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SPONGE RESEARCH DEVELOPMENTS

Experimental silicon demand by the sponge *Hymeniacidon perlevis* reveals chronic limitation in field populations

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Abstract Dissolved silicon (DSi) is a key marine nutrient. Sponges and diatoms are relevant DSi consumers, but sponges appear to have a less efficient uptake system that requires higher ambient DSI concentrations for maximum uptake. We experimentally tested whether a sponge adapted to live at the intertidal (*Hymeniacidon perlevis*) also shows such a need for high DSi. Under laboratory conditions, sponges were exposed to both the natural DSi concentration (10 μM) and much higher levels (25, 40, and 70 μM) for 36 h, being water samples taken at 6 h intervals to infer DSi uptake. Uptake rates shifted over time (particularly in high DSi treatments) and showed moderate inter-individual variability. Average DSi uptake rate at 70 μM was twice higher than those at 40 and 25 μM, which in turn were not significantly different from each other, but were twice higher than the uptake rate at 10 μM. Therefore, *H. perlevis* needs, for efficient uptake, ambient DSi concentrations two to four times higher than the maximum available in its natural habitat. From an eco-physiological point of view, it means that the skeletal growth in the populations of *H. perlevis* is chronically limited by DSi availability, a limitation that may favor sponge evolution toward skeletal slimming.

Keywords Silicate · Sponge · Porifera · Nutrient limitation · Benthic-pelagic coupling

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Introduction

Silicic acid, a biologically assimilable form of dissolved silicon (DSi), is an important ocean nutrient, being strongly involved in the control of primary production (e.g., Sarmiento & Gruber, 2006). Recent studies have suggested the idea that siliceous sponges are relevant DSi users at a large ecological scale, a role traditionally neglected by nutrient ecologists and biogeochemists and that is biasing the advance toward a realistic understanding of silicon cycling in marine systems, and particularly on continental margins

(Reincke & Barthel, 1997; Maldonado et al., 2005, 2010a, 2011; Chu et al., 2011).

One of the problems currently preventing an adequate assessment of the ecological implications of DSi utilization by sponges is the scarcity of data relative to their uptake rate. Studies are available only for the seasonal populations of the North-Atlantic demosponge *Halichondria panicea* (Fröhlich & Barthel, 1997; Reincke & Barthel, 1997), and for some sublittoral species in the genus *Axinella* (Maldonado et al., 2011). The scarce available evidence suggests that sponges appear to have a less efficient uptake system than that of diatoms, because it requires much higher ambient DSI concentrations for maximum uptake. To contribute to this issue, we have experimentally tested whether individuals of a common demosponge—*Hymeniacidon perlevis* (Montagu, 1818)—adapted to live at the intertidal zone also show such a need for high DSi.

Materials and methods

The studied species

Laboratory experiments were conducted on individuals of the halichondrid *Hymeniacidon perlevis*, a nearly cosmopolitan species, the geographical distribution of which may require further reconsideration with the help of molecular techniques for accurate discrimination of potential cryptic species and misidentifications. Previous studies on *H. perlevis* have suggested that this sponge is extremely plastic, being able to grow at either the intertidal or within the sublittoral, to feed on a wide variety of particulate sources, and to heal wounds and regenerate from small pieces at extremely rapid rates (Fu et al., 2006, 2007; Maldonado et al., 2010b). Individuals used in the laboratory experiments grew at the intertidal zone of Lingshui (38°32′33.77″N, 121°32′33.77″E; Yellow Sea, Dalian, China), where they become periodically exposed to air, also to temperatures as high as 40°C during summer low tides and as low as −10°C during winter. Nevertheless, this sponge is not a strict intertidal organism, for the largest part of the Dalian population living permanently submerged at sublittoral depths. Most of the body biomass of the intertidal individuals regresses during winter, probably by the combined effects of low temperatures and much fresh-water from heavy rainfall. The sponges are able to regrow rapidly each spring, producing appreciable biomass through summer and autumn until a new regression. They reproduce sexually each year, brooding parenchymella larvae that are released in summer (Xue & Zhang, 2009).

The uptake experiment

Upon collection from the intertidal zone during low tide (July 2008), sponges were transported to the laboratory in seawater and transferred to a 100-l aquarium of recirculating, filtered seawater with no food addition for 6 days. This acclimation period allowed healing of potential wounds and epithelium breakages caused to these cushion-shaped sponges when detaching them from the rocks. Tissue regeneration was a surprisingly rapid process in this species. For the experiment, sponges were distributed in four sets and individuals in each set exposed to a given DSi concentration (10, 25, 40, or 70 μM). DSi uptake by each individual was examined at 6 h intervals, during a 36 h period. Average DSi concentration in the sponge habitat the time of the experiments (July–August 2008) was 10 μM. Experimental DSi concentrations were obtained by using natural coastal seawater and adding sodium hexafluorosilicate (SHF) as a silicon source. Therefore, the 10 μM treatment required no SHF addition, unlike the 25, 40, and 70 μM DSi treatments. Prior to any SHF addition, seawater was filtered through 3-μm polycarbonate membranes to eliminate diatoms but allowing the pass of bacterioplankton and the smallest picoplankton, which are the main food source to these sponges (e.g., Ribes et al., 1999; Maldonado et al., 2010b).

The experiment was conducted in 5 l alimentary PVC incubation buckets that had been washed with 3% HCl and rinsed with deionized water twice prior to be filled with 4 l of seawater. For each of the four assayed concentrations, we intended to have five incubation buckets, each containing a single sponge. Nevertheless, when preparing the 40 μM DSi buckets, we added to one of the buckets the corresponding amount of SFS twice (i.e., it became 70 μM rather than 40 μM), leaving another bucket without its SFS addition (i.e., it did not become 40 μM but stayed at 10 μM). As a result of this error, which we noticed only when the results of our seawater analyses were processed 2 days later, we ended with an unequal

sample size for our DSi treatments, as it follows: $N_{10\ \mu M} = 6$, $N_{25\ \mu M} = 5$, $N_{40\ \mu M} = 3$, $N_{70\ \mu M} = 6$. To prevent that partial starvation during the experiment could negatively affect DSi uptake rates (Fröhlich & Barthel, 1997), the natural bacterioplankton coming with the seawater was supplemented by adding 10 mg (i.e., 2.5 mg l^{-1}) of freeze-dried, single-strain *Escherichia coli* culture every 18 h. This bacterium is known to be rapidly ingested and digested by this particular sponge (Fu et al., 2006; Maldonado et al., 2010b). During the experiment, the seawater in the culturing buckets was oxygenated by pumping air for 15 min every hour. Seawater temperature during the experiment varied from 19 to 21°C. As controls, we used an additional bucket for each DSi concentration treatment, which was filled with seawater at its corresponding DSi concentration but received no sponge. Control buckets were subjected to the same oxygen and food additions as the treatment buckets.

Once the sponges were transferred to the incubation buckets, we allowed them to acclimate for about 6 h, then pipetted off 10 ml of seawater from each bucket after mixing with a PVC rod for 5 min. This first set of seawater samples was used to determine DSi concentration at time "0" of experiment. Water sampling was repeated periodically every 6 h for a total of 36 h. Seawater samples were stored into sterile, acid-washed vials at 4°C for 12–48 h before analysis. Determination of DSi concentrations were conducted using a Bran-Luebbe Autoanalyzer III, following the standard colorimetric method for measuring molybdate-reactive silicate (Grasshoff et al., 1983). Chemical reactions were maintained under 37°C and the coloration change measured under 820 nm wavelength (Method: MT19 for seawater). Individual uptake at each concentration treatment and time interval was estimated by subtracting the final DSi concentration from the initial one in each bucket and correcting by the concentration change in the control bucket, caused typically by Si adsorption to bucket walls.

After the experiment, we determined the size of each assayed individual by measuring (±<5%) its displacement volume (ml), following standard protocols described elsewhere (e.g., Wilkinson & Vacelet, 1979; Maldonado et al., 2011). Subsequently, we weighed the sponges wet (g), freeze-dried them at 60°C to constant dry weight (g), and finally combusted them at 540°C for 10 h and weighed the ashes to estimate ash-free dry weight (AFDW). Significant regression equations were obtained to estimate dry weight and AFDW from volume, so that destructive sampling is no longer needed. Hourly uptake rates measured for each sponge individual were then normalized by its volume (ml) and AFDW weight (g). We have given uptake rates preferentially normalized by the volume (ml) of living sponge, as such an approach will allow subsequent inference of field DSi demands following non-destructive measurements of sponge volume through photography (Shortis et al., 2009), rulers (Maldonado et al., 2010a) or any other method avoiding specimen collection. Nevertheless, one of the two available studies to date provided DSi uptake kinetics as AFDW normalized values. Therefore, we have been compelled to implement such an AFDW normalization to make possible a reliable between-species comparison. Because uptake rate data were both normally distributed and homoscedastic, differences in mean uptake rate (integrated over the 36 h period) as a function of DSi treatments (10, 25, 40, and 70 µM) were examined using a one-way ANOVA on untransformed data, followed by sets of a posteriori pairwise Holm-Sidak tests.

Results

Our analyses indicated that DSi concentrations decreased progressively in treatment buckets over time (Fig. 1a), while concentrations were nearly steady in control buckets (Fig. 1b). Such a pattern difference between treatment and control buckets revealed both active DSi consumption by the sponges during the experiment, and minimum DSi adsorption to bucket walls.

Mean (±SD) DSi uptake rate (averaged over the 36 h period) increased with increasing DSi availability in the culturing bucket (Fig. 2), being 0.06 ± 0.02 µmol Si ml^{-1} sponge h^{-1} at the 10 µM DSi concentration, 0.15 ± 0.07 at 25 µM DSi, 0.18 ± 0.04 at 40 µM DSi, and 0.39 ± 0.07 at 70 µM DSi (see also Table 1). A 1-way ANOVA ($df = 19$, $F = 33.9$, $P < 0.001$) and subsequent Holm-Sidak pairwise comparisons revealed that average DSi uptake rate at 70 µM was significantly higher than those at 40 and 25 µM, which in turn were not significantly different from each other but higher than the uptake rate at 10 µM (Fig. 2). Differences in

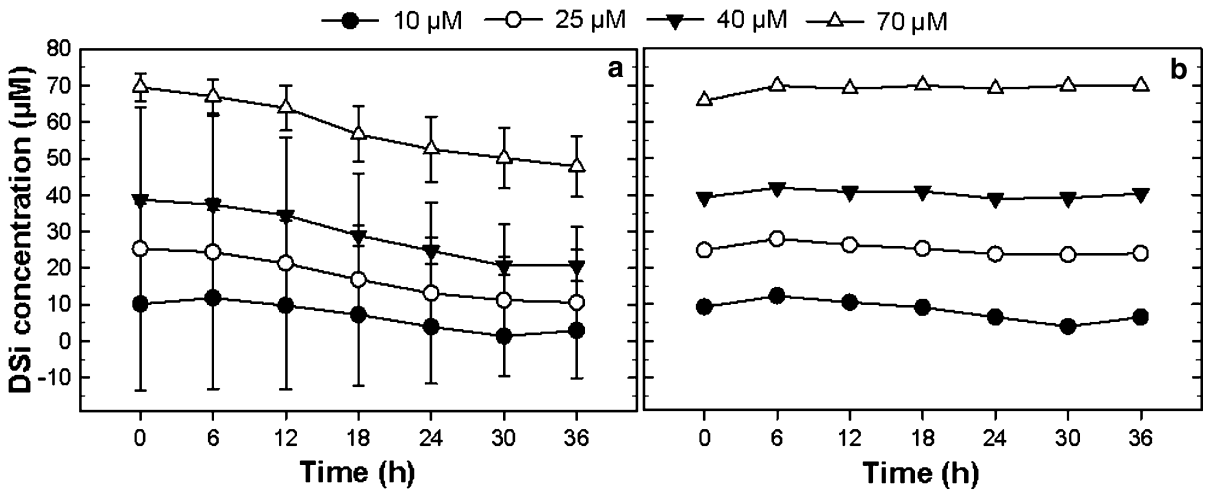

Fig. 1 Variation of mean (±SD) DSi concentrations measured at 6 h intervals within treatment (**a**) and control (**b**) culturing buckets during the 36 h experiment. Note that controls show no substantial DSi decrease over time

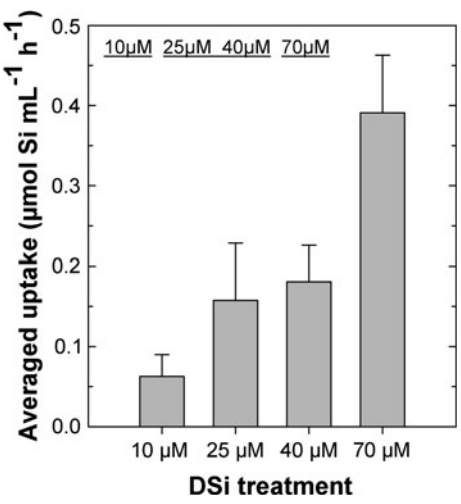

Fig. 2 Average (±SD) uptake rates (integrated over the 36 h period of experiment) in the different DSi concentration treatments. Uptake is given as µmol Si per ml of living sponge tissue and hour. At the upper left corner, DSi treatments (10, 25, 40, and 70 µM) are placed in ascending order according to the magnitude of the mean uptake responses. Treatments *underlined* by a same line are not significantly different from each other according to Holm-Sidak pairwise comparisons ($P > 0.05$) following a significant one-way ANOVA

uptake rate as a function of DSi concentration, caused the sponges to consume different Si amounts over the 36 h experiment, averaging 2.2 ± 0.9 µmol Si per ml of sponge at the 10 µM treatment, 5.6 ± 2.5 at 25 µM, 6.5 ± 1.6 at 40 µM, and 14 ± 2.5 at 70 µM.

When the time course of uptake rates was examined (Fig. 3), it was noticed that the mean rate at all four treatment concentrations varied over time, though more markedly at the higher concentrations (i.e., 40 and 70 µM). Figure 3 also shows that periods of high uptake rates alternated with periods of lower consumption rates, indicating that after a high-consumption pulse, the sponges needed to "metabolize" the taken-up DSi before engaging themselves in a new pulse of intense uptake. Such a pattern also corroborates that DSi is not entering the sponges by passive diffusion but by active transport. The magnitude of error bars (SD) also indicated moderate to large inter-individual variability in uptake rates during the experiment, irrespective of DSi concentration treatment (Fig. 3).

Discussion

To date, information on DSi uptake kinetics was available for only the demosponges *H. panicea* (Reincke & Barthel, 1997) and *Axinella* spp. (Maldonado et al., 2011). Both studies have reported DSi uptake to follow a Michaelis–Menten kinetics with saturation around 100 and 200 µM DSi, respectively. Although our experimental design cannot reveal the concentration at which *H. perlevis* reaches saturation, the data indicate that saturation shall be reached at concentrations higher than 70 µM. Interestingly, while the studies by Reincke & Barthel (1997) and Maldonado et al. (2011) suggested that the uptake rate of the sponges increases progressively with DSi

Table 1 Summary of DSi uptake rates (average ± SD) of *Hymeniacidon perlevis* in the various DSi concentration treatments

[DSi] (μM)	*Hymeniacidon* uptake rate (μmol Si g^{-1} AFDW h^{-1})	*Halichondria* uptake rate (μmol Si g^{-1} AFDW h^{-1})	*Hymeniacidon* uptake rate (μmol Si ml^{-1} h^{-1})	*Axinella* spp. uptake rate (μmol Si ml^{-1} h^{-1})
10	1.16 ± 0.50	3.42	0.06 ± 0.02	0.016
25	2.91 ± 1.33	6.76	0.15 ± 0.07	0.033
40	3.33 ± 0.84	8.94	0.18 ± 0.04	0.047
70	7.25 ± 1.34	11.62	0.39 ± 0.07	0.065

Uptake is expressed as either "μmol Si per AFDW sponge g and h" or "μmol Si per ml living sponge and h". This dual version of units allows comparison with the uptake rates predicted by the Michaelis–Menten models previously proposed for *Halichondria panicea* by Reincke & Barthel (1997) and *Axinella* spp. by Maldonado et al. (2011)

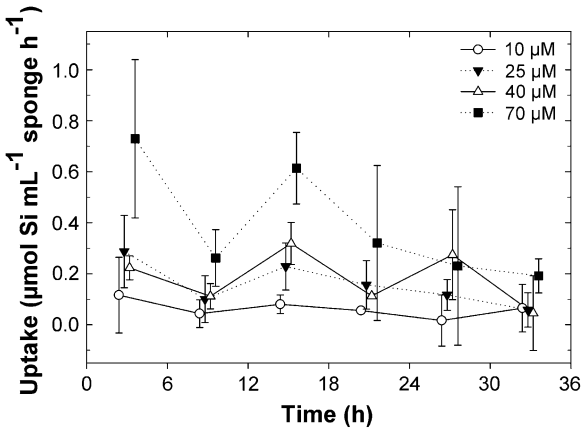

Fig. 3 Mean (±SD) uptake rates measured at 6 h intervals in each of the four DSi treatments (10, 25, 40, and 70 μM). Uptake is given as μmol Si per ml of living sponge tissue and hour

availability until reaching saturation, our data preliminarily suggest that in *H. perlevis* there could be a stepwise process, controlled by threshold concentrations above which the efficiency of the uptake system changes significantly. This suggestion is based on the realization that a DSi increase from 10 to 25 μM induced a significant increase in uptake performance, while an increase of identical magnitude between 25 and 40 μM elicited no significant acceleration in the uptake rate. On the one side, this type of response would be consistent with the idea that only above certain DSi thresholds, new groups of Si-responsive genes become activated and new types of spicule types can be produced (Maldonado et al., 1999; Krasko et al., 2000). Nevertheless, given that our sampling size was relatively low (*N* ranging from 3 to 6), we cannot discard the possibility that the ANOVA analysis lacked the power to detect differences between the 25 and 40 μM DSi treatment.

When mean uptake rates of *H. perlevis* at the different DSi treatments were compared with rates estimated for *H. panicea* (Table 1), values fell within the same range of magnitude, but being those measured in *H. perlevis* about 55% lower on average. The opposite pattern is found when *H. perlevis* uptake rates are compared with those known for some for *Axinella* species (Table 1). Consequently, all three sponge genera investigated to date appear to have uptake systems with different affinity by DSi. The reasons for these differences remain unclear. It is well known that spicule production varies over the year cycle (e.g., Bavestrello et al., 1993) and it could be that these sponges were not at the same stage of their annual growth cycle, therefore having very different DSi demands at the time of the experiments. Differences in DSi affinity could also be related to the fact that these sponges have different spicule (BSi) content (as calculated from dry weight/ash weight ratio) relative to the bulk of their respective soft tissues, accounting for about 34.4% of dry weight in *H. panicea* (e.g., Thomassen & Riisgård, 1995), 46.5 ± 8.6% in *H. peerlevis,* and 54.9 ± 11.5% in *Axinella* spp. (Maldonado et al., 2011). Another potential explanation for between-species differences in uptake rates could also be that some sponges are suspected to combine silica deposition with addition of organic skeletal materials, such as chitin (Ehrlich, 2011), in a way that is not understood yet. The formation of this skeletal composite could affect the speed at which DSi is processed and accounts for between-species uptake rate differences.

One of the most interesting aspects of the study is the fact that DSi uptake rates measured at 6 h interval showed marked shifts over time, alternating high-rate with low-rate periods (Fig. 3). The pattern was more obvious in the sponge individuals exposed to the

higher concentrations (i.e., 40 and 70 μM). Such a short-term alternation in DSi consumption rates rejects the possibility that the DSi concentration decline observed in the culturing buckets overtime could be due to passive diffusion of DSi within the sponge body. Rather, the observed pattern suggests that after a period of elevated uptake, sponges transiently reduce their consumption for some hours, probably to allow the taken-up DSi to be converted into biogenic silica. A more detailed study based on higher replication would be required to reveal patterns of periodicity, if any, and the intensity in uptake pulses. Previous studies on fresh-water sponge have shown that a 200 μm × 6–8 μm spicule can be completed in about 40 h (Weissenfels & Landschoff, 1977), although it remains unclear whether such a silicification process is either uninterruptedly continuous or stepwise. Likewise, it remains unclear how dissolved silicon is internalized by the sponges. Suggestions have been made that a sodium-bicarbonate co-transporting system could somehow be involved in taking up silicic acid from seawater (Schröder et al., 2004), which would rather be consistent with a transport enzymatic kinetic.

Our results reveal that, despite *H. perlevis* being a species evolved to growth at the intertidal zone, its uptake system appears to have been designed to consume daily much higher amounts of Si than are allowed by the natural DSi concentrations in the sublittoral zone. Our current measurements and previous work (Zhao et al., 2004) indicate that natural DSi concentration in the Dalian coastal area of the Yellow Sea range from 3 to 10 μM. South of the Dalian coast, DSi concentrations are known to range from 0.5 to 15 μM over the year, as a result of heavy seasonal rains and important runoffs by Yangtze River (about 1,000 km South from the Dalian study site) being transported North by the Kuroshio warm current (Zhang et al., 2005; 2007). Our seawater analyses ($n = 7$) indicated that DSi concentration in the sponge habitat at the time of the experiments was 10.09 ± 0.14 μM. Surprisingly, we have found that the uptake system of *H. perlevis* performs with significantly higher transport rates when DSi concentrations are at least twice to four times higher than the DSi maximum expected in their natural habitat under the most conservative approach (i.e., 15 μM). Therefore, skeletal growth in the sublittoral populations of *H. perlevis* is chronically limited by DSi availability.

Because in the genus *Halichondria* (as well as in many other halichondrids), the number of spicule types is very limited (often one or two types only), between-species taxonomic discrimination relies much on differences in spicule size. Given that optimal silicification in this species appears to require higher DSi concentration than are available in the coastal system, it cannot be ruled out that average spicule size varies drastically between populations subjected to different DSi regimes over the year, introducing an additional difficulty to correct species identification through the traditional skeletal criteria. Altogether the results of this study are consistent with previous claims of sublittoral sponges being strongly limited by DSi availability (Reincke & Barthel, 1997; Maldonado et al., 1999, 2011). The results also support the view that such a chronic limitation probably arises from the persistence in modern sponges of ancestral uptake systems that evolved in ancient oceans characterized by DSi concentrations being at least an order of magnitude higher than the maxima available in Recent oceans (Maldonado et al., 2011). It also reinforces the notion that the "current" DSi limitation is not a modern ecological process, since it probably started with the ecological expansion of diatoms during the Early Tertiary (Harper & Knoll, 1975; Maldonado et al., 1999; Lazarus et al., 2009). This process has been and it is still operating as an important environmental pressure. It has likely been forcing both the skeletal evolution of sublittoral siliceous sponges toward silica slimming and the spatial distribution of the species with high silicon needs toward high latitudes and/or aphotic zones of Recent oceans characterized by high DSi concentrations (Maldonado, 2009).

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