SUPPLEMENTARY INFORMATION (SI)

Silicon uptake by sponges: a twist to understanding nutrient cycling on continental margins

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Supplementary Information. Section 1.

In the studied Mediterranean area (41° 42' 21" N - 2° 48' 17" E and 41° 42' 25" N -2° 54' 51" E), DSi values in the water column of the continental shelf are known to stay lower than 2 μ M over the year cycle¹. Nevertheless, DSi concentrations within the narrow boundary layer of rocky substrata where sponges live have never been examined in detail. We decided to sample the water layer (1 cm thick)



Fig. S1. View of an individual of *Axinella verrucosa* in the field at 21 m depth.

directly in contact with the rocks on which sponges grow to examine the possibility that sponges were enjoining some DSi bottom enrichment relative to concentrations in the open water column above the continental shelf. Once a month, from January to December, we took seawater samples (n= 10 per month) from the boundary layer of coastal rocky walls (12-15m deep) and compared their DSi values with those of samples collected from the open water column (3 to 15 m deep) of the shelf, about 0.5 km off the coastline. The boundary layer was sampled using 50mL acid-cleaned polycarbonate syringes during scuba dives. Samples from the coastal water column were obtained using Niskin bottles from a small oceanographic boat. Each month, all



Fig. S2. Comparison of mean (±s.d.) DSi concentration at the boundary layer of rocky substrata where sponges grow and in the open water column above the continental shelf (northwestern Mediterranean coast of Spain) over a year cycle.

samples were collected during the same day in the morning and stored in refrigerated, acid-

cleaned 20 mL polyethylene vials in dark to be processed for their DSi concentration within the following 24h using a Bran-Luebbe TrAAcs 2000 autoanalyzer.

The outcome of such field monitoring corroborated that DSi concentrations stayed below 2 μ M over the year at both the boundary layer of rocks and in the remaining coastal water column (Fig. S3). Average differences between DSi values measured at the boundary layer and in the open water column were never larger than 0.2 μ M, supporting that, unlike in soft bottoms, relevant DSi inputs from the bottom do not occur.

To infer yearly DSi demand by field sponge populations, we measured the volume of siliceous sponges on the rocky sublittoral bottoms using 50x50cm polyvinylchloride sampling quadrats (n= 100). We first identified taxonomically all sponges found during scuba dives within each random quadrat and measured "in vivo" the volume (cm³) of each siliceous individual using underwater rulers, following the methodology detailed elsewhere ². The body shape of sponges was approximated to one or, more often, several geometric figures (spheres, ovals, solid cylinders, hollow cylinders, rectangular plates), and the linear parameters (length, width, diameters) were measured in situ to calculate volumes. Aspiculate and calcareous species were not considered in the quantifications.

Supplementary Information. Section 2.

To prepare the large seawater volume (100 L) required for each of the 23 DSi-concentration steps run during the experiments, we faced two practical problems that introduced some inaccuracy and caused a slight mismatch between the intended and the obtained DSi concentrations for the treatments (Table S1). First, for each DSi concentration, we had to prepare 100 L of DSi-enriched seawater split into two 50L barrels. In such large recipients, the solvent volume could not be measured with high accuracy and, additionally, some Si adsorption to the barrel walls occurred. Second, when transferring the pumps and sponges (Figs. S3-S4) from a DSi step to the following one, small volumes of water (usually with lower DSi) concentration dripped into the bottles of the new DSi treatment. Altogether, these practices typically caused that the obtained experimental DSi concentration was empirically determined in each bottle at the beginning and the end of any treatment, the minor mismatch between

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intended and assayed DSi concentrations is merely anecdotic and had no relevance on the scientific outcome.

		EXPERIMENT I		
Date	Intended DSi	Assayed DSi	Change in Dsi concentration	
	concentration	concentration	in control bottles after 48h	
	(µM)	(µM)	Average (µM)	s.s. (µM)
may-07	1	1.6	0.0	0.2
may-09	10	11.7	0.5	0.4
may-11	20	21	0.4	0.7
may-13	30	30.1	0.5	0.5
may-15	40	38.3	-0.9	1.7
may-17	50	47.6	-0.9	1.9
may-19	100	92.0	-1.7	0.5
may-21	150	143.7	-2.6	2.7
may-23	200	200.4	-2.0	2.6
may-25	resting period	1.6	XXX	XXXX
june-02	20	18.3	0.0	0.9
june-04	70	66.3	-2.3	1.2
june-06	100	91.6	-2.2	1.4
june-08	300	296.89	-1.4	1.6
june-10	450	448.9	-4.0	4.0
june-12	600	604.9	-12.3	14.0
june-14	185	186.6	-3.1	4.9
		EXPERIMENT II		
june-29	200	196.0	-3.0	4.4
july-01	300	286.0	-2.3	2.1
july-03	450	434.0	-8.2	6.1
july-05	600	593.0	-5.4	6.5
july-07	800	787.0	-19.6	12.5
july-09	850	834.0	-17.2	49.1

Table S1. Summary of the temporal development of DSi treatments during experiments I and II, differences between intended and assayed DSi concentrations at each treatment step, and average (±s.d.) changes in DSi concentration in the control bottles over each 48h treatment period.



experimental bottle. Note the pump and the rock

piece to which the sponge is attached.

Fig. S4. Partial view of the experimental setup showing 3L experimental bottles containing individuals of *Axinella spp.* and a "Micra" water pump.

To calculate an individual uptake at a given DSi step, we detracted the final DSi concentration (after 48h) from the initial value in the bottle, then corrected by the average concentration change detected in the set of the 13 control bottles, typically owing to Si adsorption on walls. At low concentrations wall adsorption was negligible, but it became more evident during steps involving high DSi concentrations (Table S1).

Uptake rates have preferably been normalized to mL of living sponge tissue (rather than AFDW), since it allows to infer DSi demand for field individuals whose volume can easily be measured by a variety of non-destructive methods (rulers, photography, waters displacement, etc). Nevertheless, to facilitate comparison with the only available study to date on sponge DSi uptake kinetics ³, we also provided uptake rates normalized by AFDW.

Supplementary Information. Section 3.

Because planktonic diatoms, unlike sponges and radiolarians radiated in ocean habitats with rapidly decreasing ambient DSi, their uptake systems appear to be particularly suited to achieve maximum efficiency at low DSi concentrations. Fossil records reveal that the earliest diatoms, known as radial centrics, had a heavy, highly silicified cell wall that initially restricted them to a benthic life style in shallow, near-shore waters ⁴. It was only when diatom enlightened their frustules that they became purely planktonic and proliferated in surface waters offshore. Therefore, benthic diatoms have evolved through history under higher DSi levels than planktonic forms, often involving increased DSi concentrations diffused from pore waters of soft bottoms. Interestingly, benthic diatoms appear to have a multiphasic uptake system that, within moderate DSi ambient concentrations (<60 μ M), displays a Michaelis-Menten kinetic with half saturation constant (54 μ M) much closer to that of sponges than to that of planktonic diatoms, while, at high DSi (60 to 300 μ M), it appears not to reach saturation⁵.

Supplementary Information. Section 4.

Modern deep-sea explorations are revealing extensive sponge populations at bathyal depths on continental margins (Fig. 5S), being of considerable importance in terms both biomass and abundance⁶⁻⁸. For instance, in fjords of British Columbia (Canada), densities as high as 240 individuals per 10 m² have been reported⁹. With few exceptions, sponges are typically long-

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lived organisms, with life spans often assumed to range from a few decades to centuries¹⁰⁻¹², and, in some cases, probably millennia¹³. Most bathyal sponges are also heavily skeletonized organisms, with their siliceous skeleton often representing 75 to 90% of the body dry weight^{8,14}. The longevity of these organisms, combined with their large size on average and highly silicified skeletons that, upon sponge death, are far more refractory to dissolution in seawater and sediments than diatom skeletons make these deep-sea sponge populations to function as benthic Si traps, retarding recycling of BSi into silicate. The available evidence suggests that the Si standing stock accumulated in bathyal and abyssal populations of heavily skeletonized sponges may be enormous, but it is unlikely to be ever quantified in global terms, given the associated logistic difficulties and costs of such a work.

A paradigmatic example of the importance of deep-sea sponge populations as Si sinks is provided by the epibathyal sponge reefs (Fig. 5c) discovered at British Columbia (Canada), which discontinuously extend over an area greater than 700 km^{2 15-16}. Reefs are built by large, heavily skeletonized hexactinellid sponges growing



Figure S5. Bathyal populations of hexactinellid sponges illustrating the potential importance of sponges as a BSi sink at bathyal depths. (a) Sericolophus hawaiicus, almost continuously covering an area of about 30km² on the Hawaiian slope (USA), (courtesy of Dr. Craig M. Young). (b) Asconema setubalensis, forming extensive fields, at 300-400 m deep, off the Canary Islands (Spain), (courtesy of Ricardo Aguilar, OCEANA). (c) Bathyal reef of hexactinellids at the Strait of Georgia (Canada), with living Aprocallistes vastus (yellow) growing on top of blackened, nondissolved siliceous skeletons of their dead congeners (courtesy of Dr. Sally Leys). All three species depicted are heavily silicified and their adult individuals reach a height ranging from 30 to about 60 cm.

on top of the non-dissolved skeletons of the dead individuals, creating large siliceous mounds that are up to 21 m in height and about 9000-year old¹⁷⁻¹⁹.

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