Title:

Dissolved organic carbon (DOC) is essential to balance the metabolic demands of North-Atlantic deep-sea sponges

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Abstract

Sponges are ubiquitous components of various deep-sea habitats, including cold water coral reefs and deep-sea sponge grounds. Despite being surrounded by oligotrophic waters, these ecosystems are known to be hotspots of biodiversity and carbon cycling. To assess the role of sponges in the carbon cycling of deep-sea ecosystems, we studied the energy budgets of six dominant deep-sea sponges (the hexactinellid species *Vazella pourtalesi*, and demosponge species *Geodia barretti, Geodia atlantica*, *Craniella zetlandica, Hymedesmia paupertas* and *Acantheurypon spinispinosum)* in an ex situ aquarium setup. Additionally, we determined morphological metrics for all species (volume, dry weight (DW), wet weight (WW), carbon (C) content, and ash-free dry weight (AFDW)) and provide species-specific conversion factors. Oxygen (O_2) removal rates averaged 3.3 ± 2.8 μmol O_2 DW_{sponge} h^{-1} (all values mean ± SD), live particulate (bacterial and phytoplankton) organic carbon (LPOC) removal rates averaged 0.30 ± 0.39 μmol C DW_{sponge} h^{-1} and dissolved organic carbon (DOC) removal rates averaged 18.70 ± 25.02 μmol C DW_{sponge} h^{-1}. Carbon mass balances were calculated for four species (*V. pourtalesi, G. barretti, G. atlantica* and *H. paupertas*) and revealed that the sponges acquired 1.3–6.6 times the amount of carbon needed to sustain their minimal respiratory demands. These results indicate that irrespective of taxonomic class, growth form, and abundance of microbial symbionts, DOC is responsible for over 90 % of the total net organic carbon removal of deep-sea sponges and allows them to sustain in otherwise food-limited environments on the ocean floor.

Introduction

The oceanic seafloor constitutes by far the largest part of Earth's surface area, covering an area of 361 million km^2, of which over 90% is found at water depths greater than 150 m (Costello et al. 2010; Ramirez-Llodra et al. 2010). However, still only a minute fraction of the deep-sea surface has been properly mapped (Mayer et al. 2018), let alone characterized in terms of biodiversity and ecology. Nevertheless, since 1848, 28 new habitats have been discovered in the deep-sea (Ramirez-Llodra et al. 2010). In the past few decades, sponges have been revealed to be ubiquitous inhabitants of many of these habitats, generally in water depths between, but not restricted to, 200–2,000 m (reviewed by Hogg et al. 2010). On the northern Atlantic continental shelf, sponges abundantly inhabit deep-sea coral reefs, form large mono-specific sponge grounds, and create sponge reefs by depositing thick spicule mats (i.e. layers of skeletal needles derived from dead and damaged sponges) (Thomson 1873; Klitgaard & Tendal 2004; Buhl-Mortensen et al. 2010; Beazley et al. 2015). In some areas, sponges can comprise up to 98 % of the total benthic biomass and sponge abundance amounts up to 24 individuals per m^2 (OSPAR Commission 2010). Deep-sea sponges are found to fulfill important ecological roles by providing habitat complexity and substrate to both mobile and sessile fauna (Klitgaard, 1995; Beazley et al. 2013; Hawkes et al. 2019). Moreover, the first estimations on respiration and organic carbon (C) uptake of deep-sea sponges (e.g., Pile & Young 2006; Yahel et al. 2007; Kahn et al. 2015) suggest that they play a crucial role in benthic-pelagic coupling.

However, due to technical restrictions inherent to deep-sea work (e.g., costly ship-based expeditions, sampling under extreme conditions), data on the ecology and physiology of deep-sea sponges is still scarce, and mostly based on specimens caught with dredges and trawls. In recent years, the increased use of remotely operated vehicles (ROVs) has provided

more opportunities to do measurements at the seafloor, and to bring up specimens from depth for laboratory experiments. The few available studies on deep-sea sponge physiology consists of a mix of in situ and ex situ studies using different direct (taking in- and out-flow water samples (Pile & Young 2006; Yahel et al. 2007; Leys et al. 2018)) and indirect (using flume experiments (Witte et al. 1997), or incubation chambers (Kutti et al. 2013, 2015; Rix et al. 2016)) methodologies. Still, metabolic rates of deep-sea sponges are only available for a limited number of species, often incomplete, and not reflecting the diversity and wide array of morphological traits found in sponges in deep-sea habitats.

Deep-sea sponges mainly belong to two classes: demosponges (Demospongiae) and glass sponges (Hexactinellidae) (Lancaster et al. 2014). Demosponges come in a wide variety of shapes and sizes, ranging from mm-thin encrusting sheets to m-wide barrels, and consist of layers of specialized cells (Simpson 1984). They occur in freshwater and marine ecosystems and their skeleton can consist of siliceous, calcium carbonate, or collagenous components (Müller et al. 2006; Ehrlich et al. 2010; Bart et al. 2019). Hexactinellids are exclusively marine, tubular, cup-, or vase-shaped, and predominantly inhabit deep-sea habitats (Schulze 1887; Mackie & Singla 1983; Leys 2007). In contrast to demosponges, their cellular structure is principally composed of massive multinucleate syncytia and their skeleton always consists of silica spicules (Bidder 1929; Mackie & Singla 1983; Leys 1999; Müller et al. 2006). Depending on the quantity and composition of associated microbes in their tissues, sponges can be further classified as having either low microbial abundances (LMA) or high microbial abundances (HMA) (Hentschel et al. 2003; Weisz et al. 2008). LMA sponges contain microbial abundances and sizes comparable to ambient seawater (~ 0.5–1 x 10^6 cells mL^{-1}), while HMA sponges can contain up to four orders of magnitude more (and generally much larger) microbes (Vacelet & Donadey 1977; Reiswig 1981; Hentschel et al. 2003). These symbionts are involved in various processes, such as C and nitrogen (N) metabolism,

synthesis of vitamins, chemical defense and horizontal gene transfer (reviewed by Pita et al. 2018).

Sponges, including deep-sea species, are well-established filter feeders, efficiently capturing and processing nano- and picoplankton (reviewed by Maldonado et al. 2012). More recently, it has been shown that many shallow-water sponges primarily rely on dissolved organic matter (DOM) as food source (reviewed by de Goeij et al. 2017). DOM, often measured in the form dissolved organic carbon (DOC), is the largest potential food source in the oceans (Hansell et al. 2009). Yet, direct evidence of DOM uptake by deep-sea sponges is still not available at present. For some species DOM uptake has been suggested (Leys et al. 2018), for others it was not found (Yahel et al. 2007; Kahn et al. 2015). However, these studies did not directly measure DOC, but derived the dissolved organic carbon fraction from the total organic carbon fraction. Direct DOC measurements are challenging, as they are performed almost within detection limits of current analytical systems. Therefore, an important question is: can deep-sea sponges utilize DOM as a food source?

Both body shape and microbial abundance are suggested to affect the capability of sponges to utilize dissolved food sources. For example, it is hypothesized that the high surface-to-volume ratio of flat, encrusting sponges is advantageous for the uptake of DOM compared to lower surface-to-volume ratio of erect, massive (e.g., ball, cylinder) growth forms (Abelson et al. 1993; de Goeij et al. 2017). Higher DOM uptake is also predicted for HMA sponges in comparison with LMA sponges, as microbes are considered to play an essential role in the processing of DOM (Reiswig 1974; Freeman & Thacker 2011; Maldonado et al. 2012; Hoer et al. 2018). However, this distinction is not always clear, as the diet of some LMA sponges also consists mainly of DOM (e.g., de Goeij et al. 2008; Mueller et al. 2014), particularly when they do not have massive growth forms (reviewed by de Goeij et al. 2017).

To quantify the metabolic- and carbon removal rates of deep-sea sponges, three aspects need to be investigated. Firstly, a wider variety of sponge biodiversity (e.g., different taxonomic classes, morphological shapes, and abundances of associated microbes) needs to be included in physiological studies. Despite the high sponge diversity present in the deep-sea, most studies currently available focused on a single sponge class or species. Secondly, the potential role of DOM in the diet of deep-sea sponges needs to be assessed. Thirdly, sponge metabolic rates are currently normalized to a variety of metrics (e.g., dry weight, wet weight, volume, or organic carbon content), which affects general ecological interpretations and that makes it difficult to compare results between studies. Therefore, the use of different standardization metrics should be addressed.

In this study, we investigated the oxygen and organic carbon removal rates of six dominant North-Atlantic deep-sea sponges with different morphological traits (three massive HMA demosponges, two LMA encrusting demosponges, and one massive LMA hexactinellid) from two different habitat types (mono-specific sponge grounds and multi-specific sponge assemblages associated with cold-water coral reefs), and composed carbon budgets. Specifically, we studied the removal rates of live particulate organic carbon (LPOC; i.e. bacterio- and phytoplankton), DOC, and dissolved oxygen (O_2) of the investigated species, using ex situ incubation experiments. We further determined different morphological metrics for the six targeted species (volume, DW, WW, carbon content, and AFDW) and provide species-specific conversion factors.

Materials & Methods

Study areas, sponge collection and maintenance

We investigated the following dominant North-Atlantic deep-sea sponge species (Fig. 1 and supplementary Table S1): *Vazella pourtalesi* (Hexactinellida; LMA; massive vase), *Geodia barretti* (Demospongiae; HMA; massive, globular), *Geodia atlantica* (Demospongiae; HMA; massive, bowl), *Craniella zetlandica* (Demospongiae; HMA; massive, globular), *Hymedesmia paupertas* (Demospongiae; LMA; encrusting, sheet) and *Acantheurypon spinispinosum* (Demospongiae; LMA; encrusting, sheet). Sponge specimens were collected by ROV during four research cruises in 2016, 2017 (two cruises), and 2018 (Fig. 2). *V. pourtalesi* specimens were collected in August, 2016, attached to their rocky substrate at ~300 m depth, during the Hudson cruise 2016-019 (Kenchington et al. 2017) at the Emerald Basin on the Scotian Shelf, Canada (44°19'8.73"N 62°36'18.49"W). Sponges were kept in the dark in a 1000-L holding tank and transported without air exposure to the Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada. In the lab, sponges were kept in the dark in a 1000-L flow-through holding tank, through which sand-filtered seawater from the adjacent Bedford Basin was continuously pumped at 7 L h^{-1}. A chiller was used to maintain water temperature at 8 °C. *C. zetlandica* specimens were collected during the Kristine Bonnevie cruise 2017610 (April 2017) at 60°42'12.5"N 4°39'09.9"E in the province of Hordaland, Norway, and kept in 14-L onboard flow-through aquaria with seawater pumped through at 120 L h^{-1}. Temperature was maintained at 8 °C. *G. atlantica* and *A. spinispinosum* specimens were collected attached to their rocky substrate during the G.O. Sars cruise 2017110 (August 2017) at the Sula reef (64°42'25.2"N 7°59'24.0"E) of the Northern Norwegian coast at depths of 250–400 m. *G. barretti* specimens were collected during the same cruise at the Barents Sea (70°47'20.8"N 18°03'47.2"E) at a depth of 272 m. The latter three sponge species were kept

172 on board the research vessel in the dark in 20-L flow-through tanks in a climate room at 6 °C.
173 North-Atlantic seawater was pumped in from a depth of 6 m at 30 L h^{-1}. *H. paupertas*
174 specimens were collected attached to rocky substrate during the G.O. Sars cruise
175 2018108(cruise code, August 2018) in the Barents Sea at 70°47'13.9"N 18°03'23.8"E. These
176 sponges were kept on board the research vessel under similar conditions as during the
177 previous year. Ex situ experiments with *G. atlantica*, *A. spinispinosum* and *H. paupertas*
178 specimens were performed on board the ship. *G. barretti* and *C. zetlandica* specimens were
179 transported without exposing them to air to the laboratory facilities at the University of
180 Bergen, Norway, where the experiments took place. In Bergen, sponges were kept in a dark
181 climate room (8 °C) in multiple 20-L flow-through aquarium systems. Each holding tank
182 contained a maximum of five sponges. Flow originated from unfiltered water pumped from
183 200 m depth from the outer fjord near Bergen at ~ 50 L h^{-1} with a temperature ranging from
184 6–8 °C. All sponges and substrate were cleared from epibionts prior to incubations.
185
186 **Incubations, sample treatment, and analysis**
187 All sponges were allowed to acclimatize for a minimum of 1 week prior to the incubation
188 experiments. Individual sponges were enclosed in flow chambers (either 2, 3, or 6 L
189 depending on sponge biomass) with magnetic stirring devices to ensure proper mixing (de
190 Goeij et al. 2013). Chambers were acid-washed (0.4 mol L^{-1} HCl) prior to the incubations and
191 kept in a water bath to maintain a constant seawater temperature during the incubations (6–9
192 °C depending on the incubation). Chambers were closed without trapping air in the system.
193 The length of every individual incubation was determined during test incubations based on
194 sponge size and oxygen consumption (ideally timed to about > 10 % to < 40 % [O_2] decrease,
195 and ranged from 2–8 h. At set time intervals depending on the incubation length (t_{sample} = 0,
196 30, 60, 90 120, 180, 240, 360 or 480 min), 85–100 mL water samples were taken with acid-

197 washed 100-mL polycarbonate syringes. Sample water volume was replaced aquarium water
198 (drawn in by suction to maintain volume and eliminate air exposure), and calculations were
199 adjusted to take these replacements into account during the analysis. Samples were
200 subdivided to analyze the concentrations of DOC and abundances of bacterio- and
201 phytoplankton. For 2 species, *C. zetlandica* and *H. paupertas*, no DOC samples were
202 analyzed, due to logistical issues.

203 Dissolved oxygen concentrations (O_2) were continuously measured during the
204 incubations with OXY-4 mini optical oxygen sensors (*PreSens*, Germany). Sensors do not
205 consume oxygen and due to their small dimensions (Ø 2 mm), flow and mass-transport inside
206 the chambers are not disturbed. O_2 concentrations were recorded every 15 s (*OXY-4-v2_30FB*
207 *software*).

208 Prior to DOC sampling, syringes, glassware and pipette tips were rinsed three times
209 with acid (8 mL 0.4 mol L^{-1} HCl), three times with milli-Q (80 mL), and twice with sample
210 water (10 mL). 20 mL of sample water was filtered (< 20 kPa Hg suction pressure) over pre-
211 combusted (4 h at 450 °C) GF/F glass microfiber (~ 0.7 μm pore-size) filter and collected in
212 pre-combusted (4 h at 450 °C) amber glass EPA vials (40 mL). Samples were acidified with 6
213 drops of concentrated HCl (12 mol L^{-1}) to remove inorganic C, and stored in the dark at 4 °C
214 until analysis. DOC concentrations were analyzed using a total organic C analyzer and
215 applying the high-temperature catalytic oxidation method (TOC-VCSH; Shimadzu) modified
216 from Sugimura and Suzuki (1988). Every 8–10 d the instrument was calibrated by measuring
217 standard solutions of 0, 42, 83, 125, 208 and 417 μmol C L^{-1}, prepared from a potassium
218 hydrogen phthalate standard (Merck 109017). Every measurement day, ultrapure (MilliQ)
219 water was used to determine the instrument blank (< 1 μmol C L^{-1}). On every measurement
220 day TOC analysis was validated with deep seawater reference (DSR) material provided by
221 the Consensus Reference Materials Project of RSMAS (University of Miami) yielding values

within the certified range of 42–45 μmol C L^{-1}. Additionally, two internal standards were prepared each measurement day using a potassium hydrogen phthalate (Merck 109017) with DOC concentration within the samples range. DOC of each sample was determined from 5–8 injections. The precision was < 4 % estimated as the standard deviation of replicate measurements divided by the mean.

Duplicate 1 mL samples for bacterioplankton and phytoplankton were fixed at a final concentration of 0.5 % glutaraldehyde for 15–30 min at 4 °C in the dark. After fixation, the samples were snap-frozen in liquid nitrogen and stored at -80 °C until further analysis. Thawed samples were analyzed using a BD-FACSCalibur flow cytometer (*Becton Dickinson*, San Jose, Calif.) with a 15 mW air-cooled argon laser (Brussaard et al. 2004). Phytoplankton were enumerated for 10 min at 80 uL min^{-1} with the trigger on Chlorophyll a, red autofluorescence (Marie et al. 1999). Phycoerythrin containing cells (e.g. cyanobacterial *Synechococcus*) were discriminated by their orange autofluorescence. Bacterial samples were diluted in sterile TE-buffer, pH 8.0 (10 mmol L^{-1} Tris, *Roche Diagnostics*; 1 mmol L^{-1} EDTA, *Sigma-Aldrich*) to avoid electronic coincidence, and stained with nucleic acid-specific SYBR Green I to a final concentration of 1 x 10^{-4} of the commercial stock (Marie et al. 1999; Brussaard et al. 2004). Samples were corrected for blanks (TE-buffer with SYBR Green I) prepared and analyzed in a similar manner as the samples. Bacterial samples were incubated in the dark for 15 min at room temperature after which samples were allowed to cool down at room temperature. Samples were analyzed for 1 min at 40 μL min^{-1}. Listmode files were analyzed using CYTOWIN freeware (Vaulot et al. 1989).

Sponge metrics

After the incubations, sponges were removed from their substrate and analyzed for volume (by water replacement) and (dripping) wet weight (WW). Then, all sponges were dried for 72

h in a drying oven at 60 °C to determine dry weight (DW). Randomly selected 1-cm^3 cubes (*n* = 6) of each massive sponge were transferred into a pre-weighed crucible and combusted at 450 °C in a muffle furnace (4 h). Combusted samples were cooled to room temperature in a desiccator and weighed (ash weight). Subsequently, ash-free dry weight (AFDW) was calculated by subtracting ash weight from DW and normalized to total volume of the original sponge specimen. The rest of the dried sponges was crushed and ground up with mortar and pestle and stored in a desiccator until further analysis.

Samples for organic C content analysis were decalcified with 4 mol L^{-1} HCl to ensure removal of inorganic C and subsequently lyophilized for 24 h in a *FD5515 Ilchin Biobase* freeze-drier. After freeze-drying, aliquots of approximately 10 mg were placed in tin-capsules and analyzed on an Elemental Analyser (*Elementar Isotope cube, (Elementar GmbH,* Langenselbold, Germany) coupled to a BioVision isotope ratio mass spectrometer *(Elementar ltd*, Manchester, UK).

Oxygen and carbon removal rates

To calculate changes in O_2 concentrations over time, a linear regression analysis was performed for each individual incubation. Resulting net O_2 removal rates were subsequently compared between sponge and seawater control incubations with a Welch's *t*-test for each species and a respective set of seawater controls.

Initial net live bacterio- and phytoplankton removal rates were calculated assuming exponential clearance of cells in incubations (Scheffers et al. 2004; de Goeij et al. 2008). The live plankton fraction was dominated by two general cell types, heterotrophic bacteria and phytoplankton, the latter represented by *Synechococcus*-like cyanobacteria. To calculate net removal rates for each plankton component, the average initial cell concentrations of all incubations were used as a starting point. Standardized data were fitted to an inverse

exponential model to calculate final cell concentrations. Final concentrations were subtracted from the initial corrected concentrations and differences were compared between treatments (sponge versus control incubations) using an unpaired t-test. Clearance rates (CR) were calculated according to Riisgård et al., 1993:

$$CR = V_w/t * ln(C_0/C_t)$$

V_w: water volume in incubation chamber (in mL)

t: duration of incubation (in min)

C_0: initial cell (bacterial or phytoplankton) concentration (in μmol C mL^{-1})

C_t: cell concentration at time point t (in cells mL^{-1})

A conservative estimate of live particulate organic carbon (LPOC) removal was obtained using established conversion factors. Heterotrophic bacterial cells were converted using 30 fg C per bacterial cell (Fukuda et al. 1998; Leys et al. 2018) and phytoplankton using 470 fg C per *Synechococcus*-type cell (Bertilsson et al. 2003; Pile & Young 2006).

Initial net DOC removal rates were calculated by applying a 2G-model (de Goeij & van Duyl 2007; de Goeij et al. 2008). This is a simplified model to describe changes in DOC concentration over time, assuming that the complex and heterogeneous DOC pool comprises two major fractions: a fast- (C_f) and slow-removable (C_s) fraction, for labile and refractory components of DOM, respectively (de Goeij et al. 2008). In an assumed well-mixed system, the fast and slow removal fractions of DOC will be consumed according to their specific removal rate constants k_f and k_s, respectively. The sum of the individual removal rates is used here to describe total DOC removal.

$$\frac{dDOC}{dt} = -(k_f C_f + k_s C_f)$$

Integrating this equation yields the function that describes the concentration of DOC as a function of time:

$$DOC(t) = C_{f,0} * e^{-kft} + C_{s,0} * e^{-kst}$$

296 Experimental data is described with the model by estimating model variables C_f and C_s using
297 a (10,000 iterations) minimalization routine (de Goeij et al. 2008). Initial DOC removal rate
298 (the flux on t = 0) was calculated from the estimated values of these variables and is given by

$$Flux_{DOC} = -(k_f C_{f,0} + k_s C_{s,0})$$

299 All fluxes of O_2 and C in μmol per different metric (sponge V, WW, DW, AFDW, or C
300 content) were corrected for controls and incubation volume.
301

302 **Carbon mass balance**

303 Total net organic carbon removal rates were estimated as the sum of net LPOC and DOC
304 removal rates. O_2 removal served as a proxy for respiration assuming a balanced molar ratio
305 of carbon respiration to net O_2 removal (1 mol C respired equals 1 mol O_2 removed), yielding
306 a respiratory quotient (RQ) of 1 (Hill et al. 2004; Yahel et al. 2003).

$$RQ = \frac{moles\ of\ C\ respired\ per\ unit\ time}{moles\ of\ O_2\ consumed\ per\ unit\ time} = 1$$

307 To establish a mass balance for the different deep-sea sponge species, the quotient
308 $\Delta O_2/\Delta TOC$ was calculated using the exponential removal rates. Carbon budgets where only
309 calculated for sponges of which we had complete sets of O_2, LPOC and DOC data (*Vazella*
310 *pourtalesi* (*n* = 4), *Geodia barretti* (*n* = 3), *Geodia atlantica* (*n* = 4), and *Acantheurypon*
311 *spinispinosum* (*n* = 3).
312

Results

Sponge metrics

Sponge characteristics (phylogeny, growth form, abundance or associated microbes) and metric conversion factors are given in Table 1. Average sponge metrics (planar surface area, volume, WW, DW, AFDW, %C) are shown in Table S1. Encrusting sponges have a one- to two-orders of magnitude higher planar surface area to volume ratio (4.2–10.0) than massive sponges (0.2–0.3) and an order-of-magnitude higher volume to dry weight ratio (21.4–22.4 and 3.3–7.3, respectively). HMA sponges show a significantly higher organic C content than LMA sponges (t = -8.13, df = 27, $p < 0.001$), Table 1), with lowest values for the hexactinelid *V. pourtalesi*.

Oxygen removal rates

The concentration of O_2 in the incubation chambers linearly decreased with time for *V. pourtalesi* (t = 4.59 , df = 7, $p < 0.01$), *G. barretti* (t = 3.69, df = 11, $p < 0.01$), *G. atlantica* (t = 5.11 , df = 5, $p < 0.01$), *C. zetlandica* (t = 3.5, df = 3, $p < 0.05$), *H. paupertas* (t = 4.38, df = 2, $p < 0.05$) and *A. spinispinosum*, (t = 7.96, df = 5, $p < 0.001$) compared to seawater control incubations. Average O_2 removal rates per species are depicted in Table 2. Examples of O_2 concentration profiles during incubations for all species and controls are shown in figure S1. O_2 removal rates for all sponges averaged 3.3. ± 2.8 μmol O_2 DW_{sponge} h^{-1} (mean ± SD throughout text unless stated otherwise), ranging from 1.0 (*C. zetlandica*) to 7.8 (*A. spinispinosum*).

LPOC removal rates

337 Bacterioplankton concentrations decreased in incubations with *G. barretti* (t = 2.44, df = 19,
338 $p < 0.05$), *V. pourtalesi* (t = 5.91, df = 9, $p < 0.001$), *G. atlantica* (t = 6.62, df = 5, $p < 0.01$),
339 *H. paupertas* (t = 2.81, df = 4, $p < 0.05$) and *C. zetlandica* (t = 4.25, df = 8, $p < 0.01$)
340 compared to seawater control incubations (Fig. 3A–E). Incubations with *A. spinispinosum*
341 showed no significant decrease in bacterioplankton compared to control incubations (t =
342 -0.72, df = 4, p = 0.51) (Fig. 3F). Average bacterial C removal and clearance rates (CR) per
343 species are presented in Table 3. Bacterial C removal rates averaged 0.25 ± 0.35 μmol C
344 DW_{sponge} h^{-1} for all species, ranging between 0.00 (*A. spinispinosum*) and 0.82 (*V. pourtalesi*)
345 (Table 3). Bacterial CRs averaged 0.69 ± 1.06 mL mL^{-1} min^{-1} for all species, ranging from
346 0.00 (*A. spinispinosum*) to 2.22 (*V. pourtalesi*).
347 Compared to control incubations, phytoplankton (i.e. S*ynechococcus*-type
348 cyanobacteria) concentrations decreased in incubations with *V. pourtalesi* (t = 5.34, df = 9 , p
349 < 0.001), *G. barretti* (t = 2.20, df = 11, $p < 0.05$) and *G. atlantica* (t = 11.92, df = 6, $p <$
350 0.001). Incubations with *C. zetlandica* (t = 1.23 , df = 3, p = 0.31) and *A. spinispinosum* (t =
351 1.56, df = 7, p = 0.16) showed no significant decrease compared to seawater control
352 incubations. Average phytoplankton C fluxes per species are presented in Table 3.
353 Phytoplankton C removal rates averaged 0.04 ± 0.07 μmol C DW_{sponge} h^{-1} for all species,
354 ranging from 0.00 (*A. spinispinosum/C. zetlandica*) to 0.15 (*V. pourtalesi*) (Table 3).
355 Phytoplankton CRs averaged 0.54 ± 0.96 mL mL^{-1} min^{-1} for all species, ranging between
356 0.00 (*A. spinispinosum*) and 1.77 (*V. pourtalesi*).
357 Combined plankton removal rates amounted to total LPOC uptake rates of, on
358 average, 0.30 ± 0.39 μmol C DW_{sponge} h^{-1}, ranging from 0.00 (*A.* spinispinosum) to 0.97 (*V.*
359 *pourtalesi*).
360
361 **DOC removal rates**

DOC concentration for incubations with four different species: *V. pourtalesi* (n = 4), *G. barretti* (n = 3), *G. atlantica* (n = 4), and *A. spinispinosum* (n = 3) fitted the 2G model and thereby showed significant removal of DOC (Fig. 4 and Supplementary Fig. S2). Unfortunately, some time-series could not be analyzed due to technical difficulties. No DOC removal occurred in the seawater controls. DOC removal rates averaged 18.70 ± 25.02 μmol C DW_{sponge} h^{-1} for all sponges, ranging from 3.70 (*G.* barretti) to 56.07 (*A. spinispinosum*) (Table 3).

Carbon mass balance

Mass balances, constructed for the four species where both LPOC and DOC were measured, showed that more than 90% of the average net TOC removal was accounted for by DOC (*V. pourtalesi* 92.0 ± 5.5 %, *G. barretti* 99.5 ± 0.5 %, *G. atlantica* 93.6 ± 8.4 %, *A. spinospinosum* 100 %) (Table 4). All species except *A. spinospinosum* also removed LPOC from the water, yet this organic carbon source accounted for less than 10 % of the net total TOC removal. Assuming a RQ of 1 in combination with exponential removal of LPOD and DOC during the incubations, we find all four species can match their minimal required carbon uptake ($\Delta O_2/\Delta TOC$ of 1.0 or lower), but only when DOC is included in the mass balance. Both HMA species show higher $\Delta O_2/\Delta TOC$ values than the two LMA species.

Discussion

This is the first study that combines measured uptake rates of dissolved oxygen (O_2), dissolved organic carbon (DOC) and live planktonic particulate organic carbon (LPOC) by multiple deep-sea sponges. We found that for the four investigated species where both LPOC and DOC were measured, DOC accounted for 92–100 % of the total organic carbon (TOC) uptake. Only when DOC is included as organic carbon source, these deep-sea sponges surpass their minimal respiratory carbon demands. Furthermore, metabolic rates, morphometrics, and conversion factors for six dominant North-Atlantic deep-sea sponge species are presented.

Oxygen and carbon fluxes

O_2 removal rates per DW of deep-sea sponges show consistency throughout literature, as most are roughly within the same order of magnitude (equal or less than factor 10 difference; Table 2). For *G. barretti*, O_2 removal rates found in this study are closely comparable with other rates reported, regardless of the method used (Kutti et al. 2013; Leys et al. 2018). When comparing respiration rates of deep-sea sponges to those reported for temperate (e.g., Thomassen & Riisgard 1995; Coma et al. 2002) and tropical sponges (e.g., Reiswig 1974; Yahel et al. 2003), rates of deep-sea sponges are consistently one to two orders-of-magnitude lower. Correspondingly, POC and DOC removal rates of deep-sea sponges are lower than those found for tropical and temperate species (e.g., de Goeij et al. 2008; Hoer et al. 2018). As depicted in figure 5, differences in O_2 and POC removal rates can be explained by the positive effect of temperature on metabolism and physiological processes (see also Clarke and Fraser 2004). DOC removal rates seem to follow a similar trend, yet due to the very limited amount of data available, the relation with temperature was not found to be

405 significant. Remarkably, O_2, POC and (specifically) DOC fluxes appear to be higher for
406 encrusting sponges compared to massive growth forms (Fig. 5). For example, the deep-sea
407 encrusting sponge *A. spinispinosum* has an order-of-magnitude higher DOC flux than
408 massive deep-sea species (56.1 μmol C h^{-1} g DW^{-1} versus 3.7–9.2 μmol C h^{-1} g DW^{-1}), as is
409 the case for encrusting tropical species (218.3–253.3 μmol C h^{-1} g DW^{-1}, de Goeij et al. 2008)
410 compared to massive tropical species (10.0–11.9 μmol C h^{-1} g DW^{-1}, Yahel et al. 2003, Hoer
411 et al. 2018). This corroborates earlier suggestions that high surface-to-volume ratios enable
412 encrusting sponges to have higher retention and removal efficiencies compared to massive
413 species (Abelson 1993; Kötter 2003; de Goeij et al. 2017).

414 In addition to morphology, higher net DOC removal rates are generally predicted for
415 HMA sponges in comparison with LMA sponges, as microbes are considered to play an
416 important role in the processing of DOM (Reiswig 1974; Freeman & Thacker 2011;
417 Maldonado et al. 2012; Hoer et al. 2018). However, both LMA species used in this study, *A.*
418 *spinispinosum* and *V. pourtalesi*, showed high uptake rates of DOC (56.1 and 9.2 μmol C h^{-1}
419 g DW^{-1}, respectively), despite their different growth forms (encrusting versus massive) and
420 different phylogeny (demosponge versus hexactinellid). Interestingly, other hexactinellids
421 were previously not found to consume DOM (Yahel et al. 2007), where in this study *V.*
422 *pourtalesi* shows the second highest DOC removal rates and DOC accounts for 90 % of its
423 TOC uptake. These difference could imply species-too-species differences or geographical
424 differences in organic carbon availability. However, Yahel et al. (2007) did not directly
425 measure DOC, but derived it from TOC analysis, potentially resulting in an underestimation
426 of actual DOC removal rates. Our results thereby add to the increasing body of evidence that
427 also sponges with low microbial abundances are capable of consuming DOC (de Goeij et al.
428 2013; Rix et al. 2016, 2017; Morganti et al. 2017).
429

430 **Interpretation of sponge metabolic rates**

431 Our understanding and interpretation of metabolic rates at both organism and ecosystem scale
432 is currently hampered by two issues. Firstly, a general lack of available oxygen and
433 (predominantly dissolved organic) carbon fluxes of deep-sea species, but also for marine
434 sponges in general (discussed in de Goeij et al. 2017). Specifically, no data are available on
435 encrusting HMA species, which raises the question whether the high fluxes of encrusting
436 LMA sponges are due to their encrusting morphology or due to their limited abundances of
437 symbionts. Secondly, the use of a multitude of metrics (e.g., m^{-2}, WW, DW, AFDW) in
438 combination with a lack of conversion factors, makes it almost impossible to compare
439 metabolic rates between different sponge species. Yet, this is crucial to upscale fluxes from
440 organism to ecosystem level in order to elucidate the role of marine sponges in
441 biogeochemical cycles and overall ecosystem functioning. But, which metric should be used?
442 In general, this may depend on the context and the research question at hand. For example,
443 when extrapolating individual fluxes to the ecosystem level, planar surface area is potentially
444 the most practical standardization metric in use (read: fast and low-cost). Abundance data in
445 deep-sea —but also in shallow-water— habitats are usually collected via 2D video surveys or
446 photo quadrants using ROV's (van Soest 2007; Roberts et al. 2009). However, 2D planar
447 surface area severely underestimates the volume and (organic) biomass of erect versus flat
448 organisms (e.g., massive versus encrusting sponges; see also discussion in de Goeij et al.
449 2017). Wet weight is another commonly used metric, but the most subjective, since the loss
450 of weight in time before measuring can significantly change within and between species.
451 Arguably the best metric to standardize metabolic rate is organic biomass (i.e. AFDW) or
452 organic carbon content, excluding ecologically inert hard constituents, such as silica spicules
453 (Rutzler, 1978). However, an increase in inorganic spicule content requires additional
454 energetic costs at the expense of organic material (McDonald et al. 2002; Carballo et al.

455 2006). Therefore, in ecological terms, volume and DW provide alternatives. Volume, similar
456 to WW, compromised by effects of large variations in shape, form and tissue densities and
457 compositions of sponges (Diaz & Rutzler, 2001). We therefore use DW here as comparative
458 measure and suggest that future physiological studies on sponges best provide a combination
459 of metrics on volume, area cover, DW, AFDW, elemental composition (e.g., C or N content)
460 and/or conversion factors between these metrics.
461
462 **Deep-sea sponge carbon budgets**
463 The contribution of DOC to the TOC uptake of the investigated sponges (92–100 %) is at the
464 high end of the range reported for shallow water sponges (56–97; see Table 1 in de Goeij et
465 al. 2017). Indirect measurements recently suggested that DOC accounts for 95 % of the TOC
466 uptake of *G. barretti* (Leys et al. 2018), which is very close to the fluxes presented here.
467 Although mass balances for all species where LPOC and DOC were measured are largely
468 positive (table 4), both HMA species show higher $\Delta O_2/\Delta TOC$ values than the two LMA
469 species. These differences might be explained by aerobic microbial processes in HMA
470 sponges, such as nitrification (Hoffmann et al. 2009) or ammonia oxidation (Mohamed et al.
471 2010), which require O_2 in addition to the O_2 demand based on carbon respiration. Moreover,
472 the organic carbon uptake needed to balance respiration requirements of HMA sponges is
473 potentially further reduced by sponge-associated chemoautotrophs using inorganic carbon
474 sources which are transferred to the sponge host (van Duyl et al. 2008; Pita et al. 2018; Shih
475 et al. 2019). A second issue with the calculating mass balances is the use of RQ value. The
476 value of 1 used in this study applies to the oxidation of simple sugars $(CH_2O)_x$. In reality,
477 proteins and nucleic acids have RQ values ranging from 0.71–0.83 (Kleiber, 1975), meaning
478 that depending on the macromolecules respired, less than 1 mole C could be needed to
479 balance consumption of 1 mole O, further reducing the amount of C needed to balance

480 respiration requirements. In addition to respiration, other processes such as growth, cell-
481 turnover and release, reproduction, and the production of metabolites require organic carbon.
482 Therefore, a complete carbon budget should include these processes. However, deep-sea
483 sponges most likely grow slowly (Leys & Lauzon 1998), and we assume that within the short
484 (2–8 h) timeframe of our incubations, growth is negligible. For several shallow water
485 encrusting sponges, a rapid cell turnover and the subsequent release of "old" cells as detritus
486 was shown (de Goeij et al. 2009, 2013; Alexander et al. 2014; Rix et al. 2016). This "loss of
487 carbon" could have a major impact on carbon budgets. However, Leys et al. (2018) reported
488 no production of new cells during experiments with *G. baretti*, suggesting minimal
489 investment in cell turnover in the investigated time frame. In contrast, Rix and colleagues
490 (2016) found that the deep-sea encrusting sponge *Hymedesmia coriacea* converted 39 % of
491 organic carbon derived from deep-sea coral mucus into detritus, and detritus production by
492 deep-sea sponges has been argued to have a major contribution to the total sedimentation rate
493 of the Greenland–Iceland–Norwegian seas (Witte et al. 1997). Concludingly, reports on deep-
494 sea sponge detritus production and cell turnover are contradictive and still very limited,
495 which does not warrant generalizations at this point. Likewise, only limited data is available
496 on the reproduction of deep-sea sponges (Spetland et al. 2007) as well as seasonal changes in
497 metabolic rates (Morley et al. 2016). Lastly, particularly HMA sponges, such as *G. barretti*,
498 are known to produce secondary metabolites for chemical defense against surface settlers and
499 grazers (Hedner et al. 2006; Sjo□gren et al. 2011). However, to our knowledge no studies
500 have been performed on the metabolic costs of this metabolite production.
501

502 **Conclusion**

503 In this study we showed for the first time that multiple deep-sea sponge species are capable of
504 consuming natural DOC, and that this DOC is essential to satisfy their minimal respiratory

carbon demand. However, although bacterio- and phytoplankton contributed only a small fraction (< 10 %) to the TOC uptake, these particulate food sources may contain valuable nutrients, such as vitamins, fatty acids, and amino acids (Putter 1925; Phillips 1984), which are essential for anabolic processes. We therefore hypothesize that DOC comprises the main carbon source for deep-sea sponges to sustain their minimal energetic requirements. But the supplementation with bacterio- and phytoplankton and possibly detritus, particularly during episodic food pulses after phytoplankton blooms, is essential to support anabolic processes such as somatic growth, reproduction, and cell turnover. The effective consumption of both dissolved and particulate food therefore allows deep-sea sponges to thrive in otherwise food-limited environments.

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773 **Figure legends**

774

775 **Figure 1. Photographs of six dominant North-Atlantic deep-sea sponge species used in**
776 **the study.** (**A**) *Vazella pourtalesi* (**B**) *Geodia barretti* (**C**) *Geodia atlantica* (**D**) *Craniella*
777 *zetlandica* (courtesy of Erik Wurz) (**E**) *Hymedesmia paupertas* (**F**) *Acantheurypon*
778 *spinospinosum*.

779

780 **Figure 2. Study area.** Sponge specimens were collected at different locations in the North
781 Atlantic during 4 different research cruises in 2016 (Emerald Basin), 2017 (Hordaland, Sula
782 Reef, Barents Sea) and 2018 (Barents Sea). The dotted line represents the Mid-Atlantic Ridge
783 (MAR).

784

785 **Figure 3. Average abundances of bacterioplankton during incubations with six deep-sea**
786 **sponge species in comparison to seawater controls incubations over time.** Sponges (blue)
787 versus seawater controls (red). A: *Vazella pourtalesi* (*n* = 7), B; *Geodia barretti* (*n* = 12), C;
788 *Geodia atlantica* (*n* = 6), F: D: *Craniella zetlandica* (*n* = 4), E: *Hymedesmia paupertas* (*n* =
789 3), F: *Acantheurypon spinospinosum* (*n* = 4). Bacterial decrease is modelled with an
790 exponential fit, shades depict 95% confidence intervals of the model. Note that x- and y-axis
791 show different ranges per species.

792

793 **Figure 4. DOC removal over time by four deep-sea sponge sponge species compared to**
794 **seawater controls in *ex situ* incubations**. A; *Vazella pourtalesi* B: *Geodia barretti* C:
795 Seawater control, D: *Geodia atlantica*, E: *Acantheurypon spinispinosum*, F: Seawater control.
796 Trend lines are given by a 2G model fit.

Figure 5. Oxygen and particulate - and dissolved carbon fluxes for tropical, temperate, and cold-water marine sponges plotted against temperature. Fluxes are log transformed. Red squares depict encrusting sponges, blue circles depict emerging/massive sponges. R^2 values are based on the linear regression of all values (encrusting + emerging). Regression lines are given by $\log(1+ \mu mol\ O2\ h^{-1}\ g\ DW^{-1}) = 0.033 * T(^oC) + 0.90$, $(\log(1+ \mu mol\ POC\ h^{-1}\ g\ DW^{-1}) = 0.017 * T(^oC) + 0.21$ and $(\log(1+ \mu mol\ DOC\ h^{-1}\ g\ DW^{-1}) = 0.041 * T(^oC) + 0.77$. All non-transformed fluxes are given in table S2.

Tables

Table 1. Conversion factors between different standard metrics for six investigated deep-sea sponges. LMA = low microbial abundance sponges, HMA = high microbial abundance sponges. Planar surface area is the surface area covered in a 2D top view, volumes are measured by water displacement in mL and the weight is given as g dry weight (DW). Conversion factors are based on average sponge metrics (planar surface area, volume, wet weight (WW), DW, ash-free dry weight (AFDW), organic carbon (C) content) for all specimens used in the experiments shown in Table S1).

Sponge species	Class	Growth Form	LMA/HMA	Planar surface area : Volume (cm^2 : mL)	Volume : Weight (mL : g dw)	organic C content (% of dw)
Vazella pourtalesi	Hexactinellid	Massive, vase	LMA	0.3	5.2	5.5
Geodia barretti	Demosponge	Massive, ball	HMA	0.3	3.3	15.9
Geodia atlantica	Demosponge	Massive	HMA	0.3	7.3	20.3
Craniella zetlandica	Demosponge	Massive, ball	HMA	0.2	4.3	20.4
Hymedesmia paupertas	Demosponge	Encrusting, sheet	LMA	10.0	21.4	12.6
Acantheurypon spinispinosum	Demosponge	Encrusting, sheet	LMA	4.2	22.4	10.9

815 **Table 2. Oxygen consumption by deep-sea sponge species (mean ± SE).** CA = Canada,

816 NO = Norway. (1) Leys et al., 2011, (2) Leys et al. 2018, (3) Kutti et al. 2013.

817

Sponge species	O_2 consumption (μmol O_2 g DW^{-1} h^{1})	O_2 consumption (μmol O_2 mL^{-1} h^{-1})	Original Location	T (°C)	Method	Reference
Vazella pourtalesi	3.4 ± 0.7	0.7 ± 0.1	Emerald Basin (CA)	6.7	Ex situ incubation	This study
Aphrocallistes vastus	-	0.1	Fraser Ridge Reef (CA)	9.0	In situ In-ex	1
Geodia barretti	1.3 ± 0.2	0.5 ± 0.1	Barents Sea (NO)	9.0	Ex situ incubation	This study
Geodia barretti	1.4 ± 0.3	0.3 ± 0.1	Langenuen fjord (NO)	8.0	Ex situ in-ex	2
Geodia barretti	1.5	-	Continental Shelf (NO)	6.9–7.4	Ex situ incubation	3
Geodia atlantica	5.8 ± 0.9	0.8 ± 0.1	Sula reef (NO)	6.0	Ex situ incubation	This study
Craniella zetlandica	1.0 ± 0.3	0.2 ± 0.1	Continental Shelf (NO)	9.0	Ex situ incubation	This study
Hymedesmia paupertas	5.8 ± 1.5	1.5 ± 0.6	Barents Sea (NO)	6.0	Ex situ incubation	This study
Acantheurypon spinispinosum	7.8 ± 0.8	0.4 ± 0.0	Sula reef (NO)	6.3	Ex situ incubation	This study

818

819

820 **Table 3. Average (± SD) net dissolved organic carbon (DOC), bacterio- and**
821 **phytoplankton carbon (BC and PC, respectively) removal rates, and bacterio- and**
822 **phytoplankton clearance rates per sponge species**. Net removal rates for bacterio- and
823 phytoplankton are based on exponential uptake during incubations, whereas net removal rates
824 for DOC are based on a 2G-model uptake. DOC = dissolved organic carbon, BC = bacterial
825 carbon, PC = phytoplankton, CR = clearance rate.

Sponge species	DOC removal (μmol C g DW^{1} h^{-1})	BC removal (μmol C g DW^{-1} h^{-1})	PC removal (μmol C g DW^{-1} h^{-1})	Bacterial CR (mL mL^{-1} min^{-1})	Phytoplankton CR (mL mL^{-1} min^{-1})
Vazella pourtalesi	9.17 ± 2.69	0.82 ± 0.41	0.15 ± 0.18	2.22 ± 1.25	1.77 ± 1.37
Geodia barretti	3.70 ± 0.26	0.02 ± 0.02	<0.01	0.15 ± 0.17	0.17 ± 0.15
Geodia atlantica	5.85 ± 5.55	0.12 ± 0.08	0.11 ± 0.13	0.08 ± 0.03	0.25 ± 0.24
Craniella zetlandica	NA	0.02 ± 0.02	0.00	0.06 ± 0.06	0.05 ± 0.11
Hymedesmia paupertas	NA	0.55 ± 0.42	NA	0.15 ± 0.10	NA
Acantheurypon spinispinosum	56.07 ± 19.92	0.00	0.00	0.00	0.00

826

827

828 **Table 4. Carbon mass balance for four deep-sea sponge species**. The mass balance was
829 based on linear fluxes of oxygen uptake, exponential net removal rates of bacterio- and
830 phytoplankton organic carbon (LPOC) and 2G-model exponential net removal rates for
831 dissolved organic carbon (DOC). Net total organic carbon (TOC) removal rates are calculated
832 as the sum of LPOC and DOC.

Sponge species	O2 (μmol h^{-1} g DW^{-1})	TOC (μmol h^{-1} g DW^{-1})	Exponential mass balance $\Delta O_2/\Delta TOC$
Vazella pourtalesi	3.19 ± 1.96	9.87 ± 2.36	0.32
Geodia barretti	1.93 ± 1.09	2.97 ± 1.87	0.65
Geodia atlantica	4.76 ± 1.76	6.07 ± 5.55	0.78
Acantheurypon spinispinosum	8.44 ± 1.04	56.07 ± 19.92	0.15

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