

Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges

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ABSTRACT: We studied the removal of dissolved organic carbon (DOC) and bacterioplankton by the encrusting sponges *Halisarca caerulea*, *Mycale microsigmatosa* and *Merlia normani* in coral reefs along Curaçao, Netherlands Antilles. Sponge specimens were collected from coral reef cavities and incubations were done on the fore-reef slope at 12 m depth. The concentrations of DOC and bacterioplankton carbon (BC) were monitored *in situ*, using incubation chambers with sponges and without sponges (incubations with coral rock or ambient reef water only). Average (\pm SD) DOC removal rates (in $\mu\text{mol C cm}^{-3}$ sponge h^{-1}) amounted to 13.1 ± 2.5 , 15.2 ± 0.9 and 13.6 ± 2.4 for *H. caerulea*, *M. microsigmatosa* and *M. normani*, respectively. The DOC removal rates by the 3 sponges were on average 2 orders of magnitude higher than BC removal rates and accounted for more than 90% of the total organic carbon removal. Total organic carbon removal rates presented here were the highest ever reported for sponges. In an additional experiment with *H. caerulea*, the fate of organic carbon was reconstructed by measuring dissolved oxygen (O_2) removal and dissolved inorganic carbon (DIC) release in a laminar flow chamber. *H. caerulea* respired 39 to 45% of the organic carbon removed. The remaining 55 to 61% of carbon is expected to be assimilated. We argue that *H. caerulea* may have a rapid turnover of matter. All 3 sponge species contained associated bacteria, but it is unclear to what extent the associated bacteria are involved in the nutrition of the sponge. We conclude that the 3 sponge–microbe associations are (related to the availability of dissolved and particulate carbon sources in the ambient water) ‘dissolved organic matter (DOM)-feeders’ and encrusting sponges are of quantitative importance in the removal of DOC in coral reef cavities.

KEY WORDS: DOC · Sponges · Nutrition · Carbon budget · Nutrient cycling · Coral reef · Reef framework cavities · Caribbean · Curaçao

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INTRODUCTION

Sponges are considered very efficient suspension feeders and their filtering capacity in combination with their abundance may have a profound impact on their environment (Gili & Coma 1998). Sponges can feed on a wide variety of types and sizes of plankton up to the capture of zooplankton (Vacelet & Boury-Esnault 1995). However, the preferred utilization of nano- and picoplankton (particles $< 2 \mu\text{m}$) by sponges has been shown both in laboratory studies (Van de Vyver et al.

1990, Riisgård et al. 1993, Turon et al. 1997) and field studies (e.g. Reiswig 1974a, Pile et al. 1996, Ribes et al. 1999, Kötter & Pernthaler 2002).

There is still debate on the qualitative and quantitative role of dissolved organic matter (DOM) in the nutrition of marine benthic animals (Wright & Manahan 1989, Thomas 1997). As early as 1872, it was suggested that DOM could be a potential food source for marine invertebrates and ever since it has remained a topic for discussion (reviewed by Jørgensen 1976). Reiswig pioneered studies on the retention of

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organic carbon by sponges (Reiswig 1971) and the vast volumes of water sponges can process over time (Reiswig 1974b). He found a discrepancy between the supply and demand of carbon in benthic suspension feeders, and DOM was proposed to be the missing link (Reiswig 1981). It is generally assumed that only sponges with sponge-associated bacteria, sometimes comprising up to half of the total biomass of the sponge, are capable of utilizing DOM (Frost 1987, Ribes et al. 1999). Sponges have been demonstrated to take up the amino acid glycine (Stephens & Schinske 1961), 0.1 μm beads (Leys & Eerkes-Medrano 2006), as well as virus particles (Hadas et al. 2006) from ambient water. Both viral particles and 0.1 μm beads easily pass a 0.2 μm filter and are therefore operationally defined as 'dissolved'. Yahel et al. (2003) were the first to show extensive removal of bulk dissolved organic carbon (DOC) by the sponge *Theonella swinhoei*. The DOC intake by this sponge accounted for more than 90% of the total organic carbon (TOC) intake.

Coral cavities, ranging from 50 to 250 dm^3 , have been identified as major sinks of organic carbon (De Goeij & Van Duyl 2007). Framework cavities in reefs of the Berau area, East Kalimantan, Indonesia and in the reefs of Curaçao, Netherlands Antilles have shown extensive removal of organic carbon. The surface of the framework exceeds that of the open reef (Richter et al. 2001, Scheffers et al. 2004) and the cavity walls are densely covered with cryptic organisms, dominated by (mostly encrusting) sponges. The natural sponge coverage in relation to the total cavity surface (including the sandy bottom) in coral cavities ranges from 10 to 27% (Wunsch et al. 2000, Richter et al. 2001, Van Duyl et al. 2006). Particle uptake from the water column by the benthic reef community is mainly accounted for by suspension feeders, such as sponges and ascidians (Ribes et al. 2005). Encrusting cryptic sponges feed effectively on ultraplankton (more than 90% accounted for by bacteria) resulting in high carbon fluxes, up to 373 $\text{mg C g ash free dry weight (AFDW)}^{-1} \text{d}^{-1}$ (Kötter & Pernthaler 2002). However, although particulate organic carbon (POC) removal rates by the cryptic habitat are considered to be high (Richter et al. 2001, Scheffers et al. 2004), DOC removal rates by coral cavities were 2 orders of magnitude higher in comparison (De Goeij & Van Duyl 2007). No efforts have been made to investigate the contribution of DOC to total carbon uptake by encrusting sponges in coral cavities.

In this study we investigate the role of 3 encrusting coral cavity sponge species, *Halisarca caerulea*, *Mycale microsigmatosa* and *Merlia normani*, in the removal of DOC and bacterioplankton carbon (BC). For *H. caerulea*, the fate of organic carbon was further studied by measuring dissolved oxygen (O_2) consumption and DIC release.

MATERIALS AND METHODS

Study area and sponge collection. The study was conducted on the Caribbean island of Curaçao, Netherlands Antilles (12° 12' N, 68° 56' W). Sponge experiments were performed at station Buoy 1 using incubation chambers on the reef flat of the fringing reefs along the leeward side of Curaçao at a depth of approximately 12 m (Fig. 1). Sponges were collected by chiseling them from overhangs and cavity walls between 15 to 25 m depth. Attached pieces of coral rock were cleared of epibionts and shaped to a maximum total volume of approximately 70 cm^3 and a sponge surface of approximately 24 cm^2 , with an average sponge thickness of 2.5 mm. Sponges were stored upside down in wire cages (20 × 20 × 15 cm; maximum of 4 pieces per cage) to protect them from sediment accumulation and predation. Cages were stored inside coral reef cavities at 15 m depth. Sponges were acclimatized for at least 1 wk prior to experiments. Pieces of sponge were kept for a maximum of 6 mo before use in an incubation experiment. They were regularly checked and when necessary the substratum was cleaned and debris removed. Sponges were re-sized by cutting them to their original surface area when overgrowth of the edges of the coral rock substratum by the sponge occurred. After each experiment 2 pieces of sponge tissue (0.5 cm^2) were cut loose from the substratum and fixed for both taxonomy and the determination of abundance of sponge-associated bacteria. Sponge and all data collection were made via SCUBA diving.

Incubation chambers. Two types of incubation chambers were used *in situ* to determine DOC and BC removal over time for the selected sponges. During the

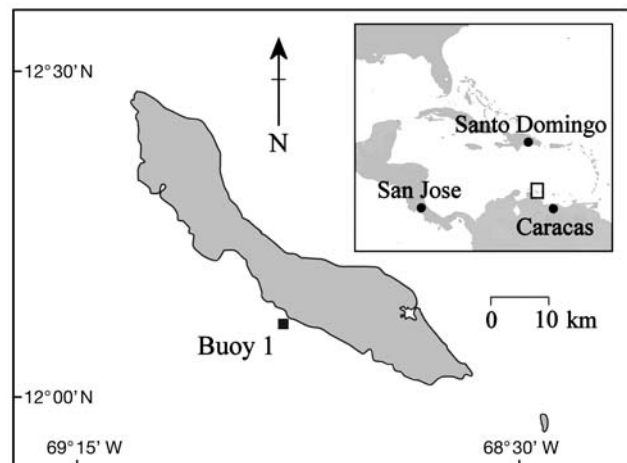


Fig. 1. Location of the Buoy 1 station on the SW coast of Curaçao. Inset: location of Curaçao in the Caribbean Sea. The long-term mean current runs from SE to NW along the island

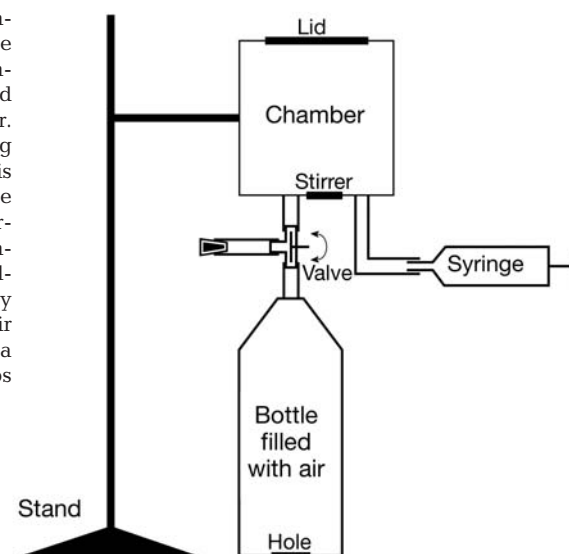
first fieldwork period (2004) we used 1.05 l Plexiglass cylinders with a magnetic stirring device, powered by a 9 V battery (Fig. 2). Sample water withdrawn from the chamber during incubation was replaced by air, supplied by an air-filled bottle attached to the cylinder. Air was supplied and flow was controlled by a 3-way valve and clamps, which were opened during sampling. Pieces of sponge (7.7 to 31.2 cm²) were placed on the bottom of the cylinder next to the magnetic stirrer and sampling point. The cylinder was sealed by two 10 cm diameter lids containing an O-ring. This incubation chamber will be referred to as Chamber 1.

To measure O₂ and dissolved inorganic carbon (DIC) fluxes in the sponge *Halisarca caerulea* we designed a portable Plexiglass flow chamber. The design of the flow chamber is given in Fig. 3. The flow chamber (35 × 8 × 12 cm) has a volume of 1.7 l and an average flow velocity of approximately 1.5 cm s⁻¹, generated by a magnetic stirrer, powered by 8 AA batteries (total 12 V). Downstream the stirrer, water passes a mesh screen with 2 mm pores, to reduce vortices and to create a quasi-laminar flow. Water was sampled downstream of the sponge, using a glass Pasteur pipette and a silicon tube. The sample water was replaced by ambient reef water through a PVC tube (2 mm in diameter and 0.5 m in length to reduce diffusive exchange) placed after the magnetic stirring device. The surface area of the sponge compartment (6.5 × 4.5 × 3 cm) relative to the volume of the chamber was scaled to the proportion of sponge surface on total cavity surface area (TSA) and coral cavity volume (CV). The empirical linear relation of CV (dm³) and TSA (m²) in coral cavities on Curaçao (for cavity volumes ranging from 86 to 248 dm³) is described as: TSA = 0.0108 × CV

+ 0.4598 (R² = 0.943, n = 7, p < 0.001) (De Goeij & Van Duyl 2007). If CV is assumed to be 1.7 l, a sponge surface area of 24.5 cm² used in the flow chamber corresponds to a sponge cover of approximately 11% of the TSA. Pieces of coral rock with sponge were shaped to the size of the sponge compartment and sponges were cut to a surface area of 24.5 cm² (6.1 cm³). The incubation chamber was sealed with a 10 cm diameter lid containing an O-ring. This laminar flow chamber will be referred to as Chamber 2. Both Chambers 1 and 2 were covered with duct tape and a black bag in order to conduct the experiments in the dark.

Sample collection. In a first series of experiments, changes in DOC concentration and bacterial abundance (BA) were measured over time in Chamber 1 to determine DOC and BC removal by the encrusting cavity-dwelling sponges *Halisarca caerulea* (n = 7), *Mycale microsigmatosa* (n = 6), and *Merlia normani* (n = 3) (Fig. 4). Incubations with only ambient reef water were performed as a blank measurement (control). In addition, incubation experiments were performed with a piece of coral rock (cleared of epibionts) to measure possible coral rock metabolism. Differences in DOC and BC removal rates between the 3 sponges were determined. Experiments were conducted between March and September 2004 between 10:00 and 12:00 h. Prior to the experiment, pumping activity of the sponges was checked and only specimens with open oscula (outflow openings) were selected for the experiments. Sponges were carefully positioned, and after closing the chamber, water samples were taken at time (t) = 0, 2, 4, 10, 30 and in some series up to 45 min. Samples for DOC and BA were taken with acid washed 100 ml polycarbonate

Fig. 2. Incubation Chamber 1. Pieces of sponge are placed in the chamber and water is mixed by a magnetic stirrer. During the sampling process, sample water is replaced by air from the bottle attached underneath the benthic chamber. The chamber is sealed from outside water by a lid with an O-ring. Air release is regulated by a 3-way valve and clamps



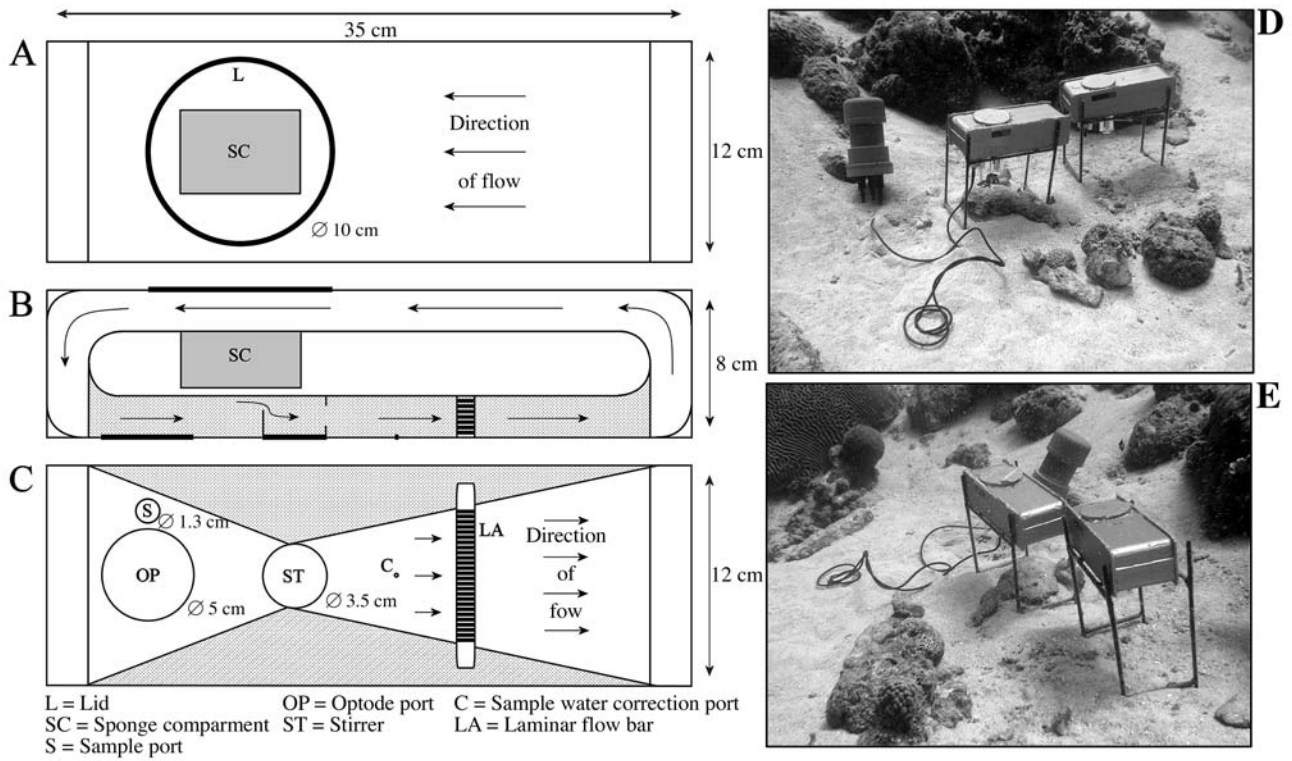


Fig. 3. Incubation Chamber 2. (A) Top, (B) side and (C) bottom views. (D,E) Flow chamber on the reef slope at approximately 12 m depth

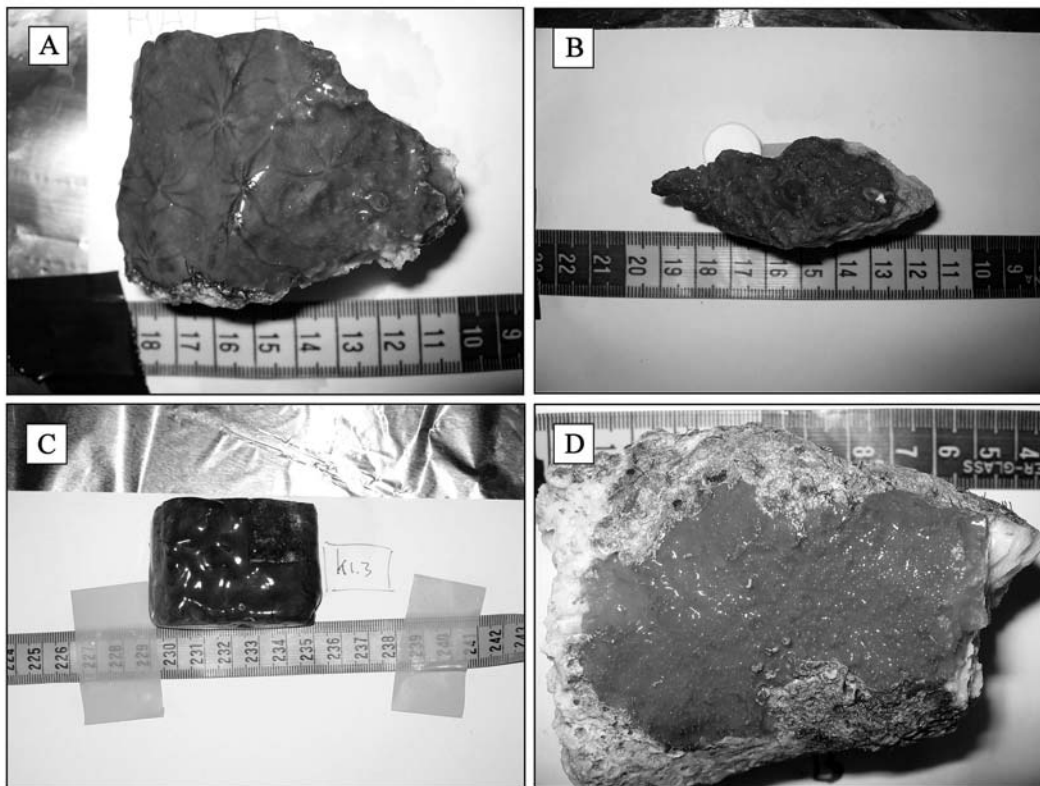


Fig. 4. (A) *Halisarca caerulea*, (B) *Mycale microsigmatosa*, (C) *Halisarca caerulea* shaped to fit sponge compartment of Chamber 2 and (D) *Merlia normani*

syringes. Syringes were stored in the dark underwater during the experiment. Sponges were monitored for pumping activity during the experiment. After the incubation experiment, syringes were kept in the dark at 4°C prior to further processing within 4 h.

In a second series of experiments, between March and June 2005 and between January and April 2006, O₂, DOC and BC uptake, and DIC release by *Halisarca caerulea* were monitored in Chamber 2. Two control experiments (with coral rock and with ambient reef water only; n = 6) were performed. Before the incubation, trapped air was carefully removed from the chambers, tubings and syringes. The flow chambers were placed on a specially designed stand and were allowed to acclimatize prior to the positioning of the sponge (Fig. 3). Before closing the chamber, time 0 (t₀) samples were taken for DOC and BA (80 ml) and O₂ (220 ml). After closing Chamber 2, samples for DOC and BA were taken at t = 5, 10, 30, 60 and in some series up to 90 min. Samples for O₂ and DIC were taken at the end of each experiment. Due to the large volumes of water needed per sample, O₂ and DIC concentration were determined only at t₀ and t_{end}. All syringes were kept underwater in the dark during the whole experiment. To determine the influence of keeping the syringes underwater during the experiments on oxygen concentration, we measured a series of t₀ oxygen samples (n = 8) that were both kept underwater during the whole experiment as well as directly processed (brought to the surface by a second diver after sampling t₀). After the incubation experiment, samples for oxygen concentration were directly processed. Samples for DOC, BA and DIC were kept in the dark at 4°C prior to further processing within 4 h.

Sample treatment and analysis. Water samples, collected for the determination of DOC, DIC, and BA, and sponge tissues for the determination of sponge-associated bacteria, were processed in the laboratory prior to transportation to the Netherlands for analysis. Samples for DOC and DIC analysis were gently (max. 20 kPa suction pressure) filtered through a 0.2 µm polycarbonate filter (Millipore, 47 mm). Prior to filtration, filters, glassware and pipette tips were washed with acid (3 × 10 ml 0.4 M HCl), 0.2 µm filtered double-distilled water (3 × 10 ml) and sample water (3 × 10 ml).

Duplicate 8 ml DOC samples were collected in pre-combusted (4 h at 450°C) glass ampoules. Ampoules were sealed immediately after acidification with 1 to 2 drops of concentrated H₃PO₄ (80%) and stored at 4°C until analysis. Measurements of DOC were performed by the high temperature combustion method, using a TOC Analyzer, Model TOC-5000A (Shimadzu). The TOC analyzer was calibrated with potassium phthalate in Milli-Q water. As an internal

control of the DOC measurements, consensus reference material provided by D. Hansell and W. Chen of the University of Miami, USA (Batch 4, 2004; 45 µmol l⁻¹, every 10 to 20 samples) was used. DOC concentrations (average ± SD) measured for the batch were 45 ± 2 µmol l⁻¹. The average analytical precision of the instrument was <3% (every sample was measured 5-fold).

DIC samples (5 ml) were transferred to glass vials (Alltech, clear screw cap 4 ml capped with a TFE liner) amended with 10 µl of a saturated HgCl₂ solution (8 g 100 ml⁻¹). Analysis of DIC was done spectrophotometrically using a continuous flow set-up run on a Technicon Traacs 80 autoanalyzer after the method of Stoll et al. (2001). The instrument was calibrated for the typical range for natural seawater (1900 to 2500 µmol l⁻¹) with Certified Reference Materials (CRMs) (Godec et al. 1992).

O₂ was measured according to the method of Winkler (1888), adjusted by Carpenter (1965) and Culbertson (1991). Winkler bottles of approximately 60 ml (volume of each bottle was precisely measured up to 10 µl) were rinsed 3 × in seawater. Sample water of 2 syringes was carefully divided over 3 Winkler bottles (overflowing approximately 40 ml). Whole bottle end point titration was determined by adding a 1% starch solution using a microburette. The coefficient of variation between the triplicate measurements was <0.5%.

Samples for BA in seawater (10 ml) were fixed in 4% paraformaldehyde (PFA), stained with acridine orange, and gently (max. 20 kPa suction pressure) filtered onto 0.2 µm black polycarbonate membrane filters (Millipore, 25 mm), which were mounted on slides and stored at -20°C.

Sponge tissues for taxonomy were fixed in 5 ml 80% ethanol. For determination of sponge-associated bacteria, sponge tissue was fixed in 3 ml 4% PFA for 30 min at 4°C. Subsequently, tissues were washed twice with 1 × PBS and stored in 5 ml 1 × PBS:80% ethanol (1:1) at -20°C. Sponge tissue was crushed in a reaction vial containing 200 µl of Lysis T (Sigma). The dissociated cells were resuspended in 200 µl of artificial sea water (ASW) and centrifuged at 2700 × g for 30 s. Supernatant was collected into a 15 ml tube. The pellet was resuspended in 10 ml ASW, and filtered through a 0.8 µm membrane filter (Millipore, 25 mm), to separate sponge cells from bacterial cells. Filters were placed on a microscopic slide with a drop of DAPI-mix to stain possible retained bacteria, and stored in the dark at -20°C. The filtrate was added to the supernatant and diluted to 10 ml with ASW, of which 2.5 ml was filtered over a 0.2 µm polycarbonate filter (Millipore, 25 mm), supported by a 0.45 µm HA filter (Millipore, 25 mm).

The filters were air dried, mounted on a microscopic slide in a DAPI-mix and stored at -20°C . Bacterial numbers were counted using an epifluorescence microscope (1250 \times). Per slide, 10 fields were counted or up to a minimum of 200 bacteria. The DAPI counts were recalculated per cm^3 sponge. Data presented in this study is only to show the presence of associated bacteria qualitatively.

Carbon uptake rates and budgets. The initial DOC removal rates by sponges were estimated by applying a 2G-model (see also de Goeij & van Duyl 2007). DOC represents a very heterogenic group of organic compounds, both in size fractions, and chemical composition as in bioavailability or biodegradability. A simplified model to describe the course of carbon over time assumes that the DOC pool is composed of 2 major fractions. In a well mixed system, the fast (C_f) and slow (C_s) removable fractions will be consumed according to their specific fast and slow removal rate constants k_f and k_s , respectively. The total DOC removal will then be described as the sum of all individual removal rates, or:

$$\frac{d\text{DOC}}{dt} = -(k_f C_f + k_s C_s) \quad (1)$$

By integrating this equation in reference to time, t , we arrive at the equation describing the concentration of DOC as a function of time:

$$\text{DOC}(t) = C_{f,0} \times e^{-k_f t} + C_{s,0} \times e^{-k_s t} \quad (2)$$

The experimental data can be described using the model by estimating the model variables $C_{f,0}$, k_f , $C_{s,0}$, and k_s using a minimalization routine. The initial uptake rate of DOC (the flux at $t = 0$) was calculated from the estimated values of these variables and is given by:

$$\text{Flux}_{\text{DOC}} = -(k_f C_{f,0} + k_s C_{s,0}) \quad (3)$$

Bacterioplankton removal rates in closed coral cavities were calculated assuming an exponential clearance of bacterial cells in a closed system with homogenous mixed water (Scheffers et al. 2004). To convert bacterial abundance to carbon biomass, a conversion factor of 30 fg per bacterial cell was used (Fukuda et al. 1998). Clearance rates (CR) were calculated according to Riisgård et al. (1993):

$$\text{CR} = (V_w/t) \times \ln(C_0/C_t) \quad (4)$$

where V_w is water volume in the incubation chamber, C_0 and C_t are the bacterioplankton concentrations at times 0 and t , calculated from the regression equation (Riisgård et al. 1993). CRs were calculated only to compare with those found in the literature.

To establish a mass balance, and to reconstruct the carbon flow for *Halisarca caerulea*, exchange rates for

DOC, O_2 and DIC were calculated from the concentration difference between t_0 and t_{end} . The TOC pool is comprised of DOC and POC. POC in tropical reef water consists mainly of phytoplankton and bacterioplankton. Phytoplankton carbon removal rates were not directly measured. The contribution of phytoplankton carbon to the total C pool in tropical waters is low and in the same order of magnitude as bacterioplankton carbon (Ayukai 1995, Yahel et al. 1998, van Duyl et al. 2002), or lower (Richter et al. 2001, Kötter 2003). For conservancy, TOC and POC removal rates used in the mass balance were calculated as follows:

$$\text{TOC} = \text{DOC} + \text{POC}, \text{ where } \text{POC} = 2 \times \text{BC} \quad (5)$$

RESULTS

DOC removal kinetics

The DOC concentration decreased exponentially with time in the presence of any of the 3 sponges, regardless of the chamber used. The 2G-model provided a fairly accurate description of the decrease in DOC concentration in the presence of the 3 sponges (Fig. 5) and was therefore applied to estimate the initial DOC removal rates for all experiments. In contrast, the concentration of DOC in the blank incubations (with ambient reef water only) did not significantly change (Fig. 5). In incubation experiments with coral rock, there was no significant removal or release of DOC (paired t -test; $t = 0.358$, $df = 5$; not significant [ns]).

Table 1 gives the volumes of the 3 incubated encrusting sponges, based on an average thickness of 0.25 cm, the DOC concentration at the start of each experiment and the initial removal rate. No significant differences in initial removal rates could be detected between the 3 species (Generalised Linear Models, GLM; $F = 1.433$, $df = 2$; ns). The average initial removal rates for the 3 sponges are given in Table 1. The average (\pm SD) initial DOC removal rate of all sponges in Chamber 1 was $14.1 \pm 2.1 \mu\text{mol C cm}^{-3} \text{ sponge h}^{-1}$. For *Halisarca caerulea*, the average initial removal rates measured in Chamber 2 ($17.1 \pm 2.4 \mu\text{mol C cm}^{-3} \text{ sponge h}^{-1}$) were higher than those observed in Chamber 1 ($13.1 \pm 2.5 \mu\text{mol C cm}^{-3} \text{ sponge h}^{-1}$) (2-sample t -test; $t = -3.076$, $df = 12$; $p < 0.025$). This may have resulted from a (seasonal) difference in DOC composition and bio-availability, the physiological status of the sponges, and from a difference in transport efficiency (different hydrodynamical conditions due to different design) between the 2 chambers. Since the design of Chamber 2 combines the surface to volume ratio of natural cavities with optimal mixing conditions, these initial DOC removal rates were considered as maximum values.

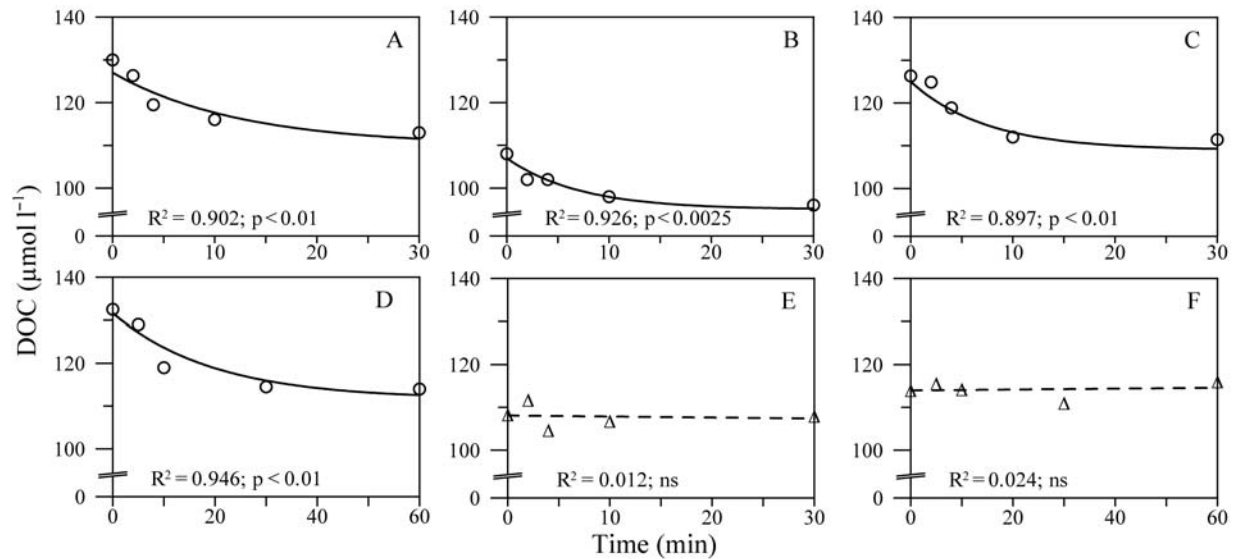


Fig. 5. *Halisarca caerulea*, *Mycale microsigmatosa* and *Merlia normani*. Exponential decrease in dissolved organic carbon (DOC) concentration with time for incubations with 3 encrusting sponges. Chamber 1: (A) *H. caerulea* Hal 2, (B) *M. microsigmatosa* Myc 2, (C) *M. normani* Mer 3. Chamber 2: (D) *H. caerulea* Hal 16. (E) and (F) are blank incubations in Chambers 1 and 2, respectively. Sponge designations correspond to those used in Table 1. Note that Chamber 1 incubations are up to 30 min and Chamber 2 incubations up to 60 min. Trend lines are given by a 2G-model fit. ns: not significant

Table 1. *Halisarca caerulea*, *Mycale microsigmatosa* and *Merlia normani*. Incubation experiments and average dissolved organic carbon (DOC) and bacterioplankton carbon (BC) removal rates, measured in 2 different incubation chambers. Values are average \pm SD. Hal: *H. caerulea*, Myc: *M. microsigmatosa*, Mer: *M. normani*; t_0 : time zero; DOC_{2G}: DOC 2G-model fit; BA: bacterial abundance; BC_{exp}: BC exponential fit; nd: no data

Sponge	Volume (cm ³)	DOC t_0 ($\mu\text{mol l}^{-1}$)	DOC _{2G} removal rate ($\mu\text{mol cm}^{-3} \text{h}^{-1}$)	BA t_0 (10^5cm^{-3})	BC _{exp} removal rate ($\mu\text{mol cm}^{-3} \text{h}^{-1}$)	Clearance rate ($\text{cm}^3 \text{cm}^{-3} \text{min}^{-1}$)
Chamber 1						
Hal 1	4.2	117	13.4	nd	nd	nd
Hal 2	7.0	131	16.4	9.2	0.3	2.3
Hal 3	6.1	94	10.6	9.1	0.6	4.3
Hal 4	5.6	nd	nd	9.5	0.7	4.8
Hal 5	6.2	nd	nd	9.9	0.6	4.0
Hal 6	6.8	105	10.7	8.1	0.5	4.2
Hal 7	5.7	130	14.3	7.9	0.6	5.2
Average			13.1 \pm 2.5		0.6 \pm 0.1	
Myc 1	7.2	108	14.0	8.8	0.5	3.9
Myc 2	5.0	109	14.4	11.0	0.9	5.5
Myc 3	7.4	144	15.1	7.4	0.4	3.9
Myc 4	1.9	154	15.3	nd	nd	nd
Myc 5	3.5	106	15.5	9.3	0.6	4.0
Myc 6	5.0	105	16.5	9.1	0.4	3.0
Average			15.2 \pm 0.9		0.6 \pm 0.1	
Mer 1	7.6	136	11.9	7.3	0.3	2.9
Mer 2	3.8	142	11.8	8.0	0.3	2.8
Mer 3	5.8	126	17.1	8.3	0.4	3.5
Average			13.6 \pm 3.1		0.4 \pm 0.1	
Chamber 2						
Hal 8	6.1	112	18.7	8.3	1.5	11.7
Hal 9	6.1	130	13.4	10.7	0.4	2.2
Hal 10	6.1	135	14.0	9.4	0.6	4.1
Hal 11	6.1	120	21.0	9.4	0.3	2.1
Hal 12	6.1	160	18.9	8.6	0.7	5.1
Hal 13	6.1	133	16.7	8.9	0.4	2.8
Hal 14	6.1	139	16.7	12.1	0.4	2.3
Hal 15	6.1	110	16.3	12.6	0.8	4.2
Hal 16	6.1	111	18.2	9.0	1.1	8.4
Hal 17	6.1	nd	nd	8.9	1.0	7.3
Average			17.1 \pm 2.4		0.7 \pm 0.4	

Bacterial clearance

BA significantly decreased exponentially with time for all sponges, whereas BA did not significantly change in the control experiments without sponges (with ambient reef water only; Fig. 6). In incubation experiments with coral rock, there was no significant removal or release of DOC (paired *t*-test; $t = 1.342$, $df = 5$; ns). Initial abundance, BC removal rate, and the clearance rates are presented in Table 1. There was no significant difference in BC fluxes measured for the 3 different sponges used in Chamber 1 (GLM: $F = 2.324$, $df = 2$; ns). Moreover, *Halisarca caerulea* BC fluxes did not significantly change between Chambers 1 and 2 (2-sample *t*-test, $t = -0.769$, $df = 14$; ns). The average (\pm SD) initial BC removal rate of all sponges in Chamber 1 was $0.52 \pm 0.16 \mu\text{mol C cm}^{-3} \text{ sponge h}^{-1}$, and for *H. caerulea* in Chamber 2 was $0.70 \pm 0.38 \mu\text{mol C cm}^{-3} \text{ sponge h}^{-1}$. The initial BC removal rates were 2 orders of magnitude lower than the initial DOC removal rates (Table 1). There was no correlation between size (volume) of the 3 encrusting sponges in Chamber 1 and the clearance rates (Pearson 2-tailed; $R^2 = 0.0196$, $n = 14$; ns).

Sponge-associated bacteria

All 3 encrusting sponges harbored sponge-associated bacteria. On average $2.1 \times 10^9 \text{ cm}^{-3}$ sponge (*Halisarca caerulea*), $2.1 \times 10^9 \text{ cm}^{-3}$ sponge (*Mycale microsigmatosa*), and $1.5 \times 10^9 \text{ cm}^{-3}$ sponge (*Merlia normani*) were

counted with DAPI. Preliminary results of the bacterial communities of the 3 sponges determined by a catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH) procedure on the filters (according to Pernthaler et al. [2002], adjusted by Teira et al. [2004]) show that DAPI-counts represented 76% (*H. caerulea*), 66% (*M. microsigmatosa*), and 60% (*M. normani*) of the CARD-FISH counts, using a probe against eubacteria (F.C. Van Duyl unpubl.). This data is only presented here to qualitatively confirm the presence of sponge-associated bacteria.

Halisarca caerulea oxygen respiration

The concentration of O_2 significantly decreased with time in the presence of *Halisarca caerulea* (paired *t*-test, $t = 8.646$, $df = 21$, $p < 0.001$). In the control experiment (incubation experiment with ambient reef water only), the O_2 concentration significantly increased with time, at an average rate of $1.4 \mu\text{mol l}^{-1} \text{ h}^{-1}$ (paired *t*-test, $t = -5.000$, $df = 3$, $p < 0.01$). In addition, the O_2 concentration of sample water directly processed on board during an experiment was significantly higher than the O_2 concentration of sample water kept underwater in the dark and processed at the end of an experiment (paired *t*-test, $t = 8.148$, $df = 6$, $p < 0.001$), yielding an average water column respiration rate of $2.7 \mu\text{mol l}^{-1} \text{ h}^{-1}$. The average (\pm SD) respiration rate for *H. caerulea*, corrected for control and water column respiration, was $2.7 \pm 0.8 \mu\text{mol O}_2 \text{ cm}^{-3} \text{ sponge h}^{-1}$, or $6.7 \pm$

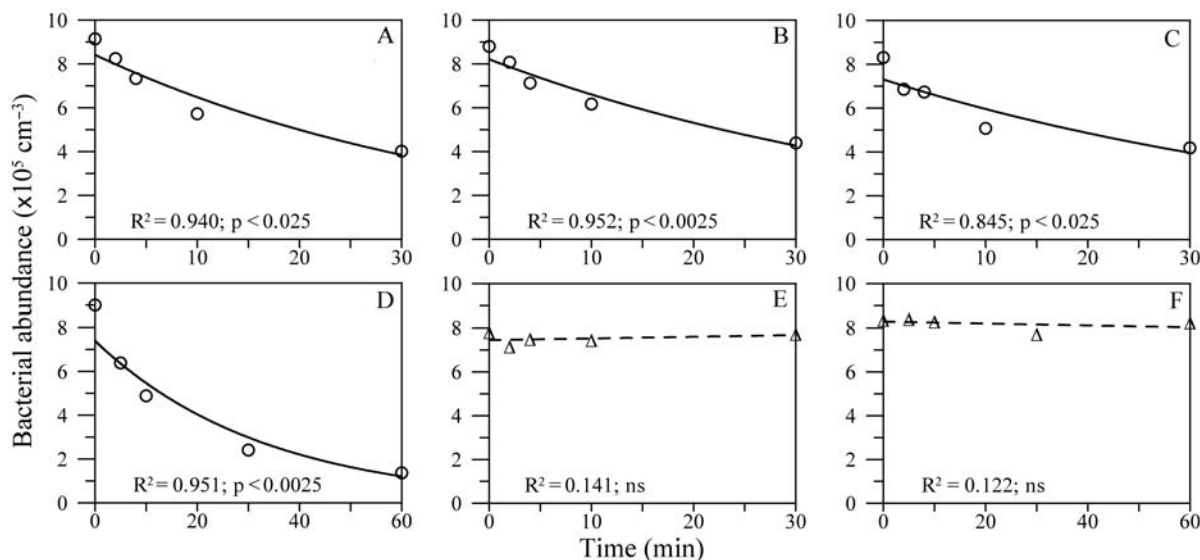


Fig. 6. *Halisarca caerulea*, *Mycale microsigmatosa* and *Merlia normani*. Exponential decrease in bacterial abundance with time for incubations with 3 encrusting sponges. Chamber 1: (A) *H. caerulea* Hal 2, (B) *M. microsigmatosa* Myc 2, (C) *M. normani* Mer 3. Chamber 2: (D) *H. caerulea* Hal 16. (E) and (F) are blank incubations in Chambers 1 and 2, respectively. Sponge designations correspond to those used in Table 1. Note that Chamber 1 incubations are up to 30 min and Chamber 2 incubations up to 60 min. Trend lines are given by an exponential fit. ns: not significant

1.9 mmol m⁻² h⁻¹ (Table 2). There was no respiration rate measured for coral rock (paired *t*-test, *t* = -1.464, *df* = 5; ns). During the sponge incubation experiments, the average drop in oxygen levels was 4.3 ± 1.2% at an average initial seawater O₂ concentration of 208 ± 7 μmol l⁻¹ (*n* = 22; range 196 to 220 μmol l⁻¹).

Halisarca caerulea DIC release

The DIC concentration significantly increased with time in the presence of *Halisarca caerulea* (paired *t*-test, *t* = -12.304, *df* = 12, *p* < 0.001). In the control incubations with ambient reef water only, the DIC concentration did not significantly change with time. However, DIC concentration significantly increased during incubations with a piece of coral rock without sponge (paired *t*-test, *t* = -3.124, *df* = 4, *p* < 0.05). This increase probably results from passive, chemical dissolution of CaCO₃, and was on average 23 μmol C l⁻¹ h⁻¹. In the presence of *H. caerulea*, the increase of DIC was on average 47 μmol C l⁻¹ h⁻¹. After correction for passive chemical dissolution of CaCO₃, the release rate (±SD) of DIC in the presence of *H. caerulea* was on average 6.4 ± 3.3 μmol C cm⁻³ sponge h⁻¹ (Table 2). The increase in DIC levels during the incubations was on average 0.3 ± 0.2% at an average seawater DIC concentration of 2073 ± 23 μmol l⁻¹ (*n* = 13, range: 2048 to 2103 μmol l⁻¹).

Halisarca caerulea mass balance

More than 90% of the TOC removal by the 3 encrusting coral cavity sponges was accounted for by DOC. Although the sponges removed bacteria very

efficiently on an absolute scale, the relative BC removal was only 2.5 to 4.1% of the TOC removal by sponges (Table 1). The contribution of POC to TOC removal was only 5.0 to 8.2%, leaving 91.8 to 95% accounted for by DOC. Table 2 shows the ΔO₂/ΔTOC and ΔDIC/ΔTOC for a selection of time series. On average (±SD), per mol organic C removed by *Halisarca caerulea*, 0.39 ± 0.12 mol of O₂ was consumed and 0.90 ± 0.43 of DIC was released (Table 2).

DISCUSSION

DOM-feeding

Sponges are opportunistic feeders and tend to select their food on the basis of availability (Pile et al. 1996, 1997, Ribes et al. 1999). Similar to oceanic waters, DOC in the oligotrophic tropical waters represents the largest fraction of TOC, with only a minor contribution from POC. TOC in the tropical reef water of Curaçao consists mainly (average ±SD) of DOC (118.4 ± 20.5 μmol l⁻¹, range 63 to 160, *n* = 46), BC (2.1 ± 0.4 μmol l⁻¹, range 1.1 to 3.1, *n* = 47), and phytoplankton carbon (PC) (measured as chlorophyll *a*) (0.9 ± 0.2 μmol l⁻¹, range 0.6 to 1.1, *n* = 41) (Van Duyl et al. 2002, De Goeij & Van Duyl 2007, this study). In our incubation experiments the DOC is clearly removed in (at least) 2 major fractions, where a large part (the slow removable fraction) of DOC is not available to the sponge in the time frame of the incubation. The residence time of water in the coral cavities (the natural environment of the sponges) is in the order of minutes (Van Duyl et al. 2006), suggesting that the slow removable fraction in our model is, on average, not readily available as a source of carbon for the cavity sponges. For the 3

Table 2. *Halisarca caerulea*. Fate of organic carbon for *H. caerulea*. The concentration of DOC, BC, O₂ and DIC were measured simultaneously per incubation, and the ΔO₂/ΔTOC and ΔDIC/ΔTOC calculated. Values are average ± SD. Note that fluxes of DOC, POC, TOC, and O₂ are removal rates and DIC is a release rate

Date	DOC	POC	TOC (μmol cm ⁻³ h ⁻¹)	O ₂	DIC	ΔO ₂ /ΔTOC	ΔDIC/ΔTOC
06 Jun 05	8.3	3.0	11.3	3.2	8.9	0.29	0.79
23 Jun 05	3.3	0.8	4.1	2.0	2.7	0.49	0.66
24 Jun 05	5.3	1.2	6.5	4.0	2.8	0.62	0.43
06 Apr 06	6.9	0.6	7.5	2.2	10.8	0.29	1.43
07 Apr 06	6.9	1.4	8.3	3.4	7.4	0.41	0.89
07 Apr 06	5.1	0.8	5.9	2.5	6.6	0.43	1.12
10 Apr 06	5.3	0.8	6.1	1.9	9.5	0.31	1.55
10 Apr 06	6.1	1.6	7.7	3.0	1.8	0.38	0.24
13 Apr 06	5.3	2.2	7.5	1.9	7.4	0.26	0.99
Average	5.9 ± 1.4	1.4 ± 0.8	7.2 ± 2.0	2.7 ± 0.8	6.4 ± 3.6	0.39 ± 0.12	0.90 ± 0.43
Average (mmol m ⁻² h ⁻¹)	14.6 ± 3.6	3.4 ± 2.0	18.0 ± 4.9	6.7 ± 1.9	16.1 ± 8.1		

encrusting coral cavity sponges studied here, the amount of DOC uptake in relation to TOC intake is comparable with values found for the sponge *Theonella swinhoei* (Yahel et al. 2003). In fact, in both studies, more than 90% of the TOC removed by the sponges is accounted for by DOC, suggesting that these species, in spite of being classified as particle feeders, are (in quantitative terms related to the availability of organic carbon sources) actually 'DOM-feeders'. This supports the suggestion by Reiswig (1974b, 1981) that DOC uptake may explain the >70% discrepancy between the particulate gain and respiratory demand of several tropical sponges.

Little is known about the uptake mechanism for DOM in sponges. It has been suggested that only sponges with large amounts of sponge-associated bacteria can utilize DOM. The 3 encrusting sponge species used in this report indeed harbor sponge-associated bacteria. Tritium labelled proline was more rapidly incorporated into symbiotic bacteria of the marine sponge *Chondrosia reniformis*, than in sponge cells (Wilkinson & Garrone 1979). However, in *Theonella swinhoei* (Magnino et al. 1999), *Verongia fistularis* (Reiswig 1981), and in the 3 sponge species presented in this report, most sponge-associated bacteria reside in the mesohyl and are not in direct contact with the passing water, and the removed DOM is likely to pass sponge cells first. Sponges feed by using flagellated cells (choanocytes) lining the choanocyte chambers, which constitute the basic pumping and filtering elements. The sponge choanocytes are functionally comparable to choanoflagellates, which are closely related with sponges (Leys & Eerkes-Medrano 2006 and references therein). Flagellates can ingest a variety of macromolecules, including carbohydrates and proteins, components of the colloidal fraction of DOM (Tranvik et al. 1993). Choanoflagellates can feed on high molecular weight molecules (Sherr 1988, Christoffersen et al. 1996) and have been demonstrated to prefer smaller sized (viral-sized) particles (50 nm latex beads) over larger bacterial-sized beads (500 nm latex beads) (Marchant 1990, Gonzalez & Suttle 1993). Sponges have been reported to take up virus particles (Hadas et al. 2006), and to remove 0.1 μm beads from ambient water (Leys & Eerkes-Medrano 2006). At least 10% of oceanic DOM is in the form of amorphous detrital particles in the size range 0.4 to 1.0 μm that easily pass the pores of the 0.2 μm filters employed in the separation of DOM and POM (Koike et al. 1990). It is possible that sponge choanocytes take up particles, to molecular weight size range, residing in the dissolved fraction mainly in colloidal form and transport part of the DOM to the sponge-associated bacteria in the mesohyl. First evidence from experiments with ^{13}C enriched DOC substrate show

that both sponge cells and associated bacteria can assimilate DOM (De Goeij et al. 2008). It is not yet clear to what extent (quantitatively and qualitatively) the sponge cells or the associated bacteria cells are involved in the utilization and metabolism of DOM, therefore 'DOM-feeding by sponges' should be more appropriately described as: 'DOM-feeding by the sponge-microbe association'.

Carbon removal rates

The total carbon removal rates by the 3 cryptic encrusting sponges presented in this study are the highest ever reported. Ingestion rates reported in the literature range from 0.08 to 1.97 g C m^{-2} sponge d^{-1} (Gili & Coma 1998 and references therein) and 0.04 to 1.80 $\mu\text{mol cm}^{-3}$ sponge h^{-1} (Yahel et al. 2003 and references therein). Assuming an average daily pumping activity of 12 h (Pile et al. 1997) yields a carbon flux of 5.15 to 6.66 g C m^{-2} sponge d^{-1} . Fluxes per volume of sponge in the present study range between 14.3 to 18.5 $\mu\text{mol C cm}^{-3}$ sponge h^{-1} . To our knowledge there is only one study on extensive DOC removal by sponges, reporting the highest total carbon intake rates at that time (Yahel et al. 2003). Since other studies do not report DOM fluxes (Reiswig 1971, Pile et al. 1996, 1997, Kötter & Pernthaler 2002), or did not find DOM retention in sponge species lacking bacterial symbionts (Ribes et al. 1999, Yahel et al. 2007), it is difficult to compare total carbon fluxes. Nonetheless, the fluxes presented here are very high compared to published values. The estimated clearance rates (in cm^3 water cm^{-3} sponge min^{-1}) of *Halisarca caerulea* (2.1 to 11.7), *Mycale microsigmatosa* (3.0 to 5.5) and *Merlia normani* (2.8 to 3.5), however, are in the range of values (\pm SD) reported in the literature, of 2.5 ± 1.7 for the sponge *Haliclona ureolus* (range 1.1 to 6.0; Riisgård et al. 1993). Kötter & Pernthaler (2002) reported average clearance rates for *H. caerulea* (6.1 ± 4.6) and *M. normani* (2.5 ± 1.1), so there is no reason to suspect abnormal clearance capacity of the 3 sponges used in this study. Rates of BC removal (in $\mu\text{mol C cm}^{-3}$ h^{-1}) for tropical encrusting sponges is on average 0.75 (Kötter & Pernthaler 2002), close to our average BC removal rates by encrusting sponges of 0.59 (Tables 1 & 2). Kötter & Pernthaler (2002) found removal rates of BC for *H. caerulea* ranging from 0.33 to 1.17 and for *M. normani* ranging from 0.59 to 0.88. We found BC fluxes of 0.60 to 2.92 and 0.62 to 0.88 for *H. caerulea* and *M. normani* respectively. Again, the feeding behavior of the 3 encrusting sponges does not seem to be out of range.

Size and body morphology of sponges can have an effect on clearance rates (Reiswig 1974b, Riisgård et al. 1993, Ribes et al. 1999), but also on the supply of

certain food fractions and, thus, the capability of feeding on different sized particles (Abelson et al. 1993), such as DOM (Yahel et al. 2003). Clearance rates have been observed to decrease with increasing sponge size (Reiswig 1974b, Riisgård et al. 1993, Ribes et al. 1999). We did not find any correlation between sponge size (in the measured size range 1.9 to 7.6 cm³) and clearance rates, but cannot exclude that larger sized sponges in the field (ranging <1 cm³ to >100 cm³, J. M. de Goeij pers. obs.) have lower clearance rates and might effect community organic carbon removal. The high surface:volume ratio of encrusting sponges as compared with massive sponges is suggested to increase their retention efficiency (Kötter 2003), and the ability of invertebrates to ingest DOC (Siebers 1982). The sheet-like body form can have a competitive edge over more massive growth forms in the particle depleted coral cavities.

Mass balance and fate of carbon

The O₂ respiration rates for *Halisarca caerulea* (in μmol O₂ cm⁻³ sponge h⁻¹) are within the range of reported values for other sponges (1.82 to 3.98 and 0.21 to 24.6, respectively) reviewed by Osinga 1999, and comparable with reported rates for *H. caerulea* (1.56 to 2.67) measured by Kötter & Pernthaler (2002). *H. caerulea* has a ΔO₂/ΔTOC of 0.39, or 39% of the ingested carbon is respired. Assuming a respiratory quotient of 1, this would yield a ΔDIC/ΔTOC value of 0.39, whereas a value of 0.90 (corrected for passive chemical dissolution) has been observed. We argue that the excess DIC release is not due to possible coral rock metabolism by epi- or infauna, since the coral rock (cleared of epibionts) did not remove or release any DOC, BC, or oxygen. Excess DIC release is attributed to *H. caerulea* respiration driven dissolution of the attached coral rock. Dissolution of CaCO₃ increases DIC by 1 mol for each mol of calcium carbonate dissolved (Gattuso et al. 1995), leaving a ΔDIC/ΔTOC for *H. caerulea* respiration of 0.45. The similarity between O₂-based respiration estimation (39%) and CO₂-based respiration estimation (45%) illustrates the accuracy for the *H. caerulea* carbon mass balance.

To determine the fate of carbon it is assumed that 1 mol of organic C removed is respired by 1 mol of O₂. The discrepancy between total organic carbon uptake and oxygen respiration can be explained by microbial processes like sulfate reduction (Hoffmann et al. 2005), or fermentation (Santavy et al. 1990). Fermentation is a common feature in benthic invertebrates (Grieshaber et al. 1994). Alternatively, or in addition, the fate of the removed organic carbon is determined by assimilation.

If it is assumed that *Halisarca caerulea* respire approximately 39 to 45% of the removed organic carbon, then 55 to 61% of the removed organic carbon can be used for growth, reproduction or the production of metabolites. The net increase of cryptic sponge biomass is not likely to be high. Competition for space is high in coral cavities (Jackson et al. 1971) and especially for the thin encrusting species, which are highly surface-dependent, and growth and mortality rates are influenced by strong space competition with neighbours (Turon et al. 1998). If more than half of the carbon uptake is assimilated by *H. caerulea*, but net growth is close to 0, then a rapid turnover of biomass is suggested. Encrusting sponges are known to have a high plasticity, or regeneration capacity, with growth rates of 2900 times the normal growth rate after tissue damage (Ayling 1983), showing that the potential for rapid cell proliferation is present. The resulting cell remnants could have been missed from our incubation measurements, because they are likely to be exported as detrital particulate carbon, which we did not measure. Both Reiswig (1971) and Yahel et al. (2003) found significant excretion of detrital material by the examined sponges (possible sponge cell material or faeces). Decomposition by the deep-sea sponge community of particles >2 μm was argued to have a major contribution to the total sedimentation rate of the Greenland–Iceland–Norwegian (GIN) seas (Witte et al. 1997).

Sponges and coral cavities

The cryptic coral reef framework is a significant sink of carbon, where most (>90%) of the removed carbon is accounted for by the dissolved fraction. The flux of carbon even exceeds the estimated gross production of the reef (De Goeij & Van Duyl 2007). But which organisms are responsible for this important carbon retention in cryptic habitats? The walls of coral cavities are covered by highly abundant groups of coelobites, such as coralline algae, ascidians, bryozoans and polychaetes. To directly link the organic carbon removal of sponges with coral cavities, qualitatively and quantitatively, the activity of other compartments of the cavity (e.g. benthic communities on cavity walls and in the sediment) has to be included in the carbon budget. The cover is, however, dominated by encrusting sponges (Wunsch et al. 2000, Richter et al. 2001, van Duyl et al. 2006). In the present study, the encrusting cryptic sponges *Halisarca caerulea*, *Mycale microsigmatosa*, and *Merlia normani* remove carbon of which the largest part (>90%) is DOC, comparable to organic carbon removal by coral cavities. It is likely that the removal of DOC by the reef framework is influenced

by the removal of DOC by cavity sponges. Thus, a thin veneer of encrusting sponges, only a few millimetres thick, may play a key role in organic carbon removal by coral cavities and thus in the overall carbon cycling of coral reefs.

Acknowledgements. We thank the CARMABI Foundation staff (Curaçao, Netherlands Antilles) and especially C. Winterdaal and B. Leysner for their hospitality and support. We thank J. W. van Dam and A. de Kluijver for their help in the field. A special thanks to S. Gonzalez for analyzing the DOC samples. K. Bakker, J. van Ooijen and E. van Weerlee are acknowledged for the analysis of the DIC samples. The laminar flow chamber was constructed by Eiso Bergsma BV, Amsterdam, The Netherlands. R. W. M. van Soest is acknowledged for sponge taxonomy. This study was financed by the Netherlands Organization for Scientific Research (NWO-WOTRO grant no. W84-547).

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Editorial responsibility: Otto Kinne,
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Submitted: May 25, 2007; Accepted: January 14, 2008
Proofs received from author(s): March 7, 2008