

Phagocytosis of microbial symbionts balances the carbon and nitrogen budget for the deep-water boreal sponge *Geodia barretti*

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Abstract

Geodia barretti is a massive nearly spherical sponge that forms dense assemblages on the continental shelf of the North Atlantic and the Norwegian Sea. We studied the metabolism of individual sponges collected using a remotely operated vehicle and maintained in large tanks with high volumes of unfiltered water brought from 160 m depth. We used direct methods (In-Ex) to measure excurrent flow rates, oxygen removed, and carbon and nutrient flux through the sponges. *G. barretti* had very low specific filtration ($0.26 \text{ mL min}^{-1} \text{ mL}^{-1}$ sponge tissue) and low respiration ($5.34 \pm 0.98 \text{ nmol O}_2 \text{ min}^{-1} \text{ mL}^{-1}$ sponge tissue; $8.44 \pm 1.51 \text{ } \mu\text{mol O}_2 \text{ h}^{-1} \text{ g C}^{-1}$) rates in comparison to other sponges. A net release of nitrogen was detected as NO_3^- . Bacteria were removed from the water filtered with up to 99% efficiency, yet comprised only 5% of the sponges' total carbon budget; the remainder consisted of dissolved organic carbon and detritus. High bacterial removal was aided by the presence of a tight gasket of cells that surrounds the collar of each choanocyte filter. A test for potential bypass canals showed removal of fluorescent microspheres until they were excreted 5–12 h after feeding. Electron micrographs showed active uptake of *Escherichia coli* “fed” to the sponge as well as phagocytosis of symbiont microbes by sponge cells in the mesohyl. These data provide the first comprehensive study of metabolism in a deep-water high microbial abundance sponge.

Sponges are widely known to be important components of benthic communities with demonstrated roles in both habitat formation and benthic-pelagic coupling (Dayton et al. 1974; Reiswig 1975; Pile et al. 1996, 1998; Gili and Coma, 1998; Yahel et al. 2003; Kahn et al. 2015). Over 40 years of studies have shown that sponges efficiently remove particulate organic carbon (POC; detritus and picoplankton), and are heavy consumers of dissolved organic carbon (DOC) where it is abundant (Reiswig 1981; Yahel et al. 2003; De Goeij and Van Duyl 2007; Maldonado et al. 2012; De Goeij et al. 2013). Many of these studies are on shallow water species but some of the densest aggregations of sponges occur in deep water (100–600 m depths) on the continental shelves of Antarctica (Barthel et al. 1991; Brey et al. 1994) the North Atlantic (Barthel and Tendal 1993; Kutti et al. 2013) and eastern Australia (Fromont et al. 2012) and these also account for the largest biomass in their habitats (reviewed in Maldonado et al. 2015).

In recent years, the removal of deep-water sponges as trawl bycatch and increased access to deep-water ecosystems by remotely operated vehicles have highlighted the abundance and therefore potential importance of sponge fauna. In regions of dense sponge cover, the whole water column up to 170 m deep can be filtered of bacteria daily, which is proposed to result in significant carbon sequestration (Kutti et al. 2013; Kahn et al. 2015). Increased turbidity caused by sediments suspended into the water column has been shown to increase the respiration and stress of sponges on the Great Barrier Reef (Bannister et al. 2012), and cause arrests of feeding in both demosponges (Reiswig 1971; Gerrodette and Flechsig 1979; Maldonado et al. 2008) and glass sponges (Hexactinellida) (Tompkins-Macdonald and Leys 2008). The extent to which sediment stress reduces the carbon sequestration and nutrient cycling and threatens the survival of sponges can only be evaluated by understanding their energy budget.

Reiswig (1971, 1981) pioneered an approach for determining the energy budget of sponges by sampling *in situ* with thermistor flow meters to determine filtration rates and collecting water expelled from the sponge to determine oxygen consumption and uptake of particulate organic carbon (POC).

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

Reiswig (1981) found that bacteriosponges or high microbial abundance (HMA) sponges, which have a vast number of prokaryotic symbionts in their tissues, have different energy budgets than sponges containing few microbial symbionts, commonly called low microbial abundance (LMA) sponges. In that study, POC (bacteria and detritus) accounted for only 14% of the energy budget to support respiration by the HMA “sponge-bacteria-polychaete” community, while other LMA sponges had a balanced budget (Reiswig 1981). The remainder of the budget for the HMA sponges and their associated microbes and polychaetes was suggested to be DOC. Using the direct (In-Ex) method to sample water filtered by a sponge Yahel et al. (2003) confirmed that dissolved organic carbon (DOC) made up over 80% of the total carbon consumed by the reef sponge “holobiont” (sponge-microbe community) *Theonella swinhoei*. DOC is high in regions of primary productivity, but on tropical coral reefs, DOC availability is particularly high for crevice communities presumably arising from mucus produced by plants, coral and other animals (Van Duyl and Gast 2001; Scheffers et al. 2005) and recent work suggests that use of DOC fuels a very high rate of tissue replacement (De Goeij et al. 2008; De Goeij et al. 2013). For deep sponges, the carbon available depends on depth and ocean habitat. On the continental shelf of the northeast Pacific, particulate carbon makes up the whole budget for the glass sponges *Aphrocallistes vastus* and *Rhabdocalyptus dawsoni* (Yahel et al. 2006; Yahel et al. 2007; Kahn et al. 2015). Here, the water comes from the deep Pacific where it is rich in nutrients and poor in particulate carbon (Whitney et al. 2005; Yahel et al. 2007). Other deep sponges, including one glass sponge *Rosella nodastrella* collected from 600 m in the NE Atlantic, are suggested to fix carbon (Van Duyl et al. 2008), however glass sponges appear specially adapted to capture the small amounts of POC available (Leys et al. 2011). Work on deep sponges is challenged by access, so *in situ* methods for direct In-Ex measurements or maintenance of sponges in high quality seawater systems (e.g., Yahel et al. 2006; Yahel et al. 2007; Kahn et al. 2015) is needed.

Geodia barretti is a deep-water boreal HMA sponge that occurs in such abundance on the shelves and fjords of the NE Atlantic that fishermen refer to the regions as “Ostur” or “cheese-bottoms” (Klitgaard and Tendal 2004). *G. barretti* can reach a meter in diameter and is found in dense assemblages of up to 0.4 m^{-2} on the Norwegian continental shelf (Klitgaard and Tendal 2004; Kutti et al. 2013). It is estimated to have 10^{10} microbes cm^{-3} of tissue (Hoffmann et al. 2009) and has been shown to have a filtration capacity of $3 \text{ L g}_{\text{dw}}^{-1} \text{ d}^{-1}$ (Kutti et al. 2013). In the last 10 years, a suite of work has shown that the *G. barretti* holobiont (sponge-microbial consortium) has a complex nitrogen budget that includes aerobic nitrification as well as anaerobic denitrification and anaerobic ammonium oxidation (anammox) (Hoffmann et al. 2005; Hoffmann et al. 2009). In addition to its ecological importance, *G. barretti* is of interest for the array of

secondary metabolites produced by its symbionts including analogs of 5-HT (serotonin) (Hedner et al. 2006; Lind et al. 2013). While it is known that sulfur oxidation, nitrification and even denitrification occur in *G. barretti* (Hoffmann et al. 2005; Hoffmann et al. 2009) that work was carried out on explants $0.3\text{--}0.4 \text{ cm}^3$ in which it was unclear there was an aquiferous system. Other work has used closed respiration chambers to calculate oxygen used and thereby carbon consumption (Kutti et al. 2013), but since sponges have been shown to reduce their filtration and uptake/excretion of dissolved nutrients in closed chamber systems (Hadas et al. 2008; Jimenez 2011), the exact carbon and nitrogen budget for whole *G. barretti* sponges is still unknown, as is the extent to which they rely on filtration of POC for their carbon and nitrogen needs.

Here, we measured water transport, respiration, and filtration using direct In-Ex methods in *G. barretti* individuals collected from 200 m and maintained in large tanks with flow-through unfiltered deep fjord water. Our findings have implications for the tolerance of this sponge to periods of reduced oxygenation as are predicted to occur in shelf waters with changes to ocean circulation, and as would occur in the case of reduced filtration caused by smothering by sediments.

Methods

Collection and maintenance of sponges

A total of 159 sponges were collected using the ROV Aglantha during cruises of the RV *Håkon Mosby* in Lungenfjord, Norway. Collections took place over 3 years in July 2011 and 2012, and June 2014. Sponges were transferred in 50 L water coolers to 1000 L flow-through tanks at the Institute of Marine Research’s deep-water aquaria facility in Austevoll, Norway. Specimens of *G. barretti* collected for this work were targeted to be between 0.25 L and 10 L in volume in order to transport them with sufficient care to the aquarium facility (Fig. 1A), but specimens ten times that size are common at the site of collection. Tanks were approximately 1 m^3 with flow-through at 2000 L h^{-1} of unfiltered seawater drawn from 160 m depths and kept in a header tank to remove potential supersaturation. Seawater temperature in the tanks was $8\text{--}9^\circ\text{C}$. The displaced volume was calculated for each sponge and the osculum area was photographed for measurement in ImageJ (NIH). A subset of 26 sponges was sacrificed for volume, wet weight (ww), and dry weight (dw). Three of the dried sponges were divided into portions, and each of them was transferred into a pre-weighed crucible and burnt at 450°C in a muffle furnace (Nabertherm LE6/11/B150, Lilienthal, Germany). Ashed samples were cooled to room temperature in a desiccator and weighed (ash weight, aw). The dw minus aw yielded ash-free dw of the samples. Another 27 pieces of sponge tissue were freeze-dried at -80°C , ground to powder and analyzed for their elemental

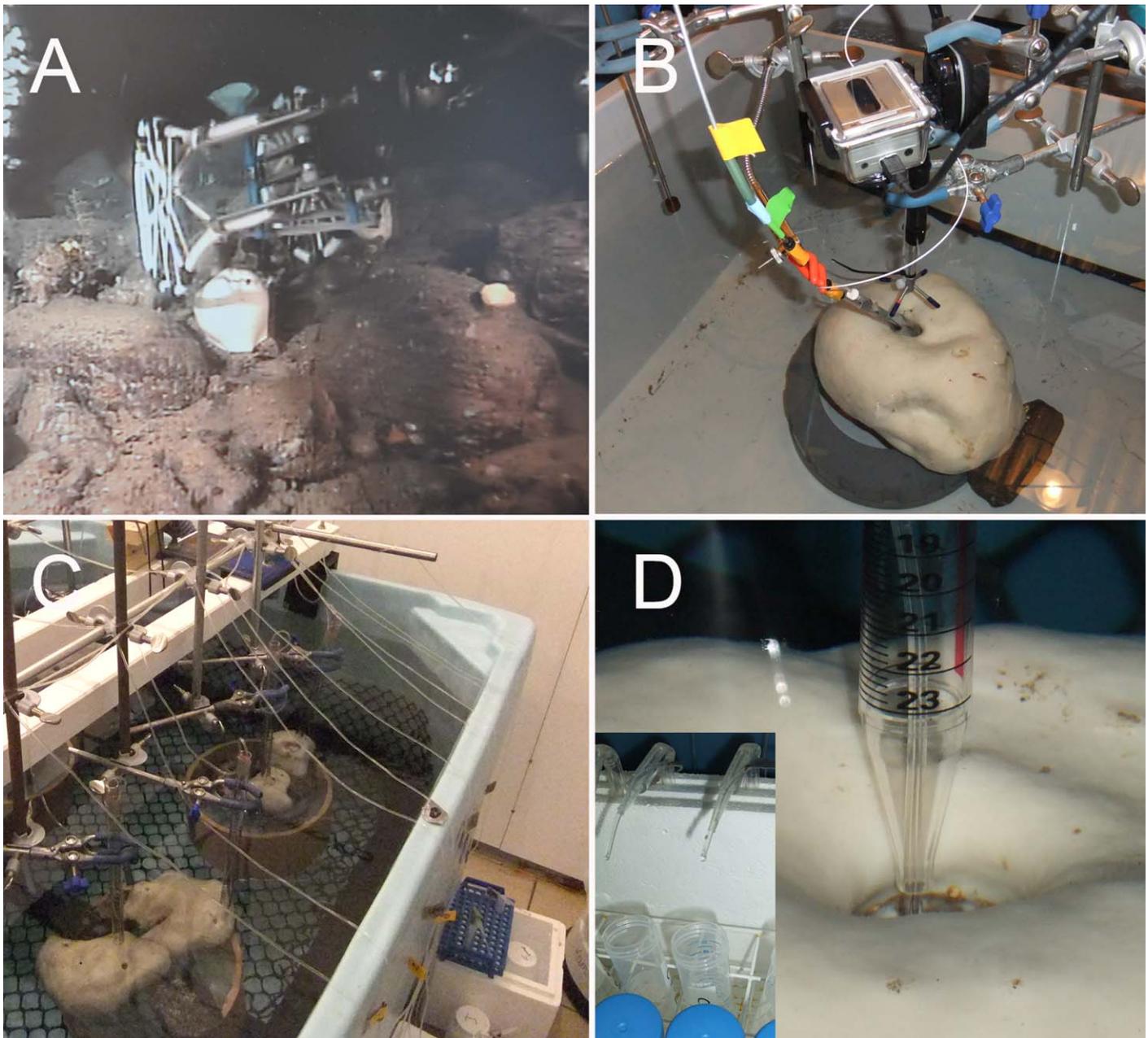


Fig. 1. *G. barretti* in situ and in the experimental setup at the Austevoll field station. **(A)** *G. barretti* being collected by the remotely operated vehicle on rocks at 200 m in the Lungenuen fjord. **(B)** Recording excurrent flow and respiration in tanks using an acoustic Doppler velocimeter and oxygen optodes. A GoPro Hero2 camera was used to monitor changes in oscules during some experiments. **(C)** In-Ex (direct) sampling of water filtered by the sponges involved positioning tubing directly in the osculum. **(D)** Water sampled from the osculum was collected at a rate lower than the excurrent velocity by dripping into tubes kept on ice (inset).

carbon contents by the OEA Laboratories (Callington, Cornwall, UK).

Pumping and respiration rates

The velocity of water exiting from the osculum, termed the excurrent velocity (cm s^{-1}) or excurrent flow, was measured from 17 individuals using a Vectrino I acoustic Doppler velocimeter (Nortek, Rud, Norway) at 10 Hz, positioned

5 cm above the sampling volume (Fig. 1B). The location of the sampling volume was visualized using fluorescein dye ($0.22 \mu\text{m}$ filtered in seawater), which illustrated the position of the excurrent flow, and a plastic cable tie was used to block, and therefore indicate, the precise location of the sampling volume. The maximum excurrent velocity (all three vectors, xyz) at that location was recorded for a minimum of 0.5 h. Excurrent flow was considered to be parabolic

(Denny 1993) and therefore average excurrent velocity across the whole osculum was calculated as half of maximum scalar velocity. Velocities were averaged to 5 min intervals for subsequent analysis and reported as mean \pm SE. Filtration rate—also called volumetric flow rate—was determined as the excurrent velocity multiplied by the area of the osculum and reported in $\text{cm}^3 \text{s}^{-1}$.

Oxygen in ambient water (tanks) and in the excurrent flow (from the osculum) was measured with needle-type oxygen optodes, 180 μm diameter (PreSens, Regensburg, Germany) calibrated to zero using sodium hydrosulfite (3 g L^{-1}) and saturated oxygen using air-saturated water. Sodium hydrosulfite and other chemicals used in this study were supplied by Sigma-Aldrich (St. Louis, Missouri) unless otherwise specified. Because *Geodia* is a HMA sponge respiration rates calculated reflect that of the holobiont (sponge + symbionts). For respiration rates, the oxygen removed by the sponge (incurrent–excurrent) was multiplied by the volumetric flow rate of the sponge and expressed as $\mu\text{mol oxygen h}^{-1}$ ($\mu\text{mol h}^{-1}$). Specific filtration and respiration rates were expressed in three ways for ease of comparison with other work: per mL of tissue or per gram dry weight of tissue or per grams of carbon ($\text{L h}^{-1} \text{mL}^{-1}$ tissue, $\text{L h}^{-1} \text{g}_{\text{dw}}^{-1}$ and $\mu\text{mol h}^{-1} \text{g}_{\text{C}}^{-1}$).

Feeding

To determine the uptake/removal of bacteria, TOC and nutrients by sponges Tygon® tubing 1/32" internal diameter (ID) was used to draw water from the excurrent or ambient water. One end of the tubing was inserted into 25 mL plastic serological pipettes that were clamped in place over the sponges to allow the tip of the tube to rest just above the opening of the osculum (Fig. 1C,D). We confirmed that sponges were filtering well for the duration of each experiment, and repeated experiments in multiple years if necessary. Excurrent samples (Ex) were collected from a total of 84 individuals in 2011 and 2012. All of these were analyzed for dissolved nutrients (NO_3^- , PO_4^{3-} , SiO_4^{4-}), 53 samples were analyzed for (NO_2^-), and 52 were analyzed for POC by flow cytometry. One ambient (In) tube sampled tank water for four to five sponges; ambient properties of the tank water are given in Table 2. The ID of the tubing set the drip rate 0.3 m below the sponges to 0.8 mL min^{-1} , slower than the volumetric flow rate. Water was collected into sampling vials placed in racks on ice. In 2014, another 29 sponges were collected and excurrent water was sampled as above and analyzed for TOC and ammonia. For TOC, silicon tubing (0.031" ID HelixMark®) was used in lieu of Tygon tubing to siphon water from the sponges. Ultrapure water (Milli-Q®, Merck Millipore, Darmstadt, Germany) was sampled from a beaker in parallel to sampling the sponge excurrent flow to confirm no contamination existed in the tubing.

To confirm and visualize capture of bacteria by choanocytes using electron microscopy (EM), three additional small

sponges were placed in a solution (approximately 10^7 cells mL^{-1}) of heat killed *Escherichia coli* and tissue pieces were removed and fixed at 30 min, 1 h, 6 h, and 12 h afterward as described below.

Water processing

Two replicates of 2 mL from ambient and excurrent water samples were fixed with 0.15% glutaraldehyde, directly frozen in liquid nitrogen, and stored at -80°C . Samples were analyzed on a BD FACSCalibur flow cytometer (Franklin Lakes, New Jersey) located at the Institute of Marine Research, Bergen, Norway, following Marie et al. (1997), to quantify heterotrophic bacteria removed by sponges. For nutrients (NO_2^- , NO_3^- , PO_4^{3-} , SiO_4^{4-}), 20 mL samples were stabilized with 200 μL of chloroform and stored at 4°C until analysis using a modified Skalar autoanalyser (Breda, The Netherlands) following standard methods (Bendschneider and Robinson 1952; Grasshoff 1965). Analytical services for nutrients were provided by the Chemistry Laboratory, Institute of Marine Research. Detection limits of NO_2^- , NO_3^- , PO_4^{3-} , and SiO_4^{4-} were $0.06 \mu\text{mol L}^{-1}$, $0.50 \mu\text{mol L}^{-1}$, $0.06 \mu\text{mol L}^{-1}$, and $0.70 \mu\text{mol L}^{-1}$, respectively. We found the ammonium (NH_4^+) removed by the sponges was too low for detection by the autoanalyzer, and therefore we carried out analysis again in 2014 following the protocol of Holmes et al. (1999). Ammonium was analyzed from 24 of the 29 sponges sampled for TOC. Samples were collected avoiding unnecessary contact with air and then 10 mL was transferred to a vial with 10 mL working reagent (WR); standards were prepared at the same time and all vials were analyzed with a fluorometer at 360/420 nm (excitation/emission). Detection limits for ammonium calculated using five replicates of tank water were $0.009 \mu\text{mol L}^{-1}$. Samples for ammonium were also collected in situ at 160 m depth using Niskin casts from the ship. These samples were mixed with WR on board in parallel with standards, and analyzed the same day in the shore laboratory. For TOC, water samples were collected into 20 mL EPA vials (Quality Environmental Containers, Beaver, West Virginia), spiked with 0.1% trace metal grade hydrochloric acid and frozen at -20°C until analysis. Samples and diluted TOC standards (109017, Merck Millipore) were injected into a Lotix combustion TOC analyzer operated with the TOC TekLink software (Teledyne Tekmar, Mason, Ohio). Through acidification and catalytic oxidation at 680°C , TOC in the samples was converted into carbon dioxide, which was then quantified by a nondispersive infrared detector. Precision was $\leq 2\%$ relative standard deviation. In initial work, we also collected water for DOC. Each of the In and Ex samples (35 mL) was filtered through a 450°C pre-combusted GF/F glass microfiber filter (pore size: $0.7 \mu\text{m}$; Whatman®, GE Healthcare, Buckinghamshire, UK). Filtered samples were processed and analyzed for DOC using the same method for TOC described above. DOC results were highly variable and therefore it was decided that

Table 1. Respiration and filtration of *G. barretti* in flow-through seawater aquaria.

	Sponge volume (mL)	Osculum area (cm ²)	Wet weight* (g _{ww})	Dry weight† (g _{dw})	Excurrent velocity (cm s ⁻¹)	Filtration rate (L h ⁻¹)	Oxygen removal (μmol L ⁻¹)	Respiration rate (μmol L ⁻¹ h ⁻¹)			
								per mL tissue			
								per g dw	per g C‡		
Average	1108	1.19	1218.14	253.79	4.63	17.61	0.022	15.10	0.32	1.38	8.44
Median	600	0.89	694.0	144.59	4.16	13.31	0.019	14.58	0.25	1.07	6.54
Min	150	0.23	169.2	35.25	2.03	3.76	0.003	9.42	0.04	0.15	0.92
Max	3500	6.64	3667.0	763.99	9.56	88.11	0.068	27.16	1.04	4.17	25.42
SE	255.1	0.35	272.92	56.86	0.59	4.8	0.004	1.16	0.06	0.25	1.51
n	17	17	17	17	17	17	17	17	17	17	17

* 8.3% of ww is ash-free dw.

† 31.4% of dw is ash-free dw.

‡ 0.164 ± 0.026 mg C per mg dry weight.

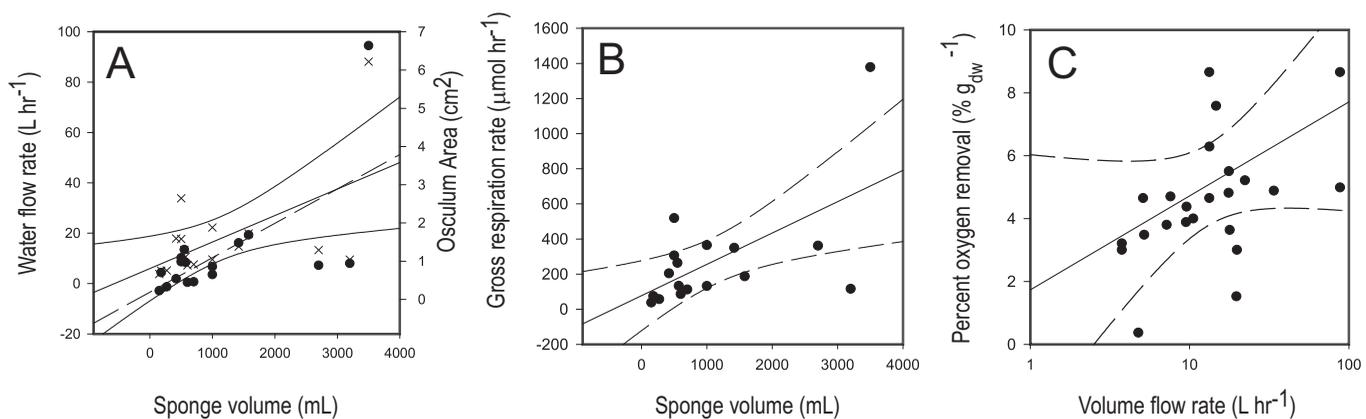


Fig. 2. Respiration and filtration rates of *G. barretti*. (A) Volume flow rates (crosses, regression shown as a solid line with 95% CI) and osculum area (circles, regression as a dashed line) varied directly with sponge volume. (B) Respiration rates varied directly with sponge volume (95% CI); the more volume processed the more oxygen consumed by sponges. (C) Oxygen removal varied directly with volume filtered by the sponge.

DOC + detritus was determined as TOC minus bacterial carbon. In other work in the nearby Trondheimsfjord (Børsheim et al. 1999), DOC has been shown to range from 55 μmol C L⁻¹ to 110 μmol C L⁻¹ (mean, 76.9 μmol C L⁻¹) at 200–400 m depth.

Bacterial cells were converted to carbon using 30.2 fg per cell and to nitrogen using a factor of 5.8 fg per cell (Fukuda et al. 1998).

Fixation for histology and EM

Tissue pieces were cut from whole sponges in seawater and immersed directly into either cold 4% paraformaldehyde for histology or into a cocktail fixative consisting of 2% glutaraldehyde, 1% osmium tetroxide in sodium acetate buffer pH 6.4 with 10% sucrose in the final volume for EM. Chemicals for EM were from Electron Microscopy Sciences

(Hatfield, Pennsylvania, U.S.A.). Fixatives were renewed after 30 min and samples were fixed overnight at 4°C and then rinsed twice, briefly, in distilled water to remove salts, and dehydrated to 70% ethanol for transport to the University of Alberta, Canada.

All samples were desilicified in 4% hydrofluoric acid in 70% ethanol for 2–3 days exchanging the medium daily until spicules were no longer evident when viewed under a stereomicroscope. For histology, samples were dehydrated to 100%, cleared in toluene and infiltrated and embedded in Paraplast wax. Sections 7 μm thick were cut on a Leica microtome, stained with Masson’s trichrome, and images captured on a Zeiss Axioskop plus microscope with a QiCam using Northern Eclipse software. For EM, samples were dehydrated to 100% ethanol, fractured in liquid nitrogen, and either embedded in epoxy (EMbed 812) for transmission EM

Table 2. Bacteria and nutrient fluxes in *G. barretti*. Differences reflect removal/production, expressed as the difference between In-Ex (negative and positive respectively). "In" samples represent ambient water of the tank coming directly from the fjord.

		Average	SD	SE	n	Min	Max
Bacteria (cells mL ⁻¹)	IN	257,224	63,704.8	15,450.7	17	196,500	345,500
	EX	20,283	15,188.1	2106.2	52	2100	66,100
	Difference	-224,571	41,322.4	5730.4	—	—	—
NH ₄ ⁺ (μmol L ⁻¹)	IN	0.0450	0.024	0.007	13	0.02	0.11
	EX	0.0208	0.012	0.002	24	0.01	0.05
	Difference	-0.0220	0.013	0.003	—	—	—
NO ₂ ⁻ (μmol L ⁻¹)	IN	0.21	0.31	0.08	14	0.05	0.94
	EX	0.11	0.20	0.03	53	0.01	1.16
	Difference	-0.15	0.30	0.04	—	—	—
NO ₃ ⁻ (μmol L ⁻¹)	IN	9.51	2.44	0.55	20	5.20	11.96
	EX	10.66	2.87	0.31	84	5.35	23.08
	Difference	1.86	1.98	0.22	—	—	—
PO ₄ ³⁻ (μmol L ⁻¹)	IN	0.85	0.10	0.02	20	0.65	1.17
	EX	0.89	0.16	0.02	84	0.15	1.47
	Difference	0.07	0.16	0.02	—	—	—
SiO ₄ ⁴⁻ (μmol L ⁻¹)	IN	6.13	0.66	0.15	20	4.99	6.64
	EX	5.93	0.66	0.07	84	4.71	6.70
	Difference	-0.06	0.13	0.01	—	—	—
TOC (μmol L ⁻¹)	IN	90.10	7.41	0.16	17	78.80	106.05
	EX	80.03	5.74	0.08	29	72.66	91.96
	Difference	10.376	4.053	0.069	—	—	—

or critical point dried and mounted on aluminum stubs for scanning EM. Thin sections were cut on a Leica Ultracut T, stained with lead citrate and uranyl acetate and viewed in a Philips Morgagni electron microscope with a Gatan camera. For scanning electron microscopy (SEM), stubs were gold coated and viewed in a Philips XL30 SEM.

Evaluating the presence of bypass routes

To determine whether *G. barretti* has potential routes by which bacteria could bypass the collar filter, fluorescent microspheres were applied to the side of sponges and the excurrent water sampled immediately and at set intervals after application. Three experiments were carried out in three different years to confirm the results. The first year a mixture of 1 μm and 3 μm latex microspheres (Fluoresbrite YG, Polysciences, Warrington, PA) was used at a final concentration of 4.55×10^8 beads mL⁻¹ in 0.2 μm filtered seawater to which fluorescein (made up as described above) had been added. A solution more concentrated than the concentration of bacteria typically found in seawater was made to compensate for mixing in the water surrounding the sponge at the time of addition. The solution was vortexed, the seawater flow in the tanks was turned off momentarily, 3 mL of the solution was pipetted onto the side of the sponge and the time it took for the first fluorescein to appear in the tubing noted. Cryovials (2 mL) were positioned under each tube and two vials of excurrent water were collected sequentially

at 2 min before the experiment and then at $t=0$ min, 5 min, 10 min, 20 min, 60 min, 120 min, 300 min, and 800 min after application of the beads. Flow in the tank was turned on gently by a second person 5 min after the experiment began. The height of the collection vials was set to generate a draw of 0.8 mL min⁻¹ (300 times slower than the average sponge filtration rate) to prevent contamination by ambient water. Fluorescein appeared in the sampling tube 30 s after it was applied to the side of the sponge. Therefore, beads that bypassed filtration by the sponge would be expected to appear during the first minute following application of the beads; the slow rate of sampling meant that the first samples (from $t=0$ to $t=10$) were sampled continuously into four sequential tubes. Water samples were kept on ice until a pause in sampling permitted fixation with 0.15% glutaraldehyde. One sample of each tube was frozen in liquid nitrogen for flow cytometry of bacteria and the other kept at 4°C for flow cytometry of latex beads. In the second and third years of this study, the solution used only 1 μm microspheres at the same concentration as above, and sampling continued to 900 min after the solution of microspheres was first applied.

When carrying the experiment out in the second and third years, because of the duration of the experiment (15 h), it could not be certain that the sampling tubes remained completely undisturbed or that each sponge kept pumping for the whole experiment. Therefore, to ensure

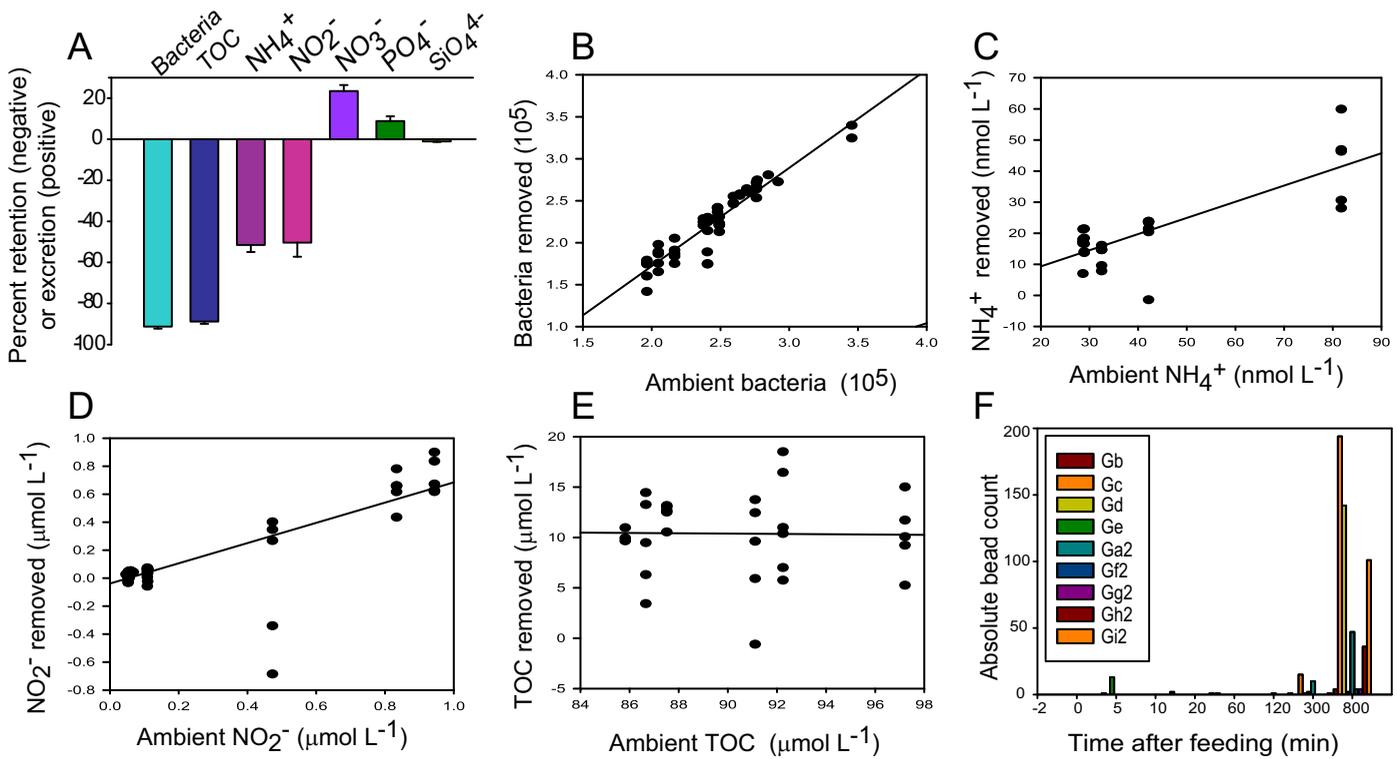


Fig. 3. Uptake and excretion by *G. barretti*. (A) Bacteria and TOC (POC and DOC) were retained by the sponge with high efficiency; ammonia and nitrite was removed from the water filtered by the sponge while nitrate and marginally also phosphate were excreted. A very small amount of silica was taken up by the sponge from the water it filtered. Percent removal or release of nutrients was calculated as [(IN-EX)/IN] × 100; negative values show removal, positive values show excretion. (B) *Geodia* removed as many bacteria as were available in the water filtered (a type I functional response). (C) TOC removal did not show a type I functional response, but for both ammonia (D) and nitrite (E) the more nitrogen available in the water the more was removed by the sponges. (F) Latex beads fed to nine sponges were largely excreted 800 min after feeding. Two individuals showed 2 and 13, respectively, 3 μm beads in the excurrent water captured at 5 min post feeding suggesting some potential for slippage through the filter, but none passed through directly with the fluorescein dye at 30 s post feeding.

Table 3. Carbon budget in *G. barretti*.

	Cost of pumping		Carbon ingested				Balance (μmol L ⁻¹)
	O ₂ removed (μmol L ⁻¹)	Carbon* (μmol L ⁻¹)	Bacteria ingested (cells mL ⁻¹)	Bacteria cells [†] (μmol L ⁻¹)	TOC (μmol L ⁻¹)	DOC + detritus (μmol L ⁻¹)	
Mean	15.10	10.79	224,571.2	0.565	10.454	9.889	-0.334
SE	1.16	0.8279	5730.38	—	0.008	—	—
n	17	—	52	—	29	—	—

* Using a ratio of O₂/C consumed for protein based catabolism of 1.4.

[†]Using 30.2 fg carbon per cell (Fukuda et al. 1998).

that all samples analyzed were from sponges that filtered throughout the whole experiment, 100 μL of each sample was mixed with SYBR Green (Life Technologies, Burlington Ontario) diluted to 1 : 10,000 for 30 min and run on a BD FACS Calibur at the Department of Oncology, University of Alberta. Only samples that showed greater than 80% filtration of bacteria were selected for analysis of microspheres. One milliliter of each sample was run at an average flow rate of 64.15 μL min⁻¹; the instrument was flushed well between samples.

Controls of seawater mixed with microspheres at the concentration applied in the experiment were also run at dilutions to determine the minimum detection level.

Results

Sponge morphometrics

Sponges were roughly spherical, ranging from 7 cm to 28 cm in diameter, and 0.25 L to 10 L in volume (N = 26)

Table 4. Nitrogen budget in *G. barretti* (All units in $\mu\text{mol L}^{-1}$).

	Uptake			Release	Balance
	Bacterial nitrogen*	NH_4^+	NO_2^-	NO_3^-	
Mean	0.093	0.02	0.15	1.86	-1.597
SE	0.00007	2.560	0.04	0.22	—
n	52	26	53	84	—

* Bacterial nitrogen calculated using 5.8 fg N per cell (Fukuda et al. 1998).

(Supporting Information Table 1). Wet weight was directly proportional to volume and dry weight was 21% of wet weight. Of the three individuals combusted to determine the organic component, ash made up 68.6% of the dry weight of *G. barretti*; 31.4% of the dry weight is organic (ash-free) ($N=3$). Tissue carbon mass was 0.164 ± 0.026 mg C per mg dry weight (mean \pm SD, $N=27$).

Pumping and respiration rates

The average excurrent velocity of sponges in the tanks was 4.63 ± 0.59 cm s^{-1} ($N=17$, range: 2.0–9.6 cm s^{-1}) (Table 1). Excurrent flow rates varied, with some sponges pumping strongly for days and others having very low excurrent flow rates at times and pumping strongly at others. The osculum in *G. barretti* lies apically and varies in shape from a flat crater to a cup-shaped atrial region closed at the apex by a sphincter (Fig. 1). Sponges with a broader osculum tended to have lower excurrent flow rates, but proportionally higher volumetric flow rates. The specific filtration rate was 0.02 ± 0.004 L h^{-1} mL^{-1} sponge tissue, and specific respiration rate was 0.32 ± 0.06 $\mu\text{mol oxygen h}^{-1}$ mL^{-1} sponge tissue, (8.44 ± 1.51 $\mu\text{mol O}_2 \text{ h}^{-1}$ g C^{-1} ; Table 1). Osculum size was directly proportional to sponge volume, and so volumetric flow (or gross transport rate) was directly proportional to sponge volume (Fig. 2A). *G. barretti* removed on average 15.1 ± 1.16 $\mu\text{mol O}_2 \text{ L}^{-1}$ filtered (Table 1) and gross respiration rates were linearly correlated with sponge size (Fig. 2B). Oxygen removal was directly proportional to the volume of water filtered (Fig. 2C).

Filtration and excretion

Ambient conditions of water brought from the fjord at the depth of collection, together with removal or excretion of nutrients and TOC by the sponges, are shown in Table 2. Sponges removed 11.5% ($\pm 4.5\%$ SD) of the TOC available. Sponges removed bacteria with an average efficiency of 91.27% ($\pm 6.93\%$ SD; range 72–99%) (Fig. 3A; Table 2) and captured more bacteria as ambient concentrations increased, a Type I functional response (Fig. 3B). Bacteria made up only 5% of the TOC consumed by the sponge. DOC plus detritus, calculated as TOC minus bacterial carbon, comprised the bulk of the total carbon (TOC removed, 10.454 $\mu\text{mol L}^{-1}$; bacterial POC removed, 0.57 $\mu\text{mol L}^{-1}$) (Table 3); TOC removal did not increase with TOC available (Fig. 3C). The nitrogen budget showed 0.02 $\mu\text{mol L}^{-1}$ nitrogen obtained in

ammonium from the ambient water, 0.1 $\mu\text{mol L}^{-1}$ from bacteria filtered, and 0.15 $\mu\text{mol L}^{-1}$ taken up as NO_2^- , also with the more removed the more available (Fig. 3A,D,E). Nitrification accounts for 1.86 μmol nitrogen released as NO_3^- for each liter of water filtered (Table 4). A small but significant amount of silicate, 0.06 $\mu\text{mol L}^{-1}$ (1%), was removed from the water filtered, and while phosphate was released, both ammonium and nitrite were removed from water filtered (Fig. 3A; Table 2).

Experiments using 1 μm and 3 μm latex microspheres to assess whether routes exist that bypass the choanocyte filter (bypass canals) showed that, for all sponges that were actively pumping, no microspheres were collected in the first water filtered by the sponges. Microspheres were excreted by the sponges after 800 min (12 h) and 87% of those excreted at 800 min were 3 μm in diameter (Fig. 3F). Of 24 sponges tested for potential bypass canals only one sponge allowed 13 and another sponge 2, three micron beads through at 5 min post bead application. During two subsequent experiments in which only 1 μm beads were applied to sponges, none were captured at any time point in excurrent samples.

Choanocyte chambers lie throughout the tissue of *G. barretti* but are small, 14 ± 0.28 μm ($n=258$ counted in SEM images) in diameter, and difficult to see with traditional histology because they lie embedded in a very dense mesohyl filled with microbial symbionts (Fig. 4A–D). They have an average of 24 choanocytes per chamber and a very obvious gasket made of several cells surrounds each collar of microvilli halfway up its height (Fig. 4D,E). There appears to be only one, or at the most two entrances to each chamber (prosopyles), and the gasket cells are connected to the single apopyle (exit) of each chamber such that water must travel through the collar microvilli to pass through the sponge (Fig. 4C–E). The distance between collar microvilli is 0.06 microns and on that filter is a mucus mesh a tenth the size (Fig. 4E). Bacteria (*E. coli*) fed to the sponges were seen on the filter and engulfed by choanocytes (Fig. 5A,B).

The mesohyl is densely packed with microbes and with a few wandering amoeboid cells. Many of the cells encountered in sections were in the process of engulfing several microbes, and in most cells at least five microbes could be seen surrounded by cell processes in a single 60 nm section

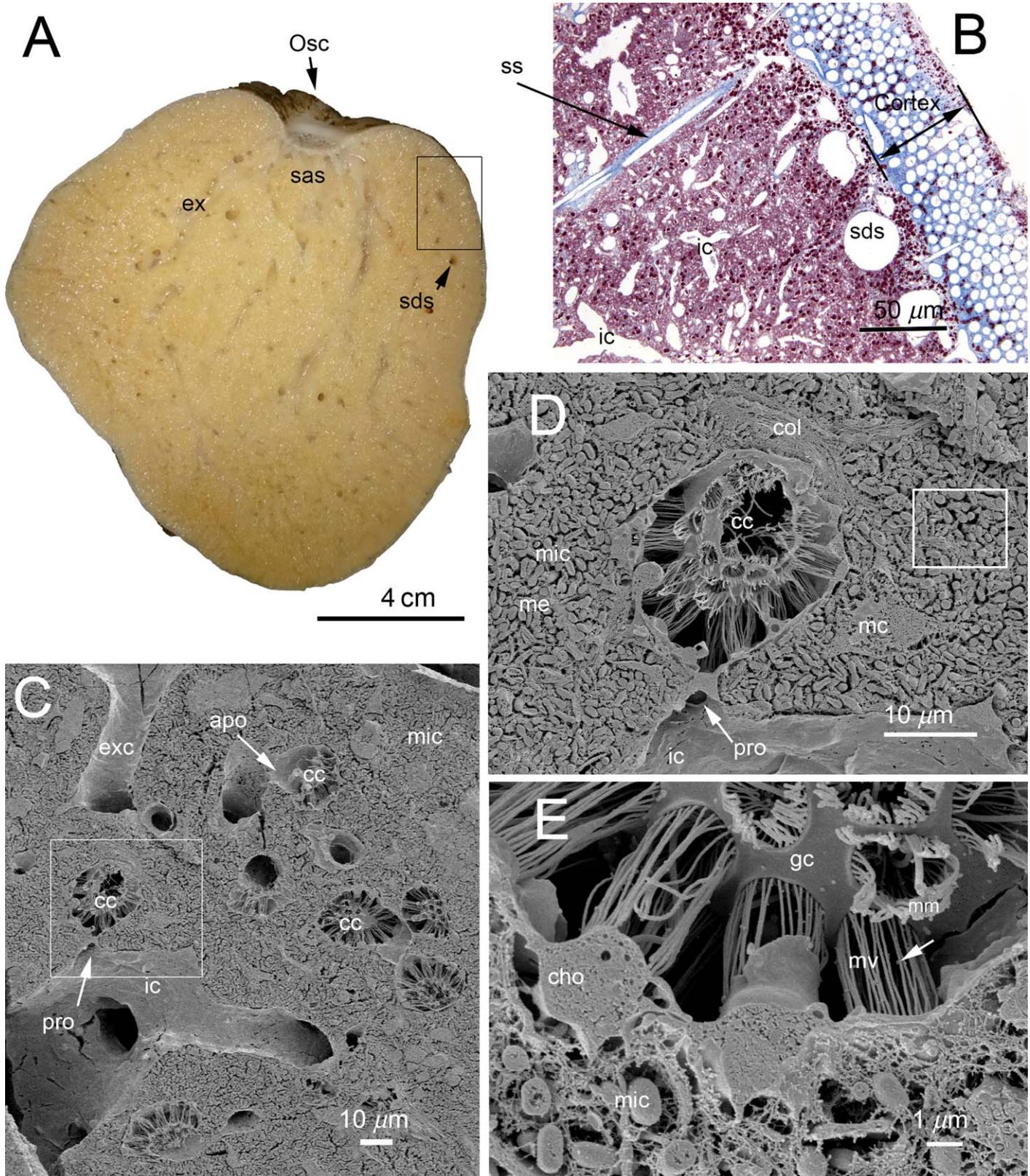


Fig. 4. The filter apparatus of *G. barretti*. **(A)** A longitudinal section through the whole sponge showing the osculum (Osc), subatrial space (sas), subdermal spaces (sds), and excurrent canals (ex). The boxed region is shown in **(B)**. **(B)** Histological section through the surface of *G. barretti* showing the collagen-dense cortex in blue and main sponge tissue in purple. A series of large canals just under the cortex form the subdermal space (sds). Incurrent canals (ic) can be distinguished from spicule spaces (ss) by the collagen in blue surrounding the latter. **(C)** Scanning electron micrograph showing the choanosome with incurrent (ic) and excurrent (exc) canals, choanocyte chambers (cc) with prosopyle entrances to chambers (pro) and apopyle exits from chambers (apo). The remaining material forms the mesohyl and consists of microbes and some amoeboid cells. **(D)** Magnification of a choanocyte chamber in **(C)**, showing the incurrent canal (ic) and prosopyle (pro), amoeboid mesohyl cell (mc) and microbes (mic) in the mesohyl. A $10 \times 10 \mu\text{m}$ box shows the dense microbial community. **(E)** Portion of a choanocyte chamber showing choanocytes (cho) with collar microvilli (mv) with gasket cells surrounding the collar (gc). Microbes (mic) lie below the choanocytes in the collagen-dense mesohyl. Arrow indicates the broken mucus mesh (mm) on the collar microvilli.

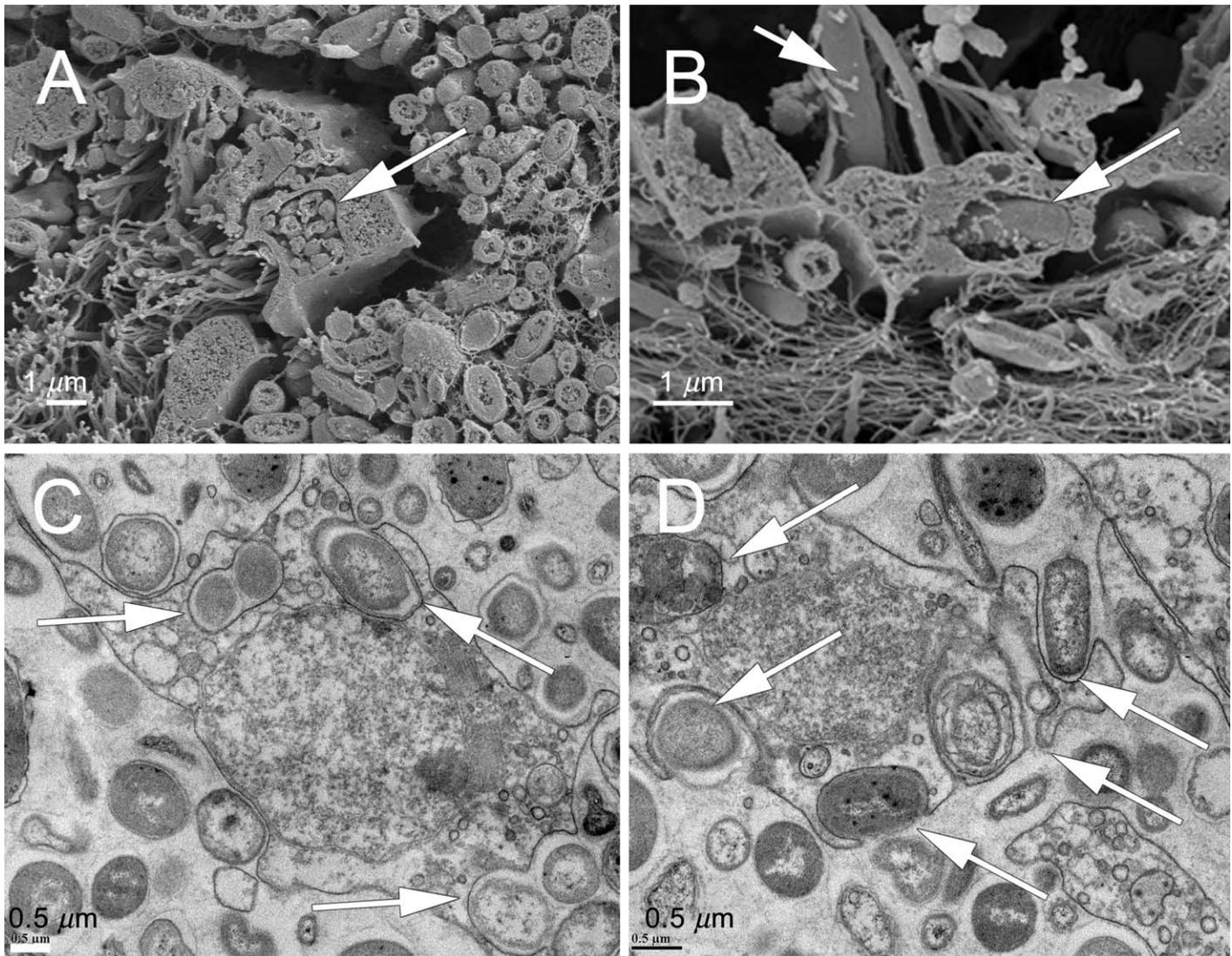


Fig. 5. Phagocytosis on microbes by sponge cells. **(A, B)** A choanocyte chamber **(A)** and choanocyte **(B)** phagocytosing microbes (arrows). **(C, D)**. Microbes (arrows) being phagocytosed in the mesohyl by sponge cells.

(Fig. 5C,D). From histological images such as that in Fig. 4B and SEM images such as Fig. 4C, we calculated that the sponge tissues (canal epithelia, choanocyte chambers, and mesohyl cells) comprised approximately 5% of the holobiont volume. We estimated that the mesohyl occupies roughly 50% of the sponge tissue (the remainder being canal spaces, choanocyte chambers, and spicule spaces). Approximately 5% of the mesohyl is taken up by wandering cells, such that 47.5% of the total sponge volume (including canal spaces) is microbes and extracellular matrix. We calculate that in $1000 \mu\text{m}^3$ there are 650 microbes (65 counted in a region $10 \mu\text{m} \times 10 \mu\text{m}$, with 10 one micron slices in that cube, Fig. 4D). In 1 mL, there are 10^9 volumes that size (10^{-9} mL), but since only 47.5% of the tissue is mesohyl with microbes, then

there would be an estimated $650 \times 10^9 \times 0.475 = 3.1 \times 10^{11}$ microbes in each mL of sponge tissue (including spicules, aquiferous canal spaces, and feeding chambers). If amoeboid cells take up 2.5% of the sponge volume, then approximately $25 \mu\text{L}$ of each cm^3 is made up of amoeboid cells. Assuming each cell is approximately $10^{-8} \mu\text{L}$ in volume (Fig. 4), then there are 2.5×10^9 amoeboid cells per cm^3 . If each of these cells phagocytoses five microbes, then 1.25×10^{10} microbes are being consumed at any given moment in 1 cm^3 or 4% of the total number of microbes. Using a conversion rate for planktonic bacteria of 30.2 fg carbon and 5.8 fg nitrogen per cell (Fukuda et al. 1998), we calculate that 30 μmol carbon and 5 μmol nitrogen are consumed in a mL of tissue at any given time.

Table 5. Comparison of metabolic activity in sponges.

	Temperature at habitat (°C)	Specific filtration rate	Specific respiration rate		Reference
		(mL min ⁻¹ ± SE) per mL tissue	(nmol O ₂ min ⁻¹ ± SE) per mL tissue	per g dw	
<i>Neogombata magnifica</i>	23	10.5 ± 1.4	37.3 ± 4.6	248	1
<i>Theonella swinhoei</i>	—	2.6 ± 1.8	23.4 ± 13	—	2
<i>Aplysina (Verongia) fistularis</i>	26–28	7.4 ± 0.4	79.09 ± 25.32	—	3
<i>Dysidea avara</i>	12–22	1.0 ± 0.8	—	161.52	2, 10
<i>Halichondria panicea</i>	14	2.7 ± 1.1	32.75	468.93	7, 8
<i>Cliona delitrix</i>	28	6.5 ± 0.33	13.8 ± 6.3	—	5
<i>Callyspongia vaginalis</i>	28	18.8 ± 3	52 ± 16.5	—	5
<i>Tethya californiana</i>	9	1.5 ± 0.112	3.8 ± 0.67	—	5
<i>Haliclona mollis</i>	9	2.1 ± 0.17	5.17 ± 1.33	—	5
<i>Neopetrosia problematica</i>	9	4.67 ± 0.83	6.33 ± 1.16	—	5
<i>Geodia barretti</i>	8	0.26 ± 0.001	5.34 ± 0.98	23.08 ± 4.13	9
<i>Aphrocallistes vastus</i>	9	3.0 ± 0.54	1.61 ± 0.29	—	6

(1) Hadas et al. (2008); (2) Yahel et al. (2003); (3) Reiswig (1981); (4) Coma et al. (2002); (5) Ludeman et al. (2017); (6) Leys et al. (2011); (7) Riisgård et al. (1993); (8) Thomassen and Riisgård (1995); (9) This work; (10) Ribes et al. (1999).

Discussion

Little work has addressed in detail the carbon and nutrient budgets of deep-water sponges. Working on them *in situ* is difficult without powerful remote operated vehicles, and maintaining them in tanks requires excellent flow-through seawater facilities. We have been able to collect *G. barretti* from deep fjords adjacent to a well-equipped marine facility and keep them in high flow rates of water collected from the deep fjord. Under these conditions, we found that compared to most sponges *G. barretti* has a low specific filtration rate (0.26 mL min⁻¹ mL⁻¹sponge tissue) and low respiration rate (5.34 ± 0.98 nmol O₂ min⁻¹ mL⁻¹ sponge tissue) (Table 5). All sponges from cold water share a similarly low specific respiration rate, and the lowest respiration rate is that of the deep-sea glass sponge (Hexactinellida) *Aphrocallistes vastus* (Table 5).

Metabolism of HMA sponges

Despite living in the boreal deep at temperatures of 8–9°C *G. barretti* has a carbon budget almost identical to that measured for the tropical reef sponge *Theonella swinhoei* (Yahel et al. 2003). Both sponges removed 11–12% of the total carbon they filtered (~ 10 μmol L⁻¹); POC removal was efficient for both (up to 95% and 99%, respectively). The small difference in amount removed (2 μmol C L⁻¹ for *T. swinhoei* and only 0.5 μmol C L⁻¹ for *G. barretti*) may reflect the difference in plankton abundance in shallow and deep water since both sponges remove more bacteria when more are available. It is also possible that our calculation of DOC underestimates the particulate carbon and pico-plankton captured by *G. barretti* since we calculated DOC as the portion of TOC

that is not bacteria, and although we could detect no other plankton cells by microscopy, we cannot rule out that detrital cells do not form a minor portion of *Geodia's* budget.

Other shallow water HMA sponges show similar efficiencies at removing POC and DOC (Mueller et al. 2014) as first proposed by Reiswig (1981). Not all species that remove DOC have large numbers of endosymbionts (e.g., *Cliona delitrix*, Mueller et al. 2014) but DOC is generally considered to be taken up by symbionts and translated into biomass that is transferred to the sponge in other ways. Electron micrographs of *G. barretti* show surprising numbers of symbiont microbes being phagocytosed by amoeboid cells in the mesohyl. We estimate amoeboid cells consume about 10¹⁰, or 4% of the symbionts at any one time. Interestingly, Jimenez and Ribes (2007) suggested that to balance the nitrogen budget of the sponges they studied (*Dysidea avara*, *Agelas oroides*, and *Chondrosia reniformis*) the sponges would have to consume 1–10% of the microbial symbionts each minute. This number was considered too high to be plausible, yet our calculations suggest *G. barretti* mesohyl cells consume that many, and possibly more, because in many cases many more than five microbes were counted being consumed by a single cell in a 60 nm thick EM section. The uptake of DOC therefore would be translated into replication of symbiont microbes for phagocytosis by the sponge.

Metabolism of deep-water sponges

Despite the similarity in carbon budgets, the specific respiration and filtration rates of *G. barretti* were 10-fold lower than those of *T. swinhoei*. The lower specific respiration and filtration rates are likely dictated by water temperature and

habitat because similarly low rates are found in the glass sponge *A. vastus*. However, *A. vastus* has a complete absence of microbial symbionts in its tissues and whereas *G. barretti* removes 15.1 μM oxygen L^{-1} filtered (similar to *T. swinhoei* 3–15 μM oxygen L^{-1}) *A. vastus* removes a mere 0.5 μM oxygen L^{-1} (Leys et al. 2011; Kahn et al. 2015).

The contrast between the metabolism of HMA and LMA sponges is seen clearly in these two species but has been known since Reiswig's seminal studies (Reiswig 1971; Reiswig 1975; Reiswig 1981). For *A. vastus*, uptake of POC forms the total carbon ingested (Yahel et al. 2007). POC, here bacteria alone, more than meets the energy required for filtration by *A. vastus* (Yahel et al. 2007). In contrast, although in *G. barretti* up to 99% of bacteria were removed from water filtered, these provided only 5% of the energy required for filtration (0.5 μmol L^{-1} bacterial carbon is ingested), the rest came from detritus or DOC (10.45 μmol L^{-1} total carbon ingested), and these together meet the requirements of filtration (10.79 μmol L^{-1} carbon consumed in filtration). Considering that *in situ* measurements from the Trondheimsfjord show DOC at 200–400 m depth to be 76 μmol L^{-1} (Børshheim et al. 1999), and we calculate ambient DOC to be 80 μmol L^{-1} , we infer that little carbon comes from detritus. We found no evidence for other plankton using flow cytometry analysis, although viruses were not investigated. Viruses are abundant in the ocean (Suttle 2007), but their nutritional significance is unknown.

The nitrogen budget also differed from *A. vastus* but showed some similarity with other HMA species. Using direct sampling, we measured a removal of ammonium by *G. barretti* (11.6 nmol mL^{-1} sponge tissue d^{-1}) and also of nitrite (79.2 nmol mL^{-1} sponge tissue d^{-1}) with a production of nitrate (982 nmol mL^{-1} sponge tissue d^{-1}). The production of nitrate we measured using direct sampling was higher than values reported previously for *G. barretti* (410 nmol L^{-1} d^{-1} , Radax et al. 2012a), but not only did those authors report a huge range in production/removal of ammonium and a net production of nitrite (150 nmol mL^{-1} sponge tissue d^{-1}), the lower values they report may reflect differences caused by the use of closed chamber systems and explants. Jimenez (2011) found that closed systems cause large differences in uptake and removal of dissolved nutrients, the differences between the numbers we report and those of Radax et al. (2012a) could be attributed to use of the incubation method in those studies. The same reasoning may be applied to the slightly lower numbers obtained for other HMA sponges including *D. avara* and *C. reniformis* (218 nmol L^{-1} d^{-1} and 319 nmol L^{-1} d^{-1}) (Hoffmann et al. 2009; Schläppy et al. 2010a). Analysis of metatranscriptomics of the *G. barretti* sponge microbiome community showed a range of nitrifying microbes in the Nitrospirae and ammonia oxidizing microbes (Thaumarchaeota) as well as a large number of sponge-specific species (Radax et al. 2012b), and fluorescent *in situ* hybridization has demonstrated their presence throughout

the tissues. It would be interesting to combine these methods to determine whether the sponge cells preferentially harvest particular symbionts.

Use of DOC and control of microbial replication

Reiswig (1981) used estimates of oxygen requirements of replication for oceanic bacteria to calculate that microbial symbionts in *Aplysina* (*Verongia*) *fistularis* could not be in exponential growth phase because the tissue was not sufficiently oxygenated. He argued that the microbes would be held in "starvation" conditions. *G. barretti* has a far lower specific respiration rate even than *Aplysina* (Table 5) and so perhaps the symbionts are also maintained in a "starvation" state by the control of oxygenation. Yet measurements of "tissue" oxygen for the HMA species *D. avara* and *C. reniformis* suggest that typically the tissues are well-oxygenated when the sponge is pumping (Schläppy et al. 2010b). Although there have been suggestions that *G. barretti* tissues become anoxic (Hoffmann et al. 2009) and our own experiments show that although tissues can go anoxic quickly when the sponge reduces its filtration, generally we find that when the sponges are undisturbed, they pump constantly for extended periods of time, and tissues are well oxygenated (SPL, ASK, RJB unpubl.). Although it has been shown that *G. barretti* can also carry out anaerobic ammonium oxidation, it is unclear how often that occurs. It seems likely that *G. barretti* maintains a balance in its tissues between aerobic and anaerobic states by activity level, reducing pumping (filtration) and thus reducing DOC uptake and at the same time deoxygenating its tissues. Symbiotic microbes could undergo various forms of metabolism depending on oxygen concentration, and the sponge then could harvest the symbionts as they are replicating. This is the first example of 'microbial farming' in a sponge and it is very similar to that done by hydrothermal vent species.

Sponge vs. microbial activity

The high concentrations of DOC in ambient water and the efficiency of uptake of DOC makes it appear as if, in contrast to the glass sponge *Aphrocallistes vastus*, *G. barretti* lives in an abundance of food. However, we found it was difficult to distinguish between the budgets of sponge filtration (pumping) and that required for microbial activity (fixation/nitrification), that is, separating the sponge's tissue activity and oxygen needs from that of the holobiont. Our estimates from electron micrographs suggest that the sponge tissues (canal epithelia, choanocyte chambers and mesohyl cells) comprise approximately 5% of the holobiont volume. Since the bacteria captured accounted for only 5% of the total energy budget we wondered whether *G. barretti* relied largely on bacteria filtered for its daily energetic needs for pumping with the remainder (that taken up by phagocytosis of symbionts or leaked from symbionts to the sponge) used for growth and reproduction. The rationale behind this hypothesis is that in two experiments in which we incubated either

small sponge explants or whole small sponges in the uridine analog EdU, we did not see production of new cells (this data is therefore not shown). This suggests to us that cells are not replaced easily, and therefore that a minimal amount of energy was put into filtration and replacement of the choanocyte filter. However, our EdU work was challenged by the difficulty of incubating such large animals in sufficient volume of labeling solution for long enough to label, and by the difficulty of locating the small choanocyte chambers in histological sections (e.g., Fig. 4). The other reason for thinking *G. barretti* may need all the particulate carbon it can obtain is the appearance of a well-placed gasket around each collar filter, which would prevent any loss of bacteria. Although some authors have suggested sponges possess channels that might bypass the filter, our experiments show no bypass canals exist. Altogether the gasket on the sponge collar, the nearly 100% filtration efficiency and absence of bypass canals found indicate that capturing bacteria is important for *G. barretti*, and yet POC still only forms 5% of the total budget.

Other sponges have a gasket made either of cells or glycolyx mesh (Weissenfels 1992; Adams 2010). Another possibility is that the tightness of the filter may not be only important to capture bacteria but rather in this sponge, it might function to slow the flow thereby allowing sufficient time for the exchange of oxygen. A less restrictive canal system would offer less resistance and so cost less energy to draw water through, but might not allow sufficient time for gas exchange and therefore reduce the oxygen available for the symbionts. Under this scenario, there must therefore be a balance between the size of the sponge and the length and narrowest dimensions of the aquiferous system required for the number of pumps to draw water through the sponge, to maintain a cost-effective filtration system.

Unquantified aspects of the budget

A complete budget would include both growth and reproduction, and although it has been possible to recollect the same individuals yearly, so far there is insufficient data to measure growth rates. Also, although we saw no evidence of reproductive structures in the specimens we collected, nor did we see signs of spawning, *G. barretti* is known to produce gametes from February to May and in some populations also again in September, and spawning has been found in June and September (Spetland et al. 2007). In that work both oogenesis and spermatogenesis took place over several months and neither oocytes nor sperm were considered to arise from choanocytes, so normal filtration would not be disrupted by spermatogenesis. Nevertheless, nutrient requirements are expected to be increased during those periods, because spawning occurred directly after peak phytoplankton blooms and carbon fallout in fjord waters (Spetland et al. 2007), and so respiration rates may differ seasonally. Given the known seasonality of *G. barretti*, in order to

properly understand the total carbon budget of populations in the North Atlantic it would be best to determine respiration and filtration rates during different months of the year, and in particular *in situ*, although that work is obviously logistically difficult.

Effect on oceanographic conditions

Under normal conditions, *G. barretti* is well-ventilated to supply oxygen and DOC/N to its symbionts. Extraction of particulate and dissolved carbon and nutrients implies that fields of this species could significantly affect the overlying water. From oxygen consumption in closed respiration chambers Kutti et al. (2013) reported use of $10.6 \pm 0.9 \mu\text{mol O}_2 \text{ d}^{-1} \text{ g}_{\text{ww}}^{-1}$ and estimated a potential removal of 60 tonnes of carbon daily for a 300 km² marine protected area. Our work on sponges kept in flow-through aquaria with water from natural depths suggest a slightly lower specific respiration ($7.68 \pm 1.44 \mu\text{mol O}_2 \text{ d}^{-1} \text{ g}_{\text{ww}}^{-1}$, range 0.96–24.96 $\mu\text{mol O}_2 \text{ d}^{-1} \text{ g}_{\text{ww}}^{-1}$) although within the range of that reported previously (Kutti et al. 2013; Kutti et al. 2015). Our direct measures of *G. barretti* filtration in flow through tanks show similar excurrent velocities to those measured previously in closed chambers in the laboratory and estimated from video of dye flow applied *in situ* using the remote operated vehicle. Our data shows an average volumetric flow rate of $17.6 \pm 4.7 \text{ L h}^{-1}$ ($N=17$) and so a total volume of water processed of $347.0 \pm 93.0 \text{ L d}^{-1} \text{ kg}_{\text{ww}}^{-1}$, compared to $260 \pm 120 \text{ L d}^{-1} \text{ kg}_{\text{ww}}^{-1}$ and $600 \pm 140 \text{ L d}^{-1} \text{ kg}_{\text{ww}}^{-1}$ (field and lab experiments, respectively) estimated previously (Kutti et al. 2013). The differences arise from the method of estimation of filtration (clearance or dye flow, vs. acoustic Doppler velocimeters) and the dimensions of the oscula measured in the lab and via video *in situ*. Nevertheless, our measurements confirm that *G. barretti* populations have a substantial effect on water column properties.

These removal rates are dependent on oxygen-rich conditions of these seas. The interplay between sponge requirements and symbiont requirements make this sponge-microbiome complex both robust in enabling it to live in unusual deep-sea habitats low in particulate carbon, but potentially weak in the face of unexpected changes in oxygenation of deep continental shelf waters.

Conclusions

Geodia barretti is a massive, HMA sponge that forms dense communities at 200–600 m depths in the boreal North Atlantic. Our calculations suggest that it has some 2.9×10^{11} microbes per milliliter of sponge and these drive its metabolism. The sponge has up to 99% retention efficiency for the ambient bacteria it filters, but those ingested account for only 5% of the sponge holobiont's total carbon needs; DOC makes up the rest of the total carbon taken up. *G. barretti* takes up ammonium and nitrite from the surrounding water and releases nitrate. It has low specific excurrent flow

rates and low specific respiration rates. The sponge produces an excess of nitrogen as NO_3^- ; its carbon requirements for metabolism just balance and phagocytosis of microbial symbionts accounts for a large portion of that budget.

Data availability statement

Data is available at the University of Alberta Education and Research Archive: <https://doi.org/10.7939/R3057D48V>

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Acknowledgments

We thank the director and staff of the Institute of Marine Research (Havforskningsinstituttet) at Austevoll and Bergen, Norway for use of facilities, the pilots of the ROV *Aglantha* and Captain and crew of the RV *Håkon Mosby* for careful collection of sponges. L. F. Lunde, M. Petersen, and J. S. Møgster analyzed the nutrient samples. G. Braybrook and N. Gerein provided technical assistance with SEM. We also thank N. Lauzon and C. M. Krogness for help with field and lab experiments, and J. de Goeij and G. Yahel and an anonymous reviewer for helpful discussions and comments. Funding was provided by the Norwegian Research Council (Grant 203894 and 225283) awarded to RJB and the University of Alberta.

Conflict of Interest

None declared.

Submitted 21 December 2016

Revised 27 May 2017

Accepted 12 June 2017

Associate editor: Rebecca Vega Thurber