In situ feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: Bulk DOC is the major source for carbon

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Abstract

The vast majority of organic matter in the world ocean is found in the dissolved pool. However, no evidence has been demonstrated for direct uptake of bulk dissolved organic matter (DOM) by organisms other than bacteria and some invertebrate larvae. The total organic carbon (TOC) is 10–30% higher in coral reefs than in adjacent open waters. The dissolved organic carbon (DOC) accounts for >90% of the TOC. Using a new in situ technique for clean sampling of the seawater inhaled and exhaled by benthic suspension feeders, we measured directly the removal of DOC in the symbiont-bearing reef sponge *Theonella swinhoei*. The sponge removed up to 26% (mean ± SD: 12% ± 8%) of the TOC (dissolved and particulate) from the water it filtered during a single passage through its filtration system. Most of the carbon gained by the sponge was from the dissolved pool (10 ± 7 μmol C L^{-1}), an order of magnitude greater than the carbon gained from the total living cells (phytoplankton and bacteria) the sponge removed (2 ± 1 μmol C L^{-1}). In *T. swinhoei,* over two-thirds of the sponge biomass consists of symbiotic bacteria, which likely play an important role in DOC uptake. Our findings indicate that the role of DOC in metazoan nutrition and the role of metazoans in DOC cycling may have been grossly underestimated.

The total organic carbon (TOC) in the ocean is divided into two major compartments: particulate (POC) and dissolved (DOC). The dissolved pool is operationally defined as the organic carbon passing through a fine filter, typically GF/F (Benner 2002; Carlson 2002). The particulate fraction consists of larger living organisms and detrital particles. The vast majority (>97%) of the organic carbon pool in seawater resides in the dissolved phase (Benner 2002). Only a small fraction of the dissolved pool is labile; the rest is thought to be refractory and unavailable for utilization by marine organisms (Carlson 2002).

A role for DOC in the nutrition of aquatic metazoans has been repeatedly proposed since the turn of the last century (Pütter 1909; Jørgensen 1976). DOC was recently suggested to explain the observed discrepancies between the supply and demand of carbon in benthic suspension feeders (Reiswig 1974, 1981; Gili and Coma 1998) and deep-sea soft-bottom communities (Smith and Kaufmann 1999). However, except for larvae of soft-bodied invertebrates (Wright and Manahan 1989), direct evidence of metazoan utilization of bulk DOC in aquatic habitats is lacking. Thus, in sharp contrast to the well-established role of DOC in the microbial loop of pelagic food webs (Carlson 2002), the role of DOC

in the nutrition of benthic animals is still unresolved (Wright and Manahan 1989; Thomas 1997).

Numerous laboratory experiments have confirmed the ability of soft-bodied metazoans, including sponges (Jaeckle 1995), to take up specific components from the dissolved pool (reviewed by Johannes et al. 1969; Jørgensen 1976; Stephens 1988; Wright and Manahan 1989; Thomas 1997; Ambariyanto and Hoegh Guldberg 1999; Roditi et al. 2000). However, the uptake of components other than dissolved glucose and free amino acids has received little attention (Wright and Manahan 1989; Thomas 1997). Because glucose and amino acids are found in trace concentrations in natural aquatic habitats, their removal is generally considered energetically insignificant (e.g., Ambariyanto and Hoegh Guldberg 1999). The very few laboratory studies that included naturally occurring DOC produced contradictory conclusions (e.g., Alber & Valiela 1995). Reiswig's (1985) attempt to quantify DOC feeding in situ with boreal glass-sponges was limited by a small data set and a variance too high to draw clear conclusions. More recently, Ribes et al. (1998, 1999) used recirculating bell jars to measure in situ the diet of a Mediterranean sponge and ascidian. Bulk DOC removal was not observed in either case. We are not aware of any direct evidence for in situ uptake of bulk DOC by benthic metazoans.

One reason for the paucity of direct empirical data for the nutritional role of DOC in benthic communities was the lack of easy, accurate DOC analyses (Sharp 2002). In the 1990s, careful reevaluation of methods and utilization of high-temperature combustion methods have resulted in much easier DOC analyses (Sharp 2002; Sharp et al. 2002). Routine oceanic DOC analyses can now be performed with reproducibility on the 2% level (Sharp 2002).

Coral reefs sustain high levels of recycling of autochthonous carbon and nutrients, driven by the high productivity of algae and zooxanthellate cnidarians and the unusually high abundance of closely interacting species. Sources for DOC in coral reefs are numerous (reviewed by van Duyl and Gast 2001). Algae excrete high proportions of their photosynthetically assimilated carbon as DOC. "Sloppy" feeding and grazing of benthic algae and animals should cause DOC leakage from broken prey (Thomas 1997), but most significant is probably the excretion of DOC by corals, frequently as mucus and free amino acids (Ferrier-Pages et al. 1998). Recently, Van Duyl and Gast (2001) ascribed the 10–15% DOC elevation they measured near the corals to a labile fraction excreted by the corals. They suggested that in reef crevices benthic suspension feeders may consume considerable amounts of this DOC.

Theonella swinhoei (class Demospongiae, order Lithistida, family Theonellidae) is a common sponge in coral reefs throughout the Indo-West Pacific. The sponge typically occurs in clusters. The body material is dense, and the barrel-shape body has a single osculum at its apex. In the Gulf of Aqaba, clusters are typically small (usually fewer than six specimens per cluster), and specimens are usually 5–15 cm in length and 3–8 cm in diameter. Similar to other dense tropical sponges (e.g., *Verongula* sp. [Reiswig 1971] and *Verongia fistularis* [Reiswig 1981]), *T. swinhoei* is actually a consortium of several symbiotic populations (Bewley et al.

1996; Magnino et al. 1999). Prokaryotic symbionts include unicellular heterotrophic bacteria (mean concentration, 20% of the sponge tissue), the unicellular, phototrophic cyanobacterium *Aphanocapsa feldmanni* (mean concentration, 15% of the sponge tissue), and filamentous bacterial symbionts (mean concentration, 40% of the sponge tissue). These filamentous bacterial symbionts contain a specific antifungal peptide (theopalauamide) and were recently proposed as a new species *Entotheonella palauensis* of the δ-Proteobacteria (Schmidt et al. 2000). In addition, large populations (>50 individuals ml^{-1}) of symbiotic polychaetes (Syllidae) typically inhabit the water channels of these sponges (Magnino et al. 1999).

In the present study, we focused on the first step in the utilization of DOC, its removal from the ambient water. Removal rates of TOC, DOC, and living phytoplankton and bacteria (hereinafter LvPOC) by the sponge were measured in situ based on the difference in the concentrations of these components between the water inhaled and the water exhaled by the sponge.

Materials and methods

The InEx technique (Yahel et al. unpubl.) was used to sample in situ the water inhaled and exhaled by the sponge. A scuba diver sampled the inhaled water by slowly (~ 0.5 ml s^{-1}) withdrawing water into a 32-ml open-ended glass tube attached at its proximal end to a syringe while holding its open (intake) end 3–5 mm from the sponge ostia (inhaling apertures). A sample of exhaled water was taken simultaneously using an identical tube held within the exhalent jet with the tube's intake end positioned ~ 2 mm above the sponge's osculum (exhaling aperture), using the excurrent jet to flush and then fill the tube. Filling time (mean $\pm$ SD: 135 $\pm$ 43 s) was determined individually for each pair so that it would last 150% of the time it took the exhalant jet to flush clear an identical tube prefilled with fluorescein dye (measured a few minutes prior to each sampling). Sampling duration was about 100 times longer than the few seconds it took the water to pass through the sponge. Thus, each InEx pair represented an integration of approximately 2 min of sponge activity (Yahel et al. unpubl. data).

All samples were collected by scuba divers at the Coral Reef Nature Reserve of Eilat, Israel. For a description of the study site, see Yahel et al. (1998, 2002). Two sampling sessions were conducted: the first in 1998 at the height of the summer stratification (10 specimens) and the second in 2000 soon after the termination of spring bloom (20 specimens). The length and diameter of each sponge was measured to the nearest 0.5 cm and converted into volume assuming a cylindrical shape. The osculum diameter was measured to the nearest millimeter. The average length of the specimens was 9 ± 2 cm, the external diameter was 5 ± 2 cm, and the osculum diameter ranged from 6 to 15 mm (Table 1).

Sample handling was performed within 2 h after each dive in a clean, open-air glass hood constructed at the seaward end of a local pier. Only precombusted glassware was used throughout the experiment, and a new set of pipettes was used for each sample. A 1-ml aliquot was withdrawn from

Table 1. Sampling times, cluster dimensions, and description of the individual specimens studied. Sampling sessions were undertaken at midday (1000–1200 h).

Sampling date and time	Cluster			Specimen			
	ID	No. specimens	Depth (m)	ID	Length (cm)	Diameter (cm)	Analyses*
15 Sep 98 1045	A	9	5	A1	8	5	DFS, FACS, TOC
				A2	7	6	DFS, FACS, TOC
				A3	9	3	DFS, FACS, TOC
27 Sep 98 1100	B	4	8	B1	10	5	DFS, FACS, TOC
				B2	10	5	DFS, FACS, TOC
	C	1	8	C1	7	5	DFS, FACS, TOC
28 Sep 98 1020	D	4	4	D1	7	2	DFS, FACS, TOC
				D2	6	3	DFS, FACS, TOC
02 Oct 98 1030	E	10	13	E1	12	4	DFS, FACS, TOC
				E2	11	5	DFS, FACS
11 Jul 00 0930†	F	4	5	F1	10	5	FACS, TOC, DOC
				F2	5	4	FACS, TOC, DOC
	G	4	5	G1	12	7	FACS, TOC, DOC
				G2	10	4	FACS
12 Jul 00 1000†	F	4	5	F1	10	5	FACS, TOC-Ex,DOC
				F2	5	4	FACS, TOC, DOC
	G	4	5	G1	12	7	FACS, TOC, DOC
				G2	10	4	FACS, TOC, DOC
24 Jul 00 1000	H	3	10	H	10	6	FACS, TOC, DOC
(17 Dec 00 O₂,	I	3	10	I1	10	8	FACS, TOC, DOC
18 Dec 00 NH₄)				I2	10	8	FACS
	J	8	10	J	6	6	FACS
25 Jul 00 1000	K	2	14	K1	8	4	FACS, TOC, DOC
				K2	7	6	FACS, TOC, DOC
	L	2	14	L1	6	4	FACS, TOC, DOC-In
				L2	6	3	FACS, TOC, DOC
26 Jul 00 1000	M	2	14	M	8	6	FACS, TOC, DOC
	N	2	14	N	10	3	FACS, TOC, DOC
	O	1	6	O	7	6	FACS, TOC, DOC
	P	8	8	P	12	8	FACS, TOC, DOC
27 Jul 00 1000	Q	2	18	Q1	10	5	FACS, TOC, DOC
				Q2	5	3	FACS, TOC, DOC
	R	2	13	R	10	5	FACS, TOC, DOC
	S	2	10	S	10	5	FACS, TOC, DOC
Average ± SD		4±3	9±4		9±2	5±2	
Range		1–10	4–18		5–12	2–8	

* DFS, due-front speed; FACS, flow cytometry; TOC, total organic carbon; DOC, dissolved organic carbon; Ex, exhaled; In, inhaled.
† Specimens F1–G2 were sampled on both 11 and 12 July 2000.

each InEx sample, preserved in 0.1% glutaraldehyde solution, and frozen in liquid nitrogen for subsequent flow cytometry analysis.

To avoid the risk of contamination associated with filtering the small InEx (32 ml) samples (Yoro 1999), the water was not filtered during the 1998 experiment; therefore, the parameter measured was TOC. The water was acidified directly in the InEx sampler using 100 μl Merck Suprapure 30% HCl, and two 5-ml aliquots were transferred to precombusted 10-ml glass ampoules. Ampoules were immediately heat sealed and stored at -80°C for subsequent TOC analysis at the laboratory of J.H.S. using high-temperature combustion oxidation with a Shimadzu 5000 analyzer. At the beginning of each day of TOC analysis, instrument performance was tested by running a set of reference DOC samples distributed globally by J.H.S. to verify accuracy (Sharp et al. 2002). The error associated with TOC measurements was

1–2 μmol C L^{-1} (coefficients of variation of repeated injection were <2%) with an average difference of TOC in duplicate ampoules of 4.0 μM C. To be conservative, a single InEx pair from 1998 with extreme TOC removal, exceeding 2 SD from the mean, was considered an outlier and omitted from further analysis.

Auxiliary POC and chlorophyll measurements were undertaken concurrently with each of the 1998 InEx sampling dives (Table 1). The samples were taken at the study site by a scuba diver at approximately the same height above bottom as the animals, using a prewashed, horizontally held Niskin bottle. In the laboratory, 4 L were filtered on a precombusted GF/F filter. Carbonate particles were removed from the filters using acidification in HCl fumes for 24 h at 32°C. POC was then measured using a NA2100 CHN analyzer (FISONS) with an absolute detection limit of 0.16 μmol C. Ambient (inhaled) DOC for the 1998 samples was therefore calculated

by subtracting the ambient POC from the measured TOC ($DOC_{In} = TOC_{In} - POC_{Ambient}$). A conservative estimate of exhaled DOC was calculated by assuming a removal of all POC except the exhaled living cells ($DOC_{Ex} = TOC_{Ex} - LvPOC_{Ex}$).

In 2000, DOC was measured directly. A set of InEx pairs was collected from the sponge as described above and analyzed with and without filtration of the water. To minimize the risk of contamination, a carbon-free filtration device with minimal internal volume was fabricated from a Millipore stainless-steel syringe filter holder (13 mm) equipped with a custom-made stainless-steel gasket. The filter holders, assembled with GF/F glass fiber filters (two filters per holder), were fitted to glass syringes, and the entire apparatus was wrapped in aluminum foil and baked for 2 h in a muffle furnace at 450°C. A separate filtration assembly was used for each sample. Sampling commenced as describe above, the syringe was filled with the sampled water, 10 ml was used to rinse the filter, and duplicate 5-ml samples were filtered directly into the ampoules (DOC samples). The filter assembly was removed, and nonfiltered water was injected into a second set of ampoules (TOC samples), resulting in eight samples for each InEx pair (two replicates of each In and Ex, filtered and unfiltered). Ampoules were heat sealed immediately after acidification (18 μl, Merck Suprapure 30% HCl) and stored at -80°C for later DOC/TOC analysis.

The DOC/TOC analyses were done with duplicate ampoules, and the number of samples required analytical runs on many days. A strict quality control was used so that precision for the analyses of all the samples could be calculated. DOC reference materials prepared by J.H.S. (Sharp et al. 2002) were run on each analysis day. For the 11 sample runs in 2000, the reference blank gave an average of -0.1 ± 1.5 μmol C L^{-1}, and the deep-ocean reference gave a value of 46.2 ± 2.3 μmol C L^{-1}. Two of the InEx pairs obtained were considered contaminated based on duplicate inconsistency (>7 μmol C L^{-1}) and were therefore omitted from the data set. The average difference between duplicate ampoules of the filtered samples was 2.7 μmol C L^{-1} ($n = 41$). Therefore, we consider the precision of our DOC analysis based on sample preparation and analysis to be on the order of ± 3 μmol C L^{-1}.

A flow cytometer (FACSort, Becton Dickinson) was used for total counts of phytoplankton and bacteria up to 8 μm, as described by Marie et al. (1997). The carbon content in the live cells counted by the flow cytometer (LvPOC) was estimated using published conversion factors (Campbell et al. 1994).

Changes in the concentration of oxygen between inhaled and exhaled water were measured for three specimens (H, I1, and J; Table 1) at dawn, midday, and dusk of 17 December 2000. InEx pairs were collected as described above using a 4-ml sampler, and oxygen concentrations were measured upon retrieval using a calibrated microelectrode (Revsbech 1989). Ammonia concentrations were measured during the following day for four specimens (H, I1, J, and K1; Table 1) using the fluorometric method of Holmes et al. (1999).

Instantaneous water transport rate through the osculum was measured a few minutes after InEx sampling using the dye-front speed (DFS) technique. This measurement was based on three to five video recordings of the movement of a dye in a transparent tube located within the excurrent jet. The diameter of each tube was similar or slightly larger than that of the sponge's osculum; thus, speed measurements were converted to rates of water transport by multiplying the tube's cross section by the DFS. This approach was corroborated through a series of laboratory measurements in a flume using artificial oscula with known flow rates (Yahel et al. unpubl.). A current meter (MicroADV, 16 MHz Acoustical Doppler Velocitometer, SonTek) was used to continuously measure the excurrent velocity for four specimens for 1–5 d each (Motro et al. unpubl.). The continuous MicroADV measurements were made in September 1999 and January 2000 at a water depth of ~5 m at the same study site.

Results

The TOC concentration at the reef's benthic boundary layer can be estimated by averaging the water inhaled by the sponges. In 1998, the average TOC in the water inhaled by the sponges was 79 ± 7 μmol C L^{-1}. After subtracting the ambient measured POC content, this leaves an average DOC of 72 μmol C L^{-1}, accounting for 91% of the TOC. In 2000, the ambient TOC concentration was slightly higher and more variable (87 ± 12 μmol C L^{-1}), but the DOC content (directly measured as 81 ± 11 μmol C L^{-1}) accounted for essentially the same proportion (93%) of the TOC as it did in 1998. The LvPOC values (calculated from the flow cytometry measurements) were also higher and more variable in 2000 than in 1998 (2.5 ± 1.3 μmol C L^{-1} and 1.9 ± 0.2 μmol C L^{-1}, respectively). However, the difference between the two sampling periods was not significant (Mann–Whitney U-test, $P = 0.10$ and 0.08 for LvPOC and TOC, respectively). Ambient POC concentration (6.3 ± 5.9 μmol C L^{-1}) calculated from the TOC minus DOC measurements of 2000 was similar but more variable than the directly measured POC in 1998 (7.2 ± 1.9 μmol C L^{-1}). Sampling in 1998 was performed at the height of the water-stratification season, whereas sampling in 2000 was performed close to the end of the spring bloom.

Most of the *T. swinhoei* we sampled removed TOC (Fig. 1), with an average removal of 12% $\pm$ 7% of the inhaled TOC (Wilcoxon matched pairs test, $T = 6.0$, $n = 29$, $P < 0.0001$). Overall, the sponge removed about 10 μmol C from every liter it pumped (Table 2). Of the total 29 paired InEx TOC samples, 24 showed removals exceeding the 3 μmol C L^{-1} estimated analytical precision, 1 showed an increase of 3.9 μmol C L^{-1}, and 4 showed no significant difference. The direct measurements of DOC also indicated a highly significant removal (Wilcoxon $T = 10.0$, $n = 20$, $P < 0.0001$). Of the 20 paired InEx DOC samples from 2000, 14 showed removals, 1 showed an increase of 3.7 μmol C L^{-1}, and 5 showed no significant difference. Our direct measurements in 2000 indicated that the DOC accounted for >90% of the TOC removed (Table 2, Fig. 2). In 1998, the sponges removed an average of 10.7 μmol C L^{-1} TOC (Figs. 1, 2; Table 2). The average particulate concentration was 7.2 μmol C L^{-1}; therefore, at least 33% of the removed TOC
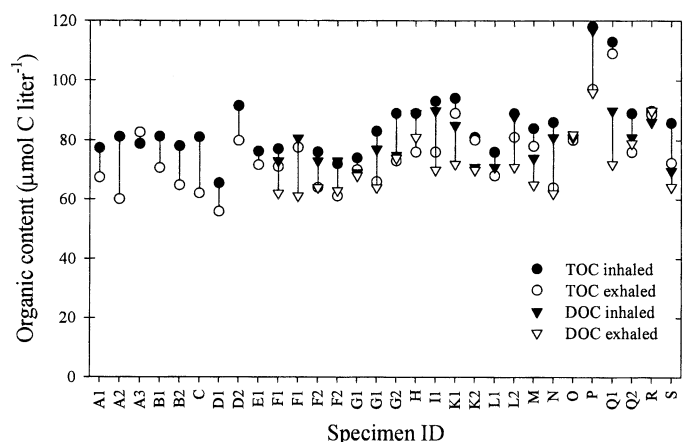
Fig. 1. The removal of organic carbon by *T. swinhoei* in a single passage of the water via its filtration system measured at the coral reef of Eilat, Israel. Solid and open symbols represent the inhaled and exhaled concentrations, respectively; TOC and DOC concentrations are averages of duplicate sample ampoules. The magnitude of the organic carbon removal is indicated by the length of the vertical lines connecting each pair of measurements. Circles represent the TOC measurements made in 1998 and 2000. Triangles represent direct DOC measurements made in 2000. Specimen IDs are indicated on the abscissa (*see Table 1*).
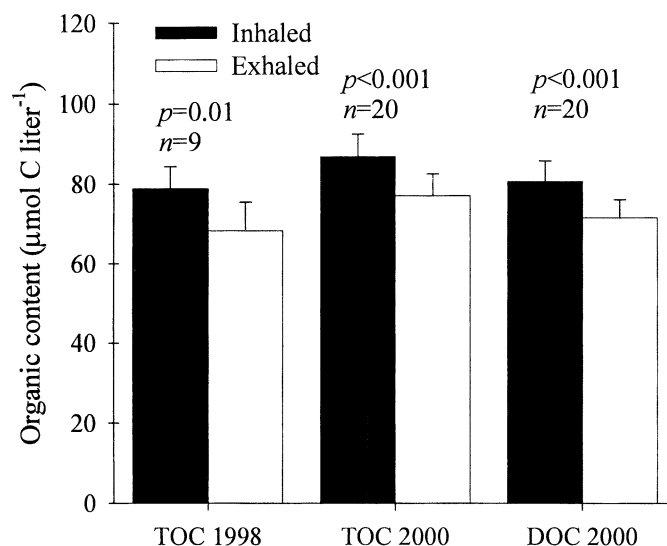

Fig. 2. Average TOC and DOC concentrations in the inhaled and exhaled water. *P* values (Wilcoxon matched pairs test) indicate the significance level of the difference in the concentration of the respective carbon pools between the inhaled and exhaled samples in *n* pairs of InEx samples. Error bars = SE.

must have been from the dissolved pool. An indication of DOC removal (cases where TOC removal exceeded the maximal ambient POC level) was evident in seven of the nine InEx pairs sampled in 1998. Thus, considerable DOC removal was demonstrated in the 1998 sampling and by the direct measurements of DOC removal in 2000 (Fig. 1).

An increase of POC in the exhaled water was observed in about one-third of the samples despite the sponge's efficient removal of nearly all the living cells. The flow cytometry analysis confirmed that most of the excreted POC was nonliving detrital particles. The POC removal (1.3 ± 6.7 μmol C L^{-1}, median = 2.3 μmol C L^{-1}) calculated as TOC removed minus DOC removed was similar to the values of LvPOC removal (2.1 ± 1.0 μmol C L^{-1}) calculated from the flow cytometer measurements.

T. swinhoei removed live phytoplankton and bacteria in all cases. Removal efficiency was remarkably high (average 85–95%, Fig. 3) except for eukaryotic cells in 1998 ($30\% \pm 11\%$). In 2000, however, eukaryotic cells were removed as efficiently as were other types of phytoplankton. Over the wide range of ambient concentrations encountered, the number of cells removed increased linearly with increasing cell

abundance, with no indication for a threshold or for saturation (Fig. 3). This pattern is similar to that reported for other sponges (e.g., Reiswig 1971; Pile et al. 1996; Ribes et al. 1999). However, in comparison to the TOC removal, the amount of LvPOC removed (maximum: 4.6 μmol C L^{-1}) was always small (Table 2, Fig. 4).

The amount of molecular oxygen removed from the water passing through *T. swinhoei* (9 μM O$_2$) was equivalent to the amount of organic carbon removed (10 μmol C L^{-1}, Table 2). This removal peaked at dawn (7% of the inhaled oxygen) and was minimal at midday (2%), when oxygen production by the photosynthetic symbionts was expected to be maximal (Fig. 5; Beer and Ilan 1998). Ammonium was also efficiently removed by the four specimens sampled ($62\% \pm 7\%$), resulting in a 204 ± 49 nmol N reduction from each liter of seawater pumped by the sponge.

The DFS measurements indicated an average excurrent speed of 7.6 ± 2.3 cm s^{-1}, corresponding to a water transport rate of 230 ± 69 ml min^{-1}. Specific water transport (normalized to the volume of the sponge) was inversely correlated with sponge size ($r = -0.87$). Large sponges (specimen volume >100 ml) pumped <2 ml min^{-1} (ml sponge)$^{-1}$, small sponges (<50 ml) pumped >4.5 ml min^{-1} (ml

Table 2. The removal of organic carbon and its components by the sponge *Theonella swinhoei* as measured in 1998 and 2000. Averages ($\pm$SD) were calculated for each year and for the grand total (*n* = number of InEx samples).

Component removed	1998 (*n*=9)	2000 (*n*=20)	Total (*n*=29)
Total organic carbon (TOC, μmol C L^{-1})	11$\pm$7	10$\pm$7	10$\pm$7
Dissolved organic carbon (DOC, μmol C L^{-1})	6$\pm$5*	10$\pm$8	
Living plankton (LvPOC, μmol C L^{-1})	1.4$\pm$0.2	2.4$\pm$1.1	2.1$\pm$1.0
% LvPOC of TOC removed	13	24	20
% DOC of TOC removed	40*	97	

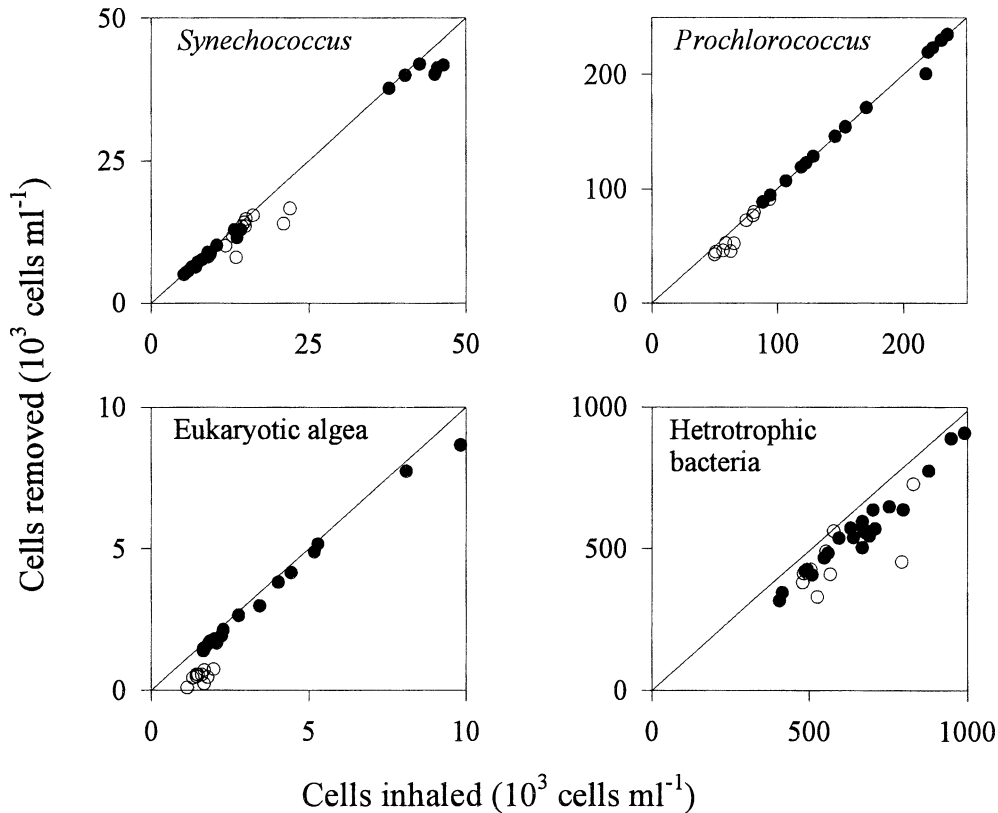* A conservative estimate of DOC removal was used by considering the POC to be fully (100%) removed by the animals.

Fig. 3.   The removal of ultraplankton (<8 μm) plotted against ambient (inhaled) concentrations. The diagonal line ($x = y$) represents 100% removal. Data are from the same InEx pairs that were used for the TOC/DOC analysis (opens symbols = 1998; solid symbols = 2000).

sponge)$^{-1}$, with an overall mean pumping rate of 2.6 $\pm$ 1.8 ml min^{-1} (ml sponge)$^{-1}$. The average excurrent speeds measured continuously with the MicroADV (7.8 $\pm$ 3.5 cm s^{-1}, $n = 4$ sponges) was in excellent agreement with the above discrete DFS measurements. Based on this agreement, the DFS measurements were used to calculate hourly integrated fluxes for matching InEx pairs (Table 3).

Discussion

DOC accounts for most of the carbon removed by the tropical symbiont-bearing sponge *T. swinhoei* from the water it pumps. This sponge is also an efficient filter feeder, removing most of the living cells from the water it pumps. The few oxygen measurements made indicated a respiratory demand of the same order of magnitude as the TOC/DOC removal (Fig. 5, Table 2). These findings confirm earlier suggestions by Reiswig (1974, 1981) that DOC uptake can reconcile the >70% discrepancy between the particulate gain and respiratory demand of several tropical sponges. Because *T. swinhoei* harbors several prokaryotes and worms, it is still unknown whether and to what extent the sponge participates in the uptake and utilization of the removed DOC.

The composition of dissolved organic matter in waters overlying coral reefs is poorly understood (van Duyl and Gast 2001), rendering speculative any discussion of the nature of the material removed from the water passing through *T. swinhoei*. The DOC content in the water exhaled by the sponge (average of 71 $\pm$ 10 μmol C L^{-1}, Fig. 1) was reduced to a level similar to the ambient DOC in the adjacent open-sea surface (68 $\pm$ 8 μmol C L^{-1}, 0–20 m depth; Häse et al. unpubl. data). However, the exhaled values were always much higher than the concentration of the refractory DOC in the deep ocean (45 μmol C L^{-1}; Benner 2002). The exhalent level is similar to that reported from the surface waters of oligotrophic oceans, presumably representing the semilabile DOC (Carlson 2002). We therefore suggest that *T. swinhoei* removes primarily the labile fraction (cf. Van Duyl and Gast 2001). Potential sources for labile DOC in coral reefs are numerous. Such sources are expected to vary over both time and space. Unlike the comparatively uniform distribution of DOC in the ocean interior (Benner 2002 and references therein), DOC in the coral reef was much more variable (Fig. 1).

The typical low surface-to-volume ratio of metazoans is thought to limit the uptake of dissolved elements from the water, to a point where mass feeding on DOC by metazoans is considered unlikely (Siebers 1982). Sponges, on the other hand, have evolved to be functionally adapted for bulk DOC removal by actively pumping water through complex filtration systems with immense internal surface areas.

At present we have no information on the mechanisms involved in the observed DOC removal. A possible role of symbiotic bacteria was suggested by Reiswig (1971, 1981).
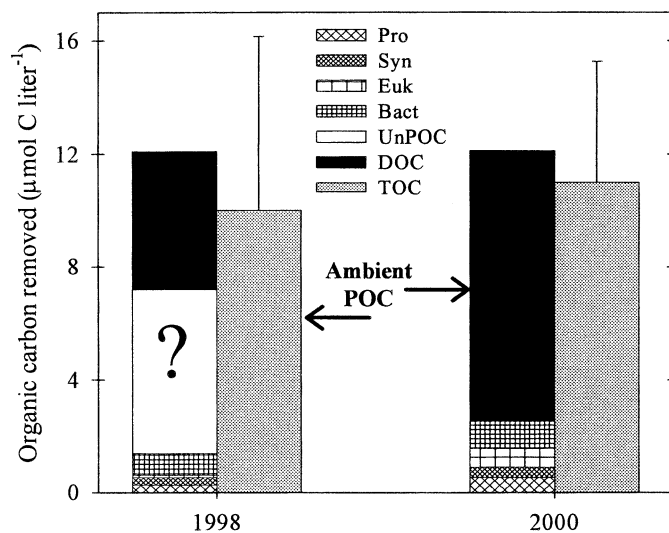
Fig. 4. The average contribution of different pools (stacked bars on the left) to the TOC (gray bars at the right) gained by the sponge *T. swinhoei* in 1998 and 2000. The unfilled region denoted by a question mark in the 1998 bar is the unresolved organic matter (UnPOC) from a conservative estimate of DOC considering remaining TOC removed as particulate detritus. Horizontal arrows indicate the corresponding ambient POC levels and thus represent an upper bound for POC removal by the sponge. Pro, *Prochlorococcus*; Syn, *Synechococcus*; Euk, eukaryotic algae <8 μm; Bact, nonphotosynthetic bacteria. Error bars = 95% confidence interval for the TOC removal.
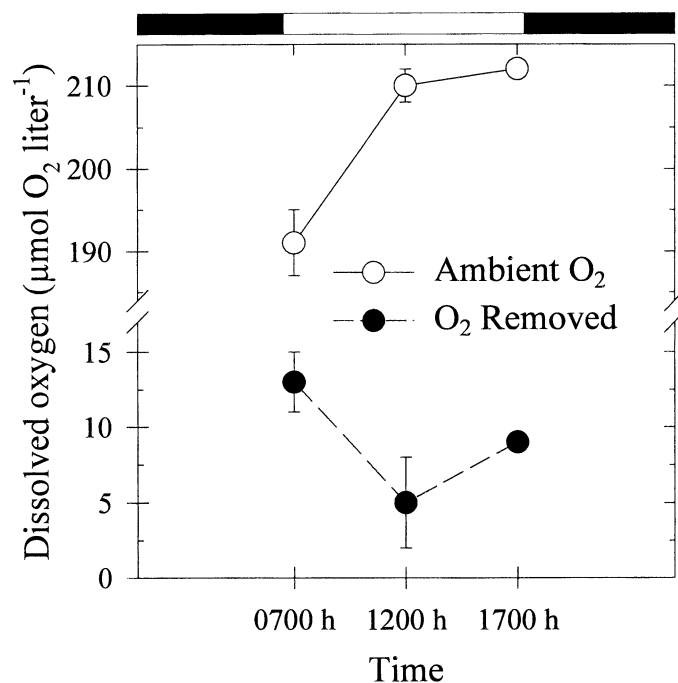

Fig. 5. The concentration of oxygen in the water inhaled and its removal by three specimens of *T. swinhoei* at dawn, noon, and dusk in a single day (17 December 2000). Data were obtained by the InEx method. The bar at the top indicates dark (solid) and light (open) time. Error bars = SE.

His findings indicated that the discrepancy between respiratory demand and particulate feeding occurred in sponges with large populations of endosymbiotic bacteria but not in asymbiotic sponges. Another indirect indication of the importance of symbiotic bacteria is the typical proximity of the endosymbiotic bacteria to the choanocyte cambers of some sponges (Wilkinson 1978; Webster and Hill 2001). Fast incorporation of labeled amino acids by the symbiotic bacteria

of the sponge *Chondrosia reniformis* further strengthens the assertion that bacteria are involved (Wilkinson and Garrone 1980). However, ultrastructural studies of *T. swinhoei* (Bewley et al. 1996; Magnino et al. 1999) and other sponges (Reiswig 1981) indicated that the bacteria are not found along the surface of the water canals but rather further inside in the sponge's mesohyl behind the pinacoderm. It is reasonable to assume that the removal of dissolved matter dur-

Table 3. Comparison of published specific metabolic activity of several sponges (mean $\pm$ SD or range) normalized to 1 ml sponge volume.

Sponge	Osculum diameter (mm)	Excurrent jet speed (cm s^{-1})	Pumping rate (ml min^{-1} (ml sponge)$^{-1}$)	Carbon gain (nmol C (ml sponge)$^{-1}$ min^{-1})	O$_2$ removal efficiency (%)	O$_2$ removal rate (nmol O$_2$ min^{-1} (ml sponge)$^{-1}$)	Source
Theonella swinhoei	6–15*	7.6$\pm$2.3	2.6$\pm$1.8	4.2$\pm$2.6			This work (LvPOC)†
					4$\pm$2	23.4$\pm$13.0	
				26.0$\pm$18.2			This work (DOC)‡
Mycale lingua	12$\pm$7	14$\pm$9.7					Pile et al. 1996
Halichondria panicea	0.15	5.6	2.7$\pm$1.1				Riisgård et al. 1993
Haliclona urceolus			2.5$\pm$1.7				Riisgård et al. 1993
Dysidea avara			1.0$\pm$0.8	0.6–9.7*			Ribes et al. 1999
Therea muricata			3.0–3.6*				Witte et al. 1997
Mycale sp.	20–100*	7.9$\pm$0.7	14.4	27.8	1.2	34	Reiswig 1974
Tethya crypta	>100	12.8$\pm$2.6	10.8	15.5	1.1	9	Reiswig 1973
Verongula sp.	108	17.3$\pm$0.6	5.1	10.5	5.6	50	Reiswig 1974
Verongia fistularis	33	10.1$\pm$0.5	7.4$\pm$0.4	9.1$\pm$1.3	5.3	79	Reiswig 1981

* Range.
† LvPOC, particulate organic carbon of living cells.
‡ DOC, dissolved organic carbon.

ing the brief (<3 s) passage of the water via the sponge filtration system would require the removing organs/organisms to be in direct contact with the flowing water. Nevertheless, the initial uptake and transport of dissolved substances in the sponge must occur across the pinacoderm cells or via the microvilli of the choanocytes. In other sponges, bacteria are also found in intracellular vacuoles (Reiswig 1981 and references therein) or concentrated in specialized bacteriocytes (Ilan and Abelson 1985). Thus, dissolved substances must first be transported across at least two cell membranes before they can come in contact with the symbiotic bacteria. The ability of sponge cells to transport and assimilate dissolved elements was shown for asymbiotic larvae of the sponge *Tedania ignis* by Jaeckle (1995). These morphological and physiological studies suggest that *T. swinhoei* may have a direct role in the uptake and transport of DOC from the water it pumps.

T. swinhoei excretes organic carbon, as do all heterotrophs, in dissolved, particulate, or both forms (Reiswig 1971; Ribes et al. 1999). The InEx technique allowed measurement of the net flux of each element. Therefore, unlike living cells (counted with the flow cytometer) our TOC/DOC measurements did not discriminate between removal and excretion of detrital particles. When measured directly, net DOC removal accounted for almost all the TOC removed (Table 2). However, the flow cytometer measurements showed that consistently ~20% of the TOC removed was LvPOC (Table 2). How is it then possible for the DOC to account for almost all the TOC removed? Moreover, a net removal of detrital particles (total POC minus LvPOC) was evident in 9 of 19 samples. This apparent discrepancy (Fig. 4) is most likely a consequence of excretion by the sponge. Furthermore, a relatively large error was inherent in the way we estimated detrital particles by subtracting two large quantities one from another: $TOC_{removed} - (DOC_{removed} + LvPOC_{removed})$. On average, the removal of POC by the sponge (1.3 ± 6.7 μmol C L^{-1}, median 2.3 μmol C L^{-1}) was similar in magnitude to LvPOC removal (Fig. 4), suggesting that the detrital removal is either small or totally masked by excretion. Thus, the role of nonliving particles in the nutrition of *T. swinhoei* remains unresolved. In contrast, detritus encompasses >90% of the carbon ingested by the temperate ascidian *Halocynthia papillosa* (Ribes et al. 1998).

Specific pumping rates of *T. swinhoei* were in the lower range of those reported for other (larger) tropical sponges but resemble those of temperate species of comparable size (Table 3). This weak pumping rate implies a rather low specific carbon gain from the particulate pool (4.2 nmol C L^{-1} (ml sponge)$^{-1}$ min^{-1}) despite the high removal efficiency. However, when the removal of both particulate and dissolved carbon (TOC) is considered, the specific organic carbon retention by *T. swinhoei* (30 nmol C L^{-1} (ml sponge)$^{-1}$ min^{-1}) is the highest ever reported (Table 3). Past studies, however, had not measured DOC removal. In the present study, the specific oxygen demand of *T. swinhoei* (23.4 nmol O$_2$ L^{-1} (ml sponge)$^{-1}$ min^{-1}) was slightly lower than its TOC gain, as expected for an organism with phototrophic symbionts (Magnino et al. 1999). Given the lack of information on the *T. swinhoei* consortium growth rate and primary production, it is not possible at this stage to estimate the relative contributions of DOC and photosynthetically derived production to body mass production of the sponge. The specific respiratory demand of three other tropical sponges (Reiswig 1974, 1981) was higher than that of *T. swinhoei* and higher than the POC intake (Table 3), further strengthening Reiswig's suggestion that a major carbon source in those sponges was dissolved organic matter.

Considering the large amount of organic material *T. swinhoei* processes, a large efflux of remineralized nutrients was expected (Gili and Coma 1998). Thus, the efficient removal of ammonium by the sponge is surprising and may be attributed to the presence of photosymbionts. Nevertheless, at this stage, our nutrient data set is too limited to allow further discussion of the nutrient dynamics in this species.

This is the first report of in situ removal of bulk DOC in invertebrates. The results of this study suggest the presence of a previously undocumented pathway of carbon flux in benthic habitats. If DOC is supplied by the sponge to symbiotic bacteria, the microbial loop in the reef would be accelerated. If, on the other hand, the DOC is directly utilized by the animal, the loop would be"short-circuited." Because most of the labile DOC in the reef is assumed to be released locally, its removal by invertebrates would represent a major pathway of carbon recycling, a key process in coral reef communities. Our preliminary data (unpubl.) suggest that other reef sponges also remove DOC. The abundance of sponges in coral reefs and their filtration capacity might have been grossly underestimated (Richter et al. 2001). These data suggest that DOC may play a major role in the trophic dynamics of coral reefs.

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