The Journal of Experimental Biology 212, 3643-3650
Published by The Company of Biologists 2009
doi:10.1242/jeb.027953

Particulate organic matter as a food source for a coral reef sponge

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Accepted 12 August 2009

SUMMARY

The ability of sponges to feed in diverse (including oligotrophic) ecosystems significantly contributes to their ubiquitous aquatic distribution. It was hypothesized that sponges that harbour small amounts of symbiotic bacteria in their mass feed mainly on particulate organic matter (POM). We examined the nearly symbiont-free (by microscopic observation) filter-feeding Red Sea sponge *Negombata magnifica* in order to: (a) study removal efficiency of naturally occurring organic particles, (b) measure the total amount of absorbed particulate organic carbon (POC) and nitrogen (PON), and (c) estimate organic carbon and nitrogen flux in this sponge. Total amount of organic carbon and nitrogen in the Gulf of Aqaba was found to be 48.46±5.69 μg l^{-1} and 6.45±0.7 μg l^{-1}, respectively. While detritus contributed 54% of POC, most PON (84%) came from planktonic microorganisms, mainly prokaryotes. Particle removal efficiency ranged from 99% (the cyanobacterium *Synechococcus* sp.) to 37% (for eukaryotic cells >8 μm). On average, *N. magnifica* ingested 480 μg C day^{-1} g^{-1} (wet mass, WM) sponge and 76.6 μg N day^{-1} g^{-1} sponge. Ingested POC balanced 85% of the sponge's energetic demand but more is needed for biomass production because it cannot digest all of the carbon. 54.4±16.1 μg N day^{-1} g^{-1} (WM) nitrogen was excreted as total ammonia nitrogen (TAN); however, nitrogen allowance should be higher because more nitrogen is deposited for sponge biomass during growth. It is hypothesized that the discrepancy in the nutritional requirements should be covered by the sponge absorbing carbon and nitrogen from sources that are not dealt with in the present research, such as dissolved organic carbon and nitrogen. This study highlights the significance of detritus as a carbon source, and prokaryotes as a PON source in sponge feeding.

Key words: Porifera, coral reef, filtration, flow cytometry.

INTRODUCTION

Sponges are ubiquitous aquatic, sessile, filter feeders. In some marine ecosystems, such as Antarctica or certain tropical reefs, they constitute a significant proportion of the benthic biomass (Barthel and Gutt, 1992; Barnes and Bell, 2002). Sponges' ability to satisfy their metabolic needs in diverse niches has a major influence on their world distribution.

Interestingly, coral reef sponges survive in frequently oligotrophic seas. Sponges are heterotrophic filter feeders, thriving on small (≤50 μm) organic particles (Reiswig, 1971a). Organic matter in coral reefs can be roughly divided into three main types (Ribes et al., 2003). The first is dissolved organic matter (DOM). Some sponges that are densely packed with symbiotic bacteria ('bacteriosponges') are known to extract considerable amounts of DOM from the water (e.g. Reiswig, 1981; Yahel et al., 2003). According to Ribes et al. (Ribes et al., 1999), insignificant amounts of DOM are also expected to be absorbed by sponges not containing large amounts of bacterial symbionts (Hadas et al., 2006), although other cases have been recently demonstrated (De Goeij et al., 2008).

The second type is live particulate organic matter (LPOM), composed of various planktonic cells. Sponge removal efficiency (RE) of some LPOM is well documented and similar among most species studied: high RE of particles of about 1 μm in size (mainly prokaryotic cells), and decreased RE of larger particle sizes (e.g. Reiswig, 1971a; Yahel et al., 2003).

The third type is non-living particulate organic matter (hereafter 'detritus'), which is a structurally and chemically diverse fraction deriving from a range of different sources (Wilson et al., 2003). Detritus significance as a food source for marine sponges is poorly documented.

Four decades ago the nutritional balance of three Caribbean sponges was comprehensively studied (Reiswig, 1971a; Reiswig, 1971b; Reiswig, 1974), comparing the amount of particulate organic carbon (POC) ingested by the sponges to their respiration rate and estimated growth rate. Reiswig concluded that one symbiont-free sponge [(*Crypto*) *Tethya crypta*] was able to meet its energetic needs by ingesting POC whereas another species (*Mycale* sp.) was just short of balancing its energetic needs (Reiswig, 1974). A similar difference was found between *Dysidea avara*, suggested to ingest only particulate organic matter (POM) (Ribes et al., 1999), and *Halichondria panicea*, which requires additional sources (Thomassen and Riisgard, 1995). Whether this inconsistency stems from a difficulty in encompassing all feed sources available for a sponge or reflects interspecies variance is unclear. In recent years there has been an accumulation of data that show that some sponge species can absorb significant amounts of dissolved organic carbon (Yahel et al., 2003; De Goeij et al., 2008). Moreover, organic nitrogen, a key nutritional element, has been overlooked and no information exists on its availability to sponges.

Two main methods have been employed to study sponge feeding. In the first method, water is sampled directly and simultaneously from the oscula (outflow) stream and close to the sponge surface just before entering its body. This method was applied *in situ* (Reiswig, 1971b; Yahel et al., 2005) and in the laboratory (Stuart and Klumpp, 1984). The second method measures removal rate of food particles resulting from filtration activity in a closed system. This method was applied in a simple aquarium system while feeding the sponges with cultured non-photosynthetic bacteria (NPB) and algae (Thomassen and

Riisgard, 1995), underwater in chambers using natural plankton (Ribes et al., 1999), and in funnels (Witte et al., 1997; Ribes et al., 2003).

The Red Sea sponge *Negombata magnifica* (Keller 1889) was used to study the nutrition of a filter feeder in an oligotrophic (coral reef) environment. This branching sponge lives (at 3–60 m depth) either exposed or inside caves (Ilan, 1995). It seldom exhibits photosynthetic activity (E.H., unpublished data), and is virtually microsymbiont-free (bacteria were seldom observed in histological sections) (Gillor et al., 2000).

The northern Red Sea has relatively low chlorophyll-a (chl-a) levels (0.1–0.5 μg l^{-1}), mostly contributed by ultraplankton (cell size <8 μm) organisms (Yahel et al., 1998). This ultraplankton fraction shows a large temporal variation in abundance and composition, due to seasonal water column mixing and stratification (Lindell and Post, 1995). The present study assesses the contribution of natural POM to *N. magnifica*'s diet, by determining the RE of the various food particles at different concentrations, measuring the amounts of POC and particulate organic nitrogen (PON) available to the sponge, determining the filtration rate, and estimating the flux of organic carbon and nitrogen to the sponge. The information obtained on sponge nutrition contributes to a greater understanding of the sponge's role in coral reef food webs and to developing artificial systems for sponge culture.

MATERIALS AND METHODS

Sponges and seawater

Thirty *Negombata magnifica* fragments were cut from 10 individual sponges growing in the northern-most area of the Gulf of Aqaba, Red Sea. These fragments had been attached to PVC plates one year prior to the laboratory experiment, as described by Hadas et al. (Hadas et al., 2005). This preparation enabled experimenting with intact sponges on inert substratum. Prior to experiments, all sponges were taken out of the water for a few seconds, placed on an electronic scale for wet mass (WM) determination (mass of the plates was recorded preceding attachment), and ranged between 50 g and 100 g. All were kept in the sea at a depth of 10 m, seaward of the Interuniversity Institute (IUI) in Eilat, Israel. Sponges were transferred (attached to plates) to the laboratory for acclimation at least 24 h prior to each experiment. The sponges were kept in the experimental aquarium with a steady flow of 1.5 l min^{-1}. All experiments were conducted at the IUI facilities during winter and spring. Seawater (22.5±0.5°C), pumped from 3 m deep, was continuously supplied to the laboratory-maintained sponges.

Water collection procedure

The sponge RE of the different POM fractions was measured by comparing particle concentrations in water samples collected simultaneously from the sponge inflowing and outflowing water (Reiswig, 1971b; Yahel et al., 2003; Yahel et al., 2005). Unlike the large uni-oscular sponges used in the cited studies, *N. magnifica* has many small (diameter 1–6 mm) oscula (E.H., unpublished data); thus, the conventional methods required adaptation.

Each 4 liter glass aquarium contained a single sponge and was supplied with fresh seawater (flow rate 1.5 l min^{-1}). The proximal ends of two capillary tubes (1 mm diameter) were directed adjacent to the sponge using a micromanipulator. The end of one tube was inserted several mm into an osculum, without contacting the sponge body. The end of the second tube was placed 1 cm from the osculum and 5 mm from the surface. The distal end of each tube was inserted into a collection vessel. *Negombata magnifica* has many small oscula, limiting the outflow amount. To ensure non-mixing of ambient with outflowing water during sampling, water collection rate was restricted to 8 ml min^{-1}. Because collection rate was slow (up to 12 h), samples were kept in the dark at 4°C to prevent changes in POM composition during this period. For each measurement the samples were analyzed in pairs of inflow/outflow. The RE (%) of the different POM fractions was calculated using Eqn 1:

$$\mathrm{RE} = 100\,(1 - C_2 \times C_1^{-1}), \quad (1)$$

where C_1 and C_2 were the cell concentrations (cell ml^{-1}) in inflow and outflow water, respectively.

Sampling procedure

To evaluate the RE of the different types of particulate matter by *N. magnifica*, all planktonic cells smaller than 50 μm were counted. Total particulate organic carbon and nitrogen content of the water flowing in and out of the sponge were also measured. To test sponge RE of different particle concentrations and types, measurements were conducted during winter and spring, when large fluctuations in plankton concentration prevail at the study site (Lindell and Post, 1995). Samples were collected when the water column was partially mixed (December), when spring phytoplankton bloom occurred (March and April), and during water stratification (June).

Terminology

No single method measures all POM in the sea. The terminology used here for the different planktonic groups is derived from the measurement methods applied and not from a standard terminology. All planktonic organisms smaller than 8 μm were termed ultraplankton, after Lindell and Post (Lindell and Post, 1995). This group consists of three cell types: the cyanobacterium *Synechococcus* sp., the eukaryotic ultraphytoplankton (EUP), and the remaining cells, here termed NPB. All cells >8 μm, whether photosynthetic or not, were termed large plankton. Non-living particles were termed detritus.

Ultraplankton counts

Ultraplankton cells (<8 μm) were identified and counted using a flow cytometer (FACScan, Becton Dickinson, Franklin Lakes, NJ, USA) equipped with a 488 nm Argon ion laser, applying procedures described by Marie et al. (Marie et al., 2000). Briefly, 1.5 ml seawater samples were fixed with glutaraldehyde (G5882, Sigma Chemical Co., St Louis, MO, USA) at a final concentration of 0.1%, frozen in liquid nitrogen and stored at –80°C until analysis. For the photosynthetic cell counts, 450 μl of sampled water were analyzed. *Synechococcus* sp. was identified based on its phycoerythrin orange fluorescence, and EUP was identified by the red fluorescence of its chlorophyll. To count the NPB, all ultraplanktonic cells were marked with the DNA stain SYBR^{TM} Green-I (Molecular Probes, Inc., Eugene, OR, USA, ref: S-7563) and 30 μl of them were then analyzed in the flow cytometer. The NPB number was calculated by subtracting the number of photosynthetic cells from the total count obtained by SYBR Green measurement. As a standard for all measurements 0.95 μm yellow–green fluorescent beads (Polysciences, Warrington, PA, USA, ref: 71825) were used. FACS list mode files were processed with the custom-designed software CYTOWIN (V. 4.1, D. Vaulot, CNRS, Roscoff, France).

Mean cell size of inflowing and outflowing EUP was compared using the flow cytometer output data. This comparison relied on the flow-cytometry signature of chlorophyll red fluorescence intensity, assuming that higher intensity is correlated to higher cell volume (Simon et al., 1994; Green et al., 2003). Although cell volume and chlorophyll content are not easily correlated because of differences caused by light adaptations, the present study

compared paired samples that included the entire cell population entering the sponge and the expelled portion of this population. Thus, under these experimental conditions red fluorescence was considered a more direct indicator of cell size than the more commonly used light side scattering (Cunningham and Buonnacorsi, 1992).

Large plankton counts

Water samples of ≤1 liter were filtered through a 5 μm polycarbonate filter (Poretics, Gorinchem, The Netherlands), using only gravitation to minimize cell destruction. Samples were stained with SYBR Green to detect non-autofluorescent cells. Following sample preparation, cells were counted using an epifluorescence microscope at a magnification of X1000 (Labophot-2, Nikon, Tokyo, Japan, equipped with a high pressure mercury lamp, model HB-10101AF). Excitation ranged between 450 nm and 490 nm, with a barrier to emission of 520 nm.

On each filter, the number of photosynthetic and non-photosynthetic organisms (recognizable by their bright-green fluorescence) was counted in 40 fields, representing the entire filter area. Non-separate cells during division were counted as one cell. Eqn 2 was used to recover the original cell concentration in the water sample:

$$C = A \times B \times V^{-1}, \quad (2)$$

where C=cell concentration ml^{-1} sample, A=the mean of all field counts, B=25,822, which is the number of fields on a filter, excluding the filter margins which were ineffective in filtration, and V=volume (ml) of water filtered.

Cell sizes in eight inflow/outflow pairs were measured to determine changes in size distribution of plankton between the sponge inflowing and outflowing water. Eighty random cells from each sample were measured using an epifluorescence microscope with calibrated ocular grid. Cell volumes were approximated by separating cells into one of two general geometric shapes: a sphere or cylinder. These volumes were divided into seven categories to test the hypothesis of selectivity in capture based on cell size. Chesson's selectivity index (Chesson, 1978) was used for the test:

$$E = (r_i - n_i) \times (r_i + n_i)^{-1}, \quad (3)$$

where E=index for selectivity, r_i,n_i=the cell abundances (in percent) in the i bin (i=1….7) in the inflow and outflow, respectively. E=0 indicates no selectivity for the i prey size, and E=1 and E=–1 mean complete selectivity for and complete rejection of the i prey size category, respectively.

POM determination

The total amount of particulate organic carbon and nitrogen in the sponge inflowing and outflowing water were directly measured with a C:N analyzer.

Water samples were collected, as described previously, from eight different sponge individuals during two weeks. Samples were filtered on pre-combusted (four hours at 550°C) and pre-weighed GF/F (Whatman, Maidstone, Kent, UK) filters until the filter clogged, usually after about 4 liters. The inflow water was pre-filtered through a 50 μm mesh size filter to eliminate the large particles that cannot enter the sponge through its ostia (incurrent openings). After filtration, the filters were dried for 24 h at 50°C and then stored at –20°C until analysis.

Prior to analysis, the filters were acidified for 8 h with hydrochloric acid fumes to eliminate any traces of the inorganic carbon prevailing in the vicinities of coral reefs. Following acidification, the samples were dried for 24 h, then weighed to determine the total organic matter on each filter. The filters were cut into halves and, after weighing one half, the filter was inserted into a tin capsule and loaded into an autoanalyzer sampler. Glycine was used as a standard and measured after every fourth sample. The amount of carbon and nitrogen was determined using a glycine standard curve.

The GF/F filter pore size is 0.8 μm. Thus, some NPB are not retained on the filter (Altabet, 1990). To estimate the amount of live POC not retained, the number of NPB cells present in the filtered water was counted using flow cytometry.

Determination of sponge filtration rate

Because RE of *Synechococcus* sp. by *N. magnifica* was nearly 100% (present study), based on Thomassen and Riisgard (Thomassen and Riisgard, 1995), it was determined that filtration rate equals the clearance rate of this microorganism. The experimental system design was based on the principles for a steady-state system, reviewed by Riisgard (Riisgard, 2001).

The experimental system comprised seven 3.5 liter aquaria, each containing a single pre-weighed (WM) sponge individual and a control aquarium with no sponge. Inside each aquarium a water pump (power of 10 l min^{-1}) ensured homogeneous mixing of the planktonic particles. Fresh seawater was constantly supplied (mean flow rate of 628±9 ml min^{-1}, determined by measuring the water volume exiting each tank per unit time). To minimize temporal variations in inflow cell concentrations, a common 20 liter central header tank supplied water to all aquaria. Following acclimation (2 h), when all sponges were active (i.e. with open oscula), tank inflow and outflow water were simultaneously sampled. The *Synechococcus* sp. cell concentration in each sample was determined using flow cytometry. Sponge clearance rate was calculated using Eqn 4 (Riisgard, 2001):

$$FR = CL = Fl\,(C_1 - C_2)\,(C_2 \times W)^{-1}, \quad (4)$$

where FR=filtration rate, CL=clearance rate (ml min^{-1} g^{-1} sponge), Fl=flow rate through the sponge tank (ml min^{-1}), C_1 and C_2 are the concentration (cells ml^{-1}) of *Synechococcus* cells in inflow and outflow water, respectively, and W=the sponge WM (g).

This measurement was assumed to be valid only if the RE of *Synechococcus* sp. was not affected by reduced cell concentrations in the steady-state system when cell concentration was lower than the ambient concentrations by about 20% (present study). To test this assumption, the inflowing cell concentration was artificially reduced and the RE of *Synechococcus* was measured. The amount of cells in the water was controlled by mixing different ratios of fresh seawater with 0.2 μm filtered seawater (Suporlife 200, PALL, Biopharmaceuticals, East Hills, NY, USA).

Nitrogen excretion

Sponge nitrogen excretion was estimated by measuring the amount of total ammonia nitrogen (TAN) it excreted in a steady-state system (Riisgard, 2001). Because the water TAN concentration was expected to be low, the highly sensitive fluorometric method described by Holmes et al. (Holmes et al., 1999) and adapted by David (David, 2002) was used.

Nine *N. magnifica* individuals attached to PVC plates were each mounted in a 1 liter metabolic chamber, constantly supplied with fresh seawater (23±1.0°C). Water flow rate was determined by measuring the chamber outflowing water volume over time. After a short acclimation (about 60 min), when most of the sponge oscula were open, the water entering and exiting the metabolic chamber was sampled simultaneously.

Water samples were each divided into four test tubes each containing 4 ml. A measured amount of 0.1, 0.2 or 0.3 ml of ammonia standard (50 μmol l^{-1}, Sigma Chemical Co.) was added to three of the test tubes; thus, used as an internal standard. Aliquots of each sample were excited at 365 nm and the emission at 492 nm was measured. The TAN concentration was determined by extrapolating the resulting regression line to the y intercept [detailed description in David (David, 2002)]. Because of this test's sensitivity to TAN contaminations, if the regression's R^2 was less than 0.99, the entire measurement was discarded.

The amount of TAN released by the sponges was calculated using Eqn 5 (Riisgard, 2001):

$$E = \frac{F(C_{out} - C_{in})}{W \times 1000}, \quad (5)$$

where E=nmol N min^{-1}, F=water flow rate (ml min^{-1}), and C_{out} and C_{in}=the TAN concentration (nmol) in the outflow and inflow water, respectively, and W is the sponge WM (g).

Conversion ratios

The carbon and nitrogen contents of bacterioplankton were calculated separately for GF/F retained and non-retained NPB (with estimated cell size of 0.056 μm^3 and 0.04 μm^3, respectively) (Lee and Fuhrman, 1987). The ratio between carbon and nitrogen amount (mass) to cell volume (μm^3) is 0.38 and 0.11, respectively, and the C:N ratio for NPB is about 3.7. Hence, the carbon and nitrogen amount for the retained NPB is 20 fg C $cell^{-1}$ and 5.4 fg N $cell^{-1}$. For the non-retained NPB, estimations are 15 fg C $cell^{-1}$ and 4 fg N $cell^{-1}$. The carbon and nitrogen amounts of *Synechococcus* sp. are 200 fg C $cell^{-1}$ and 50 fg N $cell^{-1}$ (Caron et al., 1995). From the estimates of these elements' content in phytoplankton cells we used Montagnes et al. (Montagnes et al., 1994) conversion factors, as best covering our measurement techniques. Amounts of organic carbon and nitrogen for large planktonic cells were calculated for each cell size: C (pg $cell^{-1}$)=0.109×(μm^3)$^{0.991}$ and N (pg $cell^{-1}$)=0.0172×(μm^3)$^{1.023}$ (Montagnes et al., 1994).

Statistical analysis

Statistical analyzes were performed using JMP IN (release: 5.0.1a, SAS Institute Inc., Cary, NC, USA). Statistical analysis of the RE (expressed as percentages) used an arcsin transformation of the square root of removal ratios. A probability smaller than 0.05 was considered significant unless stated otherwise. To test if the RE of the three ultraplankton cell types were mutually independent, pair-wise Pearson product-moment correlation was used. To determine differences in RE between the three ultraplankton cell types and the four sampling periods, two-way analysis of variance (ANOVA) model was used. Tukey Kramer test was used for multi-comparison analyzes.

RESULTS

Ultraplankton removal efficiency

The sponges removed all naturally occurring ultraplankton types from the water but at different RE (Fig. 1). *Synechococcus* sp. was removed at mean efficiency of 99.2±0.2% (Fig. 1A) and mean RE of the NPB cells (Fig. 1B) and EUP (Fig. 1C) were 85.7±1.3% and 84.9±2.0%, respectively.

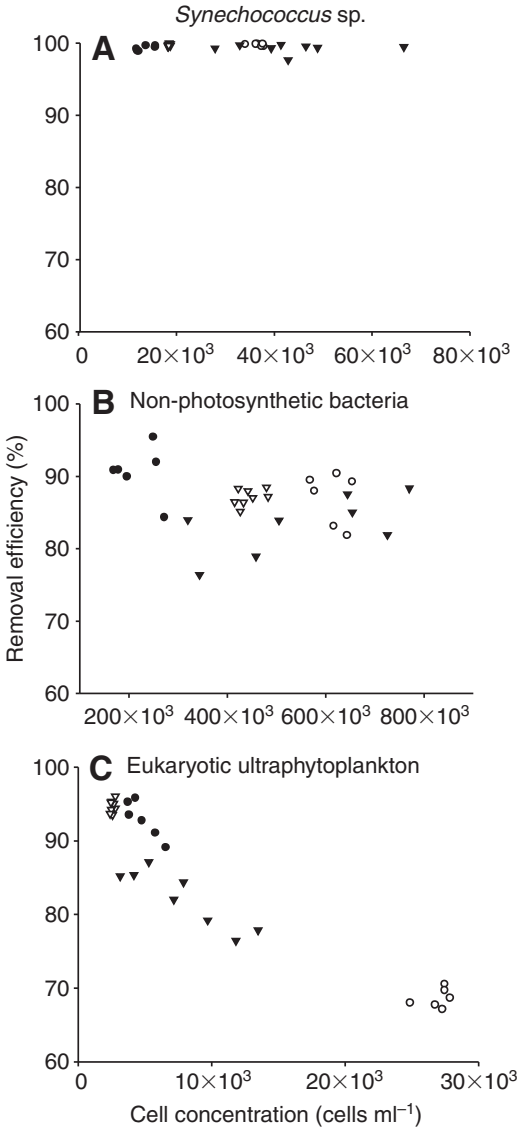

Fig. 1. Removal efficiency of *Negombata magnifica* for three ultraplankton cell types. Each point represents a value calculated from an inflow/outflow pair (see Eqn 1). (A) *Synechococcus*. (B) Non-photosynthetic bacteria (NPB). (C) Eukaryotic ultraphytoplankton (EUP). Sampling time symbols: ●, December; ○, March; ▼, April; ▽, June.

The RE of the three cell types varied independently from each other (pair-wise correlations P>0.05). Analysis of differences in RE between the three planktonic cell types revealed that *Synechococcus* sp. had significantly (P<0.001) higher RE than the other two cell types, and this remained constant through all cell concentrations (Table 1).

Table 1. Statistical analysis of removal efficiency (% ±s.e.m.) of the different cell types in various seasons by *Negombata magnifica*

Removal of:	December	March	April	June
Syn.	99.30±0.14 (A)	99.76±0.07 (A)	98.51±0.77 (A)	99.61±0.05 (A)
EUP	92.93±1.03 (B)	68.66±0.53 (E)	82.19±1.39 (D)	94.67±0.31 (B)
NPB	90.58±1.47 (B,C)	87.04±1.47 (C,D)	83.14±1.66 (D)	87.11±0.40 (C,D)

A two-way ANOVA model $F_{11,71}$=111.3, P<0.001 followed by a multiple comparison test. Different letters represent a significant difference between means.
Syn.=*Synechococcus*; EUP=eukaryotic ultraphytoplankton; NPB=non-photosynthetic bacteria.

The mean NPB cell RE was not significantly different from that of the EUP cells. However, whereas no linear regression was found between NPB RE and ambient cell concentration (N=27, R^2=0.1 P=0.09), such a relationship was found for the EUP cells (N=28, R^2=0.84, P<0.001, y=1.3–1.4×10^{-5}x).

Mean red fluorescence intensity of EUP increased significantly (7.9±1.6 fluorescence arbitrary units) in the outflowing EUP cells relative to the inflowing cells (paired t-test; N=25, t=4.8, P<0.001).

Large eukaryotic cell removal efficiency

Almost all of the large eukaryotic cells were photosynthetic (mean volume 341 μm^3 cell^{-1}). Different planktonic types dominated different seasons: small dinoflagellates in December; diatoms in March (particularly the genus *Pseudonitzchia*); and small flagellates in April.

Negombata magnifica removed large eukaryotic cells at a mean efficiency of 37.0±0.87% (Fig. 2), with great variability: from about 70% (December) to zero removal (in April for some of the examined specimens). Mean plankton cell concentration increased from December to March and decreased in April (99.1±8.2 cell ml^{-1}; 572.9±127.5 cell ml^{-1}; and 257.2±35.6 cell ml^{-1}, respectively). A comparison of RE between these sampling periods revealed a significant difference (one-way ANOVA, $F_{2,17}$=10.3, P<0.01). A multi-comparison test showed a significant difference in cell removal rates in April (19.5±2.5%) relative to those in December and March (54.6±3.9% and 36.9±2.5%, respectively), with no significant difference between the latter two sampling periods (Table 1). Although an inverse relationship exists between RE and cell concentration, calculation of algae quantities indicates that the sponges cleared from the water an average of 108 cells ml^{-1} in March, 92 cells ml^{-1} in April, and only 54 cells ml^{-1} in December.

Cell size distribution analysis showed that 60% of the algae were smaller than 20 μm^3. The selectivity index of all seven size categories was not significantly different from zero (t-test, comparison to zero as a fixed value).

POM removal

In testing the GF/F RE of ultraplankton no EUP and *Synechococcus* sp. cells were recovered in the filtered water. Conversely, 37.1±1.2% of the ambient NPB was found in the filtered water; thus, this ratio of NPB was reduced from the bacteria number that was found in the FACS analysis in order to avoid an over estimation of the contribution of this nutrient source when comparing it with the total nutrient amounts that were found in the C:N analyzer. The nutrient amounts contributed by the unretained bacteria were added to the total nutrient budget.

Total C:N analysis demonstrated that both POC and PON were significantly removed (paired t-test, N=8, t=4.9, P<0.05) by all eight sponges (Fig. 3). Mean C and N concentrations were 48.46±5.69 μg C l^{-1} and 6.45±0.7 μg N l^{-1} in inflow water and 18.84±1.74 μg C l^{-1} and 2.04±0.19 μg N l^{-1} in outflow water. A significant difference was found in the POM C:N ratio of the ambient water and the sponge outflow water (8.6±0.2 and 10.8±0.3, respectively; paired t-test, N=8, t=5.4, P<0.001).

The amount of live PON (LPON) in the inflow water was 5.4±0.24 μg N l^{-1}, comprising 84% of the total PON, while prokaryotic cells contributed 77% to the LPON pool (Fig. 4A). The amount of live POC (LPOC) in the inflow water was calculated at 22.6±1.1 μg C l^{-1}, making it 46.6% of the total amount of POC. Prokaryotic organisms contributed 69% of the LPOC (Fig. 4B).

Filtration rate of *N. magnifica*

The mean FR was 10.8±1 ml min^{-1} g^{-1} sponge (WM; N=7). Cell concentration did not differ between the control tank and ambient water, *N. magnifica* completely removed *Synechococcus* in all tested cell concentrations. A significant linear regression model was found between cell concentration and the amount of *Synechococcus* sp. removed (y=0.997x+1.1, R^2=0.99, P<0.05, N=12).

TAN excretion

TAN released by *N. magnifica* to the water was 2.7±0.8 nmol TAN min^{-1} g^{-1} (WM), equivalent to 54.4±16.1 μg N day^{-1} g^{-1} (WM, Fig. 5).

DISCUSSION

The major feeding strength of sponges stems from their nearly non-selective filtration of a wide variety of nutritional sources. Measuring, identifying and quantifying these different types of organic particles are complex, requiring many different measurement methods. Additionally, these food sources are unevenly distributed in time and space, and their nutritional value varies.

Organic carbon and nitrogen available to sponge nutrition

Nutritional value analysis showed that organic carbon and nitrogen were unevenly distributed among the different food particles. Within living particles, carbon and nitrogen were directly calculated (from the conversion factors), while the potential contribution of detritus to sponge feeding was calculated by subtracting nutrient amounts in the

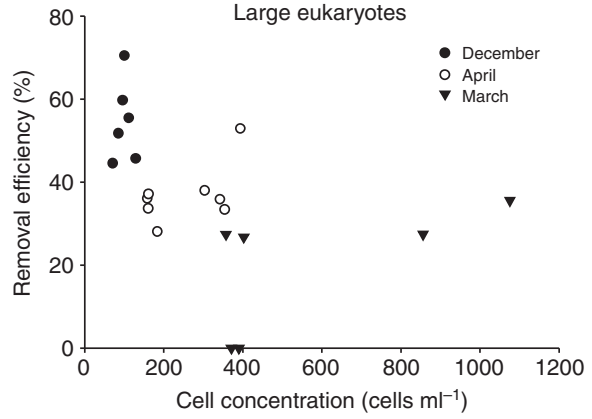

Fig. 2. Removal efficiency of the large (5–50 μm) planktonic organisms in three sampling periods by *Negombata magnifica*, N=20.

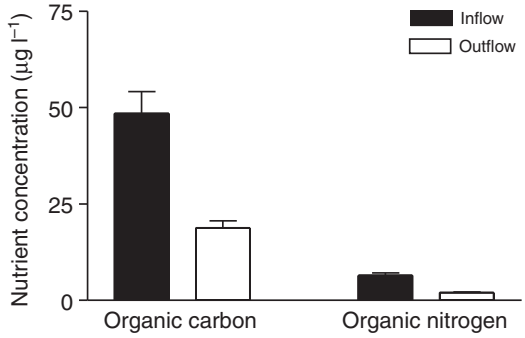

Fig. 3. Inflow and outflow organic carbon and nitrogen concentrations measured in *Negombata mgnifica* during April (N=8). Error bars indicate standard error.

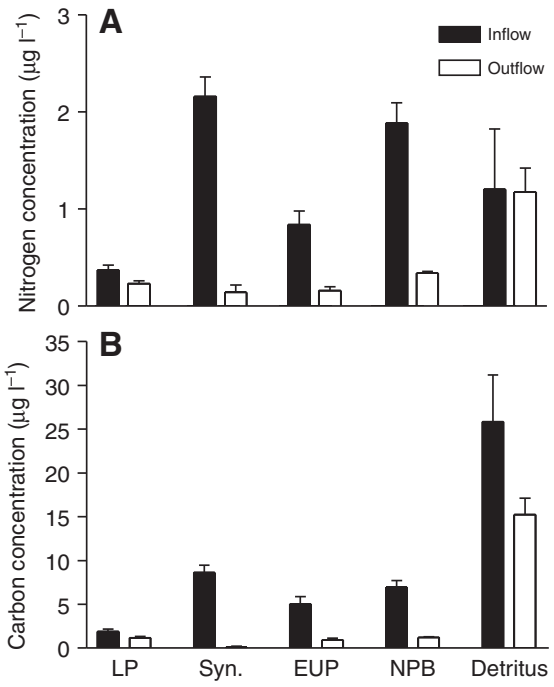

Fig. 4. The distribution of organic nutrients among the different particle types in *Negombata magnifica* inflowing and outflowing water. (A) Organic nitrogen; (B) organic carbon. LP, large plankton; Syn., *Synechococcus* sp.; EUP, eukaryotic ultraplankton; NPB, non-photosynthetic bacteria; detritus, calculated as total POM–live POM. Data represent means and standard error (N=8).

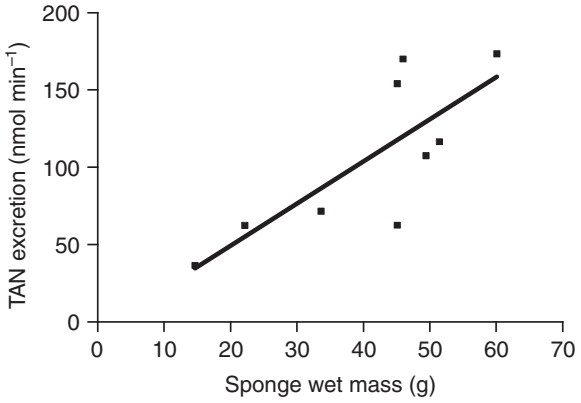

Fig. 5. Total ammonia nitrogen (TAN) excreted by *Negombata magnifica* individuals at different wet mass (WM) (at 23±1.0°C). y=2.7x–5.4, R^2=0.6, P=0.01, N=9.

live particles from total POM. We estimated that 54% of the total available POC was from detritus, almost equal to the LPOM fraction. Among LPOM, eukaryotes and prokaryotes had similar organic carbon contents. By contrast, 84% of the calculated organic nitrogen came from LPOM, with prokaryotes being the major source of PON for the sponge diet, contributing 65% of the total organic nitrogen.

As the POM pool is not totally digested by sponges, using only the plankton-to-nutrients conversion factors would lead to overestimating the actual quantity of nutrients available for sponge nutrition. Food digestibility has not been defined for sponges. We therefore used the digestibility definition as applied for fish [nitrogen digestibility = gross nitrogen intake – gross nitrogen excretion (Cho and Kaushik, 1990)]. The problem here is that some ingested particles are not digested; for example, some apparently intact algae were observed leaving the sponge. To overcome this conceptual problem, outflowing particles were considered as undigested by the sponge. Because each sample was the integration of *ca.* 12 h of sponge activity, it is suggested that the reduction in particle concentration between inflowing and outflowing water represents food particle digestibility and not any temporary status.

Because most PON (84%) was contributed by LPON, by assuming that the sponge digested the remaining 16% of PON from an unresolved source (not LPON), we could calculate the minimal nitrogen digestibility as 79% $\{[1–(\text{total PON}_{\text{outflow}}–\text{LPON}_{\text{outflow}})\times(\text{total PON}_{\text{inflow}}–\text{LPON}_{\text{outflow}})^{-1}]\times100\}$. A comparable value (78%) was found for the bivalve *Mactra veneriformis* (Hiwatari et al., 2002). Although food digestibility calculated here is within the accepted range, it is interesting that this sponge maintains a relatively high digestibility of such diverse food types. Based on nitrogen digestibility and changes in C:N ratios, we estimate carbon digestibility as at least 73.5% $[1–(1–\text{nitrogen assimilation efficiency})\times10.8\times8.6^{-1}]$.

Mechanism of POM removal

Negombata magnifica removed all unicellular planktonic organisms from the water. RE of the different cell types were not correlated to each other, unlike Witte et al.'s (Witte et al., 1997) contention for polar sponges. Prokaryotic cells in all Red Sea naturally occurring concentrations were removed at an efficiency ranging from approximately 100% for *Synechococcus* sp. to about 85% for the NPB, similar to another Red Sea sponge, *Theonella swinhoei* (Yahel et al., 2003). The difference in RE between *Synechococcus* sp. and the NPB can be attributed to different population structures; whereas the former is relatively homogeneous, the latter includes small bacteria down to a diameter of 0.4 μm (Lee and Fuhrman, 1987), possibly retained at a lower efficiency than the larger ones, similar to the RE of 0.5 μm beads by *Crambe crambe* (Turon et al., 1997). Because *Prochlorococcus* sp. cells are almost absent from the water during winter (Lindell and Post, 1995), this organism was not expected to appear in most measurements. Had the latter cell type been present in the samples, it would have been included in the NPB fraction.

The RE of EUP was concentration dependent, ranging from 65% to 90%. Analysis of the mean fluorescence intensity of the sponge inflowing and outflowing EUP, indicated that the smaller cells were retained more efficiently than the larger ones. This can explain the fluctuations found in the RE of EUP cells. The size of these cells might have changed (Arin et al., 2002) due to typical temporal variations in nutrient levels in the Gulf of Aqaba (Lindell and Post, 1995).

The ability to efficiently capture prokaryotes, the reduced ability to capture bigger EUP and larger plankton cells, and the non-selectivity toward any of the large planktonic groups, suggest that *N. magnifica*'s filtration mechanism comprises two parallel working systems.

The first system, probably operated by the flagellated choanocyte cells, specializes in capturing the smaller cells and is responsible for removing *Synechococcus* sp., NPB cells and the smaller EUP. The maximal particle size this system captures (not determined in the present study), is probably set by the prosopyle (about 5 μm) size (Weissenfels, 1992). This system's RE approaches 100% for about 1 μm particles, at all natural cell concentrations, with decreased efficiency for the smallest particles.

In the second filtration system, the aquiferous system lining pinacocytes probably capture the larger food particles (large EUP and above), as its filtration efficiency is lower than that of the former. RE varies and is partially saturated at higher natural planktonic

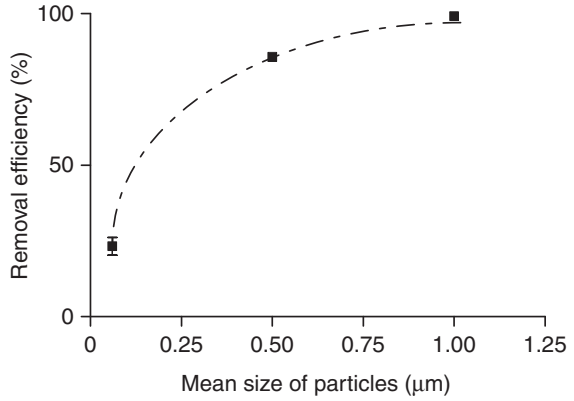

Fig. 6. Removal efficiency of three planktonic types: viruses (0.06 μm), non-photosynthetic bacteria (0.5 μm), *Synechococcus* (1.0 μm) [virus removal efficiency from Hadas et al. (Hadas et al., 2005)]. Each square represents the mean and s.e. The broken line suggests the decreased removal efficiency of food particles as a function of size.

concentrations. The pinacocytes' ability to capture small particles as NPB is probably limited. This suggested that *N. magnifica* filtration mechanism is in accordance with data obtained for *C. crambe* (Turon et al., 1997).

Detritus can be an important nutrient source (at least of organic carbon) for sponge feeding, and we hypothesize that, like live cells, its removal is size dependent. However, detrital particles cannot be separated from the sponge-generated particles (faecal pellets) retained on a GF/F filter. Therefore, RE of the ingested detritus cannot be directly measured as described for other sponges (Wolfrath and Barthel, 1989). Using an indirect calculation [$(\text{total POM})_{in}$–$(\text{total POM})_{out}$–$(\text{live POM})_{in}$+$(\text{live POM})_{out}$], we estimated that detritus ingested by the sponge contributed at least 10.6±4.8 μg C l^{-1} (approximately one-third of the ingested organic carbon). Detritus-derived organic nitrogen seems nearly negligible, because the intake was below detection level (*t*-test, t=0.05, d.f.=7, P=0.9).

Organic carbon and nitrogen flux

Negombala magnifica filtration rate was within the higher range of other sponges, whether tropical (Reiswig, 1974; Yahel et al., 2003) or temperate (Riisgard et al., 1993; Turon et al., 1997). The relatively high filtration rate might be adaptive in increasing nutrient flux into such sponges residing in oligotrophic environments.

The mean daily amounts of POC and PON absorbed by *N. magnifica* were 480 μg C day^{-1} g^{-1} (WM) sponge and 76.6 μg N day^{-1} g^{-1} (WM) sponge, including the additional GF/F filter non-retained NPB fraction. Assuming that 0.46 mg C corresponds to 1 ml of O_2, and a mean respiration rate of 37 nmol O_2 min^{-1} g^{-1} (WM) sponge (Hadas et al., 2008), the carbon amount corresponding to this respiration rate is 0.54 mg C day^{-1} g^{-1} (WM) sponge. In the present study the sponge absorbed 85% of the carbon amount required for respiration, although not all of it is digestible by the sponge, and more organic carbon is needed for the production of biomass. The amount of nitrogen excreted as TAN was found to be 54.4±16.1 μg N day^{-1} g^{-1} (WM) and more nitrogen might be excreted as nitrite and nitrate. Moreover, the nitrogen allowance should be higher because more nitrogen is deposited for sponge biomass during growth. It was previously found that *N. magnifica* can grow by up to 0.55% of its body mass per day, and protein constitutes 7.5% of its biomass (Hadas et al., 2005). These data indicate that the daily amount of nitrogen deposited in sponge biomass is 60 μg N day^{-1} g^{-1} (WM). Thus, the daily nitrogen requirement seems to be twice the absorbed amount [76.6 μg N day^{-1} g^{-1} (WM) sponge].

The discrepancy in both carbon and nitrogen budgets could be explained by sponge ingestion of nutrients undetermined in the present study, such as the colloids in the size range of between 0.8 μm and 0.2 μm that were not retained on the GF/F filters and could not be quantified (like the bacteria). Moreover, it is hypothesized that *N. magnifica* removes smaller organic matter from the water at a decreasing efficiency (Fig. 6). This overlooked nutrient source, mostly DOM (e.g. Hadas et al., 2006), can easily supply all the sponge's nutritional requirements, in potentially containing significant nutrient quantities (Yahel et al., 2003).

The nutrient budget suggested in the present study might have some potential sources of error, mainly as a result of the inability to maintain *N. magnifica* in a fully controlled environment for an extended period of time. Consequently, the data were integrated from several distinct experiments that differed in time and in sponge individuals. The dependence on a natural food source (instead of feeding the sponge with known feed particles) is probably the most significant source of error, because the quality and amount might change over time. In the future, when controlled systems for sponge culture will become available, the nutrient budget will be refined.

The present study highlights the importance of detritus as a carbon source in sponge feeding, and the significance of prokaryotic organisms as the dominant particulate organic nitrogen source in the coral reef. The apparent deficiency in the nutrient budget suggests that *N. magnifica* feeds on a broader than previously estimated spectrum of particles, and must absorb significant amounts of DOM in order to meet its nutritional requirements, despite the fact that this sponge contains very low amounts of microsymbionts. This hypothesis might also be valid for other coral reef heterotrophic suspension-feeders such as bivalves, tunicates and soft corals.

This research was supported by the Rieger Foundation, the Israel Ministry of Energy and Infrastructure, and a US-Israel BARD Foundation research grant (8708-04). We thank Prof. J. Erez (The Hebrew University, Jerusalem) for the CN analysis, Prof. J. J. Lee (City College New York, NY, USA) for identifying the main algal groups and reviewing the manuscript, Dr G. Yahel (Ruppin – The Academic Center) for sharing his unpublished results, assisting with the FACS analyzes and for fruitful discussions, Dr A. Colorni (National Center for Mariculture, Eilat, Israel) and Mr T. Katz (Interuniversity Institute for Marine Sciences, Eilat–IUI) for reviewing the manuscript, and the director and staff of the IUI for their invaluable assistance.

ABBREVIATIONS

A	mean of all field counts
B	number of fields on a filter
CL	clearance rate
C_{out}, C_{in}	TAN concentration in the outflow and inflow water, respectively
C_1,C_2	cell concentrations in inflow and outflow water, respectively
chl-a	chlorophyll-a
DOM	dissolved organic matter
E	index for selectivity
EUP	eukaryotic ultraphytoplankton
FL	flow rate
FR	filtration rate
IUI	Interuniversity Institute (in Eilat, Israel)
LPOC	live particulate organic carbon
LPOM	live particulate organic matter
LPON	live particulate organic nitrogen
NPB	non-photosynthetic bacteria
POC	particulate organic carbon
POM	particulate organic matter
PON	particulate organic nitrogen

RE	removal efficiency
r_i,n_i	cell abundances in the *i* bin in the inflow and outflow, respectively
TAN	total ammonia nitrogen
V	volume of water filtered
W	sponge wet mass
WM	wet mass

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