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In situ feeding and metabolism of glass sponges (Hexactinellida, Porifera) studied in a deep temperate fjord with a remotely operated submersible

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Abstract

Glass sponges are conspicuous inhabitants of benthic communities in the cool waters of the Antarctic and north Pacific continental shelf. We used an ROV outfitted with a new device for simultaneous sampling of water inhaled and exhaled by the sponges to provide the first data on the nutritional ecology and metabolism of two glass sponge species in their natural deep-water habitat (120–160 m). *Aphrocallistes vastus* and *Rhabdocalyptus dawsoni* were found to be mostly bacteriovores, removing up to 95% of the bacteria (median removal was 79% for both species) and heterotrophic protists (<10 μm) from the water they filter. The relatively scarce microbial cells were efficiently selected from a 'soup' of suspended clay and detritus particles (microorganisms accounted for $\sim$1% of the total ambient suspended solids). Removal of planktonic microorganisms (2.2 ± 1.3 μmol carbon [C] C L^{-1} and 0.37 ± 0.17 μmol nitrogen [N] L^{-1}) accounted for the entire total organic C uptake and ammonium excretion by both species, with no evidence for dissolved organic uptake. Similar results were obtained in laboratory experiments in which dissolved organic C was directly measured. Despite the massive siliceous sponge skeleton, silica uptake was below detection levels (0.28 μmol L^{-1}), supporting previous suggestions of low growth rates in Hexactinellida. Reported mean sponge abundances of >1 individual m^{-2} indicate that the sponge filtering activity may significantly affect the deep microbial community and benthic–pelagic mass exchange in some north–east Pacific fjords.

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Shallow-water benthic suspension feeders have an important role in the functioning of coastal and freshwater ecosystems (Gili and Coma 1998; Thorp and Casper 2003). The feeding activity of these animals can control the water column properties in shallow bays (Cloern 1982), rivers (Strayer et al. 1999), and fjords (Riisgård 1998), either directly (through grazing on the plankton community) or indirectly (by intensifying nutrient recycling or by selective removal of a specific planktonic component) (Thorp and Casper 2003). These activities are tightly coupled with hydrodynamic processes.

Whereas the nutritional ecology of shallow suspension feeders has been well studied, equivalent studies of the nutritional ecology of deep-dwelling suspension feeders (below scuba depth) are scarce (Roberts and Hirshfield 2004) and rely largely on descriptive anatomy and in vitro experiments (e.g., Fiala-Medioni et al. 1986; Witte et al. 1997; Pile and Young 1999). Our understanding of the

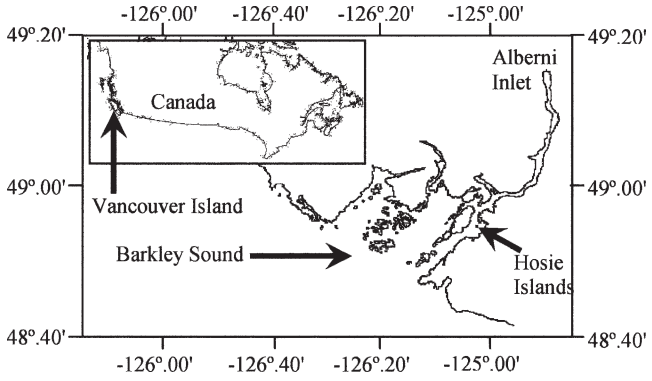

Fig. 1. Map of the study site at Hosie Islands, Barkley Sound.

diet composition and in situ metabolism of deep-dwelling suspension feeders is limited.

Glass sponges (Porifera, Hexactinellida) are common members of deep-water suspension feeding communities (Conway et al. 1991; Hughes and Gage 2004). One of the world's highest abundances of sponges occurs in fjords and continental shelf waters of the northeast Pacific (Leys et al. 2004). This region covers thousands of kilometers along the British Columbia and Alaska coastline, including several hundred fjords, many with sills that trap deep ocean water. The oceanography and planktonic and benthic communities of many of the Canadian fjords are well described (Pickard 1963; Levings et al. 1983; Leys et al. 2004 and references therein). Conspicuous inhabitants on the fjord walls are the meter-sized glass sponges (Leys et al. 2004). Outside of the fjords, at 160–240-m depths on the continental shelf, these species form the world's only present-day sponge reefs (Conway et al. 1991, 2001, 2004). During the Jurassic, similar sponges constructed vast bioherms that covered substantial parts of the north Tethys Sea (Ghiold 1991; Krautter et al. 2001).

Our objective was to study in situ the metabolism, diet composition, and waste production of two species of glass sponge, *Aphrocallistes vastus* Schulze (1886) and *Rhabdocalyptus dawsoni* (Lambe 1892), that dominate deep benthic communities in north–east Pacific fjords.

Methods

Study site—Barkley Sound is a large embayment (~550 km^2) on western Vancouver Island in British Columbia, Canada (Fig. 1). The summer thermocline is relatively weak (<3°C); however, sharp salinity gradients and strong stratification are maintained throughout the year in the upper 10–20 m by a large freshwater input, primarily in the inner sound and near the mouth of Alberni Inlet (Taylor and Haigh 1996). Turbidity is highest at the surface (680 nm, transmissivity <20% m^{-1}) and below the pycnocline (transmissivity <35% m^{-1}). Diatoms dominate the microplankton community throughout the summer, with an integrated mean biomass of 0.7–7 g carbon (C) m^{-2} (Taylor and Haigh 1996).

The pale, giant forms of the 'cloud' sponges *A. vastus* and *Heterochone calyx* are the most conspicuous feature of the fjord wall community, although 'boot' sponges *R. dawsoni* (up to 1 m in length) are also present (Fig. 2). Sponge distribution was patchy (≤5 specimens m^{-2}), with the highest abundance encountered between 120 and 160 m in depth (hereafter called "the sponge belt"). Above and below this zone, sponge abundance declined, with only *R. dawsoni* reaching the shallowest depth at ~20 m. The fjord walls also contain brachiopods and 40 other mega-fauna taxa in 27 orders.

Underwater work—The Canadian remotely operated vehicle (ROPOS; www.ropos.com) was used for in situ sampling. The ROV remained stationary during sampling on the vertical fjord walls by way of a bumper bar mounted above, and an extension box containing the SIP samplers mounted below. Four 2.5-liter Niskin bottles (General Oceanics) were attached to the upper bumper in a position to sample water close to the wall. The intake of a conductivity, temperature, and depth sensor (CTD; SBE19plus) equipped with a chlorophyll fluorometer (WETStar, Wetlabs) and a colored dissolved organic matter (CDOM) fluorometer (Wetlabs) could be positioned by the robotic manipulators at a sampling point of choice.

The in situ work was undertaken at 120–160-m depth along vertical walls of a canyon near Hosie Islands (48°54.261′N; 125°2.636′W, Fig. 1), where the deep Trevor Channel (maximum depth, 180 m) meets the mouth of Alberni Inlet. Seven sampling dives were carried out between 29 June and 03 July 2004. Each dive involved a horizontal transect toward the wall and sampling of water (in- and excurrent) and tissue from three to six sponge specimens. Sponges were selected by the ROPOS pilot based on accessibility. Whereas the initial approach sometimes stirred up the sediment, this cleared within a few minutes, and the ROPOS could be maintained in a stationary position for more than an hour without any detectable effect on turbidity or the near-wall flow (confirmed using fluorescein dye).

Cross-wall transects: Each dive began with a descent to the depth of the sponge belt. Once the wall was detected by sonar, a slow approach was made toward the wall and a Niskin bottle was triggered at ~80 m, 5 m, and 0.75 m from the wall. The vehicle was stopped at 75, 40, 20, 10, and 5 m for 1–2-min recordings with the CTD-CDOM fluorometer system running in real time. The time lapse from system inlet to sensors was measured in situ by dye injection next to the tube inlet. Near-wall (1-, 0.5-, and 0.1-m) CDOM samples were measured at least 20 min after landing. A single SIP sampler (Fig. 2A; for complete description, see Web Appendix 1 http://www.aslo.org/lo/toc/vol_52/issue_1/0428a1.pdf) with no prefiltration was used to collect a water sample at 0.1 m off the wall.

In situ sampling of sponge feeding, element uptake, and excretion: We measured directly the efficiency by which the glass sponges remove (or discharge) substances from (to) the water they filter. The pumping activity of each specimen sampled was first visualized by releasing fluorescein dye at the base of the specimen and then observing it

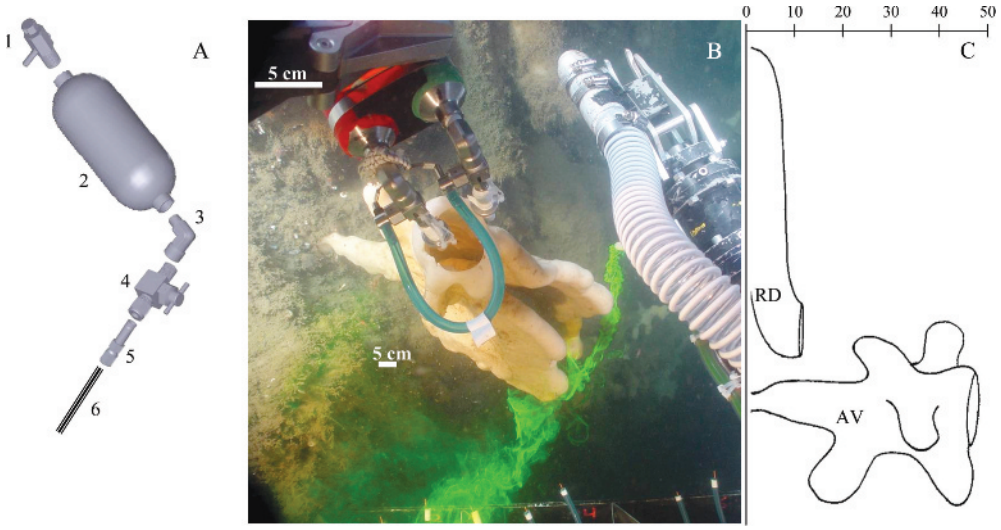

Fig. 2. In situ sampling of the water inhaled and exhaled by *Aphrocallistes vastus* at 160 m in depth. (A) The basic components of the SIP water sampler: (1) bleed valve; (2) double-ended sample cylinder; (3) street elbow; (4) quarter-turn plug valve; (5) reducer; and (6) inlet PEEK tube with external diameter of ~1.59 mm and internal diameter of ~510 μm; *see Web Appendix 1* for more details. (B) A SIP sampler is positioned by the ROPOS arm with the inlet of one sample cylinder within the osculum of the sponge, while the other arm squirts dyed seawater at the base of the sponge to visualize its pumping. The lower bumper of the ROV can be seen at the bottom of the image with the tips of five more samplers in the box. Note that the depth of the picture is ~1 m; hence, the two scale bars. The sampled sponge extends about 0.5 m off the wall and its osculum is approximately 8 cm in diameter. (C) An illustration of the profile of each sponge species against the fjord wall. AV, *A. vastus*; RD, *R. dawsoni*. Distance (cm) from the wall face is indicated along the top.

flow from the sponge osculum (Fig. 2B); then the water inhaled and exhaled by the sponge (incurrent and excurrent) was sampled simultaneously using a pair of specially developed SIP water samplers. The difference in concentrations of a substance (e.g., bacteria) between a pair of samples provides a measure of the retention (or production) of the substance by the animal (Yahel et al. 2005). An illustration of the type of data obtained by our method is given in Web Appendix 1 for ammonium excretion. In seven 8-h dives, 32 successful paired samples, out of a total of 42 (10 discarded as a result of ROPOS movement), were collected from 16 specimens of A. vastus (successful duplicate samples were obtained from 11 specimens) and five specimens of R. dawsoni. Real-time measurements of CDOM concentrations in the water inhaled and exhaled by the sponges were obtained by holding the intake of the CDOM fluorometer either within (excurrent sample) or outside (incurrent sample) the sponge osculum for 2 min. A piece of tissue was sampled from each animal by the end of the sampling session to verify its taxonomic identity.

SIP design and operation—We used a simple and inexpensive point sampler (SIP, see Fig. 2A and Web Appendix 1 for a detailed description). SIP pairs consisted of two stainless-steel sample cylinders (150 mL each) attached to a common handle. Each of the cylinders had a quarter-turn valve, and the two valves were joined by a common trigger that was normally kept closed by an elastic cord. A valve at the lower end of each cylinder allowed drainage. The paired cylinders were cleaned meticulously and flushed with helium gas at atmospheric pressure to remove oxygen and water residue. The cylinders were carefully positioned with one inlet inside the sponge osculum and the second positioned outside of the osculum adjacent to the inhalent surface. Both valves were opened simultaneously using the second ROPOS manipulator. Opening the valves allowed the external pressure difference (~1,500 kPa) to force the sampled water into the cylinder. The suction rate was ~1 mL min^{-1}, which is over an order of magnitude less than the excurrent flow from the sponge. No significant difference was found between the SIP and the Niskin water samples collected 2 m off the wall for any of the measured biological or chemical properties (doubly multivariate repeated-measures test, Wilks' $\lambda = 0.51$; $F_{5,1}$; $p = 0.929$).

Tank experiments—Whole live sponge specimens were kept in darkened tanks with flow-through seawater pumped from Bamfield Inlet (25-m depth, 2 m above bottom; see Yahel et al. in press). The water inhaled and exhaled from each sponge was repeatedly sampled via capillary tubes that were positioned in and outside of the osculum (Reiswig 1975; Yahel et al. 2005). A slow suction rate (~1 mL min^{-1}) insured no contamination of exhaled water with ambient water (Yahel et al. 2005), and pumping activity was verified with fluorescein-dyed seawater between samples.

Water processing—Within 2 h of retrieval of the submersible, SIP samplers were transferred to a clean laboratory. Both the inlet tube and the bleed valve were cleaned with about 5 mL of the sampled water by relieving the pressurized helium. The inlet tubing was replaced with a small, clean, stainless-steel nozzle that was rinsed with an additional 10 mL of the sample water, and aliquots were drawn for the different analytical procedures.

Ammonium measurements were carried out immediately following each dive according to the fluorometric method of Holmes et al. (1999). A standard calibration curve (five points in double distilled water), background fluorescence, and the matrix effect were determined separately for each dive using water collected in a Niskin bottle sampled 0.75 m from the wall.

Samples for analysis of silicic acid (dissolved silicate), phosphate, and nitrate and nitrite (hereafter called nitrate or NO_3, since the contribution of nitrite is trivial in deep oxygenated waters) were determined in duplicate using standard methods, as described by Barwell-Clarke and Whitney (1996). Samples were stored at 4°C and analyzed within 14 d of collection.

Samples for total organic carbon (TOC) were collected directly into precombusted 10-mL glass ampules, acidified with concentrated trace-metal–grade hydrochloric acid (final concentration, 0.1%), and stored at −20°C. TOC was measured in duplicate using high-temperature catalytic oxidation on a TOC-V total organic carbon analyzer. International Consensus Standard was analyzed for every 10 samples, as recommended by Sharp et al. (2002) (*see Web Appendix 1 for details*).

Total suspended solids (TSS, measured gravimetrically), particulate organic carbon (POC), and particulate nitrogen (PN) were analyzed only from the Niskin bottle samples. Sample water was filtered onto precombusted GF/F filters and stored at −80°C until analysis. Prior to POC and PN analysis, filters were dried at 65°C, acidified for 12 h by hydrochloric acid fumes, dried again, and packed into precombusted (850°C, 1-h) nickel sleeves. The samples were analyzed on an Exeter Elemental Analyzer (Control Equipment Corporation 440) standardized with acetanilide. Results were corrected for filter blanks.

For counts of free-living bacteria, duplicate 1.6-mL water samples were preserved using 0.4% glutaraldehyde (final concentration) and stored in liquid nitrogen until analysis by a flow cytometer (FACSCalibur, Becton Dickinson), as described by Marie et al. (1999). The specific parameters used are provided in Web Appendix 1.

For quantification of heterotrophic protists, 50 mL of the remaining water was preserved with 0.75% formaldehyde for microscopic analysis using 4′,6-diamidino-2-phenylindole dilactate (DAPI; D-3571, Molecular Probes), as described by Sherr and Sherr (1993) (*see Web Appendix 1 for details*).

A semiquantitative analysis of the elemental composition of the particulate matter in the water inhaled and exhaled was carried out on a subset of five unfiltered, paired water samples obtained from *A. vastus* using scanning electron microscopy (SEM) and energy dispersive X-ray microanalysis (EDX; Leppard et al. 1997). Fifty milliliters of the sample waters were fixed with 4% osmium, filtered through a 0.45-μm Millipore filter membrane, rinsed five times with 70% ethanol, air-dried (while covered to prevent accumulation of dust), and gold coated. Each filter was scanned at low magnification, and for each filter, 10 particles were selected at random and examined at high magnification. The presence of osmium was used to determine the presence of lipids (a proxy for organics; Leppard et al. 1997).

Results

Ambient water characteristics—Water below the photic zone experiences a near-permanent pycnocline (Fig. 3A) and stable environmental conditions throughout the year. The annual cycle of oceanographic conditions (Fig. 3B) was represented from data from Trevor Channel covering the period from 1954 to 2005 (http://www-sci.pac.dfo-mpo.gc.ca/osap/data/default_e.htm). Temperature varies by less than 3°C (7.5–10°C), and salinity ranges from 32.0 to 33.5. Oxygen levels are generally <75% of saturation, but from spring to fall oxygen often goes below 30% saturation (2 mL L^{-1}). In contrast to the photic zone, the region with the highest density of sponges (the sponge belt) had high levels of dissolved silicic acid (>55 μmol L^{-1}). This zone was also characterized with variable tidal currents (sometimes up to 1 m s^{-1}), with a semidiurnal periodicity, high turbidity (transmissivity <35% m^{-1}), and a high load of suspended solids (TSS, >7 mg L^{-1}; Table 1). Organic matter concentrations were low, with POC < 0.1 mg L^{-1} (<8.3 μmol L^{-1}) and DOM < 0.75 mg L^{-1} (<63 μmol L^{-1}; Table 1).

Both living and decaying phytoplanktonic cells were extremely rare in the water of the sponge belt. This was confirmed by flow cytometry and microscopic analysis as well as by the zero chlorophyll fluorescence measured throughout the study. As indicated by SEM-EDX analysis, most (64%) of the suspended particles of >5 μm were inorganic, composed of aggregated clays; a few were associated with diatom frustules and organic debris. Epifluorescence microscopy indicated that bacteria were attached to many of the aggregates. Of the particles analyzed by SEM-EDX, 96% contained aluminum or silica, and pieces of empty diatom frustules were common (20%). The organic fraction of the seston was characterized by a high proportion of living cells and high nitrogen content (C : N ratio 7.2; Table 1). Free-living bacteria (843 ± 224 × 10^3 cells mL^{-1}) and ultraplankton (<10 μm, 3 ± 4 × 10^3 cells mL^{-1}) accounted for about 40% of the POC (Table 1). While bacterial abundance was relatively homogeneous (Lloyd's Patchiness index, 1.0), the abundance of pico- and nano-heterotrophic protists was patchy (Lloyd's index, 2.2 and 3.5, respectively; Table 1). The lack of significant difference between the TOC concentrations in 2-μm filtered and nonfiltered samples (*t*-test, $p = 0.780$, $n =$ 12 and 16, filtered and nonfiltered samples, respectively) indicates that most of the POC was smaller than 2 μm. The concentrations of dissolved inorganic N and phosphorus (P) at the sponge habitat were also high: 30.2 and 2.6 μmol L^{-1} for nitrate and phosphate, respectively.

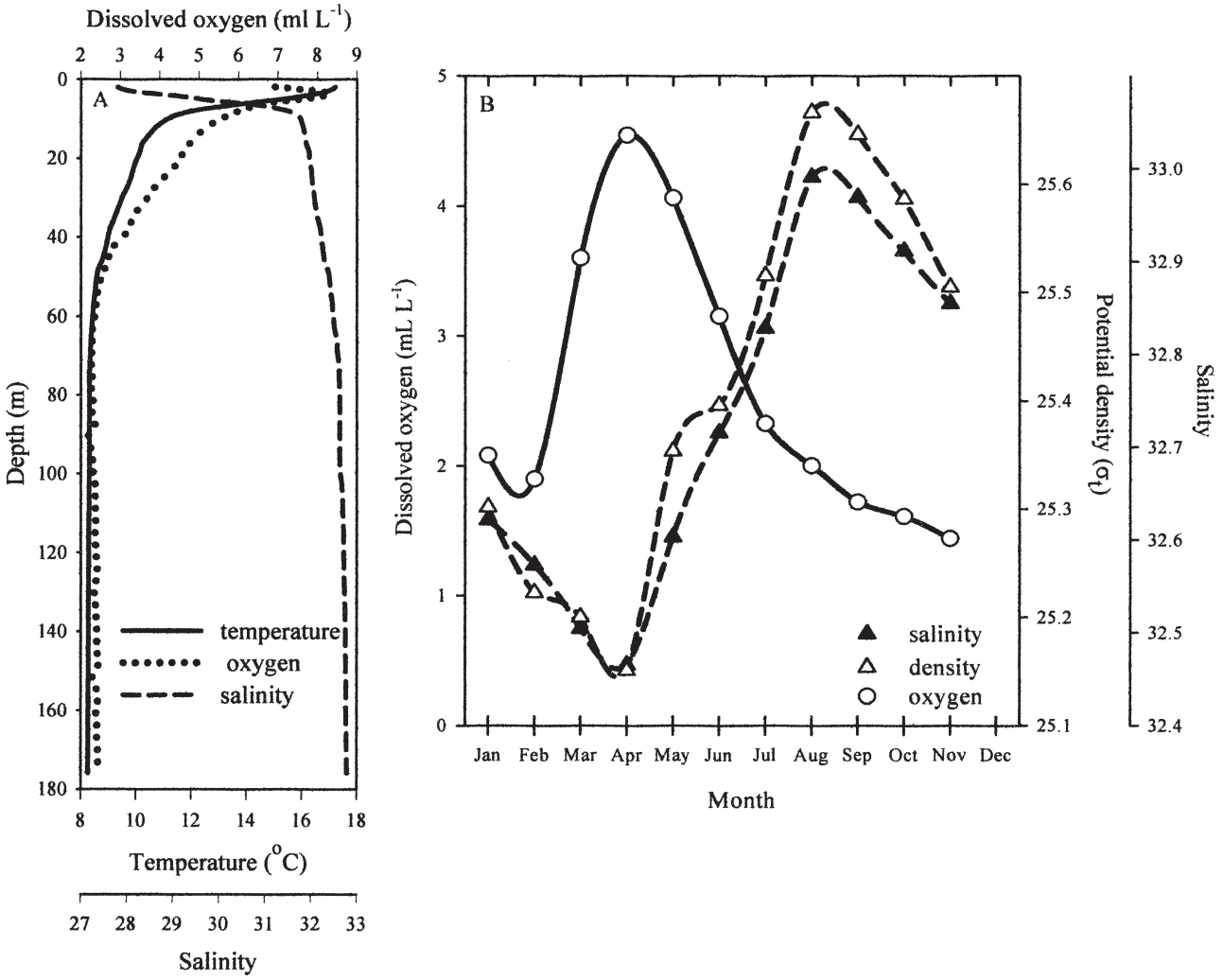

Fig. 3. (A) A profile of the water column properties at the study site (05 July 2004). Data (courtesy of R. Pawlowicz, University of British Columbia) were collected using a Seabird CTD (SBE19plus) and a Beckman/YSI oxygen sensor. (B) Annual cycle of the oceanographic conditions at the sponge habitat (125 m in depth) compiled from hydrographic profiles measured by the Institute of Ocean Sciences (IOS) between 1954 and 2005 within a box delineated by 48°50′N and 49°0′N, 125°0′W and 125°5′W. Error bars were omitted for clarity. No data were available for December.

Uptake and excretion of material by sponges—Bacteria: Glass sponges removed up to 95% of the free-living bacterial cells in each of the 31 paired water samples analyzed by flow cytometry (Fig. 4A,B). Median removal efficiency was 79% for both sponge species (average 74% ± 17% for *A. vastus* and 68% ± 29% for *R. dawsoni*). The number of cells removed increased linearly with increasing cell abundance (0.4–1.4 × 10^6 cells mL^{-1}), with no indication of either a threshold or saturation (Fig. 5E). The slope of the regression of removed over inhaled concentrations of bacteria by *A. vastus* was highly significant ($y = [0.83 \pm 0.17]x$; $R^2 = 0.79$, $p < 0.001$). The small number of *R. dawsoni* samples precluded meaningful regression analysis for this species.

The bacteria not retained by the sponges were larger (10% higher forward scatter), with a higher side scatter (31%) and higher nucleic acid content (10% higher green fluorescence) than the average bacterial population taken in by the sponges. This trend, suggestive of selective retention of smaller bacteria (*see Yahel et al. in press*), was highly significant for the side scatter for both species (two-way repeated-measures ANOVA, $F_{1,29} = 28.7$, $p < 0.001$), but only for *R. dawsoni* for nucleic acid content (Holm–Sidak pairwise multiple comparison, $p = 0.011$).

Heterotrophic protists: The distribution of heterotrophic protists was patchy, ranging from 0 to 10.5 × 10^3 cells mL^{-1}. A flagellum was detectable only in a small proportion of the cells. Where protists were present, they were removed with high efficiency by both *A. vastus* and *R. dawsoni* (up to 95% and 100%, respectively), and the median removal efficiency by *A. vastus* (80%; Fig. 6) was similar to that for bacteria. Removal of protists increased linearly with increasing cell abundance ($R^2 = 0.95$, $p < 0.001$), and the slope (0.83 ± 0.06) was similar to that for bacteria. However, the patchy distribution of these cells

Table 1. Average properties (±1 SD, median in parentheses) of the water inhaled by the glass sponges: (A) DAPI-stained ultraplankton size was measured using computerized image analysis and epifluorescence microscopy. Plankton (bacteria and heterotrophic protists) biovolume was converted to carbon (C) and nitrogen (N) using published conversion factors (*see Methods*); (B) plankton concentration (C) bulk suspended solids mass (<100 μm), its particulate organic carbon (POC) and particulate nitrogen (PN) content, and total organic carbon content (TOC, dissolved + particulate); (D) Average concentrations of dissolved inorganic silica, phosphorus, and reduced and oxidized nitrogen.

A) Plankton size	*n*	Length (major axis, μm)	Width (minor axis, μm)	Biovolume (μm^3)	Carbon content (fmol $cell^{-1}$)	Nitrogen content (fmol $cell^{-1}$)
Bacteria	17,748	0.8±0.4(0.8)	0.6±0.2(0.5)	0.15±0.19(0.08)	2.3±2.4(1.5)	0.36±0.46(0.18)
Pico-heterotrophs	6,062	2.1±0.7(2.0)	1.5±0.3(1.4)	2.7±1.8(2.0)	44±28(35)	5.6±3.6(4.3)
Nano-heterotrophs	3,501	5.9±3.3(5.1)	3.9±1.7(3.4)	78±137(30)	1,024±1,648(433)	128±206(54)

B) Plankton concentrations	*n*	Cell concentration (No. mL^{-1})	Carbon content (μmol L^{-1})	Nitrogen content (μmol L^{-1})	Lloyd's patchiness index
Bacteria	32	$843\pm224\times10^3(863\times10^3)$	1.9±0.5(2.0)	0.192±0.051(0.196)	1.07
Pico-heterotrophs	11	$2.0\pm3.1\times10^3(0.2\times10^3)$	0.1±0.1(0.1)	0.011±0.016(0.001)	2.26
Nano-heterotrophs	11	$1.0\pm1.2\times10^3(0.4\times10^3)$	1.0±1.0(0.7)	0.125±0.125(0.075)	3.46
Plankton total		$846\pm224\times10^3$	2.99±1.17	0.328±0.136	

C) Bulk properties	*n*	Total suspended solids (mg L^{-1})	Carbon content (μmol L^{-1})	Nitrogen content (μmol L^{-1})	C : N ratio
Total suspended solids	11	7.11±0.94(7.16)	7.6±1.3(7.4)	0.9±0.2(0.9)	7.2±0.5(7.2)
TOC	22		53±6(52)		

D) Inorganic nutrients	*n*	Silicic acid (μmol L^{-1})	Phosphate (μmol L^{-1})	NO_x (nitrate+nitrite) (μmol L^{-1})	NH_4 (μmol L^{-1})
	31	57.1±0.6(57.2)	2.6±0.2(2.6)	30.2±0.5(30.2)	0.124±0.091(0.084)

resulted in an overall lower average removal and higher variance (Fig. 6). The median concentration of protists in the water inhaled by A. vastus (5.3×10^{-3} cells mL^{-1}, ~0.5 m off the wall) was 24 times higher than their concentration in the water inhaled by R. dawsoni immediately adjacent to the wall.

A distinct population of perfectly spherical cells, ~3 μm in diameter, with bright-blue (DAPI) fluorescence was evident in the exhaled water of three *A. vastus* and one *R. dawsoni*. The concentration of these cells ranged from 400 to 1,200 cells mL^{-1}, and their distinct cluster was evident in the flow cytometry analysis (SYBR Green). These spherical cells were extremely rare in the inhaled water samples. Similar particles were not present in sponge tissues or in aborted SIP samples where sponge tissue blebs were suctioned from the sponge wall.

Bulk TOC: The ambient concentration of TOC was low, comparable to that of deep-sea water (Table 1), indicating that labile dissolved organic carbon (DOC) was not available for the sponges during our period of observation. Indeed, TOC removal was small and was accounted for by planktonic C removal (Table 2), indicating no net removal of DOC. Consequently, there was no detectable difference between the CDOM fluorescence in the water inhaled and exhaled by the sponges (Mann–Whitney rank sum test, $p = 0.88$, $n = 12$ sponges). For *A. vastus*, TOC removal increased linearly with TOC concentration ($y = [0.72 \pm 0.18]x - 35 \pm 10$; $R^2 = 0.77$; $p = 0.002$). In the six cases in which inhaled TOC was below 50 μmol L^{-1}, TOC was elevated in the exhaled samples (median, 5 μmol L^{-1}), indicating discharge of organic matter by the sponge. Sponge A17, which released the spherical cells, comprised the only case in which a net TOC addition to the exhaled water was measured concurrently with inhaled TOC above 50 μmol L^{-1}. Insufficient TOC data were available for *R. dawsoni* ($n = 2$ pairs). Samples with high TOC removal (>6 μmol L^{-1}) corresponded to high removal of protists. While in some cases the carbon gained from protists was >3.5 μmol L^{-1} (twice the carbon gain from bacteria), on average the removal of bacteria accounted for 61% and 78% of the carbon and 72% and 85% of the nitrogen in the planktonic diet of *A. vastus* and *R. dawsoni*, respectively.

Silicic acid: The silicic acid concentration of water in the sponge belt was comparable to that of deep-sea water, with high and relatively constant concentration (57.1 ± 0.6 μmol L^{-1}; coefficient of variation = 1%; Table 1); in contrast, surface waters contained ~15 μmol L^{-1}. There was no detectable change of silicic acid concentrations in incurrent and excurrent water samples in either species (Figs. 6, 7). Our detection limit was 0.28 μmol L^{-1} for *A. vastus* and 1.3 μmol L^{-1} for *R. dawsoni*. The 95% confidence interval for silicic acid uptake was −0.18 to 0.26 μmol L^{-1} for *A. vastus* and −1.16 to 0.60 μmol L^{-1} for *R. dawsoni*, with median differences of 0.08 and −0.27 μmol L^{-1}, n = 16 and 4 specimens, respectively.

Nitrogen and phosphorus: The ammonium concentration was markedly elevated (up to 650 nmol L^{-1}; Table 2)

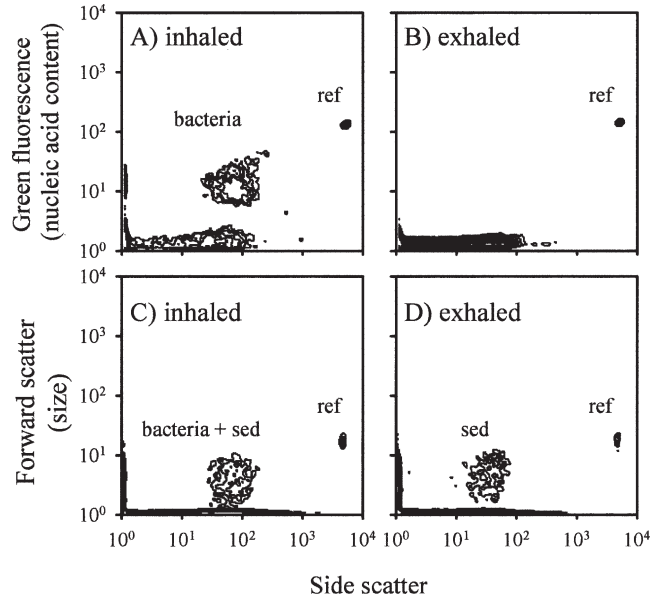

Fig. 4. An example of a flow cytometry analysis of a paired water sample (A16; 137 m in depth) obtained in situ and stained with the nucleic acid dye SYBR Green I. (A, C) Density plots of the inhaled water samples. (B, D) Corresponding plots of the exhaled water analyzed using the same settings as for the inhaled samples. Gray levels represent increasing (log 50%) particle concentrations. "ref" indicates 1.0-μm reference beads and "sed" denotes particles with very low nucleic acid content—mostly clay and inorganic debris. Axes are plotted in arbitrary units using a log scale.

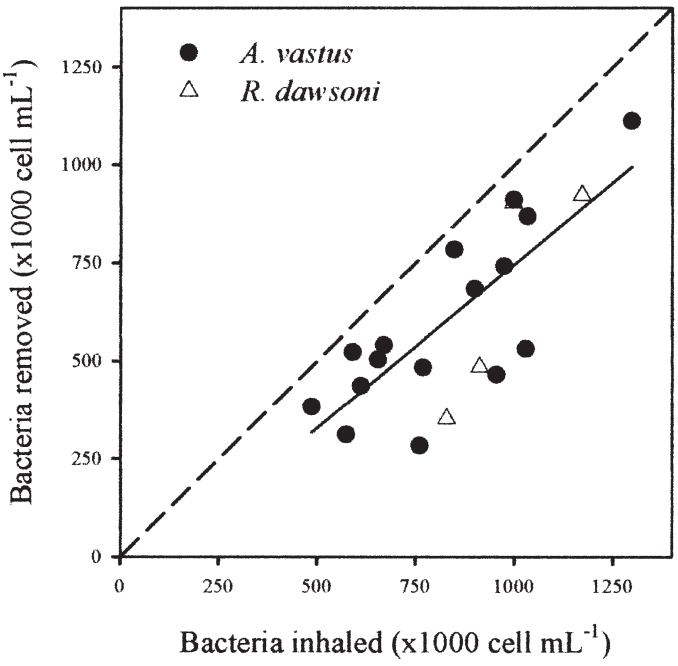

Fig. 5. The removal of bacterial cells (specimen average) plotted against ambient (inhaled) concentrations. Both sponge species responded linearly to elevated prey concentration ($R^2 > 0.79$, $p < 0.001$) within the full concentration range encountered. The diagonal dashed line represents 100% removal.

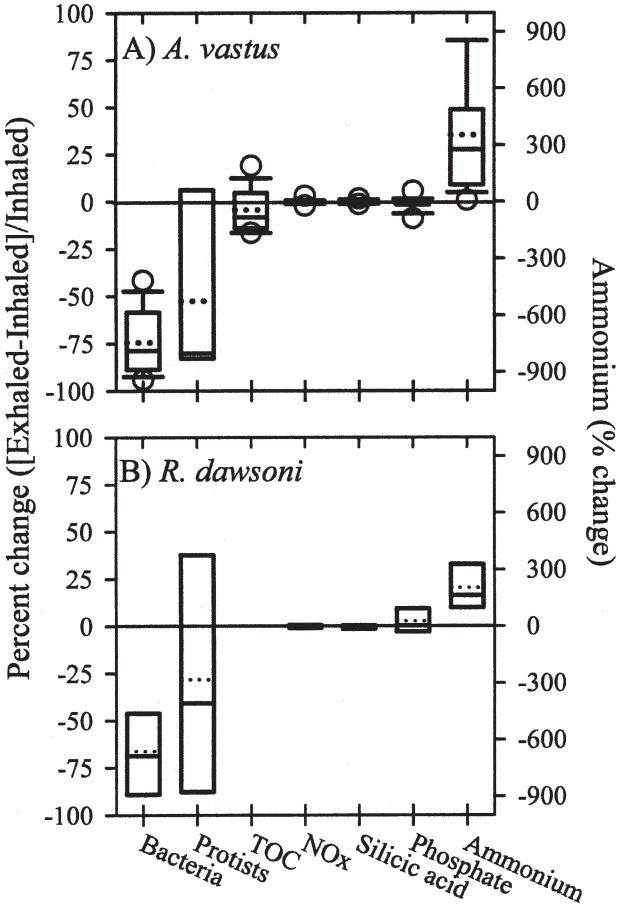

Fig. 6. Box plots summarizing the percent change of water constituents during a single passage of the water through (A) *Aphrocallistes vastus* and (B) *Rhabdocalyptus dawsoni* filtration system, in situ. Negative values indicate net removal (uptake); positive values indicate net addition to the exhaled water. Median and average values are indicated by solid and dotted lines, respectively. The box delineates lower and upper quartiles; whiskers pertain to the 10% and 90% percentiles, and all data exceeding this range are indicated by open circles. Because of its low ambient level, ammonium concentration in the exhaled water was up to 15 times higher than the inhaled values and is therefore plotted on the right axis. As a result of insufficient sample size, panel B lacks whiskers and outliers, and no box could be delineated for the total organic carbon (TOC) and nitrate + nitrite (NO_x).

in all but one of the exhaled water samples obtained from *A. vastus* and *R. dawsoni* (Web Appendix 1; Fig. 6). Excretion of ammonium was not correlated with the uptake of any potential nitrogen sources (bacteria, protists, or TOC), their ambient (inhaled) concentrations, the ambient ammonium, or total nitrogen concentration (Spearman's ranked correlation analysis, $p > 0.15$). On average, the estimated amount of nitrogen gain by ultraplankton filtration accounted for almost twice the amount of ammonium excreted by *A. vastus* but fell short of accounting for the ammonium excreted by *R. dawsoni* (Table 2). Bacterial nitrogen alone accounted for 168% ± 90% and 82% ± 46% of the ammonium excreted by *A. vastus* and *R. dawsoni*, respectively.

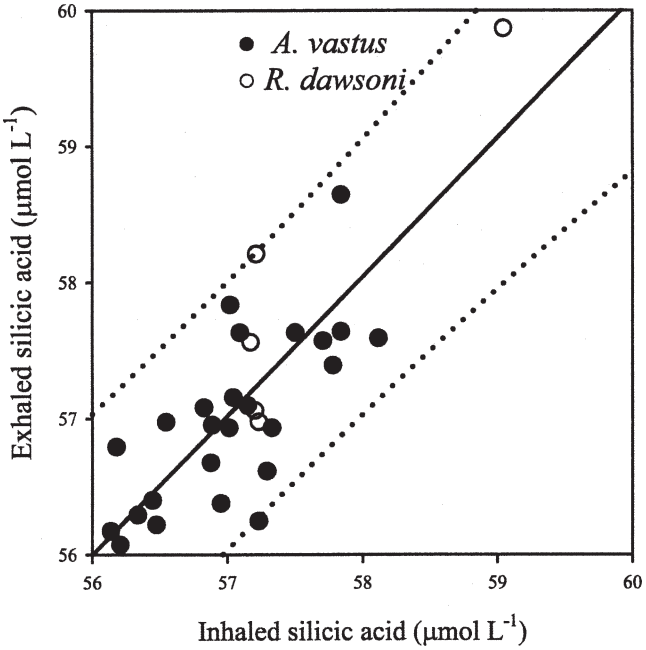

Fig. 7. The concentration of silicic acid in the exhaled water collected in situ after a single passage of the water through the glass sponges is plotted against ambient (inhaled) concentrations. Dotted lines indicate 95% prediction limits. The regression line was not statistically different from 1 (slope = 1.02 ± 0.76, $p > 0.9$, power = 1.00) and the intercept was not statistically different from 0 ($p = 0.88$).

No significant concentration shift was evident for nitrate concentrations (detection limit, 0.23 and 0.8 μmol L^{-1} for *A. vastus* and *R. dawsoni,* respectively; Table 2; Fig. 6). A slight but significant phosphorus excretion (35 ± 80 nmol L^{-1}; Wilcoxon signed rank test, $p = 0.024$) was measured for *A. vastus*.

Inorganic particles: In terms of mass, inorganic particulate matter accounted for >97% of the seston (<125 μm). In many of the samples analyzed by flow cytometry, small particles with very little fluorescence outnumbered living cells (Fig. 4A,C). In most cases, similar particles were also observed in the exhaled water (Fig. 4D). High concentrations of such particles were evident in about half of the inhaled samples. Because of the lack of specific optical properties, a robust quantification of inorganic particles was not possible with the flow cytometer. A semiquantitative analysis of particles of >5 μm (mostly aggregates) carried out using SEM-EDX analysis indicated no significant difference in the proportion of silica-containing particles in the inhaled and exhaled water (78% ± 14% and 72% ± 33%, respectively; n = 5 paired water samples), while the proportion of particles containing organic traces was reduced from 45% ± 25% to 29% ± 30%.

Tank experiments—Measurements of sponge feeding, elemental uptake, and excretion carried out in the laboratory confirmed the general patterns found in the in situ samples. Despite the differences in season and in planktonic composition and water chemical properties, both sponge species removed the small (<0.4 μm) non-photosynthetic bacteria with high efficiency (89% ± 10%, median 92%). Even though TOC concentrations in the tank were 31% higher than the TOC concentrations measured in situ (69 ± 3 and 53 ± 6 μmol L^{-1}, respectively), TOC removal by *R. dawsoni* was small (1 ± 3 μmol L^{-1}), and no significant DOC removal was measured by clean filtration of sampled waters (*t*-test, $t = -1.0$, df = 9, $p = 0.34$). Similarly, there was no evidence for silicic acid uptake in the tanks (ambient silicic acid concentrations, 23.0 ± 0.6 μmol L^{-1} in the laboratory vs. 57.1 ± 0.6 μmol L^{-1} in the field). A statistically significant excretion of silicic acid ranging from 0.3 to 1.2 μmol L^{-1} was evident in February for all five specimens of *R. dawsoni* upon transfer to the tanks. Twenty-four hours later this trend was reversed, but the difference between inhaled and exhaled concentrations was very small (0.2 ± 0.2 μmol L^{-1}) and not significantly different from zero.

Cross-wall distribution of particulates and nutrients—The concentration of most water constituents remained constant from 80 m to 5 m off the wall (Fig. 8). However, elevated ammonium concentrations and a decrease in POC,

Table 2. Removal of carbon (C) and excretion of nitrogen (N) by the two glass sponges studied. Data are averages ±SD (medians are given in parentheses). Ultraplanktonic (protists) carbon was converted to nitrogen using a C : N ratio of 8 (Menden-Deuer and Lessard 2000); bacterial carbon was converted to nitrogen using a C : N ratio of 5 (Gundersen et al. 2002); Total organic carbon (TOC) removed was converted to nitrogen using a C : N ratio of 7.2 measured for total particulate matter in this study (Table 1). *n* is the number of sponges sampled; in most cases two paired water samples were obtained from each sponge.

	Aphrocallistes vastus			*Rhabdocalyptus dawsoni*		
	n	Carbon removed (μmol L^{-1})	Nitrogen excreted (μmol L^{-1})	*n*	Carbon removed (μmol L^{-1})	Nitrogen excreted (μmol L^{-1})
Bacteria	16	1.4±0.5(1.2)	0.272±0.105(0.240)	4	1.5±0.7(1.6)	0.303±0.132(0.316)
Pico-heterotrophs	6	0.1±0.1(0.1)	0.015±0.017(0.012)	5	0.0±0.0(0.0)	0±0(0)
Nano-heterotrophs	6	0.7±1.2(0.2)	0.092±0.144(0.022)	5	0.4±0.6(0.2)	0.053±0.079(0.021)
Plankton total		2.2±1.3	0.379±0.179		1.9±0.9	0.357±0.154
TOC	13	2.3±5.6(4.4)	0.321±0.776(0.609)	2	−4.6±12.2	−0.636
NO_x (nitrate+nitrite)	16		0.016±0.196(0.012)	4		−0.029±0.280(−0.130)
NH_4	16		−0.201±0.128(−0.162)	4		−0.388±0.068(−0.404)
Total nitrogen			−0.185±0.196			−0.417±0.280

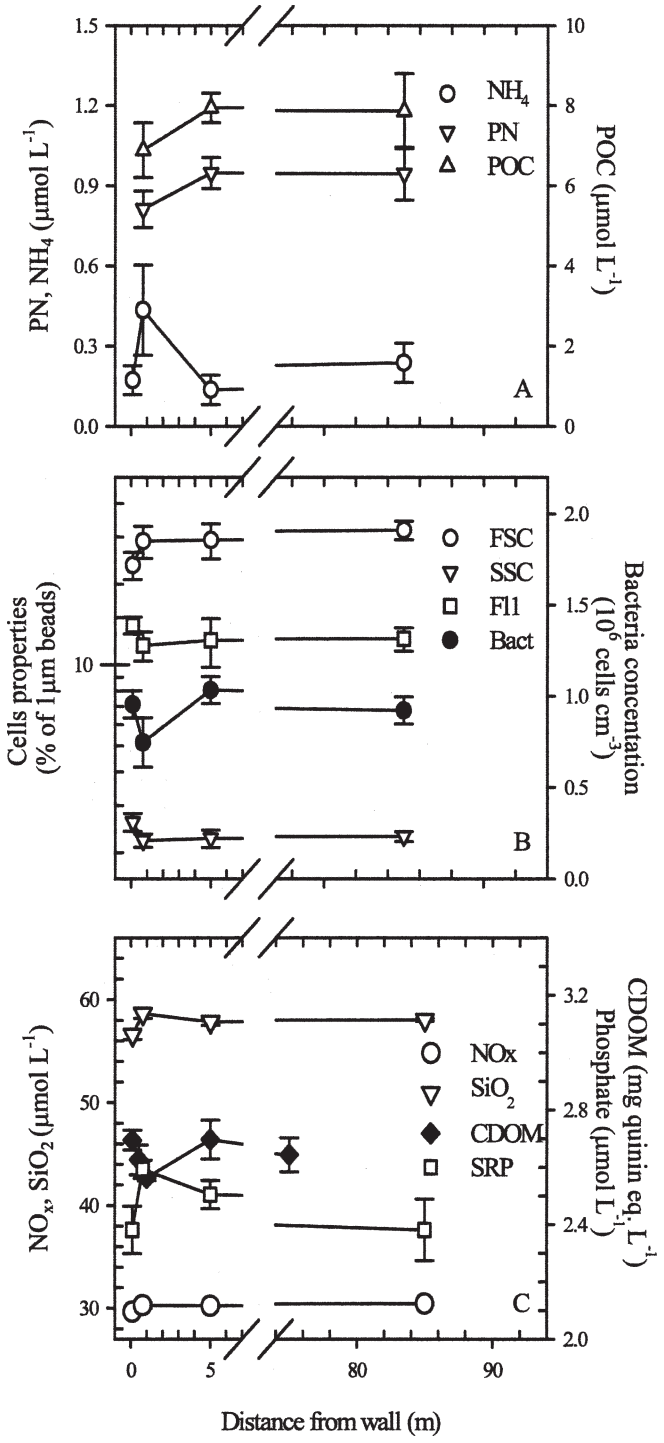

Fig. 8. Horizontal transects showing the distribution of potential food sources, skeletal material, and waste products as a function of distance from fjord walls in Barkley Sound. (A) Ammonium (NH_4), bulk particulate organic carbon (POC) <100 μm, and particulate nitrogen (PN). (B) Bacteria concentration (Bact) and average cell characteristics (FSC, SSC, Fl1) measured with the flow cytometer. (C) Silicic acid (SiO_2), nitrate + nitrite (NO_x), and phosphate concentration are plotted in open symbols. Colored dissolved organic matter (CDOM) fluorescence was measured at a higher resolution in real time using a WetLab CDOM fluorometer (filled diamond). $n = 8$ horizontal transects. Error bars, standard error (SE).

PN, and bacterial concentrations were observed 0.75 m from the wall. At 10 cm from the wall, bacteria, CDOM, and ammonium concentration were elevated again, almost to their off-wall values (Fig. 8), but the concentration of protists was reduced dramatically (data not shown).

Discussion

Water characteristics and oceanography of the sponge habitat—In their deep-water habitat on the fjord walls, the glass sponges are isolated by a permanent pycnocline from the strong seasonal oscillations experienced by shallow (<30-m) benthic communities. In addition, the downwelling/upwelling cycle along the British Columbia coast (Freeland and Denman 1982; Whitney et al. 2005*b*) causes intermediate north–east Pacific water to flow into deep coastal basins, fjords, and inlets in summer, where it can then be isolated for many months. The deep coastal sponge habitat is therefore characterized by stable, 'deep-sea–like' conditions. The planktonic composition of this region is dominated by minute heterotrophic bacteria and protists and is markedly different from the photic zone communities that are dominated by large phytoplanktonic cells (Taylor and Haigh 1996). Moreover, as indicated by our tank experiments, the nonphotosynthetic bacterial populations of these two zones are also markedly different, and some of the photic zone bacterial populations are significantly less preferred by the glass sponges (Yahel et al. in press). In contrast to abyssal or intermediate oceanic waters, the concentration of bacteria in deep fjord waters ($\sim 0.8 \times 10^6$ bacteria cm^3) is high and resembles concentrations encountered in surface waters (Nagata et al. 2000; Bendtsen et al. 2002). It is assumed that the subsidiary supply of terrestrial organic matter and fallout from coastal phytoplankton blooms in combination with resuspension processes (van der Loeff et al. 2002) result in elevated concentrations of bacteria and nano-planktonic protists in the deep fjord waters. The strong currents along the fjord walls (up to 1 m s^{-1}) could also significantly enhance food flux to the sponges.

Diet composition—Bacteriovory (e.g., Rasmont 1961; Reiswig 1975; Gili et al. 1984) and phytoplanktivory (of cells <10 μm; e.g., Pile 1997; Ribes et al. 1999; Yahel et al. 2003) are well-documented feeding strategies in shallow-water demosponges. While our tank experiments (with water from 25 m in depth) reveal that glass sponges are capable of efficiently removing both cell types (Yahel et al. in press), photosynthetic populations are completely absent in the deep sponge habitat, where both species of sponge are primarily bacteriovores.

DOC was shown to be a substantial component of the diet of the coral reef sponge *Theonella swinhoei* (Yahel et al. 2003), and a predominantly dissolved diet was also inferred for several other 'bacteriosponges' that harbor massive populations of associated (symbiotic) bacteria (Reiswig 1981). Whereas it had been previously suggested that *R. dawsoni* feeds on DOC (Reiswig 1990), no evidence for DOC uptake was found in this study. The lack of uptake of DOC by the glass sponges in deep fjord waters does not

appear to be due to the poor carbon content of the ambient water, since similar results were also obtained in the laboratory, where DOC levels were 30% higher (69 ± 3 μmol L^{-1}). Importantly, massive populations of associated bacteria do not exist in the glass sponges studied (Mackie and Singla 1983; Leys 1999).

In some shallow environments, particulate detritus can account for >90% of the carbon retained by suspension feeders (e.g., the ascidian *Halocynthia papillosa*; Ribes et al. 1998). The food web in the hexactinellid sponge habitat is inherently detrital. Whereas detritus accounts for ~60% of the POC in our study site, Barkley Sound, the carbon and nitrogen budgets indicate that the contribution of detrital particles to feeding by the glass sponges we studied is minor (Table 2). This conclusion is also supported by the lack of detectable depletion of DAPI Yellow particles (Mostajir et al. 1995) in the comparison of inhaled and exhaled water by epifluorescence microscopy.

Our results indicate that the two glass sponge species rely largely on free-living, nonphotosynthetic bacteria and nano-planktonic protists for nutrition. Similarly, removal of living cells accounted for 96% of the carbon ingested by the temperate demosponge *Dysidea avara* (Ribes et al. 1999). Additional support for this conclusion is derived from the comparison of planktonic nitrogen uptake and nitrogenous waste excretion (Table 2) and from preliminary in situ respiration measurements of oxygen consumption by *A. vastus* (~2 μmol L^{-1}; Yahel et al. unpubl. data) that are in good agreement with its planktonic carbon uptake.

The sponge removed ultraplanktonic cells in proportion to their ambient concentrations (Fig. 5) with no indication for a lower threshold or an upper limit. This pattern is similar to that seen in shallow demosponges (Ribes et al. 1999; Yahel et al. 2003) and indicates that the sponges are well adapted to utilize this food source (Coma et al. 2001). The data indicate that the hexactinellid sponges are positioned quite high in the detrital food web of their deep habitat, efficiently selecting the more nutritious living cells out of the seston and discarding both the inorganic and the less-nutritious detrital particles.

Niche partitioning between the two species—*R. dawsoni* and *A. vastus* show a clear partitioning of space in the benthos. *R. dawsoni* hangs parallel to the wall, while *A. vastus* projects perpendicularly out, up to 1.0 m from the wall face (Fig. 2C). The faster current away from the wall results in elevated food flux but increased risk of dislodgment to *A. vastus*, while *R. dawsoni*, lying adjacent to the wall, experiences sediment resuspension events (mostly induced by fish) that could clog its filtration apparatus. Indeed, our flow visualizations indicated a clear behavioral difference between the two species. Specimens of *A. vastus* pumped robustly each time they were tested with dye in situ ($n > 30$), but *R. dawsoni* specimens ceased pumping in response to the slightest disturbance (Leys and Tompkins 2004).

Ingestion of sediment—Inorganic suspended sediment is clearly a disturbance factor for the sponge, and it accounted for >97% of the ambient seston mass (Table 1). For seston dominated by coarse clay (1 μm), these levels correspond to $>5 \times 10^7$ particles cm^{-3} (Kirk and Gilbert 1990), an order of magnitude higher than the ultraplanktonic cell concentration. SEM-EDX analysis showed similar amounts of micron-size (1–10-μm) inorganic particles in the incurrent and excurrent samples, whereas flow cytometry and microscopy showed efficient removal of living cells. These data indicate that the sponges process the mostly inorganic seston, remove the living cells, and egest inorganic material into the excurrent stream. Although we did see some aggregates of particles that may be interpreted as sponge feces in exhaled water samples, it seems that most of the inorganic particles are egested individually into the excurrent water canals. Since bypassing the tight filter of the glass sponge is unlikely (Leys 1999; Yahel et al. in press), presumably all particles 0.2–20 μm in size must be individually processed by the sponge.

Silica—It is estimated that the skeleton forms nearly 80% of the dry weight of *A. vastus*; the tissue forms only a thin veneer on its surface (Whitney et al. 2005*a*). Similar proportions are estimated for lysaccine sponges like *R. dawsoni* (Barthel 1995). Considering the vast amount of silica incorporated into the glass sponge, it may seem surprising that uptake of silicic acid was below the limits of detection of our analysis, but a sensitivity analysis of the predicted silica uptake reveals otherwise. Initial results of an ongoing study of the growth rates of *A. vastus* at the study site show that small animals (<20 cm long) increase in girth and length at a rate of approximately 1–2 cm yr^{-1} (Leys et al. in press; *see also* Leys and Lauzon 1998). A sponge with an osculum of 8 cm in diameter and a pumping rate of 1 cm s^{-1} would process ~180 L h^{-1}. Considering a tissue dry mass of 24 ± 3 mg cm^{-2} (Yahel unpubl. data), the silica content is 325 ± 36 μmol cm^{-2} of sponge wall. Even at a high growth rate of 10 cm yr^{-1}, the amount of silica deposited would be only 6.85 g yr^{-1}, equivalent to a removal of 0.072 μmol L^{-1}, four times lower than our silicic acid detection limit of 0.28 μmol L^{-1}.

Excretion products and egested material—Unlike photosymbiont–containing sponges like *Theonella swinhoei* that remove a considerable amount of ammonium from the water (Yahel et al. 2003), both glass sponge species excreted their nitrogenous waste as ammonium (*see Web Appendix 1*). No evidence for sponge-mediated nitrification—reported for some tropical sponges (Diaz and Ward 1997)—was found in the glass sponges (no difference was evident in the NO_3 concentration). A small excretion of soluble reactive phosphorus was also detected (Fig. 6).

Other animals could potentially contribute waste materials to the excurrent water. Unlike *R. dawsoni*, whose outer spicule coat houses over 30 species of macrofauna (Boyd 1981), the surface of *A. vastus* is exceptionally clean. Nevertheless, several species of majiid crabs and a particular hypolytid shrimp as well as small fish (gadids and sculpins) use the large central cavity of both sponges as shelter. The excess nitrogen found in exhalent samples of *R. dawsoni* in

our study could be derived from the animals living in its ''spicule jungle'' coat (Boyd 1981).

Potential effects of sponge activity—Despite the strong currents near the walls, water sampled during multiple cross-wall transects showed a distinct signature in water properties at ~0.75 m from the fjord wall, where most *A. vastus* oscula are located (Figs. 2C, 8). Since all measured parameters were shifted in a similar fashion toward the exhaled water concentrations (compare Fig. 6 and Fig. 8), we concluded that this shift should be attributed to the feeding activity of *A. vastus*. It should be noted that, as in other British Columbia fjords (Tunnicliffe and Wilson 1988), the fjord wall was also densely populated with brachiopods and associated mobile and sessile fauna; however, *A. vastus* and its close relative *Heterochone calyx* are virtually the only animals that extend so far away from the wall. As a result of logistical constraints we could not carry out cross-wall transects on walls where sponges were absent.

Preliminary in situ measurements of *A. vastus*' pumping rate using acoustic Doppler velocimeters have revealed strong temporal variation and a high dependency of the pumping rates on the ambient flow (Yahel et al. unpubl. data). A first-order estimate of the potential impact of the glass sponge activity is developed for an average excurrent speed of 1 cm s^{-1} of a 'standard sponge' with an 8-cm osculum, assuming continuous pumping throughout the year and effective carbon removal by sponges in waters containing variable particulate carbon (Fig. 6). This standard sponge would process 4.32 m^3 of seawater d^{-1} and 30.9 g suspended solids d^{-1}, remove 0.12 g d^{-1} or 9.6 mmol d^{-1} of ultraplanktonic carbon, and excrete 1.6 mmol d^{-1} of ammonium. This processing rate is equivalent to 70% of the daily vertical flux of particulate organic matter exported from a square meter of the photic zone in a typical north–east Pacific fjord such as Jervis Inlet during the summer months (13.7 mmol organic carbon; table 3 in Timothy et al. 2003).

Taking Jervis Inlet as an example, we can consider an average fjord width of 3 km, a mean sponge-belt depth of 400 m (sponges being present from <50 m to >550 m; Leys et al. 2004), and a sponge density of 1.2 specimens m^{-2} of vertical wall (recalculated from Leys et al. [2004], only considering large- and medium-sized specimens). An imaginary 1-m–wide swath across the fjord will contain ~960 large- and medium-sized glass sponges. The estimated ultraplanktonic carbon processed in the summer months by this hexactinellid sponge population equals ~22% of the daily particulate carbon flux from the entire fjord photic zone (sediment trap data of Timothy et al. [2003]). Considering that the sponges are secondary and tertiary consumers in the detrital food web of the fjord deep water, this figure is surprisingly high. As the glass sponges are confined to the fjord wall, a large proportion of the organic matter sinking from the surface is not directly available for the sponge consumption; this matter is most likely circulated via the fjord microbial and benthic resuspension loops (van der Loeff et al. 2002) until it finally reaches the benthos via tidally driven mixing. It should be noted that our measurements represent a 'snapshot' of glass sponge metabolism in their deep fjord habitat during the sampling period in early summer. Further research is required to determine to what extent our observations can be extrapolated.

Our in situ measurements, corroborated by in-tank experiments, indicate that (1) in their deep habitat, both hexactinellid sponge species feed primarily on small ultraplanktonic cells (<10 μm); (2) there is no evidence for a significant role of dissolved or detrital components in the hexactinellid sponge diet; (3) the scarcity of nutritious living cells in the ambient water means that they must be individually selected by the sponges from the mostly inorganic seston, while unwanted particles are expelled into the excurrent stream; (4) hexactinellid sponges excrete most of their nitrogenous waste as ammonium; and (5) the potential impact of hexactinellid sponge feeding activity on the composition and ecology of deep water in the Pacific Northwest fjords is considerable.

It is extremely difficult to replicate deep-sea conditions in vitro (Bates et al. 2005). Using an ROV and a suite of in situ sampling techniques, this study provides the first data on the nutritional ecology and metabolism of two glass sponge species in their natural deep-water habitat. While our measurements were conducted at a moderate depth of 150 m, the instrumentation used was tested as deep as 1,200 m and can be easily adapted to depths of >3,000 m. Future in situ sampling at greater depths using the methods described here should shed more light on the physiology and ecology of other deep-sea benthic suspension feeders.

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