

## In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida

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### Abstract

We measured ammonium and nitrate plus nitrite fluxes from 14 common sponge species on a Florida Keys reef (Conch Reef) using a combination of incubation experiments and an in situ method that requires no manipulation of the sponge. On a 600-m<sup>2</sup> section of Conch Reef, species-specific biomass for all nonencrusting sponges was measured. The biomass data combined with species-specific dissolved inorganic nitrogen (DIN) flux rates yielded the benthic DIN flux from 14 species, and allowed us to extrapolate these data to the total nonencrusting sponge community. The species for which we measured DIN fluxes represented 85% of the nonencrusting sponge biomass in the study area and released a combined  $480 \pm 93 \mu\text{mol m}^{-2} \text{h}^{-1}$  of nitrate plus nitrite, and  $57 \pm 73 \mu\text{mol m}^{-2} \text{h}^{-1}$  of ammonium. Approximately 73% of the measured DIN flux was produced by *Xestospongia muta*, a massive barrel sponge. Of the 14 species studied, 10 hosted active nitrifying communities, and 8 hosted photosynthetic microbial associates. However, the presence of these microbial communities had no apparent effect on the magnitude of the total DIN flux. We estimate that the DIN flux for the entire nonencrusting sponge community is  $640 \pm 130 \mu\text{mol m}^{-2} \text{h}^{-1}$ .

Predicting coral reef health in the face of climate change, disease, and eutrophication requires a comprehensive understanding of nutrient and organic matter cycling in reef environments. Benthic reef organisms are known to alter suspended particulate organic matter (POM) and nutrient concentrations in water passing over the reef (Bak et al. 1998; Yahel et al. 1998). Recent studies suggest that

sponges, in particular, can be a significant sink for particulate organic matter (Ribes et al. 2005; Lesser 2006). Further, the removal of small particles and the increase in nitrate plus nitrite ( $\text{NO}_x^-$ ) concentrations observed in reef waters (Van Duyl et al. 2002; Scheffers et al. 2004) are consistent with sponge feeding and nutrient excretion (Corredor et al. 1988; Diaz and Ward 1997).

The ability of sponges to filter large volumes of water, up to 50,000 times their own volume every day (Reiswig 1971; Weisz et al. in press), combined with their associated microbial communities (Hentschel et al. 2006 and references therein) influence their biogeochemical effect on coral reefs as well as other coastal environments where they are abundant. The few existing surveys of Caribbean reefs that report both size and abundance of sponges indicate that they can outrank corals in terms of biomass (Diaz and Rützler 2001 and references therein). Despite being a dominant feature on many coral reefs, sponge populations are vulnerable to abiotic, biotic, and anthropogenic disturbances such as bleaching events, hurricanes, commercial harvesting, and algal blooms (Vicente 1990; Butler et al. 1995; Cropper and DiResta 1999). Data on long-term changes of total sponge biomass are limited in part because of the large No. of species and the morphological variability among individuals of the same species (Diaz and Rützler 2001). Given their potential to influence organic matter and nutrient concentrations on the reef,

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changes in sponge populations could have significant consequences for coral reef health.

It has been found that rapid rates of organic matter remineralization by some sponges can make them an important source of dissolved inorganic nitrogen (DIN) for Caribbean reefs (Reiswig 1981; Corredor et al. 1988; Diaz and Ward 1997). Sponges may also be responsible for a significant portion of the high rates of POM remineralization observed in coral cavities (Richter et al. 2001; Scheffers et al. 2004). Furthermore, some species host large, diverse communities of microorganisms that could affect the magnitude and/or the speciation of the DIN flux. Previous studies quantified DIN flux from a few dominant species in the sponge community; however, to our knowledge, no one has attempted to quantify the flux from the entire sponge community. Furthermore, incubation chambers (Corredor et al. 1988; Diaz and Ward 1997; Jimenez and Ribes 2007) were used in some previous studies, and these require removal of sponges from the reef substrate. Even when care is taken to avoid injury to the sponge, manipulation can change its pumping rate and possibly its metabolic rate (Reiswig 1971). The potential effect of these manipulations on sponge DIN flux has not been determined.

Here we present  $\text{NH}_4^+$  and  $\text{NO}_x^-$  fluxes from 14 common species of sponge on Conch Reef (Key Largo, Florida) measured using a combination of incubation and in situ methods. The in situ method requires no physical contact with the sponge. A net DIN flux (defined as  $\text{NH}_4^+$  plus  $\text{NO}_x^-$ ) from nonencrusting sponges at Conch Reef was achieved by combining species-specific DIN flux results with a detailed sponge biomass survey of a 600-m<sup>2</sup> area of Conch Reef. The results were utilized to compare the two methods and also to assess the overall importance of sponges relative to other DIN sources reported in the literature.

## Methods

**Study sites**—Water samples were collected from Conch Reef, located east of Tavernier Key (24°57.43'N, 80°26.82'W) in 2004–2006. Our sampling and biomass survey took place on a ridge (primarily horizontal) at approximately 15-m depth. As Conch Reef is in a no-take research sanctuary, sponges for incubation experiments were obtained from Three Sisters, a small patch reef east of Key Largo (25°01.76'N, 80°23.89'W) approximately 5 m deep.

**Sponge biomass survey**—Self Contained Underwater Breathing Apparatus (SCUBA) divers used nylon rope to establish a 20 × 30-m grid on Conch Reef marked at 1-m intervals along each side. Divers then recorded the species and dimensions of every sponge in the 600-m<sup>2</sup> area. Branching sponges of the same species that were connected were counted as a single individual. The area covered by encrusting sponges was recorded, but DIN flux was not measured from any encrusting sponges, so the calculated community flux did not include their contribution. Results are reported in terms flux per unit volume of sponge tissue or planar area of reef.

**Chlorophyll a**—Sponge tissue (visually free of epiphytes) was collected for pigment analysis from Conch Reef in January 2006. Samples were kept on dry ice until brought back to the lab, and then frozen at  $-80^\circ\text{C}$ . Chlorophyll *a* (Chl *a*) analysis was done according to Environmental Protection Agency (EPA) method 445.0 revision 1.2. All pigment extraction was conducted in a darkened lab. The outer 2 mm of sponge tissue was thawed, drained of excess water on a paper towel, and extracted with 4 mL of 100% acetone in a 15-mL centrifuge tube. This was modified from 90% acetone in the EPA method to account for the water content of the sponge (Southwell 2007). The sponge was macerated in the acetone with a glass stirring rod and sonicated (15 pulses) with a probe sonicator while submerged in an ice bath. The centrifuge tubes were then capped and allowed to incubate in the dark at  $-20^\circ\text{C}$  overnight. The sponge-acetone mixture was then resuspended by gentle stirring, and the extract was filtered through a 0.45- $\mu\text{m}$  nylon syringe filter into a borosilicate test tube. The fluorescence of the extract was measured on a Turner Designs model TD-700 with a Chlorophyll *a* optical kit. The raw fluorescence was then compared to known standards (Turner Designs) and the extracted sponge tissue was then dried and weighed.

**In situ sampling**—For the in situ method, the flux of each individual sponge was calculated as the product of its pumping rate and  $\Delta\text{DIN}$ , where  $\Delta\text{DIN}$  is defined as the difference in DIN concentration between ambient water (water immediately adjacent to the sponge) and the excurrent water (water exiting the sponge osculum). The in situ method was used whenever the sponges had an osculum large enough to reliably sample excurrent water and measure flow velocities. SCUBA divers collected paired samples of sponge excurrent water and ambient water in triplicate from 3 individuals to 5 individuals of each species studied. In order to evaluate temporal variability, triplicate samples for  $\Delta\text{DIN}$  were obtained from four of the five *Xestospongia muta* individuals at multiple time points within a 3-d period. Samples were taken in acid-washed and DI-rinsed 60-mL syringes fitted with a stopcock and a short length of narrow tubing. Excurrent samples were taken slowly ( $\sim 2 \text{ mL s}^{-1}$ ) in order to obtain only water from the excurrent plume, and the syringes were first flushed with sample before the final collection. The samples were kept on ice during transport back to the shore-based laboratory, where a 12-mL aliquot was immediately analyzed for ammonium, and the rest frozen ( $-20^\circ\text{C}$ ) in acid-washed 50-mL centrifuge tubes for analysis of nitrate plus nitrite, usually within one month.

For *Niphates digitalis*, *Ircinia strobilina*, and *Agelas conifera*, the volume-normalized mean pumping rates and standard deviations for each species (previously measured by Weisz et al. in press) were combined with the  $\Delta\text{DIN}$  measurements for each sponge individual. These pumping rates were based on measurements from 18, 8, and 12 individuals, respectively. For *X. muta*, *Aplysina archeri*, and *Aplysina lacunosa*, pumping rate measurements and  $\Delta\text{DIN}$  samples were taken contemporaneously, and so for these species we were able to pair pumping rates and  $\Delta\text{DIN}$

measurements of individual sponges. These pumping rates were measured using the same method as Weisz et al. (in press). Briefly, SCUBA divers recorded the upward movement of fluorescein dye pulses injected into the excurrent plume using underwater videography ( $n \geq 20$ ). A weighted stand with a flexible arm was used to position a ruler behind the sponge for scale. Pumping rates were calculated by multiplying excurrent water velocities by the lateral area of the excurrent plume, which was determined by releasing pulses of dye across the diameter of the osculum. Previous studies have reported periodic cessation of pumping in some individuals (Reiswig 1971). However, the individuals in this study, chosen arbitrarily, were all found to be pumping. The dimensions of the sponge individuals were recorded to calculate tissue volume.

**Incubation experiments**—The DIN fluxes from sponges with oscula too small for in situ sampling to be feasible (e.g., rope morphology) were measured using laboratory-based incubation experiments, performed in 2003–2005. At Three Sisters Reef, SCUBA divers cut pieces of sponge from healthy adults (2–8 cm<sup>3</sup>, depending on species), attached them to polyvinyl chloride (PVC) plates with plastic cable ties, and then left them on the reef to recover. Whenever possible, small individuals were used rather than subsections of a larger individual. Sponges recovered on a time scale of days to weeks, depending on species. Only sponges with healthy appearances (i.e., no visible wounds or decay) were selected for the experiments. These sponges ( $n = 3$ –6) were incubated in 2–4-L High Density Polyethylene (HDPE) plastic containers of seawater (also obtained from Three Sisters Reef) for 4–8 h, and sampled at 4–5 time points during the incubation. The water in the incubation chambers was aerated and stirred using an aquarium pump. The incubations were performed outdoors in the shade, and containers were submersed in a flow-through seawater bath to maintain ambient temperature. Two aliquots of water were taken at each time point; one was analyzed immediately for ammonium, and the other was frozen at  $-20^{\circ}\text{C}$  in an acid-washed (10% HCl) and DI-rinsed 50-mL centrifuge tube for nitrate plus nitrite analysis, usually within one month. At the end of the experiment, the dimensions of the sponges were measured, and the sponges were dried in a lyophilizer and weighed.

**Method comparison**—Agreement between the two methods for determining DIN fluxes was determined by applying both methods to *X. muta* and *A. conifera*.

**Nutrient analysis**—Ammonium was measured by fluorescence following the method of Holmes et al. (1999). Briefly, 3 mL of o-phthalaldehyde working reagent was added to 12 mL of sample in an acid-washed and DI-rinsed 15-mL centrifuge tube. Samples were incubated in the dark at room temperature for 2 h, and then analyzed using a Turner Designs fluorometer, model TD-700, fitted with an ammonium optical kit. The lower limit of quantification for ammonium measurements was found to be  $0.2 \mu\text{mol L}^{-1}$  by repeated measurement of standards. Ammonium standards were made fresh each day, and

measured at the same time as the samples. Nitrate plus nitrite ( $\text{NO}_x^-$ ) was measured using standard colorimetric methods on a QuickChem flow-through autoanalyzer (Strickland and Parsons 1972). A subset of samples from *Smenospongia aurea* and *X. muta* were analyzed without the Cd reduction column to evaluate the contribution of nitrite. The lower limit of quantification for nitrate plus nitrite was determined to be  $0.25 \mu\text{mol L}^{-1}$  by repeated measurement of standards. The standard deviations for the triplicate groups of in situ water samples averaged  $0.17 \mu\text{mol L}^{-1}$  for both  $\text{NH}_4^+$  and  $\text{NO}_x^-$ .  $\text{NO}_x^-$  was not analyzed for *N. digitalis*, but was found to be negligible in previous experiments (M. W. Southwell unpubl. data).

**Microbial community effects**—For the purposes of comparing differences in DIN fluxes among species with different types of associated microorganisms, the volume-specific rates were normalized for density (Weisz in press) and organic C content (Martens et al. unpubl. data). Density data were not available for *Aplysina fistularis*, *A. lacunosa*, *Ircinia campana*, and *S. aurea*, and so these species were not included in the organic C-normalized comparison.

**Statistical treatment**—For calculation of  $\Delta\text{DIN}$  for the in situ method, values below the lower limit of quantification were replaced with the value for that limit ( $0.2 \mu\text{mol L}^{-1}$  for  $\text{NH}_4^+$  and  $0.25 \mu\text{mol L}^{-1}$  for  $\text{NO}_x^-$ ). Because the only values affected were those from ambient samples, this replacement made our  $\Delta\text{DIN}$  values (and, thus, the fluxes) more conservative. Outliers were identified as follows: for each species, all ambient values were grouped, as were all excurrent values, and any values that were  $>1.5$  times the interquartile distance above the third quartile were discarded. For each sponge individual, the difference between the means of ambient ( $n = 2$ –3) and excurrent ( $n = 2$ –3) samples was calculated and the pooled estimate of variance for the difference between the two means was used in combination with flow measurement standard deviation to calculate a flux and standard deviation by propagation of error. Uncertainty in the sponge dimensions (based on precision of ruler measurements) was estimated to be 10%, and by propagation of error, this equals approximately 17% uncertainty in volume. The standard deviation of sponge volume measurements was, therefore, estimated to be 20%, and this value was incorporated into volume-normalized flux calculations by propagation of error. For *X. muta* individuals that were sampled multiple times, the mean  $\Delta\text{DIN}$  of all time points was used and the standard deviations of all time points were summed by propagation of error. Unless otherwise noted, uncertainties are expressed as standard deviation.

For incubation experiments, least squares regression of concentration versus time was used to calculate the flux, which was then normalized for the sponge volume and the incubation chamber volume. Flux standard deviation was based on fluxes from replicate individuals ( $n = 3$ –6) of each species. To evaluate the agreement of results from the incubation and in situ methods, we compared the mean

Table 1. Results of biomass survey from 600-m<sup>2</sup> area of Conch Reef.

	Volume (L m <sup>-2</sup> )	Abundance (Ind. m <sup>-2</sup> )
Total		6.50
Encrusting		0.69
Nonencrusting	3.60	5.91
<i>Agelas clathrodes</i>	0.01	0.03
<i>Agelas conifera</i>	0.23	0.24
<i>Agelas wiedenmeyeri</i>	0.06	0.59
<i>Amphimedon compressa</i>	0.10	1.01
<i>Aplysina archeri</i>	0.01	0.04
<i>Aplysina cauliformis</i>	0.03	0.79
<i>Aplysina fistularis</i>	0.00	0.01
<i>Aplysina fulva</i>	0.02	0.07
<i>Aplysina lacunosa</i>	0.06	0.21
<i>Callyspongia plicifera</i>	0.02	0.09
<i>Callyspongia vaginalis</i>	0.13	0.35
<i>Geodia</i> sp.	0.02	0.01
<i>Iotrochota birotulata</i>	0.00	0.09
<i>Ircinia felix</i>	0.04	0.18
<i>Ircinia strobilina</i>	0.08	0.19
<i>Mycale laxissima</i>	0.00	0.02
<i>Niphates digitalis</i>	0.05	0.54
<i>Niphates erecta</i>	0.02	0.62
<i>Pseudoceratina crassa</i>	0.33	0.47
<i>Smenospongia aurea</i>	0.02	0.02
<i>Verongula gigantea</i>	0.03	0.01
<i>Xestospongia muta</i>	2.33	0.20
Unknown	0.01	0.01

DIN, NH<sub>4</sub><sup>+</sup>, and NO<sub>x</sub><sup>-</sup> fluxes from *X. muta* and *A. conifera* obtained by both methods using a 2-tailed *t*-test assuming unequal variance. The same statistical test was also used to compare mean species-specific fluxes from sponges with and without photosynthetic microbial communities (based on Chl *a* measurements) and sponges with and without nitrifying communities (based on nitrate release). Effects of photosynthetic communities on DIN flux was also analyzed using linear regression of species mean volume- and organic C-normalized DIN fluxes versus species mean Chl *a* concentrations.

## Results

**Biomass survey**—Within the 600-m<sup>2</sup> grid on Conch Reef, we measured 3,971 sponges (3,558 nonencrusting and 413 encrusting) composed of 22 different species and found an average of 6.5 sponge individuals m<sup>-2</sup> (Table 1). The volume of nonencrusting sponges averaged 3.60 L m<sup>-2</sup> and coverage of encrusting sponges averaged 80 cm<sup>2</sup> m<sup>-2</sup> of reef substrate. *Amphimedon compressa* had the highest No. of individuals (1.01 m<sup>-2</sup>). *X. muta*, a massive barrel sponge, dominated in terms of biomass, representing 65% of total sponge biomass (Table 1).

**Chl *a* concentrations**—Chl *a* in the outer 2 mm of sponge tissue ranged from 0 μg g<sup>-1</sup> to 600 μg g<sup>-1</sup> (dry weight; Fig. 1). Chl *a* concentrations >100 μg g<sup>-1</sup> were considered to be elevated, indicative of a significant community of

internal photosynthetic cells. This threshold was chosen based on the distribution of Chl *a* concentrations in this study and in previous work (e.g., Wilkinson 1983). There was an order of magnitude difference between the Chl *a* concentrations of the lowest “elevated” species (*A. lacunosa*) and the highest “nonelevated” species (*N. digitalis*). Half of the 14 species studied had elevated Chl *a*, ranging from 177 μg g<sup>-1</sup> to 604 μg g<sup>-1</sup>. Other species contained Chl *a*, but at much lower levels, ranging from 3 μg g<sup>-1</sup> to 19 μg g<sup>-1</sup>. Low levels of Chl *a* may have been derived from particulate matter in the ambient water that was previously filtered by the sponge.

**DIN flux**—In situ flow rates, sponge volume, and ΔDIN for individual sponges appear in Table 2. DIN accumulation in the incubation treatments appeared to be linear, with no signs of leveling off over time (Fig. 2). Species means of DIN release rates among the 14 species ranged from 93 ± 57 to 311 ± 65 μmol h<sup>-1</sup> L<sup>-1</sup> (Fig. 3A) and averaged 200 ± 77 μmol h<sup>-1</sup> L<sup>-1</sup>. Multiplying these rates by the measured abundance of each species in the 600-m<sup>2</sup> study area produces species-specific benthic fluxes ranging from 0 μmol m<sup>-2</sup> h<sup>-1</sup> to 391 μmol m<sup>-2</sup> h<sup>-1</sup> (Fig. 3B). The sum of the benthic fluxes for the 14 species was found to be 530 ± 120 μmol m<sup>-2</sup> h<sup>-1</sup>, of which 57 ± 73 μmol m<sup>-2</sup> h<sup>-1</sup> was ammonium and 480 ± 93 μmol m<sup>-2</sup> h<sup>-1</sup> was nitrate plus nitrite.

**Method comparison**—For *X. muta*, the incubation method produced DIN fluxes that were lower than the in situ method (Fig. 4), although these differences were not statistically significant at a 95% level for NO<sub>x</sub><sup>-</sup> (*p* = 0.09, *t* = 2.068, *df* = 5), NH<sub>4</sub><sup>+</sup> (*p* = 0.23, *t* = 1.405, *df* = 4), or total DIN (*p* = 0.07, *t* = 2.376, *df* = 4). For *A. conifera*, the two methods produced similar fluxes for total DIN (*p* = 0.42, *t* = 0.929, *df* = 3), but more NH<sub>4</sub><sup>+</sup> (*p* = 0.01, *t* = 4.032, *df* = 5) and less NO<sub>x</sub><sup>-</sup> (*p* = 0.03, *t* = 4.230, *df* = 3) was released with the incubation method compared to the in situ method (Fig. 4).

**Effects from nitrifying and photosynthetic microbial associates**—Linear regression of species-mean Chl *a* concentrations versus species-mean DIN fluxes indicated no apparent correlation between these parameters. This was true for both volume-normalized (*r*<sup>2</sup> = 0.01, *p* = 0.76) and organic C-normalized (*r*<sup>2</sup> = 0.04, *p* = 0.54) fluxes (regression data not shown). Furthermore, mean DIN fluxes (organic C-normalized; Fig. 5) from sponges with photosynthetic microbial communities were not significantly different from sponges that lack them (*p* = 0.35, *t* = 0.980, *df* = 5). Mean DIN fluxes from species hosting nitrification were not significantly different from those that did not host nitrification (*p* = 0.51, *t* = 0.682, *df* = 4).

## Discussion

**Nonencrusting community DIN flux**—Because of the large No. of species present, it was not possible to measure DIN flux from every species observed in the biomass survey. However, by targeting the most abundant sponges,

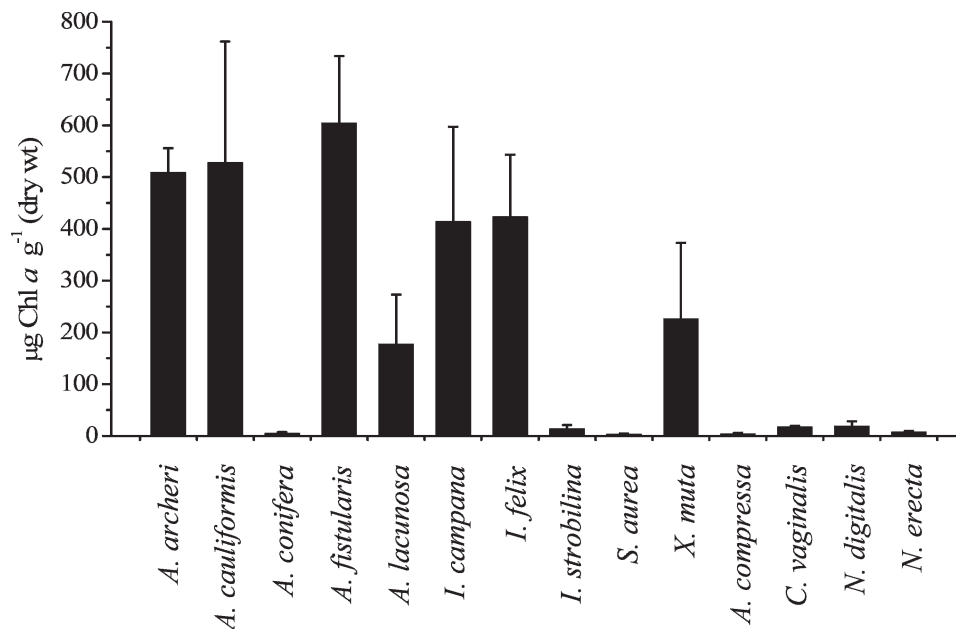


Fig. 1. Chl *a* concentrations in the outer 2 mm of sponge tissue.

the 14 species measured comprised 85% of the non-encrusting sponge biomass in the survey area. Although species composition was different from another study of Caribbean reefs, the total volume of sponge biomass was the same order of magnitude (1.2–2.1 L m<sup>-2</sup>; Wulff 2006). The 12 species for which DIN flux was not measured represented 15% of sponge biomass in the grid. If we

assume that these remaining sponges have DIN fluxes similar to the volume-normalized species average for the other 14 species (200 ± 77 µmol L<sup>-1</sup> h<sup>-1</sup>), we can estimate that these sponges are releasing an additional 110 ± 42 µmol m<sup>-2</sup> h<sup>-1</sup>. The sum of the measured and estimated fluxes, therefore, provides an estimate of the total DIN flux from the entire nonencrusting sponge community in this

Table 2. ΔDIN, flow rate, volume, and volume-normalized flux for individuals measured using the in situ method. SD = standard deviation; ND = not determined.

Species	ΔDIN (µmol L <sup>-1</sup> )				Flow rate (L h <sup>-1</sup> )		Sponge vol. (L)	Flux (µmol h <sup>-1</sup> L <sup>-1</sup> )			
	NH <sub>4</sub> <sup>+</sup>		NO <sub>x</sub> <sup>-</sup>		Mean	SD		Mean	SD	Mean	SD
<i>A. conifera</i>	-0.10	0.03	0.78	0.08	430	370	1.6	-25	-30	210	190
<i>A. conifera</i>	-0.11	0.13	0.51	0.13	280	240	1.1	-29	-39	130	130
<i>A. conifera</i>	-0.23	0.25	0.42	0.07	460	390	1.7	-94	-90	110	100
<i>A. archeri</i>	0.67	0.56	0.98	0.12	70	12	0.6	85	75	120	35
<i>A. archeri</i>	0.13	0.48	0.61	0.16	50	17	0.4	16	57	74	34
<i>A. archeri</i>	0.10	0.22	0.58	0.15	90	39	0.5	16	37	98	54
<i>A. lacunosa</i>	-0.29	0.73	1.78	0.23	20	7	0.4	-16	-27	97	40
<i>A. lacunosa</i>	0.11	0.49	0.54	0.25	90	6	0.40	24	110	120	60
<i>A. lacunosa</i>	0.45	0.56	1.09	0.42	100	26	0.9	52	67	130	65
<i>I. strobilina</i>	0.06	0.15	0.54	0.14	510	290	1.3	21	59	200	140
<i>I. strobilina</i>	0.01	0.12	0.53	0.24	1500	820	3.7	-7.5	-48	210	160
<i>I. strobilina</i>	-0.05	0.05	0.69	0.14	480	270	1.2	-28	-25	270	170
<i>I. strobilina</i>	0.18	0.13	1.26	0.14	770	440	2.0	71	67	490	300
<i>I. strobilina</i>	0.13	0.06	0.33	0.24	740	420	1.9	51	38	130	120
<i>N. digitalis</i>	0.27	0.07	ND	ND	670	450	0.6	320	240	ND	ND
<i>N. digitalis</i>	0.17	0.08	ND	ND	560	380	0.5	200	170	ND	ND
<i>N. digitalis</i>	0.20	0.03	ND	ND	370	250	0.3	240	180	ND	ND
<i>X. muta</i>	-0.06	0.27	0.41	0.36	4000	150	16	-14	-63	100	96
<i>X. muta</i>	0.03	0.31	0.81	0.20	13000	71	45	10	91	240	76
<i>X. muta</i>	-0.11	0.19	0.74	0.14	24000	120	109	-24	-46	170	45
<i>X. muta</i>	0.09	0.26	0.62	0.36	18000	130	49	33	96	220	140
<i>X. muta</i>	-0.19	0.26	1.40	0.17	1900	280	24	-15	-21	110	31

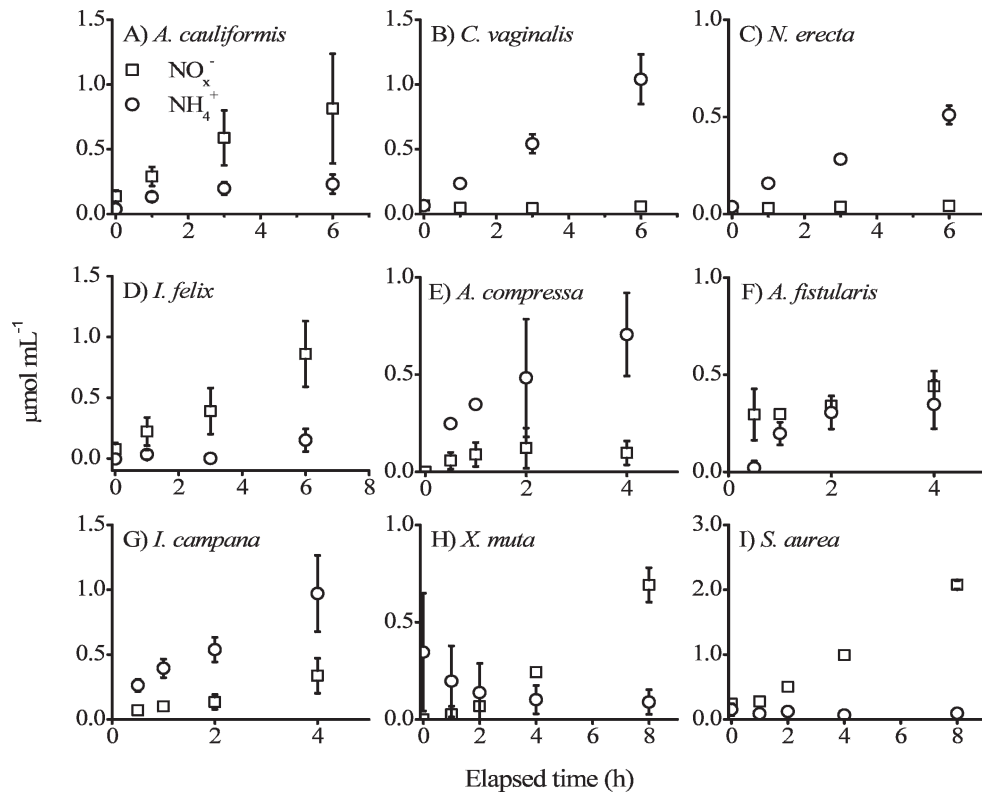


Fig. 2.  $\text{NO}_x^-$  and  $\text{NH}_4^+$  concentrations during incubation experiments. Values are normalized for sponge volume ( $\mu\text{mol mL}^{-1}$ ). (A) *A. cauliformis*, (B) *C. vaginalis*, (C) *N. erecta*, (D) *I. felix*, (E) *A. compressa*, (F) *A. fistularis*, (G) *I. campana*, (H) *X. muta*, and (I) *S. aurea*.

600- $\text{m}^2$  patch of Conch Reef of  $640 \pm 130 \mu\text{mol m}^{-2} \text{h}^{-1}$ . As previously stated, we did not include flux measurements from encrusting sponges in our study. Previous work has shown that encrusting sponges can also be a large source of DIN to the water column when their biomass is high (Corredor et al. 1988; Diaz and Ward 1997). In our biomass survey, encrusting sponges covered only 0.8% of the substrate (data not shown), but this could be an underestimate because encrusting sponges are abundant in cryptic habitats such as crevices (Richter et al. 2001; Scheffers et al. 2004), which were not included in the survey. Therefore, the estimated community total flux of  $640 \pm 130 \mu\text{mol m}^{-2} \text{h}^{-1}$  should be considered a minimum sponge community DIN flux.

Approximately 90% of the measured DIN flux was in the form of  $\text{NO}_x^-$ , a phenomenon that has been previously documented (Corredor et al. 1988; Diaz and Ward 1997; Jimenez and Ribes 2007). Nitrification has been shown to be widespread among sponges on Conch Reef, and appears to be consistent within a given species (Southwell et al. 2008). Ten species in our study released significant amounts of  $\text{NO}_x^-$ , and 8 of these released a majority of their DIN as  $\text{NO}_x^-$  (Fig. 3A). Although these eight species constituted only 33% of the 3,558 nonencrusting individuals, they represented 77% of the total nonencrusting sponge community biomass (Table 1) due to the large size of several nitrifying species. Of the species for which DIN flux was not measured, *P. crassa* had the highest biomass in the survey area (Table 1), and this sponge has also been shown

to release significant amounts of  $\text{NO}_x^-$  (Southwell et al. 2008). Therefore, given the magnitude of the DIN flux, sponge community composition likely has a profound effect on DIN speciation in the overlying water column.

**Method comparison**—We were able to employ the in situ method for 6 of the 14 species measured. This translates into 76% of sponge biomass in the survey grid, and approximately 90% of biomass for which we have flux data. Among the 12 species for which only one method was used, there was no significant difference between the species-specific fluxes obtained by the incubation method and those obtained by the in situ method. However, the direct comparison of methods from *X. muta* and *A. conifera* revealed some trends that could have important consequences for calculating community DIN flux. For *X. muta*, the flux determined from in situ measurements was twice the flux based on incubations. For *A. conifera*, the total amount of DIN released was similar, but more DIN was released as ammonium using the incubation method. This suggests that the experimental manipulation may have negatively affected the nitrifying community hosted by this sponge. Unlike *X. muta*, it was not possible to find whole individuals of *A. conifera* small enough for incubation, and cutting individuals may have isolated parts of the sponge with fewer nitrifiers, or disrupted the nitrifiers that were present, despite the time allowed for recovery.

The differences in the flux results obtained using the two methods are likely related to the effects of our physical

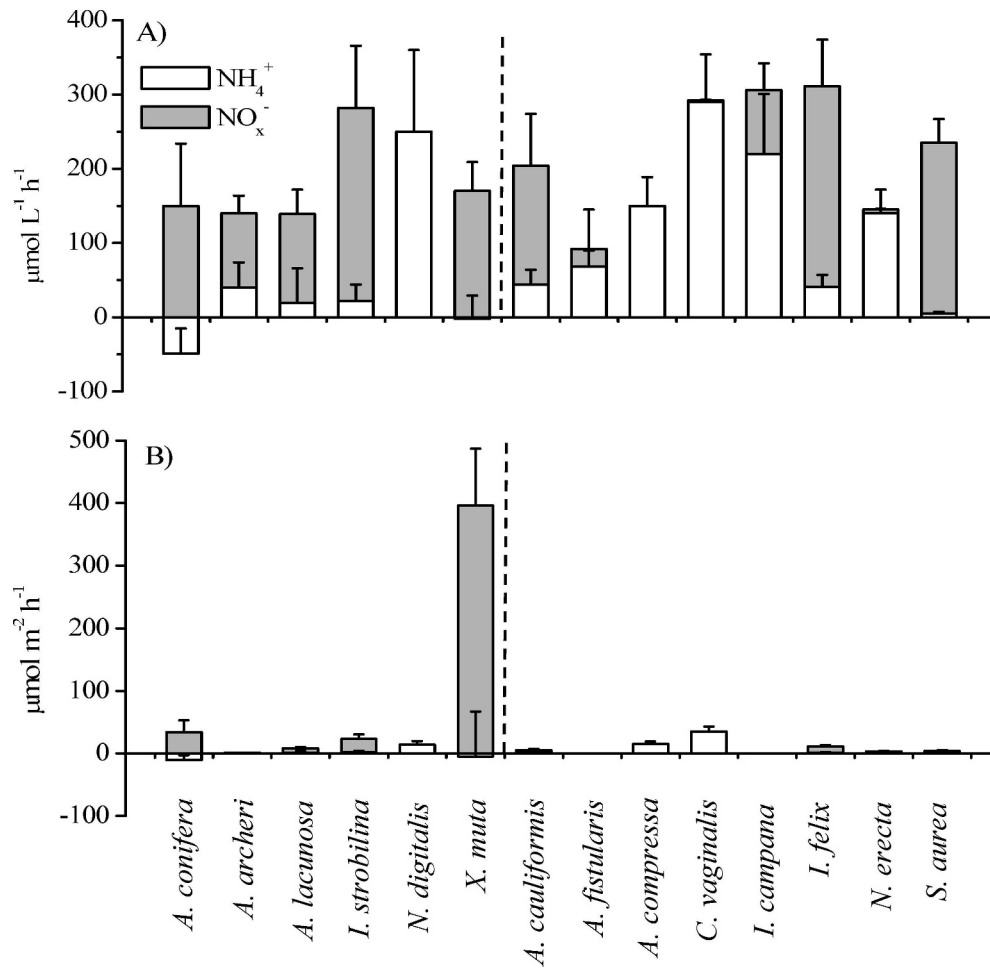


Fig. 3. (A) Volume-normalized DIN fluxes for 14 measured species on Conch Reef. (B) Benthic DIN fluxes for 14 measured species on Conch Reef. Species to the left of the dashed line were measured using the in situ method, and species to the right of the line were measured using the incubation method.

manipulations of the sponges, plus their containment in small volumes of seawater, on the metabolic rates of the sponges and their associated microbial communities. Yahel et al. (2007) compared feeding and excretion efficiencies from deep-water glass sponges using both in situ and laboratory based experiments, and found no significant difference in the results from the two methods. The difference in our results compared to Yahel et al. (2007) may be a function of the sponge types, microbial communities, and/or their sensitivity to environmental changes. Alternatively, it may be a function of the incubation methodology employed (static chambers vs. flow-through). Effects from experimental manipulation may be lessened by using larger incubation vessels (Diaz and Ward 1997), and so flow-through systems would likely further reduce these effects. However, sponges have been shown to have reduced pumping rates under laboratory conditions, even in running seawater systems (Reiswig 1974). Furthermore, environmental conditions that may affect sponge behavior and biogeochemical processes (e.g., light, temperature, nutrient concentrations) are difficult to replicate in the laboratory. Large sponges such as *X. muta*

and *A. conifera*, which can strongly influence the total DIN flux, are particularly ill-suited for incubations. *X. muta* and *A. conifera* were abundant in our study area, and so substituting the fluxes from the incubation method for these two species alone would reduce the estimated community DIN flux by 32%. For these reasons, we used results from the in situ rather than the incubation method for *X. muta* and *A. conifera* in calculating total community benthic flux. In situ sampling was not possible for all sponge types given the present technology, and so incubations were still necessary for species with small and/or numerous oscula.

*Effects from nitrifying and photosynthetic microbial associates*—The organic C-normalized fluxes revealed more interspecific variability than was apparent from the volume-normalized fluxes, especially among species that exhibit evidence of nitrifying and photosynthetic microbial communities (Fig. 5). Normalization for organic C excludes inert components such as spicules and sand and is, therefore, a more appropriate comparison of metabolic properties. Some of the interspecific variability in DIN flux

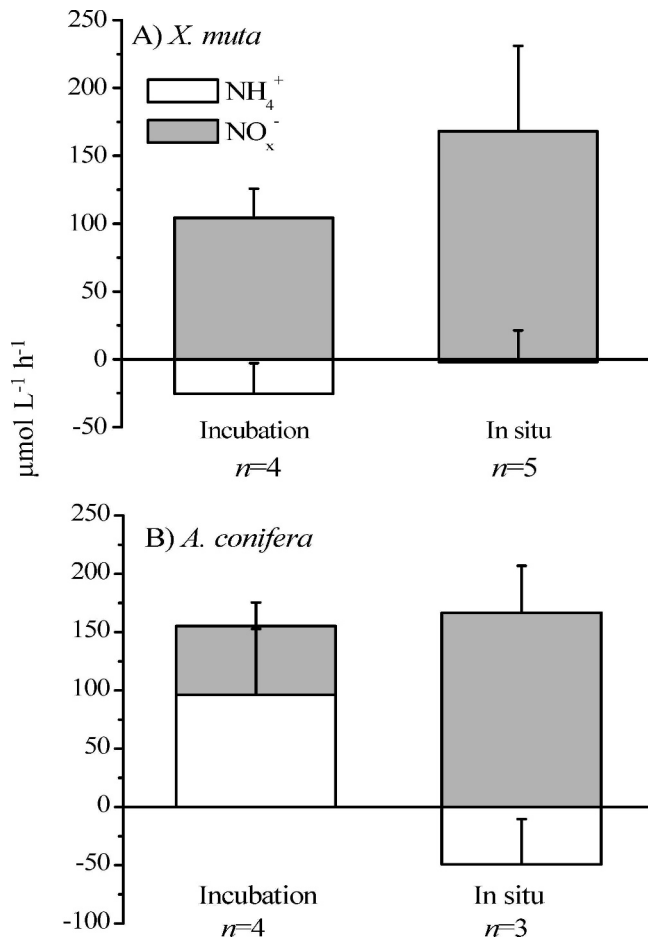


Fig. 4. Comparison of fluxes for (A) *X. muta* and (B) *A. conifera* obtained from in situ and incubation methods.

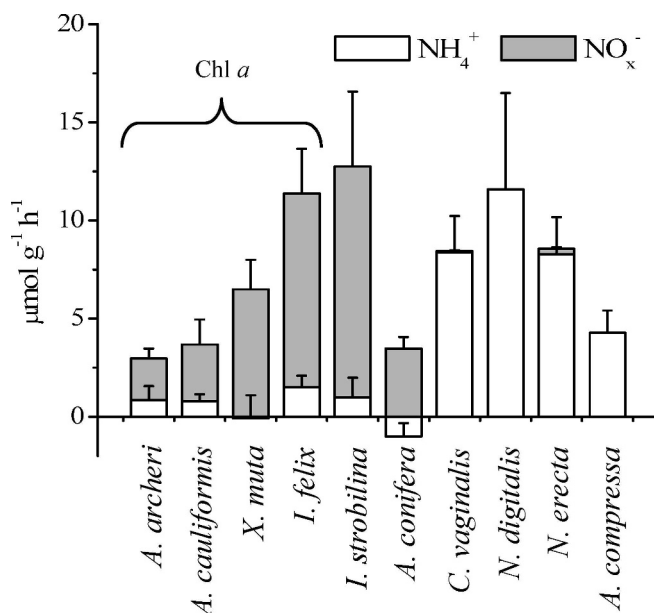


Fig. 5. DIN fluxes for 14 species on Conch Reef, normalized for organic C content. Chl *a* = species with elevated Chl *a* concentrations in the outer layer of sponge tissue (see Fig. 1).

may be due to differences in pumping (Weisz et al. in press) or metabolic rates (Reiswig 1974) among species. However, some of the interspecific variability may be caused by the sponges' microbial communities, especially if the microbial associates assimilate a portion of the waste DIN. Although both heterotrophic and autotrophic microbes are capable of assimilating DIN, the nutrient demand from autotrophic constituents such as nitrifiers and photosynthesizers should be especially important. Assimilation of waste ammonium by a sponge-associated macroalga has been demonstrated (Davy et al. 2002); if a similar relationship exists between sponges and microbial autotrophs, then sponges hosting these communities might be expected to have lower DIN fluxes compared to species that do not. However, our results indicate that DIN fluxes from sponges with nitrifying and/or photosynthetic associates were not significantly different from those lacking these microbial associates (Fig. 5). This absence of evidence is surprising, given the expected nutrient demand from autotrophic cells. Nevertheless, little is known about the N sources of sponge-hosted microbial communities, their net effect on the net DIN flux of sponges, and how this interaction varies over time.

The effects of assimilation could also be masked by concurrent processes that produce DIN. For example, the microbial communities in some sponges have been shown to utilize DOM (Reiswig 1981; Yahel et al. 2003), and this additional N source could result in higher rates of DIN release. Furthermore, it has previously been suggested that  $N_2$  fixation by sponge microbial communities could contribute to the observed high fluxes of nitrate in some sponges (Corredor et al. 1988; Diaz and Ward 1997). The 3 *Ircinia* species in this study all have high fluxes of DIN, and their tissues have low  $\delta^{15}N$  values (species means range from 0.1‰ to 1.6‰, Southwell 2007), which could indicate incorporation of newly fixed N (Fogel and Cifuentes 1993). However, direct measurements of  $N_2$  fixation rates in two species (*Aplysina cauliformis* and *Ircinia campana*) did not support this hypothesis (Southwell 2007). There may be important differences in the microbial community compositions and metabolic rates of sponge species that confound our ability to generalize about the effect of autotrophic microbial associates on DIN fluxes from sponges; nevertheless, during this study we saw no evidence for these communities acting as a sink (or source) of DIN.

*Temporal variability*—Sponges are known to slow or even stop their pumping rates periodically (Reiswig 1971; Weisz et al. in press), although all the sponges we encountered in this study were found to be pumping. It is presently unknown how reduced pumping rates might affect the DIN flux because the DIN may become more concentrated as the flow subsides. Conversely, the metabolic rate of the sponge (and possibly the associated microbes) may also decrease during this time of decreased pumping activity, reducing the production of metabolic waste. Pumping rate measurements and sample collections in this study were performed within a 30-min interval and so represent a short-term measurement of individual sponge DIN fluxes. Incubation experiments provided



Table 3. Reported values for benthic fluxes of DIN on coral reefs (per m<sup>2</sup> of projected reef area). Sponge fluxes are based on measured abundance, other substrates assume 100% coverage.

Substrate	Flux (mmol m <sup>-2</sup> d <sup>-1</sup> )	Location	Reference
Coral sediment	0.018–0.033		Corredor and Morell 1985
Coral sediment	0.01–0.1*	Great Barrier Reef	Capone et al. 1992
Coral cavities	22.3	Red Sea	Richter et al. 2001
Coral cavities	5.4±9.8	Caribbean	Scheffers et al. 2004
Sediment	0.613	Hawaii	Stimson and Larned 2000
Sponges ( <i>C. nucula</i> , <i>A. varians</i> )	12†	Caribbean	Corredor et al. 1988
Sponge ( <i>P. zeai</i> )	5.8–10.9†	Caribbean	Diaz and Ward 1997
Sponges (14 sp.)	13±2.8	Caribbean	This study
Nonencrusting sponge community (>23 sp.)	15±3.0	Caribbean	This study

\* Values are for ammonium only.

† Values are for nitrate plus nitrite only.

information over a longer time frame, yet the experiments still lasted 8 h or less. Therefore, temporal variability in DIN flux is possible. Repeated measurement of  $\Delta$ DIN from 4 *X. muta* individuals shows that this parameter varied by as much as 72% within 48 h (data not shown). However, because we do not have continuous flow data for these sponges, it is unknown whether this results in a proportional variation in DIN flux. Until appropriate studies are done to evaluate temporal variability in DIN flux, this caveat should be considered when extrapolating the DIN flux to daily (or longer) time scales.

**Implications for sponge DIN flux**—The sponge community DIN flux measured on Conch Reef is higher than rates reported in the literature for other benthic sources on coral reefs such as sediments (Table 3). Furthermore, encrusting sponges inhabiting cryptic environments appear to be important contributors to the high DIN fluxes from coral cavities (which were not part of this study; Table 3). Also, the fluxes calculated for sediments assumed 100% coverage and are, therefore, likely to be overestimates of actual benthic flux. In contrast, the fluxes from sponges were calculated based on measured local abundance. The growing body of research on N processing by sponges shows that the regeneration of nutrients by filter feeders constitutes an important component in the reef N budget.

DIN from sponges may be especially important for benthic biota, which are in close proximity to sponges. Reported rates of net benthic primary production on various coral reef substrates range from 8 mmol C m<sup>-2</sup> d<sup>-1</sup> to 39 mmol C m<sup>-2</sup> d<sup>-1</sup> (Gattuso et al. 1997; Uthicke and Klumpp 1998), and can be as high as 417 mmol C m<sup>-2</sup> d<sup>-1</sup> in coralline algae (Chisholm 2003). This implies a nitrogen requirement of 1 to 63 mmol N m<sup>-2</sup> d<sup>-1</sup>, assuming a C:N ratio of 6.6 (Redfield 1958). The nonencrusting sponge community on Conch Reef releases approximately 15 mmol m<sup>-2</sup> d<sup>-1</sup> and is, therefore, likely capable of supporting a significant amount of the benthic primary productivity. This discharge of nutrients near the benthos could have ecological consequences, as high nutrient availability may facilitate the growth of fleshy macroalgae (Littler and Littler 1984; Larned 1998), which have increased in abundance on Caribbean reefs (Done

1992). Lapointe (1997) has argued that DIN concentrations of  $\geq 1 \mu\text{mol L}^{-1}$  are associated with rapid macroalgal overgrowth, and our results show that sponge excurrent water can have concentrations as high as 5  $\mu\text{mol L}^{-1}$  (data not shown). The level of DIN enrichment near the sea floor would likely depend not only on sponge abundance, but also on vertical mixing in the water column. Nevertheless, benthic primary producers near sponges could experience higher inorganic nutrient concentrations over the long term.

The effect of long-term exposure on various coral reef organisms to low-level nutrient enrichment is still poorly understood, and high nutrient availability may have complex interactions with pathogens and other microorganisms. The connection between inorganic nutrients and coral disease incidence is controversial (Szmant 2002; Kline et al. 2006), yet there is evidence that elevated inorganic nutrient levels can increase the virulence of coral pathogens (Bruno et al. 2003) and decrease coral fecundity (Koop et al. 2001). Further, the higher rates of primary productivity likely generated by inorganic nutrient fluxes could produce more dissolved organic matter, which as been shown to be a potential factor in coral disease (Kline et al. 2006).

It is evident that, where they are abundant, sponges can play an important role in organic matter remineralization and nitrification. Therefore, changes in sponge biomass and community composition could affect POM and DOM concentrations, nutrient availability and speciation, and benthic–pelagic coupling. Such changes are indeed possible, as the response of sponges to environmental factors such as elevated temperatures and nutrient concentrations is still largely unknown (but see Holmes 2000; Ward-Paige et al. 2005). On Conch Reef, the DIN flux was dominated by *X. muta*, a massive barrel sponge that appears to be partially vulnerable to bleaching events (Vicente 1990). A recent report also observed rapid mortality in several *X. muta* individuals, possibly caused by disease (Coward et al. 2006). A widespread decline of *X. muta* could dramatically alter rates of organic matter remineralization and DIN speciation in the water column.

Our in situ measurements of DIN fluxes from sponges on Conch Reef, near Key Largo, Florida, U.S.A., reveal that sponges are a large source of DIN and represent a significant pathway for recycling of organic matter on Caribbean reefs, and presumably other benthic habitats

where sponges are abundant. The overall results of DIN flux measurements made using the in situ method were not statistically different from those obtained using the incubation method. However, data from direct comparisons between the in situ method and the incubation method suggest that the incubation methodology likely affected the speciation of DIN released from one species, and produced a lower flux of total DIN from the other species (though the difference was marginally significant). Therefore, while this study broadly supports previous findings of large sponge DIN fluxes, it also supports the use of a viable alternative method for measuring DIN flux that is nondestructive and reflects real environmental conditions.

On Conch Reef, species exhibiting evidence of nitrification represented a minority of the population in terms of the No. of individuals, but represented a majority of the population in terms of biomass. This result highlights the need for biomass and community composition measurements, in addition to abundance and areal coverage, for determining the overall biogeochemical importance of various species and for predicting the effect of the community as a whole. The few existing studies of Caribbean sponge communities over time indicate that local populations can be temporally dynamic (Wulff 2006). Yet, compared to corals, there are relatively few monitoring programs that provide information on sponge abundance, diversity, and health. Given the demonstrated role of these animals in nitrogen cycling, this lack of information could weaken our understanding of reef ecology and limit our ability to successfully maintain healthy coral reefs.

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