Vol. 156: 97–107, 1997 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published September 25

Sponge-mediated nitrification in tropical benthic communities

M. C. Diaz[1,*], B. B. Ward[2]

[1]Biology and [2]Ocean Sciences Department, A316 EMS, University of California Santa Cruz, California 95064, USA

ABSTRACT: We examined changes in the levels of dissolved inorganic nitrogen (DIN) during incubation experiments with 4 conspicuous sponge species from Caribbean coral reefs, mangroves, or seagrass beds (*Chondrilla nucula*, ?*Pseudaxinella zeai*, *Oligoceras violacea*, *Plakortis halichondroides*). DIN accumulation in the incubation water was detected for all the species, but no significant DIN concentration changes were detected in the control experiments (seawater alone). NO_2^- accumulated during all the experiments with *O. violacea* (170 to 580 nmol g^{-1} h^{-1}), while NO_3^- accumulated during most experiments with ?*P. zeai* (0 to 1033 nmol g^{-1} h^{-1}), *C. nucula* (360 to 2650 nmol g^{-1} h^{-1}), and *P. halichondroides* (0 to 320 nmol g^{-1} h^{-1}). These are the highest reported weight-specific production rates of oxidized nitrogen from benthic communities. The highest values are associated with the 3 species that possess cyanobacterial endosymbionts. Potential NO_3^- efflux rates by 2 of the species, assuming 100% areal coverage, yielded values (211 to 396 mmol m^{-2} d^{-1} for ?*P. zeai* and 242 to 413 mmol m^{-2} d^{-1} for *C. nucula*) 2 to 4 orders of magnitude higher than the most active benthic nitrification rates yet reported from the tropics. Extrapolating from incubation data (550 to 1030 nmol g^{-1} h^{-1} and biomass estimates (440 g m^{-2}), the environmental NO_3^- efflux rate of ?*P. zeai* on the Fore Reef at the Barrier Reef off Carrie Bow Cay, Belize (5.8 to 10.9 mmol m^{-2} d^{-1}) surpasses considerably the highest benthic nitrification rates reported previously (unconsolidated reef sediments: 1.68 mmol m^{-2} d^{-1}). These results strongly suggest that sponge-mediated nitrification is not uncommon in tropical marine benthic communities, and might constitute a large input of oxidized nitrogen into those habitats in which sponges abound. Our results reinforce the notion that sponges harbor and nourish microbial organisms with metabolisms that are important to the productivity and nutrient cycling in shallow benthic tropical communities.

KEY WORDS: Nitrification · Sponge associations · DIN fluxes · Benthic processes

INTRODUCTION

The level of dissolved inorganic nitrogen (DIN; ammonia, nitrite, and nitrate) in marine environments depends largely on biological processes that constitute sinks and sources for these nutrients, among which microbial transformations play a central role (Valiela 1984). Nitrification, a chemoautotrophic pathway in which reduced inorganic nitrogen (NH_4^+, NO_2) is oxidized to nitrate, is an important component of the marine nitrogen cycle (Ward 1986). This biological process is accomplished by 2 metabolic groups of bacteria, ammonia and nitrite oxidizers, and its rate is dependent on substrate concentration, light intensity, and oxygen levels (Ward 1986, Vanzella et al. 1990).

Nitrifiers require reduced forms of inorganic nitrogen (ammonium, nitrite, or hydroxyl amine), low light levels, and oxygen. As a consequence, in the ocean these bacteria are most active at the bottom of the photic zone (50 to 100 m) and in a narrow zone of surface sediment where O_2 is present (Henriksen et al. 1981, Ward 1986, Rasmussen & Jørgensen 1992). In general, ammonia and nitrite are present at submicromolar concentrations throughout the ocean. Nitrate, the end product of nitrification, accumulates in the deep ocean at concentrations of approximately 40 µM. Coastal sediments in productive waters (salt marshes, mangroves, upwelling regions) usually exhibit high rates of regeneration of DIN which result in net fluxes of DIN from the sediments to the overlying waters (Fisher et al. 1982, Capone et al. 1992, Morell & Corredor 1993).

*E-mail: diaz@cats.ucsc.edu

Our knowledge of the distribution and production rates of DIN in coastal tropical macrobenthic communities (coral reefs, mangroves, seagrass beds, etc.) is limited. It consists mainly of studies of nitrogen fixation and ammonium uptake by algae/bacterial-invertebrate associations (Webb et al. 1975, Capone 1977, Wilkinson et al. 1984), microbial transformations within coral reef sediments (Hatcher & Hatcher 1982, Seitzinger & D'Elia 1983, Corredor & Capone 1985, Capone et al. 1992) or mangrove sediments (Morell & Corredor 1993), nitrification in algal flats and tide pools (Webb & Wiebe 1975), and nitrification associated with marine invertebrates (Corredor et al. 1988, Wafar et al. 1990, Pile 1996). The association of high nitrification rates with marine invertebrates was first described by Corredor et al. (1988), who examined *in situ* fluxes of inorganic nitrogenous nutrients in 2 coral reef sponges.

Chondrilla nucula, a common Caribbean sponge, was found to release large amounts of nitrate (400 nmol N cm^{-2} h^{-1}), approximately 200 times the amount released by another sponge species, *Anthosigmella varians* (2 nmol N cm^{-2} h^{-1}). *C. nucula* harbors large populations of symbiotic cyanobacteria (Wilkinson & Vacelet 1979), while *A. varians* harbors symbiotic zooxanthellae. Corredor et al. (1988) hypothesized that sponge cells and bacteria metabolize the nitrogen contained in the organic matter filtered by the sponge to make amino nitrogen, which is released as ammonium. This would be oxidized to nitrate by symbiotic nitrifying bacteria, whose presence in the sponge, however, remained unproved. Recently, Pile (1996) reported high rates of DIN release from the sponges *Ircinia felix* and *Ircinia strobilina* from a reef in the Florida Keys (USA). The rates detected, although lower than the ones from *C. nucula* from Puerto Rico, represented rates an order of magnitude larger than those reported for coral reef sediments (Pile 1996). High release rates of ammonium and nitrate have been detected in reef corals (Wafar et al. 1990). However, the rates of nitrate release were an order of magnitude lower (5.5 to 17.3 nmol N cm^{-2} h^{-1}) than the rates detected by Corredor et al. (1988) for the sponge *C. nucula*.

Sponges show significant areal coverage and biomass in tropical marine benthic communities such as coral reefs and mangroves (Suchanek et al. 1983, Wilkinson 1983, 1987, Rützler & Feller 1996, Diaz et al. 1997). Their high water-filtration rates (Reiswig 1971) make them the most efficient filter-feeders in the sea. If nitrification were common to many marine sponges or to the most conspicuous species, they might constitute important sites of nitrite and nitrate regeneration in many habitats.

To investigate the occurrence of this phenomena among sponges from a wide range of benthic tropical communities, as well as to distinguish the regeneration of the various forms of DIN (ammonium, nitrite, and nitrate), we conducted nitrogen flux experiments with several common sponge species from Caribbean coral reefs, mangroves, and seagrass habitats. Since the high release of nitrate was first found associated with a sponge possessing cyanobacterial endosymbionts, our first goal was to determine the occurrence of this phenomenon among tropical sponges possessing this symbiont type (*Chondrilla nucula*, *?Pseudaxinella zeai*, and *Oligoceras violacea*), as compared to one lacking it (*Plakortis halichondroides*). A second goal was to differentiate the accumulation of ammonia, nitrite and nitrate. A third goal was to determine the effect of light and incubation conditions on the magnitude and direction of the DIN fluxes.

METHODS

Species selection. Three sponge species that possess symbiotic cyanobacteria (*?Pseudaxinella zeai*, *Chondrilla nucula*, and *Oligoceras violacea*) (Rützler 1990) and one lacking them (*Plakortis halichondroides*) were selected for this study.

?Pseudaxinella zeai is abundant in the Caribbean coral reefs studied here, usually at 20 to 40 m depth (Diaz et al. in press), while *Chondrilla nucula* (Chondrosiidae: Hadromerida) and *Oligoceras violacea* (Spongiidae: Dictyoceratida) predominate in relatively shallow (1 to 3 m) mangrove roots or lagoons. The species *?P. zeai* has also been referred to as *Calyx podatypa* (Oceanapidae: Haplosclerida) (Humann 1992), but recently has been tentatively assigned as an Axinellidae sponge probably of the genus *Pseudaxinella* by its skeletal composition (Alvarez et al. 1996). *Plakortis halichondroides* (Plakinidae: Homosclerophorida), a common sponge in various Caribbean coral reefs, usually has a patchy distribution with a wide depth range (3 to 50 m).

Collection and maintenance. Two sets of experiments, identically performed, were conducted in separate instances. In the first set, conducted between August 26 and September 11, 1993, sponges were collected at various localities in the eastern Bahamas (22° to 24° N, 74° to 75° W) and put in tanks of running seawater at ambient temperatures (29 to 30°C) aboard the RV 'Columbus Iselin'. In the second set, conducted between June 25 and July 7, 1995, sponges were collected on the Fore Reef off Carrie Bow Cay, Belize (16° 48' N, 88° 05' W), and maintained in tanks of running seawater within 1°C of the ambient temperatures (29 to 30°C).

Sponge specimens of *Chondrilla nucula* (3 to 26 g dry wt), *?Pseudaxinella zeai* (4 to 167 g dry wt), *Oligo-*

ceras violacea (2 to 20 g dry wt) and *Plakortis halichondroides* (5 to 9 g dry wt) were collected and put on a table of running, unfiltered seawater for a period of acclimation. Whole specimens were collected by carefully detaching them by hand from coral rubble, sand, or hard substrates. Most specimens were collected from the sand or small coral rubble, to minimize tissue damage during transplantation. A few specimens of *?P. zeai* were fragments of larger specimens, also taken by hand. Healthy-looking specimens (open oscules, active water filtration, regeneration of bruised tissues, normal coloration, and overall healthy appearance) were used for experimentation after 1 to 2 d of acclimation. Experimental specimens were sun dried in the field, and once in the laboratory they were dried in a oven (42°C) overnight and weighed.

DIN flux measurements. To investigate the flux of DIN species associated with sponges, single specimens were incubated for 6 to 12 h in acid-washed vessels containing non-filtered seawater (continuously recirculated at the rate of 0.2 l min^{-1}). The water temperature in the vessels was kept within 1°C of ambient water temperature (29 to 30°C). Marine sponges filter a great deal of water (Reiswig 1971); a 4 to 6 cm^3 sponge might filter up to 4 to 5 l h^{-1}. Because incubation volume might influence sponge activity, 2 different incubation volumes were tested for each species: 3 l, similar to the experiments (2.25 l) of Corredor et al. (1988), and 20 l, the likely approximate volume of water a 4 to 10 cm^3 sponge would filter in 5 to 6 h. To assess the effect of light, some specimens were incubated in the dark and others in the light. Light was provided with plant-light lamps attached to the underside of the vessel's enclosure (80 to 100 µE m^{-2} s^{-1}). Each species was thus subjected to 4 types of experiments (3 l/dark, 3 l/light, 20 l/dark, 20 l/light). *Plakortis halichondroides* lacks photosynthetic endosymbionts and it was only subjected to 2 types of treatments (3 l/dark and 20 l/light). Control experiments, in which only seawater was incubated from 6 to 8 h, were run under 2 types of treatments (3 l/dark and 20 l/light). Each treatment was replicated 2 to 4 times per species, each time using a different specimen. Duplicate 100 ml water samples were taken from the incubations at 1 to 2 h intervals, filtered through pre-combusted GF/F filters, and immediately sealed and frozen (–20°C) in acid-washed, plastic bottles.

Nitrite, nitrate, and ammonium concentrations were measured in duplicate for each sample by standard colorimetric methods (Koroleff 1983, Jones 1984) with a Hitachi spectrophotometer. The changes detected in the DIN concentrations (µmol l^{-1}) from the initial conditions were normalized with respect to the incubation volume and the sponge dry weight to calculate the amount of these nutrients released by the sponge during the experiments (µmol g^{-1}).

Production rates of DIN. Experimental production rates of each DIN species (µmol g^{-1} h^{-1}) were computed from the slope values of the linear regressions calculated for the change in their concentration during each experiment. A mean production rate for each nutrient was then calculated per treatment and species by averaging the slope values of all replicates per treatment (see Table 2). Potential areal production rates for each sponge species (mmol m^{-2} d^{-1}) were calculated using the minimum and maximum mean release rates from the 20 l treatments in which significant DIN concentration changes were detected ($p \leq 0.1$). Experimental production rates were converted to areal rates using weight-to-volume ratios of 0.4 g cm^{-3} for *?Pseudaxinella zeai* and 0.65 g cm^{-3} for *Chondrilla nucula*, and an average species thickness of 4 cm and 1 cm respectively. Two types of potential production rates were calculated. The first one assumed a 100% coverage by the species, and it was used to compare the experimental production rates of the species studied with published areal production rates from reef or mangrove sediments. The second one used biomass estimates (g m^{-2}) of *?P. zeai* (Diaz et al. in press) on a Caribbean coral reef to extrapolate to areal production rates by the species in its natural environment.

RESULTS

Net changes in DIN concentration during the incubation experiments

Fig. 1 shows the net hourly variation in nutrient concentration for each experiment. Control experiments showed little or no variation in DIN values (Figs. 1 & 2). Ammonia initial concentrations were variable (0.1 to 2.7 µmol l^{-1}), and net changes during the experiments included slight accumulation or disappearance of this nutrient (Figs. 1 & 2). Nitrite and nitrate remained at submicromolar levels during the control experiments (0.01 to 0.04 and 0.2 to 0.7 µmol l^{-1}, respectively) (Figs. 1 & 2). The most conspicuous change of DIN was the net accumulation of nitrite or nitrate during the experiments with the 3 species associated with cyanobacteria (*Chondrilla nucula*, *?Pseudaxinella zeai*, and *Oligoceras violacea*) (Fig. 1). These changes were 1 to 3 orders of magnitude higher than those detected in the control or the experiments with *Plakortis halichondroides*. Nitrate exhibited the largest variations during incubations of *C. nucula* and *?P. zeai*, with net increases up to 5.81 and 4.51 µmol l^{-1} h^{-1}, respectively. During all the experiments with *O. violacea* a rapid accumulation of nitrite (up to 3.33 µmol l^{-1} h^{-1}) was

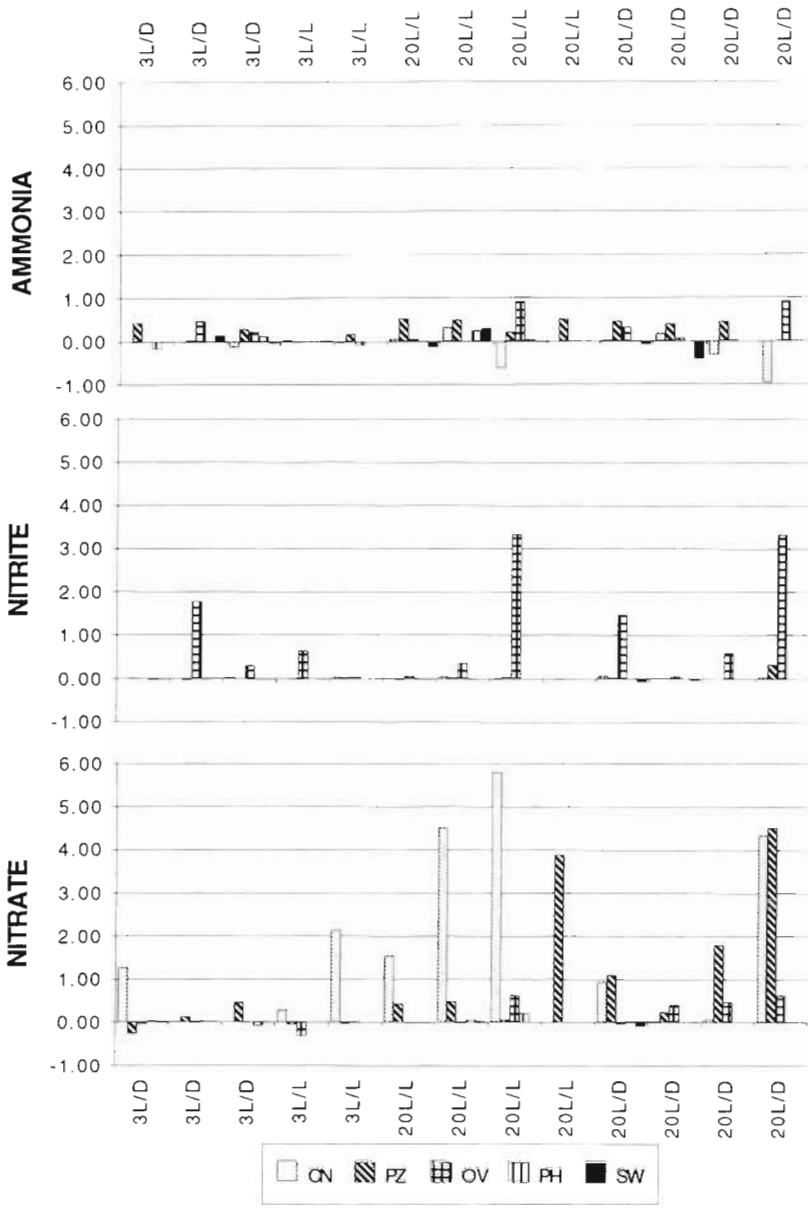

Fig. 1. Net hourly changes in ammonium, nitrite and nitrate concentrations (µmol l^{-1} h^{-1}) in time series incubations of seawater containing single sponge specimens, under 4 different treatments (3L/L: 3 l, light; 3L/D: 3 l, dark; 20L/L: 20 l, light; 20L/D: 20 l, dark). Each bar represents rates of change detected during a single experiment with *Chondrilla nucula* (CH), *?Pseudaxinella zeai* (PZ), *Oligoceras violacea* (OV), *Plakortis halichondroides* (PH), or unfiltered seawater (SW)

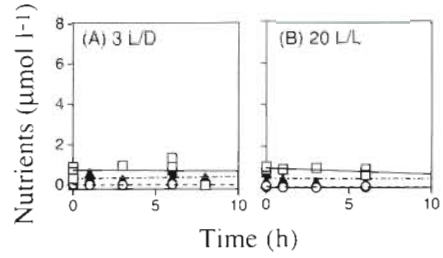

Fig. 2. NH_4^+ (□), NO_2^- (O), and NO_3^- (▲) changes during time series incubations containing unfiltered seawater only (µmol l^{-1} h^{-1}) in (A) 3 l/dark and (B) 20 l/light conditions

DIN release during time series experiments

The accumulation or disappearance of DIN during the experiments was analyzed by treatment, and shown separately for each species (Figs. 3 to 6). Each symbol in the figures corresponds to the net change in a nutrient concentration (relative to the initial conditions) per g of sponge dry weight. A regression is shown for each nutrient in each experiment. To determine the significance of these changes in nutrient concentration, the data from all experiments for a specific treatment (e.g. 3 l/ light) for each species were pooled and a correlation analysis was used to determine the significance of concentration changes with time. Table 1 shows the r^2 value for each treatment, its level of significance, and the number of experimental runs per treatment. The main trends in DIN concentration observed during these incubations are described for each species in the following sections.

Chondrilla nucula. *C. nucula* showed a consistent accumulation of NO_3^- (Fig. 3). Highly significant increases occurred during 3 l/ ($p < 0.01$) and 20 l/light ($p < 0.05$) treatments. The highest nitrate accumulations for this species were detected in the 20 l treatments. Nitrite accumulated slowly and steadily, but only significantly in the 3 l/dark treatments ($p < 0.05$). Ammonia variations were significant only in the 3 l/light experiment.

?Pseudaxinella zeai. *?P. zeai* accumulated NO_3^- significantly (Table 1) in most experiments (Fig 4). The

detected (Fig. 1), while nitrate either did not change or showed very slight variations (–0.02 to 0.63 µmol l^{-1} h^{-1}) (Fig. 1). Net changes in ammonia levels differed between runs among and within species, with a tendency to accumulate in *?P. zeai*, *O. violacea*, and *P. halichondroides* experiments, and with slight decrease in most experiments with *C. nucula* (Fig. 1).

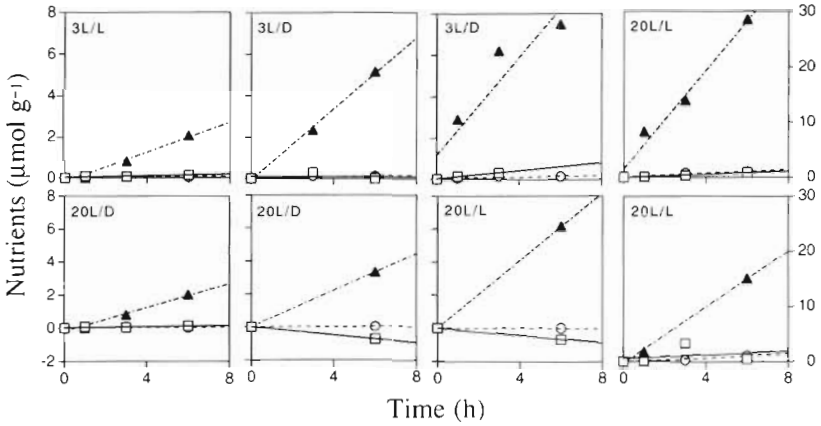

Fig. 3. DIN (dissolved inorganic nitrogen) release rates (µmol N g^{-1} dry weight) in time series incubations of unfiltered seawater containing single specimens of the sponge *Chondrilla nucula*. Symbols as in Fig. 2. Each panel represents a separate experiment with the treatment type indicated on the top left of the graph. The significance levels of the accumulation of each nutrient per treatment type are shown in Table 1

highest nitrate variations were detected during the large-volume incubations (20 l). As in *C. nucula*, the level of accumulation of this nutrient in this species varied greatly. Nitrite showed mostly submicromolar, non-significant changes, except in 1 treatment (20 l/dark). Ammonium accumulation was significant only in 1 treatment (20 l/dark, $p < 0.05$).

Oligoceras violacea. *O. violacea* accumulated NO_2^- consistently and significantly during all the experiments performed with this species (Fig. 5). This accumulation of NO_2^- was similar in magnitude to that of NO_3^- in the experiments with *Chondrilla nucula* and *?Pseudaxinella zeai*. NO_3^-, however, showed only a large and significant change in the 20 l/dark experiment with *O. violacea*. Ammonia accumulated significantly in all the treatments ($p < 0.05$ to 0.1), except in the 20 l/dark.

Plakortis halichondroides. Overall this species presented the smallest variations in DIN during the incubation experiments (Fig. 6). The 3 l experiments showed insignificant DIN variations, while the 20 l experiments showed small but significant ($p < 0.05$) accumulations of NH_4^+ and NO_3^- (Table 1).

Production rates of DIN

Table 2 presents the mean production rates (µmol g^{-1} h^{-1}) of DIN for each treatment type run on each species studied, and the mean rates of change of DIN concentration during the control experiments (µmol l^{-1} h^{-1}). NO_3^- production rates by *Chondrilla nucula* (1.02 to 2.65 µmol g^{-1} h^{-1}) and NO_2^- production rates by *Oligoceras violacea* (0.17 to 0.58 µmol g^{-1} h^{-1}) were the highest release rates detected. *?Pseudaxinella zeai* showed high nitrate production rates only in the 20 l incubations (0.55 to 1.03 µmol g^{-1} h^{-1}), while in the 3 l incubations the rates showed no significant variation. Potential areal production rates of NO_3^- (mmol m^{-2} d^{-1}), assuming 100% coverage by the species, were calculated for the most common species studied, *C. nucula* and *?P. zeai* (see 'Methods'). These potential rates were 2 to 4 orders of magnitude higher than the rates found in other benthic systems such as coastal sediments, reef and mangrove sediments (Table 3).

A more realistic production rate in nature can be derived from abundance estimations of these sponges in their habitats. Biomass estimates for *?Pseudaxinella zeai* in 7 reef zones on the Belizean Barrier Reef at Carrie Bow Cay (Diaz et al. 1997) indicate that nitrate release by this species might range from 0.12 to 10.9 mmol m^{-2} d^{-1} (Table 4). The highest release rates (5.8 to 10.9 mmol m^{-2} d^{-1}) are associated with the fore reef where the sponge had a biomass of 440 g m^{-2}

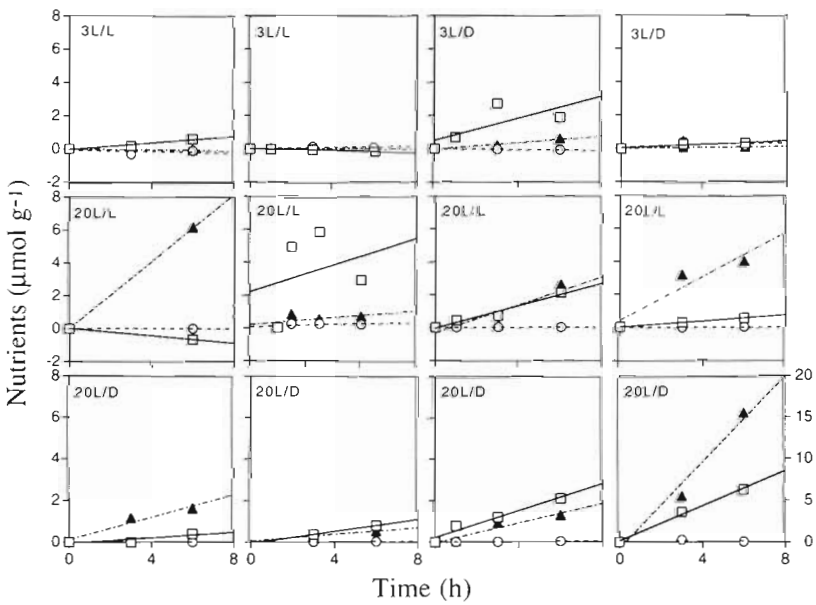

Fig. 4. DIN release rates. As Fig. 3, but for the sponge *?Pseudaxinella zeai*. Symbols as in Fig. 2

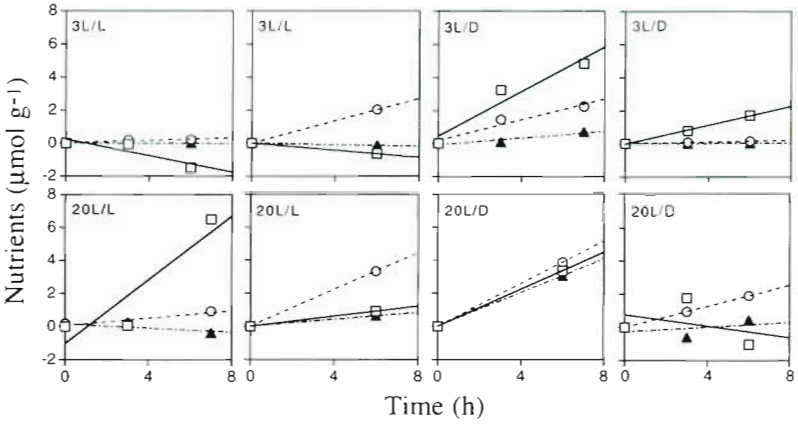

Fig. 5. DIN release rates. As Fig. 3, but for the sponge *Oligoceras violacea*. Symbols as in Fig. 2

DISCUSSION

Differential accumulation of DIN by sponges

The time series incubations performed in this study with 4 common tropical sponges showed net accumulation of DIN in the incubation water, presumably as a result of DIN release from sponge tissue. NH_4^+, a nitrogenous waste product that might be released as a product of sponge metabolism, did not accumulate significantly in most treatments in the incubation water. The highest experimental DIN release rates were observed for NO_2^- and NO_3^-. Release of nitrite or nitrate is interpreted as evidence that these sponges are active sites of nitrification, since no other process in this enclosed aerobic experimental system could account for it. This phenomenon, which was first detected in 2 Caribbean sponges inhabiting coral reefs (*Chondrilla nucula* and *Anthosigmella varians*) (Corredor et al. 1988), occurred in 3 other conspicuous tropical species (?*Pseudaxinella zeai*, *Oligoceras violacea*, and *Plakortis halichondroides*) with rates that equal or exceeded previous reports (Table 3). The levels of NO_3^- release found in this study for *C. nucula* were in the same order of magnitude as those reported before (Corredor et al. 1988).

Although the highest DIN release rates were similar (Table 2), the main form of DIN accumulated splits the studied species into 2 groups: 3 species that accumulate NO_3^- (*Chondrilla nucula*, ?*Pseudaxinella zeai*, and *Plakortis halichondroides*) and 1 that mostly accumulates NO_2^- (*Oligoceras violacea*). This distinction implies that in one group, both steps of nitrification (ammonium oxidation and nitrite oxidation) are taking place, while in the other, these 2 microbially mediated reactions are uncoupled. The net release of NO_2^- by *O. violacea* was observed both in specimens from the Bahamas and in others from Belize. Denitrification, as a sink for NO_3^-, is discounted here because anoxic or at least suboxic conditions are required for this process. The uncoupling between ammonium oxidation and nitrite oxidation is hypothesized to occur in the ocean, as a result of differ-

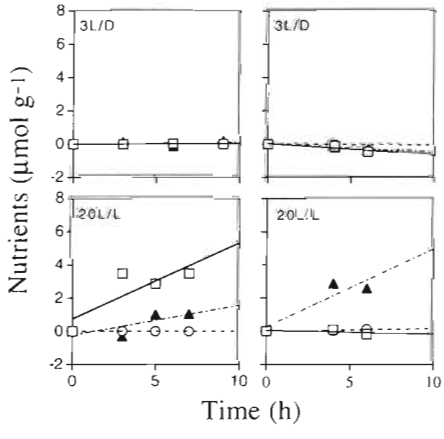

Fig. 6. DIN release rates. As Fig. 3, but for the sponge *Plakortis halichondroides*. Symbols as in Fig. 2

Table 1. Comparison of the significance of DIN changes during the time series incubations containing single sponge specimens from either *Chondrilla nucula* (CN), ?*Pseudaxinella zeai* (PZ), *Oligoceras violacea* (OV) or *Plakortis halichondroides* (PH). A linear line fit was calculated for each nutrient per treatment (r^2 values shown) by pooling the results from all the experiments run for each treatment type. r^2 values that present levels of significance higher than 90% are indicated as follows: $^{\bullet}p < 0.1$, $^{\bullet\bullet}p < 0.05$, $^{\bullet\bullet\bullet}p < 0.01$. Ne: total number of experiments per treatment; n: total number of data points

Species	Treatment	NH_4^+	NO_2^-	NO_3^-	Ne (n)
CN	3 l, light	0.661$^{\bullet}$	0.265	0.972$^{\bullet\bullet\bullet}$	1 (4)
	3 l, dark	0.038	0.687$^{\bullet\bullet}$	0.741$^{\bullet\bullet\bullet}$	2 (7)
	20 l, light	0.006	0.147	0.342$^{\bullet\bullet}$	3 (9)
	20 l, dark	0.044	0.186	0.223$^{\bullet}$	4 (13)
PZ	3 l, light	0.147	0.002	0.293	2 (7)
	3 l, dark	0.183	0.01	0.444$^{\bullet\bullet}$	2 (7)
	20 l, light	0.108	0.058	0.528$^{\bullet\bullet}$	3 (14)
	20 l, dark	0.538$^{\bullet\bullet}$	0.204$^{\bullet}$	0.229$^{\bullet\bullet}$	4 (16)
OV	3 l, light	0.672$^{\bullet\bullet}$	0.421$^{\bullet}$	–	2 (5)
	3 l, dark	0.643$^{\bullet\bullet}$	0.392$^{\bullet}$	0.459$^{\bullet}$	2 (6)
	20 l, light	0.544$^{\bullet}$	0.452$^{\bullet}$	0.002	2 (5)
	20 l, dark	0.144	0.529$^{\bullet\bullet\bullet}$	0.499$^{\bullet\bullet}$	3 (10)
PH	3 l, dark	0.049	0.14	0.01	2 (7)
	20 l, light	0.318$^{\bullet\bullet}$	0.00	0.415$^{\bullet\bullet}$	2 (7)

Table 2. Production rates of NH_4^+, NO_2^-, NO_3^- (μmol g^{-1} h^{-1}) in time series incubations of unfiltered seawater (USW) containing single sponge specimens from either *Chondrilla nucula* (CN), *?Pseudaxinella zeai* (PZ), *Oligoceras violacea* (OV) or *Plakortis halichondroides* (PH). The values are shown per treatment type, and represent the average and standard deviation of the slopes from all the experiments run per treatment type. Values from control experiments, run only with USW, represent DIN concentrations (μmol l^{-1} h^{-1}). Ne: total number of experiments per treatment; nutrient accumulation (NO_3^- for CN, PZ and PH, and NO_2^- for OV) at significance levels of $^{\bullet}p < 0.1$, $^{\bullet\bullet}p < 0.05$, $^{\bullet\bullet\bullet}p < 0.01$

Species	Treatment	NH_4^+	NO_2^-	NO_3^-	DIN	Ne
CN	3 l, light	0.019	0.006	0.36	0.385	$1^{\bullet\bullet\bullet}$
	3 l, dark	0.097 ± 0	0.02 ± 0.002	1.02 ± 0.23	1.09 ± 0.3	$2^{\bullet\bullet\bullet}$
	20 l, light	0.04 ± 0.14	0.07 ± 0.13	2.65 ± 2.61	2.76 ± 2.88	$3^{\bullet\bullet}$
	20 l, dark	0.02 ± 0.20	0.10 ± 0.13	1.55 ± 1.40	1.66 ± 1.73	$4^{\bullet}$
PZ	3 l, light	0.04 ± 0.09	0.00 ± 0.03	−0.01 ± 0.01	0.03 ± 0.05	$2^{\bullet}$
	3 l, dark	0.08 ± 0.04	0.02 ± 0.01	0.03 ± 0.02	0.13 ± 0.05	$2^{\bullet\bullet}$
	20 l, light	0.15 ± 0.20	0.01 ± 0.01	0.55 ± 0.4	0.70 ± 0.24	$4^{\bullet\bullet}$
	20 l, dark	0.48 ± 0.51	0.01 ± 0.004	1.03 ± 1.3	1.5 ± 1.85	$4^{\bullet\bullet}$
OV	3 l, light	−0.18 ± 0.1	0.58 ± 0.34	−0.01 ± 0.02	0.39 ± 0.26	$2^{\bullet\bullet}$
	3 l, dark	0.45 ± 0.27	0.17 ± 0.20	0.05 ± 0.07	0.70 ± 0.55	$2^{\bullet}$
	20 l, light	0.56 ± 0.58	0.33 ± 0.31	0.02 ± 0.12	0.92 ± 0.14	$2^{\bullet}$
	20 l, dark	0.19 ± 0.38	0.39 ± 0.24	0.57 ± 0.11	1.15 ± 0.50	$3^{\bullet\bullet}$
PH	3 l, dark	−0.03 ± 0.05	−0.02 ± 0.05	−0.02 ± 0.04	−0.06 ± 0.1	2
	20 l, light	0.22 ± 0.34	0.08 ± 0.01	0.32 ± 0.20	0.55 ± 0.13	$2^{\bullet\bullet}$
USW	3 l, light	0.1	0.01	0.01	0.12	1
	3 l, dark	0.003 ± 0.01	0.00 ± 0.01	−0.01 ± 0.02	0.02 ± 0.11	3
	20 l, light	−0.03 ± 0.05	0.00 ± 0.00	−0.01 ± 0.02	−0.05 ± 0.04	2
	20 l, dark	−0.75 ± 0.01	−0.06 ± 0.01	0.29 ± 0.07	−0.16 ± 0.09	2

ential responses of each group of nitrifiers to light intensity (Olson 1981). Photoinhibition of nitrifiers, for example, seems to affect nitrite oxidizers more strongly than ammonia oxidizers (Olson 1981, Vanzella et al. 1990). In natural samples, nitrite oxidation appears to be more sensitive to changes in substrate concentration than ammonium oxidation (Olson 1981).

None of these explanations, however, resolve the differences observed among sponge species under the same experimental conditions. Both *Oligoceras vio-*

Table 3. Comparison of production rates (nmol g^{-1} h^{-1}) and areal efflux rates (mmol m^{-2} d^{-1}) of nitrate or nitrite by marine invertebrate, and benthic communities. NA: not available. nit. rate: nitrification rate

Organism/community	DIN species	DIN (nmol g^{-1} h^{-1}) Min/max	DIN (mmol m^{-2} d^{-1}) Min/max	Source
Chondrilla nucula	$NO_2^- + NO_3^-$	428 ± 74/620 ± 145	96 ± 22.5[a]	Corredor et al. (1988)
Chondrilla nucula	NO_3^-	1023 ± 230/2650 ± 2610	242 ± 218[a]/413 ± 407	Present study
?Pseudaxinella zeai	NO_3^-	30 ± 20/1030 ± 1300	211 ± 154[a]/396 ± 499	Present study
Oligoceras violacea	NO_2^-	390 ± 240/580 ± 340	NA	Present study
Plakortis halichondroides	NO_3^-	320 ± 200	NA	Present study
Coastal sediments	NO_3^-	NA	0.73 ± 0.42	Fisher et al. (1982)
Coastal sediments	NO_2^-	NA	0.09 ± 0.06	Fisher et al. (1982)
Reef sediments	$NO_2^- + NO_3^-$ (nit. rate)	NA	1.68	Capone et al. (1992)
Reef sediments	NH_4^+	NA	0–0.096	Capone et al. (1992)
Mangrove sediments	$NO_2^- + NO_3^-$ (nit. rate)	NA	0.071 ± 0.058	Morell & Corredor (1993)
Macrofaunal tubes	NP (NO_2^-)	1.6 ± 0.4/249 ± 106[b]	NA	Mayer et al. (1995)

[a]Potential release rates assuming 100% coverage. Minimum and maximum mean production rates, from 20 l experiments with significance >90% (Table 2), were normalized to areal rates using an approximate weight-volume ratio (0.4 g cm^{-3} for *?P. zeai* and 0.65 g cm^{-3} for *C. nucula*) and an approximate specimen thickness (4 cm for *?P. zeai* and 1 cm for *C. nucula*)

[b]Values represent a range of mean values of nitrification potential (NP), measured as the accumulation of NO_2^- for various species of polychaetes, amphipods, bivalves and Anthozoa

Table 4. *?Pseudaxinella zeai*. Potential rates of nitrate release (mmol m^{-2} d^{-1}) at 7 reef zones in the barrier reef at Carrie Bow Cay, Belize. Incubation release rates from the 20 l experiments (550 to 1030 nmol g^{-1} h^{-1}) were used to calculate these potential environmental rates. Reef zones are geomorphologically distinguished (Rützler & MacIntyre 1982) and named as follows: High Spurr and Grove (I), Low Spurr and Grove (II), Inner Reef Slope (III), Outer Ridge 'inward' (IV), Ridge Top (V), Outer Ridge 'seaward' (VI), Fore Reef (VII)

Reef zone	Depth (m)	Area (m^2)	No. of ind.	Biomass (g m^{-2})	Nitrate release
I	7–10	45	4	8.9	0.12–0.22
II	15	30	0	0	0
III	20–30	60	15	87.9	1.16–2.2
IV	20–30	60	3	13.7	0.2–0.34
V	17–20	60	8	41.9	0.55–1.04
VI	20–30	90	13	58.7	0.77–1.5
VII	30–37	60	58	440	5.8–10.9

lacea and *Chondrilla nucula* were collected in mangrove lagoons under similar environmental conditions (depth, light exposure, etc.); thus a higher light intensity in the *O. violacea* habitat is unlikely to be a factor preventing nitrite oxidizers from inhabiting this species. The explanation, although not explored here, probably results from the diverse nature of microbial communities inhabiting different sponge species. Strains with different reactions kinetics, or diverse chemical interactions occurring in the sponge mesohyl, where large amounts of bacteria reside (Santavy et al. 1990, Vacelet et al. 1995), might result in different bacterial types dominating the sponge internal environment.

The difference in the DIN species accumulated in the incubation chambers for 3 of the 4 sponge species studied (nitrate for *?Pseudaxinella zeai* and *Chondrilla nucula*, and nitrite for *Oligoceras violacea*) is consistent with the idea that the microbes associated with the sponge tissues, and not in unfiltered seawater, are responsible for these transformations. Studies of the microbial associates of *C. nucula* by transmission electron microscopy reveal a mostly unidentified diverse microbial flora in the sponge mesohyl, besides the high abundance of the cyanobacterial endosymbiont *Aphanocapsa feldmani* (Sara 1966, Rützler 1990) Similar studies of *?P. zeai*, and *O. violacea* have focused on their cyanobacterial endosymbionts (Rützler 1990).

Ammonia accumulated in the experiments with *?Pseudaxinella zeai*, *Oligoceras violacea* and *Plakortis halichondroides*, suggesting that in these experiments nitrification rates were lower than the production rate of nitrogenous waste by the sponge. But during the *Chondrilla nucula* experiments ammonia either was consumed or exhibited very small increases. An efficient coupling between ammonia production by this species and subsequent ammonia and nitrite oxidation, before ammonia is released in the water, explains why net accumulation of oxidized nitrogen can occur without comparable depletion of exogenous ammonium. This idea finds support in the fact that this species showed the largest NO_3^- production rates.

It is important to consider that accumulation of ammonia in the incubation chambers might have artifactually increased nitrification rates (Olson 1981), while in nature ammonia would be lost via diffusion into the water. However, ammonia did not accumulate during the experiments with *Chondrilla nucula*. Nitrification rates estimated for this sponge were probably not stimulated by exogenous ammonium concentrations. On the other hand, these nitrite and nitrate release rates are probably underestimations of nitrification rates, since the abundant microbial community within the sponges directly consume these nutrients. Thus the release rates we measured are net accumulations resulting from production and consumption by the sponge and associated microbes. The use of isotopic tracers (^{15}N) and *in situ* measurements might be useful in understanding the dynamics and magnitude of sponge-mediated nitrification. ^{14}C tracer experiments with and without nitrification inhibitors (N-serve, acetylene) would aid in estimating the rates of productivity by nitrifying bacteria in such systems.

Light and volume of incubation effects on the release of DIN by sponges

The response of the 4 species to the 4 treatments did not show any distinct relationship between the accumulation of DIN under dark or light conditions. Since light inhibits nitrification, less accumulation of NO_2^- and NO_3^- might be expected in the experiments done in the light. Corredor et al. (1988) conducted similar experiments with *Chondrilla nucula* under natural sunlight and in the dark; they found higher but not significantly different rates of NO_2^- + NO_3^- production in the light conditions (620 ± 145 nmol N g^{-1} h^{-1} in the light and 428 ± 74 nmol N g^{-1} h^{-1} in the dark). Several matters must be addressed to understand this apparent lack of photoinhibition in sponge-mediated nitrification.

First, in our experiments the type of light used (artificial plant light) and its intensity might not affect nitrifiers. Laboratory incubations of nitrifiers under artificial irradiance (25 W m^{-2}) did not show inhibitory effects on ammonium oxidation compared to the effect of natural sunlight (Vanzella et al. 1990). Second, the lack of an inhibitory effect of light might suggest the internal localization of nitrifying bacteria in the sponge

body, which would shield these bacteria somewhat from the light. A third possibility is that the effect of light on the metabolic activity of the cyanobacteria associated with 3 of the sponges studied would affect the overall energetic condition of the sponge, altering as well its ammonium release. Thus, in the dark, nitrification would decrease as a consequence of a decrease of photosynthate available for the sponge. These 2 contrary effects of light on the sponge and its symbionts as a whole system might counteract each other and eliminate differences in the outcome of incubation experiments under different light conditions. Further studies are required to assess these possibilities.

There was an apparent effect of incubation volume on at least 2 of the species. In both *?Pseudaxinella zeai* and *Plakortis halichondroides* there were markedly lower changes in DIN, especially NO_3^- accumulation, in the 3 l incubations. Furthermore, the highest nitrite and nitrate accumulation values observed (Figs. 1, 3 & 7) occurred in the 20 l incubation experiments. This volume-effect was expected, considering the high rates of water filtration reported for a few tropical sponges (Reiswig 1971). Decreased concentration of oxygen and/or other nutrients in the low volume incubation chambers are probably the causes of this effect. This volume effect might account for the relatively higher rates of NO_3^- release found in this study for *Chondrilla nucula* compared to the rates previously reported for this species, in which 2.25 l incubation chambers were used (Corredor et al. 1988). Enclosing the sponge specimens in incubation chambers necessarily changed the flow regime around the sponges, compared to their natural setting on the reef. However, because the normal filtration activity of the sponges circulates water past the sponge tissues on the reef, as it did in the incubations, we assumed that the flow patterns and flushing rate of sponge tissue was not seriously perturbed by the incubation procedure, regardless of the volume of the incubation.

Sponges with cyanobacteria versus sponges without cyanobacteria

A clear difference in the levels of DIN accumulation was detected between the 3 species that possess cyanobacteria endosymbionts and the 1 species that lacks them. *Chondrilla nucula* and *?Pseudaxinella zeai* accumulated NO_3^- at rates 10 times higher than did *Plakortis halichondroides*. *Oligoceras violacea* accumulated NO_2^- at rates 1 to 2 orders of magnitude higher than any of the other species. Total DIN production rates for the 3 species with cyanobacteria were usually greater than that detected for *P. halichondroides*. These differences, not explained here, might reflect more robust physiology in the sponges with cyanobacteria due to the release of phostosynthate by their symbiont (Wilkinson 1980). The possibility of higher nitrification rates in these sponges due to an alternative source of ammonia from the cyanobacteria, via the fixation of dinitrogen, cannot be ruled out at this point. N_2 fixation has been measured in 2 sponges from the Red Sea that possess cyanobacterial endosymbionts (Wilkinson & Fay 1979). Attempts to detect N_2 fixation by *?P. zeai*, *C. nucula*, and *O. violacea* were made in the field, using the acetylene reduction method and previous protocols used for sponges (Wilkinson & Fay 1979, C. R. Wilkinson pers. comm.). Although N_2 fixation is expected to be advantageous if the availability of ammonium for the cyanobacteria is low, no N_2 fixation was detected in our experiments (data not shown).

Environmental nitrification rates

The comparison of potential nitrification rates associated with sponges with rates measured for other benthic components (Table 3) shows that sponges have the potential to nitrify at rates 2 to 4 orders of magnitude higher than the so-called active nitrification sites in coastal environments (sediments). A more realistic comparison can be drawn from areal production rates incorporating actual estimates of sponge abundance. Such an estimate for *?Pseudaxinella zeai* on a Caribbean coral reef (Table 4) shows mean NO_3^- efflux rates up to 10.9 mmol m^{-2} d^{-1}, a rate 1 to 3 orders of magnitude higher than most benthic rates and several times the $NO_2^- + NO_3^-$ accumulation reported for non-consolidated reef sediments (Capone et al. 1992). A similarly high $NO_2^- + NO_3^-$ production rate was reported for *Chondrilla nucula*, which occupies 12% of the surface on a reef off Puerto Rico, with mean areal nitrification rates up to 11.52 mmol m^{-2} d^{-1} (Corredor et al. 1988). The authors estimated that this nitrate production by *C. nucula* could account for 50 to 120% of the nutrients needed for the gross productivity of the reef. DIN release rates recently reported for 2 reef species (*Ircinia felix* and *I. strobilina*), in Conch reef at the Florida Keys, represented values an order of magnitude greater than those reported for coral reef sediments (Pile 1996). Sponge nitrate areal production rates estimated here and elsewhere surpass any natural rates for benthic communities so far described. These rates are estimates based on only 1 sponge species. Therefore, estimates of the nitrate release rates of major reef components would give a better approximation to the sponge contribution to reef nutrient recycling.

These results further demonstrate that sponges harbor microbes with metabolisms that are important for productivity and nutrient recycling in tropical environments. Nitrifying bacteria associated with sponges might be important as competitors (with other autotrophic organisms) for ammonium in tropical benthic communities. N_2 fixation has been postulated as a major process explaining the enigma of high biological productivity despite low inputs of N in coral reefs (Capone et al. 1992). However, mounting evidence suggests that invertebrate-mediated nitrification, through associated nitrifying microbial flora, might also bear on this paradox. The nitrate and nitrite production rates reported here suggest that organic nitrogen mineralization by the sponge metabolism, linked to sponge-mediated nitrification, constitutes a major regeneration process for these nutrients in Caribbean coral reefs and mangroves.

Acknowledgements. We are grateful to Dr Bill Fenical, and Dr Phil Crews for the invitation and funding of M.C.D. to participate in the RV 'Columbus Iselin' expedition to the Southeastern Bahamas in August 1993. We are thankful to Dr Klaus Rützler and the Smithsonian Institution (CCRE program), who supported the field work carried out in Carrie Bow Cay, Belize. Finally, we thank the UCSC Biology Department, the Dr Earl H. Myers & Ethel M. Myers Trust, and the NSF for grants awarded to B.B.W. which partially financed this project.

LITERATURE CITED

Alvarez B, van Soest RWM, Rützler K (1997) A revision of Axinellidae (Porifera: Demospongiae) of the Central-West Pacific Region. Smithson Contrib Zool (in press)

Capone DG (1977) $N_2(C_2H_2)$ fixation by macroalgal epiphytes. Proc 3rd Int Coral Reef Symp 1:337–342

Capone DG, Dunham SE, Horrigan SG, Duguay LE (1992) Microbial nitrogen transformations in unconsolidated coral reef sediments. Mar Ecol Prog Ser 80:75–88

Corredor JE, Capone DG (1985) Studies in nitrogen diagenesis in coral reef sands. Proc 5th Int Coral Reef Congr 3: 395–399

Corredor JE, Wilkinson CR, Vicente VP, Morell JM, Otero E (1988) Nitrate release by Caribbean reef sponges. Limnol Oceanogr 33(1):114–120

Diaz MC, Smith KP, Newberry AT (1997) Survey of ecologically important sponges from the Barrier Reef complex at Carrie Bow Cay, Belize. Proc 8th Int Coral Reef Symp (in press)

Fisher TR, Carlson PR, Barker RT (1982) Sediment nutrient regeneration in three North Carolina estuaries. Estuar Coast Shelf Sci 14:101–116

Hatcher AI, Hatcher BG (1982) Seasonal and spatial variation in dissolved inorganic nitrogen in One Tree reef lagoon. Proc 4th Int Coral Reef Symp 1:419–424

Henriksen K, Hansen JI, Blackburn TH (1981) Rates of nitrification, distribution of nitrifying bacteria, and nitrate fluxes in different types of sediment from Danish waters. Mar Biol 61:299–304

Humann P (1992) Reef creature identification. New World Publications, Inc, Jacksonville, FL

Jones MN (1984) Nitrate reduction by shaking with cadmium alternative to cadmium columns. Water Res 18:643–646

Koroleff T (1983) Determination of nutrients. In: Grasshoff M, Ehrhardt M, Kremlin K (eds) Methods of seawater analysis. Verlag Chemie, Weinheim, p 125–187

Mayer MS, Schaffner L, Kemp WM (1995) Nitrification potentials of benthic macrophaunal tubes and burrow-walls: effects of sediment NH_4^+ and animal irrigation behavior. Mar Ecol Prog Ser 121:157–169

Morell JM, Corredor JE (1993) Sediment nitrogen trapping in a mangrove lagoon. Estuar Coast Shelf Sci 37:203–212

Olson RJ (1981) Differential photoinhibition of marine nitrifying bacteria: a possible mechanism for the formation of the primary nitrite maximum. J Mar Res 39:227–238

Pile AJ (1996) The role of microbial food webs in benthic-pelagic coupling in fresh-water and marine ecosystens. PhD dissertation, School of Marine Science, The College of William and Mary, VA

Rasmussen H, Jørgensen BB (1992) Microelectrode studies of seasonal oxygen uptake in a coastal sediment: role of molecular diffusion. Mar Ecol Prog Ser 81:289–303

Reiswig HM (1971) *In situ* pumping activities of tropical Demospongiae. Mar Biol 9:38–50

Rützler K (1990) Associations between Caribbean sponges and photosynthetic organisms. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC, p 455–466

Rützler K, Feller C (1996) Caribbean mangrove swamps. Sci Am 274:94–99

Rützler K, Macintyre IG (1982) The habitat distribution and community structure of the barrier reef complex at Carrie Bow Cay, Belize. In: Rützler K, Macintyre IG (eds) The Atlantic barrier reef ecosystem at Carrie Bow Cay, Belize, I structure and communities. Smithsonian Institution Press, Washington, DC, p 9–45

Santavy DL, Willenz P, Colwell RR (1990) Phenotypic study of bacteria associated with the Caribbean sclerosponge *Ceratoporella nicholsoni*. Appl Environ Microbiol 56: 1750–1762

Sara M (1966) Associazioni fror Porifera e alghe in acque superficiale e litorale miarino. Ric Sci 36:277–282

Seitzinger SP, D'Elia CF (1983) Preliminary stories on denitrification on a coral reef. In: Reaka ML (ed) The ecology of deep and shallow coral reefs. Symp Ser Undersea Res NOAA 1:1–9

Suchanek TH, Carpenter RC, Witman JD, Harvell CD (1983) Sponges as important space competitors in deep Caribbean coral reef communities. In: Reaka ML (ed) The ecology of deep and shallow coral reefs. Symp Ser Undersea Res NOAA 1:55–60

Vacelet J, Boury-Esnault N, Fialamedioni A, Fisher CR (1995) A methanotrophic carnivorous sponge. Nature 377:296

Valiela I (1984) Marine ecological processes. In: Reichle DE (ed) Springer advanced texts in life sciences. Springer-Verlag, New York

Vanzella A, Guerrero MA, Jones RD (1990) Recovery of nitrification in marine bacteria following exposure to carbon monoxide or light. Mar Ecol Prog Ser 60:91–95

Wafar M, Wafar S, David JJ (1990) Nitrification in reef corals. Limnol Oceanogr 35(3):725–730

Ward BB (1986) Nitrification in marine environments. In: Prosser JI (ed) Nitrification. IRL Press, Oxford, p 157–184

Webb KL, De Paul WD, Wiebe W, Sottile W, Johannes RE (1975) Enewetak (Eniwetok) Atoll: aspects of the nitrogen cycle on a coral reef. Limnol Oceanogr 20:198–210

Webb KL, Wiebe WJ (1975) Nitrification on a coral reef. Can J Microbiol 21:1427–1431

Wilkinson CR (1980) Cyanobacteria symbiotic in marine sponges. In: Schwemmler W, Schenck, HEA (eds) Endocytobiology, endosymbiosis and cell biology. De Gruyter, Berlin, p 993–1002

Wilkinson CR (1983) Net primary productivity in coral reef sponges. Science 219:509–517

Wilkinson CR (1987) Interocean differences in size and nutrition of coral reef sponge populations. Science 236:1654–1657

Wilkinson CR, Fay P (1979) Nitrogen fixation in coral reef sponges with symbiotic cyanobacteria. Nature 279: 527–529

Wilkinson CR, Vacelet J (1979) Transplantations of marine sponges to different conditions of light and current. J Exp Mar Biol Ecol 37:91–104

Wilkinson CR, Williams DMcB, Sammarco PW, Hogg RW, Trott LA (1984) Rates of nitrogen fixation on coral reefs across the continental shelf of the central Great Barrier Reef. Mar Biol 80:255–262

This article was submitted to the editor

Manuscript received: February 6, 1997
Revised version accepted: June 25, 1997