of their skeleton. (4) The carnivorous demosponge *Asbestopluma* sp., which exhibits significant differences in silicon and oxygen isotopic composition between its hypersilicified desma spicules and its regular (i.e., non-desmoid) spicules (Hendry et al. 2015), was excluded from the dataset. (5) In the study by Cassarino et al. (2018), a total of eight sponges were considered to be of unknown class allocation but further described as having a dictyonine fused skeleton, which is exclusive to the hexactinellids in the class Hexasterophora. Consequently, we considered them all as hexactinellids in the analyses. (6) In the study by Cassarino et al. (2018), two samples initially identified as Demospongia Mycalide and Demospongia Biemnida were subsequently reported through SEM observation to have a dictyonine fused skeleton, which is a condition exclusive of the subclass Hexasterophora of Hexactinellida. Because such samples were characterized by very light $\delta^{30}Si$ values of −3.50 and −4.71, respectively, they would become outliers among the demosponges. In contrast, those $\delta^{30}Si$ values are not atypical among hexasterophorid hexactinellids. Thus, it appears that the collected samples included a pseudo encrusting demosponge growing on a hexactinellid. While the demosponge tissue was used for taxonomic identification, the hexactinellid skeleton was used for the Si isotope analyses. Consequently, these samples initially regarded as demosponges are herein reconsidered as dictyonine hexactinellids. (7) Data from Jochum et al. (2017) on the giant spicule of the hexactinellid *Monorhaphis chuni* have not been considered, since the $\delta^{30}Si$ values of that sponge silica ranged from −0.5 to −3.6, reflecting the changes in the environmental concentration of silicic acid during the last 17,000 years of continuous spicule growth. (8) Data from Riesgo et al. (2020) on three Antarctic sponges that reuse the diatom frustules to produce silica comparatively light in terms of $\delta^{30}Si_{bSi}$ values were not considered in the dataset, because that singular behavior introduces a deviation of the general pattern of silicification, also because the water samples from the sponge habitat were frozen for months prior to analysis. Freezing alters the original conditions of the seawater in terms of both silicic acid concentrations and, more critically, $\delta^{30}Si$ value. (9) Additionally, 22 of the sponges considered in previously published regression models but completely lacking any taxonomic assignation (even at the class level) were now eliminated (*see* "?" marks in Fig. 1a,b and Supporting Data File S5).

These revisions resulted in a more coherent dataset consisting of 148 species: 66 hexactinellids, 81 demosponges, and 1 homosclerophorid (Supporting Data File S6). The latter species, *Corticium candelabrum*, is the first representative of the class Homoscleromorpha for which the Si isotopic signature has been determined. Its silica showed a $\delta^{30}Si_{bSi}$ of −0.62‰, only slightly lower than that of three other species of the class Demospongiae that share habitat with it in the Mediterranean sublittoral: *Axinella damicornis* (−0.50‰), *Crambe crambe* (−0.34‰), and *Petrosia ficiformis* (−0.25‰) (Supporting Data File S4). Thus, this first datapoint would not support substantial differences in fractionation between sponges in the classes Demospongiae and Homoscleromorpha, though additional homoscleromorphs need to be examined in future studies.

In the revised dataset, the relationship between silicic acid concentration and $\delta^{30}Si_{bSi}$ or $\Delta^{30}Si_{bSi}$ was again fitted by either a hyperbolic decay model or an exponential decay model in each case (*see* Supporting Information, Statistics S3 and S4), but the strength of the regression did not increase substantially relative to the previous global dataset. The best hyperbolic decay fitting is depicted in Fig. 1c ($\delta^{30}Si_{bSi}$: $n = 148$, $R^2 = 0.683$, $p < 0.001$) and Fig. 1d ($\Delta^{30}Si_{bSi}$: $n = 148$, $R^2 = 0.501$, $p < 0.001$). Visual inspection reveals that the shape of the $\delta^{30}Si_{bSi}$ and $\Delta^{30}Si_{bSi}$ relationship follows a quasi-linear pattern at silicic acid concentrations higher than 30 μM, shifting rapidly toward a non-linear decay model at concentrations < 30 μM. The addition of demosponges from low silicic acid environments clearly pronounced the non-linear decay shape of the global regression model, with negligible participation of hexactinellid sponges in this effect. It is important to note that a few of the demosponges coming from low silicic acid shallow waters of the Caribbean Sea and the Mediterranean Sea (silicic acid < 5 μM) had silica with very low fractionation, even reaching positive $\delta^{30}Si_{bSi}$ values (Fig. 1c). This pattern was consistent across both shallow-water Mediterranean and Caribbean species, despite the fact that only the shallow Mediterranean species had strictly co-located $\delta^{30}Si_{dSi}$ seawater values, while apparent fractionation for shallow-water Caribbean species was estimated based on the nearest available data (Supporting Data File S2). In contrast to demosponges, the silica of hexactinellids—a sponge lineage found almost exclusively in relatively silicic acid-rich waters (Alvarez et al. 2017)—never reached positive $\delta^{30}Si_{bSi}$ values.

The analyses of residuals for the regressions of silicic acid concentration against both $\delta^{30}Si_{bSi}$ and $\Delta^{30}Si_{bSi}$ are nearly identical and convey the same conclusions (Supporting Fig. S1A–D; Supporting Data File S7). For the revised dataset including Demospongiae and Hexactinellida, the overall correlation between the predicted values and residuals is essentially zero, and the residuals appear at first glance randomly scattered (Supporting Fig. S1A,C), as expected for well-fitted models. However, in both regressions, the cloud of points of demospong's residuals exhibits a "funnel" shape, revealing that the scatter of these residuals, rather than being random, increases with increasing predicted values (Supporting Fig. S1A,C). The non-random pattern becomes even clearer when residuals are plotted against the independent variable (i.e., silicic acid concentration) of the regression models (Supporting Fig. S1B,-D), revealing that the pattern differs by sponge class. Demosponges show a maximum residual scatter at silicic acid concentrations between 0 and 10 μM, while hexactinellids do so between 10 and 30 μM. In both subsets, the undesirable "funnel" shape of the residuals cloud accounts for a changing error spread across silicic acid concentrations. In other words, the errors violate the assumption of homoscedasticity, indicating a suboptimal fit because the model does not fit equally well for Demospongiae and Hexactinellida.

Revitalizing the calibration proxy

From the analysis above, it appears that a significant proportion of the difference in the stable silicon isotope composition ($\delta^{30}Si_{bSi}$) between demosponges and hexactinellids may derive from differences in the ambient concentration of silicic acid and $\delta^{30}Si_{dSi}$ values of the seawater of the habitats in which these two types of sponges live (Fig. 2a; Supporting Data file S8). Indeed, a Mann–Whitney *U* test (Supporting Information, Statistics S5) confirmed that the seawater of the habitats of the demosponge subset has a median concentration of silicic acid comparatively lower (16 μM) than that of the hexactinellid subset (25 μM) with statistical significance ($U = 1370$, $p < 0.001$; Fig. 2a). Likewise, $\delta^{30}Si_{dSi}$ in the demosponge subset is significantly higher (1.31‰) than that of the hexactinellid subset (1.24‰; $U = 1574.5$, $p < 0.001$; Fig. 2b), which is in accordance with the general trend in ocean water between dissolved silicon concentrations and isotopic compositions (de Souza et al. 2014). The *U* test also revealed significant differences in the median values of $\delta^{30}Si_{bSi}$ (1.02‰; Fig. 3c) and $\Delta^{30}Si_{bSi}$ (0.81‰; Fig. 3d) between Demospongiae and Hexactinellida. Interestingly, these differences were notably larger in magnitude compared to those observed in silicic acid concentration and $\delta^{30}Si_{dSi}$ values. Collectively, these findings suggest that, while there is strong environmental mediation of biologically driven kinetic isotopic fractionation during sponge silicification, hexactinellids

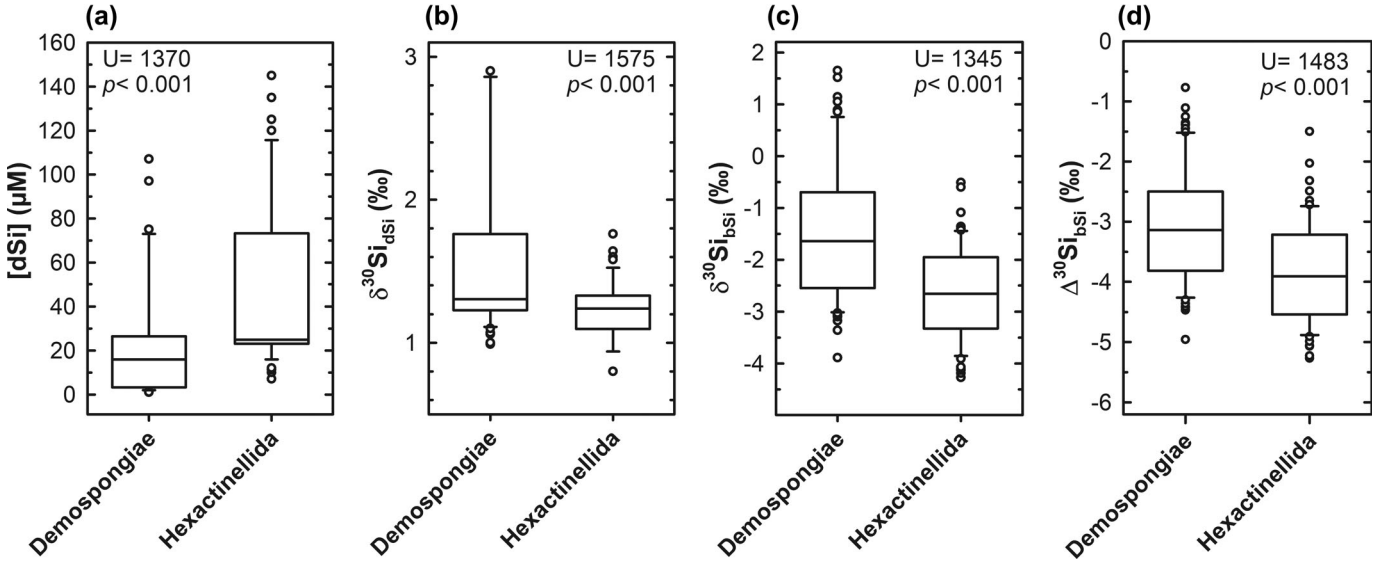

Fig. 2. Summary of statistical differences between Demospongiae and Hexactinellida in silicic acid concentration and isotopic data. Boxplots summarizing (**a**) silicic acid concentration (dSi) and (**b**) stable isotopic composition ($\delta^{30}Si_{dSi}$) values of the seawater in the habitat of demosponges and hexactinellids remaining in the revised dataset after removing problematic data points. (**c**, **d**) Respective summaries of $\delta^{30}Si_{bSi}$ and the difference between seawater and sponge stable isotopic composition ($\Delta^{30}Si_{bSi}$) values of the biogenic silica in the members of two sponge classes separately. The Mann–Whitney *U* test for differences between medians of the two sponge classes finds statistically significant between-class differences for all four parameters, but note that the relative magnitude of between-class differences is much greater for parameters related to Si isotope fractionation ($\delta^{30}Si_{bSi}$ and $\Delta^{30}Si_{bSi}$) than for parameters related to the seawater features of the sponge habitats (silicic acid concentration and $\delta^{30}Si_{dSi}$). Boxplots depict the median value of the variable within the box defined by the 25% and the 75% quartiles; error bars are the 95% confidence intervals and circles indicate each individual outlier. *See* Supporting Information (Statistics S5) for extended statistics of each of the four median comparison analyses.

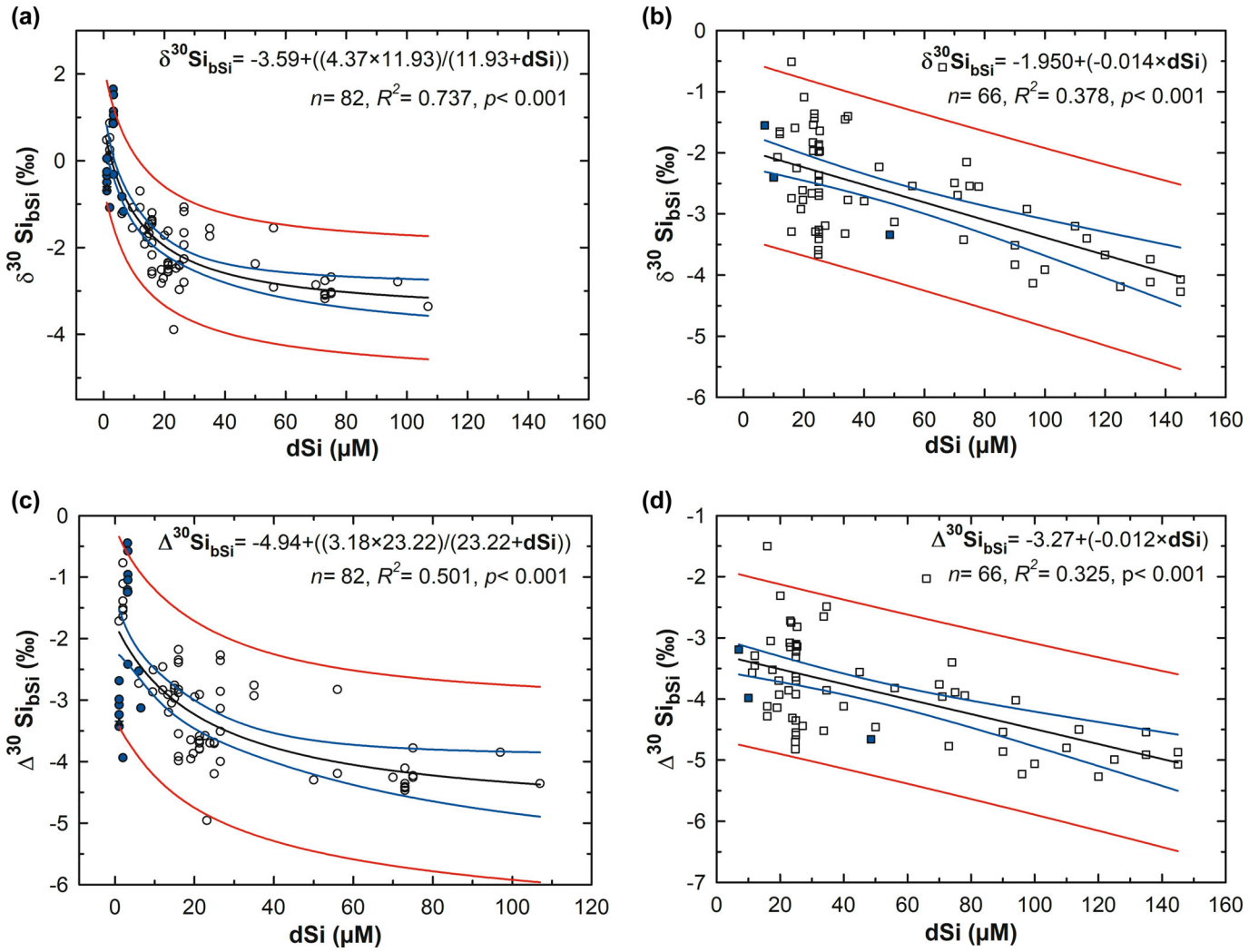

Fig. 3. Correlations between sponge stable silicon isotope compositions and dissolved silicon concentrations in seawater based on our revised datasets. (**a**) Best-fitting, 3-parameter hyperbolic decay regression "$y = y_0 + (a \times b)/(b + x)$" for the relationship between silicic acid concentrations (dSi) of the seawater and stable silicon isotopic composition ($\delta^{30}Si_{bSi}$) of the sponge silica in the revised dataset containing only demosponges. (**b**) Best-fitting linear regression model "$y = y_0 + (a \times x)$" for the relationship between silicic acid concentration (dSi) of the seawater and $\delta^{30}Si_{bSi}$ values of the sponge silica in the revised dataset containing only hexactinellid sponges. (**c**) Best-fitting, 3-parameter hyperbolic decay regression "$y = y_0 + (a \times b)/(b + x)$" for the relationship between silicic acid concentration and $\Delta^{30}Si_{bSi}$ fractionation values of the sponge silica in the revised dataset containing only demosponges. (**d**) Best-fitting linear regression model "$y = y_0 + (a \times x)$" for the relationship between silicic acid concentration (dSi) and $\Delta^{30}Si_{bSi}$ fractionation values of the sponge silica in the revised dataset containing only hexactinellid sponges. White squares, white circles, and light-green triangles respectively refer to data on hexactinellids, and non-lithistid demosponges previously published in the literature. Dark blue squares and circles are newly added hexactinellids and non-lithistid demosponges, respectively. A star symbol indicates a newly added member of the class Homoscleromorpha. The red lines indicate the 95% prediction interval and the blue lines the 95% confidence interval of the regression model (black line). *See* Supporting Information (Statistics S6–S9) for the extended statistics of each of the four regression analyses.

and demosponges may also utilize distinct mechanisms of Si isotope fractionation during their silicification (*see* Discussion).

To further investigate whether Demospongiae and Hexactinellida differ in the pattern of the relationship between silicic acid concentration and $\delta^{30}Si_{bSi}$, we split the revised dataset into Demospongiae and Hexactinellid subsets for separate analysis of their respective relationships (Supporting Data File S8). The Demospongiae dataset consisted of 82 data points (81 demosponges, and 1 homosclerophorm). It led to a silicic acid concentration vs $\delta^{30}Si_{bSi}$ relationship (Fig. 3a) that was again fitted through either a hyperbolic decay model ($n = 82$, $R^2 = 0.737$, $p < 0.001$, AICc $= -57.5$, PRESS $= 39.3$) or an exponential-decay model ($n = 82$, $R^2 = 0.747$, $p < 0.001$, AICc $= -60.6$, PRESS $= 37.8$). *See* Supporting Information (Statistics S6) for model comparison. More importantly, the relationship became more robust than in any of the combined datasets previously analyzed in this study (Fig. 1a,c). In contrast, the hexactinellid subset shifted from its previous non-

linear condition to a best-fitting linear model, although with weak association between the two variables ($n = 66$, $R^2 = 0.377$, $p < 0.001$; Fig. 3b; Supporting Information, Statistics S7). The fit of hexactinellids to a linear model may suggest differences in sampling bias in relation to habitat preference between the two classes of sponges and/or differences in Si isotopic fractionation. For both sponge classes, the relationship between ambient concentration of silicic acid and $\Delta^{30}Si_{bSi}$ (Fig. 3c,d; Supporting Information, Statistics S8 and S9) was consistently weaker than that between silicic acid concentration and $\delta^{30}Si_{bSi}$ (Fig. 3a,b). This likely reflects the propagated uncertainty associated with the calculation of $\Delta^{30}Si$, challenges in measuring seawater $\delta^{30}Si_{dSi}$, especially in low concentrations of silicic acid (e.g., Grasse et al. 2017), and the fact that the sponge and seawater samples are not always strictly co-located.

A subsequent analysis was conducted to evaluate the robustness of the differences between demosponges (exhibiting a non-linear pattern) and hexactinellids (displaying a linear pattern), regarding the relationship silicic acid concentration vs. $\delta^{30}Si_{bSi}$ or silicic acid concentration vs. $\Delta^{30}Si_{bSi}$. To further assess whether the observed between-class differences in the relationships were favored by differences in silicic acid concentration between the habitats of demosponges and hexactinellids, we considered for analysis only those demosponge datapoints corresponding to species that reside in environments with silicic acid concentrations sufficiently high to allow the occurrence of hexactinellid sponges. Because both the available dataset (Fig. 3a) and the literature (Alvarez et al. 2017) indicate that hexactinellid sponges very rarely live in environments with silicic acid values lower than 10 μM, we first explored how the silicic acid concentration vs $\delta^{30}Si_{bSi}$ relationship would perform if the hexactinellid subset ($n = 66$) is combined with only those demosponges ($n = 55$) that live in environments where the silicic acid concentration exceeds 10 μM (Supporting Data File S9). Subsequently, we repeated the test but considering only the 16 demosponges of the dataset inhabiting environments with silicic acid values higher than 30 μM, along with all 66 hexactinellid data points of the revised dataset (Supporting Data File S10).

In neither of these two restricted datasets did the relationships show increased strength ($R^2 < 0.380$ in all cases). Likewise, these datasets did not depart from the weakly supported linear pattern driven by the hexactinellid contribution (Fig. 4a–c, Supporting Information, Statistics S10–S13).

Discussion

Do hexactinellids and demosponges differentially fractionate silicon isotopes?

The analyses conducted in this study have revealed that the relationship between the environmental concentration of silicic acid and stable silicon isotopic composition (either $\delta^{30}Si_{bSi}$ or $\Delta^{30}Si_{bSi}$) follows a different trend in Demospongiae and Hexactinellida. After a revision of the demosponge dataset, it is shown that it fits robustly either a hyperbolic-decay model or an exponential-decay model, two options that consistently emerge as competing mathematical solutions to describe the relationship between silicic acid and $\delta^{30}Si_{bSi}$ (or $\Delta^{30}Si_{bSi}$). However, it should be noted there is a mechanistic rationale for using a hyperbolic-decay model, linked with isotopic fractionation associated with silicic acid uptake/efflux and silica polymerization (Milligan et al. 2004; Wille et al. 2010; Hendry and Robinson 2012). Contrary to Demospongiae, the Hexactinellida subset exhibits only a weak fit to a linear model, likely due to considerable variability arising from multiple and not well-identified sources. Therefore, the hexactinellid dataset still needs to grow in a systematic way until it can provide a coherent and explanatory message for paleoceanographic inference or other uses of the silicic acid concentration vs $\delta^{30}Si_{bSi}$ relationship.

Do the different trends in the relationship between silicic acid concentration and $\delta^{30}Si_{bSi}$ (or $\Delta^{30}Si_{bSi}$) point toward a fundamentally different fractionation process in the two major sponge groups? Many aspects of the silicification process in Demospongiae and Hexactinellida remain unclear. Nevertheless, whereas there is yet no direct demonstration that these two major groups fractionate Si isotopes differently during silicification, recent advances highlight important, between-class biological differences in the silicification process (Leria and Maldonado, under review; Shimizu et al. 2024). As is known for diatoms (e.g., Milligan et al. 2004), Si isotopic fractionation in sponges is likely the cumulative result of two processes: (i) fractionation associated with the transport of silicic acid from the ambient water to the cytoplasm of silicifying cells, and (ii) fractionation associated with the polycondensation of silicic acid into biogenic silica, which is mediated by organic molecules. The protein machinery used for silicic acid polycondensation has been shown to differ completely across sponge classes (Shimizu et al. 2024). In contrast, the machinery used for silicic acid transport—consisting of a combination of aquaglyceroporin (gAQP) channels and ArsB active transporters that are distant homologs of Ls1 and Lsi2 transporters of land plants (Supporting Text Note 2)—appears to be common to Demospongiae and Hexactinellida (Maldonado et al. 2020). Yet, the gAQP-ArsB systems of Demospongiae and Hexactinellida, though sharing a common phylogenetic origin, have followed completely distinct evolutionary trajectories, including acquisition of a novel gAQP channel by lateral gene transfer in Demospongiae, but not in Hexactinellida (Leria and Maldonado, under review).

Regarding the process of silicic acid polycondensation, the fact that Demospongiae silicify using only isoforms of the silicatein enzyme, while Hexactinellida employ three different silicifying proteins (hexaxilin, perisilin, and glassin) that are phylogenetically unrelated among them, would account for distinct Si isotopic fractionation during the step of biogenic

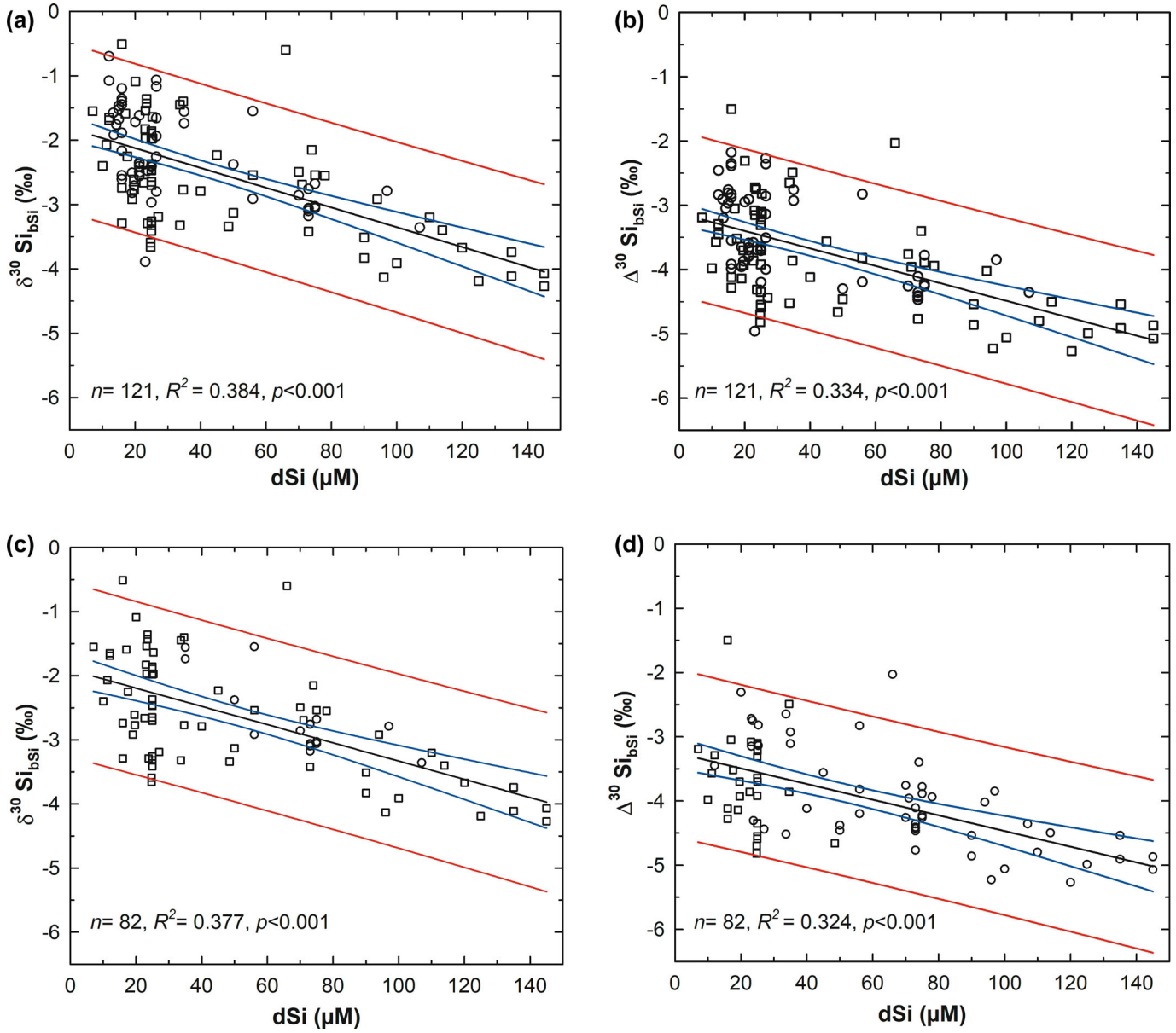

Fig. 4. Exploratory correlations between stable silicon isotope compositions and dissolved silicon concentration when constrained to high concentration values. Exploratory regression analyses combining the revised dataset of hexactinellids with data points of demosponges living in silicic acid concentrations (dSi) exceeding 10 μM (**a**, **b**) and 30 μM (**c**, **d**). The regression analyses indicate a weak relationship between the ambient concentration of silicic acid and stable isotopic compositions ($\delta^{30}Si_{bSi}$) of the hexactinellid skeletons (a, c) and even a weaker relationship between silicic acid concentration and apparent fractionation of sponge silica ($\Delta^{30}Si_{bSi}$) (b, d). In both cases, the exploratory datasets led to a statistically significant linear regression but of very low predictive capacity ($R^2 < 0.380$), which, importantly, was even weaker than that of the hexactinellids by themselves. The red lines indicate the 95% prediction interval and the blue lines the 95% confidence interval of the regression model (black line). Squares are hexactinellids and circles demosponges. Extended statistics for all four regression analyses are given as Supporting Information (Statistics S10–S13).

silica polymerization. Furthermore, differences between Demospongiae and Hexactinellida fractionation would also be consistent with previous ultrastructural studies indicating that the silica of each of the three siliceous classes exhibits notably distinct patterns of stratification and association with organic components embedded in the silica matrix (Ehrlich et al. 2007, 2016). The silica of Hexactinellida exhibits thin organic deposits intercalated between the concentric silica layers (Fig. 5a,b), while the silica of Demospongiae is highly compact, with concentric appositional layers that are rarely distinguishable from one another (Fig. 5c). Such structural differences make the silica of demosponges far more resistant to dissolution than that of hexactinellids and, therefore, more suitable for preservation in sediments (Maldonado

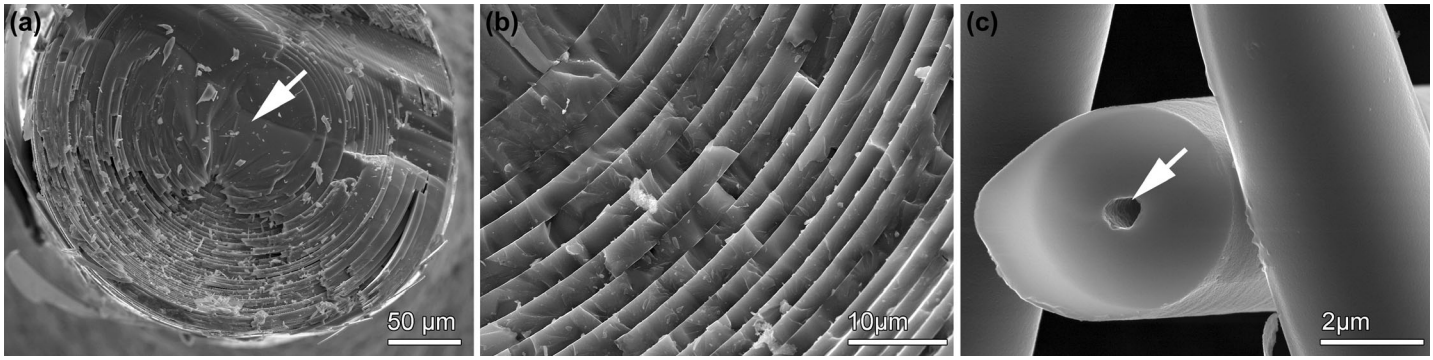

Fig. 5. Differences in silica microstructure between Hexactinellida and Demospongiae. (**a**, **b**) Cross-section of a needle-like spicule of the hexactinellid *Ijimalophus hawaiicus*, showing that the canal that should be at the center of the spicule section (arrow) is obliterated with silica and that the peripheral silica forms marked concentric layers. (**c**) Needle-like spicule of demosponge *Petrosia ficiformis* in cross section, showing the axial canal open (arrow). Note that, although picture c is at a much higher magnification than pictures a and b, it does not provide any visual evidence of concentric layering in the peripheral silica.

et al. 2022). Furthermore, the biogenic silica produced by hexactinellids is known to be bound to chitin, while that of demosponges is not (Ehrlich et al. 2007, 2016). It has recently been demonstrated that the binding of Si to organic compounds modifies the original IV-coordination of Si into VI-coordination, favoring extreme Si isotopic fractionation (Stamm et al. 2020). The complexing of the hexactinellid silica to chitin and other organic compounds could also help to explain why apparent fractionation is often larger in Hexactinellida than in Demospongiae, irrespective of additional differences in habitat.

While the biochemical step, most likely a rate-limiting one, responsible for the major component of fractionation during sponge silicification has not yet been identified, a range of biological and statistical considerations presented and discussed in this study supports the convenience of separately analyzing the Si isotopic signal in the silica of demosponges and hexactinellids.

Practical guidelines for using the proxy

The findings of this study suggest that a robust applicable model can be derived exclusively from the Demospongiae dataset. Therefore, as a general recommendation, datasets from Demospongiae and Hexactinellida should not be combined for regression analysis, except in rare cases where specific research objectives may need such an approach. Focusing solely on the Demospongiae dataset for paleoreconstruction retains considerable potential. This sponge class constitutes the majority of the more than 9,400 taxonomically described extant species, accounting for approximately 82% of species in the Phylum, compared to 7% for Hexactinellida and 1% for Homoscleromorpha. Demosponges are also ubiquitous and have a broad ecological distribution, occurring from intertidal zones to the abyssal seafloor. Likewise, demosponges built important reservoirs of silica in marine sediments ranging from sublittoral to abyssal facies (Rützler and Macintyre 1978; Bavestrello et al. 1993; Frisone et al. 2014; Murillo et al. 2016; Maldonado et al. 2019; Costa et al. 2021).

When investigating the Si isotopic composition of mixed demosponge spicules obtained from sediment samples, it is recommended to collect separately hypersilicified spicules (i.e., desmas) and regular spicules to conduct independent analyses. There is increasing evidence that desmas contain an anomalous silicon isotope fractionation compared to that of regular (i.e., non hypersilicified) demosponge spicules (Hendry et al. 2015; Fontorbe et al. 2016). The analysis of our initial global dataset (Supporting Data File S5), which contained six desma-bearing demosponges (i.e., lithistids) represented in Fig. 1a,b by light blue and green triangles, revealed that those lithistids are characterized by comparatively heavier $\delta^{30}Si_{bSi}$ and lower $\Delta^{30}Si$ values than their demosponge counterparts growing at similar silicic acid concentrations. Lithistid sponges are considered relics from sponge assemblages in Mesozoic seas, which have survived to the present day in particular habitats (Lévi 1991; Maldonado et al. 2015; Schuster et al. 2018). Because of their relict nature, a separate and independent calibration for these sponges would be desirable, as they might particularly help for inference into Mesozoic scenarios. If robust datasets for different sponge lineages were ever implemented, their respective independent analyses would provide a valuable tool for paleoinference.

There are additional limitations when applying the silicic acid vs $\delta^{30}Si_{bSi}$ relationship to paleontological questions. Firstly, current regression equations (e.g., Figs. 1c, 4a) have limited applicability to the high-silicic-acid scenarios that likely characterized early Paleozoic oceans and earlier, where concentrations are estimated by some studies to have exceeded 1000 μM (Maliva et al. 1989; Siever 1992). When these regression curves are projected to high-silicic-acid scenarios, they become asymptotic at values around 200 μM, thereby losing their predictive capacity. This holds true regardless of whether the model is based on the revised "demosponge + hexactinellid" dataset or the

"demosponges-only" dataset (Fig. 6). This model behavior is consistent with the lack of sensitiveness observed in previous models by Fontorbe et al. (2017) when attempting paleoreconstruction in high-silicic-acid scenarios. Therefore, the model proposed here for demosponges would be more applicable to scenarios within the Cenozoic and potentially the Mesozoic and earlier periods, when silicic acid concentrations in the seawater more closely resembled those of the modern ocean (Maliva et al. 1989; Fontorbe et al. 2016; Ye et al. 2021; Trower et al. 2021; Yager et al. 2025). Secondly, given the residence time of Si in seawater is likely less than 10 ky (Tréguer et al. 2021), it is possible for the isotopic composition of seawater to change through geological time, impacting the nature of the silicic acid vs $\delta^{30}Si_{bSi}$ relationship. Ocean modeling is required to address this possibility and assess the sensibility of the system over relevant timescales to changes in factors such as ocean circulation, weathering, and macroevolutionary changes (e.g., Hendry et al. 2012; Fontorbe et al. 2016).

Lastly, future improvements in the predictive capacity and accuracy of the regression models will require minimizing confounding factors and sources of uncontrolled variability wherever possible. In broad lines, the ultimate sources of variability in the isotopic signal of the sponge silica remain unclear. However, there are some approaches that can be taken when picking specimens to minimize potential noise in downcore spicule archives. As discussed above, the scatter is more marked in the class Hexactinellida than in Demospongiae, so avoiding hexactinellid spicules would be a sensible approach, regardless of any fundamental taxonomic differences in isotopic fractionation, to avoid noisy signals. Previous work has shown, for example, that a core-top calibration of spicule $\delta^{30}Si_{bSi}$ exhibits less scatter than subsampled specimens (Hendry and Robinson 2012), indicating that more robust palaeoceanographic reconstructions could be achieved by analyzing a large number of spicules per sediment horizon. It would also be advisable to pick, whenever possible, the same type of spicule to minimize variability, since differences in $\delta^{30}Si_{bSi}$ between different demosponge spicule types from the same sediment samples have also been reported (Hendry et al. 2024). Coring in areas that have hosted monospecific sponge aggregations for millennia (Murillo et al. 2016; Maldonado et al. 2017) may also offer an unparalleled opportunity to constrain variability.

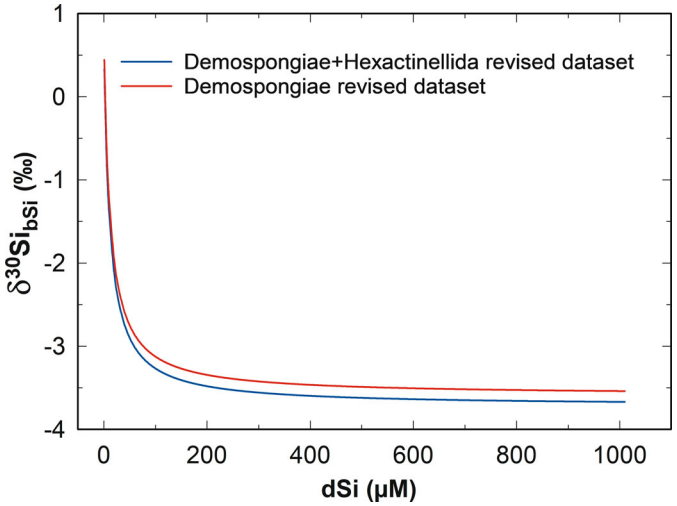

Fig. 6. Comparative performance of the hyperbolic decay models when extrapolated to high-silicic-acid scenarios. The red line represents the revised dataset that combines demosponges and hexactinellids ($n = 148$) and the blue line represents the revised dataset that contains only demosponges ($n = 85$). Both models exhibit asymptotic behavior when projected to silicic acid (dSi) concentrations exceeding approximately 200 μM. Beyond this threshold, the equations lose their capacity to generate reliable predictions.

To reduce data scattering in future expansions of the datasets, it is advisable that added data points reflect accurately the silicic acid concentration and $\delta^{30}Si_{sw}$ in the specific benthic habitat of the sponges, rather than being approximate regional values obtained from oceanographic measurements in the water column. In most previous studies (including part of the datapoints in the present study), silicic acid and $\delta^{30}Si_{SW}$ values are often derived from averaged data in regional oceanographic studies, rather than from water samples collected directly from the sponge habitat. Nevertheless, the bias introduced by using regional instead of exactly co-located seawater values—while not ideal—is likely to be relatively minor, given the comparatively small range of seawater $\delta^{30}Si$ (e.g., de Souza et al. 2015).

Another source of variability is sponge longevity. For many sponge species, the analyzed silica sample may contain a combination of spicules of varying ages, accumulated in the sponge body over decades to centuries or even millennia through the sponge's lifespan. Therefore, a given biogenic silica sample may record a complex history of changes in silicic acid concentration and $\delta^{30}Si_{dSi}$ within the sponge habitat, potentially masking sources of both within-individual and between-individual variability in $\delta^{30}Si_{bSi}$. To minimize these issues, spicule samples from small (likely younger) and large (likely older) conspecifics should not be mixed for analysis. Similarly, spicule samples from different body regions of a same individual may contain distinct isotopic signatures, and treating them either separately or in combination with a global representation of the species' skeleton could reveal or obscure different features. These effects should be further investigated systematically using model species to gain a deeper understanding of the sources of variability when interpreting the isotopic signal of sponge silica.

Conclusions

In this study, we have interrogated the relationship between the silicon isotopic composition of sponge skeletal silica ($\delta^{30}Si_{bSi}$) and the concentration of dissolved silicic acid

and its Si isotopic signal ($\delta^{30}Si_{dSi}$), on which the sponge spicule palaeonutrient proxy is based. Our new datapoints and revisions of literature data support a possible difference in this silicic acid-vs-$\delta^{30}Si_{bSi}$ relationship between demosponges and hexactinellids. This divergence may be driven by specific environmental and habitat differences, as well as complexities in how the two sponge groups fractionate silicon isotopes during silicification. Given the differences in calibration between the groups, and the more variable nature of hexactinellid fractionation, we recommend, where possible, that isotopic data from these two sponge classes not be combined for analysis. The nonlinear regression obtained for Demospongiae fits robustly either a hyperbolic-decay model or an exponential-decay model, which can have applicability in paleoinference. Yet, these regression curves become asymptotic at silicic acid concentrations above 200 μM, limiting applicability to Cenozoic and Mesozoic samples, and making application to early Paleozoic sediments challenging, given the high concentrations of silicic acid expected. The linear regression obtained for Hexactinellida shows a poor fit and is not reliable, in its current stage, for palaeoceanographic extrapolation. As such, the hexactinellid data set would benefit from careful revision and further expansion in future studies. Further work is required to provide meaningful calibrations not only for Hexactinellida, but also for desma-bearing lithistid demosponges, which may result of particular interest, given their condition of Mesozoic living fossils.

Author Contributions

Manuel Maldonado and Katharine R. Hendry designed the study and provided research funding. Manuel Maldonado was responsible for collecting sponges, cleaning the biogenic silica, conducting SEM observations, performing statistical analyses, and assembling the first manuscript. Katharine R. Hendry performed MC-ICP-MS analyses and contributed to the manuscript with text writing, editing, and scientific insights.

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Conflicts of Interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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