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FEATURE ARTICLE

Glass sponge reefs as a silicon sink

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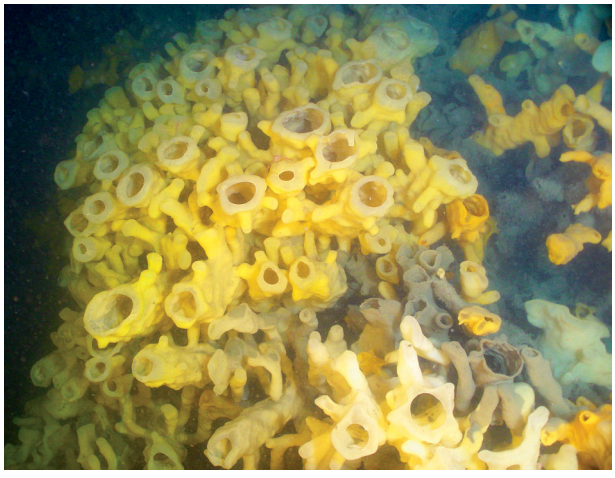
ABSTRACT: Glass sponge reefs concentrate large amounts of biological silicon (Si) over relatively small areas of the seafloor. We examined the role of glass sponges in biological silicon (Si) cycling by calculating a Si budget for 3 glass sponge reefs (Howe, Fraser, and Galiano) in the Strait of Georgia (SOG), British Columbia, Canada. The main reef-forming glass sponge *Aphrocallistes vastus* is heavily silicified, with 80% of its dry weight composed of biogenic silica (bSi). We used a combination of field sampling and surveys with a remote-operated vehicle to estimate the volume, mass, and bSi content of the reefs. BSi content ranged from 7 to 11 kg m^{-2} among reefs, amounting to a total of 915 t of bSi locked in the exposed portion of the 3 reefs. Water column measurements of dissolved Si (dSi) indicated that the SOG is a region of high dSi, with average dSi concentrations of 50 µmol l^{-1} in waters over the reefs. The skeletons of glass sponges showed very little dissolution after 8 mo immersion in seawater, as determined by changes in dSi in samples and scanning electron microscopy of the spicules. In contrast, diatom frustules, the main source of bSi in surface waters of the SOG, were ~200 times more soluble. Our calculations of Si flux suggest that glass sponge reefs can equate to 65% of the dSi reservoir (3.6×10^9 mol Si) in the SOG and represent a substantial Si sink in the continental shelf waters of the northeastern Pacific Ocean.

KEY WORDS: Glass sponge reef · Silicon cycling · Strait of Georgia · Hexactinellida, Porifera, *Aphrocallistes vastus*



Aphrocallistes vastus and *Heterochone calyx* are reef-building glass sponges, whose heavily silicified skeletons create large reservoir of silicon.
Photo: S. P. Leys, V. Tunnicliffe, and ROPOS

INTRODUCTION

Sponges are overlooked as important contributors to biological silicon (Si) cycling in the world's oceans. While the rapid growth and dissolution of diatom frustules have been shown to cycle half of the biological Si in the photic zone, with the rest sinking to deeper waters by pathways such as marine snow and fecal pellet transport (Schrader 1971, Nelson et al. 1995), recent evidence shows that sponges are major contributors to the Si budget in some continental-shelf and slope habitats (Maldonado et al. 2005,

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2010). Knowledge of the production, cycling, and preservation of biological Si in the world's oceans is useful for reconstructing the biogeochemistry of paleoenvironments (Maliva et al. 1989), but more importantly, the Si balance for modern oceans has implications for global primary productivity (Tréguer & Pondaven 2000). At the global scale, diatoms are estimated to produce about 240 teramoles (10^{12}) of Si in the oceans each year (Tréguer et al. 1995). Diatoms incorporate Si into their cell walls by polymerizing silicic acid into an amorphous hydrated form, biogenic silica (bSi; Raven & Waite 2004). After death, bSi from frustules regenerates back into the dissolved (dSi) form through a thermodynamically favored forward reaction: $SiO_{2\,(s)} + nH_2O_{\,(l)} \rightarrow H_4SiO_{4\,(aq)}$ (Williams et al. 1985). Because of the overwhelming global importance of diatoms in Si cycling, they have traditionally been considered the only important biological component when calculating marine Si budgets; all other siliceous organisms are thought to have negligible roles (Tréguer et al. 1995, Ragueneau et al. 2000).

With the growing awareness that siliceous sponges can have significant roles in bSi production on the continental shelves, it is important to empirically address the Si budgets in regions where sponges are prominent members of the benthos. Like diatoms, sponges produce a siliceous skeleton of spicules made from hydrated amorphous silica which is chemically very similar to the bSi produced by diatoms (Sandford 2003). Dissolution of diatom frustules occurs within days after cell death when exposed to the water (i.e. not buried; Kamatani 1982), whereas sponge spicules are surprisingly resistant to dissolution even after months being exposed to the water and not buried (Kamatani 1971, Maldonado et al. 2005). Maldonado et al. (2005) first quantified the large difference between long-term dissolution of diatom frustules and sponge spicules in axenic water and proposed that sponges represent an overlooked component of the oceanic Si budget.

Glass sponges (class Hexactinellida) are more heavily silicified than the remaining sponge groups, with up to 95% of their dry weight made up of spicules (Barthel 1995). Glass sponges are characteristically deep-water animals (Tabachnick 1994), but are found in shallow waters (<50 m) in a few places worldwide, in particular Antarctica (Dayton et al. 1974) and the northeast Pacific Ocean. In these habitats, the living sponges and skeletons of dead individuals can cover large areas of the seafloor and create 3-dimensional habitats for other organisms (Dayton et al. 1974, Bett & Rice 1992, Beaulieu 2001). In fjords of British Columbia, Canada, densities as high as 240 ind. 10 m^{-2} are found (Leys et al. 2004). Also on that coast, glass sponges create large reefs consisting of siliceous mounds up to 21 m high that discontinuously cover an area greater than 700 km^2. The Pacific Coast reefs are only 9000 yr old (Conway et al. 1991, 2001, 2005), but they are analogous to reefs built by now extinct siliceous sponges that once dominated the Tethys Sea during the Mesozoic (Ghiold 1991, Krautter et al. 2001). Thus, the occurrence of living reefs, which are globally unique to the west coast of North America, may reflect modern-day biogeochemical processes similar to those that determined both the success and eventual decline of the Mesozoic reefs.

If we consider that glass sponges can live for hundreds of years (Leys & Lauzon 1998, Fallon et al. 2010) and the potential of their spicules to resist dissolution, modern glass sponge reefs could represent a significant Si sink in the continental shelf waters of the north-eastern Pacific Ocean. The goal of our study was to quantify the Si flux in glass sponge reefs and determine whether they constitute a significant Si sink. We focused on 3 reefs in the Strait of Georgia (SOG), British Columbia, where earlier work had mapped the distributions of the sponges using remote operated vehicles (ROVs) and digital imagery (Chu & Leys 2010). First we quantified the amount of bSi that is locked into the skeletons of live glass sponges in each reef and the concentrations of dSi in water around the reefs. We then tested whether glass sponge skeletons dissolve (or release dSi) into seawater in bottle experiments over a period of 8 mo. The presence of bacteria has been shown to accelerate the dissolution of diatom frustules (Bidle & Azam 1999, 2001) by degrading the organic components that protect the bSi from direct contact with the seawater (Smith et al. 1995). Therefore, in contrast to the experiments performed by Maldonado et al. (2005), natural seawater containing bacteria was used in the dissolution experiments. In nature, siliceous sponge skeletons that are exposed to seawater over long periods undergo a form of diagenesis in which the negatively charged surface of the silica attracts positive ions such as Fe^{3+} and Mn^{2+} that precipitate onto the spicule, causing it to blacken (Hurd 1973). Because diagenetic skeletons have been exposed to natural dissolution conditions for a long time, we also included 'blackened skeletons' in our experiments to see if they showed increased dissolution compared to fresh spicules. Our calculations, based on measurements of bSi locked into each reef, dissolution rates, and known growth rates

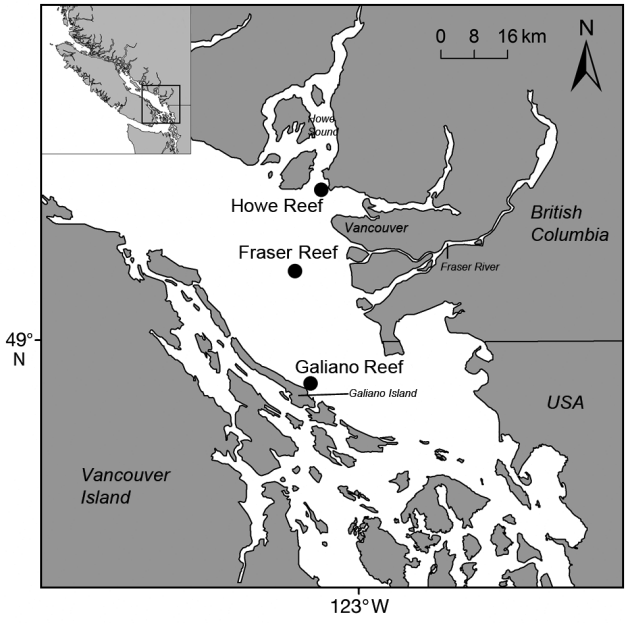

Fig. 1. Location of the 3 glass sponge reefs (●) in the Strait of Georgia, British Columbia, Canada

of hexactinellids suggest that glass sponge reefs may be significant Si sinks on the western continental shelf waters of Canada.

MATERIALS AND METHODS

Study site: Strait of Georgia

The SOG (Fig. 1) is a large estuary, approximately 1100 km^3 by volume (Pawlowicz et al. 2007) that separates the mainland of western Canada from Vancouver Island. Residence time of bottom waters is approximately 300 d, whereas the surface waters (upper 50 m) exchange approximately every 100 d (Waldichuk 1983). The estuarine circulation in the surface waters of the SOG is mainly driven by the Fraser River, which is also responsible for 65% of the freshwater flowing into the SOG (Thomson 1981, Thomson et al. 2007). The Fraser River outflow spreads over the surface of the SOG, stratifying the water column until vertical mixing is facilitated by wind (LeBlond et al. 1991). Seasonal upwelling events and intrusions of offshore waters through the southern SOG replenish deep (>100 m) and intermediate water layers (LeBlond et al. 1991, Thomson et al. 2007), causing relatively high dSi levels on the shelf (Freeland & Denman 1982, Wheeler et al. 2003, Whitney et al. 2005). Long-term monitoring of the water column properties in the SOG (www.stratogem.ubc.ca) indicates that diatoms facilitate cycling of dSi in the upper 30 m of the water column, with annual dSi concentrations fluctuating from <5 to 60 µmol l^{-1}. The largest of these dSi fluctuations coincides with diatom blooms that occur during the spring and fall; however, intermittent short-term blooms (lasting only days) can occur between March and October (Johannessen et al. 2005, Johannessen & Macdonald 2009). At depths below the upper 40 m, conditions are more temporally stable, with dSi levels remaining >40 µmol l^{-1} throughout the year. Reduced irradiance and light penetration due to low water column transmissivity are suggested to control diatom growth from October to February and limit the average photic zone depth to less than 30 m (Johannessen et al. 2006, Johannessen & Macdonald 2009).

Field sampling and the glass sponge reefs

Field work in the SOG was carried out on the Canadian Coast Guard Ship (CCGS) 'Vector' in October 2007 and the CCGS 'John P Tully' in October 2009 using the ROV ROPOS. Twelve independent sponge reef systems are known in the SOG (Conway et al. 2007), which together cover approximately 11 km^2 of the benthos at depths between 59 and 210 m; we visited 3 reefs (Fig. 1): the Fraser reef (FR: 49° 9′ 16″ N, 123° 23′ 4″ W, mean depth = 164 m), Howe reef (HR: 49° 19′ 58″ N, 123° 17′ 42″ W, mean depth = 80 m), and Galiano reef (GR: 48° 54′ 51″ N, 123° 19′ 28″ W, mean depth = 90 m). At each reef, the sponges are distributed in characteristic, highly clustered patches that can sometimes create large mounds (Fig. 2A). The extent of live and dead sponges at each reef was mapped in detail as described by Chu & Leys (2010). The closely related species *Aphrocallistes vastus* and *Heterochone calyx* are the only 2 reef-building glass sponges in the SOG. Because *A. vastus* is the dominant species (Chu & Leys, 2010), we focused on *A. vastus* for our measurements.

To determine the amount of sponge mass (tissue and skeleton) and bSi within a reef, dense patches of live individual *Aphrocallistes vastus* (Fig. 2B) were sampled using an Ekman grab (3540 cm^3). The grab was manually pushed into the sponges and closed by the manipulator arms of ROPOS. Eight grabs (HR: 2, FR: 3, GR: 3) were retrieved in 2007 and 4 additional grabs (HR: 2, FR: 1, GR: 1) in 2009. Immediately after collection, the sponge pieces were removed from the grab and frozen at –20°C until processed (Fig. 2C). Our study accounts only for the portion of a reef (live and dead sponges) that is exposed, not buried.

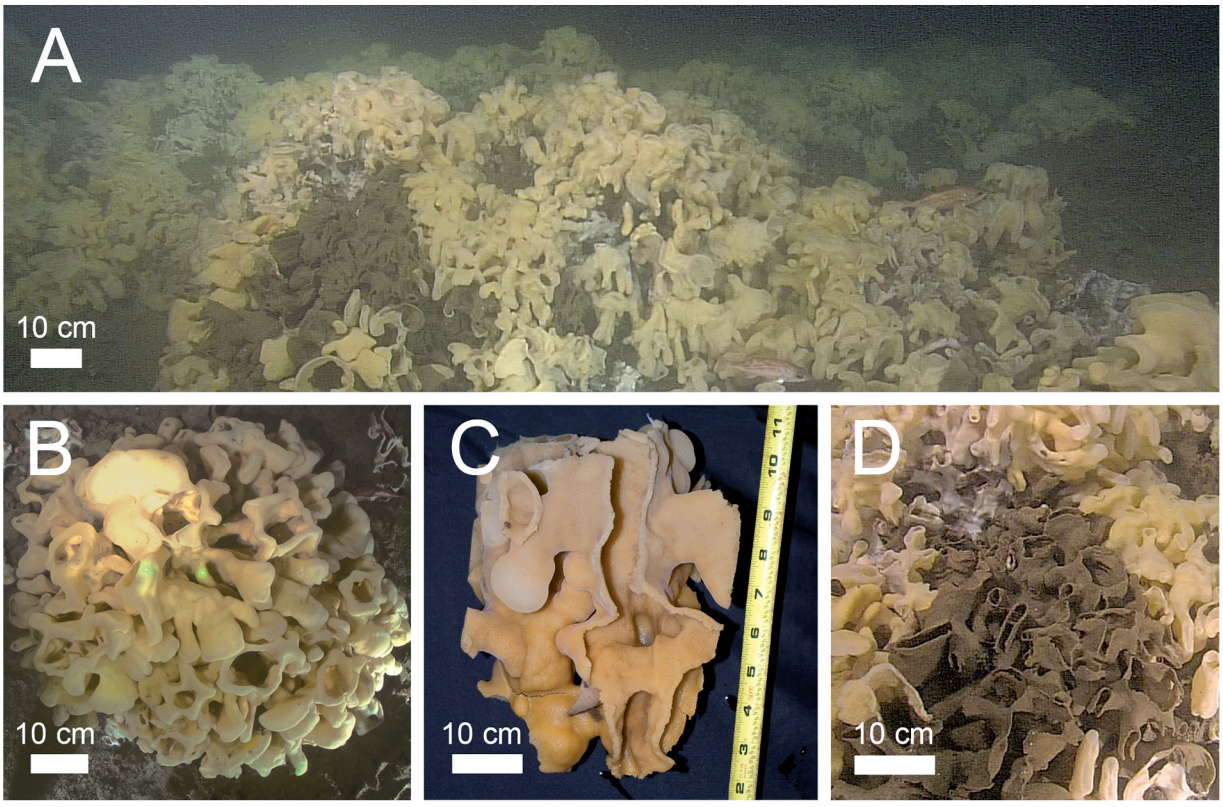

Fig. 2. Field sampling of silica at a glass sponge reef. (A) *Aphrocallistes vastus* and *Heterochone calyx* form dense bushes that cover the seafloor in patches within a reef. (B) Live sponges are cream colored. (C) Samples of live *A. vastus* were taken with an Ekman grab. (D) Dead sponges eventually 'blacken' after death but the fused skeleton persists and retains the original shape of the sponge

The extensive buried reservoir of bSi at each reef was not quantified in this study.

In 2007, water was sampled *in situ* throughout the water column for analysis of dSi levels. The sediment–water interface was sampled, ~5 cm above the bottom, using SIP water samplers (Yahel et al. 2007), which prevent resuspension of sediments and ensure a clean water sample. Water was also sampled from 2 and 10 m above the bottom (mab) using 2.5 l Niskin bottles mounted on the forward brow of ROPOS. Water samples were taken among and away from the reefs (~160 m depth, where no sponges were found). Water samples from surface and mid-water depths were collected using 5 l Niskin bottles lowered over the side of the ship. All water samples were syringe filtered through 0.22 µm Millipore membrane filters, frozen at −20°C, and analyzed for dSi within 10 d after sampling, using a Technicon AAII Autoanalyzer at the Institute of Ocean Sciences (IOS) in Sidney, British Columbia (Barwell-Clarke & Whitney 1996). Logistical constraints of submersible research limited the number of water samples we could replicate at each reef. Therefore, dSi measurements from water samples were pooled at each depth among reefs for meaningful statistical analysis. Differences in dSi concentration at the sediment–water interface among and away from sponges were determined using a Mann-Whitney *U*-test.

Seawater used for dissolution experiments was collected from a depth of ~150 m, immediately filtered through 1 to 5 µm Si-free cartridge filters to remove phytoplankton, and refrigerated in polyethylene carboys at 10°C until used.

Measurements of bSi content

The sponge skeleton from each Ekman grab was oven-dried at 60°C and weighed. To determine the bSi content of the grabs, 2 methods were used. Subsamples (n = 5 per grab) were treated with 5% hydrofluoric acid (HF) for 24 h (Maldonado et al. 2010) in pre-weighed Eppendorf tubes, centrifuged at 20 000 × *g* for 2 min (Eppendorf 5415 centrifuge), rinsed 3 times with distilled water (spun down between rinses), and oven-dried at 60°C to a constant

mass. The total bSi content of each Ekman grab was calculated from the loss in mass (percent bSi). A second set of subsamples from each Ekman grab (n = 10 per grab) was combusted at 500°C for 12 h, and the bSi content of the grabs was determined by the total ash-free dry weights of the samples (Barthel 1995). To compare the proportion of silica in skeletons of *Aphrocallistes vastus* between reefs, data were arcsine square root transformed and analyzed with a 1-way analysis of variance (ANOVA) with Tukey HSD pairwise comparisons.

The skeleton of *Aphrocallistes vastus* is composed of spicules that are loose and some that are fused to form a rigid scaffold. Loose spicules are lost with tissue when the sponge dies but the fused scaffold remains. Therefore, to determine the proportion of loose to fused spicules, 20 sponge pieces were haphazardly sampled from the remaining Ekman grab material, oven-dried, and weighed. Samples were placed in glass test tubes and washed with a small volume (<0.1 ml) of concentrated nitric acid at 95°C for 5 min to dissolve tissues. The acid was diluted with ~5.0 ml of distilled water and filtered onto preweighed 0.22 µm Millipore membrane filters. The intact fused skeleton of *A. vastus* was carefully removed with forceps and weighed. Filters were dried at 60°C and reweighed to determine the mass of loose spicules. The mass of each spicule component relative to the initial sample determined the proportions of loose and fused spicules, and the mass lost to acid digestion determined the proportion consisting of tissues.

Dissolution experiments with bSi skeletons

For bSi dissolution experiments, we used a batch design of 5 identical, independent experiments lasting 1, 2, 3, 6, and 8 mo. Each experiment consisted of 4 treatments with 8 replicates per treatment: (1) loose spicules from the tissue of freshly killed sponges (called 'loose'); (2) the fused spicule skeleton from freshly killed sponges ('fused'); (3) the fused spicule skeleton from dead sponges that was blackened by *in situ* diagenesis during long exposure to ambient natural dissolution conditions ('black'; Fig. 2D); and (4) controls with just seawater and no spicules. All spicule skeletons were cleaned in 5% sodium hypochlorite for 24 h, rinsed 3 times with distilled water, each time decanting the supernatant, and dried at 60°C until a constant mass was achieved. For each replicate, 25.5 ± 0.3 mg (n = 120) of cleaned spicules was added to capped polyethylene tubes (Corning) with 50 ml of seawater (salinity: 27 ppt). The seawater was filtered through 0.45 µm pore sized Millipore membrane filters to remove suspended diatom frustules but retain some bacteria. This filtration step was expected to skew the bacterial diversity toward smaller class sizes (<0.45 µm) and may have reduced its potential effect on bSi dissolution. Scanning electron microscopy (SEM) of the seawater confirmed the presence of small bacteria, but there were no unicellular eukaryotes such as diatoms (see 'Results'). Tubes were sealed with Parafilm and stored in the dark at 10°C for the duration of the experiments to mimic the deep-water environment of the reefs. Dissolution experiments were begun at 3 different times due to equipment and space constraints. Because the initial levels of dSi were slightly different among the 3 starting periods of our experiments (63.2, 65.7, and 64.5 µmol l^{-1}, Kruskal-Wallis test, H_2 = 14.25, $p < 0.001$), we analyzed each experiment independently of the others.

At the end of each experiment, tubes were gently inverted to mix, the pieces of fused skeletons were then carefully removed with forceps, and the concentration of dSi was measured in triplicate (SD = 0.5%) using the molybdate blue spectrophotometric method (Ultrospec 2100 pro UV/Visible spectrophotometer) at a wavelength of 810 nm (Strickland & Parsons 1972). For treatments using loose spicules, the seawater was filtered through 0.22 µm Millipore membrane filters prior to dSi measurements. Seawater pH was 8.1 at the start of the experiments and 7.5 at the end. Because the parametric assumption of heteroscedasticity was not met even after transformations, non-parametric individual Kruskal-Wallis with Dunn pairwise tests (Zar 1999) were used to examine between-treatment differences within each dissolution experiment.

To compare dissolution of sponge skeleton to diatom frustules, the diatom *Thalassiosira weissflogii* (CCMP 1336 strain; Provasoli-Guillard National Center for Culture of Marine Phytoplankton) was grown in 2 l batches using artificial seawater (salinity: 31 ppt, Instant Ocean® dissolved in distilled water) with added f/2-enriched media (Guillard & Ryther 1962) and 0.22 µmol l^{-1} sodium metasilicate. When cultures reached ~50 000 cells ml^{-1}, they were filtered onto 5 µm polycarbonate membrane filters, rinsed with distilled water into 50 ml capped polyethelene tubes, and dried at 50°C to a constant mass. Diatom frustules (n = 3) and controls of just seawater (n = 2 per experiment) were left to dissolve for 1, 2, 3, and 4 mo at 10°C. Water samples were processed and dSi concentration analyzed as above. Within each

diatom experiment, individual Mann-Whitney *U*-tests were used to test for differences in dSi between diatom and control tubes from bSi dissolution. Likewise, Kruskal-Wallis with Dunn pairwise tests were used to examine differences in dSi concentrations in diatom tubes as a function of time (1, 2, 3, 4 mo).

Microscopy

Sponge spicules and diatom frustules recovered from the dissolution experiments and untreated spicule and diatom samples were dried at 60°C to a constant mass, mounted on aluminum stubs, coated with gold, and studied using SEM (JEOL 6301F field emission microscope). To evaluate the effect of cleaning by HF and also as a visual aid of what silica etching may look like at the ultrastructural level, spicules from sponge pieces not used in our dissolution experiments were cleaned with sodium hypochlorite and then artificially etched with 5% HF for 3 min, rinsed, and prepared as above for SEM. The elemental composition of the blackened material adsorbed onto dead sponge skeletons not used in the dissolution experiments was analyzed using energy dispersive X-ray spectroscopy (EDX) with a specific resolution of 138 eV.

To determine whether bacteria were present in the seawater used for dissolution experiments, water was fixed with 5% glutaraldehyde and filtered through 0.02 µm Millipore membrane filters. Filters were post fixed in 1% osmium for 30 min, dehydrated in ethanol, and critical point dried. Filters were fixed to aluminum stubs, coated with gold, and examined by SEM.

RESULTS

Amount of sponge bSi in glass sponge reefs

Due to the amorphous shapes of the sponges we sampled, the mass of *Aphrocallistes vastus* captured by the Ekman grab was highly variable regardless of the reef sampled (Table 1). However, the proportion of silica in the sponges was quite constant in grabs from each reef, 80 to 83% (combustion method) and 88 to 92% (HF method) of the total mass, but sponges at FR had a significantly lower ratio of silica to organic tissue (combustion method: ANOVA, $F_{2,77}$ = 4.43, p = 0.015; HF method: ANOVA, $F_{2,57}$ = 11.52, p < 0.0001). The ~10% difference between the 2 methods (combustion versus HF) is most likely explained by the water content in *A. vastus* spicules (Sandford 2003), which is lost during combustion (Mortlock & Froelich 1989). Therefore, within an individual *A. vastus,* 20.7 ± 3.5% (±SD) of the sponge is made of organic tissue, with the spicule suite consisting mostly of fused skeleton (62.7 ± 3.4%) and a small component of loose spicules (16.6 ± 1.6%). For all of our calculations we used the more conservative proportions derived from combustion in our calculations (Table 1).

Sponge reefs are 3-dimensional structures which may grow to a height of 1.2 m above the seafloor (Conway et al. 2005). Given that not all sponges are 1.2 m high, we estimated the volume of the reef by using half that height (0.6 m) multiplied by the area covered by live sponge (HR: 10 242 m^2, FR: 13 774 m^2, GR: 23 432 m^2) and dead sponge (HR: 9083 m^2, FR: 6945 m^2, GR: 29 799 m^2; see Chu & Leys 2010). Using this method, we calculated that live sponges contained 17 to 27 kg of bSi m^{-3} of reef (Table 2). Live sponges contain both fused skeletons and loose spicules (bSi content, 79.3%), while dead skeletons consist of only the fused skeletons (bSi content, 62.7%). Therefore, the calculated bSi reservoirs trapped in each reef are 141 (FR), 180 (HR), and 595 (GR) t and the mass of bSi per unit area at each reef is 7.3 (FR), 8.7 (HR), and 11.2 (GR) kg m^{-2} (Table 2).

DSi in the water column

Although summer dSi concentrations in surface waters can be less than 5 µmol l^{-1}, when we sampled in October the dSi concentration in surface waters was 48 µmol l^{-1}, whereas dSi at depths greater than 50 m was slightly higher (52 µmol l^{-1}), but this difference was nonetheless statistically significant (Mann-Whitney *U*-test, U = 42, N_1 = 5, N_2 = 6, p = 0.036; Fig. 3A). Water sampled at the sediment–water interface also had slightly higher levels of dSi in areas within the sponge reef compared to areas away from reefs but without sponges (Fig. 3B; Mann-Whitney *U*-test, U = 25, N_1 = 5, N_2 = 17, p = 0.012).

Dissolution potential of sponge spicules

There was no significant change in dSi levels for treatments with either loose spicules or fused skeletons compared to the controls in any of the experiments (1, 2, 3, 6, or 8 mo; Fig. 4A–E). However, the diagenetically blackened skeleton treatments showed small but significant increases in dSi levels compared

Table 1. *Aphrocallistes vastus*. Dry sponge mass and the proportion of sponge biogenic silica (bSi) in samples from each of the 3 glass sponge reefs in the Strait of Georgia, British Columbia, Canada. An Ekman grab (3540 cm^3) was used to sample live sponges at each of the reefs, and the proportion of bSi in the sponge mass was determined from subsamples using 2 methods. The hydrofluoric acid (HF) dissolution method was done with combined 2007 and 2009 grabs. Combustion was only done with 2007 grabs. Number of grabs used in each method is in parentheses. Different letters in parentheses indicate significant differences between reefs (Tukey HSD, $\alpha = 0.05$)

Reef	Sponge mass dry weight (g)			Proportion of sponge bSi (%)					
				HF dissolution method			Combustion method		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Howe	4	60.2	35.7	20(4)	92.8 (b)	2.4	30(3)	82.1 (ab)	7.7
Fraser	4	69.3	62.3	20(4)	88.9 (a)	3.0	20(2)	80.0 (a)	2.2
Galiano	4	94.2	58.7	20(4)	92.3 (b)	2.3	30(3)	83.1 (b)	3.4

to controls (all spicule dissolution experiments, Kruskal-Wallis tests, $H_3 > 11$, $p < 0.01$) with a maximum increase in dSi levels of ~10 µmol l^{-1} after 8 mo (Fig. 4A–E) . Slightly negative dissolution values in some of the treatments indicated that there must have been some adsorption of dSi onto the insides of the experiment tubes or the substrate materials. In contrast, bottles with diatom frustules had concentrations of dSi 200 times higher after only 1 mo, and continued to release dSi even after 4 mo (Fig. 4F: Kruskal-Wallis test, $H_4 = 8.90$, $p = 0.03$).

The surface of diatom frustules showed evidence of etching already after 1 mo (Fig. 5A–B). In contrast, the surface morphology of loose spicules (Fig. 5C–E) was not obviously different from that of spicules that had been dissolving for 8 mo, having retained even the fine-scale ornamentation on the spicules (Fig. 5F–H). Similarly, on control pieces of fused skeletons (Fig. 5I), the sharp beam spikes (Fig. 5J) and exposed cross sections of broken beams (Fig. 5K) showed no appreciable difference in morphology from those that had been exposed to seawater for 8 mo (Fig. 5L–M). SEM-EDX showed that the diagenetically blackened skeletons were coated with iron and manganese (Fig. 6A). These minerals adsorb onto the fused skeleton under natural conditions *in situ* and subsequently cause other particles such as diatomaceous material (Fig. 6B and inset) to adhere to the spicules. Pre-dissolution cleaning with sodium hypochlorite removed most, but not all, of the adsorbed minerals (Fig. 6C). Even after rinses of hot nitric acid, adsorbed precipitates could sometimes remain (J. Chu pers. obs.), and thus we cannot entirely attribute the change in the dSi levels in these treatments to dissolution of sponge bSi alone.

Examination of the fused skeletons by SEM revealed pitting at the exposed fracture surfaces of broken beams. The nanoparticle structure of the spicule bSi is colloidal (Fig. 6D), which has also been observed in the structure of diatom frustules (Noll et al. 2002) and in spicules from both demosponges and glass sponges (Weaver et al. 2003, Aizenberg et al. 2005). Etching by HF showed pitting patterns at the exposed fracture surfaces of broken beams (Fig. 6E) similar to those identified in diagenetically blackened skeletons after the 8 mo dissolution experiments. The hollow cavity of the axial organic filament was also enlarged after being etched with HF (Fig. 6E, inset). In comparison, intact loose spicules etched with HF showed no change in morphology (Fig. 6F,G). Bacteria were present both in the initial sea water used for dissolution experiments (Fig. 6H) and in samples at the end of dissolution experiments (Fig. 6I).

Table 2. *Aphrocallistes vastus*. Amount of dry sponge mass, volume, and biogenic silica (bSi) found in each of the 3 glass sponge reefs in the Strait of Georgia. The volume of each reef was calculated using survey areas from Chu & Leys (2010) using a 0.6 m average reef height. The mass of bSi was calculated using proportions of bSi of 79.3% for live sponges (consisting of both spicule components) and 62.7% for dead sponges (consisting of only the fused skeleton)

Reef	Density of dry sponge mass ($kg\ m^{-3}$)	Volume of reef (m^3)		Mass of bSi (kg)		Total bSi reservoir (t)	bSi per reef area ($kg\ m^{-2}$)
		Live	Dead	Live	Dead		
Howe	17.0	6145	5450	82729	58013	141	7.3
Fraser	19.6	8264	4167	128282	51146	180	8.7
Galiano	26.6	14059	17879	296669	298302	595	11.2

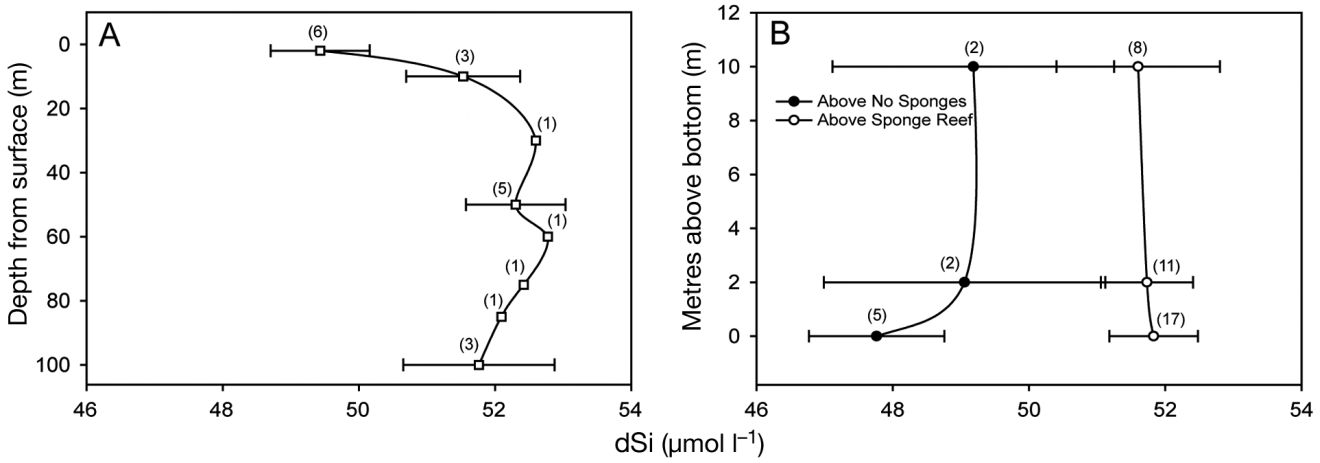

Fig. 3. Bathymetric distribution of dissolved Si (dSi) levels in the waters above and around 3 glass sponge reefs in the Strait of Georgia in October 2007 and 2009. Water samples were taken in vertical profiles at each reef, and dSi concentration measurements from each depth were pooled for analysis. Data are shown as means ± SE with sample sizes in parentheses. (A) Average dSi concentration was generally high, ranging from 48 to 52 µmol l^{-1} throughout the water column. Slightly lower dSi levels were measured in the upper 50 m of the water column, with the highest dSi levels occurring below 80 m and where the glass sponges are located. (B) At the sediment–water interface, the concentration was also slightly higher at the reefs (52 µmol l^{-1}) compared to areas away from the reefs (48 µmol l^{-1})

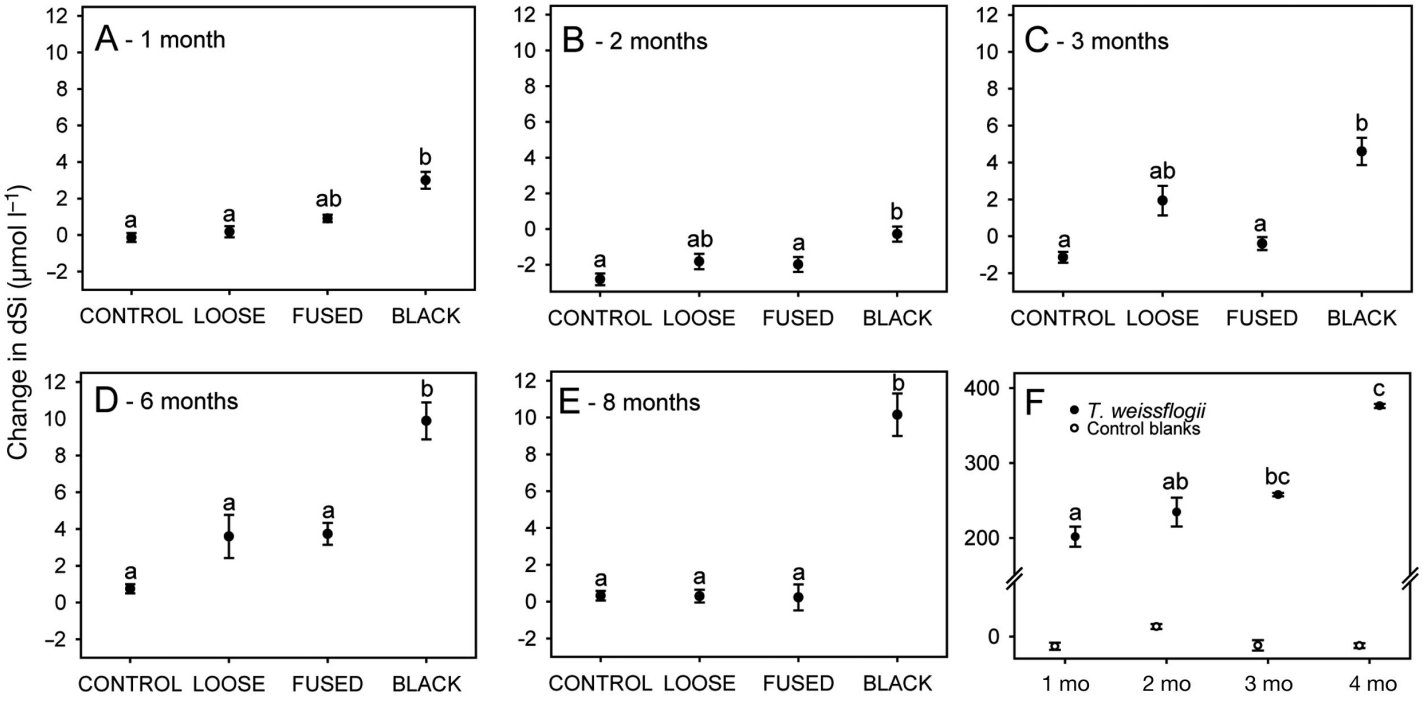

Fig. 4. *Aphrocallistes vastus*. Biogenic silica (bSi) dissolution experiments with the spicules. Independent sets of replicates were run for the duration of (A) 1 mo, (B) 2 mo, (C) 3 mo, (D) 6 mo, and (E) 8 mo. Within an experiment, different letters among columns indicate significant differences between treatments (Dunn's test, $\alpha = 0.05$). (F) One to 4 mo dissolution experiments of diatom *Thalassiosira weissflogii* frustules showed significant dissolution after only 1 mo and progressive dissolution over 4 mo (within each experiment: Mann-Whitney *U*-test, $p < 0.0001$; between experiments: Kruskal-Wallis test, $p < 0.0001$). Different letters among columns indicate differences in dissolution of frustules between experiments (Dunn's test, $\alpha \leq 0.05$). Data values are mean ± 1 SE. The slight negative values in some treatments are likely the result of adsorption of dissolved Si (dSi) onto the inside surfaces of the experimental bottles

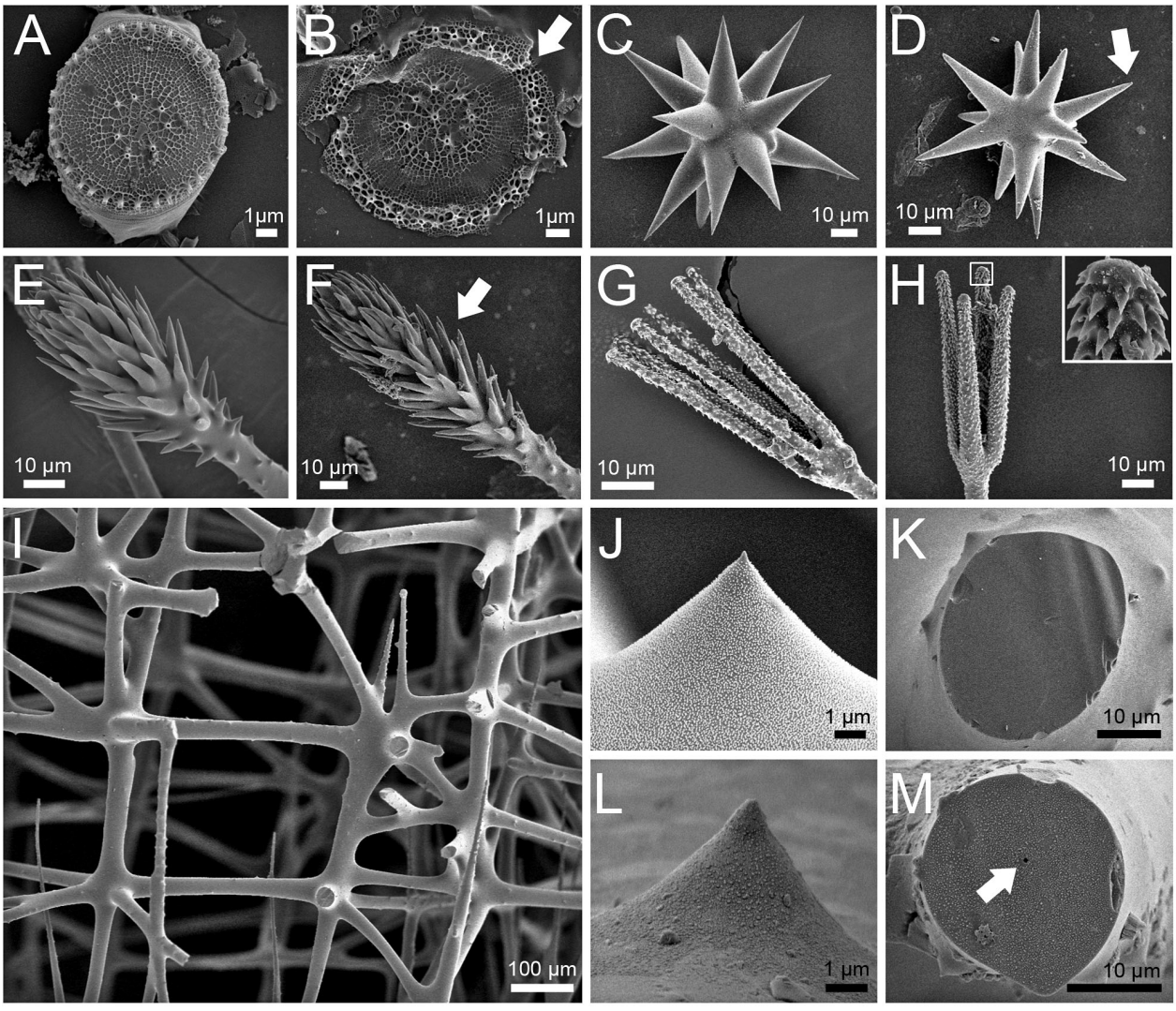

Fig. 5. Evidence of biogenic silica (bSi) dissolution in biogenic substrates observed by scanning electron microscopy. (A) Frustules of the diatom *Thalassiosira weissflogii* showed intricate surface morphology before dissolution experiments. (B) Fine details (arrow) of the surface morphology of frustules was lost to dissolution after 1 mo in seawater. (C–H) Loose spicules of *Aphrocallistes vastus* showed no signs of dissolution after 8 mo in seawater: (C) initial oxyhexaster morphology, (D) oxyhexaster retained sharp features (arrow) after 8 mo in seawater, (E) initial pinnular hexactin morphology, (F) pinnular hexactin also retained sharp features (arrow) after 8 mo in seawater, (G) initial forked scopule morphology, (H) forked scopule retained sharp features (inset) after 8 mo in seawater. (I–M) Fused skeletons of *A. vastus* showed no signs of dissolution after 8 mo in seawater: (I) initial fused skeleton, (J) initial ornamental spines on beams, (K) initial exposed cross section of a broken beam, (L) beam spine after 8 mo in seawater, and (M) exposed cross section after 8 mo in seawater. Note that the axial cavity (arrow) of the organic filament is visible

DISCUSSION

Glass sponge reefs concentrate large amounts of bSi over a relatively small benthic area because of the large size, high density, and high bSi content (80% by dry weight) of the individual sponges. Furthermore, the Si used to build the reefs is trapped for long periods because spicules are highly resistant to dissolution. Given their slow rates of growth and long-lived nature (Leys & Lauzon 1998, Fallon et al. 2010), glass sponges will continually incorporate bSi into the living portions of the reefs and thereby form significant sinks of silica.

Glass sponge reefs as large reservoirs of bSi

Aphrocallistes vastus is a heavily silicified sponge with a relative bSi content on a par with the heavily silicified demosponges and hexactinellids found in

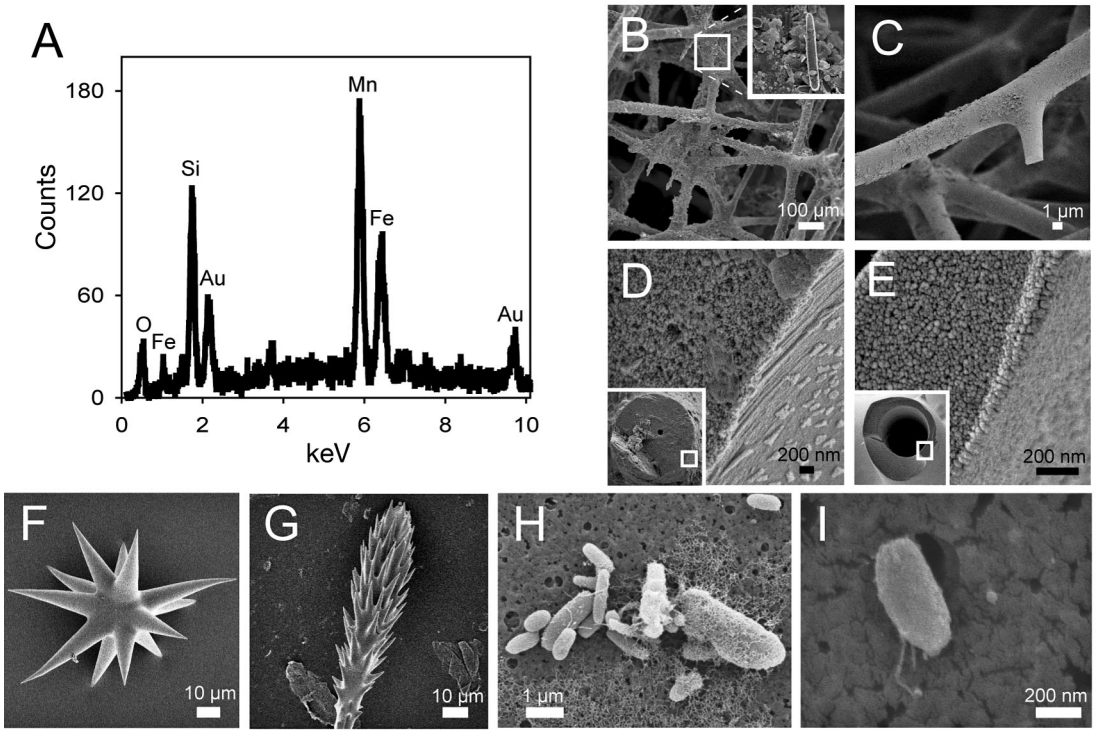

Fig. 6. *Aphrocallistes vastus*. Evidence of biogenic silica (bSi) dissolution in diagenetically blackened skeletons observed by scanning electron microscopy. (A) Energy dispersive x-ray spectroscopy (EDX) analysis showed the composition of the black coating, which consisted of adsorbed precipitates of iron (Fe) and manganese (Mn). The peak of silica is from the spicule, and the peak of the gold is from coating for scanning electron microscopy. (B) Skeletons were completely coated by precipitates which adhered diatomaceous material to the spicule (inset). (C) Most of the precipitates were removed after cleaning with sodium hypochlorite. (D) Blackened skeleton had pitting at the exposed cross sections of skeletal beams but not on the outer surface of beams after 8 mo of dissolution in sea water. (E–G) The effects of artificially etching spicules with hydrofluoric acid (HF): (E) artificially induced etching with a 3 min rinse in dilute HF showed similar patterns of etching at cross sections as the 8 mo dissolution experiment and also resulted in enlargement of the axial cavity where the organic filament previously resided. There were no obvious changes in the morphology of (F) oxyhexasters or (G) pinnular hexactins from HF rinses. (H) Bacteria were present in the initial filtered (0.45 µm) sea water used for dissolution experiments. (I) Bacteria were also present in the sea water at the end of dissolution experiments

Antarctica, where dSi levels are also elevated (Barthel 1995). The high dSi concentrations found in the demersal water layer around the sponge reefs (48 to 52 µmol l^{-1}) are comparable to the annually high dSi levels characteristic of the deeper waters (>50 m) in this region. High levels of dSi are considered one of the main factors that sustain and promote growth of siliceous sponges (Maldonado et al. 1999, Leys et al. 2004, Whitney et al. 2005). The high concentration of dSi we measured in the surface waters likely reflects dSi replenishment by a combination of both external water inputs and local dissolution of diatoms from blooms which occur in October. Slightly higher dSi (~4 µmol l^{-1}) at the sediment–water interface among reefs compared to sites away from reefs may be due to the presence of diatomaceous material trapped by the reefs. Because our observed small differences in dSi concentrations were from a single time point, additional temporal sampling would be required to determine whether the relatively high benthic dSi is persistent throughout the year in the SOG.

The amounts of bSi locked by sponge skeletons in HR (145 t), FR (180 t), and GR (595 t) equate to 2.4 × 10^6, 3.0 × 10^6, and 9.9 × 10^6 mol of Si, respectively. Our calculations take into account only the fraction of each reef that is exposed to the water column and is thus responsible for Si flux. The extensive buried portions of the glass sponge reefs (up to 21 m, Conway et al. 2005) is likely an even greater reservoir of trapped bSi, because spicules recovered from past corings of the reefs have shown no evidence of dissolution (Krautter et al. 2006), and typically only the top 0.3 m of buried sediments would experience dissolution and then still at very low rates (Hurd 1973, DeMaster 2002). If we were to take into account the buried sponges in the reefs, our calculations of the bSi reservoirs would likely be an order of magnitude higher.

Dissolution potential of glass sponge bSi

Consistent with past studies that compared the solubility between diatom frustules and sponge spicules, hexactinellid spicules are significantly more resistant to dissolution compared to diatom frustules (Kamatani 1971, Maldonado et al. 2005). The pitting observed on the diagenetically blackened spicules indicates that over the long term, sponge bSi will eventually regenerate back to a dissolved form but at a much slower rate relative to that from diatoms. A comparison of our 1 mo experiments shows a ~200-times difference in dissolution potential between glass sponge and diatom bSi. The dissolution potential for sponge bSi may be slightly lower because the small drop in pH at the end of our experiments and the potential for diatom fragments to have been attached to the diagenetic skeletons may have slightly increased our end values. Taking these factors into account, the discrepancy between the long-term dissolution potential of sponge and diatom bSi may be even higher. Considering that the diagenetic skeletons we used had already undergone extended exposure to *in situ* dissolution conditions prior to our 8 mo experiments, the rates we measured can be cautiously interpreted as being in the upper range of dissolution rates. Natural diagenetic processes occurring at the seafloor would further reduce solubility of dead sponge skeletons, because surface adsorption of positive ions Fe^{3+} and Al^{3+} to diatom bSi (Lewin 1961, Van Bennekom et al. 1991) and Al^{3+} to synthetic silica (Iler 1973) has been shown to slow their rate of dissolution. Under stable conditions, the dissolution of hexactinellid spicules likely occurs over a time scale of years, and the turnover of Si by glass sponges (from incorporation to release) would occur over decades or centuries.

The magnitude of the discrepancy in dissolution potential between glass sponge and diatom bSi highlights an overlooked mechanism in which both organisms cycle Si. A major control on bSi solubility at the seafloor is the post mortem incorporation of Al^{3+} from the siliciclastic matter found in bottom sediments (Dixit et al. 2001). Our interpretation, however, stems from experimental treatments using fresh material sampled from live specimens where both spicules and frustules were treated with identical experimental conditions. Therefore, water chemistry alone cannot account for the difference in solubility. Because diatom frustules and sponge spicules are both made of amorphous hydrated silica, the different organic subcomponents found within their silica matrix may explain the difference in dissolution potential between frustules and spicules.

In diatoms, the polysaccharide pectin makes up the organic component of the bSi matrix (Desikachary & Dweltz 1961). Bacterial activity degrades pectin, which exposes the Si to sea water and allows dissolution to occur by thermodynamic reactions. A different polysaccharide, chitin, is found in the bSi matrix within spicules of another reef-forming hexactinellid, *Farrea occa* (Ehrlich et al. 2007). Chitin itself is naturally insoluble in water (Hock 1940, Austin et al. 1981) and enhances exoskeleton insolubility when imbedded into the chitin-protein complexes of other marine invertebrates (Hunt 1970, Weiner et al. 1983). Chitinivorous bacteria, which are widely distributed in marine sediments, are responsible for degrading chitin in marine environments (Zobell & Rittenberg 1938). It is reasonable to assume that if chitin is also found in the bSi matrix within the spicules of *Aphrocallistes vastus*, then its insolubility could explain the difference in dissolution potential between sponge spicules and diatom frustules. Although we were unable to determine whether bacteria enhanced the bSi dissolution in our experiments, if chitinivorous bacteria can enhance the dissolution of sponge spicules, our hypothesized mechanism may be an important but overlooked process that promotes Si cycling in benthic waters.

The biotic and abiotic processes that affect the Si cycling in glass sponges are in stark contrast to the rapid Si turnover rates experienced by diatom-produced bSi in surface waters. For glass sponges, the combined processes of (1) optimal conditions for sponge growth, (2) long life spans, (3) resistance of their spicules to dissolution, (4) potential effects of diagenesis, and (5) the continued burial of dead sponges due to trapping of sediments would facilitate the removal of Si and create a Si sink at a sponge reef.

Silicon budget of glass sponge reefs

To quantify the Si sink created by glass sponges, we use FR to illustrate our calculations of the Si flux (Fig. 7). Two very different methods have both suggested that large glass sponges can live for 220 to 500 yr (Leys & Lauzon 1998, Fallon et al. 2010). In a population of glass sponges monitored for several years in a fjord near our study site in the SOG, glass sponges grew an average of 2 cm yr^{-1}, with the fastest growth rates observed in smaller individuals and attenuation of growth rates occurring when individuals became bigger (Leys & Lauzon 1998). Using conservative assumptions of a 1 cm yr^{-1} growth rate in only the vertical dimension of a reef, the

sponge population at FR would incorporate Si into the spicules at an average rate of 3.5×10^4 mol Si yr^{-1}. Based on our maximum observed dissolution rate (10 µmol l^{-1} over 8 mo), Si would be released at a rate of 0.083 µmol Si d^{-1} g^{-1} of dead sponge skeleton. Under favorable conditions for dissolution, the skeletons of dead sponges at FR would release an average 1.3×10^3 mol Si yr^{-1}. The rate at which Si is sequestered into the sponges is 23 times greater compared to the rate of Si released from dissolution and highlights the magnitude of the sink effect at FR. Similarly, the calculated Si sinks for HR and GR are 13 times and 9 times the rate of release, respectively. In the SOG, an average annual dSi concentration of 50 µmol l^{-1} would yield a reservoir of 5.5×10^9 mol Si in the water column. By extrapolating our calculations from FR to the approximately 11 km^2 of glass sponge reefs (Conway et al. 2007) found in the SOG, a comparable Si reservoir of 3.6×10^9 mol Si exists in the reefs, which equals 65% of the SOG dSi reservoir. This significant amount of glass sponge bSi clearly indicates that glass sponge reefs are a major component of Si cycling in the SOG.

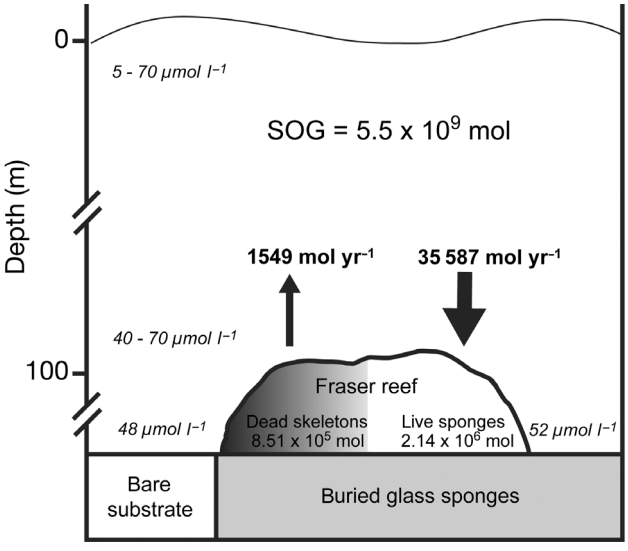

Fig. 7. Silicon (Si) budget of the Fraser glass sponge reef. A reservoir of 2.99×10^6 mol of Si is locked into the live (2.14×10^6 mol Si) and dead (8.51×10^5 mol Si) glass sponges. The rate of Si sequestering from growth (35 587 mol yr^{-1}) is 23 times greater than rate of Si released from dissolution (1549 mol yr^{-1}), and thus the reef functions as a considerable Si sink. In the Strait of Georgia (SOG), annual dissolved Si (dSi) levels fluctuate from 1 to 70 µmol l^{-1} in the surface waters (<30 m), but in deeper waters, annual dSi levels remain high (>40 µmol l^{-1}) throughout the year. October dSi concentration at the sediment–water interface was measured to be ~52 µmol l^{-1} at the reefs and ~48 µmol l^{-1} outside the reefs, over areas of bare substrate (bedrock or mud). With an average of 50 µmol l^{-1} throughout the Strait of Georgia (SOG) water column (~1100 km^3), a calculated reservoir of 3.6×10^9 mol Si exists in the SOG. Water column values for dSi were estimated from vertical profile data found at www.stratogem.ubc.ca

The area covered by glass sponge reefs in the SOG represents only a small fraction of the 700 km^2 benthic area (Conway et al. 2001) covered by all known reefs along the continental shelf of British Columbia. To determine whether glass sponge reefs affect the cycling of Si on a larger scale in the world's oceans, we can extrapolate from the above calculations. If we assume that a conservative 50:50 ratio of live to dead sponges occurs over the 700 km^2, a considerable reservoir of 9.7×10^{10} mol Si is locked into the glass sponges that sequesters 9.0×10^8 mol Si yr^{-1} and releases 7.8×10^7 mol Si yr^{-1}. This amounts to a significant Si sink, whereby 12 times more Si is sequestered than released. Although this Si budget may be negligible relative to that of the entire world's oceans (2.4×10^{14} mol Si yr^{-1}, Tréguer et al. 1995), the Si sink created by glass sponge reefs clearly represents an important biogeochemical component within large regions (10^2 km^2) of the northeast Pacific continental shelf (Whitney et al. 2005).

Ecological implications of glass sponges in the Si cycle

During the late Cretaceous, diatoms developed a more efficient mechanism of Si uptake compared to that of radiolarians and the siliceous sponges which had thrived in the dSi-rich oceans of the previous period (Maldonado et al. 1999, 2010, Lazarus et al. 2009). The dSi competition with diatoms is suggested as the cause of the disappearance of the siliceous sponge reefs from the photic zone during the Mesozoic (Maldonado et al. 1999). In the modern oceans, glass sponge reefs are unique to the dSi-rich waters of the Northeast Pacific, and here their bathymetric distributions are quite shallow (59 to 210 m) compared to glass sponge populations found elsewhere, yet they do not reach into the photic zone. If the modern sponge reef environment reflects that of their extinct Mesozoic counterpart, the rapid cycling of dSi by diatoms in the surface layer could control the shallower depth limits of the glass sponge reefs. Thus, the success of the benthic glass sponge reef system may be intrinsically linked to the primary productivity occurring in the overlying surface waters.

Global marine Si budgets rely on 2 major assumptions regarding the biological components of Si cycling: (1) benthic accumulation and eventual burial of diatom debris in the Southern Ocean is the main removal process and (2) prior to burial, dissolution of

the siliceous debris frees up 95% of the deep-water Si which circulates back up to surface waters for primary production (DeMaster et al. 1991, Tréguer et al. 1995, DeMaster 2002). In contrast, our findings suggest that dense populations of glass sponges can biologically facilitate the removal of Si at the seafloor, which may greatly reduce the amount of deep-water dSi that is returned to the surface for use by diatoms. Modern sponges are clearly important components of the Si budget in areas where they are prominent members of the benthos (Maldonado et al. 2005, 2010, this study). In Antarctica, there are dense populations of glass sponges and spicule mats from dead sponges that reach up to 2 m high (Dayton et al. 1974, Dayton 1979, Barthel 1995). Therefore, as in Northeast Pacific waters, glass sponge skeletons may be a substantial component of a large Si sink in the Southern Ocean that has yet to be quantified.

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