UNIVERSITAT DE BARCELONA

Silicon consumption by marine sponges: an empirical approach and its ecological implications

María López Acosta

María López Acosta

Silicon consumption by marine sponges: an empirical approach and its ecological implications

Silicon consumption by marine sponges: an empirical approach and its ecological implications

María López Acosta

Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals
Programa de Doctorat en Ecologia, Ciències Ambientals i Fisiologia Vegetal

Silicon consumption by marine sponges: an empirical approach and its ecological implications

Memòria presentada per María López Acosta per optar al grau de doctora per la Universitat de Barcelona

María López Acosta
Centre d'Estudis Avançats de Blanes (CEAB-CSIC)
Barcelona, Juny 2018

Director:
Manuel Maldonado Barahona
Investigador Científico CSIC
Centre d'Estudis Avançats de Blanes

Tutora:
Creu Palacín Cabañas
Professora Titular
Universitat de Barcelona

López-Acosta, M., 2018. Silicon consumption by marine sponges: an empirical approach and its ecological implications. Doctoral thesis. Universitat de Barcelona. 176 pp.

Cover design: Javier García Puig, Marta García Puig and María López Acosta.
Picture: mäerl bed of Lomergat, bay of Brest, France, from Erwan Amice.

A mi familia

Agradecimientos

A pocas horas de imprimir la tesis, no puedo posponerlo más: tengo que escribir los agradecimientos. Y los escribo con una sonrisa dibujada en mi cara, acordándome de todas las personas que me han acompañado durante estos últimos cuatro años y que han hecho posible este trabajo.

Primero quiero agradecerle *al jefe*, Manuel, por toda la confianza que ha depositado en mí y que me ha dado, por la paciencia que ha tenido conmigo, y porque ha sido una auténtica fuente de inspiración. Creo que desde pronto se notó que el "enigma" del silicio y las esponjas me cautivaba, pero no dudo que gran parte del interés vino tras escuchar tus apasionados discursos sobre el tema. Después de estos 4 años tengo la sensación de haber aprendido una barbaridad, no sólo sobre esponjas y silicio, sino también sobre otros muchos aspectos de la biología y de la ciencia. Creo que tus consejos y comentarios me han ayudado a aclarar muchos de mis argumentos científicos (no siempre formulados sencillamente en mi cabeza) y que has alimentado mi razonamiento crítico. Te agradezco la gran aportación que has hecho a mi formación como científica durante todos estos años, y la gran implicación que has mostrado sin yo pedírtelo. La verdad es que durante todos estos años que llevamos trabajando juntos (¡en unos días hará 8 años!), hemos pasado muchas horas debatiendo sobre

múltiples temas, a veces más serios, a veces más distendidos; hemos convivido mucho tiempo juntos, viajando durante semanas o meses por campañas o experimentos, y hemos compartido casi despacho, porque no se puede decir que esa pared que separa nuestros despachos sea una auténtica separación. Y en todo este tiempo, siempre me has considerado como una más. Por todo esto, y por alguna cosa más que seguro que se me olvida mientras escribo estas palabras, te doy las gracias. No dudo que aún nos quedan muchas experiencias por compartir, y espero que alguna incluya *tequila* de celebración.

Quien continúe leyendo esta tesis después de los agradecimientos se dará cuenta que la bahía de Brest es una parte clave de la misma. Y no sólo la bahía y Brest, sino todas las personas que conocí durante los casi 8 meses que estuve allí. Spécialement, je voudrais remercier à Aude, *la chef* (ou chif, ça dépend du jour). Merci, Aude, de ta patience, du temps consacré tous les jours à « mes interminables doutes », de ta confiance et, surtout, de me faire sentir comme si nous nous connaissons depuis toujours dès la première journée. Pour me prêter ton aide pour faire presque toutes les analyses de silicate pendant les expérimentes d'*uptake*. Je crois qu'on a dû analyser à la main des milliers d'échantillons pendant les mois de mon séjour ! Une folie seulement propre de ma folie particulière. Merci de toutes les occasions dans lesquelles tu m'as raccompagnée à la campagne, malgré le froid terrible ou le soleil magnifique (c'est La Bretagne!). Et merci encore de tous les bons moments en parlant "de choses et d'autres" dans ton jardin, à la voiture ou dans les couloirs de l'IUEM, d'un lieu à un autre à toute vitesse.

Ainsi qu'à Aude, je voudrais remercier à Jacques: merci de me montrer où est que je pourrais trouver des éponges dans la baie. Merci aussi de m'avoir accompagnée à la campagne les premiers jours, bien que j'aie eu besoin de toute la journée pour examiner une petite quantité d'éponges. Merci de ton commentaire, avec tout le naturel, parce que las *Tethyas* étaient dehors de l'eau et me raccompagner pour les voir quand je n'arrivais pas à y croire. Et surtout, merci de ta compréhension et ton aide chaque fois que j'arrivais à ton bureau pour te poser une question. De plus, mes remerciements aux plongeurs, Erwan, Isabelle et Thierry. Merci beaucoup parce que sans votre aide, cette thèse aurait eu impossible. Vous m'aviez raccompagnée à échantillonner chaque fois; et, où il semblait que nous avions fini, j'arrivais à nouveau en disant qu'il était nécessaire de faire une

autre plongée. Merci de votre patience. Et merci à tous de vos conseils vers la plongée. J'ai beaucoup appris, aussi des « merveilleuses conditions » de la baie de Brest. De plus, à Erwan et Thierry, vous remerciez particulièrement les photos des éponges qui illustrent et colorent cette thèse. Merci Laurent de sa patience en résolvant mes doutes sur les chambres d'incubation. Enfin et surtout, je souhaiterai montrer ma gratitude à Valérie, pas seulement pour son implication dans mon travail, aussi pour être une super compagnie tant au laboratoire qu'à l'extérieur. Mes derniers mois à Brest ils ont eu vraiment très gais après t'avoir connue!

À la fin, je ne peux pas oublier de vous remercier à Vincent, Marion, Emilie, Morgane, Briva, Jill, Aurélie, Marc L., Jacques C., Nathalie, Ewan, Aurélien, Natasha, Khaled, Romain, etc, de l'aide que vous m'aviez offert à l'IUEM et du temps que nous avons partagé quand je trouverais un peu de temps libre malgré les expérimentes. Y por último, mi más sincero agradecimiento a mis *bretones* favoritos del IUEM: Aridane y Jorge. Menuda paciencia tuvisteis escuchando mis divagaciones sobre las esponjas y los experimentos. Y cuando ya no me salían las palabras en inglés (ni obviamente en francés), siempre estabais ahí para escucharme y darme ánimos para el siguiente paso que me tocaba dar. A los dos, y a Pedro, gracias por esos domingos de mercado que tanto disfrutamos entre *huîtres et poulet noir*.

En Brest pasé 8 meses de una intensidad inimaginable, pero la gente que más me ha acompañado en este camino está "en casa". Entre esas personas, no me puedo olvidar de todos los *ceabinos* (los que fueron y los que aún son). De una manera u otra, todos formáis parte de esta tesis, ya sea por el tiempo compartido currando en los laboratorios o despachos, o por los ratos geniales que hemos compartido en la terraza a la hora de comer. Gracias a todos por las risas, las charlas, y la complicidad. Y sobre todo, gracias a mis *chicas* por la infinita paciencia y todos los buenos ratos (muchos) que hemos pasado en estos años. Gracias Cèlia, porque llevamos juntas batallando casi 8 años y siempre has estado ahí para lo que he necesitado, y no sólo de curro, sino de lo que hiciese falta: desde unas sandalias divinísimas hasta escuchar mis parrafadas vitales. Gracias también a la *newcomer*, Cris, que "acaba de llegar" pero parece que ha venido para quedarse (al menos en el despacho!). Gracias por escucharme durante estos últimos meses mis *peroratas* científicas, mostrar paciencia y complicidad. Y

gracias a mi super Marta, porque has hecho durante todos estos años del despacho "un lugar feliz", y las campañas "bajo mínimos" de Chafarinas una auténtica fiesta (a veces, literal!). Gracias a todas por haber hecho del *zulo* del despacho un lugar mejor, por ser vosotras mismas cada día y convertir "al grupo" en una pequeña familia. Vuestra complicidad y amistad traspasa las paredes del CEAB y estoy segura que cuando la vida nos haga tomar caminos alejados de aquí, nos reencontremos como si nada hubiera cambiado (excepto en lo de mirar espículas al microscopio, que seguro que no lo echaremos de menos!).

También me gustaría darle las gracias a Jana. ¿Dónde quedan aquellas interminables horas compartiendo despacho en "la comuna" las dos analizando fotos en el ordenador? Ahora ya no es tan fácil vernos, pero tus visitas fugaces aportan un chorro de energía y alegría de la que es difícil no contagiarse (i una charleta ràpida per posar-nos al dia que m'encanta!). Y también, gracias a mis dos FPIs: Rudi y Vicente. A los tres nos unió "la beca", pero desde que os conocí tuve claro que no iba a ser sólo una cuestión de coincidencia lo nuestro. Me alegra profundamente que nos hayamos cruzado "por casualidad", y me encanta poder llevaros en el recuerdo como compañeros "de promoción". Gracias por todas las charlas, de tesis y de la vida; por escucharme y aconsejarme. Creo que me va a ser imposible no acordarme de los dos cada 18 de mayo! Va a ser oficialmente nuestro día.

Gracias a todos los que han pasado por el equipo (Beril, Marie Claire, Isabel, Blanca, David, Fatu, Andrea, Vicky, Pol…) y a todos los *ceabinos* (Aitana, Carla, Carlos, Chiara, Clara, Danilo, Edu, Francesco, Gerard, Guillem, Héctor, Ibor, Joan, Joao, Jordis, Jorge, Kathrin, Leyre, Luis Fran, Mª Elena, María, Martas, Mateu, Miguel, Miquel, Mirco, Nuria, Roger, Sandra, Sara, Steffi, Vanessa, Xavis, Yaiza, y los que seguro que me estaré olvidando) que hicieron de este pueblo al sur de la Costa Brava un lugar mejor.

I would also like to thank all the workmates of the SponGES grant. In particular, I am deeply grateful to Martijn, Clea, Ulri, Furu, Chris, Joana, Barry, and Lindsay, for the great experience on board, and to Barry, Gabrielle, Sarah, Lindsay, and Ellen, for their help during our stay in the DFO facilities.

Por supuesto, no me olvido de los masterandos (of the universe!). Gracias a Aurora, Clara, Eneko, Gema, Julia, Manu, Stefano, Marta, Oscar, y Yolanda, por aportar ese punto de "lucidez" tan necesario durante todos estos años. Cada reencuentro es más épico que el anterior, y aunque no nos veamos en meses (o años), cada vez que nos juntamos es como si lleváramos viviendo juntos toda la vida. Sois la familia barcelonina, un vínculo que no dudo que será ya imposible de romper. También me gustaría agradecer a mi otra familia catalana, la calellense, por todos los buenos momentos que me han hecho pasar, convirtiendo Calella city en mi hogar. Gracias a todos (Michael, Iván, José, Ferri, Miki, Marcos C., Daniela…), y especialmente a Marcos J., por las charlas de todo un poco, y por la complicidad bióloga. También a Ana (y Miguel), gracias por todos los buenos ratitos que hemos conseguidos sacar para un concierto o una cenita.

También, me gustaría dar las gracias a todos mis *madrileños*. Muchos migrasteis hace años como yo, pero uno de mis momentos favoritos del año es (y quien lo iba a decir) volver a casa en Navidad y reencontrarme con todos vosotros: los biólogos, los ramireños, y los del Estu. Especialmente, me gustaría dar las gracias a Talía y Julia, por la paciencia infinita que tienen; porque pese a la distancia (cada una en una punta!) y el poco tiempo que podemos coincidir, nada cambia. Y aunque sean unos pocos días al año, reencontrarnos en Madrid (o donde sea) es asegurar un auténtico bienestar (más aún si es con una cervecita en la mano!). También, gracias a Nat, por su paciencia con mi vida loca, porque cada rato que sacamos para charlar por el móvil o tomarnos algo en Navidad representa que no haya pasado ni un día que me marché. También, gracias a Edu, porque sus audios por whatsapp, sin pretenderlo, me han resultado de lo más inspirador en estos últimos años. Y sobre los 20 años anteriores, creo que nada puedo decirte que no te haya dicho ya! Y también, gracias a todos los biólogos con los que empecé esta aventura en la Complu, y que ha acabado con que haya hecho una tesis! Especialmente, a mis dos biólogas favoritas, a Amaia y Bárbara.

No me olvido de mi familia (la de López y la de Acosta). Gracias por todos los buenos ratos que me habéis hecho pasar en Madrid, Barcelona o Galicia; un cumpleaños, una reunión navideña, o simplemente unas tapitas porque coincidíamos en Madrid. Y, especialmente, querría agradecerles a mis padres una infinidad de cosas. Entre ellas, gracias por inculcarme desde

pequeña un espíritu crítico para conmigo, pero no competitivo con los que me rodean. Gracias por haber tratado con total naturalidad, desde el primer día que lo dije (cuando era muy pequeña) que quería ser bióloga (siendo vosotros físicos!). Gracias porque, aunque nos pasemos días o semanas sin hablar, en un segundo nos llamamos y seguimos como si aún viviéramos juntos. Gracias por todos los veranos en Galicia: aquel tiempo entre las charcas que dejaba la marea despertaron gran parte de la curiosidad bióloga que hoy tengo. El resto sólo fue dejar que todo lo que me habías inculcado surgiera su efecto. Gracias y mil gracias. Por todo. Gracias.

Y por último, quiero dar las gracias a Marcos, *mi* compañero de viaje. No dudo que, "fuera del mundo científico", eres la persona que más sabe de esponjas y silicio del mundo! Primero, gracias por ayudarme, literalmente, en parte del curro que hice en Brest (por los pasillos a oscuras a las tantísimas llevando esponjas de un lab a otro). Y además, gracias por la eterna paciencia que tienes en todo, por aguantar mi locura cada día con total naturalidad, y por ocuparte de todo porque sí. Gracias por escuchar mis *discursos* de cinco minutos cuando llego a casa cada día sobre mi vida loca de bióloga y gracias porque, sólo estar contigo, me hace volver al mundo real. Esta tesis tiene mucho de ti. Y, sin ninguna duda, no habría resultado la misma sin tu presencia "por las cercanías".

Muchas de las cosas que he escrito hoy aquí creo haberlas dicho personalmente; si no es el caso, debería. Aquí queda escrito mi más sincero agradecimiento a todos los que me habéis acompañado durante esta tesis y a los que habéis hecho posible este trabajo.

A todos, de corazón,

Gracias.

Junio 2018

Financial support

I have had the financial support of a 4-year FPI fellowship of the Spanish Ministry of Economy and Competitiveness (BES-BES-2013-062931) and two travel fellowships also financed by the Spanish Ministry of Economy and Competitiveness (EEBB-I-15-09507 and EEBB-I-16-11550). The research conducted on this thesis has been supported by two projects financed by the Spanish Government (MEC–CTM2012-37787 and MINECO-CTM2015-67221-R) awarded to Dr. Manuel Maldonado. In addition, this research has partially been supported by the EU's project SponGES (H2020-No. 679849), also awarded to Dr. Manuel Maldonado.

Director's report

Dr. Manuel Maldonado, CSIC research scientist at the Center for Advanced Studies of Blanes (CEAB), as supervisor of the PhD thesis entitled '**Silicon consumption by marine sponges: an empirical approach and its ecological implications**',

INFORMS that the research developed by María López Acosta for her Doctoral Thesis has been organized in 7 sections, which include a General Introduction and General Discussion with Conclusions, in addition to five chapters summarizing the empirical investigations. Each of these five chapters univocally corresponds to a scientific manuscript aiming publication in international peer-reviewed journals, as it is listed below in the annex to this letter. Two chapters are already published, one is under review, and two others are currently being prepared as manuscripts for submission in the next two months.

I also CERTIFY that, although the developed research was framed in two multidisciplinary and multinational research grants involving transnational collaborative research, María López Acosta has leaded most of the empirical research presented in this Ph.D. Thesis. In particular, she has leaded field work and most laboratory experiments, sample processing, nutrient analyses, and statistical data analyses. During the elaboration of the derived manuscripts, she has also leaded and/or made outstanding contributions to data analyses, interpretation of results, paper design and writing.

Finally, I certify that research work here presented has never been used as contribution to or part of any other national or international Doctoral Thesis.

Blanes, 22 June 2018

Dr. Manuel Maldonado

List of manuscripts of this thesis, with indication of journal impact factor (IF), quartile (Q), and publication stage:

CHAPTER 1: López-Acosta, M.[1], A. Leynaert[2], and M. Maldonado[1] (2016). Silicon consumption in two shallow-water sponges with contrasting biological features. *Limnology and Oceanography* **61** (6) 2139-2150. IF (2016) = 3.383, Q = Q1/Q1. Stage: published.

CHAPTER 2: López-Acosta, M.[1], A. Leynaert[2], J. Grall[3], and M. Maldonado[1] (2018). Silicon consumption kinetics by marine sponges: an assessment of their role at the ecosystem level. *Limnology and Oceanography*. IF (2016) = 3.383, Q = Q1/Q1. Stage: in press.

CHAPTER 3: López-Acosta, M.[1], A. Leynaert[2], V. Coquille[2], and M. Maldonado[1]. Silicon utilization by sponges: an assessment of seasonal changes. *Marine Ecology Progress Series*. IF (2016) = 2.292, Q = Q2/Q1/Q2. Stage: under review.

CHAPTER 4: López-Acosta, M.[1], A. Leynaert[2], L. Chavaud[2], and M. Maldonado[1]. *In situ* determination of Si consumption rates by marine sponges: a benthic-chamber approach. To be submitted to *PLoS ONE*. IF (2016) = 2.806, Q = Q1. Stage: in preparation.

CHAPTER 5: Maldonado, M.[1], M. López-Acosta[1], L. Beazly[4], and E. Kenchington[4]. First kinetic model of silicate consumption by a hexactinellid sponge. To be submitted to *Scientific Report*. IF (2016) = 4.259, Q = Q1. Stage: in preparation.

Authors affiliations:

[1] Department of Marine Ecology, Centro de Estudios Avanzados de Blanes (CEAB-CSIC), Blanes, Girona, Spain

[2] Laboratoire des Sciences de l'Environnement Marin, UMR CNRS 6539, Institut Universitaire Européen de la Mer, Technopôle Brest-Iroise, Plouzané, France

[3] Observatoire Marin, UMS 3113, Institut Universitaire Européen de la Mer, Technopôle Brest-Iroise, Plouzané, France

[4] Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada

Summary

Silicon (Si), in its dissolved form of silicic acid (DSi), is a key nutrient in the ocean. The availability of such nutrient in marine ecosystems is regulated through its use by silica-secreting organisms, a variety of groups including protists, algae, and animals that consume DSi to build their silica (BSi) skeletons. The interest in determining how Si cycles in the ocean is high, since it interacts with the cycling of other major nutrients and ocean primary productivity. Because diatoms are the most abundant Si users, the scientific attempts to quantify the Si utilization in the ocean have been only focused on these organisms, considering negligible the role of other Si users. Over the last decades, some studies have suggested that at least another group, the siliceous sponges, are also playing a non-negligible role in the consumption of Si in marine ecosystems.

Marine sponges are conspicuous animals in benthic ecosystems. They are common across the world ocean, irrespective of the latitude and depth, being able to form enormous aggregations that may extend over large areas. Both their ubiquity and their abundance make sponges good candidates to develop relevant functional roles in marine ecosystems. Regarding the use of Si, it is surprising that, despite about 80% of the sponge species require from DSi to elaborate their skeleton, almost no information is available about how sponges consume such nutrient. In fact, before the beginning of this PhD, only few studies had investigated DSi consumption in marine sponges, with kinetic models available for only four species in two genera of

demosponges. This lack of knowledge sparked this PhD, which has been developed in the frame of three research grants: 1) "A research action to quantify fluxes and sinks of silicon through sponges: a neglected circuit within the marine cycle" (MEC–CTM2012-37787); 2) "Exploring the biological production of silica and its applications in science and technology" (MINECO-CTM2015-67221-R); and 3) WP4 —Ecosystem functions, services, and goods— in the EU's project SponGES (H2020-No. 679849). The main objective of the work was to improve the general understanding on how sponges utilize DSi, to facilitate further assessment of the quantitative role of the sponges as Si users.

Here we investigated the kinetics of DSi consumption in five sponge species: four temperate, shallow-water demosponges and, for the first time, a cold, deep-water hexactinellid sponge. We also examined the sources of between-species and between-individual variability in DSi consumption responses. Interestingly, we detected that DSi consumption kinetics can change seasonally in some species, what may have important implications when quantifying the role of sponges as Si users. Additionally, we determined for the first time the rate of DSi utilization by a sponge species *in situ*. The results significantly matched those estimated from the kinetic models obtained in the laboratory, supporting the use of long (>24h) incubations in laboratory to investigate DSi consumption kinetics in sponges, in contrast to the very short periods traditionally recommended for diatoms (< 3h). Finally, we used the empirically information gained on DSi consumption over the development of this PhD to estimate the utilization of DSi by a sponge assemblage at the ecosystem level, using as case study the bay of Brest (France).

In summary, this research showed that sponges have a noticeable role as Si users, even in a shallow-water ecosystem (the bay of Brest) where diatoms largely contribute to the phytoplankton biomass. Our results also indicated that sponges increase their role in marine ecosystems with increasing availability of DSi in seawater. Thus, sponges are predicted to play a relevant role as Si users in high-latitude and deep-water habitats, characterized by high DSi availability. All together, the siliceous sponges should be considered as Si users if we aim to accurately quantify the cycling of Si in marine ecosystems.

CONTENTS

General Introduction and Objectives

Picture: Individuals of *Cliona celata* in the rocky subtidal habitat of La Cormorandière (bay of Brest, France), from Erwan Amice.

Silicon

Silicon (Si) is the second most abundant element in Earth's crust after oxygen (O). These two elements form together the silicon dioxide (SiO_2), also known as silica, which is part of about 90% of the rocks in Earth (Wollast and Mackenzie, 1983). Humans have used this element since Paleolithic, when they created the first tools (flints) by sharping siliceous rocks. At the Bronze Age (ca. 3,000 – 1,000 BC), ancient civilizations learned how to turn sand, which is made of tiny pieces of siliceous rocks, into glass. Nowadays, Si is widely used with technological applications, particularly in medicine and material sciences (e.g., Menna et al., 1995; Schröder et al., 2007; Gordon et al., 2009; Lührs and Geurtsen, 2009; Ehrlich et al., 2016; Kumeria et al., 2017).

There is a big interest in studying Si, not only for its applications to human uses, but also for relevant biogeochemical and ecological implications to Earth's ecosystems. As a result of complex interactions among biological, chemical, and geological processes, Si is transported across the land-ocean continuum. While cycling, Si interacts with other globally important biogeochemical cycles, like that of carbon (C). For instance, by weathering (i.e., dissolution) of silicate rocks, silicic acid (DSi) is generated and CO_2 consumed. This process has a significant impact as atmospheric CO_2 sink at the geological scale (thousands of millions of years), largely influencing the global climate at such long time-scale (Berner,

1990; Struyf et al., 2009). At shorter time scales, Si has a major impact on marine primary productivity. The presence of DSi in the photic ocean favors the growth of diatoms, which are responsible of about 40% of the marine carbon sequestration (Field et al., 1998; Tréguer et al., 2018). In summary, for its applicability and biogeochemical repercussions, Si has attracted the interest of humans since the beginning of civilization.

Marine Si cycle

In the ocean, Si is a key element. Its dissolved form, the DSi, is the only form biologically assimilable by organisms. A variety of marine groups requires DSi to elaborate their siliceous skeletons made of biogenic silica (BSi); among these groups are diatoms, silicoflagellates, some species of choanoflagellates, testate amoebae, and chrysophytas, and most species of sponges and radiolarians. Since the 1960s, it is well-known that the biological activity of Si users largely controls DSi availability in seawater and the exportation of particulate BSi (i.e., the skeletons of silica-secreting organisms) to the deep-ocean. Harriss (1966) literally proposed that "primarily diatoms, silicoflagellates, radiolarian, and siliceous sponges are responsible for controlling the concentration of oceanic silica", and Calvert (1968) reported that "the deposition of empty frustules, tests and spicules [i.e., the siliceous skeletons of diatoms, radiolarians, and sponges, respectively] in bottom sediments would constitute an effective extraction mechanism for oceanic silica". Because diatoms are the most abundant Si consumers, the attempts to quantify the biological processes controlling marine Si cycle have traditionally been focused on these organisms, presuming as negligible the contribution by the other groups (Burton and Liss, 1968; DeMaster, 1981; Nelson et al., 1995; Tréguer et al., 1995; Ragueneau et al., 2000). At the end of the twentieth century, only the processes mediated through diatoms were considered relevant to the quantification of the marine Si cycle. These unicellular photoautotrophs consume DSi from the upper ocean and convert it into BSi to build the box-like valves (frustules) that protect their cell. After diatom's life, which lasts from hours to few days, BSi frustules sink in the ocean. While sinking, most of the BSi frustule gets dissolved, being again available as DSi to be consumed by Si users. The fragments of frustules that resist dissolution

reach the sea bottom, where dissolution still continues during early burial. It has been estimated that only about 8% of the BSi produced in the photic ocean by diatoms is definitively buried within the marine sediments, where extremely long mechanisms convert the buried BSi into siliceous rocks through different geological processes (Tréguer et al., 1995; Tréguer and De La Rocha, 2013).

Over the last two decades, the idea that at least another group of Si users, the sponges, is also playing a noticeable role in the marine Si cycle has gained importance (Frøhlich and Barthel, 1997; Maldonado et al., 2005; Maldonado et al., 2010; Maldonado et al., 2011; Maldonado et al., 2012b; Tréguer and De La Rocha, 2013). Yet, very little is known about the use of Si by marine sponges.

Sponges

The phylum Porifera (ca. 9,000 species), commonly alluded as sponges, is divided in 4 Classes (Van Soest et al., 2012). The Class Demospongiae is the largest and more diverse one, comprising nearly 84% of the known sponge species. The other species are distributed in the Class Calcarea (8%), Hexactinellida (7%), and Homoscleromorpha (1%). Most of the species of the Classes Demospongiae and Homoscleromorpha and all the species contained in the Class Hexactinellida use DSi to elaborate their skeletons made of BSi. Altogether, more than 80% of the known sponge species have siliceous skeletons.

Sponges are part of the marine benthic fauna since Early Cambrian, with probable origins in late Precambrian (Li et al., 1998; Love et al., 2009; Sperling et al., 2010), being the oldest extant phylum of metazoans still on Earth (Wörheide et al., 2012; Cavalier-Smith, 2017). They are sessile filter feeders that can occur in an enormous variety of marine habitats, from the intertidal to the deep sea throughout all world latitudes. They are usually patchily distributed at sea bottom, being able to form dense aggregations that may extend over large areas (Maldonado et al., 2017; Fig. 1).

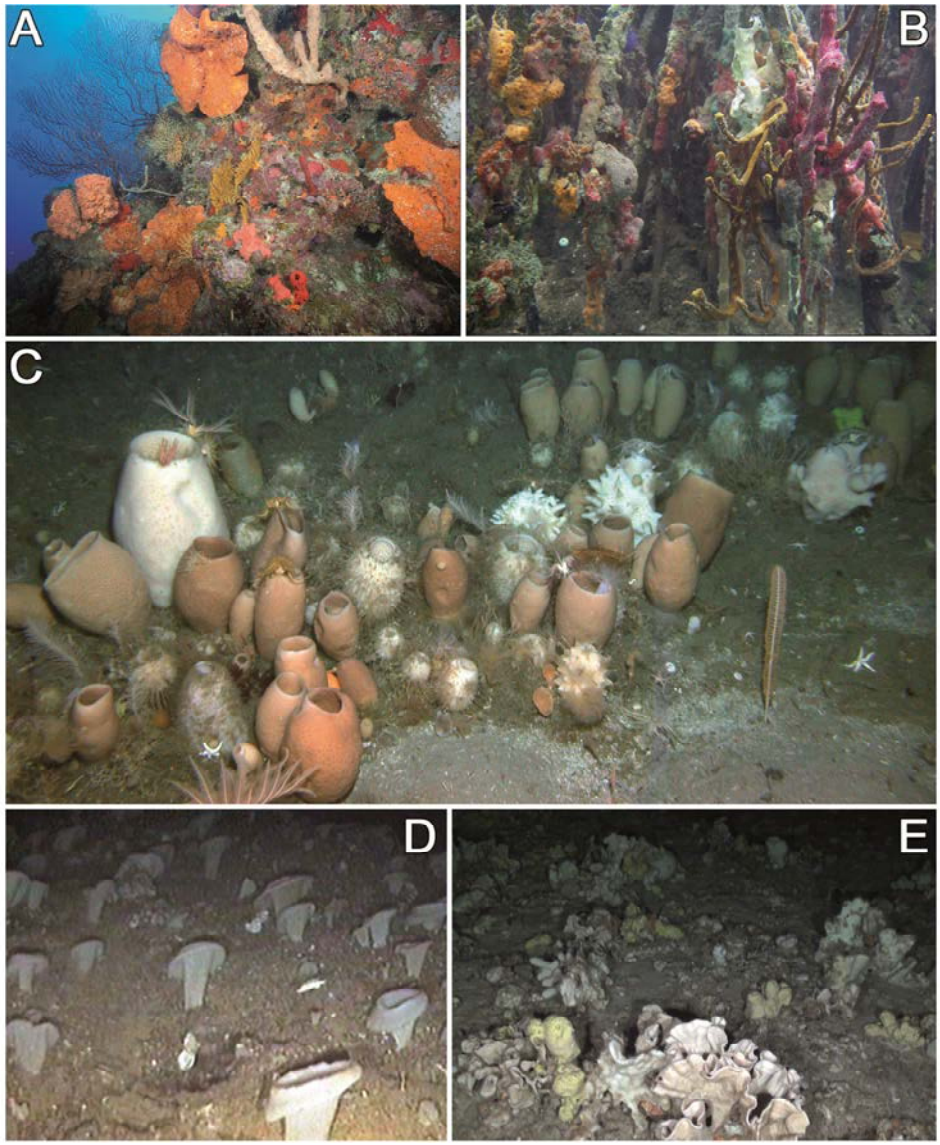


Figure 1. **Views of sponge aggregations.** (**A**) General view of a coral reef at Carrie Bow Cay (Belize), dominated by large, orange individuals of *Agelas* spp. together with a variety of smaller sponges. (**B**) General view of the very diverse sponge assemblage on some mangrove roots (Pelican Pond, Belize). (**C**) A benthic assemblage in the Southeastern Weddell Sea (Antarctica) from approximately 233 m depth, clearly dominated by sponges. (**D**) View of a field of the hexactinellid *Asconema setubalense* on bathyal seamounts around the Canary Islands (North Atlantic). (**E**) View of a sponge aggregation dominated by lithistids ("rock sponges") at the Gorringe Bank (Northeastern Atlantic, Portugal). Modified from Maldonado and co-authors (2017).

Because of their ubiquity and abundance, sponges are emerging as organisms with important functional roles in many marine ecosystems (Diaz and Rützler, 2001; Bell, 2008; Maldonado et al., 2012b; Maldonado et al., 2017). Nevertheless, the role these organisms are performing in the marine Si cycle is poorly understood, with very few empirical studies investigating the impact of sponges in the cycling of Si (Frøhlich and Barthel, 1997; Reincke and Barthel, 1997; Maldonado et al., 2005; Maldonado et al., 2010; Chu et al., 2011; Maldonado et al., 2011; Maldonado et al., 2012a). By extrapolating all available data, two review studies tried to assess the global role of sponges in the marine Si cycle, emphasizing the missing gaps that urgently need to be investigated (Maldonado et al., 2012b; Tréguer and De La Rocha, 2013). At the time this thesis started (early 2014), it appeared clear that the major parameters governing the use of Si by sponges significantly differ from those in diatoms. Nonetheless, the information regarding DSi consumption rates were available only for 2 shallow-water genera of demosponges, *Halichondria panicea* (Frøhlich and Barthel, 1997; Reincke and Barthel, 1997) and *Axinella* spp. (Maldonado et al., 2011), with the general features characterizing sponge DSi consumption largely uninvestigated. Only the investigation of the main processes involved in DSi utilization by sponges, still extensively unknown, would provide relevant information to determine the role these organisms are playing at the biogeochemical cycle of Si.

Objectives

The initial general objective behind this PhD work was to improve the general understanding on how sponges utilize DSi. An attempt has been made to characterize quantitatively the main parameters affecting and/or controlling DSi utilization by sponges. The final goal was to establish some of the basic knowledge —which was lacking before this PhD work— necessary to include the role of the sponges in future quantifications of the marine Si cycle.

The specific objectives of this thesis were:

1. **To determine DSi consumption kinetics in a variety of sponge species of ecological relevance (Chapter 1, 2, and 5)**
   We aim to provide quantitative descriptions of how sponges consume DSi, as well as identify the equations governing the process, by investigating DSi utilization in 5 sponge species: the temperate, shallow-water demosponges *Hymeniacidon perlevis*, *Tethya citrina* (Chapter 1), *Haliclona simulans*, and *Suberites ficus* (Chapter 2), and the cold, deep-water hexactinellid *Vazella pourtalesii* (Chapter 5). These five species belong to five different phylogenetic lineages.

2. **To evaluate the effects of different methodological approaches to investigate DSi consumption kinetics in sponges (Chapter 2)**
   The subject of this PhD is so little investigated that there is not even a standardized methodology for approaching quantitatively the kinetics of DSi consumption by sponges. Before the work of this thesis, DSi consumption kinetics had been investigated only twice by using methodological approaches that differed notably in the duration of incubations (7h in Reincke and Barthel, 1997 versus 48h in Maldonado et al., 2011). Because those studies resulted in kinetics significantly different, we hypothesized that the duration of the incubation might have an effect on DSi consumption rates and empirically tested such a hypothesis.

3. **To characterize the sources of variability in DSi consumption in sponges (Chapter 1, 2, 3, and 5)**
   In the scarce work available before this PhD, large between-individual and between-species differences were detected in DSi consumption rates (Frøhlich and Barthel, 1997; Reincke and Barthel, 1997; Maldonado et al., 2011). Herein we aim to determine the main sources responsible for between-species and between-individual variability in sponge DSi consumption. Also we explored the effect that seasonal fluctuations characterizing the sponge habitats may have on the patterns of DSi consumption.

4. **To measure sponge DSi consumption rates *in situ* (Chapter 4)**
   It has been discussed whether the manipulation of sponges in laboratory conditions can cause artefactual responses. In recent years, several studies attempted to determine DSi utilization by sponges in their natural habitats by measuring *in situ* the changes in DSi concentration between the ambient seawater and the seawater coming out of the sponges, but the approach was unsuccessful because the concentration changes were mostly undetectable (Perea-Blázquez et al., 2012; Morganti et al., 2017; Leys et al., 2018). Herein we have designed an alternative approach to determine sponge DSi consumption in the field, conducting *in situ* incubations that also provide a test for comparison between "laboratory" versus "field" DSi determinations.

5. **To quantify DSi utilization by sponges at the ecosystem level (Chapter 2)**
   Before the initiation of this thesis work, the information on DSi field demands by sponge populations was scarce, with estimates for only five local ecosystems (Maldonado et al., 2012). This lack of information made complicated any accurate extrapolation to the global scale. By combining the various kinetic models obtained for DSi consumption with field quantifications of sponge biomass, we have herein attempted to estimate the annual DSi utilization by a sponge assemblage at the ecosystem level, using as case study the bay of Brest (France), a shallow-water, nutrient-rich ecosystem located in temperate latitudes.

Chapter 1

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

Picture: An specimen of *Hymeniacidon perlevis* in the mäerl bed of Lomergat (bay of Brest, France), from Thierry Le Bec.

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).

Introduction

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; Maldonado et al., 2010; Maldonado et al., 2011; Maldonado et al., 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., 2017). Approximately, about 70-75% of the 8,700 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 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 (Amo and Brzezinski, 1999), 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*, *A. polypoides* and *A. 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., 2012; 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} 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 μmol Si h^{-1} g^{-1} AFDW μM^{-1}) ten times higher than that of *Axinella* spp. (V_{max}/K_m = 0.04 μmol Si h^{-1} g^{-1} AFDW μM^{-1}). 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., 2012a). 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)/dry weight (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 ± 10.58%) 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 to 15 m depth in the Bay of Brest (France) one to two weeks prior to the onset of the experiments. They were transported in seawater to the laboratory and acclimated progressively to stagnant (non-running) seawater conditions in 30L polyethylene tanks filled with seawater that was replaced every 3 days.

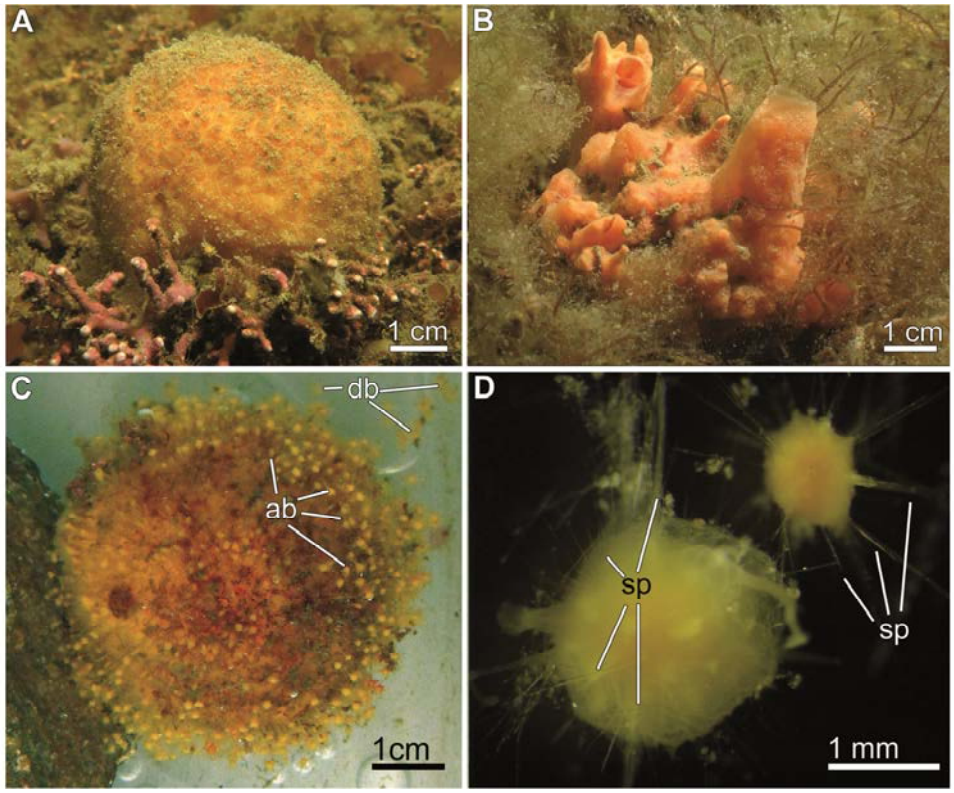


Figure 1. (**A-B**) *In situ* views of *Tethya citrina* (**A**) and *Hymeniacidon perlevis* (**B**) growing at the Bay of Brest. (**C**) View of a *T. citrina* individual producing buds in the laboratory. Most of the buds (ab) still remain attached to the body, but some have already been detached (db)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

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 to 18.2ºC. The DSi concentration was progressively increased in the experimental containers, from natural values (approx. 2 μM) until 250 to 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 to convert into silica spicules at least a good deal of the DSi taken up before they were 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, multi-compartmented 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 0.1M sodium metasilicate ($Na_2SiO_3 \cdot 5H_2O$; pH= 10) stock solution to a 100L-tank filled with filtered water and mixing with an electric pump for 18 hours to ensure complete diffusion before delivery to the experimental containers. After every 72h 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 3μm 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; Reiswig, 1971b) 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 72h step of the DSi treatment.

The sponges withstood satisfactorily the environmental conditions during each of the 72h 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 to 4 days 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 72h period and after correcting for a control treatment (see "Particular experimental setups"). Upon 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 (WW, g), dried at 60ºC to constant dry weight (DW, g), and finally combusted at 540ºC for 10 hours for ash weight (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 ash-free dried weight (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 end 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 72h periods, to the following DSi concentrations: 1.82 μM (field values), 5, 10, 20, 35, 50, 75, 100, 150, 200, and 250 μM.

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 days 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 energy-consuming and silica-using 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

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 μM. Because DSi in the natural habitat of the assayed species ranges from 0.1 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

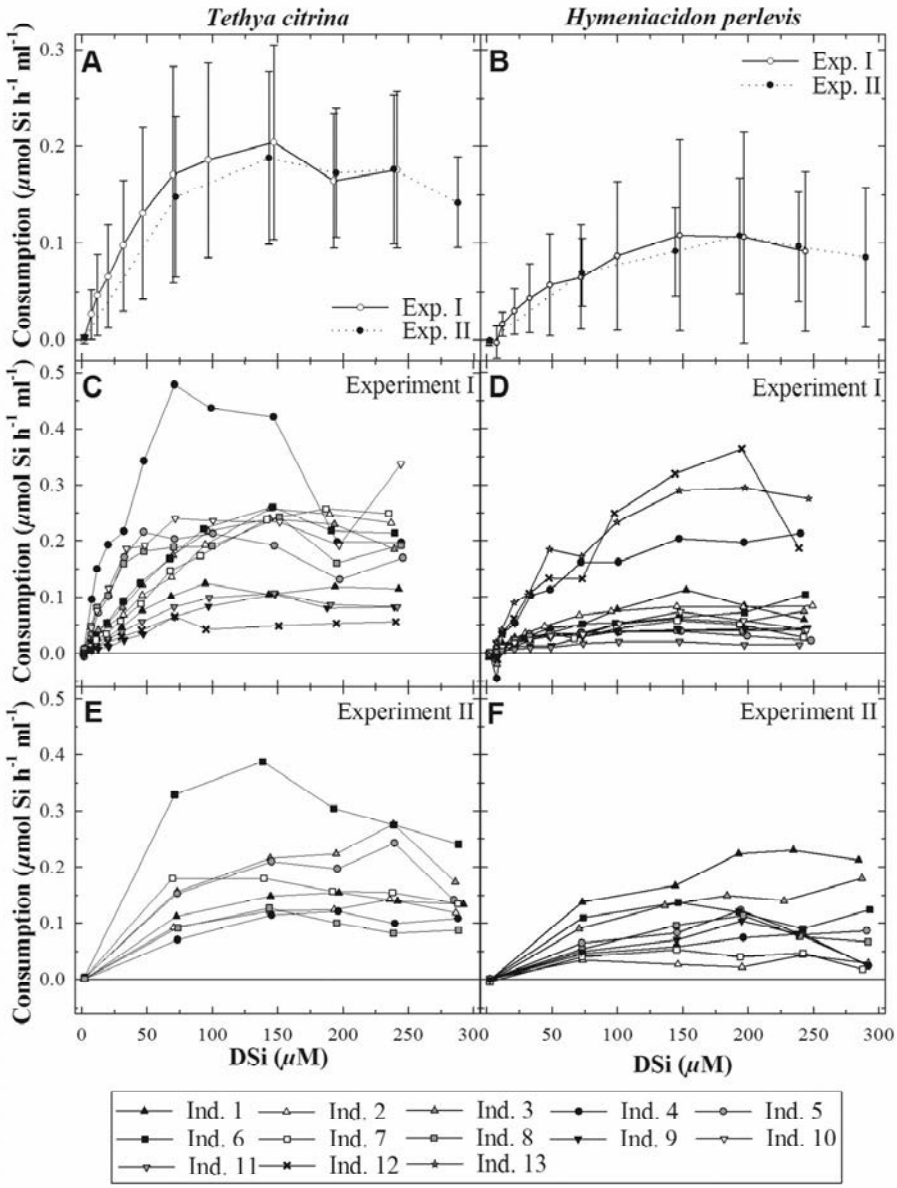


Figure 2. (**A-B**) Average (±SD) consumption rates (μmol Si h^{-1} per sponge mL) of *Tethya citrina* and *Hymeniacidon perlevis* as a function of silicic acid (DSi) concentration during experiments I and II. (**C-D**) Consumption rates of each individual of *T. citrina* (**C**) and *H. perlevis* (**D**)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

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 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 $p >> 0.05$; 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 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* (0.206 μmol-Si h^{-1} sponge-mL^{-1}) is about twice higher than that of *H perlevis* (0.127 μmol-Si h^{-1} sponge-mL^{-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 μmol-Si h^{-1} sponge-mL^{-1} concentration-μM^{-1}) in *T. citrina* than in *H. perlevis* (0.0021 μmol-Si h^{-1} sponge-mL^{-1} concentration-μM^{-1}).

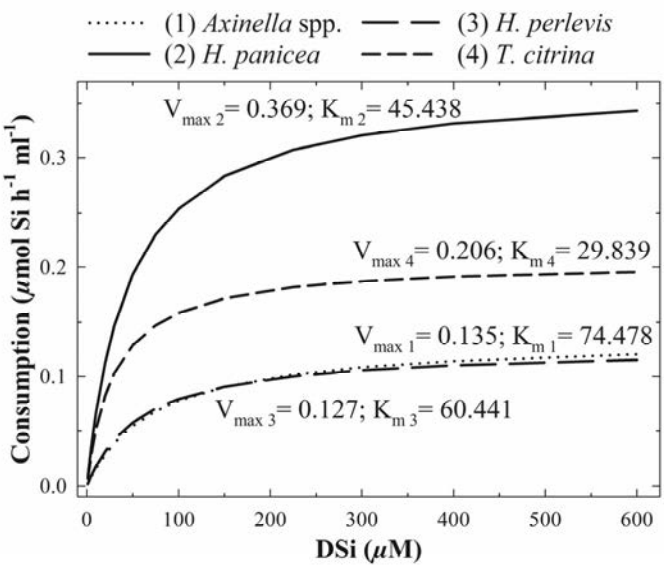


Figure 3. Comparison of the Michaellis-Menten kinetics (V= V_{max} x [DSi] / (K_m+[DSi])) fitted for silicic acid (DSi) consumption in the four demosponges investigated to date: *Axinella* spp. (Maldonado et al., 2011), *Halichondria panicea* (Reincke and Barthel, 1997), and *Hymeniacidon perlevis* and *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.

Between-individual variability

The volume of the assayed individuals ranged almost similarly in both species, from 1.8 to 24 mL in *T. citrina* and from 2 to 30 mL in *H. perlevis*. 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 the DSi consumption. Budding individuals consumed over experiment I an average of 60.83 ± 29.91 µmol DSi per sponge mL, 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.

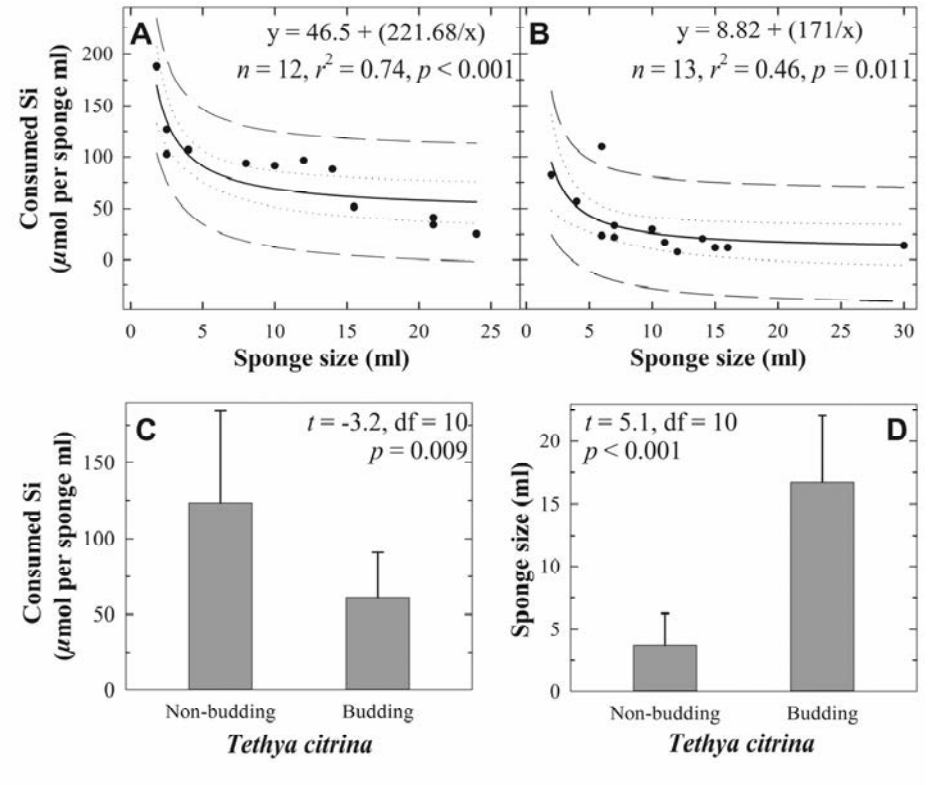


Figure 4. (**A-B**) Relationship between sponge size (mL) and total consumption of silicic acid (DSi) over the 33 days that experiment I lasted. For both *Tethya citrina* (**A**) and *Hymeniacidon perlevis* (**B**) the relationship fitted an inverse, first order, polynomial model. Dotted and dashed lines 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 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 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, those 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; Maldonado et al., 2011; Maldonado et al., 2012a; Maldonado et al., 2012b). A dramatic decrease in DSi availability in the photic ocean upon 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 kinetics 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 3h to days) with K_m values between 0.2 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 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 48h 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 μmol Si h^{-1} sponge-mL^{-1} μM^{-1}, respectively). *Tethya citrina* has an intermediate kinetics (DSi affinity of 0.0069 μmol Si h^{-1} sponge-mL^{-1} μM^{-1}), while *H. panicea* shows the highest affinity (0.0081 μmol Si h^{-1} sponge-mL^{-1} μM^{-1}). 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 36h (Maldonado et al., 2012a), the Yellow Sea individuals showed consumption rates 3.3 to 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).

Chapter 2

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

Picture: An specimen of *Haliclona simulans* on the mäerl bed of Lomergat (bay of Brest, France), from Thierry Le Bec.

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 (24h vs 48h). 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 24h 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 were 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 ± 0.19 mmol Si m^{-2} and about 6.4 x 10^6 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.

Introduction

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; Maldonado et al., 2010; Maldonado et al., 2011; Maldonado et al., 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 8,900 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 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., 7h). In contrast, the rest of published studies were based on complete individuals and longer incubation periods, but of unequal duration between studies: 48h for *Axinella* spp. and 72h 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. *Suberites ficus* is characterized by having two types of spicules: tylostiles and microrhabs. The former one are extremely abundant pin-like spicules, measuring 100-420 μm in length and 2-6 μm in width. The microrhabs are relatively scarce, tiny sticks, measuring 25 -70 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 standard deviation (SD) from the mean.

Consumption of DSi was investigated in the laboratory from mid-September to mid-October, 2016. The individuals were collected from the Bay of Brest by scuba diving (5 to 10 m depths), and acclimated to laboratory stagnant seawater conditions in 30 L polyethylene tanks for two weeks, with water replacement every other day. Seawater temperature was maintained at 15.5 ± 0.5 ºC, mimicking natural values in the field at that time of year.

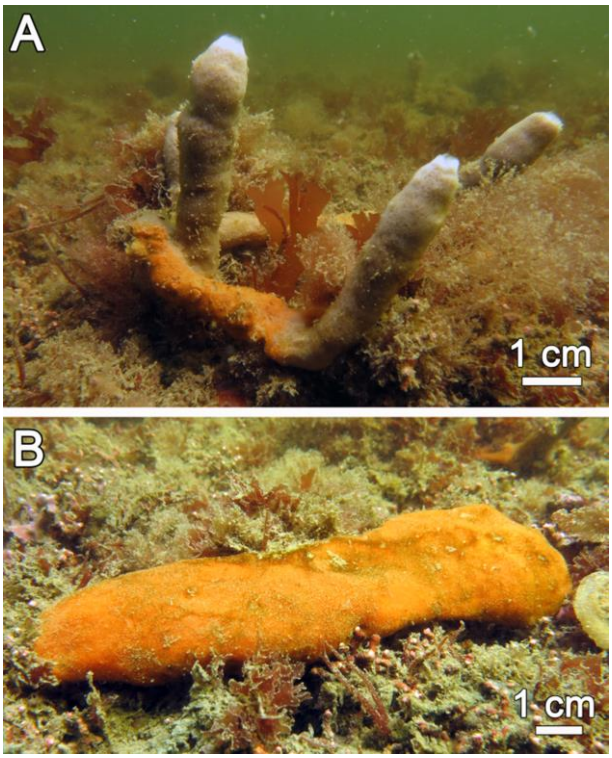


Figure 1. *In situ* views of (**A**) *Haliclona simulans* and (**B**) *Suberites ficus* at their habitat in the Bay of Brest (France).

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 48h 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, 20, 40, 70, 90, 130, 175, 210, 275, 330, 430, and 570 μM.

Experimental DSi concentrations were prepared by adding the corresponding volume of a buffered 0.1 M sodium metasilicate (Na_2SiO_3 ·

$5H_2O$; 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 18h 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 (approx. 10^6 cells/mL) of the haptophyte *Isochrysis aff. galbana* (clone T.ISO) per litre 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 concentration available to the sponges during the incubations and duration of the incubation. At each DSi concentration step, the incubation extended for 48h, but, during that period, each aquarium was sampled within minute 1, after 24h, and after 48h 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 days 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 60ºC to constant dry weight (g), and finally combusted at 540ºC for 10h for ash weight (g). Five fragments of sponge tissue per species were also dry-weighed and desilicified in 5% HF for 5 hours to estimate the average ratio of BSi content versus 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), their BSi content or their dry weight. 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, 8, 21, 44, 67, 90, 130, 175, 210, 275 μM Si) for the species *H. simulans* and 13 levels (2.8, 8, 20, 42, 65, 87, 130, 175, 210, 275, 330, 434, 566 μM Si) for *S. ficus*. The second factor of the analysis was the "Incubation Period" (i.e., "P"), with two levels, resulting from splitting each 48h 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 *Appendix – Section A* 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 statistic 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 non-parametric 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 48h periods during the entire experiment, normalizing by sponge volume to render consumption values comparable. The reason why the response in a global 48h period was preferred over those in either P1 or P2 periods is that the 48h 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 hours) 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}) were 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 48h incubation steps and searched (goodness of fit) for the equation that best fitted the average response over the entire range of assayed concentrations. Secondly, for comparative purposes, we fitted the model from the individual response data rather than from their average. In either case, the responses over 48h were preferred again over those in P1 or P2 periods because such a choice is predicted (see Results and Discussion) 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 48h-incubation steps and fitted it to a Michaelis-Menten model (goodness of fit), obtaining an average (±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 (3 data for each assayed sponge), 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 non-parametric data. Differences in the species affinity for DSi, measured as V_{max}/K_m ratio (in μmol 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 km^2 (after discounting harbours and estuaries). It is connected to the Atlantic ocean through a relatively narrow

(1.8 km wide) strait and receives fresh water and nutrients from 2 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 (Delmas and Tréguer, 1985).

The Bay can be divided into 6 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 rocky intertidal (1), rocky subtidal (2), maërl beds (3), shallow mud (4), heterogeneous sediments (5), and circalittoral coarse sediments (6). 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 x 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

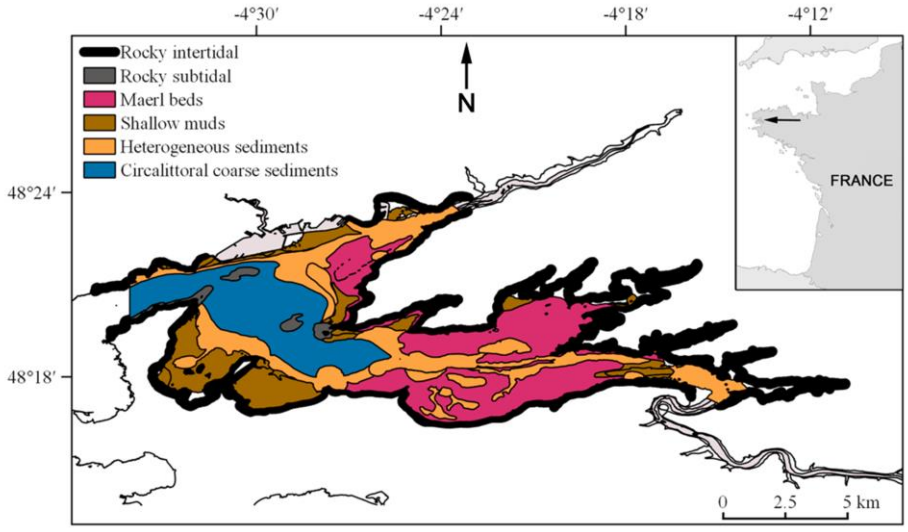


Figure 2. Map of the Bay of Brest (France), showing the six major habitats defined for this study according to their depth, substrate type, and sponge fauna. Estuaries and harbours, depicted in gray in the map, have not been considered in the study.

Bay extension) were sampled by 119, 1 x 1m quadrats during scuba dives; 3) habitats of heterogeneous sediments and circalittoral coarse sediments, both located deeper than 20 m, were sampled running an epibenthic trawl over small transects (1 m wide x 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 m^2.

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://somlit-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 estimated the average (±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 (*Suberites ficus* = -27.18 ± 25.65%; *Haliclona simulans* = -11.13 ± 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 μM in *H. simulans* and 129 μM in *S. ficus* (Fig. 3). Intuitively, those values can be interpreted as the concentration at which the

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 *Haliclona simulans* and *Suberites ficus*.

	Haliclona simulans			*Suberites ficus*		
Factor	**df**	**Statistic**	**p-value**	**df**	**Statistic**	**p-value**
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 x P	3.4	18.8	<0.001	2.4	21.4	<0.001

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 non-significant) 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*, the highest maximum velocity (V_{max}) of consumption was attained by individual #5 (0.600 μmol Si h^{-1} mL^{-1}) at 207.1 μM DSi, while the lowest V_{max} (0.224 μmol Si h^{-1} mL^{-1}), attained by individual #11, occurred at about 68.2 μM DSi (Fig. 4A). As a result, individual #5 consumed over the entire experiment per mL of sponge volume about 2.5 times (150 μmol Si mL^{-1}) more DSi than individual #11 (62 μmol Si mL^{-1}; Fig. 5A). In *S. ficus*, the highest V_{max}, reached by individual #12 at 433.2 μM DSi, was 0.865 μmol Si h^{-1} mL^{-1}, almost one order of magnitude larger than the lowest V_{max} (0.103 μmol Si h^{-1} mL^{-1}) attained by individual #8 at 178 μM (Fig. 4B). As a consequence, individual #12 consumed over 5 times (259 μmol Si mL^{-1}) more DSi per mL of sponge volume than individual #8 (41

μmol Si mL^{-1}; Fig. 5B). Therefore, differences in the size-normalized performance of the individuals were twice larger in *S. ficus* than in *H. simulans*.

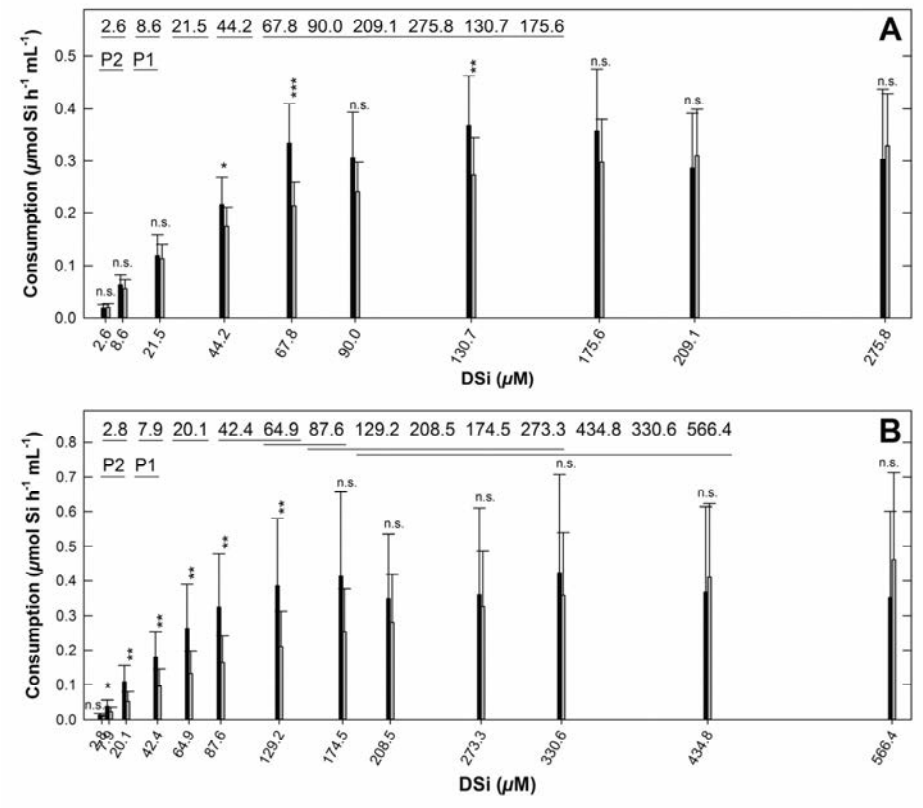


Figure 3. Average (±SD) consumption rates measured as function of silicic acid (DSi) concentration and incubation period (P1 vs P2) in (**A**) *Haliclona simulans* and (**B**) *Suberites ficus*. In the left upper corner the average values for the levels of each factor are ordered from left to right by 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 (grey bars) within each DSi concentration treatment assayed are indicated by symbols, as it follows: n.s. = non-significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

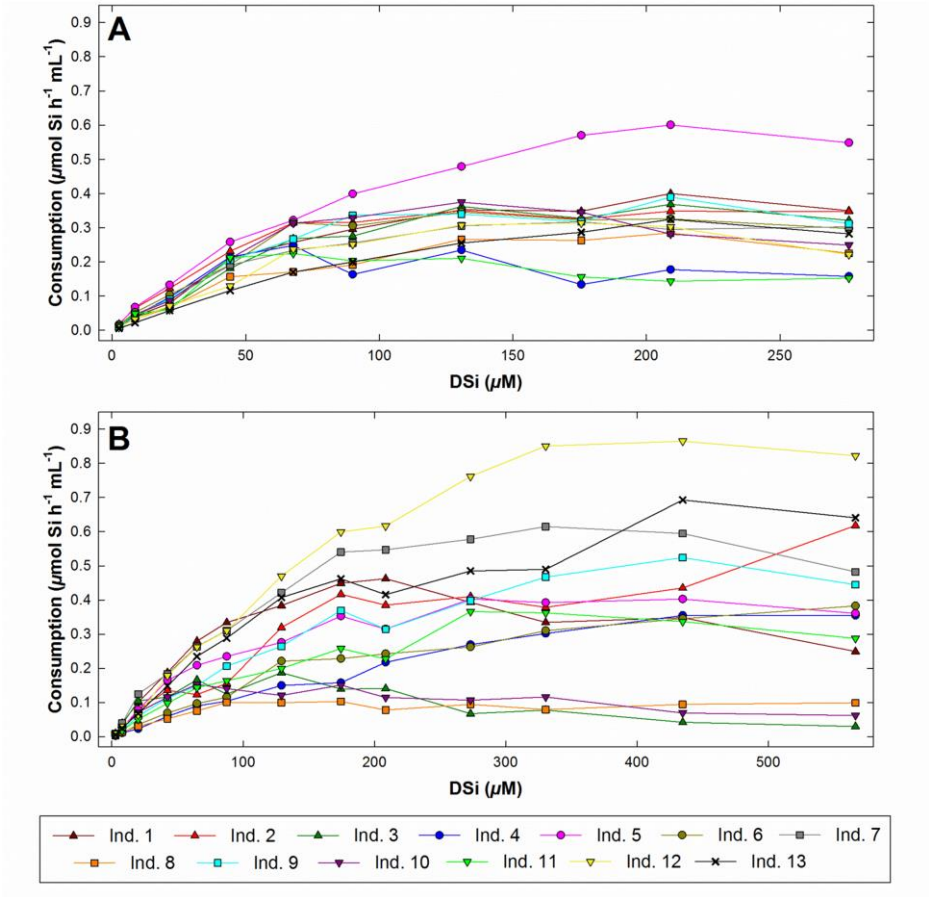


Figure 4. Consumption rates (µmol Si h^{-1} mL^{-1}) of each individual of (**A**) *Haliclona simulans* and (**B**) *Suberites ficus* as a function of the concentration (µM) of silicic acid (DSi) in seawater.

When it was examined whether sponge size could be responsible of at least part of the between-individual variability, no significant effect was found within the range of assayed sizes. When the volume of each individual was plotted versus the total amount of DSi consumed per mL of body over the experiment, neither linear nor non-linear relationships could be fitted for the data in any of the species (Figs. 5A-B). Likewise, when body size of each individual was confronted with the size-normalized maximum DSi transport rate (µmol Si h^{-1} mL^{-1}) of the individuals no significant relationship emerged for any of the species (Figs. 5C-D).

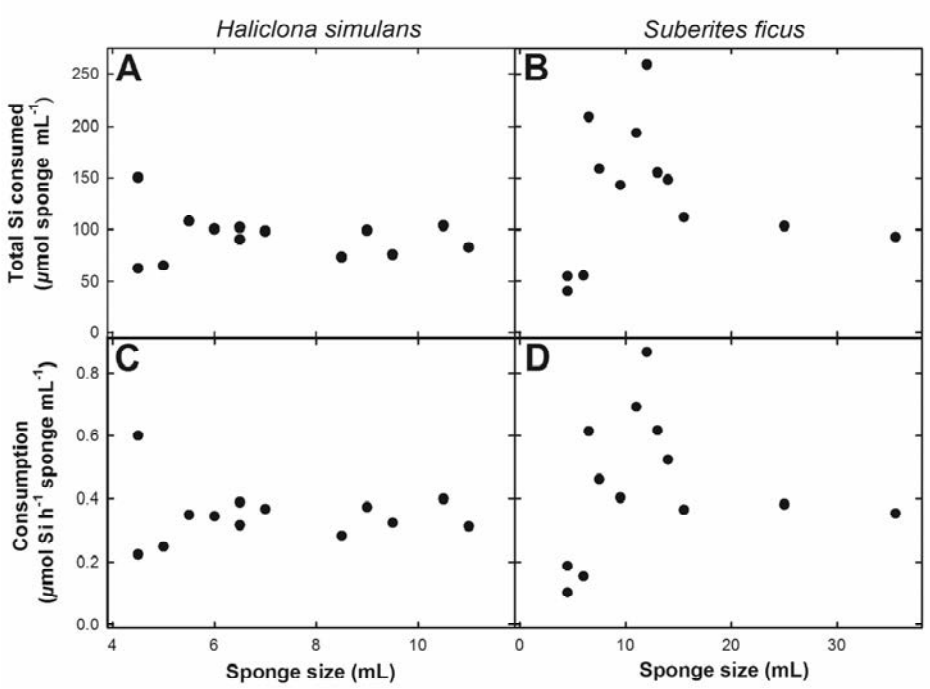


Figure 5. (**A-B**) Sponge size (mL) of each assayed individuals of (**A**) *Haliclona simulans* and (**B**) *Suberites ficus* plotted versus total Si consumed (µmol Si sponge mL^{-1}) by the individual over the entire experiment. (**C-D**) Sponge size (mL) of each assayed individuals of (**C**) *Haliclona simulans* and (**D**) *Suberites ficus* plotted versus the maximum DSi consumption rate (µmol Si h^{-1} 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 (±SD) response of the 13 individuals during the 48h 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-

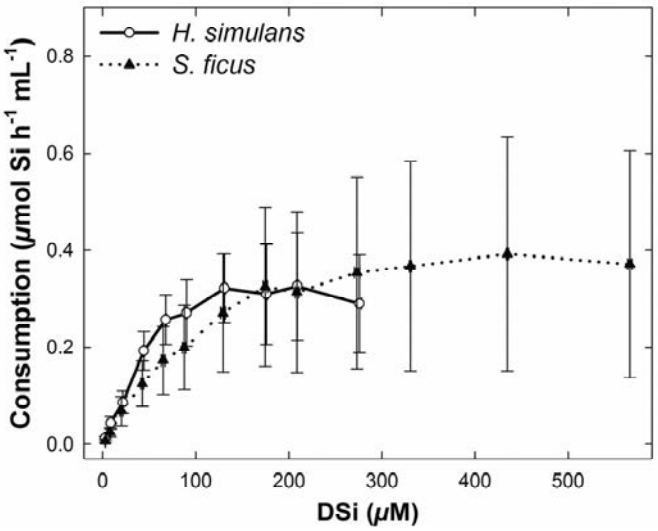


Figure 6. Average (±SD) consumption rate (µmol Si h^{-1} sponge mL^{-1}) empirically determined for individuals of *Haliclona simulans* and *Suberites ficus* as a function of their exposure to increasing concentrations (µM) of silicic acid (DSi) during 48h incubation steps.

fit analysis but based on the individual responses rather than on the averaged responses consistently led to similar conclusions (see *Appendix – Section B* for details).

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 ± 0.03 and 0.48 ± 0.02 µmol Si h^{-1} mL^{-1}, respectively), but the K_m of *S. ficus* (108.23 ± 12.12 µM DSi) was more than twice that of *H.simulans* (45.92 ± 11.98 µM 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 µmol Si h^{-1} sponge mL^{-1} μM^{-1}) than in *S. ficus* (0.004 µmol Si h^{-1} sponge mL^{-1} μM^{-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.

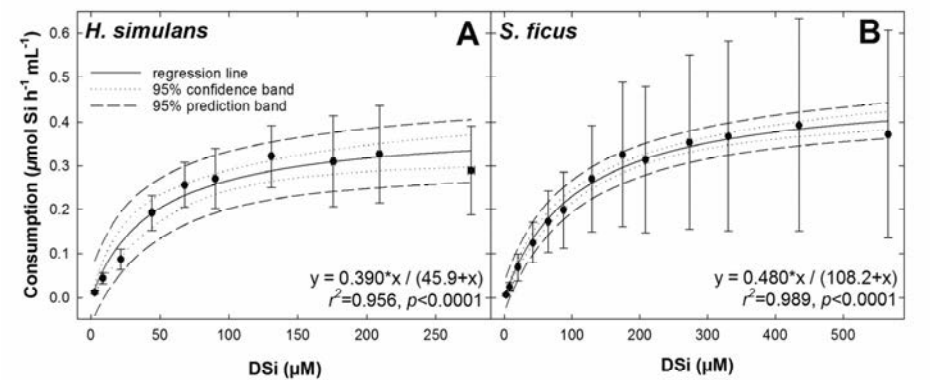


Figure 7. Summary of the statistically significant goodness of fit of the relationship between DSi availability and DSi consumption rate in (**A**) *Haliclona simulans* and (**B**) *Suberites ficus*. In both cases, the best fit was to a hyperbolic model that matches the Michaelis-Menten equation. The herein presented statistics are for the goodness of fit to the average response of the pool of individuals in each species.

Si utilization at the ecosystem level

Field surveys revealed a total of 45 siliceous sponge species in the Bay. The populations of *H. simulans* and *S. ficus* consisted of comparatively high numbers of relatively small individuals and few large ones (Figs. 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, *H. perlevis* contributes 14.6 x 10^3 m^3 to the total biomass in the Bay, almost as much as all the non-dominant sponges together (15.9 x 10^3 m^3; 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 to 9 μM over the four seasons of a year cycle (Fig. 8C), with occasional transient peaks of 15 μM 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 (Figs. 8D-E). Interestingly, the population of *T. citrina*, which amounts a global biomass in

the Bay over three-fold smaller than that of *H. perlevis*, is responsible for virtually the same DSi annual utilization, that is, about 1 megamol 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).

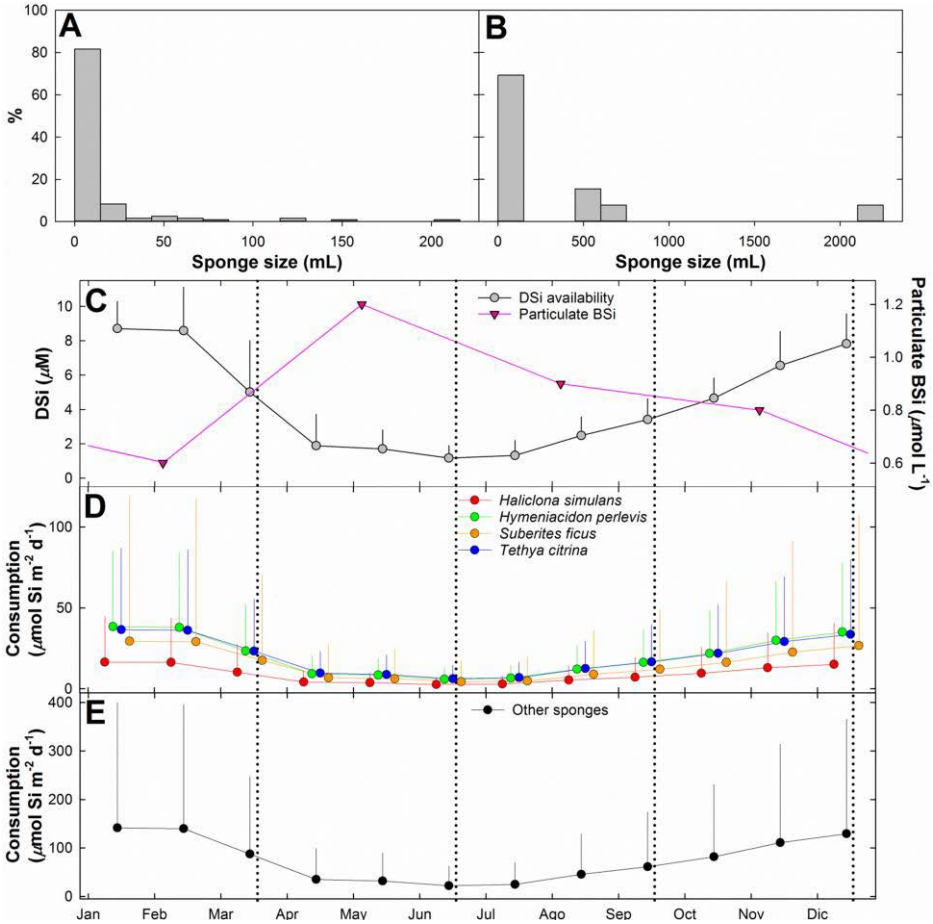


Figure 8. (**A-B**) Frequency distribution of sponge size (in volume) for (**A**) *Haliclona simulans* and (**B**) *Suberites ficus* in the Bay of Brest. (**C**) Monthly average (±SD) of DSi concentration (µM) in the Bay (taken from the SOMLIT-Brest database) along with seasonal average of diatom abundance (as particulate BSi in suspension, from Beucher et al., 2004). (**D-E**) Consumption rate (µmol Si m^{-2} 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.

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 totalled per habitats and bay. Numbers represent average ± SD values.

Species	Pa	Habitats of the Bay						Total sponge stock (10^3 m^3)	Total utilization (10^6 mol Si y^{-1})
		1	2	3	4	5	6		
		Sponge stock (mL m^{-2}) & Consumption rate (10^{-3} mol Si m^{-2} y^{-1})							
Hymeniacidon perlevis	S	86.7 ± 291.4	36.5 ± 81.3	173.9 ± 236.6	---	26.1 ± 55.7	2.4 ± 10.9	14.6 ± 17.8	---
	C	6.4 ± 7.9	2.7 ± 4.5	12.9 ± 15.3	---	1.9 ± 3.1	0.2 ± 0.5	---	1.08 ± 1.32
Tethya citrina	S	31.8 ± 122.5	98.4 ± 236.9	45.6 ± 76.3	---	25.6 ± 44.4	1.1 ± 2.2	4.8 ± 6.6	---
	C	7.1 ± 9.9	22.0 ± 38.4	10.2 ± 13.7	---	5.7 ± 7.9	0.3 ± 0.4	---	1.06 ± 1.47
Suberites ficus	S	---	---	18.6 ± 113.8	10.9 ± 33.6	92.7 ± 390.1	---	5.0 ± 15.2	---
	C	---	---	3.0 ± 11.7	1.8 ± 3.8	15.1 ± 41.7	---	---	0.81 ± 2.47
Haliclona simulans	S	---	15.8 ± 46.0	17.5 ± 39.2	---	4.3 ± 11.7	1.6 ± 4.3	1.6 ± 2.7	---
	C	---	4.6 ± 9.4	5.1 ± 8.5	---	1.3 ± 2.4	0.5 ± 0.9	---	0.46 ± 0.78
Other sponges	S	5.2 ± 27.3	3,628.9 ± 9,780.7	50.6 ± 89.4	74.0 ± 278.6	9.1 ± 23.1	21.2 ± 42.1	15.9 ± 29.0	---
	C	1.0 ± 1.8	681.1 ± 1,301.9	9.5 ± 13.3	13.9 ± 34.9	1.7 ± 3.1	4.0 ± 6.0	---	2.98 ± 5.44
TOTAL AVERAGE PER HABITAT	S	123.8 ± 338.1	3,779.6 ± 9,740.6	306.2 ± 322.1	84.9 ± 277.5	157.9 ± 383.1	26.4 ± 52.4	---	---
	C	14.5 ± 19.6	710.4 ± 1,354.1	40.7 ± 62.1	15.7 ± 38.7	25.7 ± 58.1	4.9 ± 7.8	---	---
		Total sponge stock (10^3 m^3) & Consumption (10^6 mol Si y^{-1})							
Area extension (km^2)		1.98	2.60	76.45	21.42	36.08	28.70		
ABSOLUTE TOTAL	S	0.2 ± 0.7	9.8 ± 25.4	23.4 ± 24.6	1.8 ± 5.9	5.7 ± 13.8	0.8 ± 1.50	41.8 ± 57.5	---
	C	0.03 ± 0.04	1.8 ± 3.5	3.1 ± 4.7	0.3 ± 0.8	0.9 ± 2.1	0.1 ± 0.2	---	6.39 ± 11.44

Collectively the assemblage of siliceous sponges in the Bay is estimated to consume annually 6.39 ± 11.44 megamoles of Si (Table 2). Excluding harbours 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 m^{-2} d^{-1}.

Discussion

The kinetics of DSi utilization in *Haliclona simulans* and *Suberites 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. simulans* could account for the fact that the former species produces about 50 % more silica per biomass unit (115 mg BSi 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 ever (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).

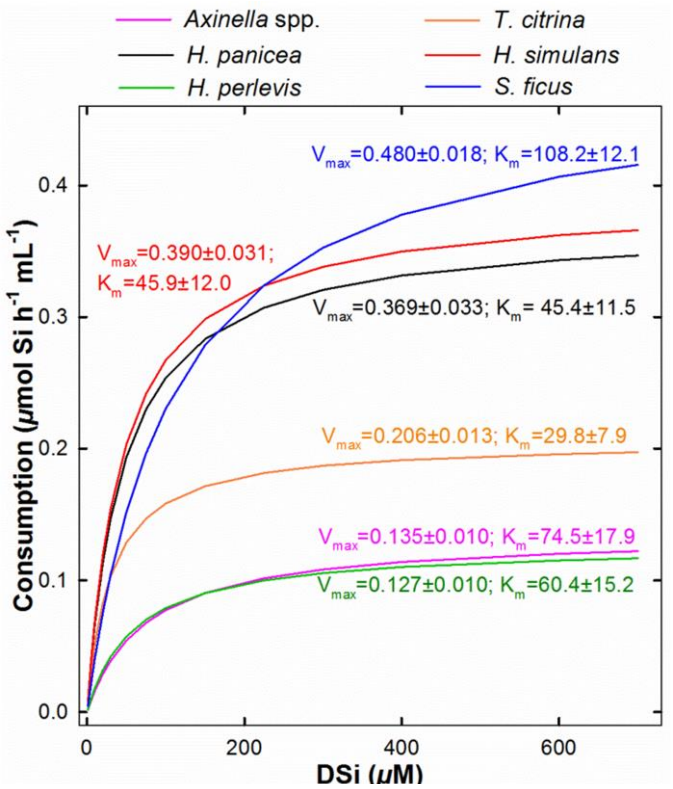


Figure 9. Summary of all DSi consumption kinetics (µmol Si h^{-1} sponge mL^{-1}) empirically established to date for sponges. The set contains only demosponges: *Axinella* spp. (Maldonado et al., 2011), *Halichondria panicea* (Reincke and Barthel, 1997), *Hymeniacidon perlevis*, *Tethya citrina* (López-Acosta et al., 2016), and *Haliclona simulans* and *Suberites ficus* (this study). In all cases, consumption kinetics fit a saturable Michaelis-Menten model: "DSi consumption = V_{max} x [DSi] / (K_m + [DSi])". Maximum velocity (V_{max}) of DSi transport and half-saturation (K_m) values are given (average±SD) in the graph for each species.

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 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 μM to 8.7 μM (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 to 108.2 μM; 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 non-saturable uptake systems, being able to shift from Michaelis-Menten saturable kinetics to non-saturable 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 (<24h) 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 to 27 % higher during the first 24h of incubation (P1) than during the subsequent 24h 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., 2017; 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 folds its own body volume per hour, depending on the species (Reiswig, 1974). All together, 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 (Frøhlich and Barthel, 1997; Reincke and Barthel, 1997; Maldonado et al., 2011; Maldonado et al., 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. non-reproductive 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 favoured 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 2,252 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 (Figs. 8A-B).

DSi utilization by sponges in the Bay averaged a rate of 0.10 ± 0.19 mmol Si m^{-2} day^{-1}. When compared with rates estimated for other shallow-water sponge assemblages (Maldonado et al., 2011; Maldonado et al., 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 4 to 9 fold 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 x 10^6 mol Si y^{-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 x 10^6 mol Si y^{-1}). Such a value results from recalculating the BSi production estimated by Ragueneau and co-workers (2005) for 180 km^2 down to 167 km^2 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.

Chapter 3

Silicon utilization by sponges: an assessment of seasonal changes

Picture: An specimen of *Tethya citrina* on the mäerl bed of Rozegat (bay of Brest, France), from Erwan Amice.

Abstract

The awareness is growing that sponges are relevant silicic acid (DSi) users, but the understanding of how their DSi consumption kinetics perform is still limited. Herein we investigated for the first time the effects that seasonal change in a temperate ecosystem (the Bay of Brest, France) have on the DSi consumption of two dominant sponge species, *Hymeniacidon perlevis* and *Tethya citrina*. The results indicated that while both species increased their rate of DSi utilization with DSi availability following a saturable Michaelis-Menten kinetics, only the kinetics of *T. citrina* shifted seasonally. This species consumed DSi at rates 99.54 ± 87.05 % higher in autumn than in summer. Surprisingly, the increase in DSi utilization did not involve an increase in net affinity for DSi, but rather augmentation of both the half-saturation concentration (K_m) and the maximum velocity of transport (V_{max}) in the kinetics. By quantifying the biomass of the sponge species in the bay and the monthly availability of DSi over a year cycle, an annual DSi consumption of 2.50 ± 3.21 x 10^6 moles Si was calculated for the populations of these two species. The oversight of the seasonal kinetic change would introduce inaccuracies of 10% into the DSi consumption budget for the bay. More importantly, because the seasonal kinetic differences increased rapidly with increasing DSi availability in the environment, the relevance of the errors could rise to over 30% in sponge assemblages characterized by higher DSi availability, as typically occurring in sponge grounds from high latitudes and deep sea.

Introduction

Silicon is an important element in ocean ecology and biogeochemistry. One of its dissolved forms, silicic acid (DSi), is consumed by a variety of marine organisms (radiolarians, diatoms, silicoflagellates, choanoflagellates, testate amoebae, cyanobacteria, sponges, seagrasses, etc). The consumed DSi is generally used to produce biogenic silica (BSi), an amorphous glass-like substance often used as skeletal material. One of the most relevant Si consumers are diatoms, which in turn are one of the ocean most important primary producers (Nelson et al., 1995; Tréguer et al., 1995). Therefore, it can be said that the DSi availability for biological consumption is a major factor in the control of ocean primary productivity. Because of the important implications of the DSi availability and its biological utilization, there is an enormous interest in modeling the biological consumption of DSi in the marine environment, at both the regional and global scales. For practical and historical reasons, all available global models are based exclusively on the properties of DSi utilization by diatoms, which are the most abundant users (Harriss, 1966; Burton and Liss, 1968; Calvert, 1968; Nelson et al., 1995; Tréguer et al., 1995; Ragueneau et al., 2000; Laruelle et al., 2009). However, there is a growing number of studies suggesting that sponges are also relevant Si users that should be incorporated in the modeling process (Rützler and Macintyre, 1978; Conley and Schelske, 1993;

Maldonado et al., 2005; Maldonado et al., 2011; Maldonado et al., 2012; Tréguer and De La Rocha, 2013), but very little is still known about the kinetics of DSi consumption by sponges. These kinetics have been characterized only for a handful of temperate shallow-water demosponges (Reincke and Barthel, 1997; Maldonado et al., 2011; López-Acosta et al., 2016). However, no assessment has been made to understand how the large seasonal changes in nutrient and food availability characterizing temperate latitudes affect the patterns of DSi consumption by sponges.

It has long been determined that the seasonal variability in some environmental factors (e.g., inorganic nutrient ratios, seawater temperature, salinity, oxygen distribution, etc) triggers drastic changes in the performance of diatoms, which may increase or decrease their rates of DSi utilization by orders of magnitude in response to those changes (Brzezinski et al., 2008; Hoffmann et al., 2008; Javaheri et al., 2015). Through evolution, sponges are expected to have also evolved to adapt their physiology to the seasonal environmental changes. However, it remains uninvestigated to date whether such seasonal changes actually affect DSi consumption by temperate sponges and what the magnitude of such changes could be, if any. A better understanding of the DSi consumption by sponges and the main factors that control the consumption process is required if we are ever to quantify in detail the role of siliceous sponges in the benthic-pelagic coupling of Si cycling at either regional or global scales.

By combining laboratory experiments and field observations, we are here investigating for the first time how seasonality affects the DSi consumption kinetics of two temperate sponges, also quantifying what the implications are in their natural populations at the ecosystem level.

Methods

The Bay of Brest as a study case ecosystem

The Bay of Brest (France; 48.31º N, 4.44º W) is a semi-enclosed marine water body, with an extension of 167.23 km^2 (estuaries and harbors excluded), a mean depth of 8 m, and a maximum depth of 45 m. It is connected to the North Atlantic Ocean through a 1.8-km wide strait, which

channels the tidal flow in and out favoring macrotides of up to 8 m tidal amplitude. In such a macrotidal system, nearly the entire water mass of the bay is renovated daily. The bay receives the inflow of two rivers that supply important loads of dissolved nutrients and particulate matter with marked seasonal patterns. The local winds are also known to mix most of the vertical water column of the bay daily (Delmas and Tréguer, 1985).

The Bay of Brest has been the subject of numerous ecological, biogeochemical and physical studies and is currently one of the best-known French coastal ecosystems, both in terms of structure and functioning (Le Pape et al., 1996; Ragueneau et al., 1996; Del Amo et al., 1997a; Del Amo et al., 1997b; Chauvaud et al., 2000). Several seawater column parameters of the bay are monitored weekly for decades and data are publically available throughout the Coastal Observation Service (SOMLIT-Brest: http://somlit-db.epoc.u-bordeaux1.fr/bdd.php). In Fig. 1, monthly averages (mean ± SD) of several basic environmental parameters of the bay are summarized for the last decade (2007 to 2016): seawater temperature (T), oxygen concentration (O_2), chlorophyll A (Chl-A), particulate organic carbon (POC), silicic acid (DSi), nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), and phosphate (PO_4^{3-}). The system clearly shows a seasonal decrease of all nutrients and oxygen from April to August-September. Such a decrease starts with the typical spring bloom of phytoplankton (Chl-A) and is coincidental with the seasonal raising of seawater temperature. From late September to April, nutrients and oxygen increase coincidentally with decreasing seawater temperature. Average values of Chl-A also decrease slowly from September until the next spring bloom. The POC, which may be used as a gross indicator of food availability for sponges, remains relatively unaltered over the year cycle. In agreement with previous studies (Quéguiner and Tréguer, 1984; Chauvaud et al., 2000), these general trends illustrate that at least two distinct environmental and ecological periods occur in the system: 1) spring-summer (April to September), characterized by progressive increase of temperature and consumption of nutrients and oxygen in the water column, and 2) autumn-winter (October-March), characterized by gradual nutrient and oxygen replenishment and decrease in water temperature and primary productivity.

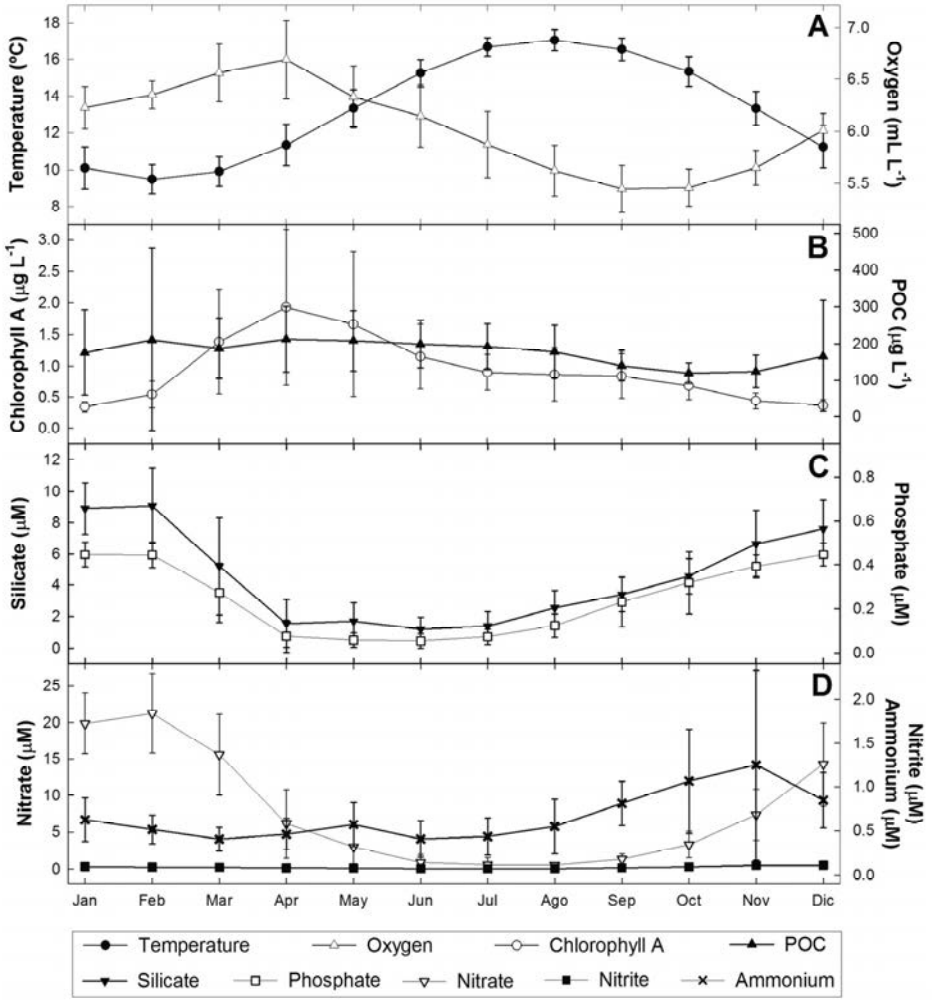


Figure 1. Monthly average (±SD) of temperature (ºC), oxygen concentration (mL L^{-1}), chlorophyll A (µg L^{-1}), particulate organic carbon – POC (µg L^{-1}), silicate (µM), phosphate (µM), nitrate (µM), nitrite (µM), and ammonium (µM) in the Bay of Brest over a year cycle. Monthly averages result from weekly measurements taken over a decade (2007 – 2016).

The Bay of Brest also hosts a rich benthic assemblage of macroalgae and macroinvertebrates, with abundance of siliceous sponges. The sponge assemblage has recently been described and quantified across the 6 habitat-like benthic compartments that are recognized in the bay (López-Acosta et

al., 2018). A total of 45 siliceous sponge species were identified and quantified, representing an average biomass of 250 ± 344 mL m^{-2}. Two species, *Hymeniacidon perlevis* (Montagu, 1814) and *Tethya citrina* (Sarà and Melone, 1965), were dominant in biomass (99.94 ± 122.20 and 32.67 ± 45.07 mL m^{-2}, respectively; López-Acosta et al., 2018), accounting collectively for 53.1% of the sponge standing stock of the bay.

Here we have selected those two dominant sponge species to examine both the effects of seasonality on their DSi consumption kinetics and the implications of those effects at the population level in the bay.

Experimental setup

To assess whether the "season" factor has an effect on how *H. perlevis* and *T. citrina* consume DSi, we investigated their DSi utilization in summer (July 2015), when natural DSi availability is low, and in autumn (October-November 2016), when DSi replenishment starts. The underlying hypothesis is that, through evolution, the sponges may have adapted their physiology (e.g. skeletal growth) to process DSi with more avidity (i.e., transport affinity) at the time of the year when its natural availability starts increasing. Being the silicification process in sponges mediated by enzymes (Shimizu et al., 1998; Krasko et al., 2000), such a change in the affinity of the sponges by the substrate (DSi) may have a profound impact on the kinetics of DSi consumption. Also these two sponge species show contrasting level of skeletonization, with the siliceous skeleton of *H. perlevis* representing about 45 ± 4 % of the dry weight of the individuals and that of *T. citrina* about 63 ± 5 % (López-Acosta et al., 2016). Such skeletal differences suggest that both species may also differ in their specific needs of DSi for building their respective skeletons.

Following the methodology detailed in (López-Acosta et al., 2016), we conducted two experiments (one in summer, the other in autumn) for characterizing the respective kinetics of DSi consumption. At each experiment, DSi consumption was investigated in 9 individuals of *H. perlevis* and 8 of *T. citrina*, along with a set of 6 controls (see below).

During the experiments, each sponge was placed into an aquarium filled with 2.78 ± 0.12 L and incubated for a succession of 72h periods under increasing DSi concentrations. The experiments started with DSi

concentrations of 2 μM in summer and 4 μM in autumn, which corresponded to the field value at the time of experiment onset. Then concentrations were progressively increased to 75, 150, 200, and 250 μM in both experiments. To determine DSi utilization during every 72h incubation, each aquarium was sampled at the beginning and at the end of each incubation period by collecting 20 mL of seawater with plastic syringes. Consumption of DSi by each sponge at a given DSi concentration step was inferred from the difference in DSi concentration between the beginning and the end of the incubation period, after correcting by the concentration changes occurred in the control aquaria, which consistently were nearly zero. The controls consisted of three aquaria filled with only seawater and three other aquaria, each filled with seawater and a piece of "maërl" similar to the one that sponges often use as substrate.

Water samples for determining DSi concentration were filtered with polycarbonate, 0.22 μm-pore, syringe-filters (Milex-GS Millipore) and stored in the fridge for only 1-4 days prior to analysis. The seawater samples from the initial and final times of each incubation were always analyzed into a single analysis run to minimize methodological variability. DSi determination was carried out by spectrophotometric analysis following the standard colorimetric method (Strickland and Parsons, 1972), with a determination precision of 5%. Samples from DSi concentrations higher than 30 μM were diluted prior to analysis.

Immediately after each experiment, we measured several morphometric parameters of the assayed individuals —volume (mL), wet weight (g), constant dry weight (g), and ash-free weight (g), which served for normalizing DSi consumption rates. Preferentially, DSi consumption was normalized by volume of seawater in the aquarium (L), time unit (h) and volume of the assayed sponge (mL). Normalization by sponge volume was preferred because it facilitates future extrapolation to field sponge populations without the need of conducting destructive sampling, but just measuring the sponges *in vivo* through rules or photogrametically (Abdo et al., 2006; Maldonado et al., 2010).

Irrespective of normalizing DSi consumption by morphometric parameters, for each species, we intended the body size range of the assayed individuals for each season to differ minimally. In both seasons the individuals of *H. perlevis* ranged similarly in volume from 1.5 to 16.5 mL and

those of *T. citrina* from 2.8 to 16 mL. The selected size ranges for these species represent the small and mean portion of their natural body size range. Very large individuals of both species were deliberately excluded because of logistic constraints for optimal maintenance of such large animals in the relative small aquaria required for the experiments.

Testing for seasonality in DSi consumption

Recent investigations in *H. perlevis* and *T. citrina* reported that DSi consumption kinetics fit a Michaelis-Menten function (López-Acosta et al., 2016). Consequently, we used non-linear goodness of fit analysis to model the DSi consumption rate as a function of increasing DSi availability to the sponges. To examine the effect of seasonality on the DSi consumption rates for each species, we carried out 3 complementary statistical approaches. In the first approach, we used a two-factor design because the experiment involved determination of DSi consumption rates as a function of both the season of the year (summer vs. autumn) and the availability of DSi (3, 75, 150, 200, and 250 μM DSi). For each season, the same sponges were exposed to increasing DSi concentrations over the experiment, but the individuals assayed in July's experiment were different from those used in October-November. Therefore, the two-factor design involves a component of "repeated measures" nested within the factor "season". Because consumption rates of both species failed the normality test (Shapiro-Wilk test) and transformations were unable to modify that condition, we approached the two-way repeated measurement test by using the ANOVA-type (ATS) statistic (Brunner et al., 2002) separately for each sponge species. Untransformed DSi consumption rates data were analyzed by the ATS test using the package "nparLD" (Noguchi et al., 2012) in R version 3.4.2. *A posteriori*, pairwise comparisons based on Brunner-Munzel (BM) tests (Brunner and Munzel, 2000) were run using the package "lawstat" in R version 3.4.2. In a second approach, we evaluated between-season differences in the total DSi consumption accumulated by each sponge species during the entire experiment (15 days). Between-season differences in the mean DSi consumed were examined separately for each species using a *t*-test on untransformed, normally distributed data. In a third approach, we examined differences between the kinetic parameters that govern the model

equations describing DSi consumption in summer and autumn for each species. To this aim, we first estimated the Michaelis-Menten model for each assayed individual, obtaining a set of individual K_m and V_{max} values for each season and species. We then examined, separately for each species, between-season differences in the average values of K_m and V_{max} parameters using the *t*-test on untransformed data, if they were normal and homoscedastic. When data did not meet either normality (Shapiro-Wilk test) or homocedasticity (Brown-Forsythe test), the non-parametric Mann-Whitney *U* test was conducted to examine the significance of between-season differences in the mean values.

Between-individual variability

In previous studies on DSi utilization by sponges the body size was identified as a source of between-individual variability in the DSi utilization rate (Maldonado et al., 2011; López-Acosta et al., 2016). Therefore, we used regression analysis to examine the relationship between the body size (in mL) of an individual and the maximum DSi transport velocity (V_{max}) it achieved during the experiment (irrespective of the DSi concentration step at which that V_{max} was achieved). For this regression, we increased the number of assayed individuals up to 34 individuals in *H. perlevis* and 33 in *T. citrina*, to assay a wider range in body size, that is, more similar to the one occurring in natural population and about twice the one previously used to determine the DSi kinetics. For both species, the body size of the assayed individuals ranged from 1 to 30 mL in volume. Because in *T. citrina* the reproductive status had been also identified as a source of between-individual variability in DSi consumption responses (López-Acosta et al., 2016), a discrimination between asexually-reproductive and non-reproductive individuals was made in the regression analyses and the potential effects of this condition examined.

DSi consumption in wild populations

To estimate daily and yearly rates of DSi consumption in the bay by the natural populations of *H. perlevis* and *T. citrina*, 3 sources of data were used: 1) the natural variability of DSi concentrations in the bay of Brest over the last decade (2007-2016; Fig. 1), which was averaged monthly over an ideal

year cycle; 2) the mean abundance (mL m^{-2}) of the selected species (*H. perlevis,* 99.94 ± 122.20 mL m^{-2}, *T. citrina*, 33.67 ± 45.07) in the natural populations (López-Acosta et al., 2018); and 3) the herein determined between-season differences in the kinetics of DSi consumption by the two concerned species. The consumption of DSi by the biomass of each species was calculated as a function of the monthly change in DSi concentration in the seawater and the corresponding species-specific kinetic shifts.

Results

DSi consumption kinetics

The two sponge species reacted similarly to the increase in DSi availability in both seasons: the mean rate of DSi consumption increased with increasing DSi availability until reaching a certain saturating concentration (Fig. 2). When these responses were modeled mathematically, the best significant model retrieved by goodness of fit for both sponges in the two seasons was consistent with a saturable Michaelis-Menten model (Fig. 3). Both the experimental data and the models suggested that the consumption rates rose with increasing DSi availability until concentrations of 150 – 200 μM DSi, values at which DSi consumption by the sponges appeared to reach saturation (Fig. 2). These saturating DSi concentrations are from one to two orders of magnitude higher than those naturally occurring in the sponge habitats over the year cycle (Fig. 1C). Consequently, the skeletal growth of the two assayed species can be said to be chronically limited by a natural shortage of DSi.

Although both species followed the same general kinetic model in the two seasons (Fig. 3), all four resulting kinetics did not always have similar values for their kinetic parameters, i.e., the maximum velocity of transport (V_{max}) and the half-saturation constant (K_m). The V_{max} characterizing the kinetic models of *Hymeniacidon perlevis* in summer and autumn were relatively similar (0.131 and 0.142 μmol Si h^{-1} sponge-mL^{-1}, respectively), suggesting that seasonal differences, if any, must be small. In contrast, in *Tethya citrina*, the V_{max} in autumn (0.429 μmol Si h^{-1} sponge-mL^{-1}) was more than twice that in summer (0.206 μmol Si h^{-1} sponge-mL^{-1}; Fig. 3A-B), suggesting seasonal effects. The inspection of K_m indicated about twice larger values in autumn

than in summer for both species (Fig. 3). Whether these numerical differences in the parameters involve statistically significant differences in the kinetics of DSi utilization will be addressed in the next section.

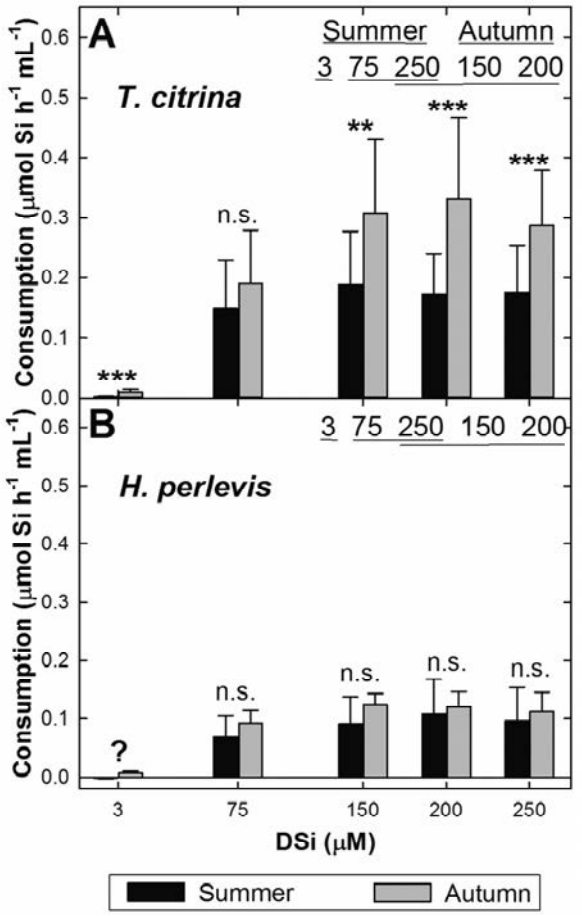


Figure 2. Average (±SD) DSi consumption rates (μmol Si sponge h^{-1} mL^{-1}) measured as a function of DSi availability during summer (black bars) and autumn (grey bars) experiments in (**A**) *Tethya citrina* and (**B**) *Hymeniacidon perlevis*. In the right upper corner of each graph, the levels of the significant factors following an ATS bifactorial test are given. Levels are ordered by increasing magnitude of their average value. Levels of a factor underlined by the same line are not significantly different from each other. The statistical significance of the pairwise tests examining differences in the consumption rate between summer (black bars) and autumn (grey bars) at each DSi concentration step are indicated, as it follows: ns= not significant, *= $p<0.05$, **= $p<0.01$, ***= $p<0.001$, ?= rates below analytical detection.

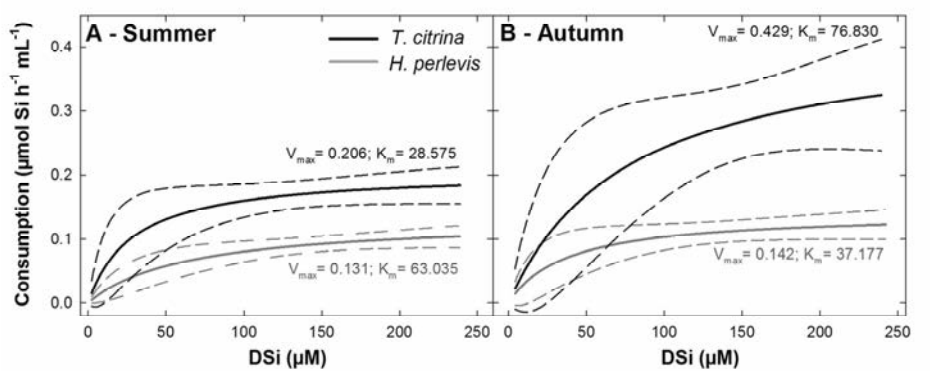


Figure 3. DSi consumption kinetics estimated by goodness of fit for the sponges *Tethya citrina* (black) and *Hymeniacidon perlevis* (grey) during the experiments in summer (**A**) and autumn (**B**) experiments. In all four cases, consumption kinetics significantly fitted a saturable Michaelis-Menten model in the form "DSi consumption = V_{max} x [DSi] / (K_m + [DSi])", with the maximum velocity of DSi transport (V_{max}) and half-saturation (K_m) values of each equation given in the figure. The statistics of the goodness of fit analyses for *H. perlevis* were r^2= 0.98 and p = 0.001 in summer and r^2= 0.97, p = 0.002 in autumn. For *T. citrina*, they were r^2= 0.98 and p = 0.001 in summer, and r^2= 0.95 and p = 0.006 in autumn. Solid and dashed lines refer to the regression line and the 95% confidence band, respectively.

Seasonal variability in DSi consumption

The results of the statistical approach 1 for each sponge species, based on a bifactorial ANOVA-type test (ATS), are summarized in Fig. 2 and Table 1. For *T. citrina* (Fig. 2A), the "season" factor was found to have a significant effect on the consumption rates, with sponges consuming DSi at an average rate that was estimated 99.54 ± 87.05% higher in autumn than in summer, when all DSi concentration steps for each season were pooled. The "DSi availability" factor also had a significant effect, increasing DSi consumption with increasing DSi availability up to a threshold concentration was achieved (Fig. 2A; Table 1). The *a posteriori* tests confirmed that such a DSi saturating threshold was the 150 µM step (Fig. 2A). The ATS also detected a significant interaction between the two main factors (Table 1). The *a posteriori* pairwise comparisons revealed that the interaction was due to the fact that DSi

Table 1. Summary of the two-factor ANOVA-type statistic (ATS) tests analyzing differences in consumption rates as a function of season and silicic acid (DSi) availability in *Hymeniacidon perlevis* and *Tethya citrina*.

	Hymeniacidon perlevis			*Tethya citrina*		
Factor	**Statistic**	**df**	**p-value**	**Statistic**	**df**	**p-value**
Season	3.6	1.0	0.058	7.2	1.0	0.007
DSi	55.1	2.6	<0.001	105.3	2.0	<0.001
Season x DSi	0.4	2.6	0.698	4.4	2.0	0.012

consumption rates were significantly larger in autumn than in summer at all the investigated DSi concentrations, but at the 75 μM step (Fig. 2A). For *H. perlevis*, the ATS statistic revealed that only the "DSi availability" factor had a significant effect on consumption rate and the pairwise *a posteriori* tests indicated that the consumption rate increased with DSi availability up to a concentration of 150 μM DSi (Table 1, Fig. 2B), in agreement with the saturating threshold value identified for *T. citrina*.

The statistical approach 2 revealed that the total amount of DSi consumed by *T. citrina* during the 15 days of the autumn experiment (78.29 ± 29.19 μmol Si mL^{-1}) was significantly higher than during the summer experiment (47.57 ± 21.04 μmol Si mL^{-1}; F = -2.4, df = 14, p = 0.030). However, *H. perlevis* did not show significant between-season differences (31.76 ± 5.32 μmol Si mL^{-1} in autumn versus 25.18 ± 13.13 μmol Si mL^{-1} in summer; F = -1.4, df = 16, p = 0.183). Therefore, the results of these *t*-tests (approach 2) come into full agreement with those of the ATS (approach 1), supporting a seasonal effect on DSi utilization in *T. citrina* but not in *H. perlevis*.

Statistical approach 3 indicated that, in *T. citrina*, there were significant between-season differences in both the V_{max} (0.229 ± 0.102 μmol Si h^{-1} mL^{-1} in summer versus 0.464 ± 0.169 μmol Si h^{-1} mL^{-1} in autumn; t=-3.4, df = 14, p=0.005) and the K_m parameters of the kinetics (49.83 ± 34.97 μM in summer versus 98.56 ± 51.44 μM in autumn; t=-2.2, df=14, p=0.044). However, in *H. perlevis*, there was not significant between-season difference in either the V_{max} (0.229 ± 0.102 μmol Si h^{-1} mL^{-1} in summer versus 0.153 ± 0.049 μmol Si h^{-1} mL^{-1} in autumn; U=32, p=0.480) or the K_m (77.07 ±

49.53 μM in summer versus 50.14 ± 36.36 μM in autumn; t=1.3, df = 16, p=0.207).

All together, the three statistical approaches fully agree in concluding that seasonality affects significantly DSi consumption in *T. citrina* but not in *H. perlevis*. Interestingly, in *T. citrina*, the kinetic differences between seasons increase with increasing DSi availability. Such a pattern is not trivial, because it means that the implications of seasonal kinetic changes will be minor for *T. citrina* populations established in environments permanently characterized by low DSi concentrations but of noticeable relevance in habitats with DSi concentrations above 50 μM (Fig. 3).

Because between-season differences do not appear to exist in *H. perlevis*, the DSi consumption kinetics for this species over the year cycle was better calculated by pooling the responses of all assayed individuals in summer and autumn (n = 18). The resulting kinetic equation (r^2= 0.92, p<0.001), characterized by a V_{max} of 0.134 μmol Si h^{-1} mL^{-1} and a K_m of 45.30 μM, is the equation line that would lie in between the two seasonal ones depicted in Fig. 3.

Between-individual variability

When Si consumption responses of each assayed individual were inspected, considerable between-individual variability was noticed within each species (Fig. 4), being consistently of larger magnitude in *T. citrina* (Fig. 4A-B) than in *H. perlevis* (Fig. 4C-D). Although the range of inter-individual variability in DSi consumption over the entire course of these experiments is difficult to be measured comparatively, some analyses were attempted. For those, we examined differences in the maximum rate of DSi consumption (i.e., empirical V_{max}) reached by the individuals during an experiment, irrespective of the DSi concentration step at which such V_{max} was attained. For instance, in *T. citrina* in summer (Fig. 4A), the individuals spanned a range in the rate of maximum DSi consumption that varied from 0.121 μmol Si h^{-1} mL^{-1} (achieved by ind. #4 at 200 μM DSi) to 0.387 μmol Si h^{-1} mL^{-1} (achieved by ind. #6 at 150 μM DSi). In autumn (Fig. 4B), they ranged from 0.190 μmol Si h^{-1} mL^{-1} (achieved by ind. #4 at 200 μM DSi) to 0.577 μmol Si h^{-1} mL^{-1} (achieved by ind. #7 at 200 μM DSi). In summary, although V_{max} values were consistently higher in autumn than in summer in *T. citrina*, the range

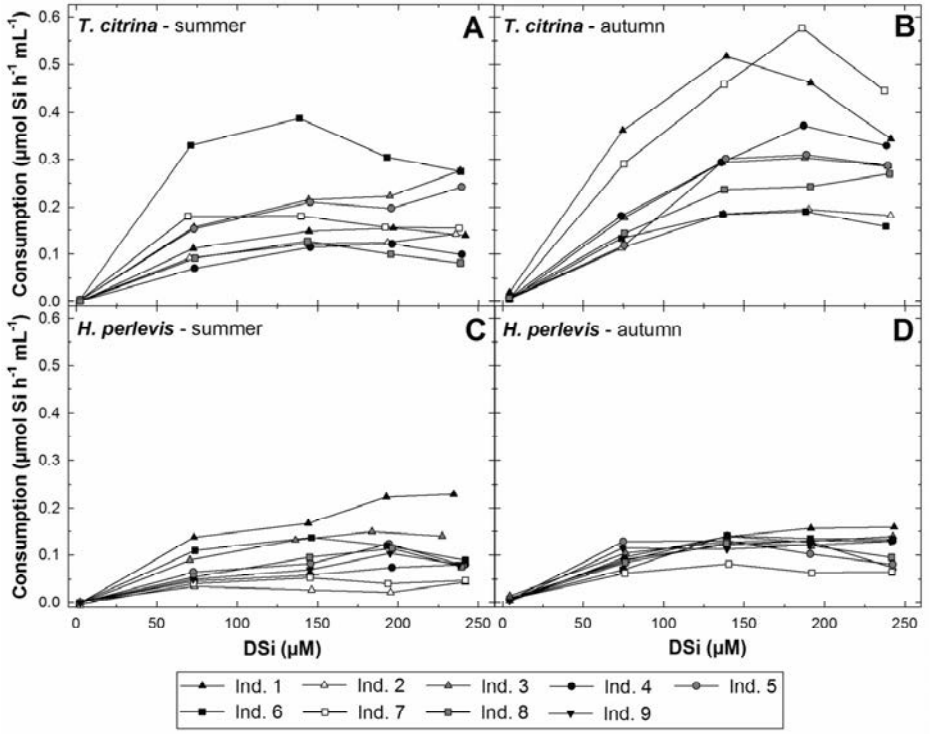


Figure 4. Consumption rate (µmol Si h^{-1} sponge mL^{-1}) as function of DSi concentration (µM) of each assayed individual of *Tethya citrina* (**A-B**) and *Hymeniacidon perlevis* (**C-D**) during summer (**A, C**) and autumn (**B, D**) experiments.

amplitude of between-individual variability was similar across seasons, the largest values being around 3-fold higher than the lowest ones, irrespective of season. Unlike in *T. citrina*, the range amplitude of DSi consumption rate in *H. perlevis* appeared to be higher in summer (Fig. 4C) than in autumn (Fig. 4D). In this case, the range spanned between the lowest and the highest values of empirical V_{max} in summer (0.044 and 0.229 µmol Si h^{-1} mL^{-1} achieved by ind. #2 and #1, respectively, at the 250 µM DSi step) was more than two folds that recorded in autumn (0.082 and 0.159 µmol Si h^{-1} mL^{-1} by ind. #7 and #1 at 150 and 250 µM DSi, respectively).

The regression analyses indicated that part of this variability in the normalized consumption rate is due to size differences between the individuals (Fig. 5). A significant —though weak— inverted, non-linear relationship was found between sponge size (in mL) and the maximum

velocity of DSi consumption (empirical V_{max}) in both *T. citrina* (n= 33, r^2= 0.24, p= 0.004; Fig. 5A) and *H. perlevis* (n= 34, r^2= 0.12, p= 0.040; Fig. 5B). Typically, the highest V_{max} was achieved by the smallest individuals. This pattern was not surprising because the smaller an individual, the more incomplete its skeletal growth and, therefore, the larger its DSi needs to complete the siliceous skeleton.

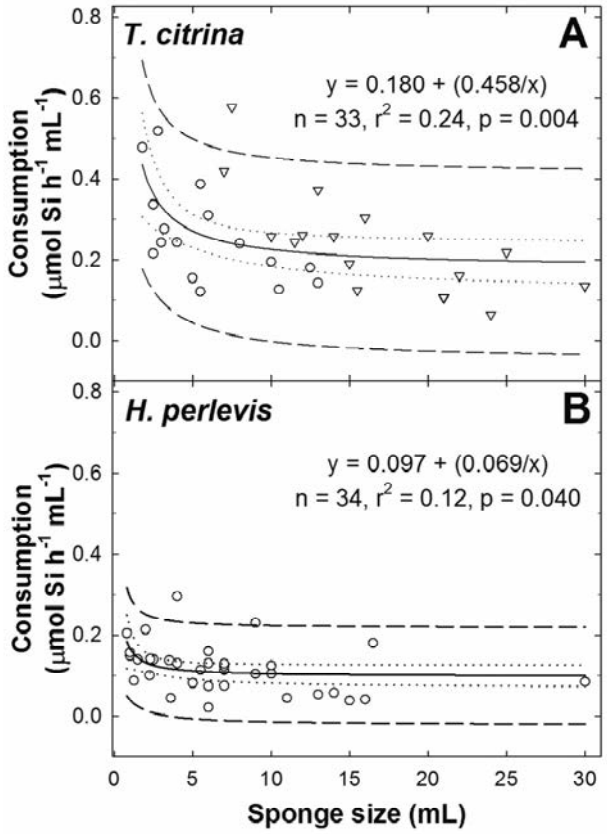


Figure 5. Relationship between sponge volume (mL) and maximum DSi consumption rate (µmol Si h^{-1} mL^{-1}) calculated for a set of 33 individuals of *Tethya citrina* individuals (**A**) and 34 of *Hymeniacidon perlevis* (**B**). The size range spanned by the assayed individuals is representative of that occurring in the wild population. In *T. citrina* (**A**), those individuals that were engaged in the production of buds for asexual reproduction are indicated by inverted triangles. Dotted and dashed lines refer to the 95% confidence band and the prediction band of the regression equations, respectively.

In the species *T. citrina*, the individuals that were budding asexually were also the biggest sponges (Fig. 5A), therefore size and asexual reproduction became confounding factors and the significance of the latter in the DSi consumption process, if any, was unable to emerge clearly from our approach.

DSi consumption in wild populations

At the Bay of Brest (France), both *H. perlevis* and *T. citrina* are abundant species, with an average biomass of 99.94 ± 122.20 and 33.67 ± 45.07 mL m^{-2}, respectively. The decadal trend in DSi concentration in the bay indicates large monthly shifts (Fig. 1), ranging from average minima of 1.2 ± 0.8 μM (in June) to average maxima of 9.1 ± 2.4 μM (in February). However, there may be transient weekly peaks of up to 20 μM from December to February, depending on year. Our kinetic models indicate that these changes impact on monthly DSi utilization rates by both sponges, but more on *T. citrina*. The effects of accommodating not only the monthly shifts in DSi availability but also the seasonal kinetic shift in DSi consumption detected for *T. citrina* are summarized in Fig. 6.

Our kinetic modelling predicts that in February, when DSi availability is maximum, both sponges show their highest DSi consumption rate (53.65 ± 65.60 μmol Si m^{-2} d^{-1} in *H. perlevis* and 35.54 ± 49.03 μmol Si m^{-2} d^{-1} in *T. citrina*; Fig. 6). However, when DSi concentration decreases to its annual minimum in June, *H. perlevis* and *T. citrina* are predicted to consume DSi at a rate about 6 times lower (8.24 ± 10.08 and 6.47 ± 8.92 μmol Si m^{-2} d^{-1}, respectively) than that in February (Fig. 6). By considering the monthly shifts in DSi availability and the seasonal kinetic adaptation uncovered in *T. citrina*, we have estimated that the populations of *H. perlevis* and *T. citrina* consume annually 1.48 ± 1.81 and 1.01 ± 1.40 x 10^6 moles of Si, respectively. Collectively, they are totaling 2.50 ± 3.21 x 10^6 moles of Si. The DSi utilization by just these two sponge species involves therefore a relatively large amount of the local Si (70.13 ± 90.19 tons of Si y^{-1}), a role that had not been considered in previous Si budget of the Bay (e.g., Ragueneau et al., 2005).

It remains unknown how many species of the bay experience seasonal changes in their DSi consumption kinetics (as *T. citrina* did) and how many

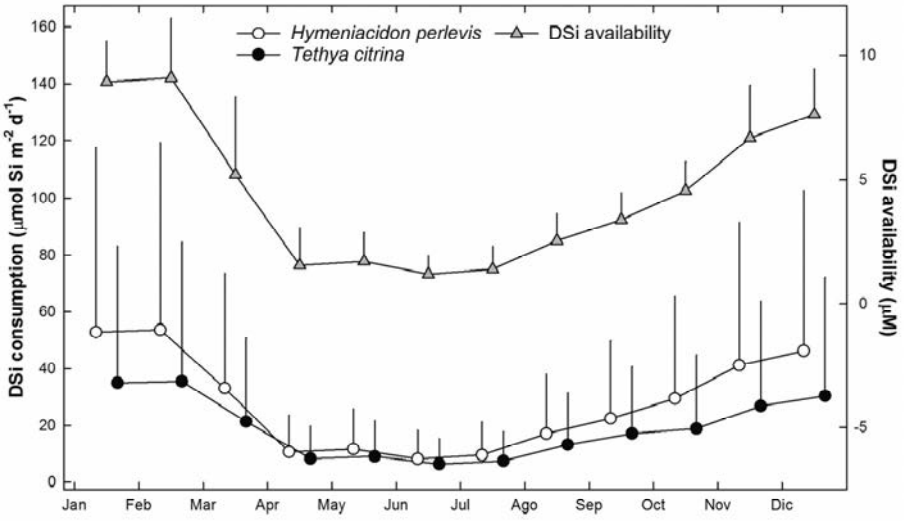


Figure 6. Monthly average (±SD) of DSi consumption rate (µmol Si m^{-2} day^{-1}) by the population of *Hymeniacidon perlevis* and *Tethya citrina* in the Bay of Brest over a year cycle. Monthly average (±SD) of DSi availability (µM) in the water of the Bay is also indicated.

do not (as *H. perlevis* did). If many species are affected seasonally, their collective effect on the global Si budget of the bay can be substantial. For instance, by neglecting the seasonal shift in the kinetics of *T. citrina* and considering only the autumn response for the calculations, we would be underestimating the Si consumed by the population in the Bay in about 9.2% (0.09 x 10^6 moles of Si y^{-1}). In contrast, by considering only the summer kinetics, we would be overestimating DSi utilization by the population in 5.1% (0.05 x 10^6 moles of Si y^{-1}). More importantly, the divergence between the summer and autumn models in *T. citrina* shown in Fig. 3 indicates that the impact of the seasonal differences will become progressively greater wherever the environmental DSi availability is high (>30 µM). Therefore, the potential effects of seasonal changes in the DSi kinetics are predicted to be of critical importance for the calculation of global Si budgets involving sponge assemblages characterized by high DSi availability, as typically happen in high latitude bottoms and in deep-sea environments.

Discussion

The DSi consumption kinetics of *Hymeniacidon perlevis* and *Tethya citrina* fitted a saturable Michaelis-Menten model irrespective of the season (Fig. 3). This pattern is consistent with that shown by all investigated sponges to date. Saturation of the DSi consumption system was identified to occur at DSi concentrations of about 150 μM, a value also consistent with all previous kinetic studies in sponges (Reincke and Barthel, 1997; Maldonado et al., 2011; López-Acosta et al., 2016). The fact that the natural availability of DSi in the sponge habitat (Fig. 1) is two orders of magnitude below 150 μM involves that these sponges can never achieve their maximum velocity for DSi utilization. Consequently, their skeletal growth is chronically limited by low availability of DSi in their habitats. A similar kinetic limitation has also been found in all sponges previously assayed (Reincke and Barthel, 1997; Maldonado et al., 2011; López-Acosta et al., 2016) and its consequences for the skeletal growth empirically demonstrated in one case (Maldonado et al., 1999).

Our results also revealed important between-individual variability in DSi consumption rates in both *H. perlevis* and *T. citrina* (Fig. 4). Irrespective of the season, the smaller specimens were consuming DSi at higher rates (about three times in average) than the bigger ones. Yet the sponge size was only responsible of 12% and 24% of the between-individual differences in DSi consumption rates in *H. perlevis* and *T. citrina*, respectively (Fig. 5).

The statistical approaches made clear that, out of the two assayed sponges, only one, *T. citrina*, experiences seasonal kinetic changes in DSi utilization. In autumn, this sponge consumed DSi at rates 99.54 ± 87.05% higher than in summer (Table 1, Fig. 2). These differences are in part explained by the availability of DSi increasing in autumn, but more importantly because the value of K_m and V_{max} parameters in the DSi consumption kinetics of this species also increase in autumn. This is the first time that such a kinetic seasonal change is demonstrated for Si users other than diatoms. The kinetic change is probably an adaptation to favor skeletal growth during those periods of the year when the natural availability of DSi is greater and enough food for the sponges still occurs in the water column.

Interestingly, despite the utilization of DSi by sponges being a process mediated by enzymes (Shimizu et al., 1998; Krasko et al., 2000), the seasonal

kinetic change in *T. citrina* did not involve a substantial change in its affinity for DSi (i.e., the putative substrate of the enzymes). It was surprising that, being the affinity for DSi mathematically defined as the "V_{max}/K_m" quotient of the Michelis-Menten parameters (Healey, 1980), the affinity for DSi was 29.1 higher in summer (7.2×10^{-3} h^{-1} mL^{-1}) than in autumn (5.5×10^{-3} h^{-1} mL^{-1}). It may be that an increase in affinity is stimulated by a decreasing DSi availability, so that it can compensate for the hindering in DSi utilization triggered by the lowering in DSi concentration. According to our data, the increase in utilization rate appears to be achieved by increasing both the half-saturation concentration (K_m) and the maximum velocity of transport (V_{max}).

The finding that at least some siliceous sponges show seasonal kinetic changes in their utilization of DSi has several implications. First, the seasonal effect is to be considered in future Si budgets involving DSi utilization by sponges. Second, the importance of the effects will be dependent on the number of sponge species in the benthic assemblages responding to seasonality. Such a knowledge can only be achieved by conducting further seasonal experiments on higher number of species. Third, the divergence between the kinetic models of the two assayed seasons increased drastically when DSi availability increased over values of 50 μM (Fig. 3). If a similar pattern is affecting sponge species from high latitudes and deep sea, the consequences for the modelling of DSi utilization can be enormous. Plenty of direct and indirect evidence indicates that dense sponge aggregations established in deep-sea and in Antarctic shelves react to periodic pulses of food and dissolved nutrients entering the sponge habitats (reviewed in Maldonado et al., 2017). Some simple calculations for the siliceous sponge assemblages on the Antarctic shelf (0.87×10^6 km^2) suggest that if those sponges would have a seasonal kinetic shift similar to the one here measured for *T. citrina*, the global impact on the regional budget of DSi utilization would be large. For instance, at the depth range of the Antarctic continental shelf, the DSi availability over a year cycle ranges from mean minimum values of about 50 μM to maxima of about 90 μM, increasing even up to 130 μM if continental slope habitats are considered (Leynaert et al., 1993; Ducklow et al., 2012). For the calculations, we will assume that the average DSi concentration from February to September is about 60 μM and the rest of the year about 75 μM. The average density of siliceous sponges

on the Antarctic shelf has been estimated as 1.27 kg wet weight m^{-2} (Gutt et al., 2013), which by applying the morphometric conversion parameters of *T. citrina* translates into an average volume of 1,550 mL m^{-2}. By extrapolating the low-DSi (summer) kinetics of *T. citrina* to that DSi availability pattern, we would come out with a global DSi consumption for the sponge assemblage of about 1.69 Tmol Si y^{-1} only on the shelf. Under the high-DSi (autumn) kinetics the estimate consumption rises to 2.32 Tmol Si y^{-1}, that is, a 30% increase in relative terms and a huge amount (0.63 Tmol Si) in absolute values. This simple example illustrates the importance of achieving a better understanding of the level at which the DSi consumption kinetics may change over the year cycle for dominant species in sponge grounds of high latitudes, deep sea and any other habitat with marked shifts in DSi availability.

Chapter 4

In situ determination of silicon consumption rates by marine sponges: a benthic-chamber approach

Picture: Detail of an individual of *Tethya citrina* (bay of Brest, France), from Thierry Le Bec.

Abstract

Sponges consume silicic acid (DSi) to build their skeletons, but only a handful of studies have attempted to quantify DSi utilization by these organisms to date. All available determinations are laboratory measurements. Here we measured for the first time DSi consumption rates of a sponge species (*Tethya citrina*) in its natural habitat (the bay of Brest, France), conducting 24h incubations in hermetic benthic chambers. Sponges consumed DSi at an average rate of 0.046 ± 0.018 μmol h^{-1} mL^{-1} when DSi availability in its habitat ranged from 4.9 to 10.1 μM. The *in situ* DSi consumption rates significantly matched the predictions of the kinetic models previously developed for this species in the laboratory, supporting the use of laboratory incubations as a simpler approach to conduct DSi-consumption experiments. We also examined the utilization of other dissolved inorganic nutrients by *T. citrina*, which are related not only with the activity of sponge cells, but also with that of the microbiome living within the sponge body. Our results indicated that *T. citrina*, a sponge species with low microbial abundance (LMA), is a net source of nitrate (-0.067 ± 0.033 μmol h^{-1} mL^{-1}), nitrite (-0.007 ± 0.003 μmol h^{-1} mL^{-1}), ammonium (-0.043 ± 0.031 μmol h^{-1} mL^{-1}), and phosphate (-0.004 ± 0.005 μmol h^{-1} mL^{-1}), in agreement with other studies conducted in LMA sponges. Those rates, some of the lowest reported for sponges, agreed with the low respiration rate recorded during our incubations, 0.35 ± 0.11 μmol O_2 h^{-1} mL^{-1}. Despite consuming those nutrients at low rates, *T. citrina* and other sponges dominating the benthic communities of the bay of Brest may be modulating the nutrient availability in demersal seawater masses. Such effects on nutrient availability are predicted to affect the dynamics of both bacterioplankton and microphytoplankton.

Introduction

Major dissolved inorganic nutrients (i.e., silicate, nitrate, nitrite, ammonium, and phosphate) are far from being at saturating concentration in the photic ocean (Paytan and McLaughlin, 2007; Raimbault et al., 2008; Tréguer and De La Rocha, 2013). Although geochemical processes may be partially responsible for the existing concentrations, a major role is attributed to processes of consumption and recycling by the microphytoplankton and the bacterioplankton (Rivkin and Anderson, 1997; Falkowski et al., 1998; Tyrrell, 1999; Ramírez et al., 2005; Zakem et al., 2018). Very rarely the role of benthic marine invertebrates has been considered as part of this biological modulation. However, there are, for instance, evidence that marine sponges are relevant in the benthic pelagic coupling of some of these dissolved inorganic nutrients (Diaz and Ward, 1997; Jiménez and Ribes, 2007; Maldonado et al., 2012b; de Goeij et al., 2017).

Most sponges consume silicate —or more strictly, silicic acid; hereafter referred to as DSi— from the seawater to produce their silica skeletons. Despite the potential impact that these organisms are suspected to have on local silicon (Si) budgets (Frøhlich and Barthel, 1997; Maldonado et al., 2005; Maldonado et al., 2010; Chu et al., 2011; Maldonado et al., 2011; López-Acosta et al., 2018a), their DSi consumption is still poorly studied.

The available measurements of direct DSi consumption by sponges (i.e., not estimated from assumed growth rates) have consistently been derived from incubations in the laboratory during several hours to days (Frøhlich and Barthel, 1997; Reincke and Barthel, 1997; Maldonado et al., 2011; Maldonado et al., 2012a; López-Acosta et al., 2016; Vicente et al., 2016; López-Acosta et al., 2018a). Some authors have also attempted to determine rates of DSi consumption in sponges *in situ* by measuring the difference in DSi concentration between the seawater going into the sponge and the seawater going out (*In-Ex* approach), but the flux of water through the sponge is so rapid and retention rate of DSi is so small that consumptions were not detectable with reliability (e.g., Perea-Blázquez et al., 2012; Morganti et al., 2017; Leys et al., 2018). Therefore, the incubation approach to determine DSi consumption is forced by logistic constraints. It has been presumed that the physiology of the sponges taken to the laboratory will perform identically to that of individuals in their natural habitats. However, such a hypothesis remains poorly tested (Southwell et al., 2008). Here we have incubated the demosponge *Tethya citrina*, but taking the chambers to their natural habitat rather than taking the sponges to the laboratory. Then, we have compared our *in situ* experimental results with those previously obtained for this species from laboratory incubations (López-Acosta et al., 2018b; under review), providing a first test for "laboratory" versus "field" DSi determinations.

A variety of studies have indicated that, in addition to DSi, sponges release and/or take up other dissolved inorganic nutrients, such as nitrate, nitrite, ammonium, and phosphate (Diaz and Ward, 1997; Bell, 2008; Maldonado et al., 2012b; Ribes et al., 2012; Keesing et al., 2013). Most of these nutrient fluxes are thought to result from a complex combination of processes that include not only the metabolism of the sponge cells but also that of the microbial communities (archaea, bacteria, cyanobacteria, dinoflagellates, etc) that can live, sometimes in large quantities, within the sponge body. Because the fluxes of those nutrients through the sponge body often happen at higher rates than the DSi flux, they have been determined efficiently by both the *In-Ex* approach (Yahel et al., 2005; Southwell et al., 2008; Morganti et al., 2016; Archer et al., 2017; Morganti et al., 2017) and laboratory incubations (e.g., Jiménez and Ribes, 2007; Southwell et al., 2008). However, N and P nutrients have never been determined using benthic

chambers *in situ* that incubate exclusively sponge individuals isolated from the rest of the bottom community. Herein, although our primary objective was to measure DSi fluxes, we have taken the opportunity of these *in situ* incubations to examine whether the assayed sponge species is a net source or sink of nitrate, nitrite, ammonium, and phosphate, discussing our results in the frame of the available previous knowledge.

Methods

Experimental setup

We investigated utilization of dissolved inorganic nutrients by the species *Tethya citrina* (Fig. 1A-B) in the bay of Brest (France). A total of nine incubations were conducted by scuba dive. Eight of them (#1 to #8) were aimed to estimate changes in nutrient concentration in the presence of a sponge individual in the chamber. A ninth incubation chamber served as a control (C), containing only seawater but no sponge. Each incubation unit was absolutely hermetic and consisted of a methyl methacrylate chamber, two sampling bottles of 1 L each, and an adjustable submersible pump connected to a flowmeter, powered by a battery (Fig. 1C; Biscéré et al., 2015). Each unit has a capacity of 7.50 ± 0.37 L of seawater. The water was circulated at 2 L min^{-1}, similar to the prevailing flow in the natural habitat of the sponge in the bay (Martin et al., 2007). Incubations lasted 24h, a period representing a balance between the duration of the battery (about 25h) and a minimum time required to detect reliably changes in nutrient concentration (particularly DSi) due to the sponge activity. In four sponge incubations (#2, 5, 6, and 7) and in the control chamber, a multiparameter probe (YSI 6920) was included in the incubation unit to record dissolved oxygen (% and mg L^{-1}), temperature (ºC), salinity (ppt), and depth (m) every minute (Fig. 1D). Dissolved oxygen was measured with two purposes. On the one side, we recorded dissolved oxygen to evaluate whether sponges where physiologically active (i.e., respiring) during the incubation period, also to detect whether leakage occurred in the chambers. On the other side, the excellent performance of the oxygen probe during the incubations allowed as to estimate with reliability oxygen utilization rates by the sponges.

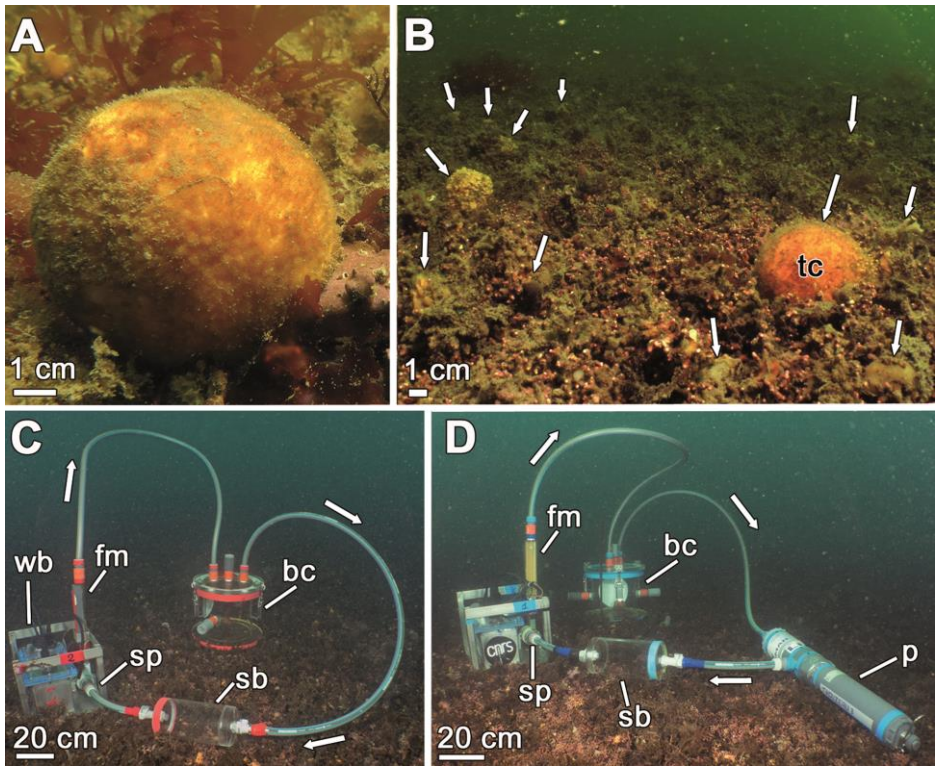


Figure 1. (**A**) View of an individual of *Tethya citrina* growing at its habitat in the bay of Brest. (**B**) General view of the maërl bed of Lomergat, with an important multispecific sponge aggregation, including *T. citrina* (tc). White arrows indicate some of the sponges occurring in the benthos. (**C-D**) Each incubation system consisted of a methyl methacrylate benthic chamber (bc), a submersible pump (sp) connected to a flowmeter (fm) powered by a waterproof battery (wb), and two sampling bottles (sb) hermetically connected to the whole system. Some incubation systems also included a multiparameter probe (p). Seawater flow direction is indicated by white arrows.

For the incubations, chambers were deployed at 6.5 m depth on the maërl bed of Lomergat (48.290º N, 4.346º W) where the sponges abound (Fig. 1B). At the time of the experiments (November 2016), photoperiod was 9:15 light:dark and seawater temperature 11.1 ± 0.5 ºC, with no substantive changes between day and night. When installing the incubation units on the seafloor special attention was paid to avoid resuspension of sediments and interstitial nutrients. Individual sponges, each attached to a

small piece of maërl, were transferred from the surrounding bottom into the chambers. Once the system was deployed and in place, the pump was turned on while keeping the benthic chamber open, circulating ambient water throughout the entire system during 15 mins. This ensured that the water to be used during the incubations was the one in the sponge habitat.

Immediately after the incubations, we transported the sampling bottles and the assayed sponges to the laboratory for nutrient analysis and biomass determination. During these 2h trip back to the laboratory, sponges were kept under seawater at all times and nutrients refrigerated. At the laboratory, sponges were first measured in volume (mL) by the water displacement method, then dried at 60ºC to constant weight (g), and turned into ash at 540º for 10h. Ash-free dry weight (AFDW; g) was also calculated by subtracting the ash weight from the dry weight.

Nutrient analyses

In addition to the utilization of DSi by the sponges, which was our primary objective, we estimated net fluxes of nitrate, nitrite, ammonium, and phosphate over the incubation period. Seawater samples were filtered through a 0.22-μm Whatman-Nucleopore polycarbonate membrane, keeping 50 mL in the fridge prior to DSi determination and freezing 200 mL for subsequent nitrate, nitrite, ammonium, and phosphate determinations. All DSi samples were processed in a single analysis, using a Shimadzu, UV-1700 PharmaSpec UV-VIS spectrophotometer and following the standard colorimetric method (Strickland and Parsons, 1972), with a determination accuracy of 5%. Samples for nitrate, nitrite, ammonium, and phosphate were analyzed using a Technicon Auto-Analyzer (3 HR, SEAL), following the method described by Tréguer and Le Corre (1975), with a determination accuracy of 1%.

Nutrient fluxes were calculated as the value of nutrient concentration in the seawater at the beginning of the incubation minus the value after 24h of incubation, being negative values interpreted as nutrient release and positive values as nutrient incorporation by the sponges. Fluxes values were also corrected by the change in concentration occurring in the control unit. Yet we understand that the incubation approach used is more reliable to estimate DSi utilization than fluxes of N and P nutrients, since modification

of the concentrations of nitrate, nitrite, ammonium, and phosphate might occur during the 24h incubation, as well as conversion of one N compound into another, as a result of re-filtration, the metabolic activity of the phytoplankton and bacterioplankton in the chambers and, more importantly, the microbiome within the sponge tissue.

Utilization of nutrients and oxygen was normalized by seawater volume in the incubation unit (L), duration of incubation (h), and sponge size (mL). We preferentially used sponge volume for normalization because it facilitates future comparisons without the need of using destructive sampling on the sponge communities. The normalized rates of DSi consumption obtained from the *in situ* incubations were compared with the consumption rates predicted from two kinetic models previously developed by us through laboratory incubations (López-Acosta et al., 2018b; under review). The two models used for the comparison incorporate the variability in the DSi consumption kinetics in *T. citrina* under different seasonal conditions (summer vs autumn).

Finally, we examined the potential pairwise relationships between the fluxes of the different nutrients in the pool of the assayed individual (n=8), using linear and non-linear regression.

Results

Oxygen utilization

Oxygen measurements revealed that the control unit slightly produced O_2 during daytime due to photosynthesis (n=5; -1.26 ± 4.37 μmol O_2 h^{-1}) while consumed oxygen during nighttime by respiration (n=18; 7.35 ± 5.54 μmol O_2 h^{-1}). This pattern suggests that the microphytoplankton and the bacterioplankton trapped in water of the chamber kept active during the incubation (Fig. 2). In the incubation units containing sponges, the phytoplankton photosynthesis was initially able to compensate the sponge respiration (n=4; -2.39 ± 1.98 μmol O_2 h^{-1}) but soon all units showed a net oxygen consumption (n=80; 13.73 ± 5.95 μmol O_2 h^{-1}; Fig. 2), a pattern probably favored because the sponges ate at least part of the available microphytoplankton in the chamber, but also because the microphytoplankton cells get damaged when going repetitively though the

water pump. This later possibility is also in agreement with the fact that the control unit produced oxygen during a first period of daylight (11:30 to 16:30) but not during the last daylight period (8:30 to 10:30).

The average oxygen consumption rate by the sponges over the incubation period and after correcting by the control was 0.35 ± 0.11 μmol O_2 h^{-1} sponge-mL^{-1} (n=4). The individual respiration rates by sponge #5, 2, 7, and 6 were relatively similar, being 0.22, 0.33, 0.38, and 0.48 μmol O_2 h^{-1} sponge-mL^{-1}, respectively. To compare oxygen utilization by sponges with that due to the plankton in the chambers, we converted utilization rates into comparable units: μmol O_2 h^{-1} within the incubation system. The comparison revealed that sponges were responsible for about 55 ± 15 % (n=4) of the total oxygen utilization, at an average rate of 6.92 ± 3.45 μmol O_2 h^{-1} (n=4) while the plankton community in the chambers was responsible of consuming the remaining 45 ± 15 % O_2 (n=4), at a rate of 5.77 μmol O_2 h^{-1}. More importantly, the data corroborated that the sponges assayed were all healthy and with similar levels of physiological activity during the incubations.

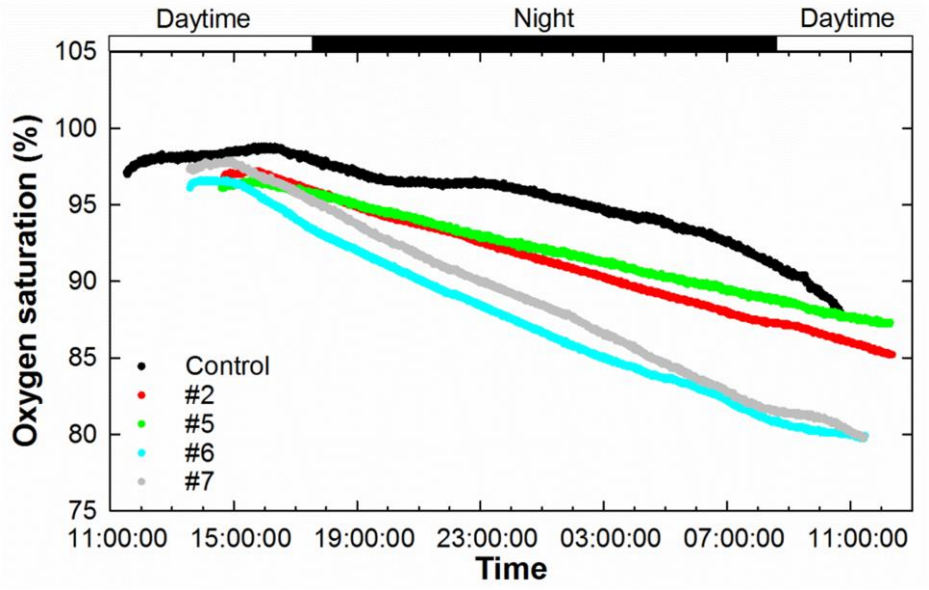


Figure 2. Oxygen saturation profiles over time of incubation. Dissolved oxygen saturation (%) variability was plotted as a function of the incubating time. Oxygen was measured every minute in five out of nine incubations: four sponge incubations (#2, 5, 6, and 7) and the control.

Silicate utilization

It took us around two weeks (from November 15 to 28, 2016) to complete the 9 incubations in the field. During those two weeks, important river discharges occurred at the bay after heavy rains, causing a progressive increase in the natural DSi availability from 4.9 to 10.1 μM (Table 1). The control unit showed no detectable change in DSi concentration during the incubation (Table 1), indicating that diatoms were scarce or non-active during the period of incubation, or even progressively killed when circulating through the water pump. However, the individuals of *Tethya citrina* consumed DSi at an average rate of 0.046 ± 0.018 μmol Si h^{-1} mL^{-1} over the incubation period (Table 1). Note that the increase in DSi in the natural habitat slightly increased the consumption rates through the different incubations over November (Table 1). This result was expected, since it has been demonstrated that DSi consumption in sponges is not linearly related to DSi availability, but related according to a hyperbolic function (see Reincke and Barthel, 1997; Maldonado et al., 2011; López-Acosta et al., 2016; López-Acosta et al., 2018a). Despite the progressive increase in DSi availability over the two weeks of field incubations, the consumption rate of the assayed individuals perfectly matched (Fig. 3) the values predicted by the models elaborated from laboratory incubations of *T. citrina* (López-Acosta et al., 2018b, under review). The consumption rate of all assayed individuals fell within the 95% confidence interval of the laboratory kinetic models for DSi consumption in *T. citrina*, irrespective of considering the kinetic model for summer or autumn. Also, the average consumption value of the set of assayed individuals fell exactly on top of the summer prediction line and its standard deviation value overlapped the autumn prediction line (Fig. 3B). Thus, the agreement between laboratory and field determinations of DSi consumptions rates is evident.

N and P utilization

The incubations indicated that all sponges were net sources of nitrate and nitrite. The availability of nitrate during the two weeks of experimentation shifted from 5.0 to 12.2 μM in the natural habitat, while the variation was minimal in the case of nitrite (from 0.2 to 0.4 μM). Individual utilization rates of nitrate ranged from -0.022 to -0.122 μmol h^{-1} mL^{-1}, and those of

Table 1. Summary of the nutrient fluxes determined during *in situ* investigation in the species *Tethya citrina*. Initial concentration (μM) of dissolved inorganic nutrients (silicic acid - DSi, ammonium - NH_4^+, nitrate - NO_3^-, nitrite - NO_2^-, phosphate - PO_4^{3-}) at each incubation system and the utilization rate (μmol h^{-1} mL^{-1}) after 24h of incubation are indicated. The size (in mL) of the assayed sponges is also indicated. Sponge nutrient utilization rates are corrected by those of the control. The average (±SD) utilization rate by the assayed sponges is indicated in the lower part of the table.

Code	Date	Sponge size	Initial nutrient level					Nutrient utilization				
			DSi	NH_4^+	NO_3^-	NO_2^-	PO_4^{3-}	DSi	NH_4^+	NO_3^-	NO_2^-	PO_4^{3-}
		(mL)	(μM)					(μmol h^{-1} mL^{-1})				
C	11.15.16	-	4.9	0.8	5.1	0.3	0.5	0.000[a]	0.003[a]	0.026[a]	0.002[a]	0.002[a]
1	11.15.16	20	4.9	0.9	5.0	0.3	0.5	0.039	-0.044	-0.022	-0.012	-0.001
2	11.22.16	18	7.8	1.8	6.1	0.3	0.5	0.035	-0.012	-0.059	-0.005	-0.004
3	11.22.16	17	7.8	1.6	6.0	0.3	0.4	0.031	0.023	-0.042	-0.002	0.001
4	11.22.16	21	7.8	1.5	5.9	0.3	0.4	0.026	-0.066	-0.049	-0.004	0.001
5	11.22.16	11	7.8	1.6	5.2	0.2	0.4	0.082	-0.079	-0.122	-0.008	-0.012
6	11.28.16	19	10.1	1.8	12.2	0.4	0.6	0.049	-0.024	-0.071	-0.011	-0.007
7	11.28.16	27	10.1	1.5	11.1	0.4	0.5	0.061	-0.075	-0.064	-0.006	-0.008
8	11.28.16	13	10.1	1.7	11.5	0.4	0.6	0.042	-0.002	-0.104	-0.009	-0.002
AVRG								0.046	-0.043*	-0.067	-0.007	-0.004
SD								0.018	0.031*	0.033	0.003	0.005

[a] Nutrient utilization rates in the control are measured in μmol h^{-1}.

* Average (±SD) ammonium utilization rate was calculated considering the seven sponges that consistently released ammonium.

nitrite, from -0.002 to -0.012 μmol h^{-1} mL^{-1}. Average utilization rates of nitrate and nitrite were -0.067 ± 0.033 and -0.007 ± 0.003 μmol h^{-1} mL^{-1}, respectively (Table 1).

Regarding the ammonium, the natural availability during the two weeks of experiments doubled, from 0.9 to 1.8 μM. All sponges were net sources of ammonium, except individual #3, which consumed this nutrient at a rate of 0.023 μmol h^{-1} mL^{-1} (Table 1). The rest of the sponges released ammonium at a rate of -0.043 ± 0.031 μmol h^{-1} mL^{-1}.

The natural concentration of phosphate shifted from 0.4 to 0.6 μM over the two-week period of the experiments. This is the only nutrient for which the sponges showed no consistent pattern in the sign of the flow, with 6 individuals being net sources (-0.006 ± 0.004 μmol h^{-1} mL^{-1}) and 2 others being net sinks (0.001 ± 0.000 μmol h^{-1} mL^{-1}). Interestingly, when the

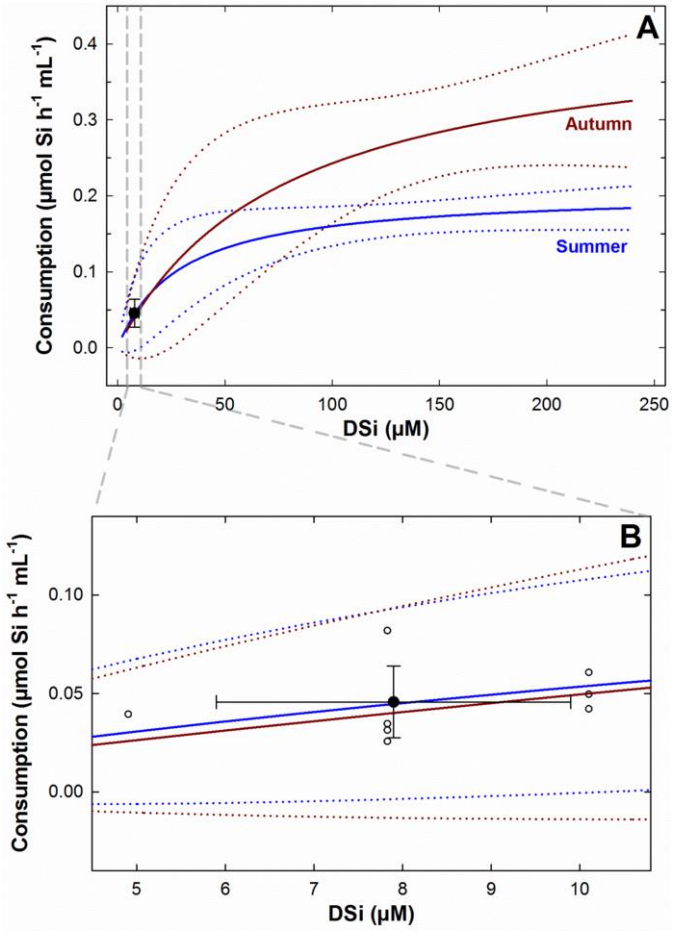


Figure 3. Comparison between field and laboratory determinations of DSi consumption rates in *Tethya citrina*. (**A**) The average (±SD) rate of DSi consumption determined in this study by *in situ* incubations perfectly matched the hyperbolic models (solid lines) determined in the laboratory for *T. citrina* in summer and autumn (López-Acosta et al., 2018b, under review). (**B**) The individual and the average (±SD) rates of DSi consumption determined *in situ* (white and black circles, respectively) are within the 95% confidence band (dotted lines) of both the summer and autumn kinetic models.

pairwise relationships between the nutrient fluxes (DSi, nitrate, nitrite, ammonium, and phosphate) were examined (Fig. 4), no statistically significant relationship was found, except for one case: the increase in the rate of phosphate release increases linearly ($p < 0.001$, $R^2 = 0.87$) with the

increase in the rate of silicate consumption (Fig. 4D). The only two individuals (#3 and 4) showing phosphate consumption were also the ones having the lowest DSi consumption rates. The biological reasons behind the relationship between the phosphate and DSi fluxes remain enigmatic.

Discussion

This study provides the first determination of DSi consumption by a marine sponge using *in situ* incubation chambers deployed in the natural habitat. The set of *Tethya citrina* individuals assayed at the bay of Brest consumed, on average, 0.046 ± 0.018 μmol Si h^{-1} mL^{-1} (Table 1). Despite a substantial rising of the natural DSi availability (from 4.9 to 10.1 μM) during the period of field incubations, all individual responses in the field fell well within the 95% confidence interval predicted by laboratory-based kinetic models for DSi consumption (Fig. 3). Indeed, field consumption was efficiently predicted by both the summer and autumn kinetic models because, under a DSi availability lower than about 15 μM (a value about the maximum DSi concentration measured in the bay at some days of the year), both summer and autumn models largely overlap (Fig. 3). This match between laboratory-based predictions and field incubations clearly corroborates that DSi consumption experiments can reliably be conducted through laboratory experiments, which simplifies enormously the logistic approach compared to field underwater incubations.

For nutrients others than DSi, some studies have been reported that artifactual nutrient utilization rates may be determined when sponges are studied out of their natural habitat and that *In-Ex* approaches may be more realistic (Ribes et al., 2000; Yahel et al., 2005; Southwell et al., 2008). Unlike for DSi, the fluxes of N and P may be due not only to the sponge cell metabolism, but also to the microbial communities within the sponge tissues. Those sponge-associated microbiomes have the capacity to readily convert one N nutrient into another, depending on the sponge species and the aerobic level of the tissue (Schlappy et al., 2010). This combination of factors may complicate the interpretation of measured net rates for N and P nutrients, particularly if incubation periods as long as those required to detect DSi consumption (i.e., 24 h or longer; López-Acosta et al., 2018a) are also used for coupled determination of N and P fluxes. However, it can be

assumed that *T. citrina* is a low microbial-abundance sponge (LMA), as it is also known for its congener *Tethya aurantium* (Gloeckner et al., 2014). Therefore, the microbiome is expected to have interfered minimally with the detected fluxes in *T. citrina*. To date, most studies on N fluxes have been conducted on shallow-water, high-microbial abundance (HMA) sponges (revised in Maldonado et al., 2012b). These sponges typically remove ammonium from seawater (as a N source for chemo and phototrophic bacteria and energy source for ammonium oxidizing bacteria and archaea)

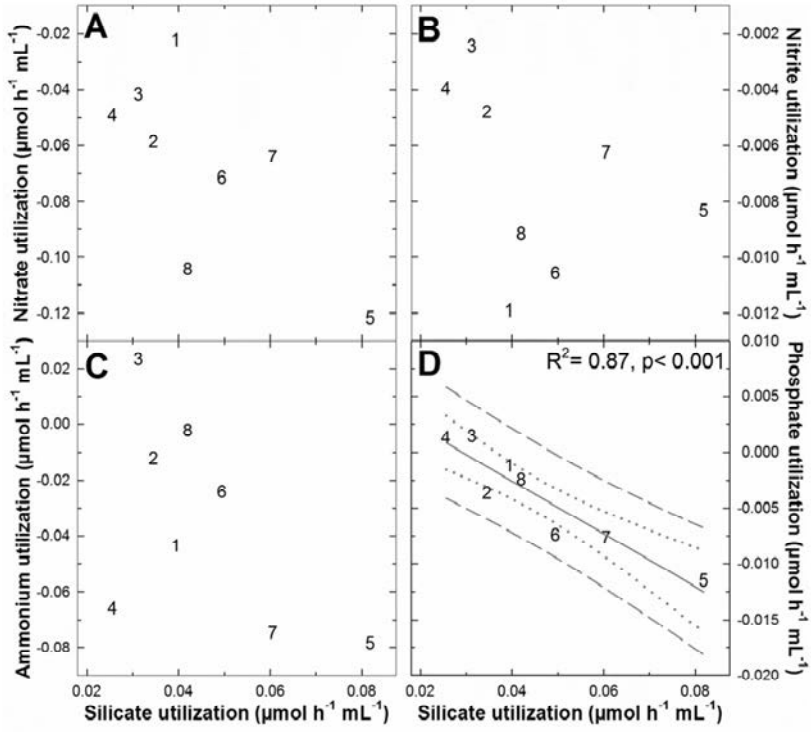


Figure 4. Pairwise relationship between the nutrient fluxes measured in *Tethya citrina*. No statistically significant relationship was found between silicate utilization rates and nitrate (**A**), nitrite (**B**), and ammonium (**C**) utilization rates. Contrastingly, the utilization of silicate by *T. citrina* was linearly related to that of phosphate (**D**), with individuals releasing phosphate at higher rates when silicate consumption rates increased. Note that although only the relationships between DSi and the other nutrients are illustrated here, the relationships between all other possible nutrient pairs have also been examined with negative results (data not shown).

and release nitrate and nitrite, as probable result of nitrification (Corredor et al., 1988). However, most LMA sponges have been identified to be mostly net sources of both nitrate and nitrite, but also ammonium (Jiménez and Ribes, 2007; Yahel et al., 2007; Southwell et al., 2008; Ribes et al., 2012; Morganti et al., 2017). Therefore, regarding the sign of the N nutrient fluxes, the results of our incubations (Table 1) fully agree with the results of previous literature on LMA shallow-water sponges. In the case of P fluxes, the few available data in the literature suggests that sponges are net sources of this nutrient (Hatcher, 1994; Jiménez and Ribes, 2007; Ribes et al., 2012; Morganti et al., 2017). Again, our results are in agreement (Table 1). The mechanisms governing the release of phosphate and its inverted relationship to DSi consumption (Fig. 4D) remains enigmatic (Taylor et al., 2007; Maldonado et al., 2012b), although potential participation of sponge-associated actynomicete bacteria could be involved (Sabarathnam et al., 2010).

The comparison of the rates at which N and P effluxes occur in *T. citrina* becomes a more complicated issue for the disparity of approaches and the irreconcilable diversity of units in which rates are expressed. We are here considering only data in which N fluxes are expressed in μmol N per hour and sponge-mL. Rix (2015), using 3h laboratory incubations for five LMA sponges, has reported average efflux rates for ammonium (-0.079 ± 0.018 μmol h^{-1} mL^{-1}) and nitrate+nitrite (-0.023 ± 0.014 μmol h^{-1} mL^{-1}) similar to the ones we have estimated in our 24h field incubations (-0.043 ± 0.031 and -0.074 ± 0.033 μmol h^{-1} mL^{-1}, respectively; Table 1). Jimenez and Ribes (2007), who incubated the LMA *Dysidea avara* in the laboratory for 6 h, reported an average release rate of -0.109 μmol NH_4^+ h^{-1} mL^{-1}, but no detectable flux for nitrate+nitrite. The two studies that used the *In-Ex* approach to measure ammonium utilization in shallow-water LMA sponges have reported releasing average rates that ranged from -0.199 ± 0.012 μmol h^{-1} mL^{-1} to -0.260 ± 0.110 μmol h^{-1} mL^{-1} (Southwell et al., 2008; Morganti et al., 2017). That method (*In-Ex*) has been also employed to determine releasing rates of nitrate+nitrite in 5 HMA (*Agelas conifera*, -0.150 ± 0.134 μmol h^{-1} mL^{-1}; *Aplysina archeri*, -0.097 ± 0.041 μmol h^{-1} mL^{-1}; *Aplysina lacunose*, -0.116 ± 0.051 μmol h^{-1} mL^{-1}; *Ircinia strobilina*, -0.260 ± 0.204 μmol h^{-1} mL^{-1}; *Xestospongia muta*, -0.168 ± 0.094 μmol h^{-1} mL^{-1}; Southwell et al., 2008). However, the same study reported a controversial disparity between

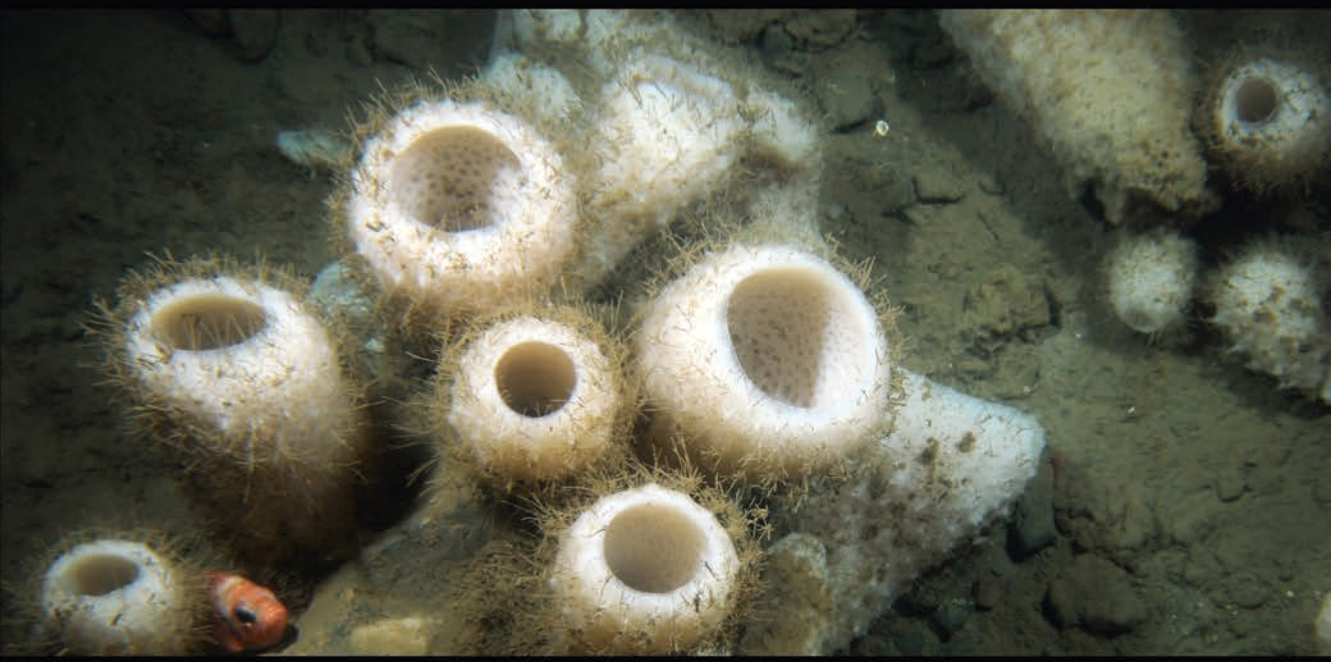
ammonium consumption and release across the individuals within the species that renders the resulting average rate unreliable for comparison (Southwell et al., 2008; see their Table 2). The above rates reported in HMA sponges are about one order of magnitude larger than the ones calculated in our species and other LMA sponges. In summary, the release rates of N nutrients in *T. citrina* are among the lowest calculated for LMA sponges, suggesting that the metabolism of both the microbiome and the sponge cells themselves must be slow, a hypothesis that also comes into agreement with the relatively low respiration rate (see below).

The monitoring of changes in oxygen concentration in the incubations units revealed that the assayed sponges consumed on average 0.35 ± 0.11 μmol O_2 h^{-1} sponge-mL^{-1}. This rate is comparable to those measured in the other two species of the same genus: 0.89 and 0.23 μmol O_2 h^{-1} sponge-mL^{-1} in *T. crypta* (Reiswig, 1974) and *T. californiana* (Ludeman et al., 2017), respectively. The respiration values known for demosponges range from 0.21 to 24.6 μmol O_2 h^{-1} sponge-mL^{-1} (reviewed in Osinga et al., 1999), so that the respiration values in *Tethya* spp. fall among the lowest ones measured for sponges to date. This result is also in congruence with previous studies indicating that respiration in LMA sponges is consistently lower than in HMA sponges (e.g., Reiswig, 1974, 1981).

Because the species *T. citrina* is one of the most abundant species in the rich sponge assemblage of the bay of Brest (López-Acosta et al., 2018a), the consumption of DSi along with the release of ammonium, nitrate, nitrite, and phosphate may modulate to some extent nutrient availability at the deep-boundary layer, particularly during periods when some of these nutrients become limiting in the water column for primary producers. In general terms, the benthic pelagic-coupling of essential nutrients mediated by sponges may therefore have a battery of still little investigated positive and negative effects on the demersal and benthic populations of bacterioplankton and microphytoplankton. Here we show that the *in situ* incubation approach can be a useful tool for further investigating those multiple ecological interactions.

Chapter 5

First kinetic model of silicate consumption by a hexactinellid sponge



Picture: Ground of *Vazella pourtalesii* in Nova Scotia, Canada; copyright of DFO.

Abstract

Sponges are silicon-consuming organisms whose role in the marine silicon (Si) cycle remains little understood because of the scarcity of studies, compared to the case of diatoms. Furthermore, the few available studies focus only on sponges of the class Demospongiae, while information is inexistent for the Class Hexactinellida, a lineage of heavily silicified sponges that usually form dense aggregations in the deep ocean and are suspected to consume large amounts of Si as silicic acid (DSi). Here the kinetics of DSi consumption is investigated for a hexactinellid sponge (*Vazella pourtalesii*) for the first time, following collection of individuals by Remote Operated Vehicle and incubation in controlled laboratory conditions. The results indicated that the rates of DSi consumption increased with increasing availability of DSi in the seawater, following a Michaelis-Menten kinetics, as it also happens in all demosponges investigated so far. The kinetic model is characterized by a V_{max} of 0.093 ± 0.015 μmol Si h^{-1} sponge-mL^{-1} and a K_m of 44.54 ± 24.37 μM DSi. The maximum velocity of DSi transport (V_{max}) is the lowest recorded to date in sponges, which indicates a relatively slow production rate for the heavy silica skeleton characterizing this species (75.4 ± 6.4 % of the dry weight). It also stresses a strong dependence of this sponge on high ambient DSi concentrations in order to achieve sensible rates of skeletal production, in agreement with previous suggestions that the availability of DSi in seawater is a factor that controls the bathymetric and latitudinal distribution of hexactinellid sponges.

Introduction

The biological consumption of silicic acid —a nutrient often alluded to inaccurately as silicate and hereafter referred to as DSi— is one of the key factors controlling the availability of this dissolved compound. Such an availability modulates both ocean primary productivity (Nelson et al., 1995; Tréguer et al., 1995) and sequestration of atmospheric CO_2 by means of both biological (Harriss, 1966; Tréguer and Pondaven, 2000) and chemical processes (Mackenzie and Garrels, 1966; Rahman et al., 2017).

So far the biological consumption of DSi in the global ocean has been modeled considering virtually only the use that diatoms —the most important DSi consumers in the ocean— make of this nutrient (Nelson et al., 1995; Tréguer et al., 1995; Laruelle et al., 2009; Tréguer and De La Rocha, 2013). Other DSi consumers, such as marine siliceous sponges, are however emerging as important contributors (e.g., Frøhlich and Barthel, 1997; Maldonado et al., 2011; López-Acosta et al., 2016; López-Acosta et al., 2018). Nevertheless, the above-mentioned studies and some others provide information only about how sponges in the Class Demospongiae use DSi. The other large Class of siliceous sponges, the Hexactinellida or "glass sponges", remains largely uninvestigated, with a single attempt to estimate

DSi consumption (Chu et al., 2011), which was not based on direct determinations of nutrient incorporation but on extrapolation from estimated growth rates. It is particularly important to start understanding how sponges in the class Hexactinellida use DSi for several reasons: 1) these sponges are expected to make a significant consumption of the DSi, since they typically live in DSi-rich, deep-sea environments and appear to use the high DSi availability to build important siliceous skeletons that represent over 80% of the sponge dry weight (Barthel and Tendal, 1993), which may consist of dense skeletal frameworks and incorporate giant (> 1 cm long) siliceous spicules; 2) hexactinellid sponges are known to form extensive aggregations with high density of individuals (Maldonado et al., 2017), so that they are expected to have an impact on the Si budgets at local and regional scales (Maldonado et al., 2010; Chu et al., 2011; Maldonado et al., 2012; Gutt et al., 2013); and 3) the available information for demosponges indicates that these sponges consume DSi following a Michaelis-Menten kinetic model (Frøhlich and Barthel, 1997; Maldonado et al., 2011; López-Acosta et al., 2016; López-Acosta et al., 2018), but it remains unknown if this kinetics also governs the process in hexactinellids. Differences in the use of DSi between demosponges and hexactinellids cannot be *a priori* discarded, since it has been found out that the silicification process in demosponges revolves around the activity of the silicatein enzyme (Shimizu et al., 1998), while that in hexactinellids appears to occur through an unrelated enzyme, the glassin (Shimizu et al., 2015).

In the frame of an H2020 research project (SponGES) aimed to investigate the biology of North-Atlantic deep-sea sponge aggregations, we are herein providing the first kinetic model for DSi consumption for an hexactinellid sponge —*Vazella pourtalesii* (Schmidt, 1870)— based on long-term laboratory incubations.

Methods

The species and its habitat

Vazella pourtalesii, commonly known as Russian hat, is a rossellid hexactinellid, known from deep-water (from about 100 to 600 m) on the northwestern Atlantic, with rare presence on the northeastern side. On

Nova Scotia (Canada), the species forms extensive aggregations at the deep region of the continental shelf (120 to 160 m deep). The aggregations are nearly monospecific (Fig. 1A), consisting of abundant vase-shaped individuals, measuring up to about 40 cm in height. Some areas on the Scotian shelf where the sponges aggregate —"*Vazella* grounds"— have recently been closed to benthic fishing to protect the sponge communities.

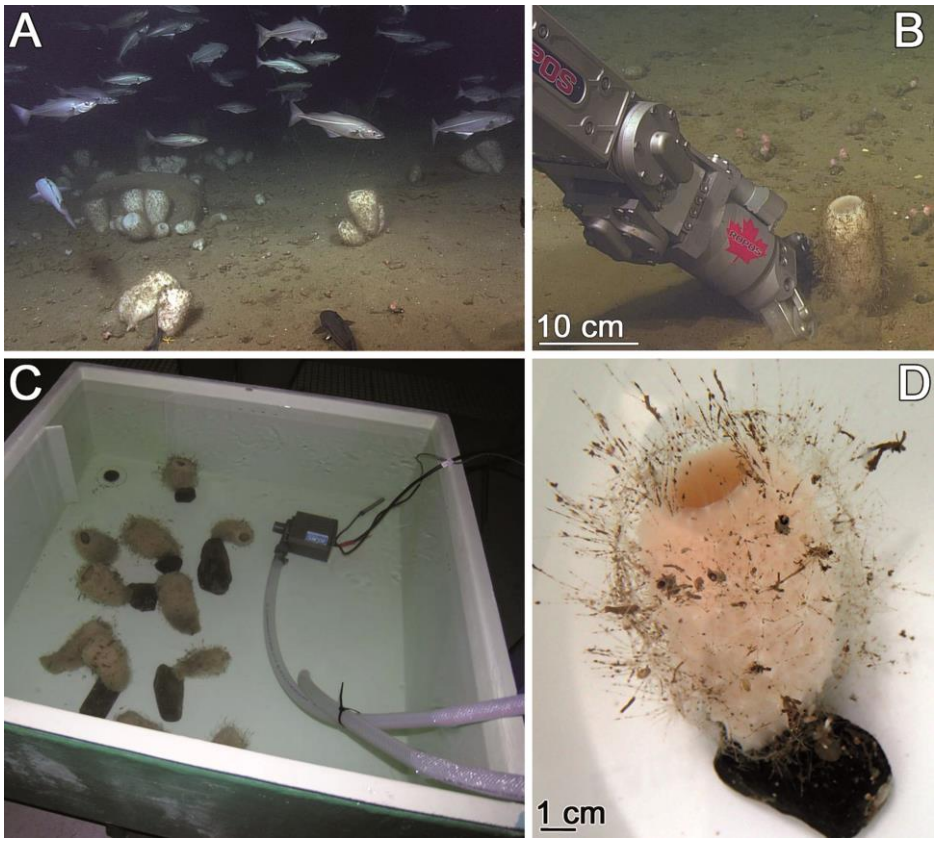


Figure 1. (**A**) General view of the sponge aggregation at Nova Scotia shelf (Canada), where the hexactinellid *Vazella pourtalesii* dominates the benthos. The picture was taken at Sambro Bank, a closure area for benthic fisheries; note the density of fishes occurring there. (**B**) Detail of the ROV-ROPOS arm collecting an individual of *V. pourtalesii* from the rock substratum to prevent any sponge damage. (**C**) View of the "resting" 360L tank used during the laboratory experiment to assess DSi consumption kinetics in *V. pourtalesii*. (**D**) Macro view of an assayed individual at the kinetic experiment within the incubating aquarium.

Kinetics of DSi consumption

For estimating the kinetics of DSi consumption, a total of 11 sponge individuals were collected using the Remote Operate Vehicle (ROV) "ROPOS" on the Canadian Coast Guard Vessel "Martha L. Black". Each sponge was collected along with the small piece of rock on which it was attached, so that the ROV manipulator arm never grabbed the sponge but just the rock (Fig. 1B). Once on board, sponges were kept for 5 days in a 700 L tank filled with seawater refrigerated to 9 ± 1 ºC (the temperature at the sponge habitat during the time of the sampling), re-circulated continuously, and exchanged every 8 hours. Upon arrival to harbor, sponges were transferred to a 360 L tank (hereafter referred to as "resting tank"; Fig. 1C) and left there for 24h for acclimation to a closed and refrigerated (9 ± 0.5 ºC) seawater system with recirculation at the Bedford Institute of Oceanography (DFO, Darmouth, Canada).

For determination of the DSi consumption kinetics (which was carried out in September 2017), consumption by each sponge was measured under increasing DSi availability until the sponges showed saturation, that is, the point at which an increase in DSi availability did not stimulate in the sponges any further increase in the rate of DSi consumption. The intended DSi concentrations were 10 (approx. field values), 30, 60, 100, 150, 200, and 250 μM. At each DSi concentration, the sponges (n= 11) were incubated separately in a polypropylene 16L aquarium for 24h (Fig. 1D). Before being subjected to a subsequent incubation step in the small aquaria, sponges were transferred to the 360-L tank for an additional resting period of 24h. The seawater used for the experiments was pumped from Halifax Bay (at 7 ºC) and filtered at 1 μm, a pore size enough small to prevent the pass of planktonic DSi users (e.g., diatoms, radiolarians, etc) but that also permit the pass of at least part of the natural sponge food, i.e., bacterio- and picoplankton. At the initiation of the resting period, 35 mL of a DSi-free, concentrated culture of approx. 10^6 cells/mL of the haptophyte *Isochrisys galbana* were added to the tank. After circulating repetitively through the re-circulation pump, it provided a homogeneous mix of particulate and dissolved organic food to the sponges. We prepared the DSi concentration needed at the experimental step by adding the corresponding volume of a buffered 0.1 M sodium metasilicate (Na_2SiO_3, pH = 10) to the 360L of filtered-seawater contained in the resting tank and mixed the water for 18h

with a submersible pump to ensure complete molecular diffusion before transferring the sponges within the tank for their resting period.

In summary, the experiment consisted on an alternation of "resting" and "incubation" periods over the two weeks that the experiment lasted. The intercalation of resting and incubating steps was mostly aimed to facilitate survival of the sponges across the battery of incubations in the relatively small incubating aquaria. Yet this approach also made very conservative the calculation of DSi consumptions because during a resting step, the sponges were already exposed to the same DSi concentration that would be assayed in the following incubation step in the small aquaria. Therefore, the determination of the DSi consumption rate in the incubating aquaria the next day was carried out after the sponges having been incorporating DSi during the previous 24h to satisfy their DSi demands. Along with the 11 assayed sponges, we used 3 control aquaria for the incubations, each containing seawater and a rock from the sponge habitat similar to the ones used by the sponges as substrate. These control bare rocks were used to correct for potential processes of either DSi release from or precipitation at the rock surface.

With this experimental design, we also sought for: 1) an incubation period (24h) long enough to detect changes in DSi concentrations due to the sponge activity, but short enough to keep the sponges in good conditions; also for 2) the total period of exposure to each DSi concentration (48h) being long enough as to reflect the entire physiological organismal response related to the silicification process, which may require activation of new sets of genes and increase in the production of the involved molecules and silicifying cells (Maldonado et al., 1999).

To determine the rate of DSi utilization by the assayed sponges at each DSi concentration step, a 50mL water sample was collected at the beginning and at the end of each 24h incubating period. Seawater samples, which were collected with acid-cleaned plastic syringes, were immediately filtered through 0.22 μm-pore, polycarbonate syringe filters (Millex-GS Millipore) and stored in the fridge not longer than 2 days prior to analysis. Samples from the same DSi-concentration step were always analyzed together into a single analysis with the Technicon AutoAnalyzer 3 (AA3, SEAL Analytical), available at the nutrient analysis service of Dalhousie

University (Halifax, Canada). Analyses were run for triplicate and followed the standard colorimetric method (Strickland and Parsons, 1972), with a determination accuracy of 5%. Samples of DSi concentrations higher than 60 μM were diluted prior to analysis with artificial seawater prepared at the same salinity that the water samples (35 PSU). The rate of DSi utilization by a sponge at a given DSi availability was inferred from the difference in DSi concentration between the initiation and the end of the incubation and after correcting by the concentration changes (often negligible) occurred in the control aquaria.

At the end of the experiment, we measured the volume (mL) of the assayed individuals and their rock substrata by water displacement; then sponges were dried at 60ºC to constant dry weight (g). Rates of DSi consumption were normalized by volume of seawater in the incubating aquaria after discounting sponge and rock volume (L), time of incubation (h), and volume of the sponge individuals assayed (mL). We preferentially expressed data normalized to sponge volume because it facilitates their future applicability to field sponge populations using ROV images without the need of collecting individuals. Normalized DSi consumption rates (in μmol Si h^{-1} sponge-mL^{-1}) were analysed by non-linear regression and fitted to the model equation better reproducing and with the highest statistical significance the empirical observations on DSi utilization relative to DSi availability.

Body size as source of between-individual variability

Previous studies focused on investigating DSi consumption in demosponges revealed important variability between individual responses of DSi utilization (Frøhlich and Barthel, 1997; Reincke and Barthel, 1997; Maldonado et al., 2011; López-Acosta et al., 2016; López-Acosta et al., 2018). Part of such variability was explained by the differences occurring in body size between the assayed sponges (Maldonado et al., 2011; López-Acosta et al., 2016). Because here we are investigating for the first time DSi consumption in hexactinellids, we explored whether the sponge size of the assayed specimens could be also explaining the between-individual variability detected during the current experiment. To this aim, we used regression analysis to examine the relationship between the body size (in mL) of each

assayed individual and the maximum velocity of DSi transport (in μmol Si h^{-1} mL^{-1}) it attained during the experiment, irrespective of the DSi concentration step at which was achieved.

Results

Kinetics of DSi consumption

Incubation of 11 individuals of *V. pourtalesii* in the laboratory under increasing concentration of DSi in the seawater (see Methods) revealed, on average, an increase in the consumption rate of the sponges with increasing availability of DSi in the seawater. The rate increase was maintained until DSi concentration reached a value of about 100 μM (Fig. 2). Over that threshold, the consumption rate became more or less constant despite further increases in DSi availability, a response indicating saturation with DSi of the consumption system of the sponges. The general course of the consumption rate with increasing DSi availability fitted the mathematical model of a Michaelis-Menten kinetics (Fig. 2; r^2= 0.83, *p*= 0.002, n= 11), with a maximum transport velocity (V_{max}) of 0.093 ± 0.015 μmol Si h^{-1} sponge-mL^{-1} and a half-saturation constant (K_m) of 44.54 ± 24.37 μM DSi. The fitting to this model involves that the velocity of maximum DSi transport occurs at concentrations of around 100 μM DSi, which is a value about an order magnitude higher than the average concentration (10 μM) occurring at North-Atlantic depths where *V. pourtalesii* is known to occur. Also the half-saturation constant of the kinetics (the concentration at which half of the maximum DSi transport is achieved) is a value about 4 times higher than the natural DSi availability to these sponges (44.54 versus 10 μM DSi). Altogether, it means that these sponge populations are suffering a chronic DSi shortage that does not allow them to unfold their full kinetic capabilities to achieve their maximum DSi consumption rates for skeletal growth. The exact skeletal consequences of this limitation by DSi availability are beyond the scope of this study, but it has been shown for demosponges that a chronic shortage of DSi shortage decreases the average size of the spicules and prevents the formation of some spicule types (Maldonado et al., 1999).

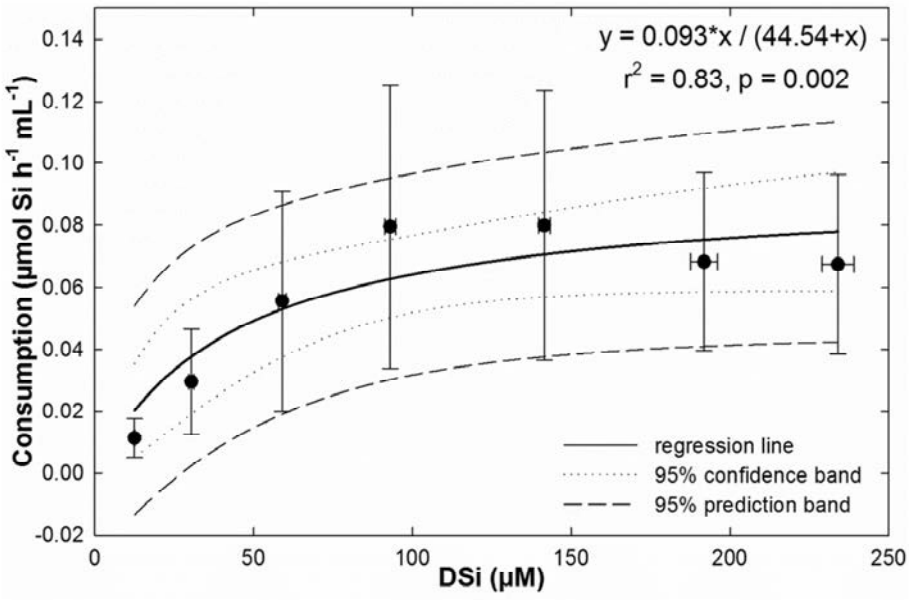


Figure 2. Silicon consumption kinetic model of the hexactinellid *Vazella pourtalesii*. The function better fitting the relationship between the average DSi consumption and the ambient DSi availability is the hyperbolic Michaelis-Menten kinetic model: "Consumption = V_{max} * [DSi] / (K_m+[DSi])", being the V_{max} the maximum velocity of DSi transport and the K_m the half-saturation parameter. Both the specific equation and the statistics of the model are indicated in the upper right corner of the graph.

Individual DSi consumption rates

The examination of the individual responses regarding saturation also indicated that when DSi availability rose over 100 µM, the rate of consumption remained virtually constant (individuals #1-8), except for three sponges (ind. #9, 10, 11), which experienced a slight decrease in their DSi consumption rate (Fig. 3). These three latter sponges were among those that consumed more DSi during the previous steps of the experiment. Thus, it is reasonable to think that, when these sponges were exposed to the saturating concentration, they still did not complete the internal processing of the large DSi amounts incorporated during earlier incubation steps and, consequently, their DSi utilization rate slightly slowed down.

Inspection of the individual DSi consumption responses revealed some between-individual differences in the maximum velocity of DSi utilization (Fig. 3). The highest V_{max} recorded, 0.166 μmol Si h^{-1} mL^{-1}, achieved by individual #9, was nearly 7 times the lowest one, 0.025 μmol Si h^{-1} mL^{-1}, achieved by individual #2. For logistic reasons the individuals assayed in the laboratory for DSi consumption do not represent the whole size spectrum of the natural population, but only a relatively modest variety of sizes in the mid range (6.5 to 11.2 cm in height and 40 to 190 mL in volume). Even so, when body size was confronted with the maximum DSi consumption rates displayed, the relationships indicate that body size differences were responsible for about half of the variability detected between the individuals, following an exponential-decay function ($n = 11$, $r^2 = 0.58$, $p = 0.006$; Fig. 4). Collectively, the group of smaller sponges (< 80 mL) attained, on average, higher V_{max} ($n = 7$, 0.108 ± 0.039 μmol Si h^{-1} mL^{-1})

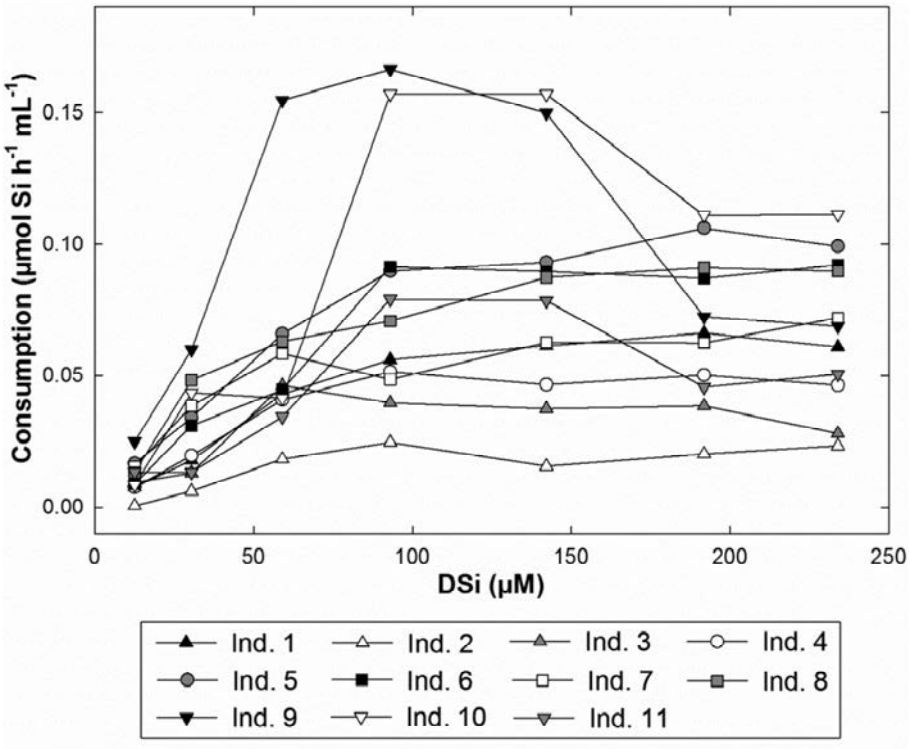


Figure 3. Consumption rates (μmol Si h^{-1} mL^{-1}) of each individual (ind.) of *Vazella pourtalesii* as a function of silicic acid (DSi) concentration (μM).

than the group of large sponges (> 110 mL; n = 4, 0.049 ± 0.019 μmol Si h^{-1} mL^{-1}), as corroborated by a t-test (t= 2.847, df= 9, p= 0.019).

Discussion

The assayed sponges of the hexactinellid *Vazella pourtalesii* consumed DSi at increasing rates when DSi concentration increased from natural levels (ca. 10 μM) to about 100 μM. When concentration increased over that DSi level, consumption rates remained virtually constant or slightly decreased (Figs. 2 and 3). Therefore, the threshold of 100 μM DSi appears to be the concentration at which the DSi consumption system of *V. pourtalesii* saturates. Interestingly, all the sponges investigated to date, which included six genera of demosponges (Reincke and Barthel, 1997; Maldonado et al., 2011; López-Acosta et al., 2016; López-Acosta et al., 2018) and one of hexactinellids (this study), revealed that, irrespective of the Class sponges belong to, these organisms saturate their consumption system at DSi concentrations that ranged from 75 to 200 μM DSi. Because those DSi concentrations are only available in deep waters of Indian, Pacific, and Southern Ocean, where average DSi concentration ranges from 100 to 125 μM (Nelson et al., 1995), most sponges would be permanently shortage by DSi when developing their BSi skeletons. Our results also corroborate this idea, being the species *V. pourtalesii* chronically consuming DSi at a rate about twenty times lower than the maximum velocity at which it is adapted to.

Large variability in DSi consumption responses has been recorded between the assayed individuals (Fig. 3). More than 50% of the variability occurring between individual V_{max} (maximum velocity of DSi transport) was explained by the sponge size (Fig. 4), with the smaller individuals consuming DSi at rates significantly higher than the bigger ones. A similar pattern had been identified in most of the demosponges investigated to date (Maldonado et al., 2011; López-Acosta et al., 2016), indicating the importance of selecting sponges with a wide range of body sizes for investigating DSi consumption kinetics, in order to capture the natural variability of responses occurring in the sponge populations.

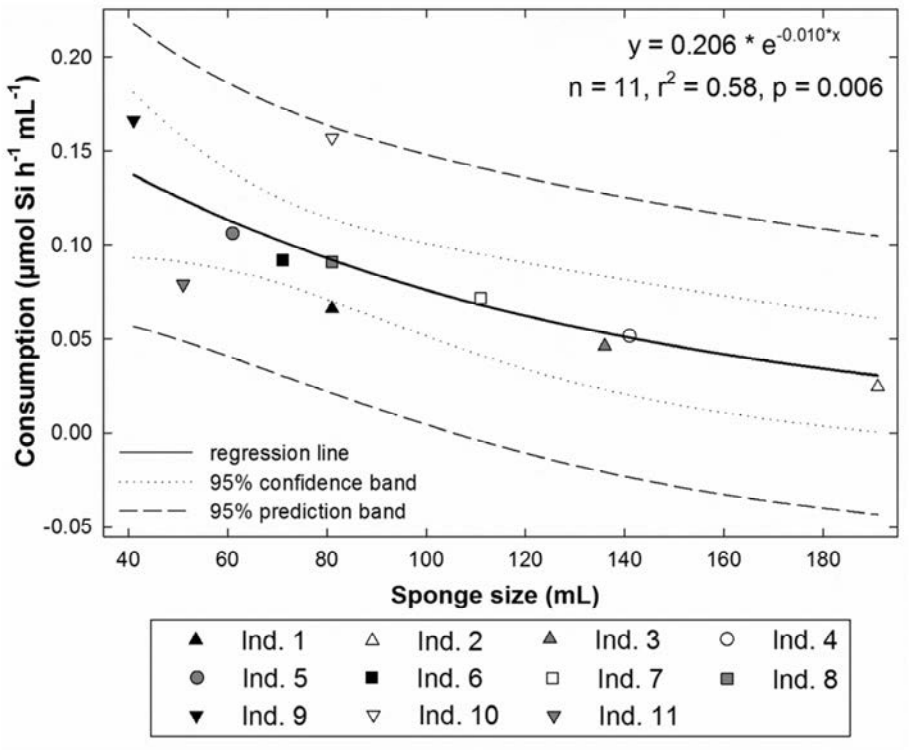


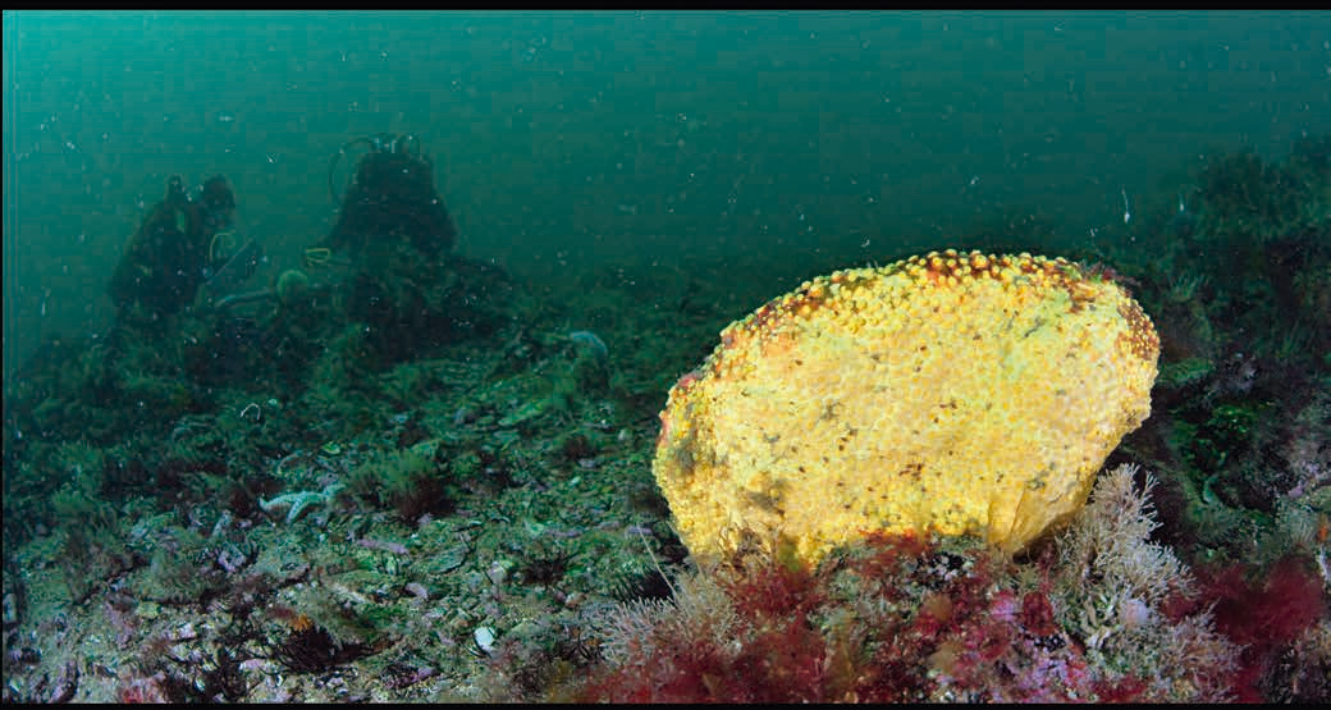
Figure 4. Relationship between sponge size (mL) and maximum velocity of DSi utilization (µmol Si h^{-1} mL^{-1}) of the 11 assayed sponges of the species *Vazella pourtalesii*. The relationship fitted the inverse function "exponential decay". The equation and the statistics of the model describing the relationship between both variables are indicated in the graph (upper right corner).

Consumption of DSi in the hexactinellid *V. pourtalesii* fitted to a saturable Michaelis-Menten kinetics (Fig. 2). This result agrees with the studies conducted in demosponges (Reincke and Barthel, 1997; Maldonado et al., 2011; López-Acosta et al., 2016; López-Acosta et al., 2018), indicating that DSi consumption in sponges is an enzyme-mediated process. That is supported by the studies of Shimizu and co-workers (1998; 2015), in which two groups of proteins participating in the polymerization of BSi were identified. The silicateines, the enzymes mediating silicification in demosponges (Shimizu et al., 1998; Cha et al., 1999), probably evolved from a lysosomal capthesin used for intracellular digestion (Riesgo et al., 2015). The enzyme mediating BSi polymerization in hexactinellids, the glassin,

shares no significant similarity with any other proteins known so far (Shimizu et al., 2015). Although both enzymes appeared to be largely unrelated, the process of DSi consumption in sponges shares a similar pattern. Despite the discoveries of Shimizu and co-workers of the enzymes mediating the BSi polymerization, it cannot be discarded that, along the pathway that an atom of Si has to do from the surrounding seawater of the sponge to finally become part of its skeleton, there may be other saturable processes that favor the saturable kinetics.

The hexactinellid *V. pourtalesii* is characterized by one of the lowest DSi-affinity indexes (*sensu* Healey, 1980, calculated as the V_{max}/K_m ratio) recorded to date (0.0021 μmol Si h^{-1} sponge-mL^{-1} μM^{-1}). Such result clearly contrast with the fact that *V. pourtalesii* is the species with the highest BSi content (about 75% of the dry weight) among the species in which DSi consumption kinetics have been investigated so far. All together, this hexactinellid appears to have a strong dependence on high DSi concentration to achieve the rates required to develop its highly silicified skeleton. Besides, our results agree with previous suggestions that the availability of DSi in the seawater is a factor that controls the bathymetric and latitudinal distribution of the sponges of the Class Hexactinellida.

General Discussion and Conclusions



Picture: An specimen of *Cliona celata* on a rocky bottom of the bay of Brest (France), from Erwan Amice.

What has been learned about DSi consumption by sponges?

Most marine sponges utilize DSi to elaborate their siliceous skeletons (Jones, 1978; Simpson, 1984; Van Soest et al., 2012). Despite this fact, quantitative information about sponge DSi utilization has been extremely scarce during decades (Frøhlich and Barthel, 1997; Reincke and Barthel, 1997; Maldonado et al., 2011; Maldonado et al., 2012a), with DSi consumption kinetics knew only for 2 sponge genera: *Halichondria panicea* (Reincke and Barthel, 1997) and *Axinella* spp. (Maldonado et al., 2011). The current work has provided kinetic models for 5 extra sponge genera: *Hymeniacidon perlevis*, *Tethya citrina* (Chapter 1 and 3), *Haliclona simulans, Suberites ficus* (Chapter 2), and *Vazella pourtalesii* (Chapter 5). The DSi consumption responses of all these genera fit to a Michaelis-Menten kinetics, a function usually used to describe enzymatic-mediated processes. Although the pathway of Si through sponges is far from being well-known, some enzymes that mediate the BSi polymerization in these organisms have been identified, being the silicateins the silicifying enzymes in demosponges (Shimizu et al., 1998; Cha et al., 1999), and the glassin, in hexactinellids (Shimizu et al., 2015).

The described Michaelis-Menten kinetics indicated that the rates of DSi consumption increased when DSi availability rose, until a DSi

concentration at which the consumption system saturates. Surprisingly, the concentrations at which saturation was achieved by the sponges (75 – 200 μM DSi) are much higher than those available in most benthic ecosystems of the world ocean. It is worth noting that the average DSi concentration in shallow waters (<200 m) is nearly 10 μM and that in deep sea, about 70 μM (Nelson et al., 1995; Tréguer et al., 1995). These relatively low ambient concentrations of DSi are preventing sponges to utilize this nutrient at the optimal conditions at which they are adapted to, with maximum velocity of DSi consumption being essentially unachieved. This "low-efficient" performance at the DSi concentrations characterizing the modern ocean has been explained by sponges having evolved their DSi consumption systems during ancient geological periods (Maldonado et al., 1999; Maldonado et al., 2011) when DSi availability in seawater was about two orders of magnitude that occurring in recent time (Maliva et al., 1989; Siever, 1992; Maliva et al., 2005). The appearance of diatoms in the Jurassic and their expansion over the Cretaceous (Sims et al., 2006) is believed to have caused a dramatic DSi depletion in the photic ocean that have reached up to recent time (Siever, 1991). That change in DSi availability would have acted as a selective pressure that led to changes in DSi utilization of the dominant Si users of that period: sponges and radiolarians (Harper and Knoll, 1975; Maldonado et al., 1999; Racki and Cordey, 2000; Lazarus et al., 2009). In sponges, the effects that such DSi shortage caused in the silica skeletons have been empirically determined, being observed either the size-reduction of some skeletal pieces (spicules) or the complete loss of some spicule categories (Maldonado et al., 1999), with suggestions that some groups may have even evolved their skeletons to organic forms that do not need any Si to be produced (Maldonado, 2009).

The comparison of the models characterizing DSi consumption kinetics in the 7 sponge genera investigated to date revealed important between-species differences (Reincke and Barthel, 1997; Maldonado et al., 2011; this thesis). For instance, the highest maximum velocity of DSi transport (V_{max}), achieved by the demosponge *S. ficus* (0.480 ± 0.018 μmol Si h^{-1} mL^{-1}), is more than 5 times the lowest one, achieved by the hexactinellid *V. pourtalesii* (0.093 ± 0.015 μmol Si h^{-1} mL^{-1}). Differences in the half-saturation parameter (K_m, that is, the DSi concentration at which half of the V_{max} is achieved) are similar, with a change of almost 4-fold between the

highest (*S. ficus*, 108.2 ± 12.1 μM DSi) and the lowest (*T. citrina*, 29.8 ± 7.9 μM DSi) K_m recorded. By comparing the V_{max}/K_m ratio, defined by Healey (1980) as the substrate affinity index, DSi affinity between species can be compared. Again, significant between-species differences occur, with the highest affinity indexes (0.0085 and 0.0081 μmol Si h^{-1} sponge-mL^{-1} μM^{-1} in *H. simulans* and *H. panicea*, respectively) being about 5 times the lowest ones (0.0018 and 0.0021 μmol Si h^{-1} sponge-mL^{-1} μM^{-1} in *Axinella* spp. and *V. pourtalesii*, respectively). Interestingly, the skeletonization level (i.e., % of BSi relative to dry weight) appears to be unrelated to the parameters characterizing the specific kinetic models. The two species with the lowest V_{max}, that is, *V. pourtalesii* and *H. perlevis*, are the species with the highest (75.4 ± 6.4 % BSi/DW) and the lowest (49.7 ± 7.1 % BSi/DW) level of skeletonization, respectively. Additionally, the affinity for the substrate did not present any relation with the BSi content ($n = 7$, $R^2 = 0.002$, $p = 0.933$). Consequently, the differences in DSi consumption kinetics occurring between species cannot be easily explained from the skeletonization level. It could be that differences in longevity and/or the growth rates developed to achieve those skeletal contents may help to explain the mismatch.

Within the species, all studies available have reported substantial levels of between-individual variability in DSi consumption rates (Frøhlich and Barthel, 1997; Reincke and Barthel, 1997; Maldonado et al., 2011; Maldonado et al., 2012a; this thesis). Several sources of between-individual variability have been investigated so far. The one explored across all the species was the body size, which was responsible of explaining from 12 to 74 % of the differences occurring between individual responses in most of the sponge species investigated (*Axinella* spp., *H. perlevis, T. citrina,* and *V. pourtalesii*). In those cases, the smaller individuals were consuming DSi at higher rates than the bigger sponges (Maldonado et al., 2011; Chapter 1, 3, and 5). Such results agree with the fact that smaller (and younger) individuals in the population need to consume nutrients at higher rates to develop their body structures (e.g., skeleton); when individuals become large (adults), slower rates of nutrient consumption are required to maintain the biomass achieved at the adulthood. The species *H. panicea*, *H. simulans*, and *S. ficus* did not show such a pattern. However, the main reason why they did not meet that general law in animal growth is that the assayed specimens in the laboratory were not including the smallest and the largest sizes of the species

size-range, which are responsible of the statistical differences (see details in discussion of Chapter 2).

The reproductive activity could also be a factor causing part of the between-individual variability in DSi consumption. For instance, individuals reproducing both sexually (*H. panicea*; Frøhlich and Barthel, 1997) and asexually (*T. citrina*; Chapter 1) were characterized by lower consumption rates than those without any sign of reproductive activity. Those results indicate that sponges would slow down consumption of DSi during the reproductive period.

There are two more sources of between-individual variability that, despite not being statistically examined during the work of this thesis, should be mentioned: food availability and seawater temperature. It has been identified that sponges can feed on a wide variety of food sources: dissolved organic matter (DOM) and particulate organic matter, which includes detritus, and bacterioplankton and microphytoplankton (Reiswig, 1975; Thomassen and Riisgård, 1995; Pile et al., 1996; Yahel et al., 2003; de Goeij et al., 2008; Maldonado et al., 2012b; de Goeij et al., 2013; Mueller et al., 2014). Frølich and Barthel (1997) determined that individuals of the species *H. panicea* consumed significantly less DSi (about one tenth) when food was limited to starvation at the laboratory. In the field, the link between food availability and DSi utilization can be found in the Antarctica, where sponges dominated the benthic communities in continental shelves and slopes. After the collapse of part of the ice shelf, a massive new input of food occurred, triggering a sudden recruitment of sponges and a huge enlargement of the sponge biomass, which implied an increase from 2-fold to 3-fold in sponge abundance and biomass over a period of only 4 years (Gutt et al., 2011; Fillinger et al., 2013; Dayton et al., 2016; Maldonado et al., 2017). Such massive increased of sponge biomass had to be necessarily accompanied by a DSi utilization in the same order of magnitude to produce the sponge silica skeleton.

Temperature was also investigated as a source of between-individual variability in DSi consumption, with specimens of *H. panicea* showing non-significant changes in DSi consumption rates when seawater temperature varied within the natural range (5 – 20 ºC) occurring in their temperate, shallow-water habitat (Frøhlich and Barthel, 1997). This suggests that

temperature is not affecting sponge DSi consumption if it varied within the range naturally occurring at the sponge ecosystems.

In this PhD work, we detected for the first time that, in a sponge species —*T. citrina*—, the DSi-consumption kinetics and the affinity for DSi can vary seasonally over the year cycle (Chapter 3). Those findings have implications for quantifying the total BSi production by sponge populations over a year period. For instance, if the massive sponge populations occurring in the Antarctica would be affected by such kind of seasonal shifts but they were not considered, a mistake of about 30% of the DSi demanded by those sponge communities would be committed (see details of the calculations in Chapter 3). In absolute terms, the mistake would imply to disregard 0.63 Tmol Si y^{-1} (1.77×10^{10} kg Si y^{-1}), what is about the 20% of the total amount of Si considered to be consumed by sponges in the world ocean according to the last revision of the marine global Si cycle (Tréguer and De La Rocha, 2013).

Seasonality has been described to influence sponge's physiology (reproduction, respiration activity, growth, etc) of both shallow- and deep-water species (Reiswig, 1973; Elvin, 1976; Leys and Lauzon, 1998; Coma et al., 2000; Coma et al., 2002; Koopmans and Wijffels, 2008). Part of the seasonal differences occurring in DSi utilization by *T. citrina* could be related to some of those physiological processes. For example, the period of sexual reproduction in this species ranges from spring to summer (Corriero et al., 1996), coinciding with the kinetics that implied slower rates of DSi utilization. Thus, it is clear that further investigation is needed to better understand the physiological shifts that marine sponges are suffering over the year cycle, including DSi consumption dynamics.

Which are the methodological concerns?

Given that the quantification of DSi utilization by sponges is essentially an empirical task, the method and the approach are usually relevant to the experimental outcome. The results in Chapter 2 revealed that the duration of the incubation period had significant effects on the calculation of DSi consumption rates, with incubations longer than 24h resulting in more conservative rates. To estimate DSi consumption in periods shorter than 24h could lead to two contrasting inaccuracies. First, very short incubations may underestimate consumption rates in sponges, likely to occur when DSi

concentration increases but is still far from saturation (i.e., 20 – 90 μM). The cause of such underestimation would be that processes initiated during silicification (activation of silicifying genes, generation of new copies of silicifying cells, etc), which need from hours to days to be completed, would be unfinished. Second, those short incubations could also overestimate consumption rates by disregarding diel physiological changes occurring in sponges that may imply a decreased in DSi demands, likely to occur when DSi concentration is nearly to saturation or exceed that threshold (i.e., ≥ 100 – 150 μM). Therefore, very short incubations (i.e., minutes or few hours) to investigate DSi consumption kinetics in sponges may generate models that will inaccurately reproduce the real responses of the sponges in their natural habitats.

It has also been discussed that investigating sponges out of their habitats may alter their physiology (e.g., Yahel et al., 2005; Southwell et al., 2008). Before this thesis, DSi consumption had been only determined in laboratory incubations (Frøhlich and Barthel, 1997; Reincke and Barthel, 1997; Maldonado et al., 2011), and field DSi consumption were not available. Recently, some authors attempted to determine DSi consumption rates by measuring changes in nutrient concentration between the ambient seawater and the seawater going out of the sponge, but differences were mostly undetectable (Perea-Blázquez et al., 2012; Morganti et al., 2017; Leys et al., 2018). Such methodology, known as *InEx* (Yahel et al., 2005; Morganti et al., 2016), aims to determine changes in the time seawater flows across the sponge body, that is, only few seconds. As we empirically identified (Chapter 2), it is necessary to measure DSi consumption rates in longer periods of time (longer than minutes to hours), hence incubations result as the best approach. Here we measured for the first time DSi consumption rates in a sponge species —*T. citrina*— at its natural habitat using incubation chambers (Chapter 4). Importantly, the rates of DSi utilization measured *in situ* were in close agreement with those resulting from the DSi consumption kinetic models calculated for the species at both summer and autumn (see Fig. 3 in Chapter 4). These results support the use of laboratory incubations to investigate DSi consumption kinetics in sponges, being a simpler and a more feasible approach than those conducted *in situ*. They also support the use of laboratory models to extrapolate DSi utilization to the sponge population level.

What is the ecological meaning of this research?

Sponges have traditionally been disregarded in the marine Si cycle, both at local and global scales (e.g., DeMaster, 1981; Leynaert et al., 1993; Brzezinski and Nelson, 1995; Nelson et al., 1995; Tréguer et al., 1995; Ragueneau et al., 2001; DeMaster, 2002; Leblanc et al., 2003; Ragueneau et al., 2005; Katz et al., 2016). In this PhD work, a special effort has been done to gain new quantitative information that may help to include sponges in subsequent estimates of global models for Si use and Si cycling in the ocean, models that so far have essentially been based on the biology of diatoms. As a regional case study, we have also used the empirically gained information on DSi consumption to estimate total utilization by the sponge populations occurring in the different habitats of the entire bay of Brest (France, North Atlantic; Chapter 2). The results indicated that the sponge community of the bay consumes on average 0.10 ± 0.19 mmol Si m^{-2} d^{-1}, what is an order of magnitude higher than the sponge assemblage of the oligotrophic Mediterranean rocky sublittoral (0.01 ± 0.01 mmol Si m^{-2} d^{-1}) and about 4 to 9 times lower than the seasonal population explosion of *H. panicea* at the Baltic sublittoral bottom (0.44 mmol Si m^{-2} d^{-1}) and the rich sponge assemblage of the Caribbean barrier reef (0.90 ± 5.00 mmol Si m^{-2} d^{-1}; Maldonado et al., 2011). When compared with planktonic diatoms occurring at the bay of Brest, sponges are responsible of about 8% of the yearly net BSi production (Chapter 2). This sounds as a secondary contribution but it has to be kept in mind that the conditions at the bay are particularly favorable to diatoms (Del Amo et al., 1997; Chauvaud et al., 2000; Beucher et al., 2004). It is also worth noting that the relative contribution of sponges as DSi users increases dramatically in habitats with high DSi availability, as indicated by the high DSi saturating concentrations detected in all specific kinetics (Reincke and Barthel, 1997; Maldonado et al., 2011; Chapter 1, 2, 3, and 5). Therefore, siliceous sponges should be considered in local, regional, and global Si budgets, if we are to improve the accuracy on those budget calculations.

Conclusions

- The kinetics of silicon consumption in all five assayed sponges (four demosponges and one hexactinellid) showed a best fit to a saturable Michaelis-Menten model, which suggests an enzyme-mediated silicification process.

- The studied species saturate their consumption systems at concentrations that range from 75 to 150 μM DSi. Because these concentrations are never available at their habitat, sponges can be said to be chronically forced to consume DSi at rates significantly lower than their maximum consumption capacity. At the relatively low DSi concentrations that characterize the modern ocean, the herein investigated sponge assemblage at the bay of Brest was found to consume DSi per m^2 at a rate that is at least one order of magnitude lower than that of planktonic diatoms.

- Our work shows for the first time that kinetics of silicon consumption may change seasonally in some species. This finding has important implications when quantifying the role of sponges in the marine Si cycle, particularly in habitats with high DSi availability, such as in high latitudes and deep-water habitats. The proportion of the species that experience seasonal shifts in kinetics in shallow- and deep-water ecosystems is still to be determined.

- Rates of DSi consumption measured *in situ* significantly matched those estimated from the kinetic models obtained in the laboratory. Unlike for diatoms, long incubations (≥ 24h) result the simplest and more accurately approach to determine DSi consumption rates in sponges.

- It was revealed that sponges show consistently important between-species and between-individual variability when consuming DSi. To consider and incorporate such variability is essential for scaling up accurately their consumption rates to population, regional, and global levels.

- Estimates of DSi consumption by the entire sponge assemblage in a temperate, shallow-water ecosystem (bay of Brest, France) revealed that these organisms play a noticeable role as DSi consumers at the ecosystem level. Similar roles are postulated to occur in other marine systems where sponges may abound in detriment of other Si users.

- All together, this research shows that sponges are Si consumers whose role in the marine ecosystems cannot be considered negligible. Such a role increases with increasing availability of DSi in demersal habitats, which is the opposite pattern showed by planktonic diatoms.

Bibliography

Picture: An specimen of *Suberites ficus* on the mäerl bed of the North Basin of the bay of Brest (France), from Erwan Amice.

Abdo, D. A., J. W. Seager, E. S. Harvey, J. I. McDonald, G. A. Kendrick, and M. R. Shortis (2006). Efficiently measuring complex sessile epibenthic organisms using a novel photogrammetric technique. *Journal of Experimental Marine Biology and Ecology* **339** (1) 120-133.

Alex, A., V. Vasconcelos, P. Tamagnini, A. Santos, and A. Antunes (2012). Unusual symbiotic cyanobacteria association in the genetically diverse intertidal marine sponge *Hymeniacidon perlevis* (Demospongiae, Halichondrida). *PLoS One* **7** (12) e51834.

Archer, S. K., J. L. Stevens, R. E. Rossi, K. O. Matterson, and C. A. Layman (2017). Abiotic conditions drive significant variability in nutrient processing by a common Caribbean sponge, *Ircinia felix*. *Limnology and Oceanography* **62** (4) 1783-1793.

Barthel, D., and O. S. Tendal (1993). Sponge spicules in abyssal and bathyal sediments of the NE Atlantic. *Deep-Sea Newsletter* **20** 15-18.

Barthel, D. (1995). Tissue composition of sponges from the Weddell Sea, Antarctica: not much meat on the bones. *Marine Ecology Progress Series* **123** 149-153.

Bell, J. J. (2008). The functional roles of marine sponges. *Estuarine, Coastal and Shelf Science* **79** (3) 341-353.

Berner, R. A. (1990). Atmospheric carbon dioxide levels over Phanerozoic time. *Science* **249** (4975) 1382-1386.

Beucher, C., P. Treguer, R. Corvaisier, A. M. Hapette, and M. Elskens (2004). Production and dissolution of biosilica, and changing microphytoplankton dominance in the Bay of Brest (France). *Marine Ecology Progress Series* **267** 57-69.

Biscéré, T., R. Rodolfo-Metalpa, A. Lorrain, L. Chauvaud, J. Thébault, J. Clavier, and F. Houlbrèque (2015). Responses of two scleractinian corals to cobalt pollution and ocean acidification. *PLoS One* **10** (4).

Brunner, E., and U. Munzel (2000). The nonparametric Behrens-Fisher problem: Asymptotic theory and a small-sample approximation. *Biometrical Journal* **42** (1) 17-25.

Brunner, E., and M. L. Puri (2001). Nonparametric methods in factorial designs. *Statistical Papers* **42** (1) 1-52.

Brunner, E., S. Domhof, and F. Langer (2002). Nonparametric analysis of longitudinal data in factorial experiments. Wiley-Interscience Publishing.

Brzezinski, M. A., and D. M. Nelson (1989). Seasonal changes in the silicon cycle within a Gulf Stream warm-core ring. *Deep Sea Research (Part I, Oceanographic Research Papers)* **36** (7) 1009-1039.

Brzezinski, M. A., and D. M. Nelson (1995). The annual silica cycle in the Sargasso sea near Bermuda. *Deep-Sea Research Part I-Oceanographic Research Papers* **42** (7) 1215-1237.

Brzezinski, M. A., C. Dumousseaud, J. W. Krause, C. I. Measures, and D. M. Nelson (2008). Iron and silicic acid concentrations together regulate Si uptake in the equatorial Pacific Ocean. *Limnology and Oceanography* **53** (3) 875-889.

Burton, J. D., and P. S. Liss (1968). Oceanic budget of dissolved silicon. *Nature* **220** (5170) 905-906.

Calvert, S. E. (1968). Silica balance in ocean and diagenesis. *Nature* **219** (5157) 919-920.

Cavalier-Smith, T. (2017). Origin of animal multicellularity: precursors, causes, consequences—the choanoflagellate/sponge transition, neurogenesis and the Cambrian explosion. *Philosophical Transactions of the Royal Society B: Biological Sciences* **372** (1713) 20150476.

Coma, R., M. Ribes, J. M. Gili, and M. Zabala (2000). Seasonality in coastal benthic ecosystems. *Trends in Ecology & Evolution* **15** (11) 448-453.

Coma, R., M. Ribes, J.-M. Gili, and M. Zabala (2002). Seasonality of *in situ* respiration rate in three temperate benthic suspension feeders. *Limnology and Oceanography* **47** (1) 324-331.

Conley, D. J., and C. L. Schelske (1993). Potential role of sponge spicules in influencing the silicon biogeochemistry of Florida lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **50** (2) 296-302.

Conway, H. L., and P. J. Harrison (1977). Marine diatoms grown in chemostats under silicate or ammonium limitation. 4. Transient-response of *Chaetoceros debilis*, *Skeletonema costatum*, and *Thalassiosira*

gravida to a single addition of limiting nutrient. *Marine Biology* **43** (1) 33-43.

Corredor, J. E., C. R. Wilkinson, V. P. Vicente, J. M. Morell, and E. Otero (1988). Nitrate release by caribbean reef sponges. *Limnology and Oceanography* **33** (1) 114-120.

Corriero, G., M. Sara, and P. Vaccaro (1996). Sexual and asexual reproduction in two species of *Tethya* (Porifera: Demospongiae) from a Mediterranean coastal lagoon. *Marine Biology* **126** (2) 175-181.

Cha, J. N., K. Shimizu, Y. Zhou, S. C. Christiansen, B. F. Chmelka, G. D. Stucky, and D. E. Morse (1999). Silicatein filaments and subunits from a marine sponge direct the polymerization of silica and silicones *in vitro*. *Proceedings of the National Academy of Sciences, USA* **96** 361-365.

Chauvaud, L., F. Jean, O. Ragueneau, and G. Thouzeau (2000). Long-term variation of the Bay of Brest ecosystem: benthic-pelagic coupling revisited. *Marine Ecology Progress Series* **200** 35-48.

Chu, J. W. F., M. Maldonado, G. Yahel, and S. P. Leys (2011). Glass sponge reefs as a silicon sink. *Marine Ecology Progress Series* **441** 1-14.

Dayton, P., S. Jarrell, S. Kim, S. Thrush, K. Hammerstrom, M. Slattery, and E. Parnell (2016). Surprising episodic recruitment and growth of Antarctic sponges: Implications for ecological resilience. *Journal of Experimental Marine Biology and Ecology* **482** 38-55.

de Goeij, J. M., H. van den Berg, M. M. van Oostveen, E. H. G. Epping, and F. C. Van Duyl (2008). Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. *Marine Ecology Progress Series* **357** 139-151.

de Goeij, J. M., D. van Oevelen, M. J. A. Vermeij, R. Osinga, J. J. Middelburg, A. de Goeij, and W. Admiraal (2013). Surviving in a marine desert: the sponge loop retains resources within coral reefs. *Science* **342** (6154) 108-110.

de Goeij, J. M., M. P. Lesser, and J. R. Pawlik (2017). Nutrient fluxes and ecological functions of coral reef sponges in a changing ocean, p. 373-410. *In* J. L. Carballo and J. J. Bell [eds.], *Climate change, ocean*

acidification and sponges: impacts across multiple levels of organization. Springer International Publishing.

Del Amo, Y., O. Le Pape, P. Tréguer, B. Quéguiner, A. Menesguen, and A. Aminot (1997a). Impacts of high-nitrate freshwater inputs on macrotidal ecosystems. I. Seasonal evolution of nutrient limitation for the diatom-dominated phytoplankton of the Bay of Brest (France). *Marine Ecology Progress Series* **161** 213-224.

Del Amo, Y., B. Quéguiner, P. Tréguer, H. Breton, and L. Lampert (1997b). Impacts of high-nitrate freshwater inputs on macrotidal ecosystems. II. Specific role of the silicic acid pump in the year-round dominance of diatoms in the Bay of Brest (France). *Marine Ecology Progress Series* **161** 225-237.

Del Amo, Y., and M. A. Brzezinski (1999). The chemical form of dissolved Si taken up by marine diatoms. *Journal of Phycology* **35** (6) 1162-1170.

Delmas, R., and P. Tréguer (1985). Simulation de l'évolution de paramètres physiques, chimiques, et de la biomasse phytoplanctonlque en période printanière dans un écosystème littoral macrotidal. *Oceanis* **11** (3) 197-211

DeMaster, D. J. (1981). The supply and accumulation of silica in the marine environment. *Geochimica Et Cosmochimica Acta* **45** (10) 1715-1732.

DeMaster, D. J. (1991). Measuring biogenic silica in marine sediments and suspended matter, p. 363-367. *In* D. C. Hurd and S. D. W. [eds.], *Marine particles: Analysis and characterization*. American Geophysical Union.

DeMaster, D. J. (2002). The accumulation and cycling of biogenic silica in the Southern Ocean: revisiting the marine silica budget. *Deep Sea Research Part II: Topical Studies in Oceanography* **49** (16) 3155-3167.

Diaz, M. C., and B. B. Ward (1997). Sponge-mediated nitrification in tropical benthic communities. *Marine Ecology Progress Series* **156** 97-107.

Diaz, M. C., and K. Rützler (2001). Sponges: an essential component of Caribbean coral reefs. *Bulletin of Marine Science* **69** (2) 535-546.

Ducklow, H., A. Clarke, R. Dickhut, S. C. Doney, H. Geisz, K. Huang, D. G. Martinson, M. P. Meredith, H. V. Moeller, M. Montes-Hugo, O.

Schofield, S. E. Stammerjohn, D. Steinberg, and W. Fraser (2012). The marine system of the Western Antarctic Peninsula, p. 121 - 159. *In* A. Rogers, N. Johnston, E. Murphy and A. Clarke [eds.], *Antarctic Ecosystems*. Wiley-Blackwell Publishing.

Ehrlich, H., R. Deutzmann, E. Brunner, E. Cappellini, H. Koon, C. Solazzo, Y. Yang, D. Ashford, J. Thomas-Oates, M. Lubeck, C. Baessmann, T. Langrock, R. Hoffmann, G. Wörheide, J. Reitner, P. Simon, M. Tsurkan, A. V. Ereskovsky, D. Kurek, V. V. Bazhenov, S. Hunoldt, M. Mertig, D. V. Vyalikh, S. L. Molodtsov, K. Kummer, H. Worch, V. Smetacek, and M. J. Collins (2010). Mineralization of the metre-long biosilica structures of glass sponges is templated on hydroxylated collagen. *Nature Chemistry* **2** (12) 1084-1088.

Ehrlich, H. (2011). Silica biomineralization in sponges, p. 796-808. *In* J. Reitner and V. Thiel [eds.], *Encyclopedia of Geobiology*. Springer.

Ehrlich, H., M. Maldonado, A. R. Parker, Y. N. Kulchin, J. Schilling, B. Köhler, U. Skrzypczak, P. Simon, H. M. Reiswig, M. V. Tsurkan, E. Brunner, S. S. Voznesenskiy, A. V. Bezverbny, S. S. Golik, I. G. Nagorny, D. V. Vyalikh, A. A. Makarova, S. L. Molodtsov, K. Kummer, M. Mertig, C. Erler, D. V. Kurek, V. V. Bazhenov, F. Natalio, A. E. Kovalev, S. N. Gorb, A. L. Stelling, J. Heitmann, R. Born, D. C. Meyer, and K. R. Tabachnick (2016). Supercontinuum generation in naturally occurring glass sponges spicules. *Advanced Optical Materials* **4** (10) 1608-1613.

Elvin, D. W. (1976). Seasonal growth and reproduction of an intertidal sponge, *Haliclona permollis* (Bowerbank). *Biological Bulletin, Marine Biological Laboratory, Woods Hole* **151** 108.

Falkowski, P. G., R. T. Barber, and V. Smetacek (1998). Biogeochemical controls and feedbacks on ocean primary production. *Science* **281** (5374) 200-206.

Fan, C. P., and D. H. Zhang (2017). On power and sample size of the ANOVA-type rank test. *Communications in Statistics-Simulation and Computation* **46** (4) 3224-3241.

Field, C. B., M. J. Behrenfeld, J. T. Randerson, and P. Falkowski (1998). Primary production of the biosphere: integrating terrestrial and oceanic components. *Science* **281** (5374) 237-240.

Fillinger, L., D. Janussen, T. Lundälv, and C. Richter (2013). Rapid glass sponge expansion after climate-induced Antarctic ice shelf collapse. *Current Biology* **23** (14) 1330-1334.

Frøhlich, H., and D. Barthel (1997). Silica uptake of the marine sponge *Halichondria panicea* in Kiel Bight. *Marine Biology* **128** (1) 115-125.

Fuller, T. L., and J. R. Hughey (2013). Molecular investigation of the invasive sponge *Hymeniacidon sinapium* (de Laubenfels, 1930) in Elkhorn Slough, California. *Aquatic Invasions* **8** (1) 59-66.

Gloeckner, V., M. Wehrl, L. Moitinho-Silva, C. Gernert, P. Schupp, J. R. Pawlik, N. L. Lindquist, D. Erpenbeck, G. Wörheide, and U. Hentschel (2014). The HMA-LMA dichotomy revisited: an electron microscopical survey of 56 sponge species. *The Biological Bulletin* **227** (1) 78-88.

Gordon, R., D. Losic, M. A. Tiffany, S. S. Nagy, and F. A. S. Sterrenburg (2009). The glass menagerie: diatoms for novel applications in nanotechnology. *Trends in Biotechnology* **27** (2) 116-127.

Gutt, J., I. Barratt, E. Domack, C. d'Udekem d'Acoz, W. Dimmler, A. Grémare, O. Heilmayer, E. Isla, D. Janussen, E. Jorgensen, K.-H. Kock, L. Sophia Lehnert, P. López-Gonzáles, S. Langner, K. Linse, M. Eugenia Manjón-Cabeza, M. Meißner, A. Montiel, M. Raes, H. Robert, A. Rose, E. Sañé Schepisi, T. Saucède, M. Scheidat, H.-W. Schenke, J. Seiler, and C. Smith (2011). Biodiversity change after climate-induced ice-shelf collapse in the Antarctic. *Deep Sea Research Part II: Topical Studies in Oceanography* **58** (1–2) 74-83.

Gutt, J., A. Bohmer, and W. Dimmler (2013). Antarctic sponge spicule mats shape macrobenthic diversity and act as a silicon trap. *Marine Ecology Progress Series* **480** 57-71.

Harper, H. E., and A. H. Knoll (1975). Silica, diatoms and Cenozoic radiolarian evolution. *Geology* **3** 175-177.

Harriss, R. C. (1966). Biological buffering of oceanic silica. *Nature* **212** (5059) 275-276.

Hatcher, A. (1994). Nitrogen and phosphorus turnover in some benthic marine invertebrates: implications for the use of C:N ratios to assess food quality. *Marine Biology* **121** (1) 161-166.

Healey, F. P. (1980). Slope of the Monod equation as an indicator of advantage in nutrient competition. *Microbial Ecology* **5** (4) 281-286.

Hoffmann, L. J., I. Peeken, and K. Lochte (2008). Iron, silicate, and light co-limitation of three Southern Ocean diatom species. *Polar Biology* **31** (9) 1067-1080.

Hoshino, S., D. Saito, and T. Fujita (2008). Contrasting genetic structure of two Pacific *Hymeniacidon* species. *Hydrobiologia* **603** (1) 313-326.

Javaheri, N., R. Dries, A. Burson, L. J. Stal, P. M. A. Sloot, and J. A. Kaandorp (2015). Temperature affects the silicate morphology in a diatom. *Scientific Reports* **5** 11652.

Jiménez, E., and M. Ribes (2007). Sponges as a source of dissolved organic nitrogen: Nitrification mediated by temperate sponges. *Limnology and Oceanography* **52** (3) 948-958.

Jones, W. C. (1978). The microstructure and genesis of sponge biominerals. *Colloques Internationaux du Centre National de la Recherche Scientifique* **291** 425-447.

Katz, T., G. Yahel, V. Tunnicliffe, B. Herut, F. Whitney, P. V. R. Snelgrove, and B. Lazar (2016). The silica cycle in a Northeast Pacific fjord; the role of biological resuspension. *Progress in Oceanography* **147** 10-21.

Keesing, J. K., J. Strzelecki, J. Fromont, and D. Thomson (2013). Sponges as important sources of nitrate on an oligotrophic continental shelf. *Limnology and Oceanography* **58** (6) 1947-1958.

Koopmans, M., and R. H. Wijffels (2008). Seasonal growth rate of the sponge *Haliclona oculata* (Demospongiae: Haplosclerida). *Marine Biotechnology* **10** 502-510.

Krasko, A., B. Lorenz, R. Batel, H. C. Schroder, I. M. Muller, and W. E. G. Muller (2000). Expression of silicatein and collagen genes in the marine sponge Suberites domuncula is controlled by silicate and myotrophin. *European Journal of Biochemistry* **267** (15) 4878-4887.

Kristiansen, S., T. Farbrot, and L. J. Naustvoll (2000). Production of biogenic silica by spring diatoms. *Limnology and Oceanography* **45** (2) 472-478.

Kumeria, T., S. J. P. McInnes, S. Maher, and A. Santos (2017). Porous silicon for drug delivery applications and theranostics: recent advances, critical review and perspectives. *Expert Opinion on Drug Delivery* **14** (12) 1407-1422.

Laruelle, G. G., V. Roubeix, A. Sferratore, B. Brodherr, D. Ciuffa, D. J. Conley, H. H. Durr, J. Garnier, C. Lancelot, Q. L. T. Phuong, J. D. Meunier, M. Meybeck, P. Michalopoulos, B. Moriceau, S. N. Longphuirt, S. Loucaides, L. Papush, M. Presti, O. Ragueneau, P. Regnier, L. Saccone, C. P. Slomp, C. Spiteri, and P. Van Cappellen (2009). Anthropogenic perturbations of the silicon cycle at the global scale: Key role of the land-ocean transition. *Global Biogeochemical Cycles* **23** (GB4031) 1-17.

Lazarus, D. B., B. Kotrc, G. Wulf, and D. N. Schmidt (2009). Radiolarians decreased silicification as an evolutionary response to reduced Cenozoic ocean silica availability. *Proceedings of the National Academy of Sciences, USA* **106** (23) 9333-9338.

Le Pape, O., Y. Del Amo, A. Menesguen, A. Aminot, B. Quéquiner, and P. Tréguer (1996). Resistance of a coastal ecosystem to increasing eutrophic conditions: the Bay of Brest (France), a semi-enclosed zone of Western Europe. *Continental Shelf Research* **16** (15) 1885-1907.

Leblanc, K., B. Queguiner, N. Garcia, P. Rimmelin, and P. Raimbault (2003). Silicon cycle in the NW Mediterranean Sea: seasonal study of a coastal oligotrophic site. *Oceanologica Acta* **26** (4) 339-355.

Leynaert, A., D. M. Nelson, B. Quéguiner, and P. Tréguer (1993). The silica cycle in the Antarctic Ocean: is the Weddell Sea atypical? . *Marine Ecology Progress Series* **96** (1) 1-15.

Leynaert, A., S. N. Longphuirt, P. Claquin, L. Chauvaud, and O. Ragueneau (2009). No limit? The multiphasic uptake of silicic acid by benthic diatoms. *Limnology and Oceanography* **54** (2) 571-576.

Leys, S. P., and R. J. Lauzon (1998). Hexactinellid sponge ecology: growth rates and seasonality in deep water sponges. *Journal of Experimental Marine Biology and Ecology* **230** 111-129.

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*. *Limnology and Oceanography* **63** (1) 187-202.

Li, C.-W., J.-Y. Chen, and T.-E. Hua (1998). Precambrian sponges with cellular structures. *Science* **279** (5352) 879-882.

López-Acosta, M., A. Leynaert, and M. Maldonado (2016). Silicon consumption in two shallow-water sponges with contrasting biological features. *Limnology and Oceanography* **61** (6) 2139-2150.

López-Acosta, M., A. Leynaert, J. Grall, and M. Maldonado (2018a). Silicon consumption kinetics by marine sponges: an assessment of their role at the ecosystem level. *Limnology and Oceanography* ***in press***.

López-Acosta, M., A. Leynaert, V. Coquille, and M. Maldonado (2018b). Silicon utilization by sponges: an assessment of seasonal changes. *Marine Ecology Progess Series* **under review**.

Love, G. D., E. Grosjean, C. Stalvies, D. A. Fike, J. P. Grotzinger, A. S. Bradley, A. E. Kelly, M. Bhatia, W. Meredith, C. E. Snape, S. A. Bowring, D. J. Condon, and R. E. Summons (2009). Fossil steroids record the appearance of Demospongiae during the Cryogenian period. *Nature* **457** (7230) 718-721.

Ludeman, D. A., M. A. Reidenbach, and S. P. Leys (2017). The energetic cost of filtration by demosponges and their behavioural response to ambient currents. *Journal of Experimental Biology* **220** (6) 995-1007.

Lührs, A.-K., and W. Geurtsen (2009). The application of silicon and silicates in dentistry: a review, p. 359-380. *In* W. E. G. Müller and M. A. Grachev [eds.], *Biosilica in Evolution, Morphogenesis, and Nanobiotechnology: Case Study Lake Baikal*. Springer Berlin Heidelberg.

Mackenzie, F. T., and R. M. Garrels (1966). Silica-bicarbonate balance in ocean and early diagenesis. *Journal of Sedimentary Petrology* **36** (4) 1075-1084.

Maldonado, M., M. G. Carmona, M. J. Uriz, and A. Cruzado (1999). Decline in Mesozoic reef-building sponges explained by silicon limitation. *Nature* **401** (6755) 785-788.

Maldonado, 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 benthopelagic coupling in the marine silicon cycle. *Limnology and Oceanography* **50** (3) 799-809.

Maldonado, M. (2009). Embryonic development of verongid demosponges supports independent acquisition of spongin skeletons as alternative to the siliceous skeleton of sponges. *Biological Journal of the Linnean Society* **97** 427-447.

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. *Limnology and Oceanography* **55** (5) 2001-2010.

Maldonado, M., L. Navarro, A. Grasa, A. González, and I. Vaquerizo (2011). Silicon uptake by sponges: a twist to understanding nutrient cycling on continental margins. *Scientific Reports* **1** 8.

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** (1) 251-257.

Maldonado, M., M. Ribes, and F. C. van Duyl (2012b). Nutrient fluxes through sponges: biology, budgets, and ecological implications. *Advances in Marine Biology* **62** 113-182.

Maldonado, M., R. Aguilar, R. J. Bannister, J. J. Bell, K. W. Conway, P. K. Dayton, C. Díaz, J. Gutt, M. Kelly, E. L. R. Kenchington, S. P. Leys, S. A. Pomponi, H. T. Rapp, K. Rützler, O. S. Tendal, J. Vacelet, and C. M. Young (2017). Sponge grounds as key marine habitats: a synthetic review of types, structure, functional roles, and conservation concerns, p. 145-184. *In* S. Rossi, L. Bramanti, A. Gori and C. Orejas Saco del Valle [eds.], *Marine Animal Forests: The Ecology of Benthic Biodiversity Hotspots*. Springer International Publishing.

Maliva, R. G., A. H. Knoll, and R. Siever (1989). Secular change in chert distribution: a reflection of evolving biological participation in the silica cycle. *Palaios* **4** 519-532.

Maliva, R. G., A. H. Knoll, and B. M. Simonson (2005). Secular change in the Precambrian silica cycle: Insights from chert petrology. *Geological Society of American Bulletin* **117** (7-8) 835-845.

Martin-Jézéquel, V., M. Hildebrand, and M. A. Brzezinski (2000). Silicon metabolism in diatoms: implications for growth. *Journal of Phycology* **36** (5) 821-840.

Martin, S., J. Clavier, L. Chauvaud, and G. Thouzeau (2007). Community metabolism in temperate maerl beds. I. Carbon and carbonate fluxes. *Marine Ecology Progress Series* **335** 19-29.

Menna, P., G. Di Francia, and V. La Ferrara (1995). Porous silicon in solar cells: a review and a description of its application as an AR coating. *Solar Energy Materials and Solar Cells* **37** (1) 13-24.

Morganti, T., G. Yahel, M. Ribes, and R. Coma (2016). VacuSIP, an improved InEx method for *in situ* measurement of particulate and dissolved compounds processed by active suspension feeders. *Journal of Visualized Experiments* (114).

Morganti, T., R. Coma, G. Yahel, and M. Ribes (2017). Trophic niche separation that facilitates co-existence of high and low microbial abundance sponges is revealed by in situ study of carbon and nitrogen fluxes. *Limnology and Oceanography* **62** (5) 1963-1983.

Mueller, B., J. M. de Goeij, M. J. A. Vermeij, Y. Mulders, E. van der Ent, M. Ribes, and F. C. van Duyl (2014). Natural diet of coral-excavating sponges consists mainly of dissolved organic carbon (DOC). *PLoS One* **9** (2) e90152.

Müller, W. E. G., M. Rothenberger, A. Boreiko, W. Tremel, A. Reiber, and H. C. Schröder (2005). Formation of siliceous spicules in the marine demosponge *Suberites domuncula. Cell Tissue Research* **321** 285-297.

Nelson, D. M., G. F. Riedel, R. Millan-Nunez, and J. R. Lara-Lara (1984). Silicon uptake by algae with no known Si requirement. 1. True cellular uptake and pH-induced precipitation by *Phaeodactylum*

tricornutum (Bacillariophyceae) and *Platymonas* sp. (Prasinophyceae). *Journal of Phycology* **20** (1) 141-147.

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 Biogeochemical Cycles* **9** (3) 359-372.

Noguchi, K., Y. R. Gel, E. Brunner, and F. Konietschke (2012). nparLD: an R software package for the nonparametric analysis of longitudinal data in factorial experiments. *Journal of Statistical Software* **50** (12) 1-23.

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. *Chemical Geology* **380** 161-171.

Osinga, R., J. Tramper, and R. H. Wijfels (1999). Cultivation of marine sponges. *Mar Biotechnol* **1** 509-532.

Paasche, E. (1973). Silicon and ecology of marine plankton diatoms. 2. Silicate-uptake kinetics in 5 diatom species. *Marine Biology* **19** (3) 262-269.

Paytan, A., and K. McLaughlin (2007). The oceanic phosphorus cycle. *Chemical Reviews* **107** (2) 563-576.

Perea-Blázquez, A., S. K. Davy, and J. J. Bell (2012). Nutrient utilisation by shallow water temperate sponges in New Zealand. *Hydrobiologia* **687** (1) 237-250.

Pile, A. J., M. R. Patterson, and J. D. Witman (1996). *In situ* grazing on plankton <10 μm by the boreal sponge *Mycale lingua*. *Marine Ecology Progress Series* **141** (1-3) 95-102.

Quéguiner, B., and P. Tréguer (1984). Studies on the phytoplankton in the bay of Brest (western Europe). Seasonal variations in composition,

biomass and production in relation to hydrological and chemical features (1981-1982). *Botanica Marina* **27** (10) 449-459.

Racki, G., and F. Cordey (2000). Radiolarian palaeocology and radiolarites: is the present the key to the past? *Earth-Science Reviews* **52** 83-120.

Ragueneau, O., E. De Blas Varela, P. Tréguer, B. Quéguiner, and Y. Del Amo (1994). Phytoplankton dynamics in relation to the biogeochemical cycle of silicon in a coastal ecosystem of western Europe. *Marine Ecology Progess Series* **106** 157-172.

Ragueneau, O., B. Quéguiner, and P. Tréguer (1996). Contrast in biological responses to tidally-induced vertical mixing for two macrotidal ecosystems of Western Europe. *Estuarine Coastal and Shelf Science* **42** (5) 645-665.

Ragueneau, O., P. Tréguer, A. Leynaert, R. F. Anderson, M. A. Brzezinski, D. J. DeMaster, R. C. Dugdale, J. Dymond, G. Fischer, R. François, C. Heinze, E. Maier-Reimer, V. Martin-Jézéquel, D. M. Nelson, and B. Quéguiner (2000). A review of the Si cycle in the modern ocean: recent progress and missing gaps in the application of biogenic opal as a paleoproductivity proxy. *Global and Planetary Change* **26** (4) 317-365.

Ragueneau, O., M. Gallinari, L. Corrin, S. Grandel, P. Hall, A. Hauvespre, R. S. Lampitt, D. Rickert, H. Stahl, A. Tengberg, and R. Witbaard (2001). The benthic silica cycle in the Northeast Atlantic: annual mass balance, seasonality, and importance of non-steady-state processes for the early diagenesis of biogenic opal in deep-sea sediments. *Progress in Oceanography* **50** 171-200.

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** (1) 19-41.

Rahman, S., R. C. Aller, and J. K. Cochran (2017). The missing silica sink: revisiting the marine sedimentary Si cycle using cosmogenic ^{32}Si. *Global Biogeochemical Cycles* **31** (10) 1559-1578.

Raimbault, P., N. Garcia, and F. Cerutti (2008). Distribution of inorganic and organic nutrients in the South Pacific Ocean - evidence for long-term accumulation of organic matter in nitrogen-depleted waters. *Biogeosciences* **5** (2) 281-298.

Ramírez, T., D. Cortés, J. M. Mercado, M. Vargas-Yanez, M. Sebastián, and E. Liger (2005). Seasonal dynamics of inorganic nutrients and phytoplankton biomass in the NW Alboran Sea. *Estuarine Coastal and Shelf Science* **65** (4) 654-670.

Reincke, T., and D. Barthel (1997). Silica uptake kinetics of *Halichondria panicea* in Kiel Bight. *Marine Biology* **129** (4) 591-593.

Reiswig, H. M. (1971a). *In situ* pumping activities of tropical Demospongiae. *Marine Biology* **9** 38-50.

Reiswig, H. M. (1971b). Particle feeding in natural populations of three marine demosponges. *Biological Bulletin, Marine Biological Laboratory, Woods Hole* **141** 568-591.

Reiswig, H. M. (1973). Population dynamics of three Jamaican Demospongiae. *Bulletin of Marine Science* **23** 191-226.

Reiswig, H. M. (1974). Water transport, respiration and energetics of three tropical marine sponges. *Journal of Experimental Marine Biology and Ecology* **14** 231-249.

Reiswig, H. M. (1975). Bacteria as food for temperate-water marine sponges. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **53** (5) 582-589.

Reiswig, H. M. (1981). Partial carbon and energy budgets of the bacteriosponge *Verongia fistularis* (Porifera: Demopongiae) in Barbados. *Pubblicazioni della Stazione Zoologica di Napoli: Marine Ecology* **2** 273-293.

Ribes, M., R. Coma, J. M. Gili, A. Svoboda, A. Julia, and J. Parera (2000). A 'semi-closed' recirculating system for the in situ study of feeding and respiration of benthic suspension feeders. *Scirntia Marina* **64** 265-275.

Ribes, M., E. Jimenez, G. Yahel, P. Lopez-Sendino, B. Diez, R. Massana, J. H. Sharp, and R. Coma (2012). Functional convergence of microbes associated with temperate marine sponges. *Environmental Microbiology* **14** (5) 1224 - 1239.

Riesgo, A., M. Maldonado, S. López-Legentil, and G. Giribet (2015). A proposal for the evolution of cathepsin and silicatein in sponges. *Journal of Molecular Evolution* **80** (5-6) 278-291.

Rivkin, R. B., and M. R. Anderson (1997). Inorganic nutrient limitation of oceanic bacterioplankton. *Limnology and Oceanography* **42** (4) 730-740.

Rix, L. (2015) Carbon and nitrogen cycling by Red Sea coral reef sponges. Thesis. Universität Bremen.

Rützler, K., and I. G. Macintyre (1978). Siliceous sponge spicules in coral-reef sediments. *Marine Biology* **49** (2) 147-159.

Sabarathnam, B., A. Manilal, S. Sujith, G. S. Kiran, J. Selvin, A. Thomas, and R. Ravji (2010). Role of sponge associated actinomycetes in the marine phosphorous biogeochemical cycle. *American-Eurasian Journal of Agriculture and Environmental Sciences* **8** (3) 253-256.

Sarmiento, J., and N. Gruber (2006). Ocean biogeochemical dynamics. Princeton University Press.

Schlappy, M. L., S. I. Schottner, G. Lavik, M. M. M. Kuypers, D. de Beer, and F. Hoffmann (2010). Evidence of nitrification and denitrification in high and low microbial abundance sponges. *Marine Biology* **157** (3) 593-602.

Schröder, H. C., D. Brandt, U. Schlobmacher, X. Wang, M. N. Tahir, W. Tremel, S. I. Belikov, and W. E. G. Müller (2007). Enzymatic production of biosilica glass using enzymes from sponges: basic aspects and application in nanobiotechnology (material sciences and medicine). *Naturwissenschaften* **94** 339-359.

Shah, D. A., and L. V. Madden (2004). Nonparametric analysis of ordinal data in designed factorial experiments. *Phytopathology* **94** (1) 33-43.

Shimizu, K., J. Cha, G. D. Stucky, and D. E. Morse (1998). Silicatein alpha: cathepsin L-like protein in sponge biosilica. *Proceedings of the National Academy of Sciences of the United States of America* **95** (11) 6234-6238.

Shimizu, K., T. Amano, M. R. Bari, J. C. Weaver, J. Arima, and N. Mori (2015). Glassin, a histidine-rich protein from the siliceous skeletal system of the marine sponge *Euplectella*, directs silica

polycondensation. *Proceedings of the National Academy of Sciences* **112** (37) 11449-11454.

Shrestha, R. P., and M. Hildebrand (2015). Evidence for a regulatory role of diatom silicon transporters in cellular silicon responses. *Eukaryotic Cell* **14** (1) 29-40.

Siever, R. (1991). Silica in the oceans: Biological-geochemical interplay, p. 287-295. *In* S. H. Scheneider and P. J. Boston [eds.], *Scientists on Gaia*. MIT Press.

Siever, R. (1992). The silica cycle in the Precambrian. *Geochimica Et Cosmochimica Acta* **56** (8) 3265-3272.

Simpson, T. L. (1984). The cell biology of sponges. Springer-Verlag.

Sims, P. A., D. G. Mann, and L. K. Medlin (2006). Evolution of the diatoms: insights from fossil, biological and molecular data. *Phycologia* **45** (4) 361-402.

Southwell, M. W., J. B. Weisz, C. S. Martens, and N. Lindquist (2008). *In situ* fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. *Limnology and Oceanography Methods* **53** 986-996.

Sperling, E. A., J. M. Robinson, D. Pisani, and K. J. Peterson (2010). Where's the glass? Biomarkers, molecular clocks, and microRNAs suggest a 200-Myr missing Precambrian fossil record of siliceous sponge spicules. *Geobiology* **8** (1) 24-36.

Strickland, J. D. H., and T. R. Parsons (1972). A practical handbook of seawater analysis, 2nd ed. Fisheries Research Board of Canada

Struyf, E., A. Smis, S. Van Damme, P. Meire, and D. J. Conley (2009). The global biogeochemical silicon cycle. *Silicon* **1** (4) 207-213.

Taylor, M. W., R. Radax, D. Steger, and M. Wagner (2007). Sponge-associated microorganisms: Evolution, ecology, and biotechnological potential. *Microbiology and Molecular Biology Reviews* **71** (2) 295-347.

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 Physiology* **146** (3) 1397-1407.

Thomassen, S., and H. U. Riisgård (1995). Growth and energetics of the sponge *Halichondria panicea*. *Marine Ecology Progress Series* **128** 239-246.

Tréguer, P., and P. Le Corre (1975). Manuel d'analyse des sels nutritifs dans l'eau de mer (utilisation de l'autoAnalyseur II), 2nd ed. Université de Bretagne Occidentale.

Tréguer, P., D. M. Nelson, A. J. Van Bennekom, D. J. DeMaster, A. Leynaert, and B. Quéguiner (1995). The silica balance in the world ocean - a reestimate. *Science* **268** (5209) 375-379.

Tréguer, P., and P. Pondaven (2000). Global change - silica control of carbon dioxide. *Nature* **406** (6794) 358-359.

Tréguer, P., C. Bowler, B. Moriceau, S. Dutkiewicz, M. Gehlen, O. Aumont, L. Bittner, R. Dugdale, Z. Finkel, D. Iudicone, O. Jahn, L. Guidi, M. Lasbleiz, K. Leblanc, M. Levy, and P. Pondaven (2018). Influence of diatom diversity on the ocean biological carbon pump. *Nature Geoscience* **11** (1) 27-37.

Tréguer, P. J., and C. L. De La Rocha (2013). The world ocean silica cycle. *Annual Review of Marine Science* **5** 477-501.

Tyrrell, T. (1999). The relative influences of nitrogen and phosphorus on oceanic primary production. *Nature* **400** (6744) 525-531.

Van Soest, R. W. M., N. Boury-Esnault, J. Vacelet, M. Dohrmann, D. Erpenbeck, N. J. De Voogd, N. Santodomingo, B. Vanhoorne, M. Kelly, and J. N. A. Hooper (2012). Global diversity of sponges (Porifera). *PLoS One* **7** (4) e35105.

Vicente, J., N. J. Silbiger, B. A. Beckley, C. W. Raczkowski, and R. T. Hill (2016). Impact of high pCO_2 and warmer temperatures on the process of silica biomineralization in the sponge *Mycale grandis*. *Ices Journal of Marine Science* **73** (3) 704-714.

Wang, X., H. C. Schroder, K. Wang, J. A. Kaandorp, and W. E. G. Muller (2012). Genetic, biological and structural hierarchies during sponge spicule formation: from soft sol-gels to solid 3D silica composite structures. *Soft Matter* **8** (37) 9501-9518.

Weissenfels, N., and H. W. Landschoff (1977). Bau und Funktion des Süsswasserschwamms *Ephydatia fluviatilis* L. (Porifera). IV. Die

Entwicklung der monaxialen SiO2-Nadeln in Sandwich-Kulturen. *Zoologische Jahrbücher* **98** 355-371.

Wollast, R., and F. Mackenzie (1983). The global cycle of silica, p. 39–76. *In* A. SR [ed.], *Silicon geochemistry and biochemistry*. Academic Press.

Wörheide, G., M. Dohrmann, D. Erpenbeck, C. Larroux, M. Maldonado, O. Voigt, C. Borchiellini, and D. V. Lavrov (2012). Deep phylogeny and evolution of sponges (phylum Porifera), p. 1-78. *In* M. A. Becerro, M. J. Uriz, M. Maldonado and X. Turon [eds.], *Advances in Marine Biology*. Academic Press.

Yahel, G., J. H. Sharp, D. Marie, C. Hase, and A. Genin (2003). *In situ* feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: bulk DOC is the major source for carbon. *Limnology and Oceanography* **48** (1) 141-149.

Yahel, G., D. Marie, and A. Genin (2005). InEx -a direct in situ method to measure filtration rates, nutrition and metabolism of active suspension feeders. *Limnology and Oceanography Methods* **3** 46-58.

Yahel, G., F. Whitney, H. M. Reiswig, D. I. Eerkes-Medrano, and S. P. Leys (2007). *In situ* feeding and metabolism of glass sponges (Hexactinellida, Porifera) studied in a deep temperate fjord with a remotely operated submersible. *Limnology and Oceanography* **52** 428-440.

Zakem, E. J., A. Al-Haj, M. J. Church, G. L. van Dijken, S. Dutkiewicz, S. Q. Foster, R. W. Fulweiler, M. M. Mills, and M. J. Follows (2018). Ecological control of nitrite in the upper ocean. *Nature Communications* **9**.

Appendix

Section A

Chapter 2: Adjustments of DSi consumption rates during P2.

As part of the DSi (from ca. 6 to 43%) initially provided at a given concentration step was consumed by the sponges during P1, the consumption during P2 started from a DSi concentration somewhat lower than that of P1, making consumption rates during P1 and P2 comparable only if a readjustment is applied, particularly at DSi concentrations below 40 μM. To render P1 and P2 rates comparable, we fitted the empirical P2 consumption of each individual to a non-linear regression model by goodness of fit and used the resulting model to project the expected DSi consumption rate at the slightly higher DSi concentration provided at the beginning of each P1 period.

Section B

Chapter 2: Comparison of average-derived and individual-derived kinetics models.

This section summarizes the statistics of the goodness of fit to a Michaelis-Menten model for the DSi consumption rates by the sponges *Haliclona simulans* and *Suberites ficus* as a function of the experimental DSi availability. For comparative purposes, Table S1 shows the statistics of the goodness of fit when the model was calculated from either the average DSi consumption rate of all individuals of each species at each DSi concentration step (averaged response; see also Figs. 6 and 7 in Chapter 2) or from the individuals responses (individual responses; see Fig. S1). Note that the value of the main parameters of the kinetics (V_{max} and K_m) remain very similar and consistently significant in both cases. In the case of the individual responses, the large dispersion of values (Fig. S1) is lowering the value of the r^2 for the model in both species. However, the relationship keeps statistically significant and still the best fitting model for the data.

Table S1. Comparative summary of the statistics for the goodness of fit to a Michaelis-Menten model for the relationship between experimental DSi availability and DSi consumption rate in the sponge species *Haliclona simulans* and *Suberites ficus*. For each species the value of the intensity of the relationship (r^2) and its statistical significance (p) is given when the model is calculated from the averaged response of all assayed individuals and from the set of individual responses. In each case, the value of the main parameters governing the model (i.e., maximum velocity= V_{max} and half-saturation constant= K_m) and their significance (p) are also listed.

	r^2	p	V_{max}	p	K_m	p
Haliclona simulans						
Averaged response	0.956	<0.0001	0.390 ± 0.031	<0.0001	45.918 ± 11.977	0.0050
Individual responses	0.714	<0.0001	0.390 ± 0.023	<0.0001	45.844 ± 8.851	<0.0001
Suberites ficus						
Averaged response	0.989	<0.0001	0.480 ± 0.018	<0.0001	108.229 ± 12.122	<0.0001
Individual responses	0.464	<0.0001	0.479 ± 0.047	<0.0001	107.346 ± 30.969	0.0007

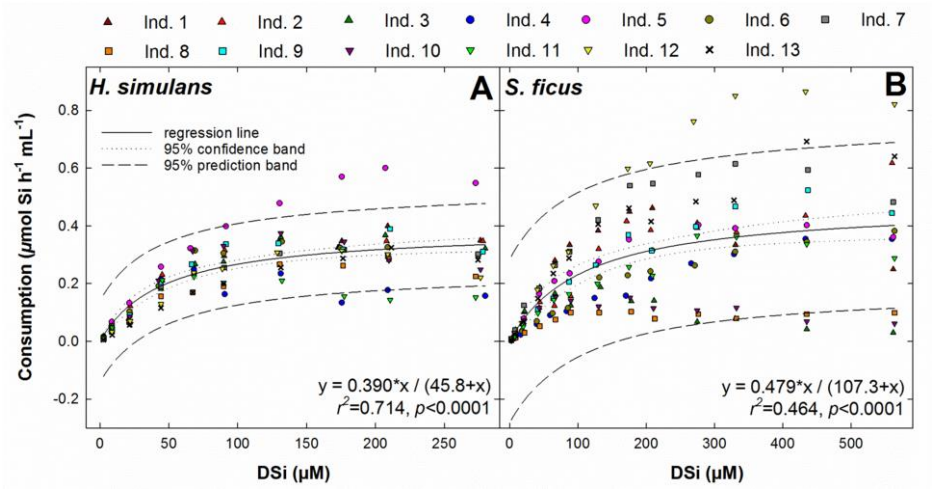


Figure S1. Summary of the statistically significant goodness of fit for the relationship between DSi availability and DSi consumption rate in *Haliclona simulans* **(A)** and *Suberites ficus* **(B).** In both cases, the best fit indicates a hyperbolic model that matches the Michaelis-Menten equation and it has been calculated from the sets of individuals (ind.) responses at each DSi concentration step. For a comparison with the model calculated from the averaged response at each DSi step, see Figs. 6 and 7 and Table S1.

Section C

As it has been indicated at each chapter, we preferred to normalize DSi consumption rates of the assayed sponges by body volume. Here are summarized the volume, wet weight, dry weight, and ash weight data (when measured) for the set of individuals investigated at each Si-consumption experiment conducted during this thesis, for future biomass conversions if needed.

Chapter 1

Experiment I		Volume	Wet Weight	Dry Weight	Ash Weight
Species	Individual	(ml)	(g)	(g)	(g)
Tethya citrina	1	15.5	17.16	3.261	2.071
	2	10.0	9.58	1.765	1.022
	3	8.0	7.31	1.249	0.822
	4	1.8	2.20	0.417	0.272
	5	2.5	1.52	0.239	0.153
	6	12.0	10.63	2.017	1.330
	7	14.0	12.32	2.635	1.743
	8	4.0	2.01	0.468	0.333
	9	21.0	14.92	2.457	1.452
	10	2.5	1.52	0.255	0.181
	11	21.0	19.37	3.657	2.410
	12	24.0	22.92	5.633	3.595
Hymeniacidon perlevis	1	7.0	6.44	0.551	0.249
	2	30.0	26.47	1.985	1.078
	3	7.0	9.46	0.899	0.403
	4	2.0	1.41	0.108	0.049
	5	15.0	13.30	1.106	0.604
	6	10.0	8.00	0.760	0.362
	7	14.0	11.94	0.898	0.440
	8	16.0	15.34	1.247	0.653
	9	11.0	4.74	0.390	0.171
	10	6.0	3.91	0.299	0.152
	11	6.0	2.75	0.219	0.107
	12	12.0	12.35	1.175	0.540
	13	4.0	3.49	0.383	0.207
Experiment II		Volume	Wet Weight	Dry Weight	Ash Weight
Species	Individual	(ml)	(g)	(g)	(g)
Tethya citrina	1	5.0	1.47	0.279	0.178
	2	13.0	6.31	1.250	0.749
	3	3.2	1.36	0.234	0.124
	4	5.5	2.33	0.479	0.302
	5	3.0	1.07	0.195	0.118
	6	5.5	5.03	1.032	0.612
	7	12.5	8.53	2.000	1.140
	8	10.5	4.52	0.790	0.492
Hymeniacidon perlevis	1	9.0	7.24	0.748	0.317
	2	3.6	1.95	0.202	0.099
	3	16.5	12.11	1.277	0.542
	4	5.0	2.14	0.161	0.072
	5	7.0	4.00	0.359	0.157
	6	3.5	2.08	0.186	0.079
	7	13.0	10.68	0.998	0.427
	8	5.5	2.25	0.183	0.080
	9	9.0	4.91	0.417	0.180

Chapter 2

Species	Individual	Volume (mL)	Wet Weight (g)	Dry Weight (g)	Ash Weight (g)
Haliclona simulans	1	10.5	4.91	0.564	0.375
	2	5.5	2.96	0.298	0.186
	3	7.0	4.90	0.467	0.292
	4	5.0	3.01	0.356	0.219
	5	4.5	2.71	0.327	0.211
	6	6.0	2.90	0.283	0.170
	7	6.5	3.25	0.358	0.221
	8	8.5	3.60	0.428	0.301
	9	6.5	3.15	0.332	0.201
	10	9.0	6.13	0.656	0.414
	11	4.5	1.51	0.154	0.094
	12	11.0	7.15	0.785	0.451
	13	9.5	6.26	0.631	0.407
Suberites ficus	1	7.5	3.04	0.653	0.475
	2	13.0	8.36	1.140	0.792
	3	4.5	1.83	0.352	0.266
	4	35.5	33.24	3.119	1.950
	5	9.5	5.28	0.842	0.589
	6	25.0	25.65	2.902	1.891
	7	6.5	4.17	0.767	0.520
	8	4.5	1.85	0.337	0.248
	9	14.0	9.84	1.372	0.954
	10	6.0	2.53	0.503	0.396
	11	15.5	11.29	1.572	1.168
	12	12.0	7.48	1.333	0.903
	13	11.0	7.75	0.962	0.634

Chapter 3

Species	Individual	Volume (mL)	Wet Weight (g)	Dry Weight (g)	Ash Weight (g)
Tethya citrina	1	2.8	2.58	0.459	0.216
	2	10.0	7.62	1.293	0.628
	3	16.0	14.16	2.395	1.237
	4	13.0	11.08	1.957	1.129
	5	6.0	4.74	0.886	0.472
	6	15.0	13.54	2.504	1.406
	7	7.5	6.22	1.317	0.679
	8	11.5	9.51	1.751	0.874
Hymeniacidon perlevis	1	6.0	3.65	0.490	0.285
	2	2.5	0.75	0.086	0.051
	3	1.5	0.57	0.062	0.030
	4	4.0	3.02	0.345	0.181
	5	6.0	4.05	0.569	0.319
	6	2.3	0.85	0.104	0.056
	7	2.3	1.15	0.142	0.069
	8	10.0	7.97	0.988	0.540
	9	7.0	4.95	0.488	0.267

Chapter 4

Species	Individual	Volume (mL)	Wet Weight (g)	Dry Weight (g)	Ash Weight (g)
Tethya citrina	1	20.0	19.74	4.173	1.807
	2	18.0	14.95	2.718	1.456
	3	17.0	12.64	2.221	1.113
	4	21.0	18.42	3.334	1.514
	5	11.0	9.52	1.703	0.825
	6	19.0	18.71	3.729	1.830
	7	27.0	26.32	4.618	2.333
	8	13.0	11.13	1.882	1.017

Chapter 5

Species	Individual	Volume (mL)	Dry Weight (g)
Vazella pourtalesii	1	81	3.60
	2	191	9.86
	3	136	6.83
	4	141	7.45
	5	61	4.75
	6	71	3.60
	7	111	7.42
	8	81	3.71
	9	41	2.19
	10	81	4.20
	11	51	2.62

Published work

Picture: Ground of *Vazella pourtalesii* in Nova Scotia (Canada), from video recorded by ROV-ROPOS.

LIMNOLOGY
and
OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 61, 2016, 2139–2150

doi: 10.1002/lno.10359

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

María López-Acosta,[1] Aude Leynaert,[2] Manuel Maldonado*[1]
[1]Department of Marine Ecology, Centro de Estudios Avanzados de Blanes (CEAB - CSIC), Girona, Blanes, Spain
[2]Laboratoire des Sciences de l'Environnement Marin, UMR CNRS 6539, Institut Universitaire Européen de la Mer, Technopôle Brest-Iroise, Plouzané, France

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),

*Correspondence: maldonado@ceab.csic.es

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

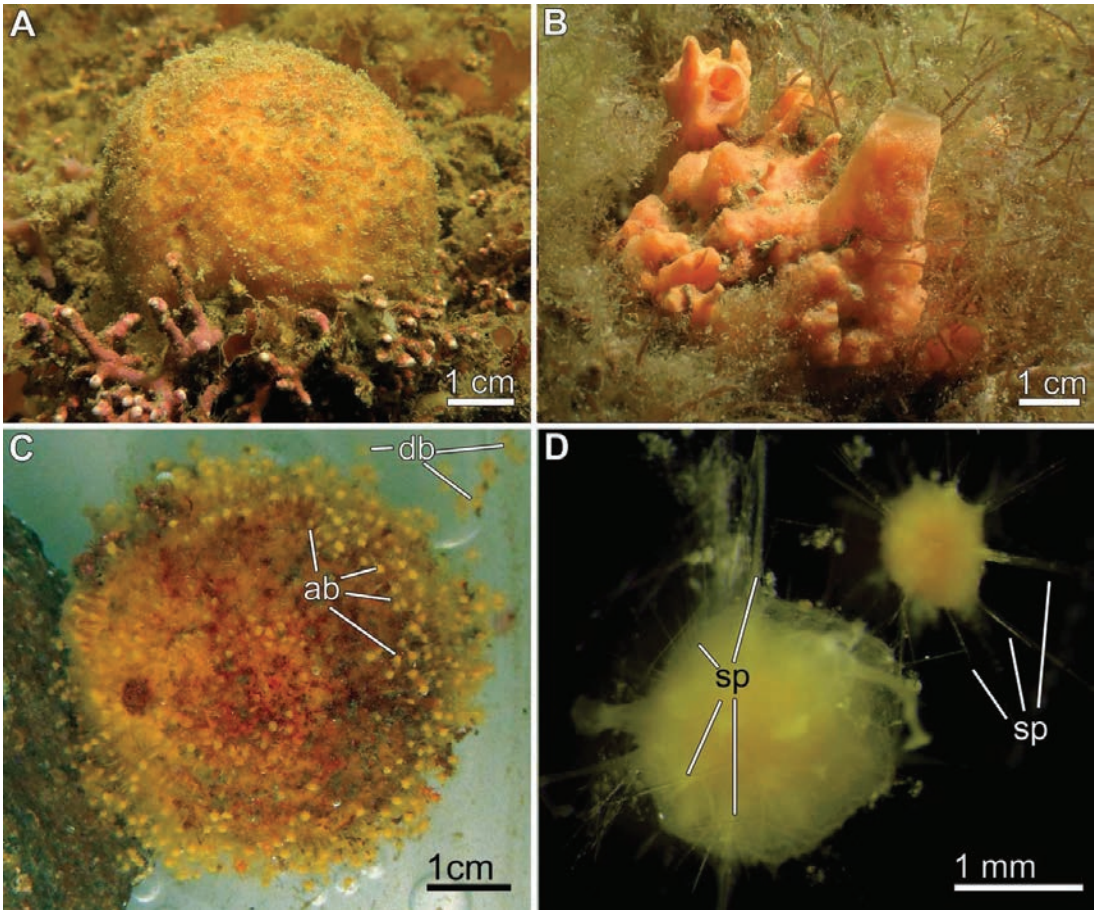


Fig. 1. (**A–B**) In situ views of *Tethya citrina* (A) and *Hymeniacidon perlevis* (B) growing at the Bay of Brest. (**C**) View of a *T. citrina* individual producing buds in the laboratory. Most of the buds (ab) still remain attached to the body, but some have already been detached (db) and rest at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

prior to the onset of the experiments. They were transported 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-

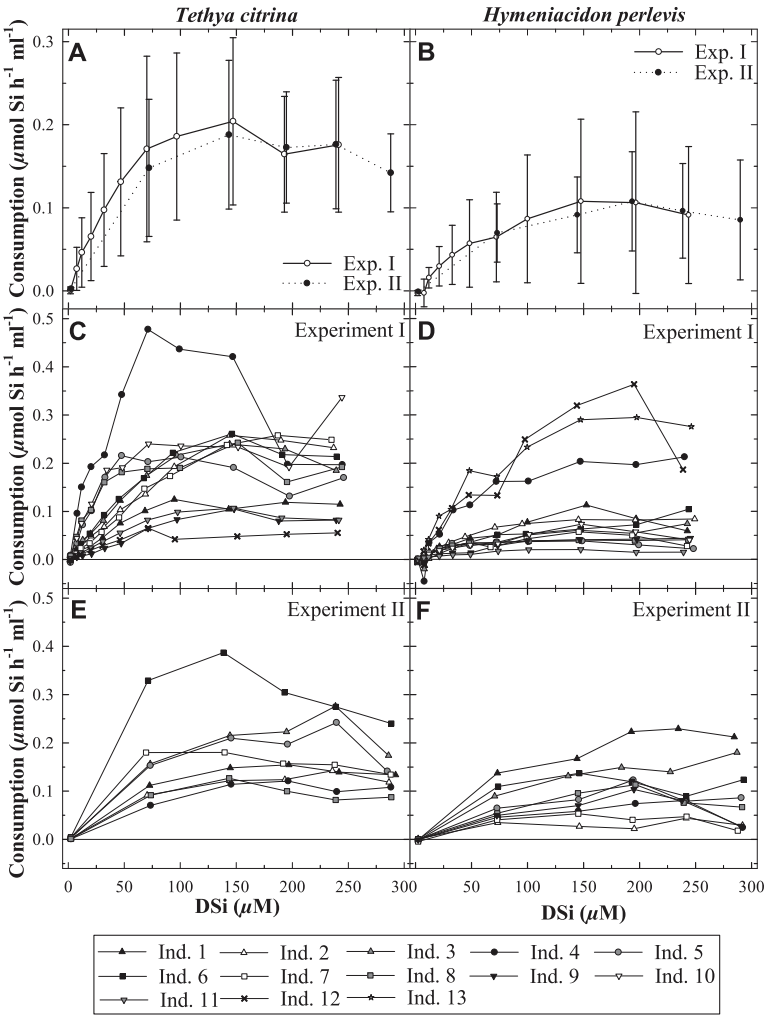


Fig. 2. (**A–B**) Average (±SD) consumption rates (μmol Si h^{-1} per sponge ml) of *Tethya citrina* and *Hymeniacidon perlevis* as a function of silicic acid (DSi) concentration during experiments I and II. (**C–D**) Consumption rates of each individual of *T. citrina* (C) and *H. perlevis* (D) as a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

individual variability was noticed (Fig. 2D), with the highest 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

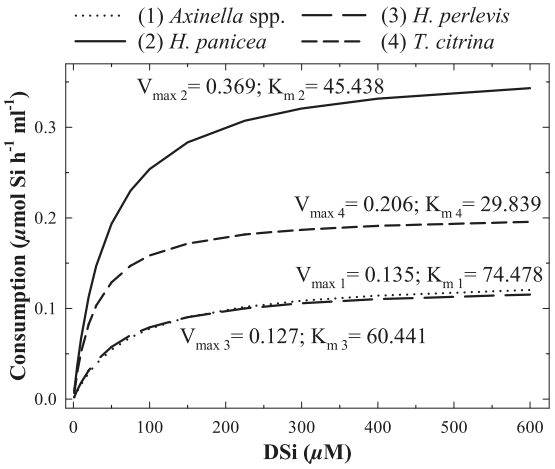


Fig. 3. Comparison of the Michaellis-Menten kinetics ($V = V\text{max} \times [\text{DSi}] / (K\text{m}+[\text{DSi}])$ fitted for silicic acid (DSi) consumption in the four demosponges investigated to date: *Axinella* spp. (Maldonado et al. 2011), *Halichondria panicea* (Reincke and Barthel 1997), and *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.

average saturating concentration of 150 μM with only an 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

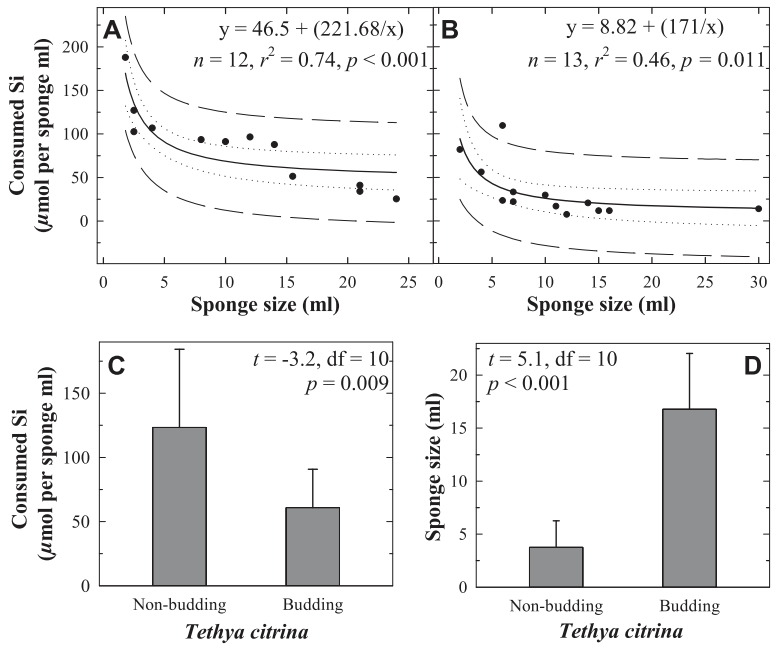


Fig. 4. (**A–B**) Relationship between sponge size (ml) and total consumption of silicic acid (DSi) over the 33 days that experiment I lasted. For both *Tethya citrina* (A) and *Hymeniacidon perlevis* (B) the relationship fitted an inverse, first order, polynomial model. Dotted and dashed lines refer 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 ($\sim$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).

References

Alex, A., V. Vasconcelos, P. Tamagnini, A. Santos, and A. Antunes. 2012. Unusual symbiotic cyanobacteria association in the genetically diverse intertidal marine sponge *Hymeniacidon perlevis* (Demospongiae, Halichondrida). PLoS One **7**: e51834. doi:10.1371/journal.pone.0051834

Amo, Y. D., 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

Barthel, D. 1995. Tissue composition of sponges from the Weddell Sea, Antarctica: Not much meat on the bones. Mar. Ecol. Prog. Ser. **123**: 149–153. doi:10.3354/meps123149

Conley, D. J., and C. L. Schelske. 1993. Potencial role of sponge spicules in influencing the silicon biogeochemistry of Florida lakes. Can. J. Fish. Aquat. Sci. **50**: 296–302. doi:10.1139/f93-034

Conway, H. L., and P. L. 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

Cha, J. N., K. Shimizu, Y. Zhou, S. C. Christiansen, B. F. Chmelka, G. D. Stucky, and D. E. Morse. 1999. Silicatein filaments and subunits from a marine sponge direct the polymerization of silica and silicones in vitro. Proc. Natl. Acad. Sci. USA **96**: 361–365. doi:10.1073/pnas.96.2.361

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

DeMaster, D. J. 1991. Measuring biogenic silica in marine sediments and suspended matter, p. 363–367. *In* D. C. Hurd and S. D. W. [eds.], Marine particles: Analysis and characterization. American Geophysical Union.

Ehrlich, H. 2011. Silica biomineralization in sponges, p. 796–808. *In* J. Reitner and V. Thiel [eds.], Encyclopedia of geobiology. Springer.

Ehrlich, H., and others. 2010. Mineralization of the metre-long biosilica structures of glass sponges is templated on hydroxylated collagen. Nat Chem. **2**: 1084–1088. doi:10.1038/nchem.899

Frøhlich, 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

Fuller, T. L., and J. R. Hughey. 2013. Molecular investigation of the invasive sponge *Hymeniacidon sinapium* (de

Laubenfels, 1930) in Elkhorn Slough, California. Aquat. Invasions **8**: 59–66. doi:10.3391/ai.2013.8.1.07

Harper, H. E., and A. H. Knoll. 1975. Silica, diatoms and Cenozoic radiolarian evolution. Geology **3**: 175–177. doi:10.1130/0091-7613(1975)3<175:SDACRE>2.0.CO;2

Harriss, R. C. 1966. Biological buffering of oceanic silica. Nature **212**: 275–276. doi:10.1038/212275a0

Hoshino, S., D. Saito, and T. Fujita. 2008. Contrasting genetic structure of two Pacific *Hymeniacidon* species. Hydrobiologia **603**: 313–326. doi:10.1007/s10750-008-9295-2

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. Longphuirt, 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

Maldonado, M. 2009. Embryonic development of verongid demosponges supports independent acquisition of spongin skeletons as alternative to the siliceous skeleton of sponges. Biol. J. Linn. Soc. **97**: 427–447. doi:10.1111/j.1095-8312.2009.01202.x

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. González, and I. Vaquerizo. 2011. Silicon uptake by sponges: A twist to understanding nutrient cycling on continental margins. Sci. Rep. **1**: 1–8. doi:10.1038/srep00030

Maldonado, M., and others. 2016. Sponge grounds as key marine habitats: A synthetic review of types, structure, functional roles, and conservation concerns p. In press. *In* S. Rossi, L. Bramanti, A. Gori and C. Orejas [eds.], Marine animal forest. Springer. doi:10.1007/978-3-319-17001-5_24_1

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

Maliva, R. G., A. H. Knoll, and R. Siever. 1989. Secular change in chert distribution: A reflection of evolving biological participation in the silica cycle. Palaios **4**: 519–532. doi:10.2307/3514743

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

Müller, W. E. G., M. Rothenberger, A. Boreiko, W. Tremel, A. Reiber, and H. C. Schröder. 2005. Formation of siliceous spicules in the marine demosponge *Suberites domuncula*. Cell Tissue Res. **321**: 285–297. doi:10.1007/s00441-005-1141-5

Nelson, D. M., G. F. Riedel, R. Millan-Nunez, and J. R. Lara-Lara. 1984. Silicon uptake by algae with no known Si requirement. 1. True cellular uptake and pH-induced precipitation by *Phaeodactylum tricornutum* (Bacillariophyceae) and *Platymonas* sp. (Prasinophyceae). J. Phycol. **20**: 141–147. doi:10.1111/j.0022-3646.1984.00141.x

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. Glob. Biogeochem. Cycles **9**: 359–372. doi:10.1029/95GB01070

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

Ragueneau, O., D. B. Varela, P. Tréguer, B. Quéguiner, and Y. Del Amo. 1994. Phytoplankton dynamics in relation to the biogeochemical cycle of silicon in a coastal ecosystem of western Europe. Mar. Ecol. Prog. Ser. **106**: 157–172. doi:10.3354/meps106157

Ragueneau, O., and others. 2000. A review of the Si cycle in the modern ocean: Recent progress and missing gaps in the application of biogenic opal as paleoproductivity proxy. Global Planet. Change **26**: 317–365. doi:10.1016/S0921-8181(00)00052-7

Reincke, T., and D. Barthel. 1997. Silica uptake kinetics of *Halichondria panicea* in Kiel Bight. Mar. Biol. **129**: 591–593. doi:10.1007/s002270050200

Reiswig, H. M. 1971a. *In situ* pumping activities of tropical Demospongiae. Mar. Biol. **9**: 38–50. doi:10.1007/BF00348816

Reiswig, H. M. 1971b. Particle feeding in natural populations of three marine demosponges. Biol. Bull. Mar. Biol. Lab. Woods Hole **141**: 568–591. doi:10.2307/1540270

Riesgo, A., M. Maldonado, S. López-Legentil, and G. Giribet. 2015. A proposal for the evolution of cathepsin and silicatein in sponges. J. Mol. Evol. **80**: 278–291. doi:10.1007/s00239-015-9682-z

Sarmiento, J., and N. Gruber. 2006. Ocean biogeochemical dynamics. Princeton Univ. Press.

Shimizu, K., J. N. Cha, G. D. Stucky, and D. E. Morse. 1998. Silicatein alpha: Cathepsin L-like protein in sponge biosilica. Proc. Natl. Acad. Sci. USA **95**: 6234–6238. doi:10.1073/pnas.95.11.6234

Shimizu, K., T. Amano, M. R. Bari, J. C. Weaver, J. Arima, and N. Mori. 2015. Glassin, a histidine-rich protein from the siliceous skeletal system of the marine sponge *Euplectella*, directs silica polycondensation. Proc. Natl. Acad. Sci. USA **112**: 11449–11454. doi:10.1073/pnas.1506968112

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

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

Tréguer, P. J., and C. L. De La Rocha. 2013. The world ocean silica cycle. Annu. Rev. Mar. Sci. **5**: 477–501. doi:10.1146/annurev-marine-121211-172346

Wang, X. H., and others. 2011. Advances in research on siliceous sponge spicules: Novel insight into the understanding of biomineralization mechanisms and bionic applications. Acta Geoscientica Sinica **32**: 129–141.

Wang, X., H. C. Schroder, K. Wang, J. A. Kaandorp, and W. E. G. Muller. 2012. Genetic, biological and structural hierarchies during sponge spicule formation: From soft solgels to solid 3D silica composite structures. Soft Matter **8**: 9501–9518. doi:10.1039/c2sm25889g

Weissenfels, N., and H. W. Landschoff. 1977. Bau und Funktion des Süsswasserschwamms *Ephydatia fluviatilis* L. (Porifera). IV. Die Entwicklung der monaxialen SiO_2-Nadeln in Sandwich-Kulturen. Zool. Jarhrb **98**: 355–371.

Acknowledgments

The authors thank J. Grall, E. Amice, T. Le Bec and I. Bihannic for their invaluable help during underwater field work and for pictures 1A-B. The staff maintaining the SOMLIT-Brest database is thanked for making public their information on nutrient distributions at the Bay of Brest. This research was funded by two grants of the Spanish Ministry of Economy and Competitiveness (CTM2012-37787 and CTM2015-67221-R) awarded to MM and an associated FPI fellowship to ML-A.

Submitted 21 December 2015
Revised 27 April 2016
Accepted 26 May 2016

Associate editor: Rebecca Vega Thurber