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Silicon consumption in two shallow-water sponges with contrasting biological features

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Abstract

There is growing awareness that to improve the understanding of the biological control of silicon (Si) cycling in the oceans, the biogeochemical models need to incorporate Si users other than diatoms. In the last decades, siliceous sponges are coming into sight as important Si users, but the scarce quantitative information on how they use Si is hindering the assessment of their role. We are here investigating Si consumption kinetics in two demosponge species (*Tethya citrina* and *Hymeniacidon perlevis*) that have contrasting biological features while inhabiting at the same sublittoral habitat. In laboratory experiments, we have determined that both species share some common traits when incorporating Si from seawater: (1) saturable Michaelis-Menten kinetics; (2) maximum velocity of Si consumption occurring at high silicic acid (DSi) concentrations (~150 μM) that are not available in shallow waters of the modern oceans; (3) the ability to increase consumption rates rapidly and predictably in response to increasing DSi availability; and (4) half-saturation constants that indicate an affinity for DSi lower than those of diatom systems. Across the four sponge species investigated to date, the affinity for DSi varies about 4.5 times. Our results also suggest that at least part of that between-species variability reflects the skeletonization level of the species. Within a given species, there are also between-individual differences in the DSi demand, which appear to reflect the particular physiological condition of each individual (i.e., body size, reproductive vs. non-reproductive stage).

To know the details of the biogeochemical cycling of silicon (Si) in the ocean is of great interest because the biological availability of this element has a major impact on marine primary productivity and because its cycling intertwines with that of other relevant elements, such as carbon. There are a variety of marine organisms that require Si to build their skeletons, such as diatoms, sponges, radiolarians, choanoflagellates, silicoflagellates, and some life stages of chrysophyceans and testate amoebas. It was long established that Si cycles in the ocean largely under biological control (Harriss 1966; DeMaster 1981; DeMaster 1991; Nelson et al. 1995; Tréguer et al. 1995). The traditional view held that diatoms, which use Si to elaborate a protective siliceous case external to their cells, were the only virtual responsible for such biological control at a global scale (Ragueneau et al. 1994; Tréguer et al. 1995; Ragueneau et al. 2000). Over the last 20 years the idea has grown that at least another group of organisms, the siliceous sponges, are also players to be considered at both local (Conley and Schelske 1993; Reincke and Barthel 1997) and global scales (Maldonado et al. 2005, 2010, 2011, 2012b; Tréguer and De La Rocha 2013).

Sponges are benthic ubiquitous organisms occurring with moderate to high abundance on continental shelves, slopes, and seamounts, being also present at mid ocean ridges, abyssal plains, and even hadal bottoms (reviewed in Maldonado et al. 2016). Approximately, about 70–75% of the 8700 known sponge species are siliceous, that is, they use Si to elaborate their skeletons. Depending on the groups, the relative contribution of the silica skeleton to body dry weight (DW) can range from a modest percentage up to 95% in the most heavily skeletonized deep-sea and polar species (Barthel 1995; Maldonado et al. 2005). A comprehensive assessment of the role of sponges in the Si cycle is however difficult to be currently achieved because some basic aspects of the use of Si by sponges are still ill known. To help alleviating this lack of knowledge, we have investigated the consumption of silicic acid ($Si(OH)_4$), hereafter referred to as DSi, by sponges. This weak acid is the most abundant dissolved form of Si in seawater (Del Amo and Brzezinski 1999),

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

and it is regarded to be the main Si compound biologically assimilable by the Si-using organisms.

To date, kinetics equations describing DSi utilization are available for only two genera in the class Demospongiae, despite that information being pivotal to infer local and regional DSi demand by sponge populations. One of the kinetics was reported by Reincke and Barthel (1997) for Baltic-Sea individuals of *Halichondria panicea*, the other by Maldonado et al. (2011) for Mediterranean individuals of *Axinella damicornis* and *Axinella verrucosa*. Interestingly, in both cases, the Si consumption conformed to an hyperbolic function that fitted the Michaelis-Menten kinetics, which is typical of saturable processes, including enzyme-mediated reactions. Those independent studies were in turn in agreement with the discovery that siliceous demosponges — unlike all others silicifying organisms— condense DSi into siliceous spicules (i.e., biogenic silica= BSi) through a silicifying enzyme, the silicatein (Shimizu et al. 1998; Cha et al. 1999). Silicatein has probably evolved from a lysosomal capthesin used for intracellular digestions (Riesgo et al. 2015). The molecular control of silicification in sponges is complex and still ill known, with a variety of enzymes and proteins that participate and interact with each other at different steps, such as glassin, galectins, silintaphins, chitin, and collagen (Ehrlich et al. 2010; Ehrlich 2011; Wang et al. 2011; Shimizu et al. 2015).

The available studies also agree that the sponge systems for DSi incorporation from seawater saturate at concentrations between 100 and 200 μM DSi. These values are about two orders of magnitude higher than the nutrient concentrations available in the natural habitats of the assayed sponges and even unachievable in most shallow waters of modern oceans. Despite sharing that chronic DSi shortage, the consumption kinetics of the two investigated sponge genera notably differ in their specific parameters. The species *Halichondria panicea* has a maximum velocity of DSi transport (V_{max}= 19.33 μmol Si h^{-1} g^{-1} ash-free dried weight [AFDW]) that is an order of magnitude higher than that of the *Axinella* spp. (V_{max}= 1.74 μmol Si h^{-1} g^{-1} AFDW) and an affinity for DSi (V_{max}/K_m= 0.42) more than twenty times higher than that of *Axinella* spp. (V_{max}/K_m = 0.02). These differences have been hypothesized to result from consumption rates of *Axinella* spp. being experimentally measured on complete individuals, while those in *H. panicea* being measured on body fragments cut down prior to the experiment. That wounding would have forced the sponges to regenerate some body parts and their associated BSi skeleton during the experiments, probably increasing abnormally their DSi demands (Maldonado et al. 2011; Maldonado et al. 2012b). Differences also may result from *Axinella* spp. being slow-growing, long-living organisms adapted to a consistently oligotrophic Mediterranean environment in terms of DSi and food availability, while the population of *H. panicea* in the nutrient-rich Baltic Sea consists of seasonal individuals that are born in spring, growing fast for a few weeks, and then dying in late autumn to winter (Frøhlich and Barthel 1997; Reincke and Barthel 1997). It is likely, therefore, that the individuals of *H. panicea*, apart from being regenerating their wounded body parts, are locally adapted to process high amounts of DSi and food during their short life span. These major differences in both DSi utilization kinetics and general biology of the two sponges investigated to date make evident the need of additional experimental data. More comparative information is required to gain a deeper understanding about how DSi utilization systems of sponges are functioning and which are the levels of between-species variability. Here we are investigating DSi consumption by two common, shallow-water demosponges with contrasting biological features: *Tethya citrina* (Sarà and Melone, 1965) and *Hymeniacidon perlevis* (Montagu, 1814).

Methods

Selected species

The species *Tethya citrina* (fam. Tethyidae; Fig. 1A) and *Hymeniacidon perlevis* (fam. Halichondriidae; Fig. 1B) were selected for the experiments because of four major reasons: (1) both species are common at many shallow-water habitats in the Atlantic-Mediterranean region and also occur themselves or have sister species worldwide, facilitating that the estimated kinetics of DSi utilization can reliably be extrapolated across equivalent sublittoral systems of other oceans; (2) unlike most other sponges, they are able to withstand the harsh conditions of tidal pools and even transient air exposure at low tides, being therefore foreseen as well suited to cope with laboratory handling; (3) they show contrasting "organic tissue vs. silica skeleton" ratios within their bodies, what, in turn, suggests different dependence on DSi availability. The species *T. citrina* is relatively skeletonized (ash weight [AW]/DW ratio = 62.91 ± 4.50%), elaborating a skeleton that combines tiny (<100 μm) aster-like spicules and large (up to 1.5 mm or larger) needle-like spicules; *H. perlevis* is an opportunistic species with a nearly worldwide biogeographic distribution, which has a moderate skeleton content (AW/DW ratio = 44.84 ± 4.14%) derived from the production of a single, mid-size (approx. 150–475 of μm) type of needle-like silica spicules; (4) these sponges show contrasting reproductive strategies, with individuals of *T. citrina* producing large amounts of highly skeletonized asexual buds all year around (Fig. 1C,D), while no similar energy-demanding and, presumably, silica-consuming process takes places in *H. perlevis*.

General design and conceptual framework

Two consecutive experiments were conducted in laboratory conditions, following a shared general design. Sponges were collected without physical damage by scuba diving at 3–15 m depth in the Bay of Brest (France) one to two weeks prior to the onset of the experiments. They were transported

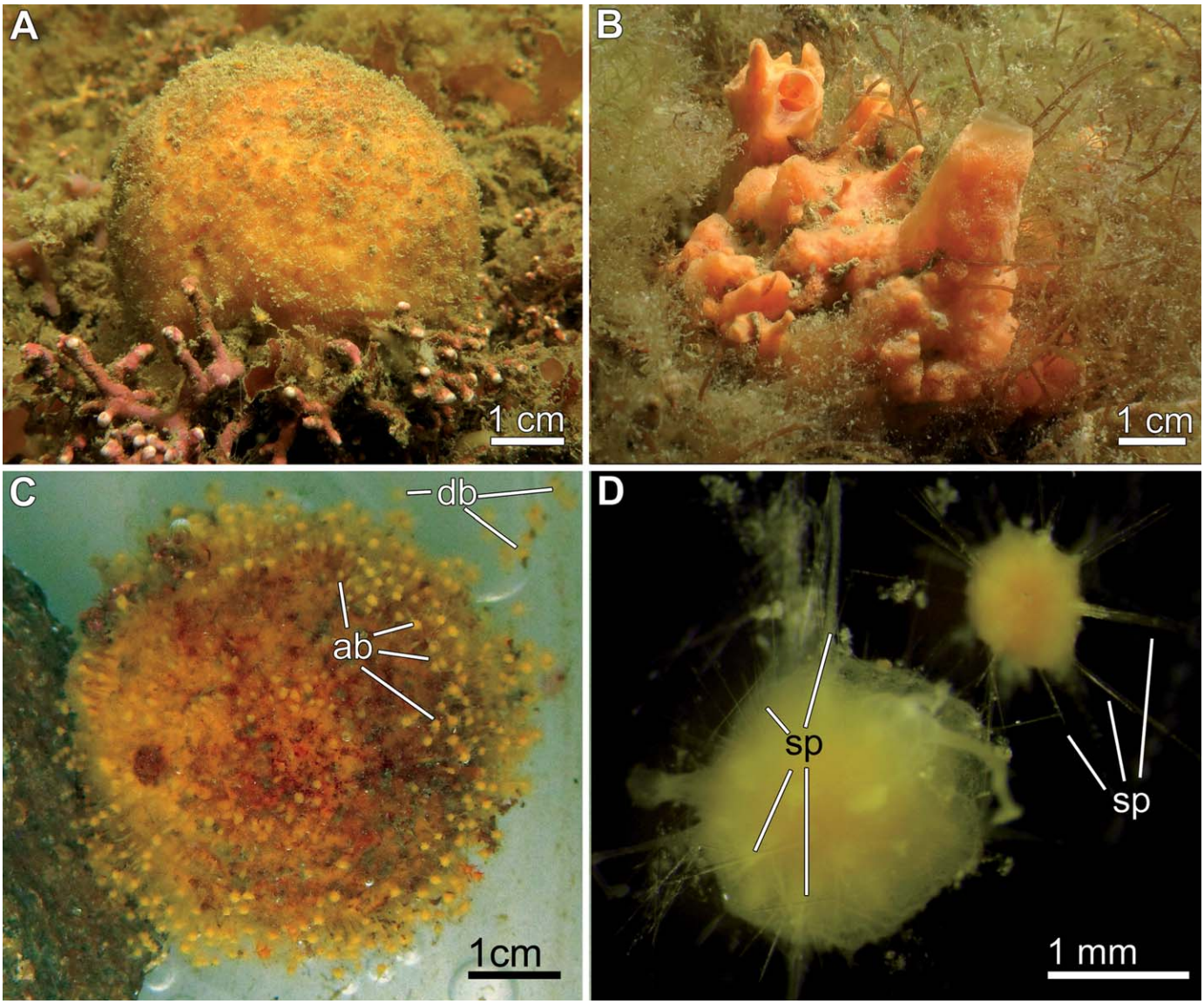

Fig. 1. (**A–B**) In situ views of *Tethya citrina* (A) and *Hymeniacidon perlevis* (B) growing at the Bay of Brest. (**C**) View of a *T. citrina* individual producing buds in the laboratory. Most of the buds (ab) still remain attached to the body, but some have already been detached (db) and rest at the container bottom. (**D**) Detail of two detached buds of different size, bearing many silica spicules (sp).

in seawater to the laboratory and acclimated progressively to stagnant (non-running) seawater conditions in 30 L polyethylene tanks filled with seawater that was replaced every 3 d.

For the experiments, each individual was placed into a food-grade polycarbonate container filled with 2.67 ± 0.04 L of filtered seawater. Containers were maintained in a temperature-controlled room, so that seawater temperature ranged minimally, from 17.8°C to 18.2°C. The DSi concentration was progressively increased in the experimental containers, from natural values (approx. 2 μM) until 250–300 μM, concentrations at which the analyses consistently evidenced saturation of the DSi incorporation system in the sponges. Because at least 40 h are known to be needed to build a 200 μm-long spicule (Weissenfels and Landschoff 1977), we opted for a 72 h period to estimate DSi consumption at each concentration. That time period was considered long enough to allow the sponges converting into silica spicules at least a good deal of the DSi taken up before they being transferred to a subsequent step in the DSi treatment. Consequently, this study has to be understood as an attempt to determine the kinetics of net DSi "consumption" by the sponges. These "consumption" rates, which conservatively integrate the changes in the physiological rhythms of the sponges over several daily cycles, are different from the DSi "uptake" kinetics determined for diatoms. "True uptake" in diatoms, as interpreted by Thamatrakoln and Hildebrand

(2008), involves determination of the amount of DSi incorporated across their single-cell membrane system and during a very brief time period (minutes). The short incubation is required to prevent that passive DSi leakage from the cell to the seawater by gradient-facilitated diffusion causes an artefactual underestimation of the actual DSi transport rate. Therefore, our approach deals with DSi consumption rates, not uptake rates. The concept of "true uptake," as it has been defined for diatoms, makes no much biological sense in the case of sponges for a variety of reasons, being the most substantive that sponges are multicellular, multicompartmented organisms. Unlike in diatoms, the DSi has to be transported not only across a single cell-membrane barrier, but through several cell and tissue compartments. DSi has to be transported from seawater into (or across) the epithelial cells, then into the intercellular matrix of the mesohyl, which is a lax, parenchyma-like tissue under homeostatic control making possible the physiological regulation of the internal conditions of the sponge body. From the mesohyl matrix, DSi should be transported into the sclerocytes, which are the silicifying cells. Once there, the DSi is stored into the silicification vesicle to be enzymatically polycondensated. Nevertheless, because many of the sponge skeletal pieces to be elaborated are larger than the size of a sclerocyte cell, the DSi is packed in the sclerocyte cytoplasm into smaller, membrane-bound vesicles (silicasomes) and then actively exported back to the mesohyl matrix for extracellular silicification (Müller et al. 2005; Maldonado et al. 2012b; Wang et al. 2012). Given the number of cells and tissues involved and the fact that the mesohyl is an homeostatically controlled intercellular matrix (rather than mere seawater), the possibility that the DSi internalized into the sclerocyte leaks by passive diffusion back to the ambient water is remote. Therefore, the concept of a "true uptake" affected by diffusional leakage when measured for periods longer than a few minutes mostly applies to single-celled silicifying organisms directly surrounded by seawater, but it makes no biological sense for the internal silicifying cells of sponges. Additionally, it has been proved in at least one sponge species that increasing DSi concentration in seawater stimulate the production of new types of skeletal pieces (Maldonado et al. 1999). It also means that DSi availability controls the activation of different sets of silicifying genes and the production by mitosis of new populations of silicifying cells. These are both processes that require more than minutes (probably several hours to days) to be completed and they would never be captured in very short incubations.

In our experiments, DSi concentrations were prepared by adding the corresponding volume of a previously prepared 0.1 M sodium metasilicate ($Na_2SiO_3 \cdot 5H_2O$; pH= 10) stock solution to a 100 L-tank filled with filtered water and mixing with an electric pump for 18 h to ensure complete diffusion before delivery to the experimental containers. After every 72 h step, seawater was replaced, replenishing the containers with new filtered seawater at the new DSi concentration. During water replacement, each sponge was maintained within a small (180 mL) "transferring" container to avoid exposure to air. At all steps seawater was filtered at 3 μm, a pore size virtually excluding diatoms and other silicon users (silicoflagellates, radiolarians, choanoflagellates, etc). Absence of contaminating Si users in the experimental containers was further corroborated by periodic microscopy inspection of seawater subsamples stained in Lugol's iodine. The 3 μm pore size is known not to exclude bacterioplankton and the smallest picoplankton, which helped to supplement the sponges with part of their natural food diet during the experiments (Maldonado et al. 2011). Because sponges are known to require large daily amounts of food (Reiswig 1971a,b) and because DSi utilization rates are suspected to be detrimentally affected by starvation (Frøhlich and Barthel 1997; Maldonado et al. 2011), the feeding of each sponge individual was supplemented by addition of 100 μL of a DSi-free culture (approx. 10^6 cells/mL**)** of the haptophyte *Isochrysis aff. galbana* (clone: T-ISO) at each 72 h step of the DSi treatment.

The sponges withstood satisfactorily the environmental conditions during each of the 72 h steps of the experiments, with no casualties. Nevertheless, prior to the experiments, five individuals of *T. citrina* (out of 25) and two of *H. perlevis* (out of 24) died during the acclimation process.

DSi analyses and consumption rates

DSi concentrations were determined by collecting a 20 mL water sample after mixing manually with a plastic stick to homogenize any potential gradient built in the treatment containers. Water samples were filtered with 0.22 μm-pore, syringe filters (Millex-GS Millipore) and kept in the fridge for 1–4 d before analysis. Initial and final samples of each DSi-treatment step were processed in a same analysis, using a Perkin Elmer, Lamba 25 UV-VIS spectrophotometer and following a standard colorimetric method (Strickland and Parsons 1972), with a determination accuracy of 5%. Seawater samples from experimental steps with DSi concentrations higher than 20 μM were diluted prior to analysis.

Incorporation of DSi from seawater by the sponge in each container was estimated from the difference in DSi concentration between the beginning and the end of a given 72 h period and after correcting for a control treatment (see "Particular experimental setups"). On completion of the experiments, the volume (ml) of the assayed individuals was determined by the water displacement method. Sponges were then processed for wet weight (g), dried at 60°C to constant DW (g), and finally combusted at 540°C for 10 h for AW (g). Consumption of DSi (μmol) was then normalized by time unit (h), volume of seawater in the container (L), and volume of the sponge individual (ml) or its AFDW (g). We have preferentially normalized data by sponge volume because it facilitates their future application to field sponge populations without the need of collecting any individual or conducting

destructive approaches (Maldonado et al. 2011). Normalized data on DSi consumption were analyzed by non-linear regression and fitted to the equation that reproduced better the kinetics and with the highest statistical significance.

Particular experimental setups

Following the above-described methodology, we performed two consecutive experiments, one from mid-May to the end of June (experiment I) and another (experiment II) over July, 2015. In experiment I, we characterized the DSi consumption kinetics of *T. citrina* and *H. perlevis* using 12 and 13 individuals, respectively. Each sponge was exposed, in consecutive 72 h periods, to the following DSi concentrations: 1.82 μM (field values), 5, 10, 20, 35, 50, 75, 100, 150, 200, and 250 μM (see Supporting Information in Tables S1 and S2 for slight average deviation between intended and assayed DSi concentrations).

To investigate whether acclimation to gradually increasing DSi concentrations might have an effect on the consumption kinetics, particularly at the high experimental DSi levels, we conducted a slightly modified approach (experiment II) using an additional set of sponges (8 individuals of *T. citrina* and 9 of *H. perlevis*). In experiment II, the sponges were subjected to changes of greater magnitude between consecutive DSi concentration steps (i.e., 2.28, 75, 150, 200, 250, and 300 μM), being therefore exposed to very high DSi concentrations after only minimal time at intermediate concentrations. For each of the two experiments, we also sampled a set of seawater-filled treatment containers ($n_{control} = 5$) that included no sponge and served as controls to correct the spectrophotometric DSi determinations for spurious color absorbance.

Differences between experiments I and II in the average uptake rate of the sponges at a given DSi concentration (i.e., natural concentration, 75, 150, 200, and 250 μM) were examined by Mann-Whitney U tests.

Assessment of between-individual variability

In the frame of experiment I, it was investigated whether sponge size is a significant source of between-individual variability in DSi consumption. Separately for each species, a regression analysis examined whether there is a relationship between sponge size (ml) and total amount of consumed DSi (μmol per sponge ml) during the 33 d that experiment I lasted. Because some of the assayed individuals of *T. citrina* were producing during the experiment large amounts of skeletonized asexual buds ($n = 7$) while the others ($n = 5$) were not, it was tested whether such an activity had a detectable impact on DSi consumption. To that aim, we used a "t" test to examine whether the group of individuals actively producing buds consumed a significantly different DSi amount than the group of non-budding individuals. Prior to analysis, data were corroborated to be homoscedastic and normally distributed. We also investigated whether sponge size was related to the ability of an individual to produce buds. To this aim, differences in mean size between the group of budding ($n = 7$) and non-budding individuals ($n = 5$) were examined using also a *t*-test analysis.

Results

Experimental consumption rates

Raw data of experiments I and II are provided as Supporting Information in Tables S1 and S2.

In experiment I, the relationship between DSi availability and average (±SD) DSi consumption rate of both sponge species showed similar general patterns (Fig. 2A,B, solid line). Consumption rates increased almost linearly from the low natural DSi concentration to about 75 μM in *T. citrina* and 50 μM in *H. perlevis*. Above those DSi concentrations, subsequent increases in DSi availability induced progressively smaller and smaller positive increments of the consumption responses, until eliciting no increase at all, indicating that the DSi concentration saturating the consumption system was reached. Interestingly, saturation, which is also the stage at which the maximum velocity of DSi consumption takes place, was reached in both species at a similarly high concentration, around 150 Si μM. Because DSi in the natural habitat of the assayed species ranges from 0.1 μM to 15 μM at the Bay of Brest over the year cycle (SOMLIT-Brest database: http://somlit-db.epoc.u-bordeaux1.fr), it can be stated that the skeletal development of these sponges is restrained by a chronic DSi shortage: the DSi availability is one to two orders of magnitude lower than the optimum required by the sponges. At the low natural DSi concentrations, the consumption values were so small that sometimes they fell below the detection limit of the spectrophotometric approach, yielding slightly negative, artifactual consumption values for some of the individuals (Fig. 2C–F).

There was however between-species differences in the functioning of the consumption system (Fig. 2A,B). On average, maximum velocity of DSi transport in *T. citrina* (0.204 ± 0.101 μmol Si h^{-1} mL^{-1}) was about twice higher than that in *H. perlevis* (0.108 ± 0.099 μmol Si h^{-1} mL^{-1}). The inspection of the individual responses within each species (Fig. 2C,D) also indicated large between-individual variability in both the magnitude of the utilization response to a given DSi concentration and the concentration value at which V_{max} is achieved. In *T. citrina* the highest V_{max} was attained by individual #4 (0.478 μmol Si h^{-1} mL^{-1}) and it was reached at a DSi concentration of 75 μM (Fig. 2C), which strongly departs from the average saturating concentration (~150 μM) obtained for the bulk of the assayed conspecifics. The lowest V_{max} (0.065 μmol Si h^{-1} mL^{-1}), attained by individual #12, was an order of magnitude lower than that in individual #4, and also took place at 75 DSi μM (Fig. 2C). In *H. perlevis* a similarly broad range of between-individual variability was noticed (Fig. 2D), with the highest

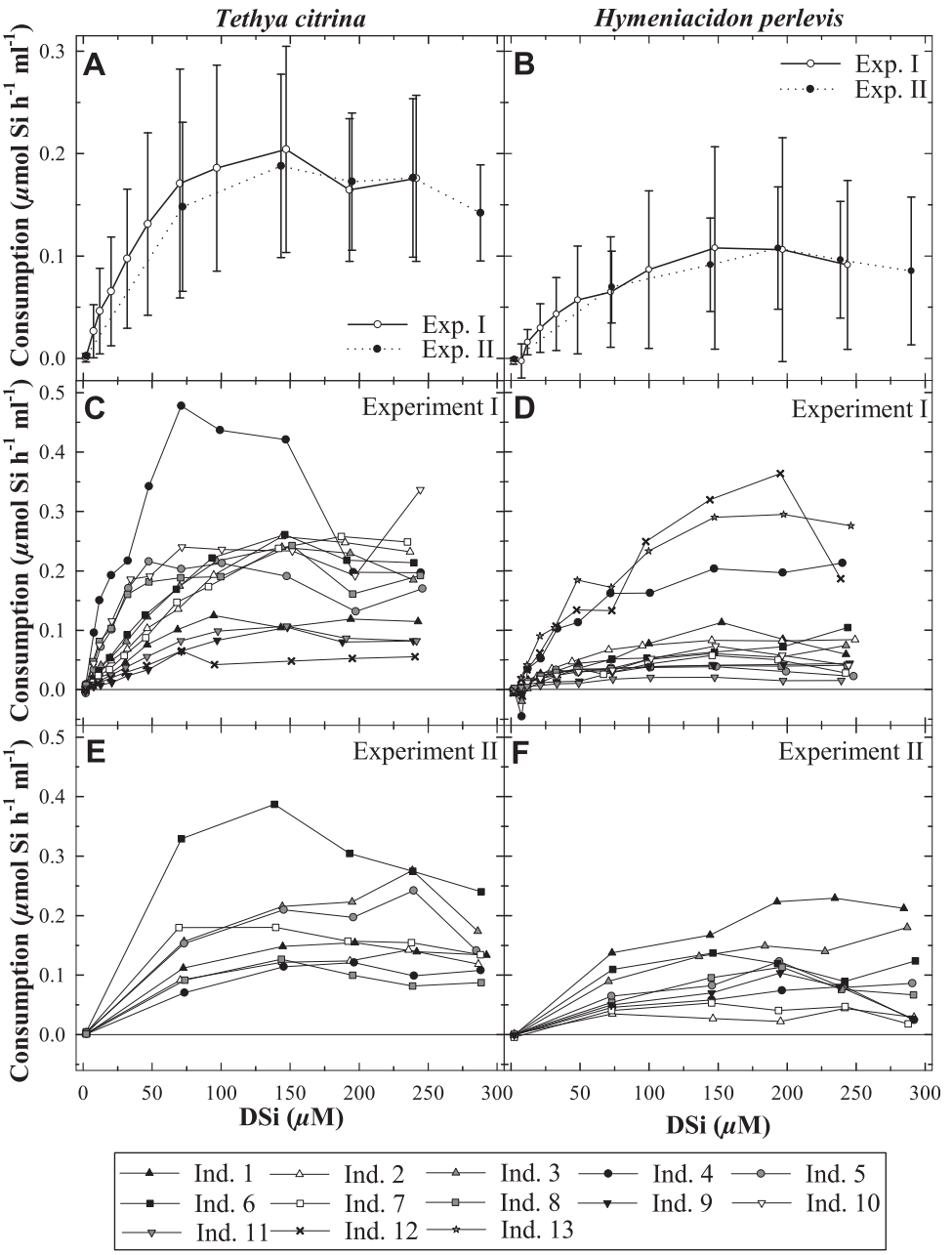

Fig. 2. (**A–B**) Average (±SD) consumption rates (μmol Si h^{-1} per sponge ml) of *Tethya citrina* and *Hymeniacidon perlevis* as a function of silicic acid (DSi) concentration during experiments I and II. (**C–D**) Consumption rates of each individual of *T. citrina* (C) and *H. perlevis* (D) as a function of silicic acid (DSi) during experiment I. (**E–F**) Consumption rates of each individual of *T. citrina* (E) and *H. perlevis* (F) as a function of silicic acid (DSi) during experiment II.

V_{max} of 0.364 μmol Si h^{-1} mL^{-1} attained by individual #12 at 200 μM DSi, and the lowest V_{max} of 0.021 reached in 150 μM DSi by individual #11. Some of the *H. perlevis* individuals attained the V_{max} at 200 and even 250 μM DSi.

Figure 2A,B (solid vs. dotted lines) shows that there were no substantial differences in mean DSi consumption rate at any of the assayed DSi concentrations between experiment I and II (all Mann-Whitney U test's $p \gg 0.05$; statistics not shown). Therefore, experiment II corroborated that the sponges were able to consistently reproduce the average consumption kinetics of experiment I, even though in this second assay they were not gradually brought into the high DSi concentration treatments. Rather the sponges experienced quite dramatic changes in DSi concentration, being transferred from a low natural value of about to 2 μM to their average saturating concentration of 150 μM with only an

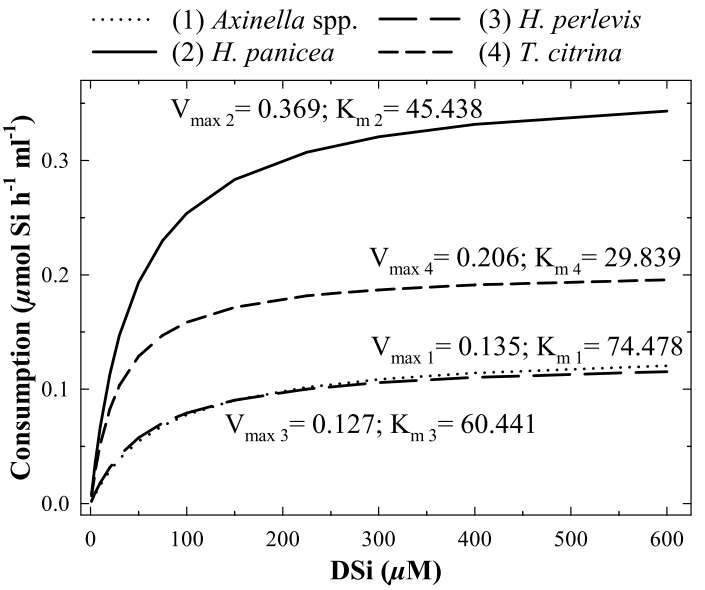

Fig. 3. Comparison of the Michaellis-Menten kinetics ($V = V\text{max} \times [\text{DSi}] / (K\text{m} + [\text{DSi}])$) fitted for silicic acid (DSi) consumption in the four demosponges investigated to date: *Axinella* spp. (Maldonado et al. 2011), *Halichondria panicea* (Reincke and Barthel 1997), and *Hymeniacidon perlevis* and *Tethya citrina* (this study). Consumption is expressed as μmol Si per hour and ml of sponge. Maximum velocity of DSi transport (*V*max) and half-saturation (*K*m) parameters of each species are given in the graph.

intermediate step at 75 μM (Fig. 2A,B, dotted line). These results indicate that the sponge consumption system is able to react rapidly and efficiently to drastic changes in ambient DSi concentrations and that the response is predictable. Experiment II also showed for the two assayed species a level of between-individual variability in V_{max} and saturating DSi concentration (Fig. 2E,F) similar to those in experiment I.

Consumption modeling

A non-linear regression analysis for all individual consumption data of experiments I and II pooled together indicates that the DSi utilization kinetics significantly fits a Michaelis-Menten's like function for both species (Fig. 3; $r^2 = 0.919$, $p < 0.001$ for *T. citrina*; $r^2 = 0.942$, $p < 0.001$ for *H. perlevis*). The equations confirmed that, for both species, the consumption system saturates around 150 μM DSi. Nevertheless, despite these two sponges sharing habitat and experiencing identical DSi natural concentrations at the Bay of Brest, their kinetic equations showed distinct half-saturation (K_m) and V_{max} parameters (Fig. 3). The V_{max} of *T.citrina* (0.21 μmol-Si h^{-1} mL-sponge^{-1}) is about twice higher than that of *H perlevis* (0.13 μmol-Si h^{-1} mL-sponge^{-1}), while the K_m of *T. citrina* (29.84 μM) is about half of that in *H. perlevis* (60.44 μM). This parameter combination results in a specific DSi affinity index (calculated as the V_{max}/K_m ratio) being more than three times higher (0.0069) in *T. citrina* than in *H. perlevis* (0.0021).

Between-individual variability

The volume of the assayed individuals ranged similarly in both species, from 1.8 mL to 24 mL in *T. citrina* and from 2 mL to 30 mL in *H. perlevis* (Supporting Information in Table S3). For both sponge species, there was a significant, inverse, non-linear relationship between sponge size and total amount of DSi consumed during experiment I (Fig. 4A,B), with the smallest sponges using two to four times more DSi than the largest individuals, depending on the species.

The relationship between sponge size and DSi consumption is almost twice more intense in *T. citrina* ($r^2 = 0.74$; $p < 0.001$) than in *H. perlevis* ($r^2 = 0.46$; $p = 0.011$). As some individuals of *T. citrina* were producing large amounts of skeletonized buds during the experiment (Fig. 1C,D), it was worth testing whether such a budding activity could have an effect on DSi consumption. Budding individuals consumed over experiment I an average of 60.83 ± 29.91 μmol DSi, while non-budding ones consumed 123.38 ± 37.94 μmol DSi on average (Fig. 4C). The associated *t*-test corroborated that the budding activity reduced by half the DSi consumption with statistical significance. Nevertheless, such a conclusion is not biologically straightforward, since a subsequent *t*-test also revealed that budding individuals were about five times larger on average than non-budding ones (Fig. 4D). Therefore, it appears that a combination of confounding factors (i.e., being small and non-budding) is responsible for high DSi consumption in *T. citrina*, leading to the detected between-individual differences in consumption. Likewise, the accumulative effect of both factors in only *T. citrina* causes the relationship between size and DSi consumption to be more intense in this species than in *H. perlevis*.

Discussion

The two assayed species, *T. citrina* and *H. perlevis*, show consumption kinetics that fitted a saturable Michaelis-Menten model, in agreement with previous uptake studies in the demosponges *H. panicea* (Reincke and Barthel 1997) and *Axinella* spp. (Maldonado et al. 2011). Collectively, these results are also in congruence with the discovery that the polycondensation of DSi into skeletal BSi in demosponges is a process enzymatically controlled (Shimizu et al. 1998; Cha et al. 1999; Riesgo et al. 2015). We cannot discard that the saturable kinetics may also be favored by additional saturable components of the silicification process other than the enzymatic step. The pathway of DSi from seawater until becoming silica within the sponge body is complex and involves several ill-known transport steps across the sponge epithelia, the intercellular medium of the mesohyl, and the sclerocytes, each of one might also have functional restrictions at some point.

The experiments also revealed that the consumption systems saturate at DSi concentrations (~150 μM) that are one to two orders of magnitude higher than DSi average availability at

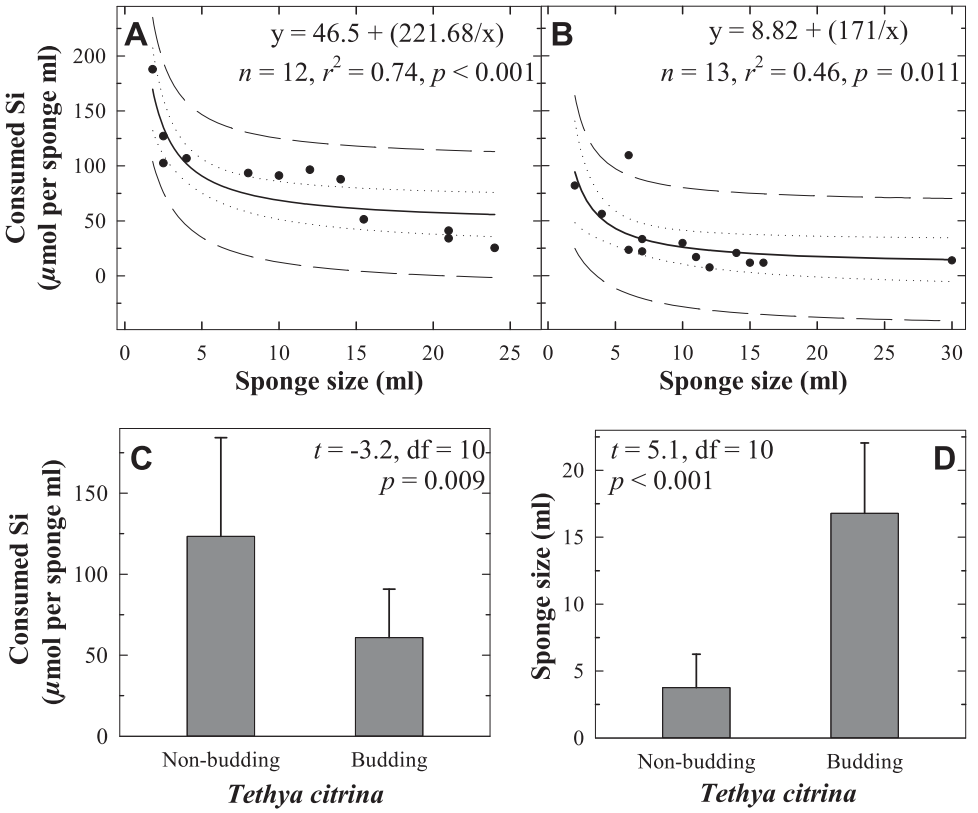

Fig. 4. (**A–B**) Relationship between sponge size (ml) and total consumption of silicic acid (DSi) over the 33 days that experiment I lasted. For both *Tethya citrina* (A) and *Hymeniacidon perlevis* (B) the relationship fitted an inverse, first order, polynomial model. Dotted and dashed lines refer to the 95% confidence band and the prediction band, respectively, of the regression equations. (**C–D**) Summary of *t*-tests examining differences in mean DSi consumed over experiment I (C) and in mean body size (D) between budding and non-budding individuals of *T. citrina*.

the natural habitat of the sponges (i.e., decadal DSi average concentration: 4.47 ± 3.24 μM; SOMLIT Brest database: http://somlit-db.epoc.u-bordeaux1.fr). Indeed, the very high DSi concentrations at which the sponges saturate are rarely available in shallow waters of the modern oceans and are found only in particular deep-sea environments or at very high latitudes (Nelson et al. 1995; Sarmiento and Gruber 2006; Tréguer and De La Rocha 2013). Such a chronic DSi limitation has been reported to affect in some or other way all five shallow-water demosponge species examined to date (Frøhlich and Barthel 1997; Reincke and Barthel 1997; Maldonado et al. 1999, 2011, 2012a, 2012b). A dramatic decrease in DSi availability in the photic ocean on the Cretaceous expansion of diatoms (Harper and Knoll 1975; Maliva et al. 1989) appears to be the most likely cause of the current DSi limitation of modern sponges (Maldonado et al. 1999; Maldonado 2009). Interestingly, most modern planktonic diatoms have consumption systems saturating not much higher than at 10 μM DSi (Paasche 1973; Conway and Harrison 1977; Martin-Jézéquel et al. 2000), which grossly corresponds with the average concentration of DSi in the modern ocean (Tréguer and De La Rocha 2013). By keeping DSi concentrations in the photic ocean relatively low, diatoms favor their own uptake kinetic based on high affinity for DSi while forcing sponges to inefficiently take up DSi at concentrations far below their kinetic optimum.

It is not easy to compare the kinetics of diatoms and sponges in terms of V_{max}/K_m ratios for two major reasons. First, in the case of diatoms, short-term incubations (minutes) have been used to infer "uptake" kinetics, which accurately reflects active transport across the diatom cell membrane before diffusion starts restoring the DSi concentration equilibrium with the extracellular environment (seawater). In contrast, in the case of sponges, kinetics do not strictly reflect "uptake," but net DSi "consumption" that is measured over a period of days to integrate the effect of potential changes in the diel cycle of the physiological activity of the sponges. Second, the V_{max} of the kinetics of diatoms and sponges are measured in different units: those of diatoms are normalized to their BSi content, while those of sponges are referred to body volume. To bring the V_{max} values into common units by normalizing sponge V_{max} by BSi content would only solve the mathematical approximation but not the biological weakness behind the approach. The production of a complete BSi skeleton in diatoms and sponges shows drastic biological differences: diatoms are

unicellular entities with life spans of a few days, while decades to centuries are required for the sponges to produce their BSi, also including processes of shedding and production of new spicules without any detectable variation in total body volume. Therefore, the most sensible approach is to limit comparisons to the K_m values of their respective kinetics for just DSi consumption (i.e., specifically excluding "uptake" data of diatoms from the comparison). In this context, the K_m value can be defined as the DSi concentration at which the consumption process removes DSi from seawater at half its maximal rate and therefore is related to the affinity of the compound for the process. The comparison reveals that most planktonic diatoms have consumption kinetics (i.e., measured in incubations from 3 h to days) with K_m values between 0.2 μM to 8.7 μM (Paasche 1973; Conway and Harrison 1977; Kristiansen et al. 2000; Martin-Jézéquel et al. 2000), which are clearly smaller than those of sponges (29.8 μM to 74.5 μM, see Fig. 3). The low K_m values characterizing DSi consumption kinetics of diatoms come into stark contrast with the much larger K_m values typically obtained for diatom "true uptake" kinetics (i.e., derived from incubations of minutes that are not affected by diffusional processes), being K_m values above 50 μM DSi common in most benthic species and some planktonic ones (Nelson et al. 1984; Martin-Jézéquel et al. 2000; Thamatrakoln and Hildebrand 2008; Leynaert et al. 2009). Indeed, some planktonic diatoms and most benthic ones have been shown to have a non-saturable uptake system, since they are able to shift from Michaelis-Menten saturable kinetics to non-saturable models when exposed at very high DSi concentration and under particular physiological conditions (Thamatrakoln and Hildebrand 2008; Leynaert et al. 2009). Two competing explanations have been proposed to address the shift in uptake kinetics of diatoms at high DSi availability: (1) the shift would take place above a threshold DSi concentration at which uptake stops being actively mediated by Si-transporters and it is replaced by passive diffusion; (2) the shift would take place when a threshold DSi concentration would activate new families of putative, ill-known Si transporters based on a high K_m. The possibility that any of those two factors are also responsible for the sponges saturating at very high DSi concentrations is nil. Experiments about DSi consumption using control containers that hosted a non-siliceous sponge have revealed no detectable decrease in the DSi concentration of the containers after 48 h treatments, even when concentrations were higher than 200 μM DSi (M. Maldonado, unpubl.). Those results therefore prove that passive diffusion of DSi into the sponges is not affecting our quantification of DSi utilization. It is also worth noting that the kinetics of DSi utilization in all examined sponge species to date does reach a saturation stage without any major shift in the kinetic equations. In the case of diatoms, the shift in the uptake kinetics is thought to help them to take maximum advantage when high DSi concentrations may transiently be encountered in their local systems. Our results in experiment II show that the consumption systems of *T. citrina* and *H. perlevis* —despite having much lower affinity for DSi than diatoms and despite not shifting kinetics— are also suited to react rapidly and predictably to large increases in DSi availability. This is probably the consequence of the sponge DSi utilization systems being originally designed to perform with maximum velocity under DSi concentrations much higher than the average characterizing most shallow-water habitats in the modern oceans. Both experiment I and II showed that once the individuals reached their saturating concentration, subsequent exposure to a higher DSi concentration did not elicit a net DSi consumption similar to the one yielded at the previous saturating concentration, but smaller consumption (Fig. 2A–E). In contrast, one would expect the sponges to consume equally at saturating and supersaturating concentrations, that is, at their V_{max} in both cases. The smaller net consumption above the saturation threshold suggests that the sponges are probably converting into silica some of the already internalized and transiently stored DSi (i.e., DSi transported into sclerocytes, silicasomes, etc. during the previous DSi treatment step).

Our study has also found a noticeable between-species differences in consumption kinetics, with the more skeletonized *T. citrina* achieving a twice higher maximum velocity of transport and three times higher affinity for DSi than the less skeletonized *H. perlevis* (Fig. 3). When the consumption kinetics (standardized to sponge volume) of these two species studied herein were compared with those available in the literature for the long-living, slow-growing *Axinella* spp. and for the seasonal, opportunistic *H. panicea* (Fig. 3), it was found that *H. perlevis* and *Axinella* spp. have nearly identical kinetics. They have almost overlapping equations, with a similarly low DSi affinity (V_{max}/K_m ratio of 0.0021 and 0.0018, respectively). *Tethya citrina* has an intermediate kinetics (DSi affinity of 0.0069), while *H. panicea* shows the highest affinity (0.0081). Note here that the kinetics parameters of *H. panicea*, originally normalized to AFDW (Reincke and Barthel 1997), have been re-normalized by volume for appropriate comparison. To that aim, we collected individuals from the Bay of Brest and established their relationship AFDW vs. volume by lineal regression ($R^2 = 0.930$, $p = 0.002$, $n = 6$). Altogether, the global between-species comparison reveals that the affinity for DSi ranges about 4.5 times across the four sponges investigated to date. In the case of *H. panicea*, the high affinity appears to reflect the need of an explosive skeletal growth during the short, seasonal life span of the individuals in the Baltic Sea. Besides, the experiments in which the consumption of *H. panicea* was determined probably yielded artificially increased DSi utilization values, as previously noticed (Maldonado et al. 2011). In the case of *T. citrina*, the comparatively high DSi affinity may be an adaptation responding to the need of elaborating a relatively silicified skeleton. The consumption of *H. perlevis* showed

comparatively low performance. The results for *H. perlevis* in our study contrast with those obtained for intertidal individuals of this same species in the Yellow Sea (Dalian, China). When exposed at 10 (field DSi concentration), 25, 40, and 70 μM DSi during 36 h (Maldonado et al. 2012a), the Yellow Sea individuals showed consumption rates 3.3–5.7 times higher than the ones expected according to the *H. perlevis* kinetic equation obtained from the Brest population. In the current stage of knowledge, local adaptations in the performance of the sponge DSi utilization systems cannot be ruled out. Another plausible explanation is that the world-wide distributed *H. perlevis* is actually a cryptic species complex, as it would be supported by the Dalian specimens being larger, thicker, and fleshier than the Brest individuals. The taxonomic species limits of *Hymeniacidon* spp. are currently hard to be delineated with any accuracy, having their species high "intraspecific" genetic variability and being their natural distributional ranges accidentally intermixed world-wide by aquaculture global trading (Hoshino et al. 2008; Alex et al. 2012; Fuller and Hughey 2013).

Our study also detected between-individual variability in the DSi consumption responses. At least part of that variability in both *T. citrina* and *H. perlevis* is inversely related to the sponge size, in full agreement with previous reports in other sponge species (Maldonado et al. 2011). In the case of *T. citrina*, the production of buds also appeared to be a source of inter-individual variability, with budding individuals consuming about half of the DSi demanded by the individuals not engaged in budding (Fig. 4C). Nevertheless, to ascertain the net impact of the budding effect on DSi consumption is not an easy task, since the "budding" effect is confounded with the "size" effect: only the largest assayed sponges produced buds (Fig. 4D). It was surprising that, contrary to our expectations, the individuals producing skeletonized buds consumed on average less DSi than the non-budding individuals. This unexpected result suggests that the spicules being incorporated into the buds are not elaborated during the budding process. Rather, they would be parental spicules elaborated in advance and later incorporated into the growing cell mass of the buds. Therefore, the reason why skeletal growth slows down during budding is that the sponges probably deviate their energy into mitotic processes to produce the cell masses required for the buds. This suggestion is also in line with a previous report that individuals of *H. panicea* engaged in sexual reproduction (i.e., forming non-skeletonized oocytes) had lower DSi demands than their non-reproducing counterparts in the laboratory assays (Frøhlich and Barthel 1997).

In summary, the available information suggests that the DSi consumption kinetics of sponges share some features across species:

(1) a saturable Michaelis-Menten model; (2) maximum velocity of DSi transport occurring at high DSi concentrations (~150 μM) that are never available in their natural habitat; and (3) half-saturation constants that suggest much lower affinity for DSi than those of diatom systems. The consumption kinetics can also vary between and within sponge species as a function of at least skeletonization level and the particular physiological condition (i.e., body size and reproductive vs. non-reproductive stage).

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