


Silicon consumption kinetics by marine sponges: An assessment of their role at the ecosystem level

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Abstract

The silicic acid (DSi) is a dissolved nutrient used by diverse marine organisms to build their skeletons of biogenic silica (BSi). This consumption, mostly due to diatoms, largely determines the availability of DSi in the photic ocean. Yet growing evidence suggests that Si consumers traditionally disregarded, such as the siliceous sponges, may also play a role. This study investigated the kinetics of DSi utilization by two demosponges as a function of both DSi availability and duration of the incubation period (24 h vs. 48 h). Consumption increased with increasing DSi availability following a saturable Michaelis–Menten kinetics. *Haliclona simulans* saturated at about 70 μM ($K_m = 45.9$) and *Suberites ficus* around 130 μM ($K_m = 108.2$). Forty-eight hour incubations yielded more conservative consumption rates than 24 h incubations, particularly when DSi availability was far below saturation. DSi concentrations in the sponge natural habitats (0.2–15 μM) were consistently much lower than required for efficient elaboration of the BSi skeleton, suggesting a chronic DSi limitation. The DSi consumption kinetics was combined with quantifications of sponge biomass in the Bay of Brest (France), which was used as case study. In this system, sponges consume daily $0.10 \pm 0.19 \text{ mmol Si m}^{-2}$ and about $6.4 \times 10^6 \text{ mol Si}$ yearly. This activity represents 7.6% of the net annual BSi production in the Bay, a figure overlooked in previous nutrient balances based only on diatoms. Since the world marine Si cycle does not yet incorporate the contribution of sponges, its global BSi production budget may also be underestimated.

Silicic acid (DSi), the only biologically assimilable dissolved form of silicon (Si), is a pivotal nutrient to ocean primary productivity. Its availability facilitates the growth of diatoms, which polycondensate DSi to elaborate their skeletons of biogenic silica (BSi). Diatoms are fundamental primary producers in the ocean food web, also the main DSi consumers and BSi producers in the photic ocean, largely determining the interplay between particulate (i.e., BSi) and dissolved (i.e., DSi) forms of Si in the marine biogeochemical cycle of this element (DeMaster 1981; Nelson et al. 1995; Tréguer et al. 1995). Over the last decades, the concern is rising that at least another group of Si-using organisms, namely marine siliceous sponges, may also play a relevant global role regarding the conversion of DSi into BSi in the ocean (Reincke and Barthel 1997; Maldonado et al. 2005, 2010, 2011, 2012b; Tréguer and De La Rocha 2013; López-Acosta et al. 2016).

Sponges are abundant and even dominant organisms in many marine benthic communities, both in shallow-water and deep-sea habitat (e.g., Maldonado et al. 2017). Approximately, about 80% of the 8900 known sponge species have silica skeletons, produced from the silicic acid dissolved in the seawater. Unlike in the case of diatoms, the sponge DSi consumption consistently deals with the DSi pool in demersal water masses rather than that in the open water column. Despite the potential of sponges as DSi users, quantitative approaches to their functional role are scarce. The scarcity of this basic information is, in turn, preventing the understanding of their function within the global marine Si cycle.

To date, the kinetics of DSi consumption has been evaluated only for six sponge species: *Halichondria panicea* (Reincke and Barthel 1997), *Axinella damicornis*, *A. polypoides*, *A. verrucosa* (Maldonado et al. 2011), and *Hymeniacidon perlevis* and *Tethya citrina* (López-Acosta et al. 2016). All species show consumption kinetics that fit a saturable Michaelis–Menten model. Yet large between-species variability has been noticed in the value of the parameters that govern that Michaelis–Menten kinetics, that is, maximum DSi transport velocity (V_{max}) and half-saturation constant (K_m), defined as the DSi

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

concentration at which the half of the V_{\max} is attained. For instance, the V_{\max} of DSi transport in *H. panicea* is three times higher than those in *H. perlevis* and *Axinella* spp., while *T. citrina* shows an intermediate value. The affinity for DSi (defined as " V_{\max}/K_m " ratio, sensu Healey 1980) varies across species as well, with *H. panicea* having, for instance, an affinity for DSi 4.5 times higher than that of *Axinella* spp.

The sources of such a variability remain largely unidentified. It could arise from species-specific adaptations, from individual physiological capabilities, and even from differences in the methodological approach followed by each of the three independent studies published to date. For instance, in the study with *H. panicea*, the individuals were cut in pieces prior to the experiment and their DSi consumption rates determined from relatively short incubations (i.e., 7 h). In contrast, the rest of published studies were based on complete individuals and longer incubation periods, but of unequal duration between studies: 48 h for *Axinella* spp. and 72 h for *H. perlevis* and *T. citrina*.

By investigating DSi utilization in two sponge species during two different incubation periods, this study seeks to improve the knowledge of the sources of variability in DSi utilization. By applying the obtained kinetic models to quantifications of sponge abundance in the field, the study also provides a first assessment of the functional role of these organisms as DSi users at the ecosystem level, considering the ecological system of the Bay of Brest (France) as case study.

Methods

Selected species

Silicon consumption rates were determined for the demosponges *Haliclona simulans* (Johnston, 1842) in the family Chalinidae and *Suberites ficus* (Johnston, 1842) in the family Suberitidae (Fig. 1).

The two herein assayed species were selected for three major reasons. First, these two species show contrasting levels of skeletonization, with the silica content in the skeleton of *S. ficus* being about 50% higher than that in *H. simulans*; such a difference suggests potential between-species differences in DSi requirements for skeletal growth. Second, the two species can be collected without physical damage along with a piece of the detachable hard substrate to which they are naturally attached to; this facilitates successful transference from the field to the experimental aquaria. Third, the two assayed demosponges belong to phylogenetic lineages different from each other but also different from all others investigated to date.

Skeletally, *H. simulans* is characterized by having only one type of needle-like siliceous spicules, oxeas, which are 120–150 μm in length and 5–12 μm in width. *S. ficus* is characterized by having two types of spicules: tylostiles and micro-rhabs. The former one is extremely abundant pin-like spicules, measuring 100–420 μm in length and 2–6 μm in width. The

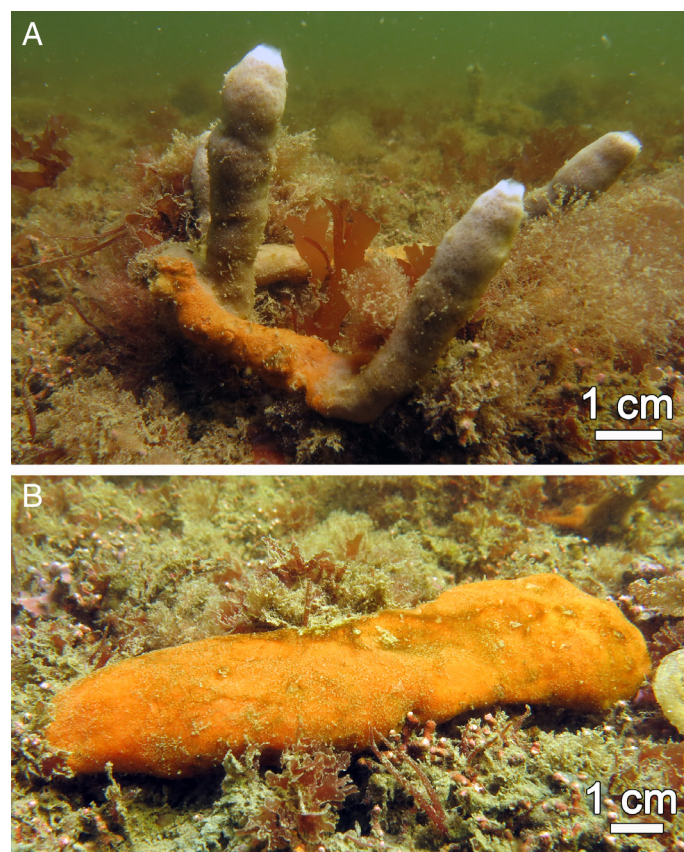


Fig. 1. In situ views of (A) *H. simulans* and (B) *S. ficus* at their habitat in the Bay of Brest (France).

microrhabs are relatively scarce, tiny sticks, measuring 25–70 μm and 2–3 μm in length and width, respectively.

General experimental design

All average values given in this study represent means and its associated errors are 1 SD from the mean.

Consumption of DSi was investigated in the laboratory from mid-September 2016 to mid-October 2016. The individuals were collected from the Bay of Brest by scuba diving (5–10 m depths), and acclimated to laboratory stagnant seawater conditions in 30-liter polyethylene tanks for 2 weeks, with water replacement every other day. Seawater temperature was maintained at $15.5 \pm 0.5^\circ\text{C}$, mimicking natural values in the field at that time of year.

The experiments involved 13 independent sponge individuals of each species. Each individual was placed into a polycarbonate incubation aquarium filled with 2.90 ± 0.06 L of filtered seawater and incubated for a succession of 48 h steps. At each successive step, the DSi concentration in the seawater was increased respect to the previous step. The experiment ended when the increase in DSi concentration elicited no subsequent increase in the consumption rate, that is, saturation had been reached. Intended DSi concentration treatments for the successive incubation steps were 2.7 μM (i.e., natural value

at the time of experiment), then, 10 μM , 20 μM , 40 μM , 70 μM , 90 μM , 130 μM , 175 μM , 210 μM , 275 μM , 330 μM , 430 μM , and 570 μM (see Supporting Information S1 for slight deviation between intended and practiced DSi concentrations).

Experimental DSi concentrations were prepared by adding the corresponding volume of a buffered 0.1 M sodium metasilicate ($\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$; pH = 10) stock solution to 200 L of 1 μm -filtered seawater stored in a graduated plastic barrel. Two electric pumps mixed the solution for 18 h to ensure complete molecular diffusion and homogeneous DSi concentration before delivering to the smaller incubation aquaria. The pore size used for seawater filtration excluded planktonic silicon users (such as diatoms, radiolarians, and silicoflagellates) from the treatment water while still allowing the pass of at least part of the bacterioplankton on which the sponges feed. Additionally, we provided supplementary food to the sponges by adding 50 μL of a culture ($\sim 10^6$ cells mL^{-1}) of the haptophyte *Isochrysis aff. galbana* (clone T.ISO) per liter of seawater at the beginning of each incubation period.

All 13 individuals of each species were subjected to the DSi treatments at the same time, along with two sets of controls. One control set consisted of three aquaria, each containing a small piece of maërl, similar to the one typically used as substrate by the assayed sponges. The other control set consisted of three aquaria filled with only seawater but neither sponge nor substrate.

Consumption rates as a function of DSi and incubation duration

The experiment was designed to examine differences in the rate of DSi consumption as a function of both DSi concentrations available to the sponges during the incubations and duration of the incubation. At each DSi concentration step, the incubation extended for 48 h, but, during that period, each aquarium was sampled within minute 1, after 24 h, and after 48 h to determine the change in the DSi concentration. Each sampling involved collection of a 20 mL water sample using acid-cleaned plastic syringes. Water samples were filtered through 0.22 μm pore, syringe filters (Millex-GS Millipore) and stored in the fridge not longer than 2 d prior to analysis. DSi was determined manually, following the standard colorimetric method, with 5% accuracy (Strickland and Parsons 1972). Samples with DSi concentrations higher than 30 μM were diluted prior to analysis.

The rate of DSi utilization by a sponge during a given period was inferred from the difference in DSi concentration in the aquarium between the beginning and the end of the concerned period and after correcting values by the concentration changes (often nil) occurred in the control aquaria. When the experiment was over, we measured the volume (mL) of the assayed individuals by water displacement. Sponges were then wet weighed (g), dried at 60°C to constant dry weight (g), and finally combusted at 540°C for 10 h for ash weight (g). Five fragments of sponge tissue per species were also dry-

weighed and desilicified in 5% hydrofluoric acid (HF) for 5 h to estimate the average ratio of BSi content vs. organic content per species (Maldonado et al. 2010). Finally, consumption rates of DSi were normalized by volume of seawater in the container (L) and time unit (h), and referred to either the volume of the sponge individuals (mL) and their BSi content or their dry weight (Supporting Information S2). We have preferentially expressed data referred to sponge volume because it facilitates their future applicability to field sponge populations without the need of any further destructive collection of individuals.

Differences in DSi consumption rate as a function of DSi availability and duration of the incubation were tested using a two-factor approach. One of the examined factors (hereafter referred to as “DSi”) was availability of DSi to the sponges in the cultures, containing 10 levels (2.6 μM , 8 μM , 21 μM , 44 μM , 67 μM , 90 μM , 130 μM , 175 μM , 210 μM , 275 μM) for the species *H. simulans* and 13 levels (2.8 μM , 8 μM , 20 μM , 42 μM , 65 μM , 87 μM , 130 μM , 175 μM , 210 μM , 275 μM , 330 μM , 434 μM , 566 μM) for *S. ficus* (see Supporting Information S1).

The second factor of the analysis was the “Incubation Period” (i.e., “P”), with two levels, resulting from splitting each 48 h incubation step into a first and a second 24 h period (hereafter, “P1” and “P2,” respectively). With this factor design, we intended to test whether the decrease in DSi concentration measured in the aquaria when sponges are transferred from a lower to a higher DSi concentration mostly derives from an active DSi utilization or rather from a passive chemical adsorption. In the latter case, the process should just last minutes (Oelze et al. 2014), with DSi consumptions predicted to be very high during P1 and virtually nil during P2 (see Supporting Information S3 for details in calculation of consumption rates during P2).

The two factors (i.e., “DSi” and “P”) of this two-way experimental design involved repeated exposure of the same sponge individuals to the treatment levels over time, leading to a statistical model of “repeated measures”. Untransformed data of DSi consumption rate for *H. simulans* were normal (Shapiro–Wilk test) but heteroscedastic (Brown–Forsythe test), becoming non-normal but homoscedastic after log transformation. Untransformed data for *S. ficus* were non-normal and homoscedastic, but transformation was unable to alter those properties. Altogether, because of these data properties, a parametric two-way repeated measures ANOVA could not be run. As an alternative approach, the ANOVA-type statistic (ATS) was used. This is a robust, rank-based statistic designed to perform nonparametric analyses in multifactorial designs, including the interaction of factors (Brunner et al. 2002). Unlike the parametric ANOVA, it tests the hypothesis of the equality of marginal distributions rather than the equality of means and it is robust against outliers, random missing values, and small sample size (Brunner and Puri 2001; Shah and Madden 2004; Nussbaum 2014; Fan and Zhang 2017). The ATS analyzing

differences in Si consumption rates as a function of DSi concentration and incubation period on untransformed data was conducted using the package “nparLD” (Noguchi et al. 2012) in R version 3.4.2. Data for each sponge species were analyzed separately. Pairwise, a posteriori comparisons based on Brunner–Munzel (BM) test (Brunner and Munzel 2000) were run using the R package “lawstat” in R version 3.4.2.

Between individual responses and body size

Previous studies have indicated important between-individual variability in the rate of DSi utilization within a species (Frøhlich and Barthel 1997; Maldonado et al. 2011; López-Acosta et al. 2016). We examined whether differences in body size could be behind such a variability. First, we calculated the amount of DSi consumed by each individual across all the 48 h periods during the entire experiment, normalizing by sponge volume to render consumption values comparable. The reason why the response in a global 48 h period was preferred over those in either P1 or P2 periods is that the 48 h incubation period was predicted to incorporate more realistically potential shifts in the physiological capability and biological rhythms of the sponges when consuming DSi. These relatively long incubations are preferred for sponges, in stark contrast with the short-time (minutes to less than 3 h) traditionally preferred to estimate DSi uptake in diatoms (see López-Acosta et al. 2016 for an extended discussion).

The relationship between body size (volume) of each individual and both the DSi consumed during the entire experiment and its maximum DSi consumption rate (i.e., V_{\max}) was examined using regression analysis.

Modeling the DSi consumption

To model the kinetics of DSi consumption in each species, we followed two different approaches. First, we averaged the response of the 13 individuals at each of the 48 h incubation steps and searched (goodness of fit) for the equation that best fitted the average response over the entire range of assayed concentrations. Second, for comparative purposes, we fitted the model from the individual response data rather than from their average. In either case, the responses over 48 h were preferred again over those in P1 or P2 periods because such a choice is predicted (see “Results” and “Discussion” sections) to lead to more conservative and resilient models.

A tentative test to assess statistically the differences between the equations obtained for *H. simulans* and *S. ficus* was conducted. First, we used the DSi consumption curve measured for each assayed individual during the 48 h incubation steps and fitted it to a Michaelis–Menten model (goodness of fit), obtaining an average (\pm SD) value for K_m and V_{\max} parameters of each of the individuals ($n = 13$) of each species. To be able to incorporate “error propagation” in the analyses, we considered a data set consisting of not only the average value of V_{\max} and K_m for each individual, but also two additional values, “average + SD” and “average – SD”, which

incorporate the “error effect.” By this procedure, we obtained a data set of 39 individual values for the K_m and V_{\max} parameters (three data for each individual), which incorporates the propagated error. Then, between-species differences in mean K_m and V_{\max} values were respectively examined using Mann–Whitney U test for nonparametric data. Differences in the species affinity for DSi, measured as V_{\max}/K_m ratio (in $\mu\text{mol Si h}^{-1}$ sponge mL^{-1} concentration μM^{-1}), were also tested using the Mann–Whitney U statistic. The kinetic models obtained for *H. simulans* and *S. ficus* were also discussed in the light of those others previously known in the literature.

Si utilization at the ecosystem level

The models of DSi consumption were used to make a first assessment of the amount of DSi utilized yearly by the sponge communities in the Bay of Brest. The Bay is a shallow (mean depth = 8 m; maximum depth = 45 m), semi-enclosed basin of about 167.23 km² (after discounting harbors and estuaries). It is connected to the Atlantic ocean through a relatively narrow (1.8 km wide) strait and receives freshwater and nutrients from two main rivers. The Bay can be defined as a macrotidal environment (maximum tidal amplitude = 8 m), its entire seawater mass being well-mixed daily by important tidal currents and local winds (e.g., Delmas and Tréguer 1985).

The Bay can be divided into six major habitat-like zones (hereafter referred to as “habitats”; Fig. 2), according to depth and nature of the substrate and regarding the occurrence of sponges, namely (1) rocky intertidal, (2) rocky subtidal, (3) maërl beds, (4) shallow mud, (5) heterogeneous sediments, and (6) circalittoral coarse sediments. The sampling effort carried out within a given habitat to estimate sponge abundance was approximately equivalent to its representativeness relative to the total Bay extension, with diverse sampling techniques being used for different habitats, as it follows: (1) the rocky intertidal was sampled through 23, random quadrats (1 × 1 m) during the spring low tides; (2) the rocky subtidal, the shallow mud, the maërl beds, and the heterogeneous sediments (all four habitats being shallower than 20 m depth and accounting for most of the Bay extension) were sampled by 119, 1 × 1 m quadrats during scuba dives; (3) habitats of heterogeneous sediment and circalittoral coarse sediment, both located deeper than 20 m, were sampled running an epibenthic trawl over small transects (1 m wide × 5–10 m long; $n = 28$). In all cases, each of the sponges found within the quadrats or in the trawls was counted and its volume measured by rulers (Maldonado et al. 2010). Counts and volume values in each habitat were finally normalized to m².

Weekly values of DSi concentration in the seawater of the Bay for the last dozen years (2005–2016) were obtained from SOMLIT-Brest database (<http://somalit-db.epoc.u-bordeaux1.fr/bdd.php>). By knowing the course of DSi availability over a dozen years and the consumption kinetics for each of the four dominant species in the Bay of Brest—*S. ficus* and *H. simulans*: this study; *T. citrina* and *H. perlevis*: López-Acosta et al. 2016—, we

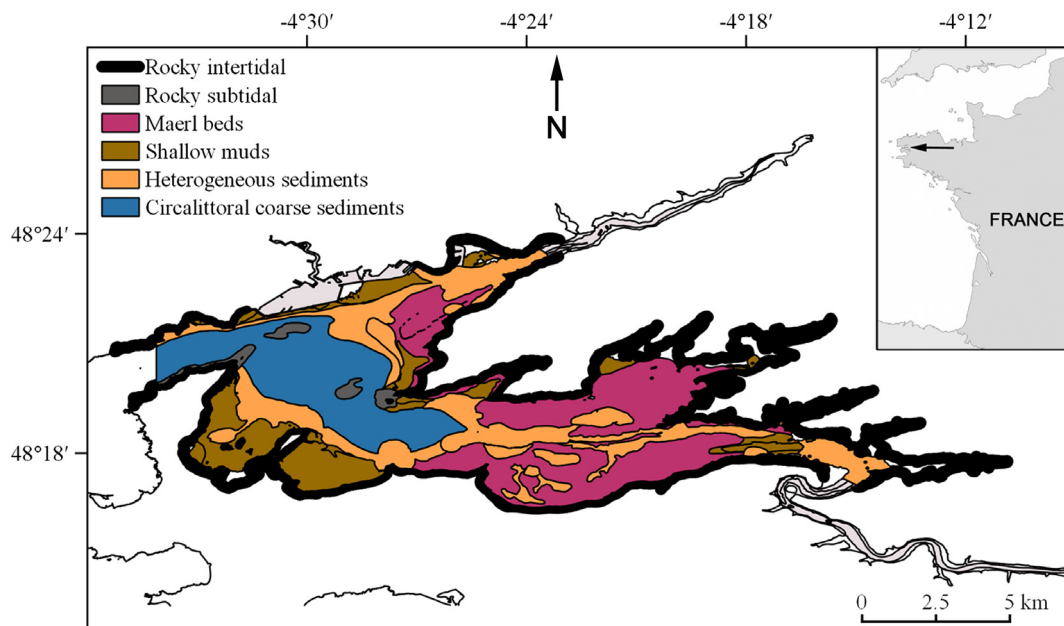


Fig. 2. Map of the Bay of Brest (France), showing the six major habitats defined for this study according to their depth, substrate type, and sponge fauna. Estuaries and harbors, depicted in gray in the map, have not been considered in the study.

Table 1. Statistic summary for the ANOVA-type (ATS) tests used to analyze differences in DSi consumption rates as a function of silicic acid concentration (DSi) and incubation period (P) for *H. simulans* and *S. ficus*.

Factor	<i>H. simulans</i>			<i>S. ficus</i>		
	df	Statistic	<i>p</i>	df	Statistic	<i>p</i>
DSi	1.7	106.9	<0.001	1.3	52.0	<0.001
P	1.0	23.0	<0.001	1.0	28.5	<0.001
DSi × P	3.4	18.8	<0.001	2.4	21.4	<0.001

estimated the average (\pm SD) annual DSi consumption in the Bay by these sponges. Additionally, by quantifying the biomass per area unit of the rest of sponge species in the Bay and by applying the DSi consumption kinetics resulting from averaging the models of the four most abundant species, the global DSi utilization by all the sponges in the Bay was calculated.

Results

Consumption rates as a function of DSi and incubation duration

The ATS revealed that the incubation period (P factor) had statistically significant effects on the calculated consumption rate (Table 1; Fig. 3), with sponges consuming during P2 slightly less DSi than during P1. By pooling the differences between P1 and P2 in the average consumption across all steps of the experiment, we determined that both sponge species consumed less during P2 than during P1 (*S. ficus* = $-27.18\% \pm 25.65\%$; *H. simulans* = $-11.13\% \pm 15.29\%$). The ATS also revealed that sponges increased the DSi consumption rate progressively with increasing DSi availability, but only up

to a certain threshold concentration (Fig. 3). The a posteriori tests suggested that such a threshold would be represented by a DSi concentration of $67 \mu\text{M}$ in *H. simulans* and $129 \mu\text{M}$ in *S. ficus* (Fig. 3). Intuitively, those values can be interpreted as the concentration at which the DSi consumption system of each species reaches saturation. The ATS analysis also detected a significant interaction between the main factors (Table 1). The pairwise comparisons revealed that it is due to the fact that the consumption rate during P1 is significantly larger than during P2 for nearly all DSi concentrations below the saturation value in *S. ficus* (Fig. 3B), but this difference disappeared (i.e., it became statistically nonsignificant) at higher concentrations. A similar effect, but less clear, is seen for *H. simulans*, in which the consumption rate during P1 was significantly larger than during P2 at only two DSi concentration steps (Fig. 3A).

Between individual responses and body size

The inspection of individual DSi consumption rates in response to the DSi availability indicated large between-individual differences in both species (Fig. 4). In *H. simulans*,

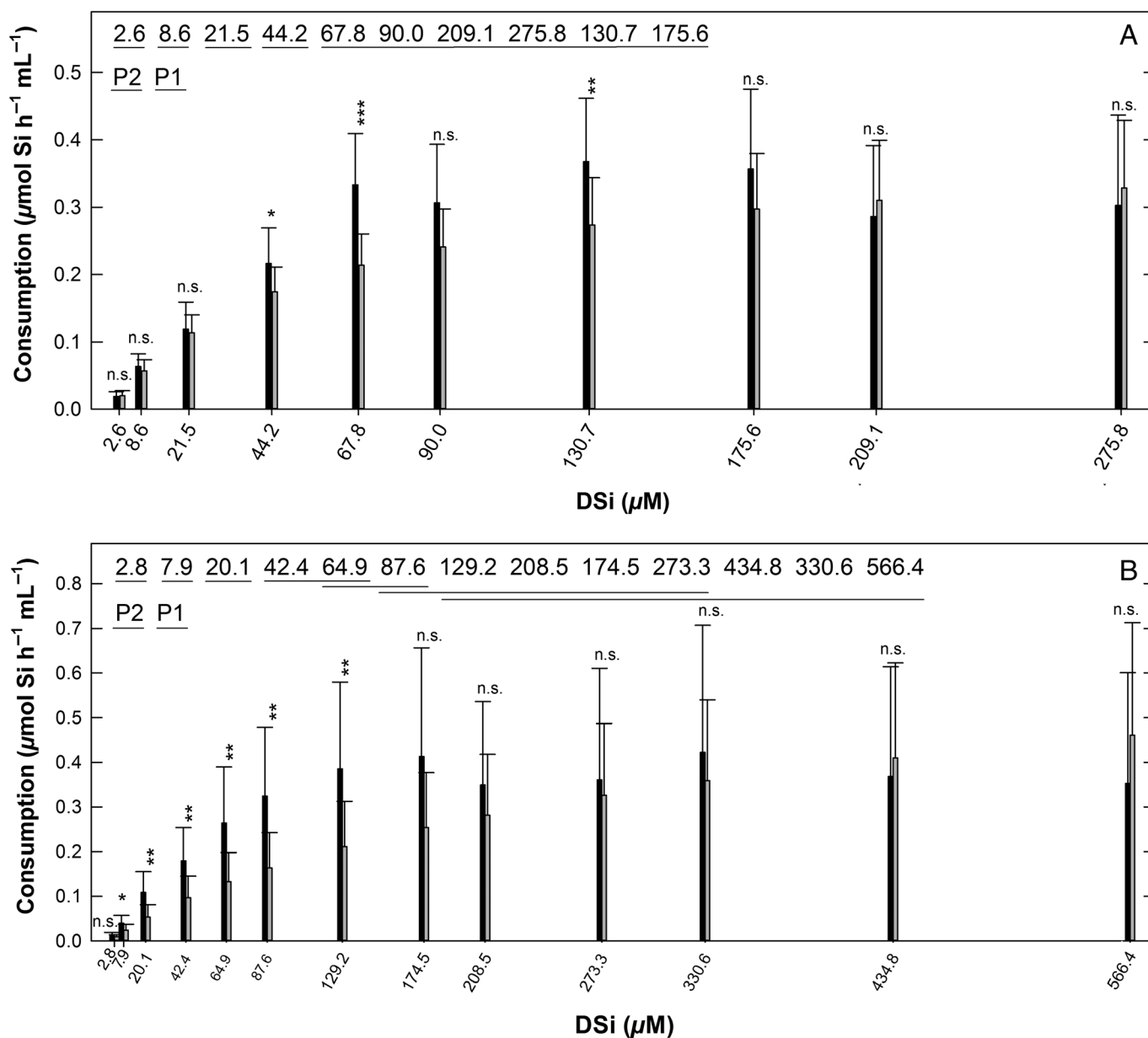


Fig. 3. Average (\pm SD) consumption rates measured as function of silicic acid (DSi) concentration and incubation period (P1 vs. P2) in (A) *H. similans* and (B) *S. ficus*. In the left upper corner, the average values for the levels of each factor are ordered from left to right by increasing magnitude. Levels of a factor underlined by the same line are not significantly different from each other according to the results of an ANOVA-type test (ATS) and the associated pairwise, a posteriori, Brunner–Munzel’s tests (Table 1). The significance of differences in consumption rates between P1 (black bars) and P2 (gray bars) within each DSi concentration treatment assayed are indicated by symbols, as it follows: n.s., nonsignificant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

the highest maximum velocity (V_{max}) of consumption was attained by individual #5 ($0.600 \mu\text{mol Si h}^{-1} \text{ mL}^{-1}$) at $207.1 \mu\text{M}$ DSi, while the lowest V_{max} ($0.224 \mu\text{mol Si h}^{-1} \text{ mL}^{-1}$), attained by individual #11, occurred at about $68.2 \mu\text{M}$ DSi (Fig. 4A). As a result, individual #5 consumed over the entire experiment per mL of sponge volume about 2.5 times ($150 \mu\text{mol Si mL}^{-1}$) more DSi than individual #11 ($62 \mu\text{mol Si mL}^{-1}$; Fig. 5A). In *S. ficus*, the highest V_{max} , reached by individual #12 at $433.2 \mu\text{M}$ DSi, was $0.865 \mu\text{mol Si h}^{-1} \text{ mL}^{-1}$,

almost one order of magnitude larger than the lowest V_{max} ($0.103 \mu\text{mol Si h}^{-1} \text{ mL}^{-1}$) attained by individual #8 at $178 \mu\text{M}$ (Fig. 4B). As a consequence, individual #12 consumed over five times ($259 \mu\text{mol Si mL}^{-1}$) more DSi per mL of sponge volume than individual #8 ($41 \mu\text{mol Si mL}^{-1}$; Fig. 5B). Therefore, differences in the size-normalized performance of the individuals were twice larger in *S. ficus* than in *H. similans*.

When it was examined whether sponge size could be responsible of at least part of the between-individual

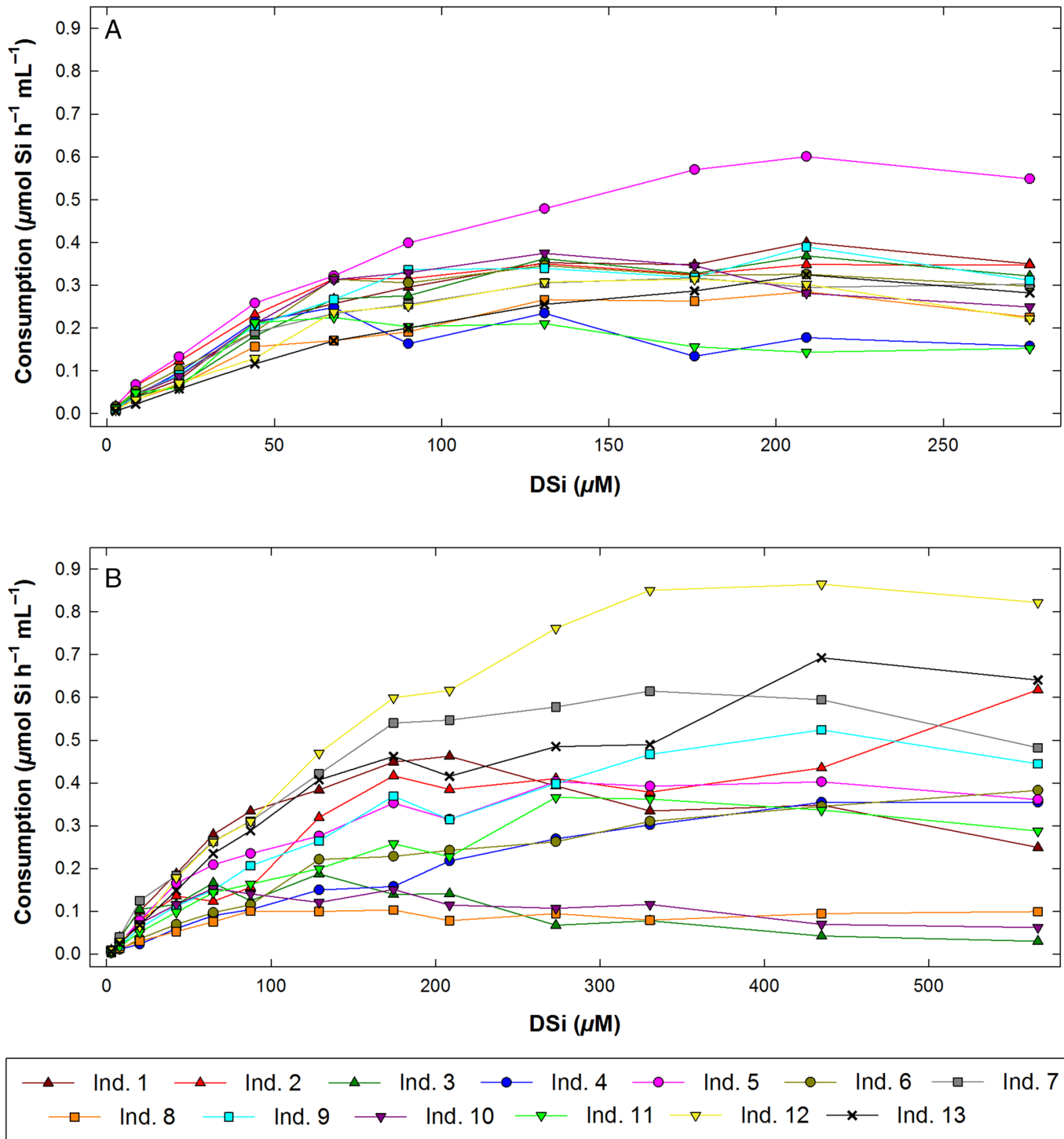


Fig. 4. Consumption rates ($\mu\text{mol Si h}^{-1} \text{ mL}^{-1}$) of each individual of (A) *H. simulans* and (B) *S. ficus* as a function of the concentration (μM) of silicic acid (DSi) in seawater.

variability, no significant effect was found within the range of assayed sizes. When the volume of each individual was plotted vs. the total amount of DSi consumed per mL of body over the experiment, neither linear nor nonlinear relationships could be fitted for the data in any of the

species (Fig. 5A,B). Likewise, when body size of each individual was confronted with the size-normalized maximum DSi transport rate ($\mu\text{mol Si h}^{-1} \text{ mL}^{-1}$) of the individuals, no significant relationship emerged for any of the species (Fig. 5C,D).

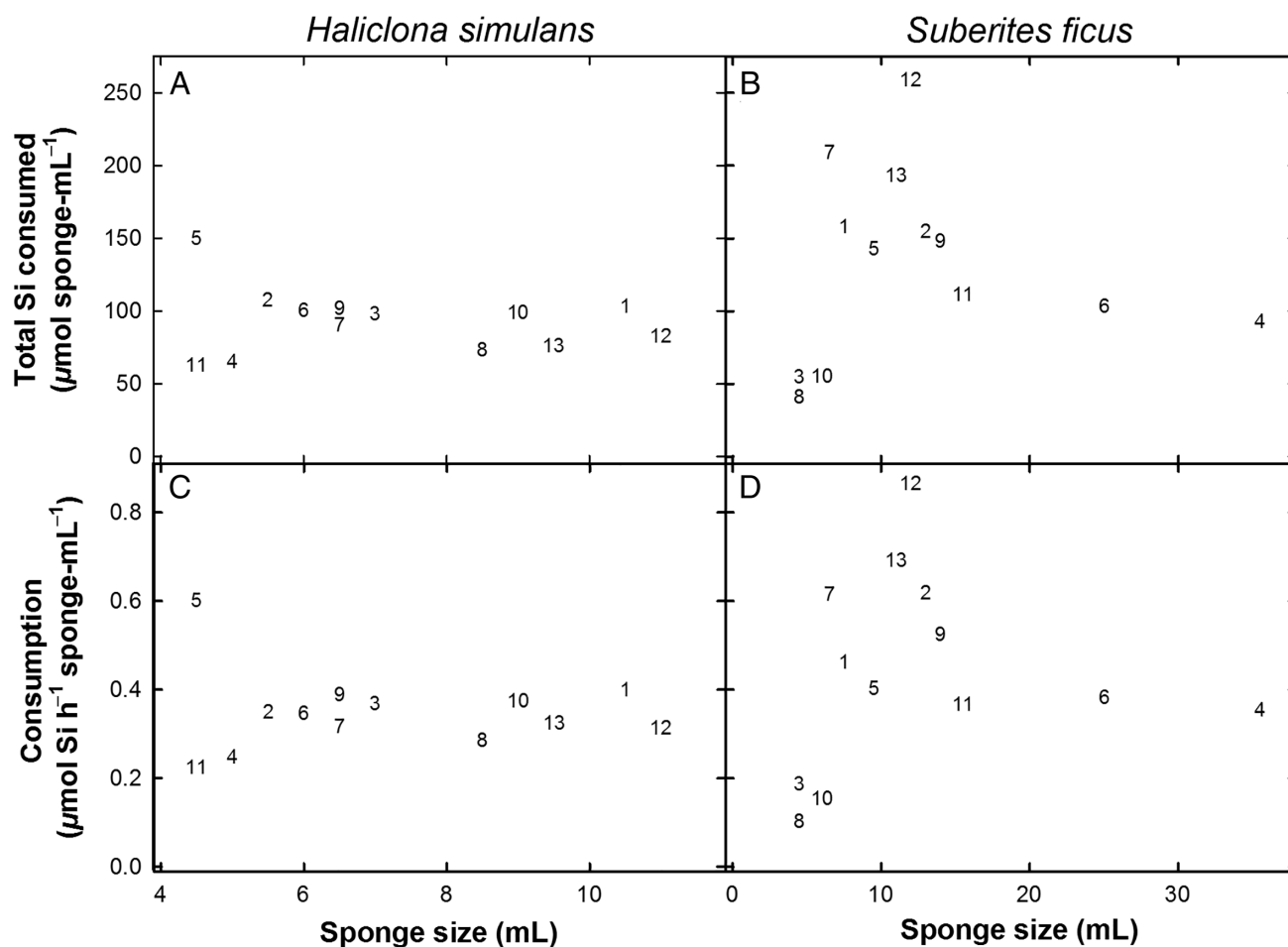


Fig. 5. (A, B) Sponge size (mL) of each assayed individuals of (A) *H. simulans* and (B) *S. ficus* plotted vs. total Si consumed ($\mu\text{mol Si sponge mL}^{-1}$) by the individual over the entire experiment. (C, D) Sponge size (mL) of each assayed individuals of (C) *H. simulans* and (D) *S. ficus* plotted vs. the maximum DSi consumption rate ($\mu\text{mol Si h}^{-1} \text{ sponge mL}^{-1}$) recorded for each individual at any step over the experiment. Numbers indicate the identity of the individual associated to each symbol.

Modeling the DSi consumption

The average (\pm SD) response of the 13 individuals during the 48 h incubation steps revealed a common general trend in DSi consumption kinetics in both species, with consumption rate increasing with DSi availability, first linearly and then asymptotically, until reaching a DSi concentration at which the consumption rate does not longer increases with increasing DSi availability (Fig. 6). The goodness-of-fit analysis based on the average responses shown in Fig. 6 corroborated that the DSi utilization process fitted a saturable Michaelis–Menten function in both species ($r^2 = 0.956$, $p < 0.0001$ for *H. simulans*; $r^2 = 0.989$, $p < 0.0001$ for *S. ficus*; Fig. 7A,B). A similar goodness-of-fit analysis but based on the individual responses rather than on the averaged responses consistently led to similar conclusions. Thus, for the sake of simplicity, the individual-derived model and its statistics are summarized only as Supporting Information S4.

Although the two species shared a common general kinetic model, there were subtle differences. For the models obtained

from the averaged response, the V_{max} in *H. simulans* and *S. ficus* were quite similar ($0.39 \pm 0.03 \mu\text{mol Si h}^{-1} \text{ mL}^{-1}$ and $0.48 \pm 0.02 \mu\text{mol Si h}^{-1} \text{ mL}^{-1}$, respectively), but the K_m of *S. ficus* ($108.23 \pm 12.12 \mu\text{M DSi}$) was more than twice that of *H. simulans* ($45.92 \pm 11.98 \mu\text{M DSi}$). The affinity for DSi during the consumption process, calculated as the V_{max}/K_m ratio, was twice as high in *H. simulans* ($0.008 \mu\text{mol Si h}^{-1} \text{ sponge mL}^{-1} \mu\text{M}^{-1}$) than in *S. ficus* ($0.004 \mu\text{mol Si h}^{-1} \text{ sponge mL}^{-1} \mu\text{M}^{-1}$).

When we tested statistically for between-species differences in the kinetics considering the propagated error for the models obtained from the individual responses, statistically significant differences were found in all the parameters characterizing the kinetics, that is, the V_{max} ($n = 39$, $U = 510$, $p = 0.013$), the K_m ($n = 39$, $U = 418$, $p < 0.001$), and the V_{max}/K_m ratio ($n = 39$, $U = 323$, $p < 0.001$). These tests consistently support that *H. simulans* has a higher affinity for DSi than *S. ficus*, particularly at low DSi concentrations.

Si utilization at the ecosystem level

Field surveys revealed a total of 45 siliceous sponge species in the Bay. The populations of *S. ficus* and *H. simulans* consisted of comparatively high numbers of relatively small individuals and few large ones (Fig. 8A,B). These two species, along with *T. citrina* and *H. perlevis*, are dominant species in biomass (volume) in the Bay (Table 2), representing collectively about 62% of the total sponge standing stock. Indeed,

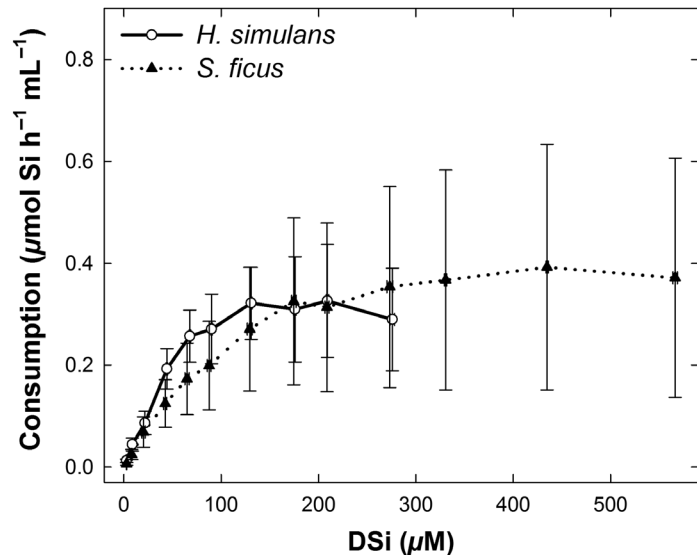


Fig. 6. Average (\pm SD) consumption rate ($\mu\text{mol Si h}^{-1}$ sponge mL^{-1}) empirically determined for individuals of *H. simulans* and *S. ficus* as a function of their exposure to increasing concentrations (μM) of silicic acid (DSi) during 48 h incubation steps.

H. perlevis contributes $14.6 \times 10^3 \text{ m}^3$ to the total biomass in the Bay, almost as much ($15.9 \times 10^3 \text{ m}^3$) as all the nondominant sponges together (Table 2). The largest sponge biomass in absolute numbers corresponded to the maërl bottom (habitat 3), followed by far by the rocky subtidal bottom (habitat 2). The lowest sponge biomass occurred at the rocky intertidal belt of the Bay (habitat 1).

The average of monthly DSi availability for the last dozen years at the natural habitat ranged from $1 \mu\text{M}$ to $9 \mu\text{M}$ over the four seasons of a year cycle (Fig. 8C), with occasional transient peaks of $15 \mu\text{M}$ during the winter of some years. Seasonal shifts in DSi availability in the seawater of the Bay caused changes in the capacity of the sponges to consume DSi (Fig. 8D,E). Interestingly, the population of *T. citrina*, which amounts a global biomass in the Bay over threefold smaller than that of *H. perlevis*, is responsible for virtually the same DSi annual utilization, that is, about 1 Mmol Si (Table 2). The DSi utilization by *S. ficus* and *H. simulans* ranked behind those two. The remaining DSi consumption was due to the populations of the 41 other siliceous species of sponges occurring in the Bay. By habitats, the consumption resulted from a combination of the average sponge density and habitat extension (Table 2). The largest annual consumption happened in the maërl beds (habitat 3), followed by the rocky subtidal (habitat 2).

Collectively the assemblage of siliceous sponges in the Bay is estimated to consume annually 6.39 ± 11.44 Mmol of Si (Table 2). Excluding harbors and estuaries (gray zones in Fig. 2), it can be concluded that the sponge assemblage of the Bay consume DSi at an average rate of 0.104 ± 0.190 mmol Si $\text{m}^{-2} \text{d}^{-1}$.

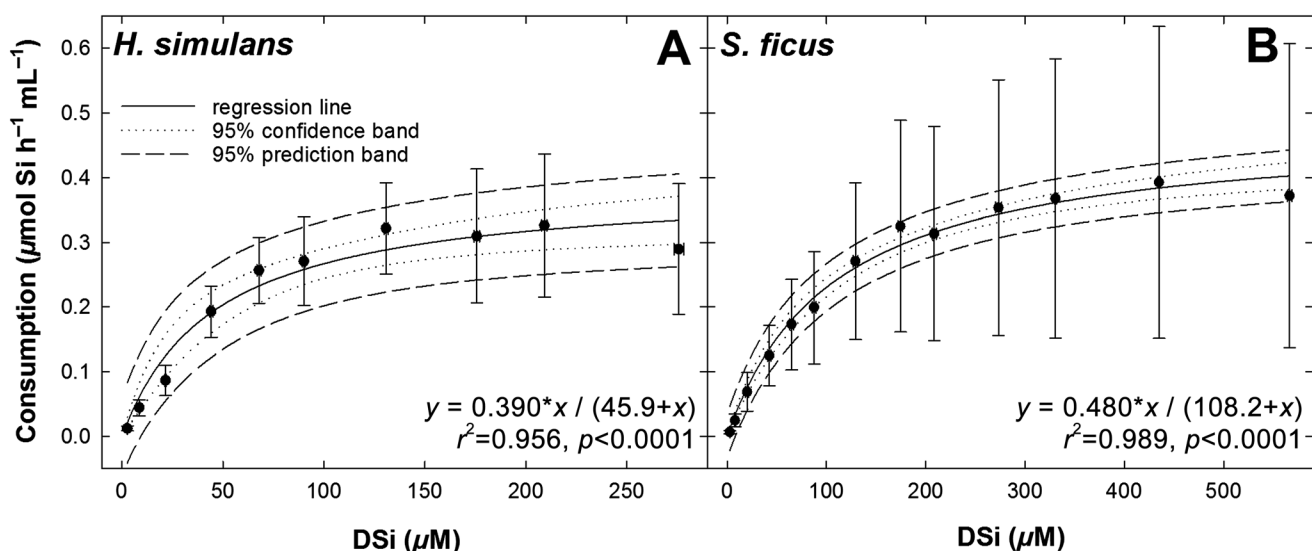


Fig. 7. Summary of the statistically significant goodness of fit of the relationship between DSi availability and DSi consumption rate in (A) *H. simulans* and (B) *S. ficus*. In both cases, the best fit was to a hyperbolic model that matches the Michaelis–Menten equation. The herein presented statistics are for the goodness of fit to the average response of the pool of individuals in each species. The statistics for the models fitting the individual responses themselves are given as Supporting Information Fig. S1.

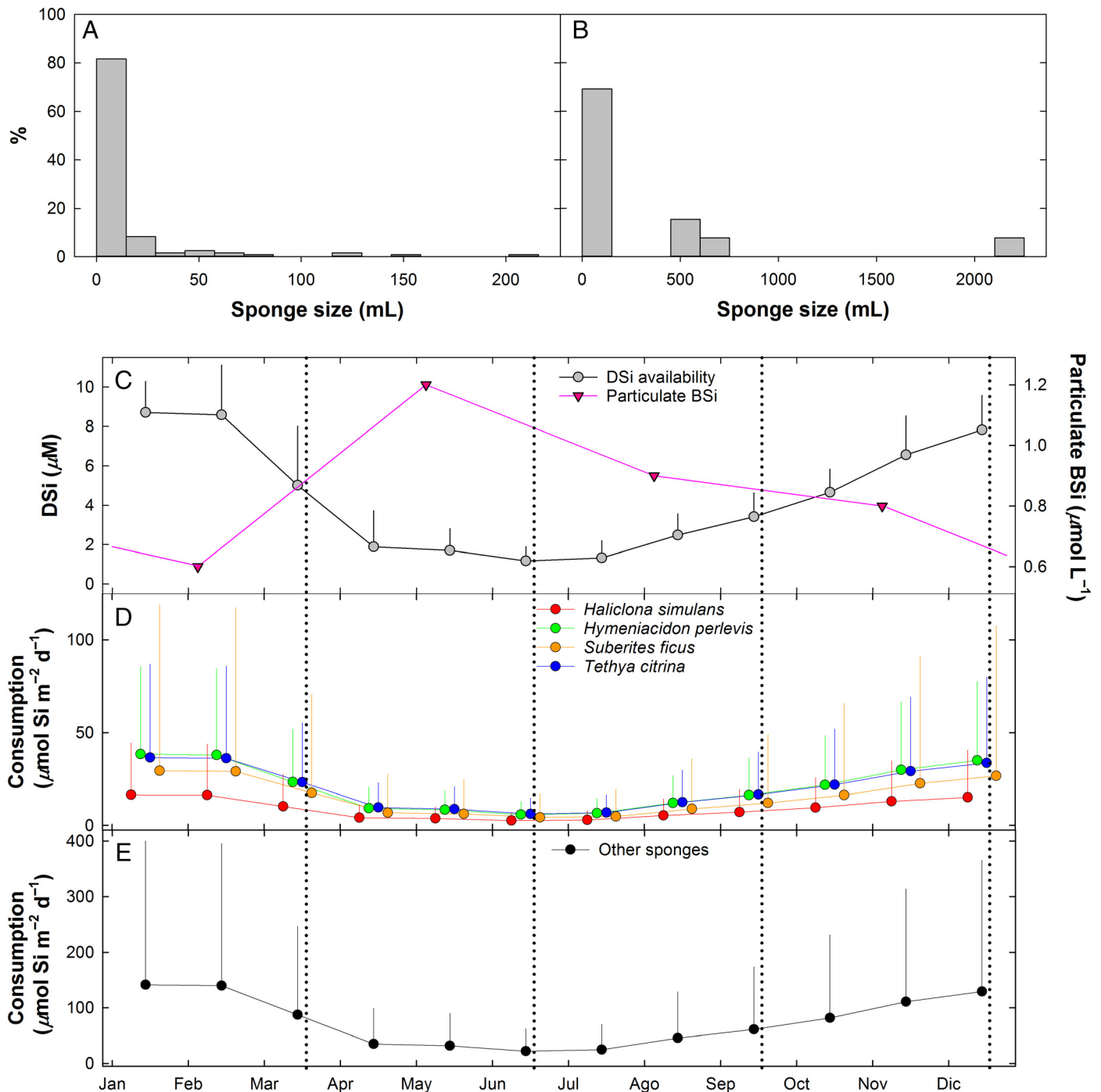


Fig. 8. (A, B) Frequency distribution of sponge size (in volume) for (A) *H. similans* and (B) *S. ficus* in the Bay of Brest. (C) Monthly average (\pm SD) of DSI concentration (μM) in the Bay (taken from the SOMLIT-Brest database) along with seasonal average of diatom abundance (as particulate BSi in suspension, from Beucher et al. 2004). (D, E) Consumption rate ($\mu\text{mol Si m}^{-2} \text{d}^{-1}$) by the populations of the four most abundant species in the Bay of Brest (D) and the rest of 41 other sponge species (E). Vertical dotted lines define seasons.

Discussion

The kinetics of DSI utilization in *H. similans* and *S. ficus* fit a saturable Michaelis–Menten function, in agreement with all previously investigated demosponges. A comparative plot of all the available kinetic models to date (Fig. 9) reveals that the

two herein assayed sponges appear to be the most rapid in DSI consumption, featuring the highest V_{max} values known to date. The V_{max} in *S. ficus* being slightly higher than that of *H. similans* could account for the fact that the former species produces about 50% more silica per biomass unit (115 mg BSi

Table 2. Summary of sponge standing stock (S), given as volume, and DSi consumption (C), given by m^2 and year in each habitat. These two parameters (Pa) are specified per habitat over a year for each of the dominant species and the rest of the sponge fauna, and then totaled per habitats and bay. Numbers represent average \pm standard deviation values.

Species	Pa	Habitats of the Bay						Total sponge stock ($10^3 m^3$)	Total utilization ($10^6 mol Si yr^{-1}$)
		1	2	3	4	5	6		
<i>Sponge stock ($mL m^{-2}$) and consumption rate ($10^{-3} mol Si m^{-2} yr^{-1}$)</i>									
<i>H. perlevis</i>	S	86.7 \pm 291.4	36.5 \pm 81.3	173.9 \pm 236.6	—	26.1 \pm 55.7	2.4 \pm 10.9	14.6 \pm 17.8	—
	C	6.4 \pm 7.9	2.7 \pm 4.5	12.9 \pm 15.3	—	1.9 \pm 3.1	0.2 \pm 0.5	—	1.08 \pm 1.32
<i>T. citrina</i>	S	31.8 \pm 122.5	98.4 \pm 236.9	45.6 \pm 76.3	—	25.6 \pm 44.4	1.1 \pm 2.2	4.8 \pm 6.6	—
	C	7.1 \pm 9.9	22.0 \pm 38.4	10.2 \pm 13.7	—	5.7 \pm 7.9	0.3 \pm 0.4	—	1.06 \pm 1.47
<i>S. ficus</i>	S	—	—	18.6 \pm 113.8	10.9 \pm 33.6	92.7 \pm 390.1	—	5.0 \pm 15.2	—
	C	—	—	3.0 \pm 11.7	1.8 \pm 3.8	15.1 \pm 41.7	—	—	0.81 \pm 2.47
<i>H. simulans</i>	S	—	15.8 \pm 46.0	17.5 \pm 39.2	—	4.3 \pm 11.7	1.6 \pm 4.3	1.6 \pm 2.7	—
	C	—	4.6 \pm 9.4	5.1 \pm 8.5	—	1.3 \pm 2.4	0.5 \pm 0.9	—	0.46 \pm 0.78
Other sponges	S	5.2 \pm 27.3	3628.9 \pm 9780.7	50.6 \pm 89.4	74.0 \pm 278.6	9.1 \pm 23.1	21.2 \pm 42.1	15.9 \pm 29.0	—
	C	1.0 \pm 1.8	681.1 \pm 1301.9	9.5 \pm 13.3	13.9 \pm 34.9	1.7 \pm 3.1	4.0 \pm 6.0	—	2.98 \pm 5.44
Average per habitat	S	123.8 \pm 338.1	3779.6 \pm 9740.6	306.2 \pm 322.1	84.9 \pm 277.5	157.9 \pm 383.1	26.4 \pm 52.4	—	—
	C	14.5 \pm 19.6	710.4 \pm 1354.1	40.7 \pm 62.1	15.7 \pm 38.7	25.7 \pm 58.1	4.9 \pm 7.8	—	—
<i>Total sponge stock ($10^3 m^3$) and consumption ($10^6 mol Si yr^{-1}$)</i>									
Area (km^2)		1.98	2.60	76.45	21.42	36.08	28.70		
Total	S	0.2 \pm 0.7	9.8 \pm 25.4	23.4 \pm 24.62	1.8 \pm 5.9	5.7 \pm 13.82	0.76 \pm 1.50	41.8 \pm 57.5	—
	C	0.03 \pm 0.04	1.8 \pm 3.5	3.1 \pm 4.7	0.3 \pm 0.8	0.9 \pm 2.1	0.1 \pm 0.2	—	6.39 \pm 11.44

mL^{-1}) than *H. simulans* (75 mg BSi mL^{-1}) when building the skeleton. However, the most silicified sponge occurring in the Bay, *T. citrina* (145 mg BSi mL^{-1}), is known to have a V_{max} about twice smaller (Fig. 9) than those of *S. ficus* and *H. simulans* (López-Acosta et al. 2016). Consequently, although the comparison of all available data in the literature shown in Fig. 9 does not rely on a test for detecting statistically significant differences between all possible species pairs, it appears that obvious differences do occur between some species and that their causes cannot be easily explained from the V_{max} and the BSi content of the sponges.

Interestingly, the half-saturation constant characterizing *S. ficus* is the largest recorded to date (which suggests the lowest affinity for DSi), while that of *H. simulans* falls within the range of previously studied species (Fig. 9). In both species, the DSi consumption system performs with maximum velocity at a relatively high saturating DSi concentration (about 67 μM in *H. simulans* and 129 μM in *S. ficus*), being these concentration values that are never available in the natural habitats. The DSi limitation affects specially to the most active individuals in DSi consumption, such as either individual #5 of *H. simulans* (which developed its V_{max} at 207 μM DSi) or individual #12 of *S. ficus* (which did it at 433 μM DSi). All together, these new data agree with previous sponge kinetics (Reincke and Barthel 1997; Maldonado et al. 2011; López-

Acosta et al. 2016) in supporting the notion that, despite between-species variability, the DSi consumption systems of demosponges appear to be evolutionarily designed to perform with maximum speed in oceans characterized by much higher DSi concentrations than the ones available in the modern ocean (Maldonado et al. 1999).

To compare the DSi consumption kinetics of sponges and diatoms is not easy for several reasons. First, short-term incubations (minutes) have been used to infer “uptake” kinetics in diatoms, which accurately reflects active transport across the diatom cell membrane before diffusion starts restoring the DSi concentration equilibrium with the extracellular environment. Unlike for diatoms, sponge kinetics do not strictly reflect “uptake,” but net DSi “consumption” measured over a period of days that integrate the potential changes in the diel cycle of the physiological activity of the sponges (see López-Acosta et al. 2016). Second, the V_{max} 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 be biologically meaningless. Diatoms are unicellular organisms that build their BSi content in just hours and live only for a couple of days. In contrast, sponges are long-lived multicellular animals that store the BSi produced by thousands of cell

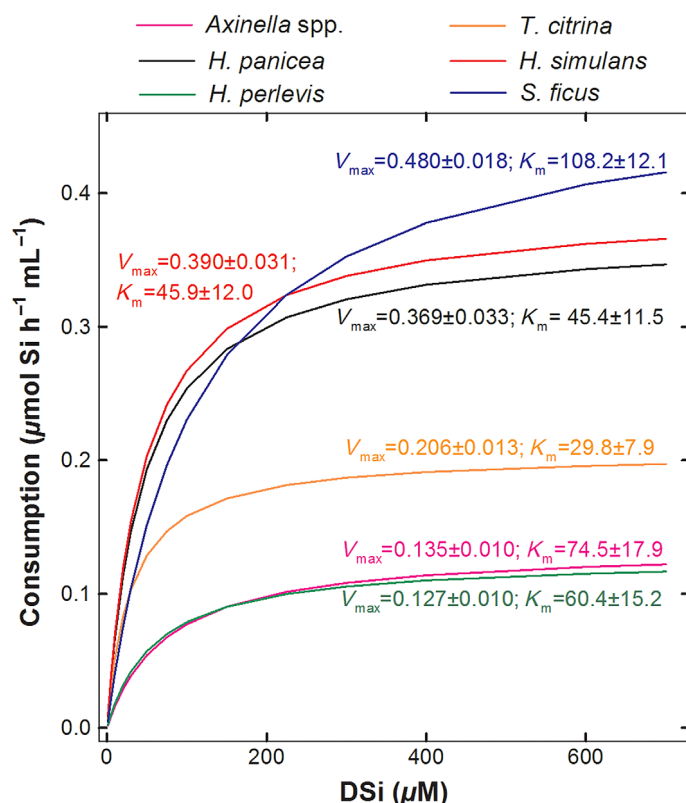


Fig. 9. Summary of all DSi consumption kinetics ($\mu\text{mol Si h}^{-1}$ sponge mL^{-1}) empirically established to date for sponges. The set contains only demosponges: *Axinella* spp. (Maldonado et al. 2011), *H. panicea* (Reincke and Barthel 1997), *H. perlevis*, *T. citrina* (López-Acosta et al. 2016), and *H. simulans* and *S. ficus* (this study). In all cases, consumption kinetics fit a saturable Michaelis–Menten model: “DSi consumption = $V_{\text{max}} \times [\text{DSi}] / (K_{\text{m}} + [\text{DSi}])$ ”. Maximum velocity (V_{max}) of DSi transport and half-saturation (K_{m}) values are given (average \pm SD) in the graph for each species.

generations for years, decades, and even centuries. Therefore, the “unicellular values” of diatoms would never be comparable to the “multicellular values” of sponges, even if those values are apparently brought to the same mathematical units. In this scenario, only the inspection of K_{m} values (defined as the DSi concentration at which the consumption process removes DSi from seawater at half its maximal rate) would have biological sense. By considering only diatom studies measuring DSi consumption rather than short-term “uptake”, the comparison reveals that most planktonic diatoms feature kinetics with K_{m} values between $0.2 \mu\text{M}$ and $8.7 \mu\text{M}$ (Paasche 1973; Conway and Harrison 1977; Kristiansen et al. 2000; Martin-Jézéquel et al. 2000). These values are markedly smaller than those of sponges (29.8 – $108.2 \mu\text{M}$; see Fig. 9), indicating that most planktonic diatoms perform with maximum consumption velocity at DSi concentrations much lower than those required for sponges. The situation is completely different if benthic diatoms are considered, since they have been shown to have nonsaturable uptake systems, being able to shift from Michaelis–Menten saturable kinetics to

nonsaturable models when exposed at very high DSi concentration (Thamatrakoln and Hildebrand 2008; Leynaert et al. 2009). Laboratory experiments have revealed that a non-saturating kinetics at high DSi concentrations may also occur in at least some planktonic diatoms (Del Amo and Brzezinski 1999; Shrestha and Hildebrand 2015).

Our results support, in agreement with previous sponge studies (Frøhlich and Barthel 1997; Maldonado et al. 2011; López-Acosta et al. 2016), that the measured DSi consumption rates derive from active DSi utilization and not from a mere chemical adsorption of DSi into the sponge body. If the latter were the case, a high consumption rate would have occurred during P1 and virtually no consumption during P2. Such a situation never happened (Fig. 3) over the several DSi concentration steps of our experiments. It has also been pointed out that kinetics of DSi uptake in diatoms must be determined only through brief incubation periods (minutes) to prevent that passive DSi leakage from the cell to the seawater may cause an artifactual underestimation of the actual DSi transport rate (Thamatrakoln and Hildebrand 2008). Unlike in diatoms, no evidence of DSi leakage from the multicellular body of sponges has ever been found (reviewed in López-Acosta et al. 2016). Indeed, incubation times much longer than minutes are required for sponges. Long incubations ensure that DSi transport across the different cell and organelle compartments of these multicellular organisms has been completed and storage concentrations built. Periods longer than a day may also be needed for sponges to activate the specific silicifying genes and to generate the appropriate numbers of gene copies and cell types involved in processing the increasing DSi concentrations. The idea of increasing DSi concentrations by activating sets of genes and cells that were inactivated at low DSi concentrations was long suggested (Maldonado et al. 1999). It was based on the demonstration that skeletal pieces absent in wild populations were produced under increased DSi availability in the laboratory. For the above reasons, too short incubations (< 24 h) could lead to artifactual situations and inaccurate DSi consumption rate determinations either by default or by excess: (1) underestimation of rates, because the sponges do not have enough time to unfold all the molecular and cellular mechanisms involved in the silicification process; (2) overestimation of rates, as suggested by the fact that we found (Fig. 3) consumption rates at some DSi concentration steps being 10–27% higher during the first 24 h of incubation (P1) than during the subsequent 24 h period (P2).

The possibility that a substantial decrease in DSi concentration along the flow pathway inside the sponge may occur is unlikely. Indeed, there have been attempts to measure empirically such a decrease in DSi concentration. However, the concentration decrease along flow is so small that the approaches have consistently failed to detect differences in the DSi concentration between the seawater going into the sponges and that going out of their bodies (Perea-Blázquez et al. 2012;

Morganti et al. 2016; Leys et al. 2018). The retention rate of DSi (i.e., amount of silicate consumed every time a liter of seawater passes through the sponge body) appears therefore to be extremely low for most sponges. This is probably due to the fact that sponges need to pump at relatively high speeds to gather enough food from the seawater. It was long quantified that individuals of shallow-water demosponges can filter a seawater volume equivalent to 60- and 800-fold its own body volume per hour, depending on the species (Reiswig 1974). Altogether, it means that the seawater stays within most demosponges from just a few seconds to a few minutes, a residence-time range also expected in our assayed demosponges.

In agreement with all previous sponge studies (Fröhlich and Barthel 1997; Reincke and Barthel 1997; Maldonado et al. 2011, 2012a; López-Acosta et al. 2016), our results show important levels of between-individual variability in the DSi consumption responses. While in the demosponges *Axinella* spp., *T. citrina*, and *H. perlevis* (Maldonado et al. 2011; López-Acosta et al. 2016), the between-individual variability was significantly related to sponge size and/or the particular physiological stage (i.e., reproductive vs. nonreproductive condition), a similar pattern has not been retrieved herein. Because recognizable symptoms of reproduction were detected neither in *H. simulans* nor in *S. ficus* at the time of the experiments, we could not decide on their reproductive condition. At the first sight, the absence of correlation between body size and DSi consumption in the two assayed species (Fig. 5) would be in conflict with the above-mentioned studies. It would also go against the general notion that the smaller, non-fully-grown sponges aspiring to reach their maximum body size would consume DSi at higher rates than larger individuals that are already fully grown from a skeletal point of view. However, the absence of relationship between body size and DSi consumption in the assayed species may be an artifactual result (type error II) that we unwarily favored because consistently selected for relatively small sponges, avoiding the largest and fully grown individuals in the field populations to facilitate the logistics of sponge manipulation and maintenance in the laboratory. This suspicion is supported by two facts. The average size of the lab assayed individuals of *H. simulans* was 7.2 ± 2.2 mL, while our field data revealed a mean size of 14.4 ± 29.7 mL in the population and the occurrence of individuals as large as 216 mL (Fig. 8A). The selected specimens of *S. ficus* involved even larger differences with the field population, being the average lab size 12.6 ± 8.9 mL and the mean field size 327.9 ± 630.3 mL, with some individuals as large as 2252 mL (Fig. 8B). The fact that *H. simulans* and *S. ficus* featured the highest V_{\max} recorded among demosponges to date also supports the idea that our experiments were based on non-fully-grown sponges particularly avid for DSi to complete their skeletal growth. Interestingly, this much higher abundance of relatively small individuals also appears to be the predominant pattern of size distribution in the natural populations of both species (Fig. 8A,B).

DSi utilization by sponges in the Bay averaged a rate of 0.10 ± 0.19 mmol Si m⁻² d⁻¹. When compared with rates estimated for other shallow-water sponge assemblages (Maldonado et al. 2011, 2012b), the Bay of Brest assemblage consumes at a rate that is an order of magnitude higher than the oligotrophic Mediterranean rocky sublittoral (0.01 ± 0.01 mmol Si m⁻² d⁻¹), but fourfold to ninefold lower than those respectively registered during the seasonal population explosion of *H. panicea* at the Baltic sublittoral bottoms (0.44 mmol Si m⁻² d⁻¹) and for the rich sponge assemblages of the Caribbean barrier reef (0.90 ± 5.00 mmol Si m⁻² d⁻¹).

The average DSi consumption rate by the sponge assemblage in the Bay of Brest is an order of magnitude lower than that measured for the diatom communities in the Bay (Ragueneau et al. 2005), which ranges from an average of about 1 mmol Si m⁻² d⁻¹ during months of lowest light availability to about 4 mmol Si m⁻² d⁻¹ during the months of maximum photoperiod. On average, the siliceous sponges in the Bay consumed around 6.39×10^6 mol Si yr⁻¹, which represents some 7.6% of the yearly net BSi production. The rest of the net BSi production would be owed to diatoms (77.76×10^6 mol Si yr⁻¹). Such a value results from recalculating the BSi production estimated by Ragueneau et al. (2005) for 180 km² down to 167 km² of Bay extension, then detracting the diatom BSi dissolution determined by Beucher et al. (2004). The nature of the kinetics of DSi consumption in sponges suggests that the relatively low contribution of sponges to the Bay probably derives from the fact that they need much higher DSi concentrations than those available in the Bay during the year to perform with maximum velocity. Actually, diatom proliferation appears to be responsible for maintaining DSi relatively low all year around, particularly during the well-illuminated months (Fig. 8C), competitively preventing sponges to develop a larger contribution.

Since the silicon budget previously established for the Bay of Brest overlooked about 8% of the net annual BSi production by disregarding the sponge role, it cannot be ruled out that similar or even larger errors are likely to affect the budget of the global marine Si cycle, which does not yet incorporate the potential contribution of sponges.

References

- Beucher, C., P. Tréguer, R. Corvaisier, A. M. Hapette, and M. Elskens. 2004. Production and dissolution of biosilica, and changing microphytoplankton dominance in the Bay of Brest (France). *Mar. Ecol. Prog. Ser.* **267**: 57–69. doi:10.3354/meps267057
- Brunner, E., and U. Munzel. 2000. The nonparametric Behrens-Fisher problem: Asymptotic theory and a small-sample approximation. *Biom. J.* **42**: 17–25. doi:10.1002/(SICI)1521-4036(200001)42:1<17::AID-BIMJ17>3.0.CO;2-U
- Brunner, E., and M. L. Puri. 2001. Nonparametric methods in factorial designs. *Stat. Pap.* **42**: 1–52. doi:10.1007/s003620000039

- Brunner, E., S. Domhof, and F. Langer. 2002. Nonparametric analysis of longitudinal data in factorial experiments. Wiley.
- Conway, H. L., and P. J. Harrison. 1977. Marine diatoms grown in chemostats under silicate or ammonium limitation. IV. Transient response of *Chaetoceros debilis*, *Skeletonema costatum* and *Thalassiosira gravida* to a single addition of the limiting nutrient. *Mar. Biol.* **43**: 33–43. doi:10.1007/BF00392569
- Del Amo, Y., and M. A. Brzezinski. 1999. The chemical form of dissolved Si taken up by marine diatoms. *J. Phycol.* **35**: 1162–1170. doi:10.1046/j.1529-8817.1999.3561162.x
- Delmas, R., and P. Tréguer. 1985. Simulation de l'évolution de paramètres physiques, chimiques, et de la biomasse phytoplanctonique en période printanière dans un écosystème littoral macrotidal. *Oceanis* **11**: 197–211.
- DeMaster, D. J. 1981. The supply and accumulation of silica in the marine environment. *Geochim. Cosmochim. Acta* **45**: 1715–1732. doi:10.1016/0016-7037(81)90006-5
- Fan, C. P., and D. H. Zhang. 2017. On power and sample size of the ANOVA-type rank test. *Commun. Stat. Simul. Comput.* **46**: 3224–3241. doi:10.1080/03610918.2015.1082585
- Fröhlich, H., and D. Barthel. 1997. Silica uptake on the marine sponge *Halichondria panicea* in Kiel Bight. *Mar. Biol.* **128**: 115–125. doi:10.1007/s002270050075
- Healey, F. P. 1980. Slope of the Monod equation as an indicator of advantage in nutrient competition. *Microb. Ecol.* **5**: 281–286. doi:10.1007/BF02020335
- Kristiansen, S., T. Farbrot, and L.-J. Naustvoll. 2000. Production of biogenic silica by spring diatoms. *Limnol. Oceanogr.* **45**: 472–478. doi:10.4319/lo.2000.45.2.0472
- Leynaert, A., S. N. Longphuir, P. Claquin, L. Chauvaud, and O. Ragueneau. 2009. No limit? The multiphasic uptake of silicic acid by benthic diatoms. *Limnol. Oceanogr.* **54**: 571–576. doi:10.4319/lo.2009.54.2.0571
- Leys, S. P., A. S. Kahn, J. K. H. Fang, T. Kutti, and R. J. Bannister. 2018. Phagocytosis of microbial symbionts balances the carbon and nitrogen budget for the deep-water boreal sponge *Geodia barretti*. *Limnol. Oceanogr.* **63**: 187–202. doi:10.1002/lno.10623
- López-Acosta, M., A. Leynaert, and M. Maldonado. 2016. Silicon consumption in two shallow-water sponges with contrasting biological features. *Limnol. Oceanogr.* **61**: 2139–2150. doi:10.1002/lno.10359
- Maldonado, M., M. C. Carmona, M. J. Uriz, and A. Cruzado. 1999. Decline in Mesozoic reef-building sponges explained by silicon limitation. *Nature* **401**: 785–788. doi:10.1038/44560
- Maldonado, M., M. C. Carmona, Z. Velásquez, A. Puig, A. Cruzado, A. López, and C. M. Young. 2005. Siliceous sponges as a silicon sink: An overlooked aspect of the benthopelagic coupling in the marine silicon cycle. *Limnol. Oceanogr.* **50**: 799–809. doi:10.4319/lo.2005.50.3.0799
- Maldonado, M., A. Riesgo, A. Bucci, and K. Rützler. 2010. Revisiting silicon budgets at a tropical continental shelf: Silica standing stocks in sponges surpass those in diatoms. *Limnol. Oceanogr.* **55**: 2001–2010. doi:10.4319/lo.2010.55.5.2001
- Maldonado, M., L. Navarro, A. Grasa, A. Gonzalez, and I. Vaquerizo. 2011. Silicon uptake by sponges: A twist to understanding nutrient cycling on continental margins. *Sci. Rep.* **1**: 30. doi:10.1038/srep00030
- Maldonado, M., H. Cao, X. Cao, Y. Song, Y. Qu, and W. Zhang. 2012a. Experimental silicon demand by the sponge *Hymeniacidon perlevis* reveals chronic limitation in field populations. *Hydrobiologia* **687**: 251–257. doi:10.1007/s10750-011-0977-9
- Maldonado, M., M. Ribes, and F. C. Van Duyl. 2012b. Nutrient fluxes through sponges: Biology, budgets, and ecological implications. *Adv. Mar. Biol.* **62**: 114–182. doi:10.1016/B978-0-12-394283-8.00003-5
- Maldonado, M., and others. 2017. Sponge grounds as key marine habitats: A synthetic review of types, structure, functional roles, and conservation concerns, p. 145–183. *In* S. Rossi, L. Bramanti, A. Gori, and C. Orejas [eds.], *Marine animal forests: The ecology of benthic biodiversity hotspots*. Springer International Publishing.
- Martin-Jézéquel, V., M. Hildebrand, and M. A. Brzezinski. 2000. Silicon metabolism in diatoms: Implications for growth. *J. Phycol.* **36**: 821–840. doi:10.1046/j.1529-8817.2000.00019.x
- Morganti, T., G. Yahel, M. Ribes, and R. Coma. 2016. VacuSIP, an improved in-ex method for in situ measurement of particulate and dissolved compounds processed by active suspension feeders. *J. Vis. Exp.* **114**: e54221. doi:10.3791/54221
- Nelson, D. M., P. Tréguer, M. A. Brzezinski, A. Leynaert, and B. Quéguiner. 1995. Production and dissolution of biogenic silica in the ocean: Revised global estimates, comparison with regional data and relationship to biogenic sedimentation. *Global Biogeochem. Cycles* **9**: 359–372. doi:10.1029/95GB01070
- Noguchi, K., Y. R. Gel, E. Brunner, and F. Konietzschke. 2012. nparLD: An R software package for the nonparametric analysis of longitudinal data in factorial experiments. *J. Stat. Softw.* **50**: 1–23. doi:10.18637/jss.v050.i12.
- Nussbaum, E. M. 2014. *Categorical and nonparametric data analysis: Choosing the best statistical technique*. Taylor & Francis.
- Oelze, M., F. von Blanckenburg, D. Hoellen, M. Dietzel, and J. Bouchez. 2014. Si stable isotope fractionation during adsorption and the competition between kinetic and equilibrium isotope fractionation: Implications for weathering systems. *Chem. Geol.* **380**: 161–171. doi:10.1016/j.chemgeo.2014.04.027
- Paasche, E. 1973. Silicon and the ecology of marine plankton diatoms. II. Silicate-uptake kinetics in five diatom species. *Mar. Biol.* **19**: 262–269. doi:10.1007/BF02097147
- Perea-Blázquez, A., S. Davy, and J. Bell. 2012. Nutrient utilisation by shallow water temperate sponges in New Zealand. *Hydrobiologia* **687**: 237–250. doi:10.1007/s10750-011-0798-x

- Ragueneau, O., L. Chauvaud, B. Moriceau, A. Leynaert, G. Thouzeau, A. Donval, F. Le Loc'h, and F. Jean. 2005. Biodeposition by an invasive suspension feeder impacts the biogeochemical cycle of Si in a coastal ecosystem (Bay of Brest, France). *Biogeochemistry* **75**: 19–41. doi:[10.1007/s10533-004-5677-3](https://doi.org/10.1007/s10533-004-5677-3)
- Reincke, T., and D. Barthel. 1997. Silica uptake kinetics of *Halichondria panicea* in Kiel Bight. *Mar. Biol.* **129**: 591–593. doi:[10.1007/s002270050200](https://doi.org/10.1007/s002270050200)
- Reiswig, H. M. 1974. Water transport, respiration and energetics of three tropical marine sponges. *J. Exp. Mar. Bio. Ecol.* **14**: 231–249. doi:[10.1016/0022-0981\(74\)90005-7](https://doi.org/10.1016/0022-0981(74)90005-7)
- Shah, D. A., and L. V. Madden. 2004. Nonparametric analysis of ordinal data in designed factorial experiments. *Phytopathology* **94**: 33–43. doi:[10.1094/PHYTO.2004.94.1.33](https://doi.org/10.1094/PHYTO.2004.94.1.33)
- Shrestha, R. P., and M. Hildebrand. 2015. Evidence for a regulatory role of diatom silicon transporters in cellular silicon responses. *Eukaryot. Cell* **14**: 29–40. doi:[10.1128/ec.00209-14](https://doi.org/10.1128/ec.00209-14)
- Strickland, J. D. H., and T. R. Parsons. 1972. A practical handbook of seawater analysis, 2nd ed. Fisheries Research Board of Canada.
- Thamatrakoln, K., and M. Hildebrand. 2008. Silicon uptake in diatoms revisited: A model for saturable and nonsaturable uptake kinetics and the role of silicon transporters. *Plant Physiol.* **146**: 1397–1407. doi:[10.1104/pp.107.107094](https://doi.org/10.1104/pp.107.107094)
- Tréguer, P., D. M. Nelson, A. J. V. Bennekom, D. J. DeMaster, A. Leynaert, and B. Quéguiner. 1995. The silica balance in the world ocean: A reestimate. *Science* **268**: 375–379. doi:[10.1126/science.268.5209.375](https://doi.org/10.1126/science.268.5209.375)
- Tréguer, P. J., and C. L. De La Rocha. 2013. The world ocean silica cycle. *Ann. Rev. Mar. Sci.* **5**: 477–501. doi:[10.1146/annurev-marine-121211-172346](https://doi.org/10.1146/annurev-marine-121211-172346)

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Conflict of Interest

None declared.

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