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Sponges and ascidians control removal of particulate organic nitrogen from coral reef water

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Abstract

We studied removal rates of plankton and total particulate organic nitrogen (PON) by benthic reef communities from the overlying water in a large experimental flume. The flume was filled with mixtures of coral and coral rubble, and biomass of plankton was measured as water was recirculated over the experimental benthic community. All planktonic particle types, picoplankton, nanoplankton, microplankton, and total PON, decreased in concentration at rates proportional to their biomass. The mean first-order rate constant for the decrease in particle concentration was $96 \pm 61 \times 10^{-6}$ m s^{-1}, corresponding to PON uptake of 10 mmol N m^{-2} d^{-1}. *Synechococcus* sp. and heterotrophic bacteria were the major sources of PON. Particulate organic nitrogen removed by rubble and live coral assemblages was directly related to sponge and ascidian biomass (number and area) on the coral and coral rubble. Uptake of PON was about the same as the previously measured uptake of dissolved inorganic nitrogen into these coral reef communities, making it an important flux of nitrogen into the reef.

Particle feeding by coral reef benthos is considered an important pathway for carbon and nutrients in coral reef ecosystems. Coral reef benthos captures zooplankton and large diatoms (Glynn 1973; Sebens et al. 1996). More recently, picoplankton and bacteria are considered significant sources of carbon and nutrients: (1) Buss and Jackson (1979) demonstrated ''reef-fouling'' communities remove picoplankton; (2) Moriarty et al. (1985) argued there must be intense grazing of bacteria by reef communities to remove the rapidly growing cells; and (3) depletion of microbial communities by coral reef benthos has been reported in the Great Barrier Reef (Ayukai 1995), the Red Sea (Yahel et al. 1998), and the Caribbean (Gast et al. 1998). It is now recognized that small plankton (<10 μm) typically account for over 80% of the total particles removed by reef communities (Yahel et al. 1998; Richter et al. 2001; Ribes et al. 2003). This suggestion of the importance of small plankton is consistent with general observations that picoplankton (0.2–2 μm) and nanoplankton (2–20 μm) are the major components of plankton and the largest contributors to planktonic production (Ducklow 1990). Thus it is suggested that picoplankton, nanoplankton, and microplankton may be significant sources of carbon and nutrients to reef communities. It is not evident how particles of different sizes are removed from the water column and which organisms within reef communities are responsible for the removal of cells.

Typical coral reefs have a thin windward fringe of live coral, backed by extensive reef flats comprised of consolidated pavement, coral rubble, and sediment. Coral rubble (dead coral fragments) is an abundant hard substrate on coral reefs (e.g., Gischler and Ginsburg 1996). Kaneohe Bay, Oahu, Hawaii, is typical, where more than 70% of the total reef flat area consists of coral-rubble fields (Cheroske et al. 2000). The extent to which these benthic assemblages remove particles from the water is still poorly known.

Incorporation of particles from the water to the benthos can occur in three different ways. First, passive settling or trapping into the benthos; second, passive adsorption to sticky mucus and settling of that aggregated mucus (Alldredge and Silver 1988); and, third, capture by benthic sus-

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pension feeders. In coral reef communities, cnidarians—mainly symbiotic anthozoans—are the dominant animal group, at least in terms of biomass. For 30 yr it has been suggested that bacteria might constitute a food source for some reef-building coral species (Sorokin 1973; Bak et al. 1998; Ferrier-Pagès et al. 1998); however, the extent to which this occurs in nature has not been addressed. Thus, understanding the importance of small particles for coral reef nutrition, especially at natural concentrations, remains scant.

Coral reef communities include a wide variety of fauna that live under or between coral and coral rubble (''associated fauna''). Most of the associated fauna are either known or suspected to graze on small particles (picoplankton and nanoplankton), including bivalves, gastropods, sponges, ascidians, and polychaetes (*see* Gili and Coma 1998 for a review). Yet, there are no quantitative assessments of the different mechanisms of particle removal.

In the series of experiments reported in this paper, we first determined the capacity of coral-rubble assemblages (dead coral reef with associated algae and fauna hereafter called rubble) to remove particulates from recirculating water in a large (24 × 0.4 × 0.4 m) experimental flume. Second, we set up several manipulative experiments to elucidate the mechanism that produced the observed decrease in particle concentration over the different experimental reef assemblages. These experiments verified the relationship between benthic filter-feeder biomass and the rate of particle removal. Third, to distinguish the role of live corals and associated fauna in the removal of cells smaller than 2 μm, we set up an experiment and showed that sponges and ascidians on the corals and rubble were the organisms responsible for the removal of particles.

Methods

Experimental assemblages—Coral and rubble were put in a 24-m long recirculating flume; the flume has a 12-m length in one direction and a return parallel 12-m length in the opposite direction. Each assemblage covered 2.1 m^2 of the flume bottom on one side of the flume, with an empty flume bottom on the return side of the flume. There were four types of experimental assemblages; (1) all rubble; (2) all bleached coral skeletons; (3) mixtures of rubble with bleached coral skeletons; and (4) rubble mixed with live coral. These experimental communities or assemblages are described following. Coral, rubble, and the associated fauna attached to these substrates were collected from the Point reef flat (1–1.5 m deep) Coconut Island, Kaneohe Bay, Oahu, Hawaii. Rubble is defined as a community of unstable dead coral fragments (Stoddart 1969) and is a common benthic habitat classification used by the National Oceanic and Atmospheric Association (NOAA). To avoid damage to the experimental organisms, only dislodged or very loosely attached coral and rubble heads were collected. All experimental coral assemblages had approximately the same composition of coral, which was as follows: *Porites compressa* covered about 79% of the coral planar surface area and was the dominant species (mean coral head volume, 1,595 ± 834 ml), *Montipora capitata* covered about 12% (mean coral head volume, 918 ± 639 ml), and *Pocillopora damicornis* covered about 9% (mean coral head volume, 578 ± 501 ml), reflecting the natural composition of corals in Kaneohe (Hunter 1988). All rubble assemblages had proportions of the three dominant coral species similar to those of coral assemblages (*P. compressa*, mean rubble head volume, 1,793 ± 884 cm^3; *M. capitata*, mean rubble head volume, 1,136 ± 1,031 cm^3; *P. damicornis*, mean rubble head volume, 560 ± 328 cm^3). The rubble had green filamentous algae on top and ascidians and sponges on the bottom and in the cavities. The coral and rubble were submerged in a bucket and placed in the experimental flume, only 10 m from the collection site.

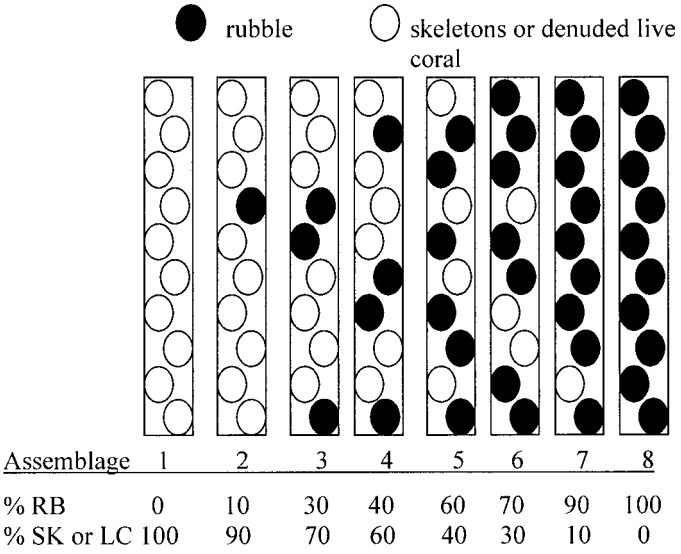

Fig. 1. Eight different assemblages used in the mixture of rubble skeleton (RB-SK) and rubble-denuded live coral (RB-LC).

(1) Rubble assemblages: To investigate the capability of the rubble communities to remove planktonic organisms, we constructed rubble assemblages that were composed of dead coral heads with algae and diverse associated fauna. Three independent rubble assemblages were constructed (RB 1–3).

(2) Skeleton assemblages: To test the effects of complex, rough nonliving benthos in the removal of plankton, we constructed assemblages composed of clean, sun-bleached, and dried coral skeletons—controls (CTRL). These assemblages had the same planar surface area as the rubble assemblages. Several independent skeleton assemblages were constructed.

To test whether live coral or associated fauna are the grazers of plankton, we used a regression-based experimental design, based on two different mixed assemblages (Fig. 1).

(3) Mixture rubble skeletons: Assemblages with eight different mixtures of rubble and skeletons (called RB-SK 1–8). RB-SK 1 (0% rubble) can be considered as a control.

(4) Mixture rubble–live corals: Assemblages with eight different mixtures of rubble with living coral heads that were manually scraped clean of associated filter-feeding fauna (called RB-LC 1–8). We cleaned undersides of coral and rubble because it was easy to manipulate the biomass without damaging the coral heads. Boring fauna were not manipulated because it would have destroyed the coral heads. There was no apparent evidence of bioerosion infesting live

coral heads. All experiments were run one per day (one experimental flume available) during 3 months. The order of the runs of the different experiments was randomly selected.

Biomass of associated fauna—Abundances of sponges, bryozoans, and colonial tunicates that were associated with rubble were estimated by their planar area using a 30 by 25-cm quadrate divided into 750 squares of 1 cm^2. Solitary ascidians and actinians were counted as individuals. All associated fauna were classified to order, except the most abundant species, which were identified to species. The taxa recorded included sponges, tunicates, cnidarians, and bryozoans but did not include bivalves and polychaetes because of the limitations of the nondestructive methods used to assess the abundance of associated fauna.

Experimental procedure—The experimental assemblages were maintained in the flow-through open system mode for 4 d before experiments and between experiments. At the beginning of each experiment the volume of water in the flume was completely replaced, then the inlet and outlets were closed and the water recirculated over the experimental community for 6 h, from 0900 h to 1500–1600 h (we did not address nocturnal feeding). Temperature was measured at 10-min intervals in the flume and on the reef flat where the assemblages were collected. Water samples were collected for particulate organic nitrogen and naturally occurring particles, five to seven times throughout the 6-h experimental period (samples were taken at 50-min intervals). Five liters of water were collected from the flume with a Niskin bottle. Water samples were screened with a 335-μm net to remove larger particles then immediately preserved for further analysis (*see Particle Assessment Protocol*).

All the experiments were performed at a single water velocity of 23 cm s^{-1}. This water velocity is an average wave velocity over the Kaneohe Bay Barrier Reef (Cheroske et al. 2000) and represents a moderate water velocity over many reef flats in the Indo-Pacific (Atkinson et al. 1981). Water velocity was measured by timing a neutrally buoyant drogue as it passed over the full length of the assemblage at least 10 times. Water recirculated over the experimental assemblages about 200 times during the 6-h experiments. Change in height of the water was also measured in each experiment using pitot tubes and a vernier scale to the nearest 0.1 mm as described in Baird and Atkinson (1997).

The change in head is a measure of the loss of energy due to friction by the bottom (Bilger and Atkinson 1992; Baird and Atkinson 1997). From the change in head (k) and the water velocity (U_b), we calculated two parameters, (1) a friction coefficient to describe the effect of the rough bottom on the flow,

$$c_f = 2ghk/U_b^2 \quad (1)$$

where g is acceleration from gravity (which is 9.8 m s^{-2}), h is the height of the water, k is the slope of the water above the assemblage, and U_b is the bulk velocity (*see* Baird and Atkinson 1997); and (2) the Reynolds number of the flow:

$$\mathrm{Re} = U_b 4h/\nu \quad (2)$$

where U_b is the bulk velocity, h is the height of the water, and ν is the kinematic viscosity of seawater at 25°C (0.94 10^{-6} m^2 s^{-1}).

Topographic relief was estimated by laying a chain (link length 0.5 cm) along the surface of the assemblage and calculating the ratio of the length of this chain to the planar length of the assemblage four times for each assemblage (Loya 1978). In principle for these experiments, we tried to design the experiments so the flow conditions would be similar for all experimental assemblages.

Particle assessment protocol—We used flow cytometry to quantify picoplankton cells. Water samples (2 ml) were fixed with 1% paraformaldehyde + 0.05% glutaraldehyde (final concentration) and frozen in liquid nitrogen; afterwards, they were stored at −80°C or in dry ice. For determination of bacterial and picoeukaryote abundance we used a Coulter Epics 753 flow cytometer (Coulter Electronics) equipped with two 5-W argon lasers and a micro-sampler-delivery-system. The flow cytometer was set up for UV (220 mW) and 488 nm (1 W) colinear analysis. Hoechst 33342 was used to stain DNA. Five parameters were collected in list mode and analyzed with custom-designed software (Cytopc by Daniel Vaulot): red fluorescence (from chlorophyll *a*), orange fluorescence (from phycoerythrin), blue fluorescence (from DNA stained with Hoechst 33342), and forward- and right-angle light scatter signals. For statistical purposes, sample size for analysis was chosen to provide more than 10,000 events per sample (Ribes et al. 1999). Nanoeukaryote abundance was determined on 20-ml subsamples stained with DAPI (4′,6 Diamidino-2-phenylindole) and filtered through a 0.2-μm filter (Nucleopore). Stained cells were directly enumerated using epifluorescence microscopy. Cell sizes of heterotrophic bacteria, *Synechococcus* sp., picoeukaryotes, and nanoeukaryotes were measured on the same filters. To quantify phytoplankton and ciliate cell numbers, 350-ml water samples were preserved with Lugol's solution (10% final concentration). Subsamples of 100 ml were transferred to settling chambers, and the major groups of nanophytoplankton and microphytoplankton were quantified using an inverted microscope. Cell sizes (length and width) were measured using an ocular micrometer. Cell biovolumes were estimated from the length and width measurements, assuming the nearest geometrical shape.

Total particulate organic nitrogen was measured by filtering 1-liter water samples and then by adding 10% HCl to dissolve carbonate on precombusted glass fiber filters (Whatman GF/F 1825 025). Filters were kept frozen at −80°C until analysis. Prior to analysis, filters were dried at 60°C for 24 h and analyzed with a C : H : N analyzer (Perkin-Elmer 240). Particulate organic nitrogen (PON) measurements included both detrital and live nitrogen. Detrital PON was estimated as the difference between total PON (C : H : N analysis) and total live nitrogen (estimated from cell counts and measurements).

Carbon (C) and nitrogen (N) content were estimated using conversion factors from the literature. For heterotrophic bacteria, 9.3 fg C $cell^{-1}$ and 1.8 fg N $cell^{-1}$ (Gundersen et al. 2002); for *Synechococcus* sp., 192 fg C $cell^{-1}$ and 21 fg N $cell^{-1}$ (Heldal et al. 2003); for picoeukaryotes and nanoeukaryotes, 183 fg C μm^{-3} and 26.1 fg N μm^{-3} (Caron et al.

Table 1. Physical description of the flume experiments for the assemblages (A): control (CTRL), rubble (RB), dilution of rubble with skeletons (RB-SK), and dilution of rubble with denuded live coral (RB-LC). Temperature (T) range (min–max) in °C. U_b (cm s^{-1}) is the water velocity. Reynold numbers (Re × 10^3) and friction coefficient (c_f) values, Re and c_f are dimensionless. TR is the mean topographic relief.

A	% rubble	T_{min} (°C)	T_{max} (°C)	U_b (cm s^{-1})	Re	c_f	TR
CTRL	0	25.2	27.6	22	228	0.048	1.4
RB 1	100	24.3	28.2	25	243	0.052	1.4
RB 2	100	27.8	30.7	22	220	0.047	1.7
RB 3	100	27.6	29.3	22	224	0.044	1.8
RB-SK 1	0	25.2	27.6	22	220	0.047	1.6
RB-SK 2	15	24.8	27.6	24	231	0.041	1.6
RB-SK 3	30	24.6	24.8	24	235	0.041	1.6
RB-SK 4	45	24.8	27.1	23	226	0.048	1.7
RB-SK 5	60	24.7	25.8	23	224	0.044	1.7
RB-SK 6	75	24.7	28.3	24	232	0.047	1.5
RB-SK 7	90	24.7	28.1	23	224	0.046	1.6
RB-SK 8	100	25.3	28.0	23	222	0.051	1.6
RB-LC 1	0	28.0	32.9	23	229	0.042	1.4
RB-LC 2	15	27.8	31.7	22	213	0.043	1.7
RB-LC 3	30	28.0	32.4	22	212	0.045	1.8
RB-LC 4	45	27.9	31.9	22	215	0.050	1.8
RB-LC 5	60	28.1	30.7	22	211	0.064	1.8
RB-LC 6	75	28.0	31.7	22	214	0.041	1.7
RB-LC 7	90	28.0	31.2	22	214	0.050	1.7
RB-LC 8	100	27.7	29.3	22	219	0.042	1.8

1995). For phytoplankton, biovolume (V, μm^3) was converted to carbon weight using the equation pg C $cell^{-1}$ = $0.109 \times (\mu m^3)^{0.991}$ and to nitrogen using the equation pg N $cell^{-1}$ = $0.0172 \times (\mu m^3)^{1.023}$ (Montagnes et al. 1994). The content of phosphorus was estimated assuming an N : P ratio of 16 : 1.

Results

Mean water temperatures of the flume experiments varied between 24.3°C and 32.9°C, similar to temperatures on the Point reef ($\chi^2 = 0.73$, $p > 0.05$; mean = 27.9 ± 2.6 and 26.8 ± 1.9 for flume and reef, respectively; Table 1). The water in the flume warmed 0.2°C to 4.4°C during each experiment. All experiments were performed at a constant velocity (23 ± 1 cm s^{-1}), so Reynolds numbers only varied from 211,000 to 243,000 (Table 1). Friction factors, c_f, of the communities varied from 0.041 to 0.052 giving turbulent flows and supporting rapid vertical mixing in the flowing water.

In the experiments with rubble assemblages (No. 1, RB 1–3; *see Methods*), concentrations of all plankton types (i.e., heterotrophic bacteria, *Synechococcus* sp., picoeukaryotes, nanoeukaryotes, diatoms, dinoflagellates, and ciliates) and total PON decreased in all the experiments (Fig. 2a–f). *Prochlorococcus* sp., which is the dominant planktonic group offshore, was not present in the ambient water inside the Bay (Ribes et al. pers. comm.). The decrease in concentration of each particle type through time (t) was exponential (e^{-kt}), indicating that the rate of removal slowed as the concentration of particles decreased (Scheffers et al. 2004). Thus, the rate constant for the removal rate can be calculated by plotting ln (cell ml^{-1}) versus time and determining the slope in units s^{-1}. This slope multiplied by the water volume : planar surface area (m^3 m^{-2}) gives a rate constant in units of m s^{-1}, S (Table 2). Planar surface area was used for normalization because it is the convention to describe transport between surfaces and fluids (Bilger and Atkinson 1992). The water volume is the volume of the whole flume, and the surface area is the planar surface area of the experimental assemblage (2.1 m^2). S multiplied by concentration of plankton (number of cells m^{-3}, Ribes et al. 2003), gives an uptake rate in numbers of cells per meter squared per second. This number can be easily converted to numbers of cells per meter of reef per day, or, when conversion units of carbon, nitrogen, or phosphate per cell are used, these numbers can be converted to removal of particulate nutrients per day. Thus using a rate constant, which is a speed, makes it easy to compare the relative speed of particle removal for different experiments and different assemblages of benthos.

In rubble assemblages, mean S from the three assemblages varied by a factor of 13 between 19 and 240 × 10^{-6} m s^{-1} (mean ± SD, 96 ± 64 × 10^{-6} m s^{-1}; or 2–21 m d^{-1}; Table 2), *Synechococcus* sp. was the most efficiently removed particle type. The slopes of ln concentration versus time for the assemblages of dead coral skeletons, which were used as controls (CTRL; *see Methods*) were not significantly different than zero (Fig. 2a–f; Table 2). These results clearly indicate that particle removal was due to living benthic organisms, not physical trapping in baffles or cavities within the control community and not predation by plankton. Based on the rate constants determined in our experiments, a square meter of rubble can deplete 780 ± 190 × 10^9 heterotrophic bacteria per hour, 190 ± 44 × 10^9 *Synechococcus* sp. per hour, 6 ± 2 × 10^9 picoeukaryotes per hour, 7 ± 3 × 10^3 nanoeukaryotes per hour, and 4 ± 2 × 10^2 microplankton cells per hour. Assuming the C and N content of each cell stated in the Methods, a square meter of rubble community could obtain from these planktonic cells 95 mmol ± 2 mmol C d^{-1} (1.15 ± 0.02 g C d^{-1}) and 10 ± 0.2 mmol N d^{-1}. This calculated amount based on numbers of cells is the same amount measured by PON uptake (Table 3). Because picoplankton cells (heterotrophic bacteria, *Synechococcus* sp., and picoeukaryotes) represented over 90% of the carbon and nitrogen (based on estimates of C and N conversion factors in Table 3), we only considered these three cell types in the experiments of particle removal in mixed assemblages of coral, dead skeletons, and live rubble.

In both sets of experiments conducted with mixed assemblages (No. 3, RB-SK 1–8; and No. 4, RB-LC 1–8), concentrations of all picoplankton cells decreased with time in all the experiments (Fig. 3). The decrease in concentration of each particle type was exponential as described for the rubble assemblages (No. 1, RB 1–3) (Fig. 2). The rate constant S in units of m s^{-1} varied from 0 to 250 × 10^{-6} m s^{-1} (100 ± 70 SD × 10^{-6} m s^{-1}). In experiments with both skeletons (No. 3, RB-SK 1–8) and denuded live coral (No. 4, RB-LC 1–8), the removal rate of picoplankton increased as the percentage of rubble increased (Fig. 4; Table 4). There were no significant differences in the regression slopes of S

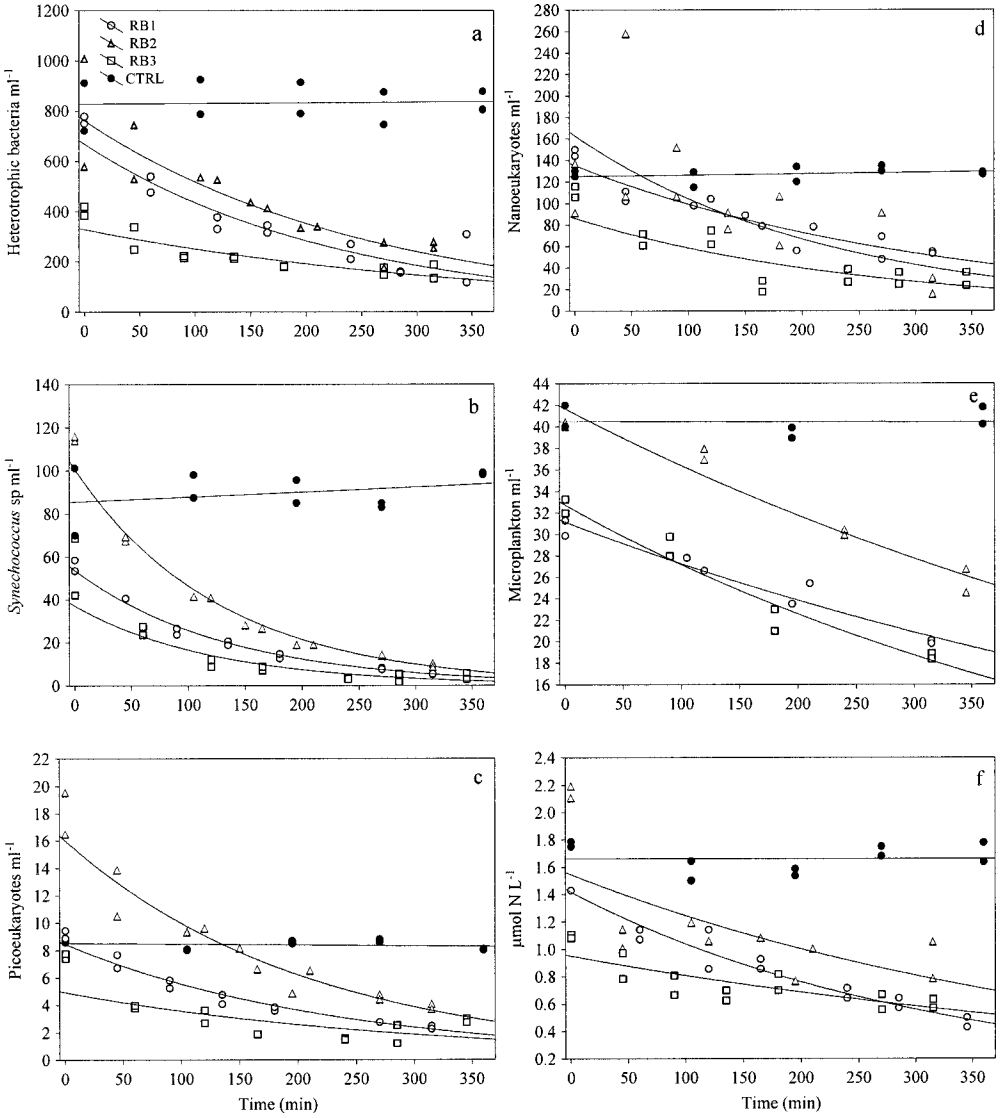

Fig. 2. Rubble and skeleton assemblages: concentration of (a) heterotrophic bacteria, (b) *Synechococcus* sp., (c) picoeukaryotes (in 10^3 cells ml^{-1}), (d) nanoeukaryotes, (e) microplankton, and (f) total PON versus time. Note the curvature, showing rate of removal slows down as concentration decreases.

Table 2. Regression analysis between ln Cp (particle concentration) and time for each rubble (RB) assemblage and the control (CTRL). Rate constant, S (10^{-6} m s^{-1}), and the lower and upper range of the 95% confidence limits of the regression. HB, heterotrophic bacteria; Syn, *Synechococcus* sp.; Pk, picoeukaryotes; Nk, nanoeukaryotes; Mic, microplankton including diatoms, dinoflagellates, and ciliates; and PON, particulate organic nitrogen. Controls are not significantly different that zero. All others are significant to $p < 0.01$.

Particle type	CTRL			RB 1				RB 2				RB 3			
	S	Lower	Upper	S	Lower	Upper	r^2	S	Lower	Upper	r^2	S	Lower	Upper	r^2
HB	−25	−37	−13	−81	−104	−59	0.80	−120	−140	−100	0.82	−80	−96	−64	0.77
Syn	−16	−40	8	−151	−188	−114	0.80	−240	−251	−230	0.98	−220	−228	−212	0.99
Pk	−6	−18	6	−57	−93	−21	0.44	−140	−152	−128	0.94	−120	−128	−112	0.97
Nk	−2	−7	3	−76	−109	−42	0.58	−62	−74	−50	0.87	−140	−179	−101	0.48
Mic	0	−4	3	−19	−23	−15	0.94	−26	−31	−21	0.84	−40	−48	−32	0.94
PON	0	−7	7	−57	−64	−49	0.95	−60	−88	−33	0.47	−32	−45	−19	0.65

Table 3. Total removal of cells, carbon (g C m^2 d^{-1}), and nitrogen (mmol N m^2 d^{-1}) for rubble communities RB 1–3. Abbreviations as in Table 2. Total POC, PON, calculations from total particulate organic carbon and nitrogen.

	Cells removed (m^{-2} h^{-1})	g C removed (m^{-2} d^{-1})	mmol N removed (m^{-2} d^{-1})
HB	$776\pm189\times10^9$	0.17 ± 0.04	2.4 ± 0.6
Syn	$202\pm13\times10^9$	0.93 ± 0.06	7.3 ± 0.5
Pk	$423\pm103\times10^7$	0.03 ± 0.001	0.3 ± 0.1
Nk	$602\pm112\times10^5$	0.005 ± 0.001	0.1 ± 0.01
Mic	$432\pm100\times10^4$	0.01 ± 0.003	0.2 ± 0.04
Live particles	—	1.15 ± 0.02	10 ± 0.2
Total POC, PON	—	1.14 ± 0.04	10 ± 3

versus percentage rubble for dead skeletons (No. 3: RB-SK 1–8) and denuded live coral (No. 4, RB-LC 1–8) (analysis of covariance on *S*% rubble, two-way, cells nested in mixture; $p < 0.0001$), indicating corals did not remove picoplankton at faster rates than dead skeletons. Because dead skeletons also did not remove plankton, living corals do not remove significant quantities of picoplankton

Sponges were the dominant active suspension feeders in the associated fauna. In the rubble experiments, RB 1, 2, 3 (all rubble; *see Methods*), sponges covered surface areas ranging between 2220 and 2370 (2310 ± 80 SD) cm^2 per assemblage. The species *Biemna fistulosa* accounted for more than 60% of this surface. Tunicate abundance consisted of 12–15 (13 ± 2) cm^2 of colonial tunicates and 150–180 (160 ± 17) solitary ascidians per assemblage. *Brotryllus* sp. and *Polyclinum* sp. were the dominant colonial species and *Phallusia nigra*, *Herdmania momus*, and *Ciona intestinalis*

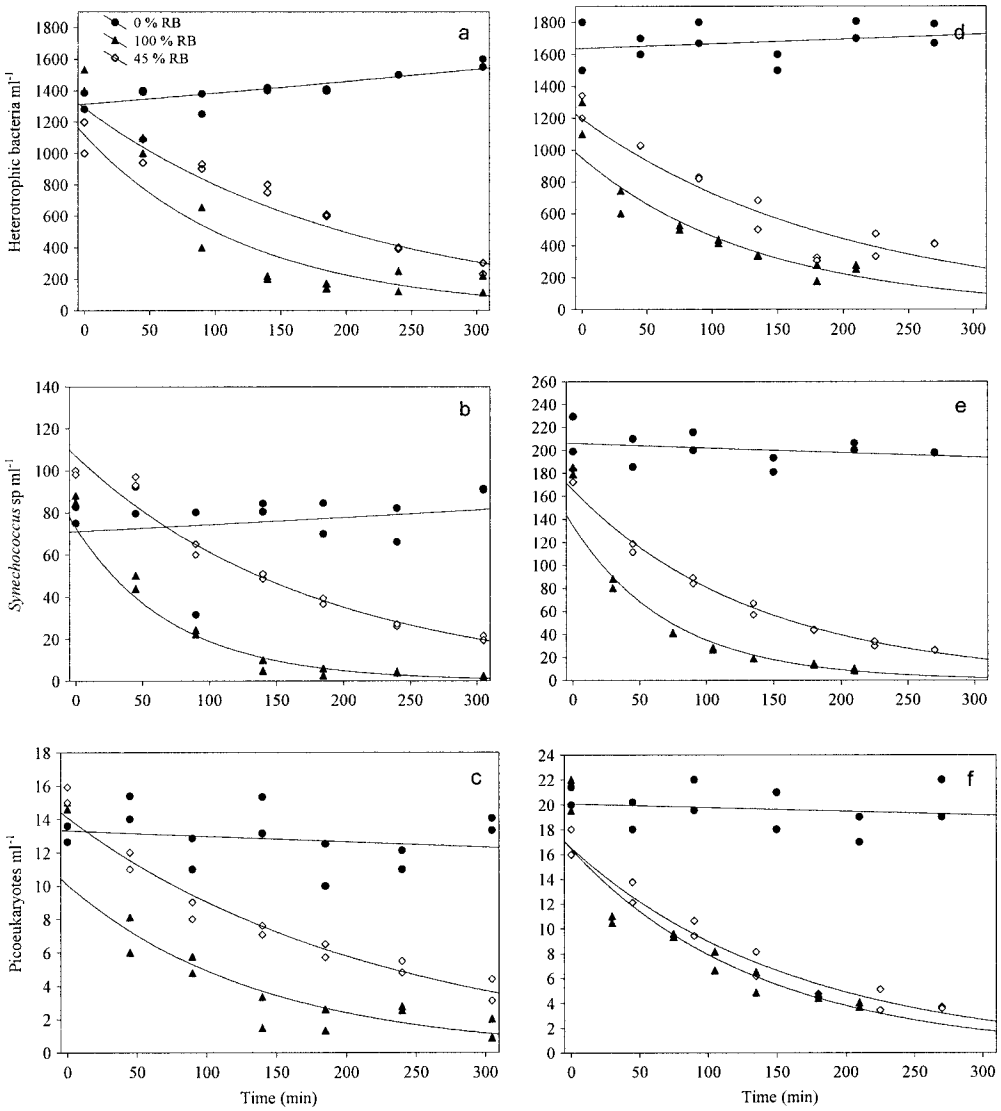

Fig. 3. Mixture experiments of rubble (0%, 45%, and 100% rubble) with denuded live coral and with skeletons. Concentration of (a) heterotrophic bacteria, (b) *Synechococcus* sp., (c) picoeukaryotes (in 10^3 cells ml^{-1}), (d) nanoeukaryotes, (e) microplankton, and (f) total PON versus time.

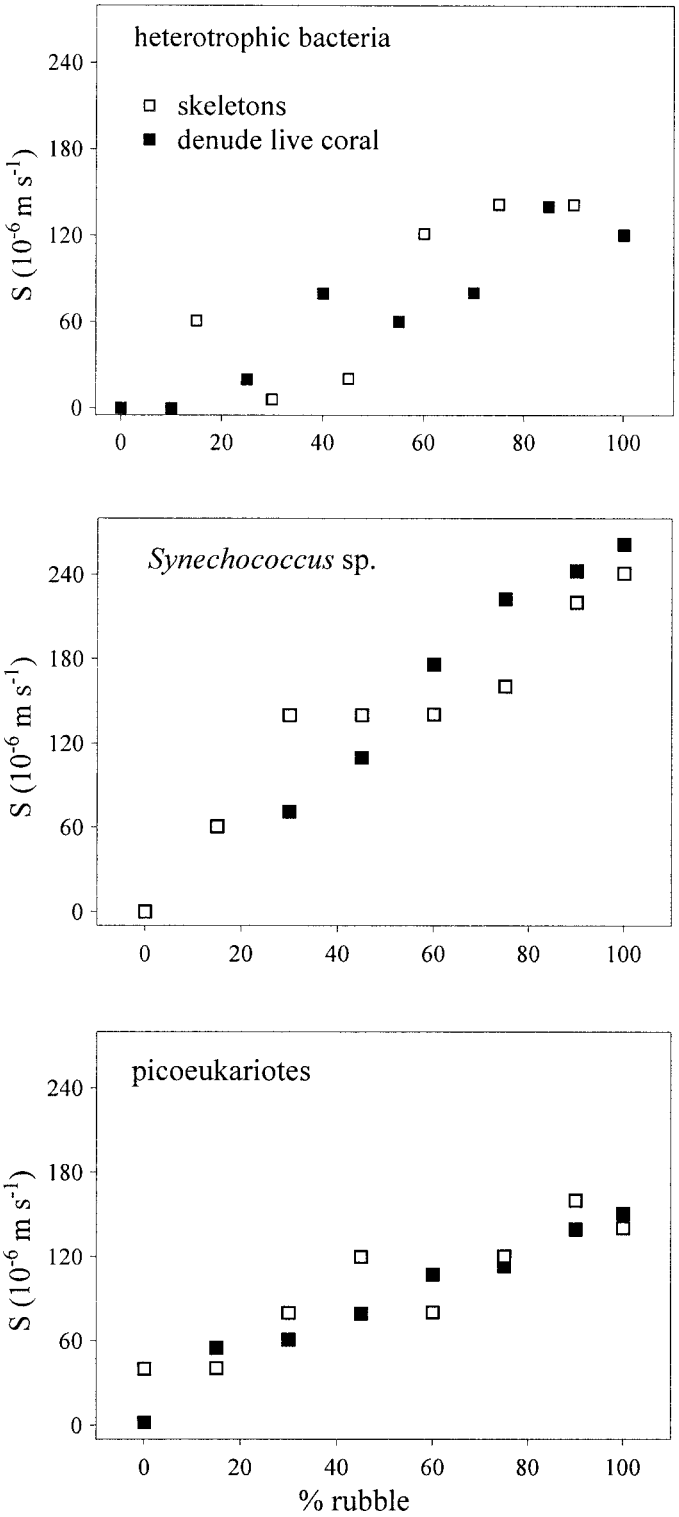

Fig. 4. The first-order rate constant for removal of picoplankton, *S*, versus the percentage of rubble for the two mixed assemblages (rubble skeletons, RB-SK 1–8; rubble-denuded live coral, RB-LC 1–8).

Table 4. Linear regression slopes and intercepts of *S* ($\times 10^{-6}$ m s^{-1}) versus percentage of rubble. All probabilities are < 0.05.

Particle type	Slope	Intercept	r^2	*n*
RB-LC				
Heterotrophic bacteria	1.4	−4.5	0.87	8
Synechococcus sp.	2.1	39	0.86	8
Picoeukaryotes	1.1	44	0.78	8
RB-SK				
Heterotrophic bacteria	1.5	1.5	0.65	8
Synechococcus sp.	2.7	4.8	0.98	8
Picoeukaryotes	1.4	19	0.96	8
RB-LC and RB-SK				
Heterotrophic bacteria	1.4	−1.8	0.76	16
Synechococcus sp.	2.4	23	0.92	16
Picoeukaryotes	1.2	32	0.85	16

were the dominant solitary ascidians. Actinians were the most abundant passive suspension feeder in the associated fauna, ranging between 20 and 80 (47 ± 31) individuals per assemblage. *Aiptasia pulchella* was the dominant actinian species. Very few bryozoans were found. Given that assemblages were 2.1 m^2, these values translate into an average value of 1,100 cm^2 of sponges per m^2 planar surface area, 6.2 colonial tunicates per m^2 planar surface area, and 76.2 solitary ascidians per m^2 planar surface area.

Coral rubble generally had green filamentous algae on top and ascidians and sponges on the bottom and in the cavities. For experiments using mixtures of rubble skeletons, and mixtures of rubble-denuded live corals (No. 3, RB-SK 1–8; and No. 4, RB-LC 1–8), the total area of sponge and number of ascidians in each assemblage were positively correlated with percent rubble (Fig. 5; cm^2 sponges m^{-2} reef $= -8.4 + 11.6$ (percentage rubble), $r^2 = 0.87$, $p < 0.0001$, $n = 16$; N ascidians m^{-2} reef $= 1.56 + 0.67$ (% rubble), $r^2 = 0.72$, $p < 0.0001$, $n = 16$).

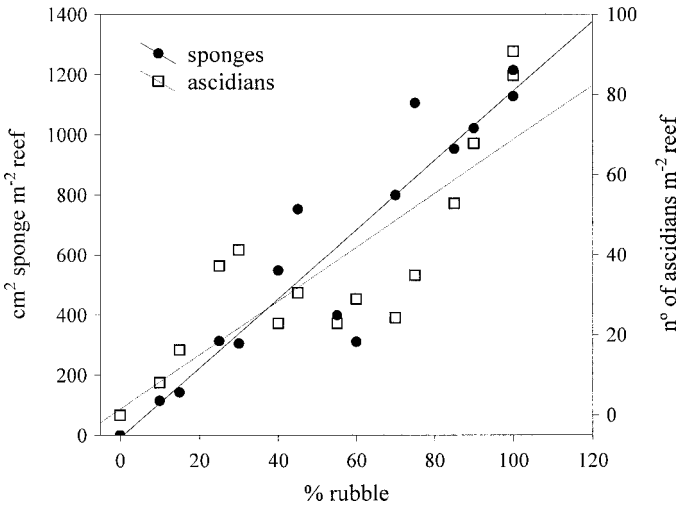

Fig. 5. Area of sponges and numbers of ascidians m^{-2} of reef versus the percentage of rubble. These data are from experiments of rubble mixed with live coral (RB-LC 1–8) and rubble mixed with dead coral skeletons (RB-SK 1–8), *see Fig. 1.*

Table 5. Multiple regression parameters to calculate rate constants, S (10^{-6} m s^{-1}), for removal of the following particles given area of sponge and number of ascidians m^{-2} of reef. $S = a$ (cm^2 sponges m^{-2} reef) + b (no. of ascidians m^{-2} reef) + c, where c is a constant, $n = 16$. S times the number of cells in the water column results in the removal rate of cells in numbers of cells m^{-2} d^{-1}. All probabilities are < 0.05.

Particle type	a	b	c	r^2	S (10^{-6} m s^{-1})	Element (mmol m^{-2} reef d^{-1})		
						C	N	P
Heterotrophic bacteria	0.1	−0.29	19	0.9	72	10	2	0.1
Synechoccoccus sp.	0.18	−0.1	41	0.9	157	48	5	0.3
Picoeukaryotes	0.1	0.11	36	0.9	108	3	0.4	0.03
Total						**61**	**7**	**0.5**

Discussion

These results clearly show that active uptake of particles by benthic suspension feeders is the major mechanism for particle removal from the water. Passive trapping of particles and adsorption by mucus and predation by planktonic communities are only minor pathways for particle removal. Rate constants (S, m s^{-1}) for particle removal were on average 1.5 times higher for rubble communities ($96 \pm 61 \times 10^{-6}$ m s^{-1}, 2–18 m d^{-1}) than for live coral communities ($62 \pm 25 \times 10^{-6}$ m s^{-1}; 3–8 m d^{-1}; Ribes et al. 2003). The removal of particles <2 μm, however, is directly related to the cover of ascidians and sponges, not coral cover. It is very likely that the positive correlation between S (for all plankton types) and rubble is regulated by sponge and ascidian biomass (or percentage cover). These experiments were not designed to evaluate the difference in efficiency between sponge and ascidian filtering. An inspection of Fig. 4 reveals that sponge cover is more closely correlated to percentage rubble ($r^2 = 0.87$) than numbers of ascidians ($r^2 = 0.72$). Consequently, a two-variable linear regression between S (for different types of plankton) and sponge and ascidian biomass indicates that the correlation between S and rubble is primarily driven by sponge cover (''a'' coefficient; Table 5), not numbers of ascidians (''b'' coefficient; Table 5). The differences in filter efficiency for sponges and ascidians need experimental validation; nevertheless, these results clearly indicate the filter-feeding component of the community (sponges and/or ascidians) is controlling the removal of planktonic organisms. Thus, it does not matter whether the bottom cover is coral or rubble; it is the sponges and ascidians associated with this substrate that control the removal rates of plankton. Corals do take up bacteria (Sorokin 1973) but at rates much less than active suspension feeders (Bak et al. 1998; Ferrier-Pagès et al. 1998; Houlbrèque 2004). Without these associated fauna, coral reef ecosystems would probably not remove significant quantities of picoplankton.

Temperature can affect the filtering rate of sponges and ascidians (Riisgård et al. 1993), so it is important to consider the effects of temperature on the rates constants. The maximum increase in temperature during a single experiment was 4.4°C. Assuming a Q_{10} of 2 (Frost 1987; Riisgård et al. 1993), the actual rate would only decrease by 23% ([SN observed/ln2(1/10)($t_1 - t_2$)] = SN actual; where N is the concentration of cells and S is the rate constant as discussed above). The average change in temperature during single experiments is 2.9 ± 1.2 (Table 1); thus, on average we can consider the effects of temperature—if there are any at all—to be confined to within <10%. To test whether temperature may have affected the outcome of the statistics, all data were recalculated assuming a Q_{10} of 2. We recalculated S values for both sets of experiments conducted with mixed assemblages removing the possible temperature effect on S calculation. There were no changes in the statistical results, so the observed and the actual (assuming $Q_{10} = 2$) regression slopes of S versus percentage rubble for dead skeletons (No. 3, RB-SK 1–8) and denuded live coral (No. 4, RB-LC 1–8) were not significantly different (No. 3, RB-SK 1–8, *Synechococcus* observed slope = 2.7, actual slope = 2.1; No. 4, RB-LC 1–8, *Synechococcus* observed slope = 2.1, actual slope = 1.8). Thus we conclude that temperature had a very minor effect on the results; sponge and ascidian biomass are the major determinants of rates of particle removal.

More than 70% of the total amount of carbon removed by uptake of cells (0.95–1.50 g C m^{-2} d^{-1}) was from autotrophic cells (0.71–1.14 g C m^{-2} d^{-1}), such as *Synechococcus* sp., photosynthetic picoeukaryotes and nanoeukaryotes, and microphytoplankton. These values are similar to the values reported from studies of microplankton in water mass flowing over reef flats. Richter and collaborators estimated an uptake of 0.89 ± 0.05 g C m^{-2} d^{-1}, from the phytoplankton by a coelobite community dominated by sponges (Richter et al. 2001). Other workers reported somewhat higher values of carbon uptake from phytoplankton (1.1–2.0 g C m^{-2} d^{-1}, Fabricius et al. 1998; Yahel et al. 1998). Given the variability in reef and microbial community species composition and the conversion factors used, the similarity of calculated carbon uptake values is surprising.

In terms of nitrogen, our reported values from the phototrophic fraction of plankton (≈8 mmol N m^{-2} d^{-1}) are lower than the values reported by Richter and collaborators (22 mmol N m^{-2} d^{-1}). Total PON rates are all about the same as uptake of inorganic nitrogen species such as ammonia and nitrate (<10 mmol N m^{-2} d^{-1}; Atkinson and Falter 2003), indicating uptake of particulate nitrogen through active feeding of suspension feeders can be the dominant pathway for uptake of nitrogen in the reef systems. Furthermore, it has been recently observed that even rubble without apparent macrofauna can remove significant amounts of phytoplankton from the overlying water, which was attributed to the grazing activity of minute suspension feeders inhabiting the outer centimeters of these surfaces (Yahel et al. in press).

The C and N removal rate is S multiplied by the concen-

tration of cells (number of cells per m^3) for each particle type multiplied by the amount of carbon and nitrogen per cell. Natural particles in Kaneohe Bay were dominated by prokaryotes: heterotrophic bacteria was 23×10^5 cells ml^{-1}, *Synechococcus* sp. was 2.6×10^5 cells ml^{-1}, picoeukaryotes was 15×10^3 cells ml^{-1}, nanoeukaryotes 200 cells ml^{-1}, microplankton (ciliates, diatoms, and dinoflagellates) were a very small proportion of the particles, with only 10–30 cell ml^{-1} (Ribes et al. 2003). *Prochlorococcus* sp. was not found during the experiments and is generally uncommon in Kaneohe Bay (Ribes et al. pers. comm.).

We can estimate the amount of carbon, nitrogen, and phosphorus removed from the water column normalized to sponge area and ascidian number by taking values of *S* from the two variable linear regressions (Table 5) and multiplying by concentration of cells (number of cells m^{-3}) in water and amounts of carbon, nitrogen, and phosphorus in different cells. For average sponge and ascidian abundance in live coral and rubble assemblages (668 cm^2 sponges m^{-2} reef; 46 ascidians m^{-2} reef), these calculations clearly show that *Synechococcus* sp. was the major source of particulate carbon, nitrogen, and phosphorus (48 mmol C m^{-2} d^{-1}, 5 mmol N m^{-2} d^{-1}, and 0.3 mmol P m^{-2} d^{-1}; Table 5), compared with heterotrophic bacteria (one-third of *Synechococcus* sp.) and picoeukaryotes ($<$ 10% of *Synechococcus* sp.). The combined fluxes from these three plankton types gives 61 mmol C m^{-2} d^{-1}, 7 mmol N m^{-2} d^{-1}, and 0.5 mmol P m^{-2} d^{-1} (Table 5). The particulate nitrogen and phosphorus removal rates reported here are about the same as uptake of dissolved inorganic compounds such as nitrate, ammonia, and phosphate ($<$10 mmol N m^{-2} d^{-1} and $<$1 mmol P m^{-2} d^{-1}; Atkinson and Falter 2003), though the removal of carbon is relatively small compared with gross primary production (61 compared to 500–1,000 mmol C m^{-2} d^{-1}). Thus it is possible that with the constant remineralization of these particles, through the grazing of filter feeders, concentrations of some dissolved nutrients could increase over shallow reef flats with high water residence times.

Water samples collected over the Kaneohe Bay Barrier reef during 1979 to 1981 ($n = 421$) showed a significant increase in ammonia, no significant change in nitrate, and a decrease in phosphate (Atkinson 1987). The decrease in phosphate is at a mass transfer rate (Falter et al. 2004). The increase in ammonia concentration corresponds to an efflux from the reef of about 2.5 mmol N m^{-2} d^{-1}, 35% (2.5/7) of the estimated particulate removal by filter feeders in the coral-rubble experiments, and half of the excretion rate measured for microatolls in the Great Barrier Reef (4.3 mmol NH_4 m^{-2} d^{-1}; Steven and Atkinson 2003). Thus, regenerated dissolved nitrogen from filter feeders can easily be the source of nitrogen that elevates ammonia over the Kaneohe Bay reef flat.

We can draw two conclusions from this research: (1) particle removal is controlled predominantly by ''biomass'' (area and numbers) of filter-feeding sponges and ascidians and (2) particulate flux of nitrogen and phosphate can be major sources of nutrients to the reef where filter feeders are abundant. This may contribute to understanding the apparent lack of nutrient removal from the water mass (e.g., Odum and Odum 1955) and the export of dissolved nutrients from some reefs (Delesalle et al. 1998; Hata et al. 1998), a crucial issue in current understanding of the food-web dynamics and biogeochemical cycle of coral reef ecosystems.

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