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CHAPTER THREE

Nutrient Fluxes Through Sponges: Biology, Budgets, and Ecological Implications

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Contents

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Abstract

Marine sponges are able to process a variety of carbon (C), nitrogen (N), phosphorous (P), and silicon (Si) dissolved compounds, in addition to the particulate C, N, and P obtained through regular feeding. While Si fluxes through sponges are exclusively related to the elaboration of their skeleton of biogenic silica, C, N, and P fluxes derive from a complex combination of metabolic processes that include feeding, respiration, egestion, excretion, as well as hosting of large microbial populations within the sponge body. Because of the remarkable abundance of sponges in many benthic marine communities, they have the potential to impact the availability of the compounds they take up and release, affecting the benthic–pelagic coupling and cycling rates of chemical elements that are crucial to determine growth of bacterioplankton and primary producers at the ecosystem level. Unfortunately, our knowledge and understanding of the magnitude of the sponge-meditated nutrient fluxes and their ecological implications depends much on the compound type (i.e. C, N, P, or Si). Herein, we review the available knowledge on the subject with emphasis on recent developments.

Key Words: carbon balance; carbon metabolism; DOC uptake; nitrogen balance; nitrogen metabolism; phosphorous balance; POM uptake; silicate; silicon balance; silicon metabolism

1. Introduction

Sponges are ubiquitous marine organisms, occurring with moderate to high abundance on continental shelves, slopes, abyssal plains, and even hadal bottoms. Because of their ubiquity and abundance, sponges are now understood as an ecologically important benthic group that plays a variety of functional roles in marine ecosystems: (1) They have long been recognized to influence the structure of benthic communities through substrate occupation. Sponges participate in processes of both spatial exclusion of competitors (Rützler, 1970; Sarà, 1970; Thacker *et al.*, 1998; and references therein) and cooperative spatial interactions, being relevant habitat builders that add complexity to the communities by favouring increased abundance and diversity of other organisms (Dayton *et al.*, 1974; McClintock *et al.*, 2005; Wulff, 2008). (2) In carbonated ecosystems, such as coral reefs, sponges are thought to influence the carbonate framework through processes of both cementation (Wulff, 1984, 2001) and bioerosion (Diaz and Rützler, 2001). (3) The suspension-feeding activity of sponges (mostly on bacterioplankton and microphytoplankton) has also been vindicated as a significant trophic link between the benthos and the overlying water column, being suspected to impact the benthic–pelagic coupling of particulate

carbon fluxes at a diversity of scales (e.g. Reiswig, 1971b; Pile, 1997; Gili and Coma, 1998; Ribes *et al.*, 1999b). (4) Yet, sponges have a further functional role that remains relatively understudied despite its multiple ecological and biogeochemical derivations. During the past decades, the notion has emerged that sponges may influence the availability of dissolved nutrients, such as dissolved carbon (Yahel *et al.*, 2003; De Goeij *et al.*, 2008b), various nitrogen compounds (Corredor *et al.*, 1988; Bayer *et al.*, 2007; Jiménez and Ribes, 2007), and silicate (Reincke and Barthel, 1997; Scheffers *et al.*, 2004; Maldonado *et al.*, 2005, 2010a). These dissolved nutrients exert a major impact on primary production and their use by the phytoplankton is, in turn, responsible for establishing interconnections of much ecological, environmental, and biogeochemical relevance between C, N, P, and Si cycles. Therefore, it is urgent to identify the level at which sponges affect the availability and cycling of those chemical elements. Here, we review the available information to date with the objective of providing a better global understanding of the role of sponges as sources, sinks, and cyclers of a variety of relevant C, N, P, and Si dissolved and particulate compounds, usually referred to as "nutrients".

1.1. Measurement of nutrient fluxes

Nutrient fluxes in sponges are commonly evaluated through estimates of uptake rates and efflux rates in individual organisms, then extrapolated to the population or community level whenever possible; however, comparison among methods is not always straightforward (Riisgård, 2001; Yahel *et al.*, 2005). Different methodologies can be used, but not all of them are suitable for certain organisms. Based on Yahel *et al.* (2005), methods can be classified as indirect or direct techniques.

1.1.1. Indirect techniques

They have been widely used in research of nutrition and physiology of filter feeders (Wafar *et al.*, 1990; Díaz and Ward, 1997; Ribes *et al.*, 2000; Pile *et al.*, 2003; Jiménez and Ribes, 2007). For these techniques, animals are placed in a vessel with little or no renewal of water. Changes in compound concentration (cells, coloured beads, dissolved radioactive label, excretion products, etc.) in the surrounding water over time are attributed to the animal's activity and are used to quantify production and/or removal rates (Riisgård, 2001). The comparison with a control situation (same vessel without organisms) is used to correct for changes in compound concentration not due to the organisms' activity. Indirect methods cannot differentiate pumping rates and retention efficiency, so in feeding studies the term "clearance rate" is used, that is, volume of water cleared of particles (assuming an efficiency in the removal of 100%) per time unit (Riisgård, 2001). Incubation in closed vessels is one of the most popular indirect

methods used in the study of diverse benthic suspension feeders, such as bivalves (Roditi and Fisher, 1999; Petersen *et al.*, 2004), sponges (Díaz and Ward, 1997; Ribes *et al.*, 1999b; Jiménez and Ribes, 2007; Bayer *et al.*, 2008; De Goeij *et al.*, 2008a; Van Duyl *et al.*, 2008), corals (Ribes *et al.*, 2000), and ascidians (Ribes *et al.*, 2000).

There are several problems related to the use of closed systems for the study of feeding and excretion of suspension feeders. In particular, decline in oxygen and/or food concentration and accumulation of excretion compounds in the incubation water are reported to alter-feeding behaviour and pumping (Díaz and Ward, 1997; Yahel *et al.*, 2005; Hadas *et al.*, 2008). Other biases may be induced by re-filtration of the incubation water, which can cause underestimates of removal rates if sampling times are not well adjusted to the pumping rates of the studied animal (Ribes *et al.*, 2000; Yahel *et al.*, 2005).

In flow-through methods (also an indirect technique), animals are placed in a vessel with a continuous water flow, and their activity measured by determining differences in compound concentration between inflow and outflow water. The flow-through method would overcome some of the problems associated with incubations (i.e. stagnation), as water is renewed during the experiments. Some of the shortcomings of this methodology are that all water flowing through the chamber has to be in contact with the filter-feeder and that re-filtration has to be avoided. Also, choosing the best flow rate in the chamber is not trivial: flow rate has to be low enough to detect the organism signal in the outflow but high enough to avoid re-filtration. Additionally, flow rate can have an effect over the regular pumping behaviour (Yahel *et al.*, 2005; Hadas *et al.*, 2008), with unusual flows either stimulating or inhibiting pumping.

1.1.2. Direct techniques

They imply sampling water immediately upon completing a single run through the organism's filtration system. Changes in compound concentration between the inflow and the outflow, combined with organisms' pumping rates (measured with Dye Front Speed method, flowmeters, dye-video records, etc.) result in ingestion/uptake or production rates (Reiswig, 1971a,b, 1974; Savarese *et al.*, 1997; Yahel *et al.*, 2005; Weisz *et al.*, 2008). Problems associated to indirect methodology, such as stagnation or flux in the vessel, are avoided with the application of direct methodology. As concentrations are measured directly from inflows and outflows, and not from ambient water, direct techniques facilitate "*in situ*" approaches both in the field (Reiswig, 1971a; Pile *et al.*, 1996; Yahel *et al.*, 2003; Southwell *et al.*, 2008b) or in tanks with high renovation rates (Hadas *et al.*, 2006; Yahel *et al.*, 2006). Also with the application of direct methodology, retention efficiency and pumping rate are clearly discriminated, allowing more detailed studies of nutrition, including food selectivity (Pile *et al.*, 1996;

Yahel *et al.*, 2006). Despite the advantages of the direct techniques (Riisgård, 2001, 2004; Yahel *et al.*, 2005), they are not free of limitations, as the organisms must have a well-defined excurrent aperture to allow reliable direct sampling without contacting the animal tissue. Also, sampling suction rate has to be much lower than organism excurrent flow rate to avoid contamination of the outgoing-water sample with ambient water (Yahel *et al.*, 2005). These requirements make the In-Ex method unsuitable for organisms with extremely low pumping rates, those with an excurrent aperture < 1 mm, and those lacking well-defined outflows.

Different methodologies have been applied indiscriminately to sponges and their comparisons that can result in conflicting estimates of uptake and/or release rates and consequently in large differences when extrapolated as community-scale fluxes. Although some efforts have been done to evaluate the effect of different methods for some particular organisms, such as, for instance, mussels (Riisgård, 2001; Yahel *et al.*, 2005), few methodological comparisons have been performed for sponges (but see Southwell *et al.*, 2008b).

1.2. Nutrient availability: Particulate and dissolved compounds

It has been documented that marine sponges are able to remove and return a variety of both particulate and dissolved "nutrients" and related compounds, that is, those known or suspected to interfere with primary production and bacterioplankton growth.

1.2.1. Particulate nutrients

Particulate organic matter (POM) can be generally divided into live particulate organic matter (LPOM) composed of various planktonic cells, and non-living particulate organic matter (hereafter "detritus"), which is a structurally and chemically diverse fraction deriving from several sources (Ribes *et al.*, 2003; Wilson *et al.*, 2003; Hadas *et al.*, 2009). Detritus significance as a food source for marine sponges is poorly documented, but in some cases it can contribute up to 50% of particulate organic carbon (POC; Hadas *et al.*, 2009). Sponges can retain LPOM from viruses (< 0.2 μm) to larger cells as phytoplankton, but, due to the features of their filtration system, they are particularly efficient in retaining 0.2–2 μm picoplankton cells (Pile *et al.*, 1996; Ribes *et al.*, 1999b; Hadas *et al.*, 2006; Yahel *et al.*, 2006).

Regular particle capture can take place at three functional sites inside sponges. Large particles (> 50 μm) that do not enter the ostia are taken up at the surface by epithelial pinacocytes. Smaller particles (< 50 μm) that enter the ostia are taken up by pinacocytes lining the canal walls. The smallest particles (< 5 μm) are often engulfed by the choanocytes in the

choanochambers. After capture by pinacocytes and choanocytes, food particles in incipient digestion stage are passed to the mesohyl cells by transcytosis. Once inside the mesohyl, they are phagocytosed mostly by archaeocytes, which complete digestion and deliver the resulting assimilable compounds to other cells. The general perception from the earliest studies on sponge feeding is that particle uptake by sponges is highly efficient at the 1–2 μm size range but largely unselective (Reiswig, 1971a; Pile *et al.*, 1996; Ribes *et al.*, 1999b). Nevertheless, more recent studies have shown that contribution of different planktonic groups may differ among sponge species due to variation in choanocyte numbers, feeding methods (Leys and Eerkes-Medrano, 2006), and complexity of aquiferous systems (Weisz *et al.*, 2008).

Table 3.1 shows retention efficiency of various picoplankton cells by several sponges. Some species, such as *Callyspongia* sp. from southwestern Australia, exhibited consistently negative selectivity for LDNA bacteria (i.e. smaller and less active heterotrophic bacteria) and positive selectivity for *Synechococcus* spp.; HDNA bacteria (i.e. bigger and more active heterotrophic bacteria) experienced neutral or positive selection (Hanson *et al.*, 2009). In the Mediterranean demosponge *Spongia officinalis,* it has also been found a positive selection on *Synechococcus* spp. and picoeukaryotes (Topçu *et al.*, 2010). Additional experimental evidence of selective feeding on particular functional categories of external bacteria and bacterial symbionts has been reported in the Mediterranean demosponge *Aplysina aerophoba* (Wehrl *et al.*, 2007). Contrary to expectations, the hexactinellid *Aphrocallistes vastus* was found to select against *Synechococcus* spp. during July (Yahel *et al.*, 2006; 2007). This negative selection was intriguing, as *Synechococcus* spp. and *Prochlorococcus* spp. appear to be the most preferred food type by most marine demosponges examined to date (Pile *et al.*, 1996; Pile, 1999; Ribes *et al.*, 1999b; Van Duyl *et al.*, 2002; Yahel *et al.*, 2003; 2005). More recently, Maldonado *et al.* (2010b), through a combination of laboratory experiments and transmission electron microscopy study, assessed the potential of the marine sponge *Hymeniacidon perlevis* to feed on three pathogenic microbes common in coastal waters: two bacteria (*Escherichia coli* and *Vibrio anguillarum*) and the marine yeast *Rhodotorula* sp. All three microbes were ingested by the sponge, but selectively, at different rates and using different cellular mechanisms. Such differences in the ingestion and digestion pathways led to large differences in the effectiveness of the sponge to remove a particular microbial type from the ambient water.

1.2.2. Dissolved nutrients and sponge-associated microbes

Many studies have shown that the ability of sponges to remove and/or release large quantities of organic and inorganic dissolved compounds may be related to photoautotrophy and chemoautotrophy processes mediated by sponge-associated microbial communities.

Table 3.1 Retention efficiency (%) of heterotophic bacteria (Hb), *Synechococcus* spp. (Syn), *Proclorococcus* spp. (Pro), nanoeucaryotes (Nk), and pikoeucaryotes (Pk) by sponge species from different locations

			Retention efficiency (%)					
Sponge	Location	Bacterial type	Hb	Syn	Pk	Nk	Pro	Source
Spongia officinalis	Mediterranean		48	77–89	77–87	41–64	–	Topçu *et al.* (2010)
Callyspongia sp.	NW Australia	LDNA	40	86	–	–	–	Hanson *et al.* (2009)
		HDNA	85					
Negombata magnifica	Red Sea		83–90	98–99	65–90	>55	–	Hadas *et al.* (2009)
Theonella swinhoei	Red Sea		85–95		20–40		–	Yahel *et al.* (2003)
Rhabdocalyptus dawsoni	NE Pacific	LDNA	92	80–86	84–91	100	NP	Yahel *et al.* (2006, 2007)
		HDNA	89–97					
Aphrocallistes vastus	NE Pacific	LDNA	84	68	82	80	NP	Yahel *et al.* (2006, 2007)
		HDNA	82					
Sericolophus hawaiicus	Central Pacific		47–54			–	–	Pile and Young (2006)
Dysidea avara	Mediterranean		98	99	91	–	87	Jiménez (2011)
Chondrosia reniformis	Mediterranean		98	96	92	–	93	Jiménez (2011)
Agelas oroides	Mediterranean		79	94	94	–	93	Jiménez (2011)
Neopetrosia problematica	NE Pacific		80	90	98	–	NP	Jiménez (2011)
Haliclona mollis	NE Pacific		88	91	98	–	NP	Jiménez (2011)
Haliclona sp.	NE Pacific		83	98	99	–	NP	Jiménez (2011)
Neopetrosia vanilla	NE Pacific		77	82	100	–	NP	Jiménez (2011)
Sycon coactum	NE Pacific		81	79	98	–	NP	Jiménez (2011)
Thethya leysae	NE Pacific		91	90	91	–	NP	Jiménez (2011)
Halocliona sp.	Red Sea		68	93	92	–	93	Jiménez (2011)
Diacarnus sp.	Red Sea		65	100	98	–	100	Jiménez (2011)
Theonella swinhoei	Red Sea		90	100	98	–	100	Jiménez (2011)
Niphates rowi	Red Sea		69	98	95	–	98	Jiménez (2011)

LDNA, low DNA heterotrophic bacteria; HDNA, high DNA heterotrophic bacteria; NP, not present; –, data not available.

Most marine sponges establish a persistent association with microorganisms (archaea, bacteria, cyanobacteria, yeasts, dinoflagellates, diatoms, etc.), being in many cases a symbiosis, as understood by Moya *et al.* (2008): long-term association between two or more organisms of different species that is integrated at the behavioural, metabolic, or genetic level. Microbial associates often occur intercellularly in the sponge mesohyl (Fig. 3.1), although small populations of "microrganisms" may also occur intracellularly

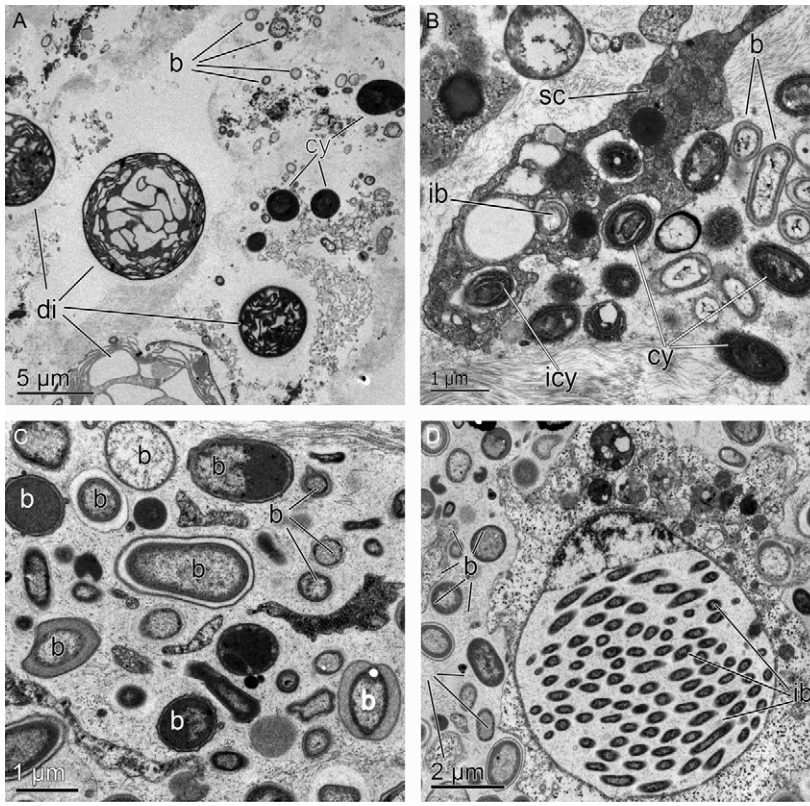

Figure 3.1 Transmission electron microscopy showing microbial communities in the tissues of marine sponges. (A) Detail of the mesohyl of the demosponge *Ircinia variabilis*, showing intercellularly associated dinoflagellates (di), cyanobacteria (cy), and bacteria (b). (B) Detail of the mesohyl of the demosponge *Chondrilla nucula,* showing abundance of intercellular bacteria (b) and cyanobacteria (cy) in the vicinity of a sponge cell (sc), which has incorporated intracellularly some cyanobacteria (icy) and bacteria (ib). (C) Abundance and diversity of intercellular bacteria (b) in the mesohyl of the demosponge *Aplysina cavernicola*. (D) Micrograph of *A. cavernicola* showing abundance and diversity of intercellular bacteria (b) in the mesohyl, but also a sponge cell, the nucleus of which contains abundant intranuclear bacteria (ib), recalling a virus infection.

(Fig. 3.1D). The acquisition of these microbes can be by either environmental transmission, that is, recruiting the microbes from the surrounding water or vertical transmission, that is, transference of the paternal microbes to the progeny via their incorporation in gametes and early embryo stages (reviewed in Maldonado, 2007). Whether one or both acquisition pathways operate for a given sponge species will largely determine the diversity and specific composition of its associated microbial community, as well as the level at which these microbial associates resemble the microbial community in the surrounding ambient seawater. It appears that a given sponge species may contain a mixture of generalist and specialist microorganisms (Taylor *et al.*, 2004).

Based on the density of hosted microbes, sponges have been categorized as either "high-microbial abundance" (HMA; former bacteriosponges) or "low-microbial abundance" (LMA) sponges. HMA sponges are characterized by a microbial population exceeding in 2–4 orders of magnitude, the average microbial density in seawater. In contrasts, LMA sponges host microbial densities of 10^5–10^6 microbes g^{-1} sponge wet weight, which fall in the range of natural bacterial concentration in seawater (Vacelet and Donadey, 1977; Wilkinson, 1978; Reiswig, 1981; 10^5–10^6 microbes g^{-1} sponge wet weight; Hentschel *et al.*, 2006) and also reflect approximately the phylogenetic diversity of the natural microbial assemblage (Schmitt *et al.*, 2007). HMA and LMA are considered as two basic life strategies resulting from evolutionary processes with contrasting characteristics. LMA species have well-irrigated tissues, high pumping rates, and low-diversity microbial communities. Contrarily, HMA species are characterized by a dense tissue, low pumping rates, and high concentration and diversity of microbial associates that are different from the ones occurring in the surrounding water column (e.g. Siegl *et al.*, 2008; Weisz *et al.*, 2008).

Microbial symbionts are hypothesized to contribute to the health and nutrition of sponges in different ways, including production of defending antibiotics, acquisition of limiting nutrients, and processing of metabolic waste (Hoffmann *et al.*, 2005; Taylor *et al.*, 2007; Siegl *et al.*, 2008). Although consumption and remineralization of POM by sponges may have an important role in the ecosystems where they are abundant, the association of sponges with microbial communities cause their functional role to be more complex than mere heterotrophy. While a wide array of phylogenetically and physiologically diverse microbes associated with sponges has been described, their exact role in nutrient cycling remains poorly known. Most of our current understanding about the microbial diversity associated with marine sponges is based on 16S rRNA gene library construction, functional genes surveys, and metagenomics, which are used to attempt unravelling the metabolic routes integrated in those of the sponge metabolism. These metabolic pathways include a variety of processes with alternative energy (photo- or chemotrophic) and carbon

(hetero- or autotrophic) sources under different oxygen conditions (Vacelet *et al.*, 1995, 1996; Hoffmann *et al.*, 2005, 2009; Hentschel *et al.*, 2006; Taylor *et al.*, 2007).

2. Use of Carbon by Sponges

Sponges are remarkable suspension feeders that remove substantial amounts of organic matter from the passing water. Wherever they are abundant, they are suspected to play an important role in organic matter processing and benthic–pelagic coupling. Here, we review the knowledge on carbon fluxes through sponges and highlight recent developments, anticipating that to quantify realistically their impact on the carbon flux at the ecosystem level, we still need further insight in the carbon metabolism of different functional groups of sponges.

2.1. Carbon metabolism: The carbon balance

The carbon balance of sponges has challenged sponge scientists for more than 40 years. Compiling carbon balances of sponges confront us with the fact that the basic physiology of sponges is still not fully understood. Of essential aspects, such as (1) the ingestion of total organic carbon, (2) its assimilation and excretion, and (3) the role of sponge-associated microbes in the sponge growth, we know surprisingly little more than when Henry Reiswig reviewed the literature on the subject and presented his pioneering energy budgets of several tropical sponges some 30 years ago (Reiswig, 1974, 1981). He conducted the first *in situ* studies on particulate feeding by marine sponges, on water transport through the aquiferous canals, and on respiration and energetics of sponges, shortly followed by Frost's work (1976, 1978) on fresh water sponges. Since then, main findings for improving our understanding of carbon metabolism of sponges comprise:

1. The low energy costs of pumping and filtration in sponges compared with other benthic invertebrates and the large effect of temperature on filtration rate (Riisgård *et al.*, 1993).
2. The high energy cost of net growth in sponges compared with other benthic suspension feeders (Thomassen and Riisgård, 1995).
3. A better understanding of the particulate diet and selective filtration of particles by sponges (Pile *et al.*, 1996, 1997; Turon *et al.*, 1997; Ribes *et al.*, 1999b; e.g. Maldonado *et al.*, 2010c).
4. Demonstration of bulk dissolved organic matter (DOM) uptake by sponges *in situ* (Yahel *et al.*, 2003; De Goeij *et al.*, 2008b).
5. Demonstration of direct consumption of DOM by both sponge cells and sponge-associated prokaryotes (De Goeij *et al.*, 2008a).

6. Demonstration that HMA sponges pump per unit volume 52–94% less water through their aquiferous system than LMA sponges (Weisz *et al.*, 2008).
7. Realization that sponges may experience rapid cell turnover and choanocyte shedding (De Goeij *et al.*, 2009).

The discovery of bulk uptake of DOM by tropical sponges is an important breakthrough in carbon balance studies (Yahel *et al.*, 2003; De Goeij *et al.*, 2008a). DOM is a large source of organic matter in the aquatic environments and consumption may solve imbalances between carbon consumption and respiration by sponges. Another challenge in sponge carbon metabolism is improving our understanding of energy expenditure on net production by HMA and LMA sponges (Thomassen and Riisgård, 1995; Weisz *et al.*, 2008; De Goeij *et al.*, 2009).

This review focuses on carbon ingestion, growth, respiration, and waste disposal of sponges. Literature is screened for ingestion and respiration data of different sponge species, also in relation to their microbial abundance. The hypothesis that will be tested is whether mismatches between ingestion and respiration in studies that measured POC and/or plankton uptake and did not measure dissolved organic carbon (DOC) uptake, are on average larger in HMA than LMA sponges. In addition, the carbon balance of several sponge species are discussed in the light of recent findings.

2.1.1. Carbon balance equation

Reiswig (1971a, 1974, 1981) was the pioneer putting up carbon balances based on *in situ* measurements for different sponge species. It took decennia to confirm or refute with new measurements some of his interpretations and suggestions (Thomassen and Riisgård, 1995; De Goeij *et al.*, 2008b; Hadas *et al.*, 2008; 2009; Koopmans *et al.*, 2010). The carbon balance in its most simple form is described as:

$$I = P + R + E.$$

It comprises ingestion (I) of carbon, its use for biomass production (P) and respiration (R), and disposal of waste (E), which includes excretion as well as egestion. The P and R terms together represent the assimilation (A). Ingestion is defined as the incorporation of carbon in the sponge and, therefore, not the total amount of organic matter entering the sponge aquiferous system with the water inflow. After ingestion, the carbon is used for biomass production and generation of energy. Biomass production is defined as growth in volume, size, or weight, including gonadal development (net synthesis of organic compounds). Respiration is the metabolic process through which organic matter is oxidized with release of energy. The oxidation of organic matter for energy coincides with the release of carbon dioxide under aerobic conditions in the sponge (=respiration). The

net growth efficiency (NGE) is determined as $P/(P+R)$. Since P in relation to R is only sporadically measured in sponges, as far as we know, I/R is used as a measure of metabolic efficiency. Besides carbon loss through respiration, there is organic matter excretion and egestion by the sponge. Waste organic carbon is disposed of and consists of detrital material (DOC as well as POC), comprising digested as well as indigested material. Determination of the ingestion of undefined fractions of organic matter (such as detrital POC and DOC) is biased by the excretion/egestion of organic matter by the sponge. What is measured in most studies is ingestion minus "excretion + egestion" ($I-E$) and it is referred to as the net ingestion rate. However, the E term does not usually cover the full excretion/egestion, because part of the waste disposal by sponges is via faecal pellets, which may settle on the bottom and become unavailable for suspension feeders (Witte *et al.*, 1997).

2.1.2. Carbon ingestion

Organic matter enters the sponge via diffusion, epithelial pinocytosis, and phagocytosis, all facilitated by water pumping at transport rates ranging from 0.002 to 0.84 $cm^3\ cm^{-3}$ sponge s^{-1} (Simpson, 1984). Particle uptake is constrained by the maximum diameter of sponge ostia, generally 50 μm. The main retention and ingestion sites are the choanocyte chambers, although pinacocytes are also able to engulf and pinocyte particles. Sponges have traditionally been regarded as little selective in their particle uptake and therefore incorporating organic particles, regardless of their nutritional value (e.g. Reiswig, 1971a; Wolfrath and Barthel, 1989; Turon *et al.*, 1997). Nevertheless, there is growing evidence that at least some sponges perform a size-independent selection of their food (Frost, 1976, 1980; Van de Vyver *et al.*, 1990; Yahel *et al.*, 2006; Maldonado *et al.*, 2010c).

Carbon capturing rates are thought to be dependent on a wide variety of factors that differ between sponge species, such as body size and morphology (Vogel, 1977, 1978), feeding strategy, such as filtering, carnivorism, methanoptrophy, and others (Vacelet *et al.*, 1996; Vacelet and Duport, 2004; Leys and Eerkes-Medrano, 2006), complexity of the aquiferous system (Reiswig, 1971a; Vacelet and Donadey, 1977; Turon *et al.*, 1997; Weisz *et al.*, 2008), etc. Ingestion of organic carbon also depends on its availability in the ambient water, water transport rate through the sponge, particle-size retention efficiency (e.g. heterotrophic bacterioplankton, cyanobacteria), life-cycle stage, environmental conditions, etc. (Gerrodette and Flechsig, 1979; Riisgård *et al.*, 1993; Ribes *et al.*, 1999a).

Sponges ingest plankton or living particulate organic carbon (LPOC) with preference for the nano- and picoplankton. The highest particle retention efficiencies are found for particles between 0.1 and 10 μm in size. Retention efficiency can rise to 100% for plankton size classes ranging in diameter from 0.2 to 6 μm (e.g., Thomassen and Riisgård, 1995; Pile, 1997; Ribes *et al.*,

1999b, see also Table 3.1). Plankton ingestion rates are part of the POC removal rates of sponges listed in Table 3.2, with plankton ingestion rates (LPOC) varying from 0.04 to 1.17 μmol C cm^{-3} h^{-1}.

Detrital particulate organic carbon (POC_{det}) is also retained by sponges and accounts for the fraction of POC minus LPOC (Reiswig, 1971b; Witte *et al.*, 1997; Yahel *et al.*, 2003; Hadas *et al.*, 2009). POC_{det} ingestion is not always easy to assess. Retention efficiencies of detrital POC are biased by faecal production of the sponge (Reiswig, 1971b; Stuart and Klumpp, 1984; Kowalke, 2000). Re-filtration of suspended waste products of the sponge may particularly occur in closed systems. Based on *in situ* measurements, Hadas *et al.* (2009) ascribe 1/3 of the ingested POC by the reef sponge *Negombata magnifica* to detritus, implying that detritus can form an important part of the diet. Sponges are regarded as opportunistic feeders within their specific particle size spectrum (Coma *et al.*, 2001). Ingestion of detritus is, therefore, dependent on the size of the detritus particle and the preferred size spectrum of the sponge. Total POC ingestion ($LPOC + POC_{det}$) range from 15 to 232 μmol C g DW^{-1} h^{-1} and from 0.252 to 1.4 μmol C cm^{-3} h^{-1} for the sponges in Table 3.2. The POC ingestion rates do not differ between temperate and tropical sponges. Low POM concentrations in oligotrophic waters appear to be compensated by sponges showing either higher filtration rates or increased consumption of DOM.

DOC has long been considered as a food source for sponges. The composition of natural DOM is basically unknown and comprises minute particles, as well as truly dissolved DOM. The DOC size fraction in ambient water is operationally defined as the organic carbon passing through a GF/F (nominal pore size 0.7 μm) or a 0.2-μm pore size filter. Therefore, small particles, such as viruses (<0.2 μm diameter) and 0.1 μm beads, fall in the size fraction of natural DOM. These small size fractions have been reported to be retained by sponges (Hadas *et al.*, 2006; Leys and Eerkes-Medrano, 2006). There are also some indications that colloids and aggregates in the DOM fraction may be consumed by sponges (Reiswig, 1990; Alber and Valiela, 1995), but there are no uptake rates available to date. The capacity for retention and uptake of truly DOM, such as glucose, amino-acids palmitic acid by sponges, or fluorescently labelled DOM in laboratory experiments, has been known for quite some time (Stephens and Schinske, 1961; Schmidt, 1970; Weissenfels, 1976; Wilkinson and Garrone, 1979; Jaeckle, 1995), but experimental evidence for bulk uptake of DOC by sponges was missing until recently. On the tropical sponge *Theonella swinhoei,* Yahel *et al.* (2003) provided the first quantitative assessment of *in situ* retention of ambient bulk DOC. The DOC uptake by *T. swinhoei* is on average 1.56 μmol C cm^{-3} h^{-1}, equalling the highest recorded plankton uptake rates by sponges (Table 3.2). Similarly, De Goeij *et al.* (2008b) reported DOC removal from ambient water at even higher rates by the encrusting coral cavity sponges *Halisarca caerulea*, *Mycale*

Table 3.2 Overview of metabolic rates of different sponge species measured under *in situ* environmental conditions

Sponge	Host type	T (°C)	DOC	POC	WTR	CR_{POC}	I_{DOC}	I_{POC}	R	I_{POC}/R	$I_{POC+DOC}/R$	FTE	Source
Per g DW													
Haliclona oculata	LMA	19	–	40.83		5.67	0	231.53	95.54	2.45		0.06	Koopmans *et al.* (2010)
Halichondria panicea	LMA	14	–	16.67[a]	1.7	1.7	–	28.33	26.75[b]	1.06		0.06	Thomassen and Riisgård (1995)
Dysidea avara	LMA	17	–	32.25	0.06	0.65	0	20.96	9.25	2.28		0.07	Ribes *et al.* (1999b), Coma *et al.* (2002), Turon *et al.* (1997)
Negombata magnifica	LMA	22.5	–	4		4.2	–	16.8	14.88	1.13		0.28	Hadas *et al.* (2008a,b)
Mycale laxissima	LMA	29	–	5.33	12.29	12.29	–	65.55	25.26	2.59		0.49	Reiswig (1973, 1974)
Tectitethya crypta	LMA	30	–	7.08	5.35	3.72	–	26.35	6.64	3.97		0.56	Reiswig (1973, 1974)
Verongula reiswigi	HMA	29	–	5.33	3.05	2.77	–	14.77	27.39	0.54		0.1	Reiswig (1973, 1974)
Aplysina fistularis	HMA	27	–	4.47	3.15		–	3.87	33.64	0.11			Reiswig (1981)
Spongilla lacustris				0.075				30.31					Frost (1978), Karchenko and Lyashenko (1986)
Per cm^3													
Theonella swinhoei	HMA				0.16	0.156	1.56	0.252	1.404	0.18	1.29	0.11	Yahel *et al.* (2003)
Halisarca caerulea	L/HMA	27	127	5.3		0.248[c]	17.1	1.4	2.7	0.52	6.85		De Goeij *et al.* (2008a)
Aplysina fistularis	HMA	27	–		0.44		–	0.545	4.74	0.12		0.09	Reiswig (1981)
Aplysina aerophoba	HMA	18	–	–			–	0.003	1.25	0.0026			Wehrl *et al.* (2007): 20 fg C/bacterium; 1 cm^3 sp = 1.0469 g WW, from Reiswig (1981)
Per g AFDW													
Mycale acerata	HMA	1.8				0.18			0.00393			45.8	Kowalke (2000)
Isodictya kerguelensis	–	1				0.22			0.00156			140.9	Kowalke (2000)

T, temperature in degree Celsius; DOC, dissolved organic carbon in µmol C l^{-1}; POC, particulate organic carbon in µmol C l^{-1}; I, ingestion of DOC or POC in µmol C h^{-1}; R, respiration in µmol O_2 h^{-1}; WTR, specific water transport rate in l h^{-1}; CR, clearance rate in l h^{-1}; I/R, ingestion in µmol C. µmol O_2^{-1}; FTE, filtration efficiency (convection requirement) in L µmol O_2^{-1}; –, not measured.

[a] Bacterial ingestion rate.

[b] Maintenance respiration.

[c] Chl a plus bacterium-based POC.

microsigmatosa, and *Merlia normani,* which ranged from 11 to 21 μmol C cm^{-3} h^{-1}. Interestingly, more than 90% of the diet of these sponges consists of DOM.

Sponges in clear shallow water often have photosynthetic properties and harbour zooxanthellae and cyanobacteria (Rützler, 1990; Schönberg *et al.*, 2005; Thacker, 2005; Erwin and Thacker, 2007; Usher, 2008). Sponges may benefit by their photosynthetic associates and carbon metabolism therefore varies between sponges with and without photosynthetic symbionts (Thacker, 2005; Weisz *et al.*, 2010). In addition, there are sponges harbouring chemolithoautotrophic prokaryotes fixing CO_2 (Vacelet *et al.*, 1995; Van Duyl *et al.*, 2008; Hoffmann *et al.*, 2009; Nishijima *et al.*, 2010; Siegl *et al.*, 2011). So there are various pathways potentially contributing to the total carbon acquisition, but organic carbon is generally the most important food source for sponges. Therefore, the emphasis of this review will be on net heterotrophic feeding of sponges.

2.1.3. Assimilation (respiration and production)

Respiration by sponges was reviewed by Osinga *et al.* (1999). *In situ* oxygen consumptions of sponges, as obtained from bulk measurements range from 0.21 to 24.6 μmol O_2 cm^{-3} sponge h^{-1} (Osinga *et al.*, 1999 and references therein). Oxygen consumption is markedly species specific, but it varies with temperature, pumping activity, and with physiological processes, such as growth and reproductive stage (Coma *et al.*, 1995, 2002). Consistent differences in oxygen consumption are not apparent between temperate and tropical sponges (Osinga *et al.*, 1999; Coma *et al.*, 2002; De Goeij *et al.*, 2008b), while polar and boreal sponges tend to show lower respiration rates on average (Witte and Graf, 1996; Kowalke, 2000; Gatti *et al.*, 2002; Hoffmann *et al.*, 2005). Higher transport- and size-specific respiration rates in HMA than in LMA sponges are expected and, accordingly, Reiswig (1974, 1981) reported higher oxygen demands by bacteriosponges (HMA sponges: *Aplysina fistularis*, *Verongula reiswigi*) than those by LMA sponges (*Tectitethya crypta* and *Mycale laxissima*, previously reported as *Tethya cripta and Mycale* sp. by Reiswig in 1974). The mesohyl in HMA sponges is packed with microbes, coinciding with a denser mesohyl than in LMA sponges (Vacelet and Donadey, 1977; Weisz *et al.*, 2008). This may be a reason for enhanced respiration rates in HMA sponges, because bacteria often have a specific respiration (lower NGE or growth yield, less than 10–25%) higher than that of eukaryotic organisms (Del Giorgio and Cole, 2000). In addition, HMA sponges, characterized by more complex aquiferous systems, have a comparatively larger gas exchange surface (Vacelet and Donadey, 1977; Boury-Esnault *et al.*, 1990). Whether respiration is higher on average in HMA than LMA sponges remains to be elucidated.

Respiration comprises both the respiration for growth/reproduction and the respiration for basal metabolic maintenance. Energy costs of growth and

maintenance have been determined for few sponges. The maintenance costs of sponges appear to be high in relation to total respiration, when compared to other benthic invertebrates (e.g. Coma *et al.*, 2002). This is not primarily due to the pumping activity, because studies demonstrate that the energy costs of water pumping do not restrict benthic suspension-feeding animal distribution (Jørgensen *et al.*, 1990; Petersen and Riisgård, 1992; Riisgård *et al.*, 1993). There is apparently always enough potential energy available in natural waters to fuel pumping activity. Pumping, as a part of the maintenance costs, accounts on average for 0.85% in *Halichondria panicea* and 25% in *N. magnifica* of total respiration (Thomassen and Riisgård, 1995; Hadas *et al.*, 2008). Hadas *et al.* (2008) report high maintenance costs, including water pumping activity requirements for *N. magnifica*, accounting for 74% of total respiration. During field experiments with *H. panicea* at 14 °C and ambient POC concentration of 1.7 mg C l^{-1}, maintenance respiration (26 µmol O_2 g DW^{-1} h^{-1}) virtually equals ingestion (24.08 µmol POC g DW^{-1} h^{-1}), leaving no organic carbon for net growth (Thomassen and Riisgård, 1995). Moreover the respiratory costs associated with net growth may be high in sponges. Thomassen and Riisgård (1995) related the specific respiration to the specific growth rate of the sponge and found a linear relation to conclude that the respiratory cost associated with the net synthesis of new tissue in *H. panicea* was 139%. This means that 239 units carbon are required to net produce 100 units of biomass. This is surprisingly high in comparison with other invertebrates (12–40%; e.g. Vahl, 1984; Nielsen *et al.*, 1995; Petersen *et al.*, 1995; Clausen and Riisgård, 1996). Sponges apparently have a higher energy demand for growth than other invertebrates (Thomassen and Riisgård, 1995). These studies indicate that rapid growth in size is probably not a key strategy in sponges. These organisms appear to invest relatively more energy in maintenance than in net body growth compared with other invertebrates.

Surprisingly few studies address sponge biomass production in combination with respiration (but see Reiswig, 1974, 1981; Thomassen and Riisgård, 1995; Koopmans *et al.*, 2010). Growth of sponges is usually measured as increase in volume, weight, or area. Sponges are generally considered as slow growing (e.g. Pansini and Pronzato, 1990). Volume increases of less than 5–60% y^{-1} have been recorded *in situ* for temperate and tropical sponges (Reiswig, 1973; Hoppe, 1988: 7.7–19.4 y^{-1}; Koopmans and Wijffels, 2008: 0.3–3% day^{-1}; McMurray *et al.*, 2008: 52% y^{-1}). Growth of cold-water sponges can be particularly slow, with growth rates ranging from 0.003% to 0.07% y^{-1} (Dayton, 1979; Leys and Lauzon, 1998; Van Duyl *et al.*, 2008). Nevertheless, rapid volume increases have been reported for various farmed sponges, such as *Latrunculia wellingtonensis* and *Polymastia croceus*, which grew respectively 960% and 730% of their initial volume on average in only 6 months (Duckworth and Battershill, 2003). Young stages of sponges show high growth rates, doubling their

volume in only a few days (e.g. Maldonado and Young, 1999) and small individuals usually grow faster than larger individuals of the same species (Reiswig, 1973; de Caralt *et al.*, 2008). Rapid volume increase has also been noticed in sponge individuals transplanted out of their original microhabitat or habitat (Wilkinson and Vacelet, 1979; Maldonado and Young, 1998). Seasonal variation in growth rate is known, with lower growth rates generally during the cold season (Barthel and Theede, 1986; Ribes *et al.*, 1999b; Garrabou and Zabala, 2001; Koopmans and Wijffels, 2008). Temperature, food availability, and life-cycle stage are also known to influence growth rates. Reshaping, shrinkage, fission, and fusion are also processes affecting growth rates and that vary seasonally with the life cycle of the sponge and the predation pressure (e.g. Garrabou and Zabala, 2001; Tanaka, 2002; de Caralt *et al.*, 2008). Shrinkage rates may equal or nearly equal growth rates leading to major individual reshaping but no substantial net growth. For instance, in *Corticium candelabrum*, maximum monthly shrinkage rate was measured at 0.15 $month^{-1}$ while maximum growth rate was 0.19 $month^{-1}$ (de Caralt *et al.*, 2008). Sponges regenerating damaged body parts show much faster growth than undamaged sponges, demonstrating a large growth potential (Ayling, 1983; Duckworth, 2003; Walters and Pawlik, 2005; Koopmans *et al.*, 2010).

Little is known about differences in growth between HMA and LMA sponges. Microbes, in general, have lower growth efficiencies than higher organisms, usually less than 20%. Therefore, heterotrophic microbes living in association with the sponge may claim a substantial portion of the energy supply to the sponge holobiont, depending of their growth rate (Van Duyl *et al.*, 2008). The carbon they process may not be directly available for the sponge, but sponges may subsequently use the microbially fixed carbon for sponge cell growth. Evidence for such a trophic transfer in sponges is still limited (Wilkinson and Garrone, 1979; Hentschel *et al.*, 2006). Phototrophic and chemoautotrophic microbes associated with the sponge fix dissolved inorganic carbon (DIC) and the photosynthate may come available for the sponge host. Nutrient translocation of photosynthates, such as glycerol and organic phosphate, from cyanobacterial symbionts to sponge hosts have been demonstrated (Wilkinson, 1979). Contributions of phototrophic cyanobacteria to the carbon assimilation account for up to 80% of the carbon budget of the sponge (Wilkinson, 1983; Wilkinson and Cheshire, 1990; Thacker, 2005; Usher, 2008). Cyanosponges are probably faster growing than sponges without photosynthetic symbionts (Wilkinson and Cheshire, 1990). Thacker (2005) found that the specialized filamentous cyanobacterial symbiont *Oscillatoria spongiarum* benefits its sponge host *Lamellodysidea chlorea* with respect to growth. Cyanobacteria associated with sponges may also be commensals (e.g. *Synechococcus spongiarum* in *Xestospongia exigua*) that do not benefit the host sponge (Thacker, 2005; Lopez-Legentil *et al.*, 2008). Recently also trophic transfer of C from

sponge-associated zooxanthellae (*Symbiodinium* spp.) to sponge cells has been confirmed for certain sponges (Weisz *et al.*, 2010).

Inorganic carbon fixation by chemolithoautotrophs tend to be low in dark habitats (Van Duyl *et al.*, 2008; Hoffmann *et al.*, 2009). Van Duyl *et al.* (2008) argue that the carbon incorporation rate of DOC and DIC in the dark by sponge-associated microbes of *Higginsia thielei* and *Rossella nodastrella* of cold-water coral reefs represented approximately 10% to the total net carbon production of the sponge. Values of $\delta^{13}C$ and $\delta^{15}N$ measured by Vacelet *et al.* (1996) in a carnivorous demosponge with vertically transmitted methanotrophic symbionts revealed that the sponge derives a substantial portion of its nutrition from its symbionts.

In addition to measuring growth directly, the energy available for growth can be determined from a bioenergetic budget. Physiological responses of sponges can be converted to energy equivalents and used in the balanced energy equation to calculate the production (Widdows and Johnson, 1988), which is then called the scope for growth (SFG). Energy in the ingested food and the absorption efficiency of the energy from the food need to be assessed. A problem with this approach is that there are, as far as we know, no absorption efficiencies available for sponges. Typical non-bivalve absorption efficiencies for benthic suspension feeders are 58.5% (Newell *et al.*, 1982), but whether this is applicable to sponges is unknown. Trussell *et al.* (2006) derive SFG assuming an absorption efficiency of 100%, based on the low energy expenditure on ammonia release by the sponge *Callyspongia vaginalis*. In this review, we focus, however, on the carbon balance and not on the energy balance.

2.1.4. Excretion and egestion

Excretion in sponges occurs through both the outgoing-water flow and the external surface of the body. Special amoebocytes disintegrate in the mesohyl and their granules and vesicles are expelled through the exhalant canals (Simpson, 1984). Likewise, amoeboid cells charged with large membrane-bound inclusions have been seen to squeeze between the epithelial cells until reaching either the lumen of the aquiferous canals or the external surface, where they discharge their contents or even discard themselves out of the sponge body. This putative cell excretion system has been reported from adult sponges and larval stages (Vacelet, 1967; Maldonado, 2009).

Several authors have also reported elimination (i.e. egestion) of pellets of undigested materials and detrital particles (Reiswig, 1971b; Weissenfels, 1976; Stuart and Klumpp, 1984; Witte *et al.*, 1997; Yahel *et al.*, 2003; De Goeij *et al.*, 2009), as well as net DOM efflux (Ribes *et al.*, 1999b). Wolfrath and Barthel (1989) made a detailed study of the production of faecal pellets by *H. panicea*. Feeding experiments with indigestible beads and algal cultures demonstrated production of 15–55 μm in diameter, oval to rounded faecal pellets consisting of densely packed aggregates surrounded by a thin, fragile,

membrane-like envelope. Aggregates harboured remnants of up to 560 digested algal cells. In addition to remnants of digested food, faecal pellets can contain residue of digested food as well as indigestible materials. Indigestible material may, however, also be egested as single particles without envelope, which pass more rapidly through the sponge. To date the only estimates of "excretion" rates are from Reiswig (1971b). He reported negative clearance rates of detritus for three tropical sponges ranging from 0.042 to 0.382 mg POC m^{-3}, comprising approximately 1–5% of the ingested particles, though the real level of detrital particle production remained unclear. Sharp and sudden declines in the retention efficiency of 1-μm sized particles in Antarctic sponges and of 2- to 4.5-μm sized particles in temperate and tropical sponges has been interpreted as indirect evidence of detrital particle elimination, probably break-down products of digestion in that size range (Stuart and Klumpp, 1984; Kowalke, 2000). Nevertheless, rates were not derived. The closest to estimating a particulate excretion rate for sponges may be the work by De Goeij *et al.* (2009). They report rapid proliferation of choanocytes in *H. caerulea*. Choanocyte turnover had a periodicity of about 6 h in steady state conditions, with "old" cells being replaced by new cells and aggregates of detrital material (probably remains of shred cells) being expelled to the exhalant canals. Considering the limited growth of *H. caerulea*, the average cell proliferation rate of 13.8 ± 0.9 μmol C cm^{-3} sponge h^{-1} nearly equals cell shedding. Egestion of digested and undigested detrital particulate material from the mesohyl is probably low in this sponge considering the fact that it mainly feeds on DOM (De Goeij *et al.*, 2008b). The rate of choanocyte proliferation may therefore be a reasonable estimate for egestion in the carbon balance of *H. caerulea*.

2.2. Carbon balance in HMA and LMA sponges (and POC vs. DOC)

Imbalances in carbon budgets between ingestion and respiration have been reported for several HMA and LMA sponge species (Reiswig, 1974, 1981; Thomassen and Riisgård, 1995; Hadas *et al.*, 2008, 2009). Discrepancies have been ascribed to potential underestimation of carbon ingestion. For sponges in Table 3.2, the I_{poc}/R index ranges from 0.003 to 3.97. For sponges with an I_{poc}/R smaller than 1, insufficient particulate carbon is most likely assimilated to meet the CO_2 release. In those cases, respiration also exceeds carbon ingestion. DOC is usually invoked as the missing carbon source. Considering recent studies of tropical sponges, in which DOC consumption was evident and substantial in all four sponge species tested (Yahel *et al.*, 2003; De Goeij *et al.*, 2008b), it is likely that other sponge species rely on DOC consumption as well. Microbes are pre-eminently DOM consumers and may mediate DOM consumption in sponges. The general assumption therefore is that HMA sponges are better equipped to

feed on DOM than LMA sponges. Of the examined DOC-feeding sponges, *T. swinhoei* is an HMA sponge (Yahel *et al.*, 2003). The DOM-feeding *H. caerulea*—hosting 2.1×10^9 microbes cm^{-3} sponge, according to DAPI counts (De Goeij *et al.*, 2008a)—could be regarded as an HMA sponge, *sensu* Hentschel *et al.* (2003). However, electron microscopy examination by Vacelet and Donadey (1987) found only few bacteria in the mesohyl of this sponge. Absence of mid-chain-branched fatty acids, as well as low % of bacterium-specific fatty acids (< 10%), in *H. caerulea* compared to *Chondrilla caribensis* (Van Duyl *et al.*, 2011) would also classify *H. caerulea* as an LMA sponge (Hochmuth *et al.*, 2010). The DOM-feeding sponges *M. microsigmatosa* and *M. normani* have amounts of associated microorganisms comparable to that in *H. caerulea* (De Goeij *et al.*, 2008a), but it remains unclear whether these sponges have to be categorized as LMA or as HMA. Anyway, they contain approximately 10^9 prokaryotes cm^{-3} sponge, which is 2–3 orders of magnitude higher than in ambient seawater (De Goeij *et al.*, 2008a). For several LMA sponges with prokaryote concentrations up to 1 order of magnitude higher than in seawater (i.e. *Dysidea avara*, *Haliclona oculata),* net DOC uptake could not be established (Ribes *et al.*, 1999b; Koopmans and Wijffels, 2008). Whether the LMA sponges *H. panicea* and *N. magnifica* feed on DOM besides POM remains to be examined. Imbalances have been reported for these sponges, as well as for HMA sponges (Reiswig, 1974; Thomassen and Riisgård, 1995; Hadas *et al.*, 2008, 2009). The emerging view is that the extent of the mismatch between ingestion of POC and respiration tends to be larger in HMA than in LMA sponges (Table 3.2).

To investigate whether DOM is primarily assimilated by sponge-associated microbes or by sponge cells, De Goeij *et al.* (2008b) conducted a labelling study with *H. caerulea*. Labelling of the sponge with diatom derived ^{13}C-DOC demonstrated instantaneous incorporation of ^{13}C in bacterium-specific as well as sponge-related fatty acids of *H. caerulea*, implying uptake of ambient DOC by both sponge cells and sponge-associated microbes (De Goeij *et al.*, 2008b). Although there are doubts about the HMA or LMA status of *H. caerulea*, it is evident that sponge cells take up DOM directly from the ambient water, as do the sponge-associated prokaryotes. Since most microbes in typical HMA sponges dwell in the mesohyl, there is no direct contact between the ambient DOC and the microbes. DOC needs to pass the pinacocyte and/or choanocyte epithelia before being accessible for the microbes. Therefore, it is unlikely that DOM uptake is restricted to sponge-associated microbes and to HMA sponges.

Between-species comparison of sponge metabolic rates in Table 3.2 shows that several sponges show evident imbalances between POC ingestion (I_{poc}) and respiration (R). The I_{poc}/R index is smaller than 1 for 6 of the 12 sponges. This may indicate carbon shortage for maintenance and growth assuming a respiratory quotient of 1 (mol CO_2. mol O_2^{-1}). All species in

Table 3.2 with I_{poc}/R smaller than 1 are clearly HMA sponges, except *H. caerulea*. Sponges with I_{poc}/R just above 1, such as the LMA sponges *N. magnifica* and *H. panicea,* may need additional carbon to account for detrital waste disposal and for net production of biomass in case they grow (Thomassen and Riisgård, 1995; Hadas *et al.*, 2008, 2009). Sponges cannot digest all the ingested carbon (Witte *et al.*, 1997). Exclusion of DOC uptake by *T. swinhoei* and *H. caerulea* from the equation results respectively in I_{poc}/R values of 0.18 and 0.52, showing imbalances comparable with those for *V. reiswigi* and *A. fistularis* (Reiswig, 1974, 1981). Adding the consumed DOC by these sponges, the index exceeds 1 and comes into balanced conditions. The clearance efficiency of total organic matter ($I_{poc+doc}/R$) for *T. swinhoei,* however, remains still quite low compared to *H. caerulea*, being 1.29 versus 6.85. Carbon uptake may still be underestimated because *T. swinhoei* also acquires carbon via its photosynthetic symbionts (Magnino *et al.*, 1999). Likewise, *A. fistularis* and *V. reiswigi.* (Reiswig, 1981) may have also acquired carbon from their associated photosymbionts. Lowering the respiratory quotient (RQ) to 0.7 would alleviate the imbalances found for *N. magnifica* and *H. panicea*. The RQ of a specific sponge is determined by the elemental composition of its food and the physiological state, which may differ between sponge species and growth phases. Sponges mainly feeding on DOC are expected to have higher RQs than sponges feeding on algae or actively growing sponges (e.g. Roy *et al.*, 1999). Lower values have been used for calculations on sponges (e.g. Koopmans *et al.*, 2010), but since we do not know the composition of the diet and physiological state of the sponges, we are maintaining an RQ equal to 1. Summarizing, we can conclude that HMA as well as LMA sponges probably take up DOC. HMA sponges, however, may rely more on DOC as a source of carbon than LMA sponges.

2.3. Between-species carbon balance differences

Studies in which total ingestion (DOC and POC), growth, and respiration have been measured synchronously *in situ* or under comparable conditions in sponges are scarce. The carbon metabolism of seven different sponge species for which at least three variables of the equation $I = P + R + E$ were comparatively quantified is summarized in Table 3.3. None of the studies provide sufficient data to check whether fluxes measured together really meet the balance over a certain time period. Always an unknown flux remained, which was subsequently estimated on basis of the measured fluxes. For the tropical sponges studied by Reiswig (1981), the shortage of carbon ranged from 0.34 μmol C h^{-1} cm^{-3} sponge in *M. laxissima* to 2.9 in *A. fistularis*. In the balance, no account is taken of exuded material that is no longer available for re-filtration. Therefore, the total amount actually required may even be much higher for all sponges, except *H. caerulea*. For

Table 3.3 Carbon balance equations for five tropical and two temperate sponges

Sponge	Host type	$I-E_{partial}$	P	R	$I_{shortage}$	E_{rest}	NGE	%	$R/(I-E_{partial})$	DOC%	Source
Tropical species (µmol C h^{-1} cm^{-3})											
Tectitethya crypta	LMA	0.93	0.03	0.46		0.44	0.06	6	0.49	0.0	Reiswig (1971b, 1973)
Mycale laxissima	LMA	1.45	0.11	1.67	0.34		0.06	6	1.16	18.8	Reiswig (1971b, 1973)
Verongula reiswigi	HMA	0.63	0.02	2.48	1.87		0.01	1	3.94	74.7	Reiswig (1971b, 1973)
Aplysina fistularis	HMA	0.48	0.16	3.22	2.90		0.05	5	6.77	85.9	Reiswig (1981)
Halisarca caerulea	L/HMA	18.50	2.00	2.70		13.8[a]	0.43	43	0.15	92.4	De Goeij *et al.* (2008a, 2009)
Temperate species (µmol C h^{-1} g DW^{-1})											
Haliclona oculata	LMA	231.50	7.60	95.54		128.36	0.07	7	0.41	0.0	Koopmans *et al.* (2010)
Halichondria panicea	LMA	51.64	13.81	37.93	0.10		0.27	27	0.73	0.2	Thomassen and Riisgård (1995)

$I-E_{partial}$, net ingestion of organic carbon; P, net growth in biomass; R, respiration (RQ = 1); E_{rest}, detrital material not available for recirculation; NGE, net growth efficiency (= $P/(P+R)$); shortage, imbalances between Ingestion and assimilation; $R/(I-E_{partial})$, index estimating the amount of the organic matter ingested which was lost in respiration; DOC%, DOC contribution values to total. Grey cells contain calculated values.

[a] Rate of cell shedding.

this sponge, choanocyte shedding (De Goeij *et al.*, 2009) is assumed as a reliable estimate of particle excretion.

Comparing net growth rates measured over long time scales with oxygen consumption measurements yields NGEs of 0.6–6% (derived from data in Reiswig, 1973; 1974; Table 3.3). The growth efficiency of *H. oculata* based on volume increase in time is 7%, assuming an RQ of 1 or 10% for an RQ of 0.75 (Koopmans and Wijffels, 2008; Koopmans *et al.*, 2010). Higher efficiencies of 20–30% have been found at growth rates of 1–4% day^{-1} in laboratory and field experiments (Thomassen and Riisgård, 1995). The NGE of *H. caerulea* is high (43%) when compared to other sponges. It is tempting to assume that the high NGE in *H. caerulea* is due to the efficient and rapid growth rate of choanocytes. De Goeij *et al.* (2009) reported high choanocyte proliferation rates coupled to shedding of old cells in *H. caerulea*. Rapid cell renewal may be a maintenance strategy similar to that in higher animals. Therefore, cell proliferation rate is listed under excretion in Table 3.3, implementing the still undemonstrated assumption that proliferation rate should equal cell shedding rate. Whenever the cell proliferation exceeds shedding, sponge net growth may take place. Whenever sponges invest energy in cell renewal, their net growth may be low. This process probably takes the bulk of the energy delivered to *H. caerulea,* not leaving much energy for net growth in size (De Goeij *et al.*, 2008a). Average production of *H. caerulea* calculated at 2 μmol C cm^{-3} sponge h^{-1} on the C balance equation is probably an overestimate. Further research is needed to establish the role of choanocyte renewal in sponge metabolism and growth process.

2.4. Ecological significance of carbon use

Sponges ingest 29–1970 mg C m^{-2} sponge day^{-1} (Gili and Coma, 1998 and references therein) and 0.04–18.5 μmol C cm^{-3} sponge h^{-1} (De Goeij *et al.*, 2008a and references therein). Wherever sponges are an important component of the benthic community, they withdraw substantial amounts of organic matter from the ambient water, playing a potentially relevant role in the benthic–pelagic coupling of C cycling (Reiswig, 1971b; Lesser, 2006). The pelagic microbial food web represents the main food source for sponges throughout their entire bathymetric and latitudinal range of occurrence (Yahel *et al.*, 2005; Pile and Young, 2006; Bell, 2008). Picoplankton is frequently depleted in water layer overlying coral reefs (Ayukai, 1995; Gast *et al.*, 1998; Yahel *et al.*, 1998; Richter *et al.*, 2001; Van Duyl *et al.*, 2002; Genin *et al.*, 2009; Monismith *et al.*, 2010), an effect largely due to sponges (Richter and Wunsch, 1999; Scheffers *et al.*, 2004). Also DOM depletion in coral reef waters and in coral reef cavity waters has been reported (De Goeij and Van Duyl, 2007; Nelson *et al.*, 2011). The diet of various reef sponges consists for more than 90% of total net carbon uptake of

DOM (Yahel *et al.*, 2003; De Goeij *et al.*, 2008a), accounting for DOC depletion in cavities (De Goeij and Van Duyl, 2007). At least part of DOM removed from the ambient water by DOM-feeding sponges is converted to detrital POM (De Goeij *et al.*, 2008a; 2009). Moreover, more than 50% of the carbon ingested by reef sponges has a clear reef signature with respect to $\delta^{13}C$, $\delta^{15}N$, and fatty acids, suggesting that these sponges obtain the bulk of their food from benthic production (Van Duyl *et al.*, 2011).

Benthic primary producers, such as macroalgae, seagrasses, and corals, produce substantial amounts of DOC and mucus during the daylight period (e.g. Haas *et al.*, 2010; Naumann *et al.*, 2010). This organic matter presents an important source of energy for sponges in the benthic boundary layer and the benthos of shallow-water habitats (e.g. Wild *et al.*, 2004; Behringer and Butler, 2006; Granek *et al.*, 2009; Van Duyl *et al.*, 2011). In Fig. 3.2, a schematic impression of the main net fluxes of carbon in shallow-water habitats is given. Sponges capture plankton, POC_{det}, and DOC, and fuel the detrital pathways by enriching their surrounding benthic environment with waste organic matter. As such, sponges attract commensals and support the

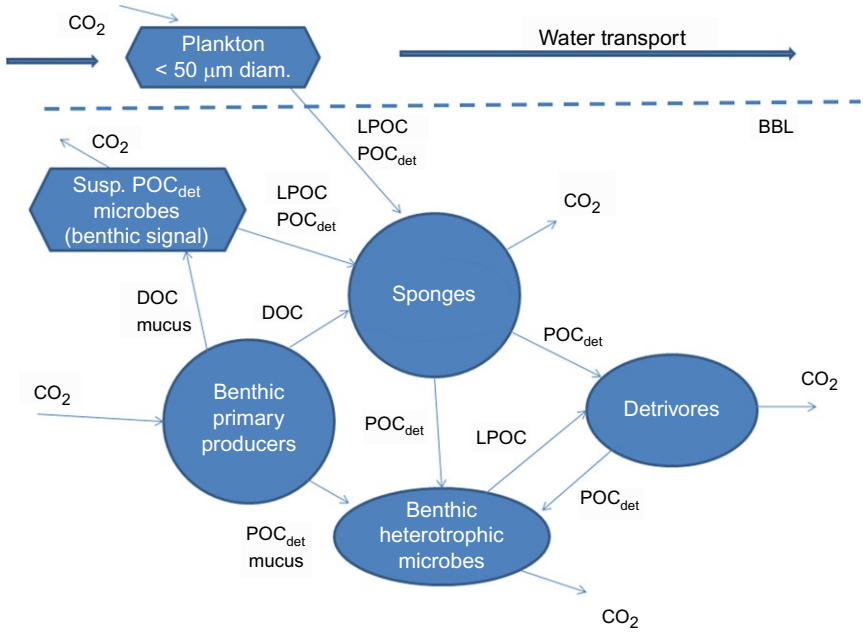

Figure 3.2 Schematic outline of major net fluxes of organic carbon through sponges. Arrows indicate flux of organic carbon to and between state variables representing standing stocks of organic matter and CO_2 pools. Hexagons refer to pelagic state variables, while circles and ellipses refer to benthic state variables. BBL, benthic boundary layer.

increase in biomass of detritivores and benthic microbes (e.g. Queric *et al.*, 2008). Bacterioplankton in the benthic boundary layer may obtain a "benthic signal" indicating that the microbes receive nutrition via benthic release (Nelson *et al.*, 2011; Van Duyl *et al.*, 2011). In aphotic deep waters, sponges rely mainly on bacterioplankton and POC_{det} (e.g. Yahel *et al.*, 2007). It is evident that sponges play a key role in many ecosystems by trapping organic matter from both the pelagic and the benthic compartment, transferring organic matter to the benthic compartment. As a result, sponges enrich benthic communities with organic matter in the form of sponge biomass and by their detrital POM waste disposal.

3. Use of Nitrogen and Phosphorous by Sponges

The nitrogen cycle is highly and thoroughly associated with microbes. Essential and unique steps in the nitrogen cycle are performed by a variety of bacteria, archaea, and eukaryotes (Ward *et al.*, 2007). The nitrogen cycle controls the availability of nitrogenous nutrients, hence markedly influencing the biological productivity in marine systems. Main steps of the nitrogen cycling are: (1) nitrogen fixation, (2) nitrification, (3) denitrification, (4) anaerobic ammonium oxidation (anammox), and (5) remineralizarion. The three last processes are carried out under suboxic conditions. While there are some processes that *per se* do not involve input or loss of nitrogen, as for example nitrification and remineralization, others imply either direct acquisition (nitrogen fixation) or release (denitrification, anammox) of nitrogen. Detection and quantification of all these processes are important for understanding the role of sponges as nitrogen sources or sinks in ecosystems.

In relation to the N cycle, the emerging notion in recent years is that sponges have to be understood as a "consortium" integrating the metabolic reactions of the sponge cells and those of the microbial associates. Rapid pumping rates of sponges indicate that large volumes of water charged with dissolved and particulate nutrients are transported through the body on a nearly constant basis and, somehow, interact with the dense microbial communities in the sponges, offering a high potential for rapid rates of biogeochemical processes. The application of oxygen-sensitive microelectrodes in the tissues of different sponge species showed remarkable oxygen deficiencies in their mesohyl during non-pumping periods (Schläppy *et al.*, 2007; Hoffmann *et al.*, 2009). Consequently both anaerobic and aerobic microbial processes can be expected in sponge tissues. Different steps of the nitrogen cycle have been identified in the same sponge species, suggesting that nitrogen cycling in sponges goes through a complex network of

metabolic pathways (Hoffmann *et al.*, 2009). Most studies to date are based on the detection and description of microbial components that could mediate different nitrogen cycling steps (Taylor *et al.*, 2007 and references therein). Few of them included data on resulting fluxes and, overall, no consistent pattern has clearly emerged on the role of marine sponges as sink or source of a particular nitrogen compound. Herein, we review the nitrogen cycle processes noted in marine sponges.

3.1. Nitrogen fluxes in sponges

3.1.1. Remineralization of POM

This process involves the conversion of the particulate organic nitrogen filtered by the sponge to dissolved organic nitrogen and ammonia (also to DOC and phosphate; Fig. 3.3). Nevertheless, if associated microorganisms

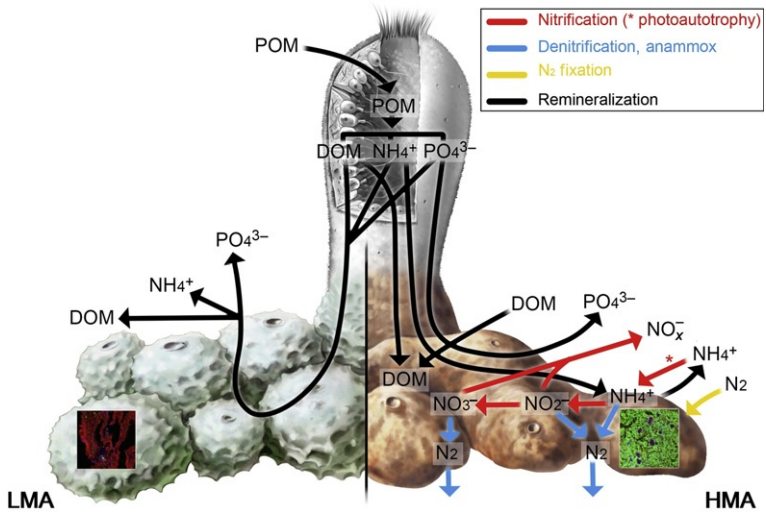

Figure 3.3 Common and specific nutrient fluxes and pathways in marine sponges related to their microbial components abundance. LMA, low-microbial abundance species; HMA, high-microbial abundance species. Remineralization includes the removal of particulate organic matter (POM) by all sponge species. As result of POM metabolism, dissolved organic matter (DOM), ammonia (NH_4^+), and phosphate (PO_4^{3-}) will be generate inside the sponge. Those compounds will be released directly to the water column by LMA species but would be processed through nitrification, photautotrophy, denitrification, and/or anammox in HMA species. External uptake of NH_4^+ and release of NO_x^- will appear in HMA species as well as uptake of dissolved organic compounds. Pictures are confocal microscopy FISH images with universal bacteria probes (bright green area) showing the relative abundance of symbiotic bacteria in each sponge type.

are abundant (HMA sponges), dissolved components can also be uptaken directly from the seawater and transformed by them (Scheffers *et al.*, 2004). Remineralization of POM is a common nutrient step in many sponges, irrespective of the abundance and composition of their associated microbial communities. In this sense, availability of POM was a major determinant of growth rates in three Caribbean species with different microbial abundance (i.e. *C. vaginalis* is LMA, *Agelas conifera* and *A. fistularis* are HMA), pointing out the importance of particulate nutrients in sponge metabolism (Lesser, 2006). Ammonia excretion has also been detected in many sponge species, irrespective of their microbial abundance (Table 3.3; Fig. 3.5), showing that marine sponges can act as a net ammonia source through remineralization of particulate material. The relative importance of different ingested particles as nitrogen source may be related to the reported sponge ability for food selection. Nevertheless, due to differences in food particle size and C:N content, a positive selection over a particular food type does not always imply a net nitrogen source. For instance, whereas cyanobacteria and picoeukaryotes were the major food source in terms of particle abundance for the Mediterranean sponge *S. officinalis*, isotopic analyses revealed that most of the carbon (and probably also nitrogen) retained in terms of biomass came from nanoeukaryotic cells (Topçu *et al.*, 2010). Due to its size, *Synechoccocus* is often the dominant prokaryotic contributor to sponge carbon fluxes (Ribes *et al.*, 2005; Pile and Young, 2006), even though it is often much less abundant than heterotrophic bacteria (Ribes *et al.*, 1999a). The heterotrophic cells, however, are better sources of nitrogen than autotrophic picoplankton due to their lower C:N ratio (Wheeler and Kirchman, 1986; Geider and La Roche, 2002; Bertilsson *et al.*, 2003). Detritus may also contribute to the acquisition of organic nitrogen by sponges, with values ranging from trace levels hard to detect (Yahel *et al.*, 2007) to about 20% (Hadas *et al.*, 2009).

3.1.2. Nitrification

This consists of the oxidation of ammonia to nitrite and nitrate for energy purposes and is mediated by two phylogenetically distinct groups: the ammonia oxidizing bacteria or archaea and the nitrite oxidizing bacteria. Sponge-mediated nitrification (Table 3.4) has been detected in many Caribbean sponges (Corredor *et al.*, 1988; Díaz and Ward, 1997; Southwell *et al.*, 2008a), in several sublittoral Mediterranean sponges (Jiménez and Ribes, 2007; Bayer *et al.*, 2008; Schläppy *et al.*, 2010; Ribes *et al.*, 2012), in *Geodia barretti* from the North Atlantic coast (Hoffmann *et al.*, 2009), and in two species inhabiting a deep-water coral mound (Van Duyl *et al.*, 2008). Also, vertical transmission of ammonium oxidizing archaea has been described in *Luffariella variabilis* and *Rophaloeides odorabile*, two sponge species from the Great Barrier Reef (Steger *et al.*, 2008). Ammonium and nitrite are suspected to accumulate within the sponge body during periods

Table 3.4 Dissolved nitrogen and phosphorous fluxes in marine sponges from different locations

Sponge	Location	Host type	Phototrophic components	NH_4^+	NO_x^-	PO_4^{3-}	DON	Source
µmol L^{-1}								
Agelas conifera	Florida keys	HMA	Low Chl a	**0.15**	−0.57	–	–	Southwell *et al.* (2008a), Weisz (2006)
Aplysina archeri	Florida keys	HMA	High Chl a	−0.3	−0.72	–	–	Southwell *et al.* (2008a), Weisz (2006)
Aplysina lacunosa	Florida keys	HMA	High Chl a	−0.09	−1.14	–	–	Southwell *et al.* (2008a), Weisz (2006)
Ircinia strobilina	Florida keys	HMA	Low Chl a	−0.07	−0.67	–	–	Southwell *et al.* (2008a), Weisz (2006)
Niphates digitalis	Florida keys	LMA	Low Chl a	−0.21	–	–	–	Southwell *et al.* (2008a), Weisz (2006)
Xestospongia muta	Florida keys	HMA	High Chl a	**0.05**	−0.86	–	–	Southwell *et al.* (2008a), Weisz (2006)
Aphrocallistes vastus	North Pacific	LMA	NP	−0.16	0	−0.04	0	Yahel *et al.* (2007)
Rhabdocalyptos dawsoni	North Pacific	LMA	NP	−0.4	−0.13	−0.04	0	Yahel *et al.* (2007)
Dysidea avara	Mediterranean	LMA	DGGE	**NS**	NS	−0.02	−0.48	Ribes *et al.* (2012)
Chondrosia reniformis	Mediterranean	HMA	DGGE	**1.69**	−0.99	−0.02	**NS**	Ribes *et al.* (2012)
Agelas oroides	Mediterranean	HMA	DGGE	**0.57**	−0.99	−0.04	**0.01**	Ribes *et al.* (2012)
µmol g DW^{-1} h^{-1}								
Aplysina cauliformis	Florida keys	HMA	High Chl a	**0.5**	−1.7	–	–	Southwell *et al.* (2008b)
Smenospongia aurea	Florida keys	HMA	Low Chl a	0	−1.8	–	–	Southwell *et al.* (2008b)
Niphates erecta	Florida keys	LMA	Low Chl a	−1.5	0	–	–	Southwell *et al.* (2008b)
Dysidea avara	Mediterranean	LMA	DGGE	−0.65	0	−0.05	–	Jiménez and Ribes (2007)
Agelas oroides	Mediterranean	HMA	DGGE	0	−0.36	−0.06	–	Jiménez and Ribes (2007)

Chondrosia reniformis	Mediterranean	HMA	DGGE	**0.64**	−0.7	0	–	Jiménez and Ribes (2007)
Ircinia oros	Mediterranean	HMA	–	0	−0.23	0	–	Jiménez and Ribes (2007)
Aplysina aerophoba	Mediterranean	HMA	Cyanos	**0.3**	−0.77	−0.03	–	Jiménez and Ribes (2007)
Aplysina aerophoba	Mediterranean	HMA	Cyanos	**0,012** ⋆1	−0.33	–	–	Bayer *et al.* (2008)
				−0,73 ⋆2	−0.23	–	–	Bayer *et al.* (2008)
Chondrilla nucula	Caribbean	HMA	Cyanos	**0.35**	−0.6	–	–	Corredor *et al.* (1988)
Anthosigmella varians	Caribbean	HMA	Zoox	**0.27**	−0.02	–	–	Corredor *et al.* (1988)
Chondrilla nucula	Caribbean	HMA	Cyanos	−0.019	−0.37	–	–	Díaz and Ward (1997)
Pseudaxinella zeai	Caribbean	HMA	Cyanos	−0.04	0	–	–	Díaz and Ward (1997)
Oligoceras violacea	Caribbean	HMA	Cyanos	0.18	−0.57	–	–	Díaz and Ward (1997)
Plakortis halichondroides	Caribbean	HMA	–	−0.1	−0.02	–	–	Díaz and Ward (1997)
Aplysina sp.	W. Australia	–	–	−0.69	0	−0.005	**0.21**	Hatcher (1994)
Iotrochota baculifera	W. Australia	–	–	−0.52	0	−0.004	**0.39**	Hatcher (1994)
Haliclona cymiformis	GBR	–	Rhodophyte	**2.4**	**0.1**			Davy *et al.*, (2002)
µmol cm^{-3} h^{-1}								
Geodia barreti	North Atlantic	HMA	NP	**0.067**	−0.024	–	–	Hoffmann *et al.* (2009)
Dysidea avara	Mediterranean	LMA	DGGE	−0.109	0	−0.009		Jiménez and Ribes (2007)
Agelas oroides	Mediterranean	HMA	DGGE	0	−0.127	−0.02		Jiménez and Ribes (2007)
Chondrosia reniformis	Mediterranean	HMA	DGGE	**0.13**	−0.157	0		Jiménez and Ribes (2007)
Ircinia oros	Mediterranean	HMA	–	0	−0.046	0		Jiménez and Ribes (2007)
Aplysina aerophoba	Mediterranean	HMA	Cyanos	**0.104**	−0.24	−0.008		Jiménez and Ribes (2007)
Higginsia thielei	North Atlantic	HMA	NP	−0.042	−0.001			van Duyl *et al.* (2008)
Rossella nodastrella	North Atlantic	HMA	NP	−0.002	−2.5 10^{-5}			van Duyl *et al.* (2008)
µmol h^{-1} L^{-1}								
Agelas conifera	Florida keys	HMA	Low Chl a	**49**	−170	–	–	Southwell (2007)

(*continued*)

Table 3.4 (*continued*)

Sponge	Location	Host type	Phototrophic components	NH_4^+	NO_x^-	PO_4^{3-}	DON	Source
Aplysina archeri	Florida keys	HMA	High Chl a	−40	−100	–	–	Southwell (2007)
Aplysina lacunosa	Florida keys	HMA	High Chl a	−19	−120	–	–	Southwell (2007)
Ircinia strobilina	Florida keys	HMA	Low Chl a	−18	−370	–	–	Southwell (2007)
Niphates digitalis	Florida keys	LMA	Low Chl a	−260	NP	–	–	Southwell (2007)
Xestospongia muta	Florida keys	HMA	High Chl a	**2**	−170	–	–	Southwell (2007)
Aplysina cauliformis	Florida keys	HMA	High Chl a	−44	−160	–	–	Southwell (2007)
Aplysina fistularis	Florida keys	HMA	High Chl a	−68	−24	–	–	Southwell (2007)
Amphimedon compressa	Florida keys	LMA	Low Chl a	−150	0	–	–	Southwell (2007)
Callyspongia vaginalis	Florida keys	LMA	Low Chl a	−290	−2	–	–	Southwell (2007)
Ircinia campana	Florida keys	HMA	High Chl a	−220	−86	–	–	Southwell (2007)
Ircinia felix	Florida keys	HMA	High Chl a	−41	−270	–	–	Southwell (2007)
Niphates erecta	Florida keys	LMA	Low Chl a	−140	−5	–	–	Southwell (2007)
Smenospongia aurea	Florida keys	HMA	Low Chl a	−5	−230	–	–	Southwell (2007)

HMA, high-microbial abundance species; LMA, low-microbial abundance species; DON, dissolved organic nitrogen; Low and high Chl a, refers to Chl a measurements as indication of phototrophic components; DGGE, denaturing gradient gel electrophoresis used to detect phototrophic components; NP, not present; –, not measured; NS, not significant. Positive values (bolded) mean removal, negative values mean excretion; negative values are effluxes; *1, April measurements; *2, May-September measurements. Grey colour highlights LMA species.

of pumping arrest. Conversion to nitrate would be a solution for eliminating these compounds, which can be toxic to the sponge if accumulated above a certain concentration threshold (Steger *et al.*, 2008).

The most evident nitrogen flux in sponges mediating nitrification is a net production of NO_x^- (i.e. either nitrite and/or nitrate). Production of NO_x^- is a common feature in HMA species, irrespective of habitat and biogeographic area, which is in contrast with the low rates or even lack of nitrification in LMA species (Table 3.4, Fig. 3.3). In this sense, Southwell *et al.* (2008a) showed high rates of nitrification in HMA sponges of coral reefs systems, in agreement with previous work on a limited number of Caribbean species (Corredor *et al.*, 1988; Díaz and Ward, 1997). Beside NO_x^- production, nitrification can also result in NH_4^+ uptake (Fig. 3.4), being this combined nitrogen flux (sink of NH_4^+ and source of NO_x^-) specially evident in HMA species (Table 3.4). A seasonal variability can exist in those fluxes, as reported in the Mediterranean *A. aerophoba* (Bayer *et al.*, 2008). In this species, it was also demonstrated that ammonia addition stimulated nitrification, the sponge becoming an "ammonium source" during summer months. Nitrate release (excretion) was also significantly stimulated by ammonium in the spring months, while in August, addition of ammonium did not result in enhanced nitrate release (Bayer *et al.*, 2008). NH_4^+ uptake by sponges can also be related to photoautotrophy, as suggested by the presence of phototrophic microorganisms in the sponge exhibiting that ability (Table 3.4). This is mostly evident in HMA sponges.

3.1.3. Nitrogen fixation

This involves the conversion of ambient dinitrogen (N_2) to ammonia by sponge-associated cyanobacteria (Wilkinson and Fay, 1979) and/or heterotrophic microorganisms (Shieh and Lin, 1994). Sponge-mediated nitrogen fixation was first reported in several species in the Red Sea (Wilkinson and Fay, 1979) and years later in a tropical species of *Halichondria* sp. (Shieh and Lin, 1994). Detection of nitrogen fixing bacteria expressing *nifH* genes has been described in several sponges from Key Largo, Florida (Mohamed *et al.*, 2008). Nitrogen fixation by sponge-associated microorganisms may contribute to complete sponge nitrogen needs in environments with low nitrogen availability (Taylor *et al.*, 2007). Nitrogen fixation results in a net removal of nitrogen from the water column and it is mostly detected in HMA species (Fig. 3.3).

In situ studies showed that nitrogen fixation rates in several sponges of Florida Bay were very low relative to the ambient water, also compared to DIN (NH_4^+ and NO_x^-) production rates (Southwell, 2007). The studied species (*Aplysina cauliformis*, *A. conifera,* and *Ircinia felix*) are all HMA. The former one showed a maximum N_2 fixation about 2.5 $\mu g\ g^{-1}\ day^{-1}$. Yet

this figure was much lower than DIN release rates (430 $\mu g\ g^{-1}\ day^{-1}$). Southwell (2007) concluded that sponge-mediated nitrogen fixation in Caribbean reefs probably contributes significantly neither to the sponge nutrition nor to nitrogen inputs to the reef. Nevertheless, the result may be different in other geographical regions with stronger nitrogen limitation.

3.1.4. Denitrification and anammox

Denitrification is the conversion of nitrate to N_2 by associated bacteria or archaea. Denitrification in sponges was recently discovered in *G. barretti* (Hoffmann *et al.*, 2009), then in the Mediterranean species *D. avara* and *Chondrosia reniformis* (Schläppy *et al.*, 2010). Recovery of two groups of *NirS* gene (encoding for nitrite reductase) suggest that denitrification activity is generally performed by more than a group of bacteria (Hoffmann *et al.*, 2009). Anammox (anaerobic denitrification) reaction is the conversion of ammonia and nitrite to N_2. Microorganisms that carry out this reaction are bacteria from the phylum *Planctomycetales*. First evidence of anammox activity in sponges was described in *G. barretti* and associated anammox bacteria in its tissue (Hoffmann *et al.*, 2009). Anammox bacteria have also been detected in *Ircinia strobilina* and *M. laxissima* (Mohamed *et al.*, 2010). Denitrification occurs under aerobic conditions but anammox requires hypoxic conditions. Combination of both aerobic and anaerobic mesohyl portions are common in HMA species and Hoffmann *et al.* (2009) showed evidence of anammox and denitrification in the same animal host (i.e. *G. barretti* from North Atlantic). The resulting nitrogen flux is then a net N_2 production. This production could be fuelled by providing the microorganisms with sponge metabolic waste products (a way to clean the sponge tissue from noxious compounds), and/or with inorganic nitrogen (NH_4^+) taken up from the environment, adding to the effect of nitrification and photoautotrophy in HMA species (Fig. 3.3). Reported nitrogen release due to denitrification and anammox in *G. barretti* was 1 or 2 orders of magnitude lower than that by nitrification: nitrification = 0.024, denitrification = 0.0038, and anammox = 0.00013 $\mu mol\ cm^{-3}\ h^{-1}$ (Hoffmann *et al.*, 2009).

Few studies have included the measurement of dissolved organic nitrogen in marine sponge nitrogen fluxes. Data available for seven sponges including LMA and HMA species from the Mediterranean, North Pacific and Western Australia suggest that LMA species are characterized by either DON excretion or a neutral balance between inhaled and exhaled water, while a net DON uptake appears to characterize HMA species (Table 3.4). Interestingly, average DON incorporation by *H. caerulea* is 0.001 $mmol\ cm^{-3}\ h^{-1}$. This sponge harbours nitrifiers, but only shows average net nitrification after ammonia addition (Fleur C. van Duyl, in preparation).

3.2. Phosphorous fluxes in sponges

Phosphorous is an essential nutrient required by all organisms for biological synthesis and energy transfer processes (Tyrreell, 1999; Benitez-Nelson, 2000). In aquatic environment, budgets of total phosphorous results from the addition of three major phosphorous compounds: (1) dissolved inorganic phosphate, which is usually measured as phosphate (PO_4^{3-}); (2) dissolved organic phosphorous (DOP); and (3) particulate phosphorous. DOP is the dominant form (Dyhrman *et al.*, 2007). Since Taylor *et al.* (2007) devoted just a couple of lines to phosphorus cycling in marine sponges in their extensive review on sponge-associated microorganisms, not much advance has been attained in the understanding of sponge-mediated P fluxes. The few available measures come from some Mediterranean and North Pacific species, in which fluxes of PO_4^{3-} have been assessed (Table 3.4). Both LMA and HMA species acted as net PO_4^{3-} sources. This outcome is consistent with Taylor and co-workers' assumption that sufficient P is obtained by the sponges from the diet.

Recent work on *Dendrilla nigra* has shown that actinomycetes isolates were able to solubilize phosphate compounds, making this element available both for the host and favouring a faster P cycling (Sabarathnam *et al.*, 2010). However, "*in vivo*" studies are needed to evaluate the potential role of such microbe-mediated mechanisms in phosphate accumulation and solubilization by marine sponges.

3.3. Ecological significance of nitrogen and phosphorous use

Marine sponges are able to act either as sink or source of a variety of nitrogen compounds, which is more diverse in HMA species. Some general patterns can be summarized. Marine sponges act as a sink of POM. Their grazing impact on the small plankton fraction can be important to the point that picoplankton depletion layers have been registered above benthic communities where sponges were abundant (Savarese *et al.*, 1997). As a result of POM oxidation, release of inorganic dissolved compounds, such as ammonia and phosphate plus organic dissolved compounds, is expected. Nevertheless, the pathways followed after oxidation of organic matter is highly dependent on the abundance and type of the associated microbial communities. LMA sponges act as a net source of NH_4^+, PO_4^{3-}, and DOM. The scenario in HMA species is more complex due to the larger variety of the hosted microbial partners. Despite only few available data, it can be suggested that HMA sponges share the ability to act as a PO_4^{3-} source, irrespective of the amount and type of associated microbes. HMA sponges also represent a source of NO_x^-, although the magnitude of this flux appears to be substantially affected by seasonal variability. On the other side, HMA

sponges are a sink of NH_4^+, as a result of either nitrification processes or photoautotrophy. The few data available on DON fluxes suggest that HMA species are a sink of these nitrogen compounds. To date, the available data indicate that sponges are neither a significant N_2 source nor a sink.

Being that sponges are one of the most abundant and widespread group of benthic organisms in a wide variety of marine systems, their activity can potentially increase local concentrations of NH_4^+, PO_4^{3-}, and DON, nutrients often limiting for primary production and bacterial growth. This effect would be more likely to occur in LMA-dominated sponge assemblages. In contrast, the metabolic activity of HMA sponges does not appear to represent a potential source of compounds stimulating primary production or bacterioplankton growth. Rather, these sponges will compete with planktonic organisms for limiting nutrients and DON. Most of studies relating bacterial productivity to inorganic and organic nutrient inputs derived from benthic activity have been performed in coral reef systems. Differences are found between studies, depending on the nutrient limiting bacterial growth at each site. DOM produced through mucus released by corals was responsible of enhancing activity in the bacterioplankton adjacent to corals (Ferrier-Pages *et al.*, 2000; Van Duyl and Gast, 2001; Wild *et al.*, 2008). Inorganic nutrients from the remineralization of ingested compounds by suspension feeders living in the reef also enhanced bacterial activity in reef crevices (Gast *et al.*, 1998; Torreton *et al.*, 2000; Richter *et al.*, 2001; Van Duyl and Gast, 2001; Scheffers *et al.*, 2005). A lack of studies from other geographical sites (including temperate and cold systems) prevents any generalization on the effects of sponge activity on plankton productivity.

4. Use of Silicon by Sponges

4.1. Biological role of silicon structures

About 75% of the extant sponges species (most Demospongiae and all known Hexactinellida) use Si to build a siliceous skeleton. It consists of discrete pieces (known as spicules; Fig. 3.4A and B), pieces fused into rigid networks or a combination of both elements. The siliceous skeleton may represent up to a 90–95% of the body dry weight, depending on the species (e.g. Barthel, 1995; Maldonado *et al.*, 2010a). Spicules range in size from 1 μm to about 2–3 m, depending on the species. They also show a large variety of shapes and ornamentation details (reviewed in Hartman, 1981; Uriz *et al.*, 2003; Uriz, 2006). Often small spicules ($\sim < 100$ μm) are termed microscleres, being larger spicules known as megascleres. Both shape and size of spicules are relatively constant within species, a condition also conserved through the geological time scale, as revealed by the fossil record (e.g. Hinde and Holmes, 1892; Mostler, 1976; Wiedenmayer, 1994; Vacelet *et al.*, 2009).

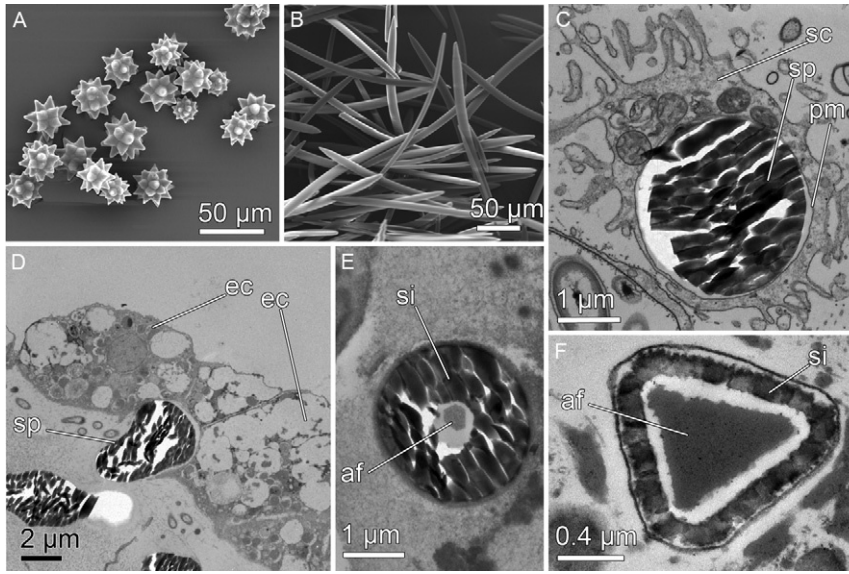

Figure 3.4 (A) Star-like spicules of the demosponge *Chondrilla nucula*. (B) Needle-like spicules of the demosponge *Petrosia ficiformis*. (C) Sclerocyte (sc) of *P. ficiformis* elaborating a siliceous spicule (sp) within an intracytoplasmic vesicle limited by the plasmalemma (pm) membrane. (D) Epithelial cell (ec) of the homosclerophorid *Corticium candelabrum* in the process of extruding to the mesohyl a sponge spicule (sp) that have been produced intracellularly. (E) Transversal section of a young spicule of *P. ficiformis*, showing a hexagonal axial filament (af) surrounded by a moderate amount of silica (si). (F) Transversal section of a young spicule of the demosponge *Crambe crambe*, showing a triangular axial filament (af) surrounded by an incipient silica layer (si).

Conserved spicule sizes and shapes could have resulted from particular adaptations to specific functions, which in most cases, have not been elucidated yet. Spicules are assumed to strengthen the mesohyl, helping to support the growth of soft parts and reinforcing the epithelia (pinacoderms) to avoid collapse of the aquiferous subectosomal spaces and canals. In many sponges, spicules form either a dense cortex at the surface or sharpened protruding palisades, thought to provide mechanical defences that deter potential predators. Spicules protruding from the body are also believed to protect the inhalant areas of the sponge surface from silt accumulation and clogging, particularly in deep-sea habitats. Some sponges also use giant spicules (centimetres to metres) to form rooting systems and stalks through which sponges attach to the seafloor, particularly in soft bottoms. In carnivorous sponges, siliceous micro-hooks that are finely and diversely micro-ornamented protrude from the body to hook the appendices of the microcrustaceans that will serve as food to these non-filtering sponges

(Vacelet and Duport, 2004). Additionally, siliceous spicules are excellent light transmitters that facilitate illumination of the subectosomal body regions, allowing phototrophic symbionts (i.e. cyanobacteria and zooxanthellae) to grow successfully in relatively deep regions of the sponge body for enhanced mutualistic or symbiotic relationship (Cattaneo-Vietti *et al.*, 1996; Brummer *et al.*, 2008). In sponge larval stages, spicules are thought to be involved in control of weight mass distribution and buoyancy (reviewed in Maldonado, 2006). Nevertheless, beyond the above generic functions postulated for sponge spicules, it remains largely unknown whether adaptation is the reason for the highly conserved nature of spicule size, shape, and ornamentation.

4.2. Cytology of silicon use: Intracellular versus intercellular modes

Siliceous skeletal pieces are elaborated by sponge cells (Fig. 3.4C and D), which are somehow able to take up the silicic acid ($Si(OH)_4$) dissolved in seawater and polymerize it into biogenic silica (SiO_2). Therefore, Si fluxes through sponges, unlike those of other dissolved nutrients, are thought to be exclusively mediated by sponge cells, being unrelated to the composition and density of sponge-associated microbial populations. Hereafter, we will refer to silicic acid as dissolved silicon (DSi) and to biogenic silica as BSi. In the past decades, the understanding of the mechanisms involved in spicule production has improved notably, but many unknowns still remain.

Plenty of observational evidence through light and electron microscopy during the twentieth century has corroborated that siliceous spicules start being elaborated intracellularly by amoeboid, free-moving cells that occur in the sponge mesohyl, known as sclerocytes (Simpson and Vaccaro, 1974; Simpson, 1978, 1984; Wilkinson and Garrone, 1980; Imsiecke *et al.*, 1995; Custodio *et al.*, 2002; Leys, 2003). Spiculogenesis begins within an intracellular vesicle, with the synthesis of a protein filament (termed axial filament; Fig. 3.4D and E) that is subsequently mineralized by condensation of silica around it. The protein making the axial filament is mostly silicatein, an enzyme that somehow directs polymerization of silicic acid into biogenic silica (Shimizu *et al.*, 1998; Cha *et al.*, 1999). The membrane of the vesicle, known as silicalemma (Simpson, 1984), has long been suspected to mediate in the condensation of silica around the axial filament. In most cases, each sclerocyte secretes only one spicule. Nevertheless, there are reports of sclerocytes secreting two or several spicules simultaneously (Imsiecke *et al.*, 1995; Custodio *et al.*, 2002), or even multiple spicules arranged in parallel in dense packs (Wilkinson and Garrone, 1980).

By investigating homosclerophorid sponges, it was recently found that spicules are produced intracellularly not only in the sclerocytes (Fig. 3.4C) but also within the epithelial cells (Fig. 3.4D), that is, exopinacocytes and, to a lesser degree, endopinacocytes (Maldonado and Riesgo, 2007). The

unconventional epithelial origin and the particular internal structure of those homosclerophorid spicules—with two relatively thick, concentric organic layers intercalated among silica layers in addition to a central axial filament—strongly suggests that silicification in homosclerophorid sponges does not follow the standard described for other siliceous sponges. Therefore, since it cannot be discarded that the ability for silicification may have evolved independently more than once in the phylum, a careful re-examination of the hitherto monophyletic state attributed to biosilicification within the phylum Porifera is needed.

Most of studies reporting intracellular production of sponge spicules come from observations on microscleres or small megascleres (Simpson and Vaccaro, 1974; Simpson, 1978; Wilkinson and Garrone, 1980; Imsiecke *et al.*, 1995; Custodio *et al.*, 2002; Maldonado and Riesgo, 2007). Therefore, it remains unclear how the large spicules (several mm to cm) characterizing many demosponges and hexactinellids could be produced intracellularly and it has long been suspected that some sort of extracellular BSi condensation could occur during the building of the largest megascleres (Simpson, 1984). In at least some Hexactinellida, it has been corroborated that relatively large spicules are formed intracellularly, within multinucleate giant sclerocytes, named sclerosyncytia (Mackie and Singla, 1983). From TEM observations in the demosponge *Crambe crambe,* it has been proposed the alternative view that production of megascleres in most demosponges could occur intercellularly (Uriz *et al.*, 2000). This claim was based on the realization that axial filaments of *C. crambe* were initially produced intracellularly in a membrane bounded vesicle of the sclerocytes and subsequently exocytosed to the intercellular medium. There, DSi condensation would take place somehow within an external "pocket" formed around the silicatein filament by pseudopodia emitted by the sclerocyte membrane (i.e. the plasmalemma).

More recently, another mechanism has been proposed, mostly from accumulative research on silicification in primmorphs of the demosponge *Suberites domuncula*. It postulates that silicification starts intracellularly and is completed extracellularly through mechanisms different from the "sclerocyte pockets" suggested by Uriz and co-workers (reviews by Schröder *et al.*, 2008; Wang *et al.*, 2011b). This latter model vindicates that, at the cellular level, sponge silicification involves three major steps: (1) intracellular production of the axial filament and initiation of BSi condensation within the silicalemma-enveloped vesicle, (2) exocytosis of the unfinished spicule to the intercellular milieu (the mesohyl), and (3) completion of BSi deposition onto the external surface of the exocytosed spicule. This secondary BSi deposition is hypothesized to result from the release of concentrated DSi and silicatein enzymes previously stored in vesicles by one or several sclerocytes congregated in the vicinity of the exocytosed spicules. Such an intercellular silicification phase would occur directly onto the spicule surface without the need of intimate contact between the growing spicule and the membrane of nearby sclerocytes. A fourth extracellular silicification step has recently been proposed. It

postulates that at least one of the sclerocytes in the vicinity of the newly exocytosed spicule emits a pseudopodium-like evagination of its membrane that enters the axial canal and deposits a silica layer, reducing considerably the lumen of the axial canal (Wang *et al.*, 2011b).

4.3. Molecular aspects of silicon use

As at the cellular level, many molecular aspects of DSi use by sponges still remain obscure. The available information has been somewhat repetitively reviewed in the past years (Schröder *et al.*, 2008; Müller *et al.*, 2009; Wang *et al.*, 2011a), because new findings that complicate the global picture are published continuously (Schloßmacher *et al.*, 2011; Wiens *et al.*, 2011). A major step in this field was the realization that the axial filament of spicules mostly consists of an enzymatic protein (silicatein) that directs polymerization of DSi into BSi (Shimizu *et al.*, 1998; Cha *et al.*, 1999). So far, two isoforms of silicatein (alpha and beta) have identified (Cha *et al.*, 1999). It has been shown *in vitro* that silicatein can use synthetic tetraethoxylene as substrate (Cha *et al.*, 1999), but, given that this type of compound is not naturally available to the sponges, the natural substrate for the enzyme remains enigmatic. It has preliminarily been suggested that silicic acid–sugar complexes might be possible natural candidate substrates for silicatein (Schröder *et al.*, 2008). The particulars of reaction mechanisms leading to the production of BSi from DSi in sponges are still under debate. A model postulating the basic reactions of enzymatically mediated BSi deposition starting from a synthetic alkoxide substrate was originally proposed by Cha *et al.* (1999), but no significant progress on the putative reactions regarding natural substrates has been achieved since.

In contrast, some progress has been attained relative to the mechanisms through which silicatein is produced. The silicatein gene renders an inactive 36-kDa translation product, which is cleaved twice before becoming a mature 23-kDa enzyme (Müller *et al.*, 2005). Pos-translational modifications also include multiple phosphorilations and a dehydroxilation step (Müller *et al.*, 2005; Schröder *et al.*, 2006). Silicatein monomers are able to self-assemble into oligomers, then into longer protein filaments by mechanisms still under debate (Murr and Morse, 2005; Schröder *et al.*, 2008).

Silicatein is not only the main component of axial filaments. It has also be demonstrated to occur on the spicule surface of intercelullar spicules (Müller *et al.*, 2006). Extracellular aggregation of silicatein onto the surface of "unfinished" spicules is postulated to control the extracellular silica condensation required to complete the spicule formation, according to the Müeller–Schröder's silicification model (Müller *et al.*, 2005; Schröder *et al.*, 2006). Although silicateins are the predominant proteins related with sponge silicification, a number of additional proteins, such as Galectin-2, Silintaphin-1, and Silintaphin-2, have been identified so far. Galectin is

thought to serve as a structural matrix for the assembly of silicatein molecules (Schröder *et al.*, 2006, 2008). Silintaphin-1 appears to facilitate the assemble of silicatein-alpha multimers and somehow binds to those multimers to enhance BSi condensation around the silicatein scaffold (Wiens *et al.*, 2009; Schloßmacher *et al.*, 2011). The role of the recently discovered Silintaphin-2 (Wiens *et al.*, 2011) still remains unclear.

It also remains little understood the mechanism responsible for binding and transporting DSi from seawater to the cytoplasm of the silica-secreting cells. To date, the only enzymes properly identified in connection with DSi condensation belong to the silicatein family (Shimizu *et al.*, 1998; Cha *et al.*, 1999), but neither this enzyme nor other known components involved in the process of DSi polymerization inside or outside the sclerocytes cells are having a demonstrated role in internalization of DSi from ambient seawater. Suggestions have been made that a sodium-bicarbonate co-transporting system could somehow be involved in taking up silicic acid from seawater. Although active DSi uptake in diatoms has been shown to be supplemented by some passive diffusion across the cell membrane (Thamatrakoln and Hildebrand, 2008), a similar diffusion process is unlikely in sponges, because silica-secreting cells of sponges (sclerocytes) occur typically at the inner mesohyl regions of the sponge body. As a result, silicifying cells are typically isolated from ambient seawater by epithelial cell layers and dense intercellular deposits of collagen and other macromolecules.

4.4. Ecology of silicon use by sponges

Most DSi in the ocean is thought to be consumed by marine plankton (i.e. diatoms, radiolarians, choanoflagellates, silicoflagellates), particularly by diatoms (Nelson *et al.*, 1995; Tréguer *et al.*, 1995; Sarmiento and Gruber, 2006). Diatoms use DSi to build their extracellular siliceous skeleton (frustule) of BSi, which supports cell growth. High levels of DSi in seawater typically stimulate growth of diatom populations, which in turn decreases DSi, nitrate, and phosphate levels in surface waters and facilitate transfer of atmospheric carbon dioxide to the ocean, hence connecting silicon to carbon, phosphate, and nitrogen cycles (Siever, 1991; Harrison, 2000; Planavsky *et al.*, 2010). Because of these cycle interconnections, there is strong interest in predicting the interplay between DSi and BSi budgets and many efforts have been made during the last decades to unravel the "diatom" route of Si through the oceans.

There had always been suspicions that the contribution of sponges to the marine Si cycle, even if never quantified in global terms owing to its technical complexity, could be of some importance (e.g. Harriss, 1966; Siever, 1991). A small group of studies investigating the contribution by siliceous sponges (Rützler and Macintyre, 1978; Conley and Schelske, 1993; Reincke and Barthel, 1997; Maldonado *et al.*, 1999, 2005, 2010a)

has already alerted the notion of a Si cycle exclusively revolving around diatoms may incur an unadvisable oversimplification. Here, we review the knowledge on Si fluxes through sponges, highlighting recent developments.

4.4.1. Experimental silicon uptake rates

The most serious limit to our understanding of the magnitude of Si turnover through sponges is the current lack of information regarding uptake kinetics. There are only two available studies to date. One study was conducted on the demosponge *H. panicea* (Reincke and Barthel, 1997), the other on several species in the genus *Axinella* spp. (Maldonado *et al.*, 2011). Both studies have agreed that DSi uptake rates increase with increasing ambient DSi availability according to a hyperbolic function (Fig. 3.5), which is the model characterizing ligand-binding kinetics with one site saturation (i.e. Michaelis–Menten kinetics). That kinetics is also in full agreement with the independent discovery of silicatein as the enzyme responsible for silica deposition in sponges (Cha *et al.*, 1999).

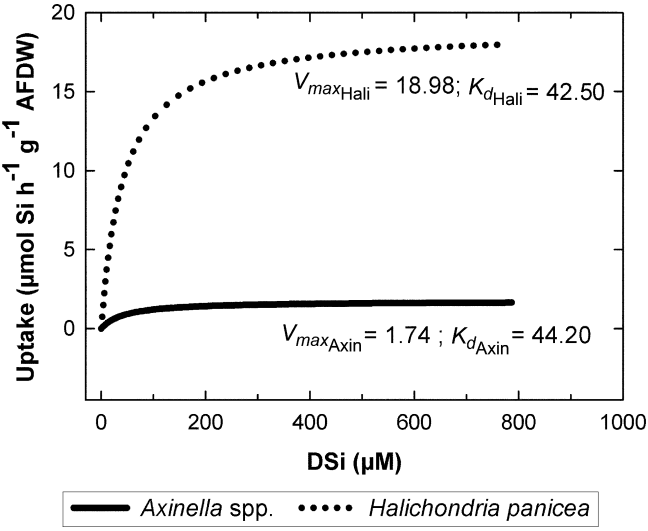

Figure 3.5 Comparative DSi uptake kinetics resulting from a reanalysis of raw experimental data for the demosponges *Halichondria panicea* (from Reincke and Barthel, 1997) and several *Axinella* species (from Maldonado *et al.*, 2011). For comparative purposes, uptake is expressed as µmol Si hour^{-1} and ash-free dry weight gram of sponge. Note that the half-saturation value (K_d) is relatively similar in both sponge genera, while maximum velocity of DSi transport (V_{max}) is much greater in *H. panicea*. This latter difference is assumed to result from uptake rates of *Axinella* spp. being measured on histologically undisturbed, complete individuals, while *H. panicea* uptake rates were measured on body fragments cut down for the experiment and inadvertently induced to regenerate much soft tissues and BSi skeleton at accelerate rates, probably biasing the uptake experiment.

According to Reincke and Barthel's (1997) experiments (Fig. 3.5), *H. panicea* has an uptake kinetics with a half-saturation constant (K_d) of 46.41 μM DSi and a saturated uptake rate (V_{max}) of 19.33 μmol DSi h^{-1} g^{-1} of sponge ash-free dry weight (AFDW). Experiments on *Axinella* spp. (Fig. 3.5) by Maldonado *et al.* (2011) indicated a K_d of 74.47 μM and a V_{max} of either 0.13 μmol h^{-1} sponge ml^{-1} or 1.74 μmol h^{-1} g^{-1}, if expressed as AFDW for comparative purposes. Surprisingly, saturated uptake rate for *H. panicea* was 19.33 μmol Si h^{-1} g^{-1} AFDW, an order of magnitude higher than that measured for *Axinella* spp. Such differences in V_{max} could be explained by both sponge genera having very different affinity by DSi. An alternative explanation is that *H. panicea* uptake measurements were not taken from complete sponge individuals but from explants, that is, regenerating pieces that had been obtained for the experiment by fragmenting larger sponges (Reincke and Barthel, 1997). It is well known that sponges regenerate rapidly large portions of their body (including the corresponding production of new silica skeleton), accomplishing regeneration at rates that are up to 2900 times the undisturbed growth rates (Ayling, 1983).

It is also worth noting that the *H. panicea's* uptake system saturates at ambient DSi concentrations around 100 μM DSi and that of *Axinella* spp. does it at about twice higher concentration (i.e. 200 μM), indicating that affinity for DSi may vary between sponges species. Interestingly, DSi uptake in both *H. panicea* and *Axinella* spp. becomes more efficient when DSi is provided as much higher concentrations (1 to 2 orders of magnitude higher) than the natural values (1–10 μM DSi) characterizing the sublittoral ecosystems where these sponges live. It has also been shown (Maldonado *et al.*, 2012) that DSi uptake rates by the intertidal demosponge *H. perlevis* increases when exposed to increasing DSi concentrations, with average DSi uptake rate (μmol Si mL^{-1} fresh sponge h^{-1}) at 70 μM being significantly higher (0.39 ± 0.07) than those at 40 and 25 μM (0.18 ± 0.04 and 0.15 ± 0.07, respectively), which in turn were not significantly different from each other, but were higher than the uptake rate at 10 μM (0.06 ± 0.02). In agreement with *H. panicea* and *Axinella* spp., these results support that the uptake system of *H. perlevis* performs with significantly higher efficiency when DSi concentrations are at least two- to fourfold higher than the DSi maximum expected in the natural habitat under the most conservative conditions (i.e. 10–15 μM). Consequently, all investigated sublittoral sponges are suffering a severe, chronic limitation by DSi (< 10 μM) in their natural habitats. Another example of severe DSi limitation has been experimentally provided by long-term exposure of the shallow-water demosponge *C. crambe* to DSi concentrations higher (30 and 100 μM) than those in its natural habitats (about < 2 μM). Those high concentrations induced secretion of not only longer and thicker spicules but also additional spicule types that are never produced in wild populations

(Maldonado *et al.*, 1999). Such a response suggests that the genetic systems controlling Si uptake and silicification are up-regulated by threshold DSi concentrations higher than those naturally available to the sponge.

Altogether, these findings on DSi uptake kinetics open the possibility that extant sponge species belonging to lineages that diversified before the decline in DSi availability caused by the ecological expansion of diatoms during the late Cretaceous and Early Tertiary (about 100 to 50 mya) may still silicify through uptake systems originally suited to deal with the high DSi concentrations that seemingly characterized pre-Tertiary oceans (Maldonado *et al.*, 2011). From an eco-physiological point of view, it means that the skeletal growth in the sublittoral populations of many Recent demosponges is chronically limited by DSi availability. The idea that low DSi concentrations in shallow waters of Recent oceans might limit sponges was originally disregarded (e.g. Hartman, 1981; Vacelet, 1988; Pisera, 1997), as it was assumed that their uptake systems had progressively adapted during the post-Cretaceous DSi crisis. In contrast, it seems that such an adaptation was never achieved by many sponges. Consequently, the reduced DSi availability characterizing modern oceans still operates as an important environmental pressure probably favouring evolutionary changes in these sponges towards either weakening their siliceous skeletons (Maldonado *et al.*, 1999; Maldonado, 2009) or tuning their silicification systems for higher efficiency at low DSi concentrations.

4.4.2. Silicon field demands

Information on DSi field demands by sponge populations is extremely scarce because of the technical complexity of the approaches. Any estimate of yearly DSi demands by sponge populations have to be taken as tentative, because calculations will be affected by several sources of variability for which corrections are difficult to implement. For instance, laboratory estimates of sponge DSi uptake on hourly or daily basis may not incorporate appropriately the biological variability inherent to the uptake process. It is likely that DSi uptake by sponges occurs neither as a continuous process nor at a constant rate, even if ambient DSi concentrations would be kept constant. DSi uptake is also linked to processes governing sponge growth, which in turn are strongly affected by seasonality (e.g. Stone, 1970a,b; Elvin, 1976; Turon *et al.*, 1998), individual age and size (e.g. Dayton *et al.*, 1974; Barthel, 1989), incidence of predation and grazing (e.g. Ayling, 1983), food availability (e.g. Frøhlich and Barthel, 1997), and species-specific "BSi/organic tissue" ratios. The effects of some of these parameters on DSi uptake rates may be dramatic. For instance, Ayling (1981) observed that some encrusting sponges, which had experimentally been injured to simulate predation, regenerated the lost tissues at rates between 22 and 2900 times the undisturbed growth rate, depending on species. Therefore, Si uptake and BSi production will be very different in two conspecific populations experiencing

different levels of predation or grazing. Similarly, Frøhlich and Barthel (1997) demonstrated that sponges starved for 1 week showed Si uptake rates only 15% as large as those shown by non-starved sponges.

Despite problems, some tentative estimates have been produced. Field surveys along a relatively oligotrophic, sponge-poor Mediterranean rocky sublittoral revealed that siliceous sponges average 0.34 ± 0.52 L m^{-2} and ambient DSi 0.73 ± 0.44 µM (Maldonado *et al.*, 2011). At that ambient DSi concentration, sponge uptake rate is predicted to average $1.31 \pm 0.79 \times 10^{-3}$ µmol Si h^{-1} sponge ml^{-1} (according to equation for Mediterranean sponges in Fig. 3.5). It means that the sponge fauna per m^2 of habitat at that portion of Mediterranean rocky coast use yearly about 3.9 ± 5.9 mmol DSi, which represents yearly about 21.4 ± 32.7% of the average DSi available in a 30-m overlying water column and about 10.7 ± 16.3% in a 50-m deep-water column of that coastal ecosystem, if there is no replenishment. Similarly, it has been estimated that the abundance of siliceous sponges across a Mesoamerican continental shelf (Belize) averages 2.6 ± 14.3 L m^{-2} of bottom (Maldonado *et al.*, 2010a). Mean yearly DSi concentration in the 25-m deep-water column of such shelf ecosystem is about 3.6 ± 0.6 µM, and the sponge communities are predicted to consume DSi at an average rate of about $14.5 \pm 0.35 \times 10^{-3}$ µmol h^{-1} sponge ml^{-1} (according to the equation in Fig. 3.5). It means that yearly sponge uptake is about 332 ± 1826 mmol DSi m^{-2}, and that it would virtually deplete the shallow Belizean shelf of DSi once every 98.9 ± 83.7 days, if there is no DSi replenishment.

For a comparison (Table 3.5), the estimated Si consumption rate by the poor (in volume) sponge fauna of the Mediterranean sublittoral would be about 0.01 ± 0.01 mmol Si m^{-2} day^{-1}, while that of the richer Caribbean sponge assemblages would be 0.90 ± 5.00 mmol Si m^{-2} day^{-1}. By using available DSi uptake data for seasonal Baltic populations of the sponge *H. panicea* (Reincke and Barthel, 1997) and the mean biomass (20 ml m^{-2}) in the less favourable sponge habitat (Barthel, 1988), a conservative average consumption of 0.44 mmol Si m^{-2} day^{-1} during Baltic summer months may also be arrived at. From raw data in Dayton *et al.* (1974) and Dayton (1979), Maldonado *et al.* (2005) deduced that BSi production by the demosponge *Homaxinella balfouriensis* during an astonishing population bloom from 1968 to 1975 occurred at rates of about 22.94 mmol Si m^{-2} day^{-1} (Table 3.5). Surprisingly, this high value is not far behind the annual BSi production rate of the whole diatom assemblage in the Ross Sea, estimated between 37 and 93 mmol Si m^{-2} day^{-1}, according to mean and maximum production rates, respectively (Ragueneau *et al.*, 2000). Importantly, according to Dayton's abundance data (1979), the BSi production rate of the sponge *H. balfouriensis* would have experienced an additional twofold increase in the study area from 1975 to 1977, which, if considered in the calculations, would have lead to an even higher annual average rate of Si consumption.

Table 3.5 Rates of Si use by communities of planktonic diatoms and sublittoral sponge communities in various marine systems

Habitat/system	Mean Si demand (mmol Si m^{-2} day^{-1})
Planktonic diatoms	
Coastal upwelling	90
Other coastal conditions	15
Southern ocean	15
Deep ocean	2.3
World ocean average	1.6–2.1
Sublittoral sponges	
Outer shelf of Belize	0.9
Baltic sublittoral bottoms	0.44
Mediterranean rocky bottoms	0.01
Antartic demosponge population bloom	22.9
Bathyal sponges	
Canadian hexactinellid reefs	3.52

Diatom Si demands were originally measured as BSi production rates (Nelson *et al.*, 1995; Tréguer *et al.*, 1995; Ragueneau *et al.*, 2000), demands by sublittoral non-Antarctic sponges have been derived from Si uptake rates (Reincke and Barthel, 1997; Maldonado *et al.*, 2011), demands by Antarctic sublittoral demosponges inferred by Maldonado *et al.* (2005) from Dayton (1979), and demands by bathyal hexactinellids inferred from estimated from growth rates (Chu *et al.*, 2011).

At present, it is impossible to estimate with any accuracy global Si consumption by sponges in the world oceans—as will be the case for decades to come owing to the extremely variable distribution of sponge biomass on the continental margins at depths that prohibit extensive measurements of individual Si contents per bottom area. With the exception of the transitory population bloom of the Antarctic demosponge *H. balfourensis*, the Si consumption rates inferred for sponges to date (Table 3.5) are somewhat smaller than the average BSi production by diatoms in the global ocean, estimated at 1.6–2.1 mmol Si m^{-2} day^{-1} (Nelson *et al.*, 1995; Ragueneau *et al.*, 2000). They are also lower than the average diatom demand estimated in diverse marine systems. Nevertheless, sponge abundance is so important in some sublittoral areas that, despite their chronic DSi limitation, DSi incorporation at the community level is anticipated to have some relevance, particularly at local or regional level.

If the most conservative DSi consumption rates determined for sublittoral, non-Antarctic demosponges (Table 3.5) are standardized per unit area of bottom (according to the sponge abundances measured at the Mediterranean, the Baltic, and the Caribbean communities) and these figures extrapolated over the entire continental shelf (22×10^6 Km^2) of the earth, a first, very tentative estimate of the global Si consumption by sponges can be inferred, falling somewhere between 8.6×10^{10} and 7.3×10^{12} mol Si $year^{-1}$

(Maldonado *et al.*, 2011). This figure is still about 2– 4 orders of magnitude smaller than the 2.0 to 2.8 $\times 10^{14}$ mol Si year^{-1} estimated for diatoms (Nelson *et al.*, 1995; Tréguer *et al.*, 1995). Nevertheless, should DSi consumption by the dense sublittoral Antarctic populations and the many sponge communities that are being discovered at bathyal depths following the advent of ROVs and manned oceanographic submersibles (Richardson and Young, 1987; Rice *et al.*, 1990; Genin *et al.*, 1992; Maldonado and Young, 1996; Leys *et al.*, 2004; Maldonado *et al.*, 2005; Chu *et al.*, 2011) incorporated into the calculations, a generous—though unlikely to be ever accurately quantified—increase of the yearly global Si sponge consumption might be arrived at.

The information available to infer sponge DSi demands in bathyal and abyssal communities (where ambient DSi is not limiting) is even scarcer than that on sublittoral bottoms. To our knowledge, only the study by Chu *et al.* (2011) has addressed this issue, using a combination of field sampling and surveys with ROVs to investigate field populations of large and heavily skeletonized hexactinellid sponges (mostly *A. vastus*). These sponges are able to grow on top of non-dissolved skeletons of dead conspecifics, creating large siliceous mounds up to 21 m in height and about 9000 year old (Conway *et al.*, 1991, 2001; Chu and Leys, 2010). These sponge reefs are unique epibathyal habitats, so far known only from British Columbia (Canada), where they discontinuously extend over an area greater than 700 km^2 (Whitney *et al.*, 2005; Conway *et al.*, 2007). Because in this Pacific area annual DSi levels fluctuate in surface waters (< 30 m) from 1 to 70 μM, but remain higher (about 50 μM) throughout depths (150–240) where sponge reefs occur, DSi is thought not be limiting to the sponge reefs. Under the assumption of a 1-cm year^{-1} growth rate in only the vertical dimension of reefs, the sponge populations of the 700-km^2 reef system were estimated to incorporate Si into their skeletons at an average rate of 1.3×10^6 mol Si km^{-2} year^{-1} (Table 3.5), leading to a current standing stock of 1.4×10^8 mol Si km^{-2} accumulated as BSi skeletons. These figures are thought to represent an important pool at the regional scale of the northeast Pacific continental shelf (Chu *et al.*, 2011).

4.4.3. Silicon in silica standings stocks

Only recently has the magnitude of sponge BSi standing stocks started being evaluated. A first assessment for a Caribbean sublittoral population of *Chondrilla nucula* in reef habitats and for a sublittoral Mediterranean population of *C. crambe* revealed that either population accumulated amounts of Si in the form of skeletal BSi that were about 200–300 times those available as DSi in the water column of their respective habitats (Maldonado *et al.*, 2005). The local population of *C. nucula* contained about 67.4 tons BSi km^2 of habitat and *C. crambe* about 81 kg BSi per linear km of rocky sublittoral.

To date, only a study has compared the relative contribution of BSi standing stocks by sponges and diatoms in a continental shelf ecosystem and

it concluded, contrary to expectations, that sponges (and not diatoms) comprise the largest standing stock of BSi in the regional pool (Maldonado *et al.*, 2010a). Collectively, BSi in the various sponge communities was about 88.6% of the total Si pool in the studied Mesoamerican shelf ecosystem, while diatoms represented only 4.2%, being the remaining Si (7.2%) as ambient DSi. Such sponge dominance was not attributable to impoverished environments in terms of either nutrients or phytoplankton, but to high sponge abundance. On average, siliceous sponges in that shelf showed a mean BSi content of $0.3\,6 \pm 2.7$ kg m^{-2}, but large individuals of some species were estimate to contain up to 28 kg BSi each.

The few available, deep-sea studies strongly suggest that below the mixed layer of the ocean, where diatoms do not proliferate, sponge standing BSi stocks may also be significant. It has been shown that Si standing stocks by dense populations of bathyal hexactinellid *Sericolophus hawaiicus* average 12.6 g Si m^{-2}, which means that the sponges retain an amount of Si per m^2 of bottom that is equivalent to that contained in 2.6×10^6 l of ambient water from their habitat, an amount equivalent several times to that contained in the entire 400-m high water column above the sponge population (Maldonado *et al.*, 2005). These data are a very conservative estimate of total BSi content trapped in that bathyal sponge bed, because the abundant masses of large spicules (30–40 cm) released to the seafloor after sponge death were not considered. More importantly, the population of *S. hawaiicus* is not an isolated case of sponge abundance at bathyal depths. Recent surveys of 9-km abyssal transects at a depth of 4100 m off the California coast have shown occurrence of 2418 heavily silicified hexactinellid stalks at densities of 0.18–0.33 m^{-2} (Beaulieu, 2001). Dense populations of heavily silicified demosponges and hexactinellids are also known from continental slopes of several North Atlantic locations (Rice *et al.*, 1990; e.g. Barthel *et al.*, 1996; Maldonado and Young, 1996), and many of these populations are reported to accumulate on the bottom large amounts of BSi skeletons released after sponge death (Bett and Rice, 1992; Barthel and Tendal, 1993). Siliceous spicules are also extremely abundant in both Arctic and Antarctic bottoms at diverse depths, where they form mats up to 2 m thick (Koltun, 1968; Dayton *et al.*, 1974; Van Wagoner *et al.*, 1989). Of particular interest to illustrate the issue of BSi retention by sponge skeletons below the photic layer are the epibathyal reefs of hexactinellid sponges in the Hecate Strait and the Strait of Georgia in British Columbia (Conway *et al.*, 2001; Krautter *et al.*, 2001). In these habitats, the abundance of living hexactinellids can reach 240 individuals in 10 m^2 (Leys *et al.*, 2004). Because discrete reefs can be up to 19 m high and 2–10 km in extension and they are discontinuously scattered over an area greater than 700 km^2 (Conway *et al.*, 2001; Krautter *et al.*, 2001; Leys *et al.*, 2004), it can easily be deduced that these sponge systems function as huge Si traps. Estimates of BSi content in only three discrete reefs reported values ranging from 7 to 11 kg m^{-2},

amounting to a total of 915 tons of BSi locked in only the exposed portion of reefs, which extended for about 0.047 km^2 (Chu *et al.*, 2011). Because discrete reefs are discontinuously scattered over an area greater than 700 km^2, it was deduced that glass sponge reefs equate to 65% of the DSi reservoir (3.6×10^9 mol Si) in that area and represent a substantial silicon sink in the continental shelf waters of the northeastern Pacific Ocean.

It is also worth stressing that the skeletal Si incorporated by most kinds of sponges will be locked in their bodies for their entire lifetime, a period which may extend from decades to millennia, in striking contrast with the retention period by the short-lived diatoms, which is only days.

4.4.4. Dissolution and burial of silicon through sponges

Because seawater is highly undersaturated with Si, it is predicted that any siliceous skeleton exposed to seawater will readily dissolve into silicic acid, following a thermodynamically favoured reaction. The rapid postmortem dissolution observed for diatom frustules (e.g. Bidle and Azam, 1999) clearly fits such a theoretical prediction. At the global scale, diatoms are estimated to produce yearly about 240×10^{12} mol of BSi in the world oceans (Tréguer *et al.*, 1995). Nevertheless, after a few days of planktonic life, diatoms die and their BSi skeletons dissolve, releasing DSi. Dissolution is so rapid that about 50% of the skeletal BSi produced yearly by diatoms dissolves back into DSi before sinking below the photic layer (~200 m). Dissolution continues once frustule fragments have reached the ocean bottom, so that it is estimated that only about 3% of the skeletal BSi produced yearly by diatoms is finally buried in the marine sediment and exported to the geological cycle. Because the features of diatom BSi favours a rapid turnover between DSi and BSi states, the idea of a rapid BSi–DSi re-cycling has traditionally been extrapolated to the functioning of the global Si cycle (Nelson *et al.*, 1995; Tréguer *et al.*, 1995; Sarmiento and Gruber, 2006). Nevertheless, several experimental approaches have revealed that dissolution dynamics of sponge BSi are far slower than that of diatom BSi. For instance, by exposing acid-cleaned frustules and a variety of sponge spicule to abiotic seawater for 8 months, Maldonado *et al.* (2005) demonstrated that frustules dissolve about 75%. In contrast, three types of sponge spicules resisted dissolution similarly (0–5%), despite having marked differences in surface area. Therefore, surface area by itself cannot account for the drastic differences in dissolution dynamics between frustules and spicules. Studies by Katamani (1971) and Chu *et al.* (2011) have consistently agreed that major differences in dissolution rates between diatom frustules and sponge spicules also occur when the solvent is either alkaline solutions or non-aseptic seawater.

The reasons for these large difference in the dissolution dynamics remain unclear. Katamani (1971) reported infrared absorption near 940 cm^{-1} by diatom frustules, an absorption peak that is missing in sponge spicules. This

peak indicates either water molecules trapped in the interstitial voids of the BSi polymeric network or hydroxyl groups in various states of association. Nevertheless, such minor differences, even if favouring dissolution of frustules relative to spicules, would be insufficient to explain the large differences detected in dissolution kinetics (Katamani, 1971). Substantial incorporation of fourfold-coordinated Al into the sponge BSi during silicification could be responsible for the very different dissolution kinetics. This element is known to drastically reduce dissolution rate of BSi skeletal remnants when it is incorporated in trace amounts from the sediment (Lewin, 1961; Dixit *et al.*, 2001). More recently, structural polysaccharides, such as chitin, have been shown to be incorporated into the concentric BSi layers of the sponge spicules (Ehrlich *et al.*, 2007), and it could be at least partially responsible for stabilizing the BSi matrix against dissolution. Chitin itself is naturally insoluble in water (Hock, 1940; Austin *et al.*, 1981) and enhances exoskeleton insolubility when imbedded into protein complexes of other marine invertebrates (Hunt, 1970; Weiner *et al.*, 1983).

Whatever the mechanism that sponges have developed to prevent passive dissolution of their spicules, such ability has resulted in multiple adaptive advantages. Spicule resistance to dissolution allow sponges to project large spicules out of the body forming "roots" for attachment, long stalks that elevate the sponge body for enhanced filter feeding, velvety surfaces that smooth water flow around the body, lacerating walls that deter predators, long palisades that prevent filtering surfaces from clogging, and minute hooks that capture microinvertebrates.

The resistance of sponge spicules to dissolution may also have major implications for Si cycling dynamics. According to the available data on spicule dissolution, it appears that BSi cycles into DSi through sponges over the timescale of decades to centuries, while that cycling takes only days to years when going through diatom frustules. The diatom-based model of the Si cycle postulates that 32–39% of the total burial of BSi is concentrated along continental margins owing to coastal diatom populations (Ragueneau *et al.*, 2000; DeMaster, 2002). Nevertheless, the persisting neglect of the impressive sponge populations characterizing continental shelves and upper slopes strongly suggests that global Si burial values may be underestimated. For instance, Rützler and MacIntyre (1978) reported that sponge spicules (and not diatom frustules) are the main component of particulate silica in Caribbean reef sediments. Likewise, sponge spicules have been shown to dominate the sediments of some Mediterranean coastal areas (Bavestrello *et al.*, 1996; Bertolino *et al.*, 2012). In Arctic and Antarctic bottoms at various depths, sponge spicules form mats up to 2 m thick (Koltun, 1968; Dayton *et al.*, 1974; Van Wagoner *et al.*, 1989). Dense populations of heavily silicified demosponges and hexactinellids are known from continental slopes of several North Atlantic locations (Rice *et al.*, 1990; Barthel *et al.*, 1996; Maldonado and Young, 1996), and many of these populations

are reported to accumulate on the bottom large amounts of BSi skeletons released after death of the sponges (Bett and Rice, 1992; Barthel and Tendal, 1993).

The available evidence suggests that the magnitude of Si burial through sponges at the global scale may be more important than traditionally thought, even if no global quantification can be provided currently because of insufficient knowledge of global patterns of sponge BSi content in sediments. Unfortunately, most on-going research efforts towards BSi quantification in sediments and burial rates are guided by the extended notion that DSi removal on continental margins derives from production and burial of BSi primarily by diatoms and radiolarians. In contrast, the recent findings relative to sponges support the idea that these organisms may account for puzzling gaps in the current Si cycle model, particularly missing Si sinks on continental margins. For instance, after the recent realization that early estimates of BSi accumulation in the Southern Ocean and Antarctic deep sea were about 35% overestimated (DeMaster, 2002), the widely accepted model of steady-state balance for the marine Si cycle became unbalanced, because a Si sink equivalent to approximately one quarter of the global BSi burial is now missing. Because in order to bring back the cycle into its assumed balance an additional BSi sink should be identified, it has been proposed that BSi accumulation by diatoms on continental margins should account for most of the "missing" BSi burial (DeMaster, 2002; Ragueneau *et al.*, 2010). Admittedly, reliable direct estimates of diatom Si retention on continental margins are still lacking (Ragueneau *et al.*, 2010). Current data on sponge DSi demands, along with those already available on BSi standing stocks in sponge populations, suggest that much of the "missing" BSi could correspond to BSi stocks in extant populations of long-live sponges and in skeletons being buried in continental margins.

4.5. The role of sponges in the ocean silicon cycle

The current model of the marine Si cycle is summarized in Fig. 3.6. In brief, it describes that about 2.4×10^{14} Si mol of DSi are removed yearly from the photic ocean layer. This consumption is exclusively attributed to Si uptake of diatoms, which use it to build their BSi frustules. The model also predicts that about 50% of the BSi produced in the photic layer rapidly dissolves, being returned to the photic ocean as DSi for subsequent diatom new BSi production. The remaining 50% of BSi is exported to the non-photic ocean. Therefore, in a steady-state Si cycle, it can be assumed that the diatom BSi standing stock is about 1.2×10^{14} mol Si. Approximately 75% (9×10^{13}) of the sinking BSi that escapes from the photic layer upon diatom death, dissolves as DSi during its slow gravity transit through the aphotic water column, with only about 25% of the sinking BSi reaching the bottom as particulate material (BSi rain). From the BSi arriving at the bottom (i.e.

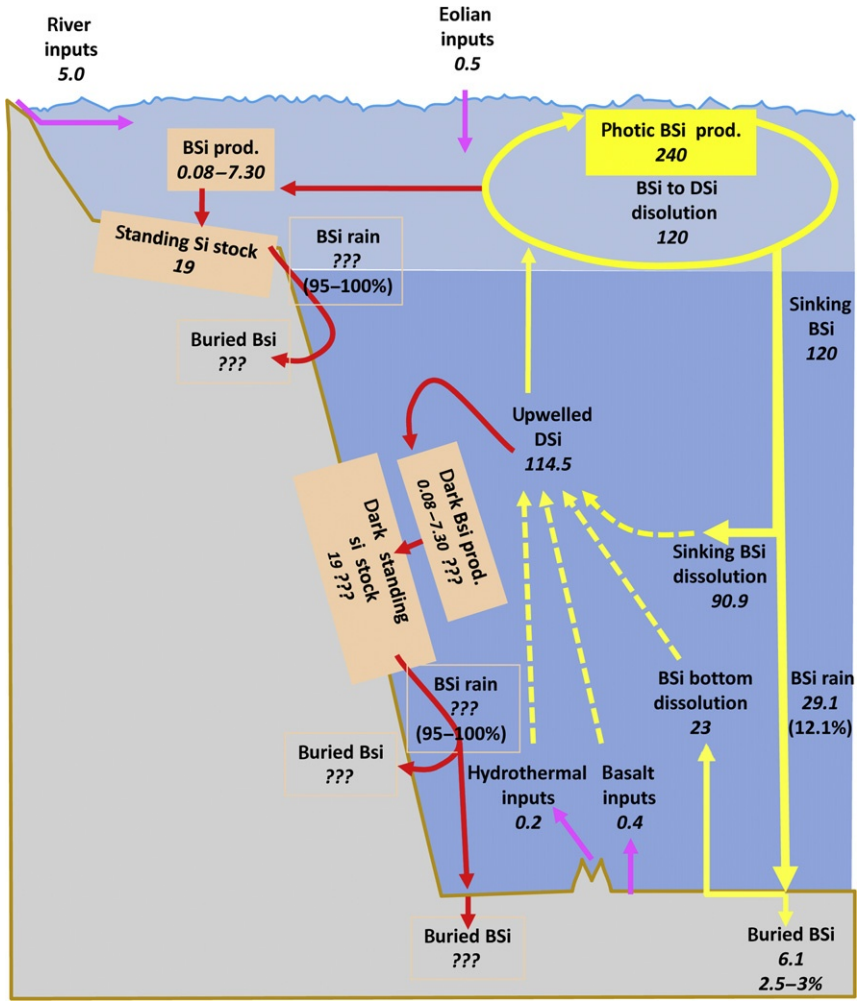

Figure 3.6 Global biogeochemical cycle of Si in the world ocean (as redrawn from Tréguer *et al.*, 1995), in which putative sponge contributions have been incorporated. Purple arrows refer to input Si fluxes to the ocean. Yellow arrows and boxes refer to diatom-mediated budgets and fluxes. Sponge budgets are given in orange boxes and putative fluxes referred by red arrows. Numbers in italics refer to budgets and fluxes calculated on a global yearly basis and expressed in mol Si $\times 10^{12}$. "Percentage values" for BSi rain and burial refer to total yearly BSi production estimated for either diatoms or sponges. Unknown or doubtful budgets and fluxes are indicated by a question mark (?). Note that most sponge-mediated processes concentrate around continental margins.

25% of sinking BSi or about 12% of the annual BSi production in the photic layers, i.e. 29.1×10^{12} mol Si), about four-fifths (i.e. about 10% of the BSi production in photic layers) dissolves and is returned as DSi to the deep-ocean water. Therefore, only about 3% (6.1×10^{12} mol Si) of the yearly BSi production in the photic ocean is finally buried in the sediments and exported to the geological cycle (Nelson *et al.*, 1995; Tréguer *et al.*, 1995; Sarmiento and Gruber, 2006). Because this model is largely based on diatom contribution, it assumes that: (1) DSi removal takes place exclusively in the photic ocean and deep-BSi production does not occur; (2) BSi rapidly cycles into DSi, because frustules dissolved readily when exposed to seawater after diatom death; and (3) only about 12% of the yearly BSi production arrives in particulate form to the marine sediments and only a small fraction (3%) is finally buried.

However, these cycle assumptions are not met when DSi is processed through sponges, for two major reasons: (1) Important populations of heavily silicified sponges occur at bathyal depths on continental margins (and to a lesser extent on abyssal plains), accounting for some DSi removal and BSi production in aphotic environments; (2) rates of sponge BSi return into DSi is predicted to be dramatically slower than that estimated for diatoms, because of greater sponge longevity and greater resistance of spicules to dissolution. It is likely that a large (> 3%) percentage of the yearly sponge BSi production becomes finally buried in the sediments. It could also be that a significant fraction of the Si burial attributed to diatom frustules indeed corresponds to sponge spicules in sediments, particularly on continental margins.

Figure 3.6 provides a summary of sponge-mediated Si fluxes that can tentatively be estimated under the current state of knowledge. The Si standing stock in siliceous sponges of the relatively sponge-poor rocky Mediterranean sublittoral averages 0.27 ± 0.94 mol Si m^{-2}, while it is 5.00 ± 45.07 mol Si m^{-2} in the sponge-reef Caribbean sublittoral (Maldonado *et al.*, 2010a, 2011). A preliminary tentative Si standing stock of about 19×10^{12} mol Si in sponges can be arrived at for earth continental shelves. This figure would be based on the tentative, conservative assumptions that (1) only about a third of the earth continental shelf area (22×10^6 km^2) harbours sponge populations and (2) abundances in half of the sponge-populated area are equivalent to those in the poor Mediterranean and, in the other half, equivalent to those in the Caribbean. Likewise, DSi demand by sponges on shelves has tentatively been estimated to fall somewhere from 0.08 to 7.30 Tmol Si (Maldonado *et al.*, 2011) and similar figures could be expected globally for sponges at bathyal and abyssal communities. Given that sponge BSi is highly reluctant to dissolution in seawater, nearly 95–100% of the BSi yearly released upon sponge death (whatever its magnitude), is assumed to reach the sediments. The rates of sponge BSi

burial remain unknown, but a large fraction of the spicules reaching the bottom is likely to be buried before experiencing significant dissolution (e.g. Bertolino *et al.*, 2012), except in particular, highly carbonated environments, such as coral reefs (e.g. Rützler and Macintyre, 1978).

Therefore, the role of sponges is likely to affect current estimates of several Si partial budgets in the global Si cycle model, namely:

(1) DSi demands on continental shelves are slightly larger than currently predicted.
(2) There is "dark DSi consumption" due to sponges growing on the aphotic regions of the continental margins, where no diatom BSi production occurs.
(3) Large amounts (nearly 100%) of produced BSi is delivered directly to the sediments (BSi rain) upon sponge death.
(4) Rates of Si burial on continental margins are likely greater than predicted. The magnitude of this burial could even challenge the idea that the marine Si cycle is at a perfect steady balance.

More importantly, the ecological importance of sponge DSi demands, BSi production, and Si burial does not derive strictly from the magnitude of these figures in the global cycle, but rather from the fact that these processes largely concentrate on continental margins. Therefore, if we are to understand adequately Si fluxes in some coastal regions, we may need a new conceptual Si model, involving three major Si pools: (1) Si in rocks and deep sediment layers that cycles on geological time scales; (2) Si in diatoms that cycles comparatively rapidly and is connected to primary production processes; and (3) Si in sponges that cycles apart from primary production and does so at a much slower (but still undetermined) rate as compared with that of diatoms.

It also deserves attention that the fact that all DSi used by sponges on continental shelves is at the expense of the stock available for diatoms. The DSi progressively accumulated into sponge BSi on continental shelves (and slopes) is taken away from the primary production circuit for a long period, given the longevity of most sponges and the low levels of dissolution characterizing sponge skeletons. By disregarding this sponge DSi-sequestering process while considering exclusively diatom-related DSi and BSi stocks, the current biogeochemical models may be overestimating the real levels of connection between Si and C cycles on continental margins.

5. Concluding Remarks

- Sponges are opportunistic suspension feeders that process rapidly large volumes of water and incorporate particulate and dissolved nutrients.

They are able to harvest carbon as POC, DOC, and inorganic carbon. They are also able to incorporate organic nitrogen and organic phosphorous through feeding on planktonic cells and detritus. Siliceous sponges (i.e. most Demospongiae and all Hexactinellida) are also able to incorporate inorganic silicon from its dissolve silicic acid form. While Si fluxes are exclusively related to the elaboration of their skeleton of biogenic silica, C, N, and P fluxes derive from a complex combination of metabolic processes that include feeding, respiration, egestion, excretion, as well as hosting of large microbial populations within the sponge body. Sponge-associated microorganisms mediate in most C and N fluxes. Such a metabolic integration complicates the assessment of C and N balances through the sponges, particularly in sponges hosting high microbial density. In the case of N fluxes, the situation may even be more complex, for aerobic and anaerobic microbial processes co-occurring in the same sponge. Because of these major differences between the metabolic pathways, through which each of the concerned elements (i.e. C, N, P, Si) is processed, the available information on the sponge-meditated fluxes is very different in each case. For C and Si, there have been attempts to establish individual balances to infer subsequently fluxes at the population or community level and further assess the role of sponges in element cycling at local, regional, or global scales. For N and P, the situation is more complicated, as most studies are still attempting to unravel the basic metabolic pathways, as well as understanding the basis of environmental and between-species variability in fluxes. Therefore, the available information does not allow scaling up to large ecological sceneries.

- The study of C flux clearly shows that sponges have a C metabolism that differs in many aspects from the typical known in other invertebrates. This probably results from sponges being simple and ancient multicellular organisms that are evolutionarily close to the colonial organization stage. Due to the action of associated microbes, it is not surprising that C ingestion rates are often exceeded by C respiration rates. The most likely explanation is that total carbon ingestion rates may have been underestimated somehow. DOM is now an acknowledged food source for sponges that have to be incorporated into C balances and diet studies. Sponge cells along with sponge-associated microorganisms have been reported to assimilate DOC. HMA sponges, however, appear to rely more on DOM as a carbon source than LMA sponges. Whether sponges consume DOM probably depends on the naturally available amounts of DOM versus picoplankton cells. Most of the carbon assimilated by sponges appears to be used for maintenance and is respired. Costs for maintenance are relatively high in sponges compared to the ingestion, leaving not much energy for net growth in size. Moreover, the costs of growth also appear to be higher than in other invertebrates. These are probably constrains maintaining sponge growth usually at slow rates,

despite the potential for growth being surprisingly high in these organisms. The impact that sponge populations have on their environment in terms of C flux remains to be accurately quantified. The qualitative effects of their activity are a net withdrawal of organic carbon from the passing water, accumulation of biomass, and excretion and biodeposition of detrital organic matter. Additionally, DOM consuming sponges may convert DOM into POM conserving energy within the system.

- Regarding N, the role of sponges is much related to the activity of their microbial associates. Microbes with the potential to mediate in each of the steps known in N cycling (i.e. nitrogen fixation, nitrification, denitrification, and anammox) have been molecularly identified in marine sponges. Nevertheless, there is a serious lack of studies quantifying the resulting N fluxes. Sponges, irrespective of being HMA or LMA, act as a source of NO_x^- (due to nitrification). HMA species appear to act consistently as a DON and NH_4^+ sink (potentially related to phototrophic activity and nitrification). Nevertheless, the role of HMA species in N_2 cycling is not that consistent, as they can be a N_2 sink (through nitrogen fixation) or a N_2 source (through denitrification or anammox). HMA sponges may theoretically compete with the phytoplankton and the bacterioplankton for limiting nutrients, such as DON and NH_4^+, while LMA species are more likely to release nutrients that are limiting to primary production. Nevertheless, preliminary assessments of these processes suggest that they contribute significantly neither to sponge nutrition nor to induce relevant changes in concentration of N compounds in the ambient water of the sponge habitats.
- The few available data on PO_4^{3-} fluxes indicate that marine sponges generate a net efflux of this nutrient, irrespective of their HMA or LMA condition. This suggests that regular heterotrophic feeding is providing the sponges with more than enough P to cover their metabolic needs. Despite phosphate being a limiting nutrient for phytoplankton in many local environments, the ecological relevance of the net P efflux by sponges remains uninvestigated.
- Regarding Si, the available information for shallow-water demosponges reveals that they incorporate Si following a Michaelis–Menten kinetic, a process that operates with maximum efficiency when ambient Si concentrations are about 1–2 orders of magnitude higher than natural availability. Consequently, all sponges investigated to date are strongly limited by Si ambient availability. This condition reveals a marked lack of adaptation of the sponge Si uptake system to the low ambient Si concentration characterizing modern oceans, a situation compensated for by other better-adapted Si consuming organisms, such as diatoms. The exact molecular and cellular pathway through which Silicon is transported to the inner of the silica-secreting cells for polycondensation remain poorly known, but it appears to use mechanisms different from those in diatoms

and other Si using organisms. The yearly Si consumption by sponges on continental shelves has tentatively been estimated somewhere between 8.6×10^{10} and 7.3×10^{12} mol Si year^{-1} and the accumulation of Si in their skeletons at 19×10^{12} mol Si. These figures may double when the contributions by sponge populations on continental slopes and deeper bottoms are incorporated into calculations. Therefore, sponges represent a modest, though non-negligible, contribution (probably around 15%) to the global marine Si budget. Because sponges may live for decades, centuries, and even millennia, and because the dissolution of their siliceous skeletons is extremely slowly, sponge populations on continental shelves and slopes function as transitory traps that slow down Si re-cycling around continental margins, favouring also a significant net export to the sediments that awaits quantification. The ecological importance of sponge Si consumption and net export does not derive strictly from the magnitude of their rates in global terms, but rather from the fact that the effects are accumulative and largely concentrated on continental margins. All Si incorporated by sponges on continental shelves and slopes is at the expense of the stock available for diatoms, being taken away from the primary production circuit for a long period. The lack of quantitative data on this sponge Si-sequestering process favours a conceptual biogeochemical model overestimating the real levels of connection between Si and C cycles on continental margins.

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