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Sponges as a source of dissolved inorganic nitrogen: Nitrification mediated by temperate sponges

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Abstract

We evaluated rates of carbon and nitrogen ingestion from particulate organic matter and dissolved inorganic nitrogen excretion by six common sponge species from Mediterranean sublittoral rocky bottom habitats. Clearance rates varied from 4 cm^3 dry weight (dry wt)$^{-1}$ min^{-1} to 41 cm^3 dry weight (dry wt)$^{-1}$ min^{-1}, depending on cell type and sponge species. Carbon and nitrogen ingestion rates of picoplankton by the different sponges ranged from 0.17 μg C g dry wt^{-1} min^{-1} to 1.5 μg C g dry wt^{-1} min^{-1} and 2 nmol N g dry wt^{-1} min^{-1} to 13 nmol N g dry wt^{-1} min^{-1}. Although excretion compounds and rates varied between sponge species, most of the species exhibited the ability to excrete significant amounts of nitrate with nitrification rates of 3–13 nmol N g dry wt^{-1} min^{-1}. Only one species (*Dysidea avara*) excreted a significant amount of ammonium. This is the first account of nitrification by temperate sponges. Particulate nitrogen ingestion and dissolved inorganic nitrogen excretion did not balance in most cases, suggesting that Mediterranean sponges may be using alternative sources of organic nitrogen. Sponge activity could have a relevant role in remineralization of organic nitrogen in oligotrophic marine coastal zones.

Benthic-pelagic coupling includes processes working in both directions: (1) planktonic production providing organic matter that settles on the seabed and/or is consumed by benthic communities and (2) nutrient fluxes from the seabed fueling planktonic primary production (Nixon 1981; Asmus and Asmus 1991). Quantification of nutrient flow between plankton and benthos is critical to characterize coupling and to understand biogeochemical cycles in coastal zones. Shallow water conditions and high water motion in coastal zones accelerates the flow of nutrients between plankton and benthos, making the study of benthic trophodynamics (i.e., the flow of energy and materials) particularly relevant to understanding the biological dynamics of coastal systems (*e.g.*, Lopez et al. 2006).

Most of the studies of benthic-pelagic coupling have been conducted in soft bottom systems, primarily estuaries (Middelburg and Soetaert 2005). Under certain circumstances, plankton dynamics in shallow bays can be controlled by the activity of benthic suspension feeders (Riisgård et al. 1998). Furthermore, nutrient fluxes at the sediment–water interface can influence or regulate the nutrient composition of the water column, and benthos behave as either sinks or sources of inorganic nitrogen (N), phosphorus (P), and silicon (Nixon 1981). In some cases, nutrient remineralization mediated by macrozoobenthos (*e.g.*, Magni et al. 2000) and in the sediment can supply a large proportion of autotrophic nutrient demand (Middelburg and Soetaert 2005). However, despite the current understanding of nutrient dynamics in estuaries, the role of benthic suspension feeders dominating rocky bottom habitats in nutrient dynamics has received little attention.

Three general properties of sponges suggest that they may contribute to the comprehension of nutrient dynamics in coastal ecosystems. First, sponges are ubiquitous marine benthic suspension feeders with great importance in terms of abundance and structure in many rocky bottom habitats (*e.g.*, Uriz et al. 1992; Ballesteros 2006). Second, they filter large volumes of water. Some sponge populations in the Caribbean are able to filter the equivalent of a 40-m-deep water column daily (Reiswig 1974), and filtration of 60–900 times their volume per hour has been documented in different areas (Reiswig 1974; Ribes et al. 1999*a*; Yahel et al. 2003). In addition, sponges have high retention efficiencies of picoplankton (between 75–99%; Reiswig 1975; Wilkinson 1978; Pile et al. 1996). Third, sponges exhibit an heterogeneous diet because although picoplankton cells are the main food source for sponges, they can

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Fig. 1. Experimental setup used to conduct the incubation experiments.

also take up dissolved organic carbon (Yahel et al. 2003) and feed on other planktonic groups such as nanoplankton and microplankton, although at a lower efficiency (Ribes et al. 1999*a*). Consequently, these characteristics of sponges (abundance, high filtration capacity, and heterogeneous diet) potentially enable them to play a relevant role in benthic-pelagic coupling and biogeochemical cycles in coastal areas (Richter et al. 2001; Ribes et al. 2005).

Most marine sponges contain nonphotosynthetic bacterial associations, ranging from very few bacterial cells to populations that can sometimes occupy >40% of the sponge tissue volume (Wilkinson 1978, 1984; Imhoff and Stöhr 2003). Despite the effort invested during the last years to the study of the associations between sponges and microorganisms, the role of microorganisms is still an unknown aspect of sponge biology. Because bacteria are potential consumers of the inorganic and organic dissolved nutrients released after oxidation of particulate organic matter ingested by sponge cells, a bacterial effect on remineralization might be expected in some systems. In fact, the nitrification capacity of tropical sponges has been examined and was related to the association between bacteria and sponge (Diaz and Ward 1997). Although the presence of dense bacteria populations living in the intercellular spaces of Mediterranean sponges was observed by Vacelet and Donadey (1977), to our knowledge, no data on nitrification mediated by sponges have been reported in temperate seas.

To contribute to the knowledge of the role of benthic communities in biogeochemical nutrient cycles in coastal zones dominated by benthic suspension feeders, we assessed the balance between particulate organic nitrogen (PON) uptake rates and excretion rates of six common sponges widely distributed in Mediterranean sublittoral rocky bottom habitats: *Agelas oroides, Dysidea avara, Chondrosia reniformis, Axinella polypoides, Ircinia oros*, and *Aplysina aerophoba* (Uriz et al. 1992; Ballesteros 2006). We focused on two main goals: (1) the quantification of the compounds excreted by these Mediterranean sponges and (2) the assessment of the nitrogen balance of the water processed by the sponges and the estimation of the remineralization efficiency by comparing excretion rates with PON ingestion rates.

Material and methods

Experimental procedure—Sponge specimens were collected by scuba diving from the coralligenous community (Ballesteros 2006) at 20-m depth on the Montgri Coast (Catalan coast; NW Mediterranean Sea, 42°3′N, 3°13′E) except for *A. aerophoba*, which was collected at 10-m depth. Whole specimens were removed, together with a piece of substrate free of other organisms, and cleaned of any macroepibionts. Sponges remained at the Experimental Aquaria Zone of the Institute of Marine Science in 125-liter tanks with a flow-through system of fresh seawater renewing the total volume every 15 min. For each experimental run, six cylindrical aquaria (7 liters) were filled with seawater, gently stirred to reduce gradients, and placed in a flume with running seawater to maintain a constant temperature (Fig. 1). Four of the aquaria were used to incubate individual sponges, and two were used as controls. Two hundred milliliter water samples from each aquarium were taken every 30 min during the first 3 hours and every hour during the next 3 hours. Plankton was quantified at time 0, 30, 60, 90, 120, 150, and 180 min, and dissolved nutrients were analyzed at time 0, 60, 120, and 180 min. polyvinyl chloride (PVC) tubing was fixed to each aquarium for water sampling. Experiments for all species excepting *A. oroides* were conducted in September 2005 between 09:00 and 19:00 h (*A. oroides* experiments were performed in July 2005). Temperature was recorded in all tanks during each experimental run.

Picoplankton cell assessment protocol—We used flow cytometry (FACSCalibur, Becton Dickinson, 488 nm excitation laser) to quantify picoplankton (Heterotrophic bacteria, *Prochlorococcus* sp., *Synechococcus* sp., and picoeukaryotes) following the methods of Gasol and Moran (1999). Water samples (three replicates of 2 mL each) were fixed with 1% paraformaldehyde + 0.05% glutaraldehyde (final concentration) and frozen in liquid nitrogen. They were stored at −80°C or in dry ice until final processing. An amount volume of 1.0 μm Polysciences Fluoresbrite yellow-green beads from an stock solution that had been precalibrated with Becton-Dickinson True-Count beads was added immediately before processing in each sample. The number of beads enumerated in each

sample run was used to determine the sample volume processed and thus the cell abundance. Logical gating in Becton-Dickinson Cell Quest software was used to separate different cell types. Heterotrophic bacteria were stained with Syto 13 (Molecular Probes) at 2.5 μmol L^{-1} and run at a flow rate of approximately 18 μL min^{-1}. They were detected by their signature in a plot of sidescatter versus green fluorescence. For the detection of *Prochlorococcus* sp., *Synechococcus* sp., and picoeukaryotes, we ran the sample again at a flow rate of approximately 44 μL min^{-1}. *Synechococcus* sp. were detected by their signature in a plot of orange fluorescence versus red fluorescence. The signature for *Prochlorococcus* sp. was low red fluorescence and no orange fluorescence, and picoeukaryotes had high red fluorescence and no orange fluorescence.

Nutrient analyses—Samples for nutrient analysis were collected in acid-rinsed, 50-mL plastic bottles. Samples were frozen and measured for inorganic nutrients with an Alliance autoanalyzer following the method of Grasshoff et al. (1983). Parameters measured were NH_4^+ (ammonium), NO_3^- ($+NO_2^-$) (nitrate), and PO_4^{3-} (phosphate).

Particulate nitrogen—Total particulate water column nitrogen (PON) was measured by filtering 250 mL of water on precombusted GF/F glass fiber filters. The determination of total PON was made by the oxidation method protocol of Pujo-Pay and Raimbault (1994). Nitrate concentrations in the digested samples were measured with an Alliance autoanalyzer following the method of Grasshoff et al. (1983). Particulate nitrogen was measured at the beginning and at the end (after 3 hours) of each experiment.

Sponge biomass estimation—Each sponge specimen was photographed, and sponge area was determined from the photographs using image analysis software (NIH-image). Afterwards, sponge specimens were dried at 100°C to a constant weight (minimum 24 h).

Ingestion rates—Because of the fast decrease in cell concentration during the first 30 min (*see* Results), ingestion rates were calculated from the first (time 0) and second sample (time 1, after 30 min). Exponential growth and the clearance of cells were assumed (Frost 1972; Ribes et al. 1999*a*). Cell growth rates were computed as k (min^{-1})

$$k = \ln(C_1/C_0)/t_1 - t_0 \quad (1)$$

where C_0 and C_1 were the cell abundance in the aquaria at the initial time t_0 and at the final time t_1. The clearance rate CR (volume swept cm^3, g dry wt $sponge^{-1}$ min^{-1}) was computed as

$$CR = V \times g/B \quad (2)$$

where V is the volume of the aquaria in cm^3, B is the sponge biomass in g of dry weight, and g is the grazing coefficient (min^{-1}), computed as

$$g = k_c - k_o \quad (3)$$

where k_c is the cell growth rate in the control aquaria, and k_0 is the apparent picoplankton growth rate in the aquaria with sponge. Ingestion rate I (cells ingested g dry wt^{-1} min^{-1}) is

$$I = CR \times C \quad (4)$$

where C is the initial cell abundance (cell mL^{-1}) in the aquaria.

Carbon (C) and N content were estimated using conversion factors from the literature: for heterotrophic bacteria, 9.3 fg C $cell^{-1}$ and 1.8 fg N $cell^{-1}$; for *Synechococcus* sp., 192 fg C $cell^{-1}$ and 21 fg N $cell^{-1}$; for *Prochlorococcus* sp., 37 fg C $cell^{-1}$ and 4 fg N $cell^{-1}$; for picoeukaryotes, 183 fg C μm^{-3} and 26.1 fg N μm^{-3} (Ribes et al. 2005 and references therein).

Excretion rates—Excretion rates ([E] nmol g dry wt^{-1} min^{-1}) were calculated using Eq. 1–4, in which C_0 and C_1 were nutrient concentrations at time 0 and at the final time. Final time was the first sample where a significant change in nutrient concentration was detected (first 60 min in most cases). The excretion rate constant ([ER] volume of water processed cm^3 g dry wt^{-1} min^{-1}) was calculated as CR in Eq. 2.

Results

Environmental parameters—Water temperature during the experiments ranged between 21.5°C and 23.1°C (Table 1). These temperatures were within the natural range of temperature in the field where sponges were collected (L'Estartit meteorological station). Initial cell abundances ranged from 7.43–10.3 $\times$ 10^5 heterotrophic bacteria mL^{-1}, 1.60–34.8 $\times$ 10^4 *Synechococcus* sp. mL^{-1}, 2.94–34.1 $\times$ 10^3 *Prochlorococcus* sp. mL^{-1} (*Prochlorococcus* sp. was not detected during the *A. oroides* experiment, which was performed 2 months earlier), and 6.91–253 $\times$ 10^2 picoeukaryotes mL^{-1} (Table 1). For all groups, these values were within the natural range of abundance at the site of collection (Ribes et al. 1999*b*). Total PON varied from 0.60 μmol N L^{-1} to 1.32 μmol N L^{-1}; and picoplankton accounted for 12–57% of the PON at the beginning of the experiments (Table 1). Dissolved nutrients ranged from 0.87–2.81 μmol L^{-1} of ammonium, 1.25–2.99 μmol L^{-1} of nitrate, and 0.25–0.46 μmol L^{-1} of phosphate (Table 1). Oxygen concentration was always fully saturated or supersaturated, and during the incubations, it never decreased by >10% from initial concentration. The sponge area of the specimens used in the experiments ranged from 34 cm^2 to 87 cm^2, representing a range of mean biomass from 6.3 g dry wt to 31 g dry wt (Table 1).

Ingestion rates—Abundance of all cell types decreased in all experiments (Fig. 2). Because cell abundance after the first 30 min decreased between 17% and 75% of the original concentration (Fig. 2), we calculated clearance and ingestion rates using the first and second samples (initial and after 30 min). Cell growth rates calculated for each sponge species in the control and the sponge aquaria

Table 1. Environmental parameters at the beginning of the experiments, mean±SD.*

Species	*A. oroides*	*D. avara*	*C. reniformis*	*I. oros*	*A. aerophoba*	*A. polypoides*
Temperature (°C)	21.5±0.45	23.1±0	22.1±0.11	22.4±0.04	22.4±0.26	22.6±0.15
Dry weight (g)	31.0±5.8	8.8±4.0	7.5±2.07	15.5±6.5	20.6±7.6	6.3±3.2
Area (cm^2)	87.0±10.7	50.8±18.5	34.1±3.7	72.3±44.2	66.1±23.0	74.6±42.6
HB×10^5 (mL^{-1})	10.2±1.1	10.3±2.4	7.61±0.20	7.43±0.27	8.18±0.33	6.67±0.32
Syn×10^4 (mL^{-1})	34.8±3.9	1.61±0.31	2.51±0.11	1.60±0.22	25.3±4.2	1.81±0.18
Pro×10^3 (mL^{-1})	–	3.63±1.07	5.63±0.32	2.94±0.62	34.1±2.0	3.18±0.56
Pk×10^2 (mL^{-1})	223±26	6.91±1.66	55.6±25.3	11.1±2.7	253±42	13.6±1.6
PON	1.24±0.30	1.32±0.15	0.87±0.36	0.60±0.11	1.21±0.21	0.98±0.14
% N picoplank	57	12	17	20	46	12
NH_4^+	1.12±0.27	2.81±0.47	1.08±0.15	0.87±0.19	1.77±0.37	0.96±0.25
NO_3^-+NO_2^-	2.38±0.17	2.99±0.25	1.34±0.10	1.25±0.25	2.67±0.36	2.07±0.36
$PO_4^{=}$	0.25±0.02	0.46±0.05	0.37±0.07	0.33±0.02	0.41±0.07	0.32±0.02

* HB, Heterotrophic bacteria; Syn, *Synechococcus* sp.; Pro, *Prochlorococcus* sp.; Pk, picoeukaryotes. PON, particulate organic nitrogen measured by filters; % N picoplank, percentage of the PON accounted by HB, Syn, Pro, and Pk. PON, NH_4^+, NO_3^-+NO_2^-, and $PO_4^{=}$ in μmol L^{-1}.

showed significant differences in all of the sponge species and cell types (Two-tailed Wilcoxon test, $p < 0.005$), indicating cell removal by the sponges (Fig. 3). CR varied from 4 cm^3 g dry wt^{-1} min^{-1} to 41 cm^3 g dry wt^{-1} min^{-1} depending on the cell type and sponge species, but did not differ among cell types within any sponge species (Table 2). C and N ingestion rate ranged from 0.17 μg C g dry wt^{-1} min^{-1} to 1.5 μg C g dry wt^{-1} min^{-1} and 2 nmol N g dry wt^{-1} min^{-1} to 13 nmol N g dry wt^{-1} min^{-1}. *A. aerophoba* exhibited the highest C and N ingestion rates and *I. oros* the minimum (Table 2). It was not possible to calculate ingestion rates for *A. polypoides* because of a mistake in the sample preservation process.

N ingestion was also calculated from filters (total PON analysis) to account for an integrated estimate of nitrogen consumption from all particle sizes. PON samples were taken at the beginning and at the end (180 min) of the incubations. The consumption of PON varied from 0.27 nmol N g dry wt^{-1} min^{-1} to 1.71 nmol N g dry wt^{-1} min^{-1} (Fig. 4). N ingestion from total PON analysis showed lower

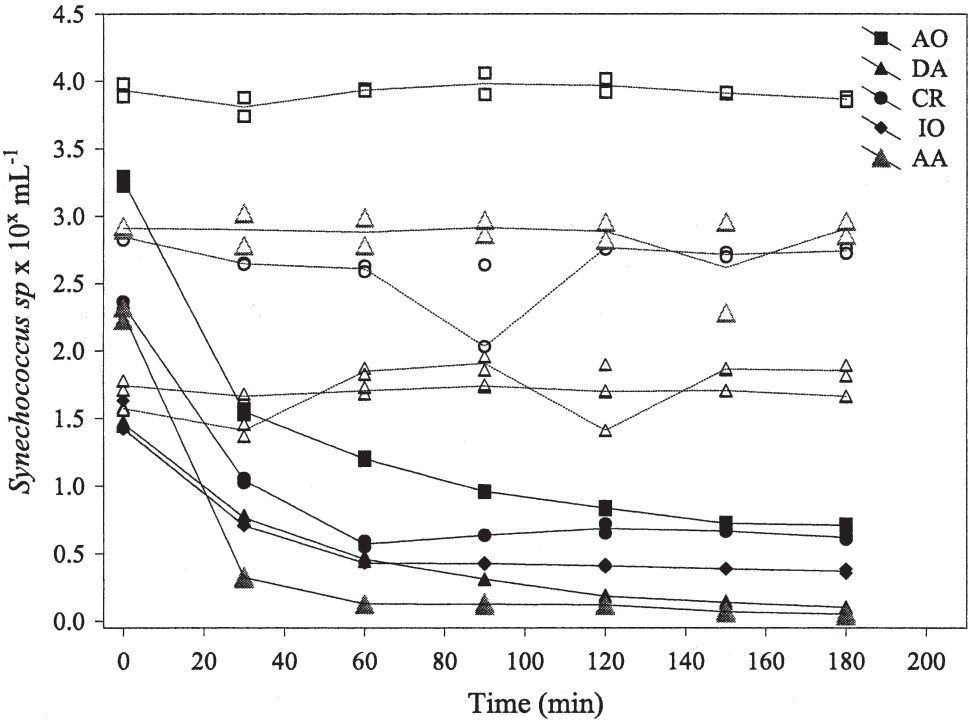

Fig. 2. Change in concentration of the aquaria with sponges (2) and controls (2) of picoplankton (*Synechococcus* sp. as an example) in cells mL^{-1} versus time in minutes. AO and AA values in *Synechococcus* sp. × 10^5 mL^{-1}, DA, CR, IO values in *Synechococcus* sp. × 10^4 mL^{-1}. AO: *A. oroides*, DA: *D. avara*, CR: *C. reniformis*, IO: *I. oros*, AA: *A. aerophoba*. Closed and open symbols represented sponges and controls respectively for each species.

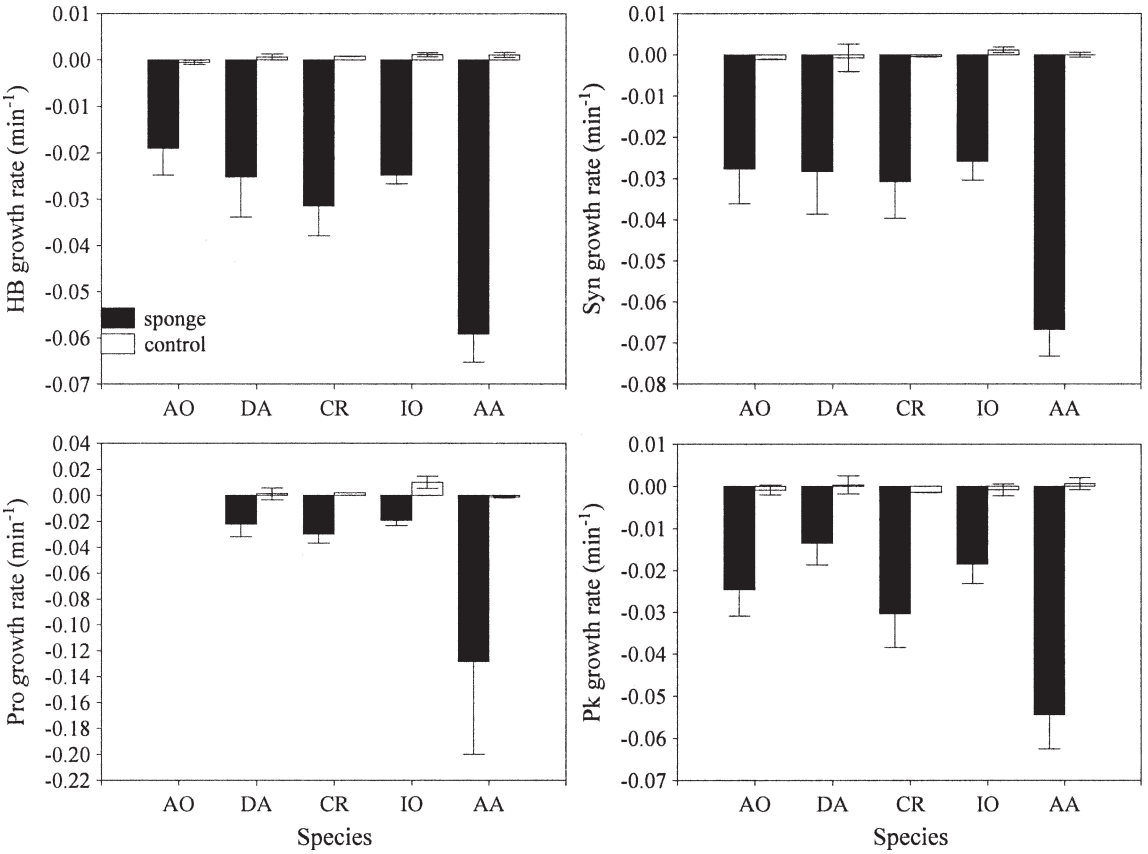

Fig. 3. Growth rate of picoplankton (mean ± SE) in the aquaria with sponges and controls for each sponge species. AO: *A. oroides*, DA: *D. avara*, CR: *C. reniformis*, IO: *I. oros*, AA: *A. aerophoba*. In all cases picoplankton growth rates were significantly different between sponge and control aquaria. HB: Heterotrophic bacteria; Syn: *Synechococcus sp*; Pro: *Prochlorococcus* sp.; Pk: Picoeukaryotes.

values (between 3 times in *I. oros* and 50 times in *A. aerophoba*) than N ingestion calculated from picoplankton (Fig. 4).

Excretion rates—Significant differences in the increase or decrease rates in dissolved nutrients between aquaria with sponges and controls were found for all of the species. *D. avara* was the only species with a significant increase (interpreted as excretion) of ammonium in the aquaria with sponges (Fig. 5). *C. reniformis* and *A. aerophoba* showed significant decrease (interpreted as consumption) of ammonium in the aquaria with sponges. Phosphate was excreted by *A. oroides*, *A. aerophoba*, and *D. avara*. All species but *D. avara* showed significant nitrate increases (Fig. 5). Excretion rates (E, nmol g dry wt^{-1} min^{-1}) ranged from nondetectable values to <1 nmol phosphate g dry wt^{-1} min^{-1}, 11 nmol ammonium g dry wt^{-1} min^{-1} (*D. avara*), and 13 nmol nitrate g dry wt^{-1} min^{-1} (*A. aerophoba*) (Table 3).

The ratio between nitrogen ingestion and excretion rates, considering nitrogen ingestion either from picoplankton or from total PON, was lower than one in most cases. This lack of balance was observed in *A. oroides*, *C. reniformis*, *D. avara*, and *I. oros* (Fig. 6).

Carbon and nitrogen fluxes—Evaluation of sponge abundance in the system is an important factor to determine the implications of sponge nutrient flux in functioning of the system. However, the evaluation of sponge densities in the field was outside the scope of this work. Therefore, we used data from the literature to conduct a first estimate on sponge nutrient flux. Data on sponge coverage from different rocky bottom locations in the NW Mediterranean ranged between 707 cm^2 sponges m^{-2} and 2,187 cm^2 sponges m^{-2} (Bibiloni 1990; Uriz pers. comm.). Given that the six studied species are among the most common species on rocky bottom communities, we assumed that mean carbon and nitrogen ingestion and excretion rates derived from these species could be considered as representative for flux estimation (Tables 2, 3). Under these assumptions, Mediterranean sponges would be able to remove between 0.18–0.54 g C m^{-2} d^{-1} and 1.6–5 mmol N m^{-2} d^{-1} from the water column. Nitrogen would be returned by the sponges to the water column with a flux of 2.5–7.9 mmol N m^{-2} d^{-1}, mostly as nitrate.

Discussion

Excretion and nitrification—Water temperature, sponge size, food, and oxygen concentrations have been proposed as the main factors controlling excretion rates (*e.g.*, Frost 1987). We avoided the confounding effects of these factors on excretion rates by conducting the experiments within the

Table 2. Clearance rate (CR) is the volume swept clear biomass^{-1} or area^{-1} time^{-1} and I (mean±SD) is the carbon and nitrogen ingested per biomass (g dry weight)$^{-1}$ or area (cm^2)$^{-1}$ time^{-1}. Biomass in g dry weight and area in cm^2 sponge.*

	CR (cm^3 weight or area^{-1} min^{-1})		I (ng C weight or area^{-1} min^{-1})		I (nmol N weight or area^{-1} min^{-1})	
	(g dry wt)	(cm^2 sponge)	(g dry wt)	(cm^2 sponge)	(g dry wt)	(cm^2 sponge)
D. avara						
HB	−25.2±22.8	−4.40±3.91	241±218	42.1±37.4	3.33±3.01	0.58±0.52
Syn	−29.7±26.0	−5.19±4.49	92±80	16.0±13.9	0.72±0.63	0.13±0.11
Pro	−26.9±27.1	−4.72±4.64	3.61±3.64	0.63±0.62	0.03±0.03	0.01±0.01
Pk	−14.8±13.7	2.52±2.25	2.68±2.48	0.46±0.41	0.03±0.03	0.01±0.004
TOTAL			339±304	59.2±52.2	4.10±3.69	0.72±0.63
A. oroides						
HB	−4.1±2.1	−1.44±0.72	38.5±20.2	13.6±6.8	0.53±0.28	0.19±0.09
Syn	−5.9±3.1	−2.08±1.04	393±206	139±70	3.07±1.61	1.09±0.54
Pro	–	–	–	–	–	–
Pk	−5.1±2.2	−1.85±0.86	29.9±12.9	10.8±5.0	0.31±0.13	0.11±0.05
TOTAL			461±238	163±81	3.90±2.01	1.38±0.69
C. reniformis						
HB	−33.1±16.6	−6.88±3.17	235±117	48.7±22.4	3.24±1.62	0.67±0.31
Syn	−31.9±20.1	−6.51±3.96	154±97	31.3±19.0	1.20±0.76	0.24±0.15
Pro	−32.6±17.7	−6.29±3.70	6.79±3.69	1.31±0.77	0.05±0.03	0.01±0.01
Pk	−30.8±18.9	−30.8±18.9	44.8±27.5	44.8±27.5	0.46±0.28	0.46±0.28
TOTAL			440±244	126±70	4.95±2.67	1.38±0.74
I. oros						
HB	−12.9±3.8	−3.07±1.43	123±36	29.3±13.6	1.70±0.50	0.41±0.19
Syn	−12.6±2.6	−3.19±1.86	38.9±8.2	9.86±5.73	0.30±0.06	0.08±0.04
Pro	−14.6±6.4	−3.52±1.98	1.96±0.85	0.47±0.27	0.02±0.01	0.004±0.002
Pk	−8.2±4.8	−2.24±1.75	1.48±0.88	0.40±0.32	0.02±0.01	0.004±0.003
TOTAL	−24.1±5.6		165±41	40.1±19.7	2.03±0.53	0.49±0.24
A. aerophoba						
HB	−21.8±5.3	−6.71±1.43	166±40	51.1±10.9	2.29±0.55	0.71±0.15
Syn	−24.1±5.6	−7.44±1.55	1173±271	361±75	9.16±2.12	2.83±0.59
Pro	−40.7±34.5	−13.7±13.8	51.3±43.5	17.3±17.5	0.40±0.34	0.13±0.13
Pk	−19.7±6.0	−6.12±1.86	131±39.7	40.5±12.3	1.33±0.40	0.41±0.13
TOTAL			1520±342	470±104	13.18±2.99	4.08±0.91

* HB, heterotrophic bacteria; Syn., *Synechococcus* sp.; Pro, *Prochlorococcus* sp.; Pk, Picoeukaryotes.

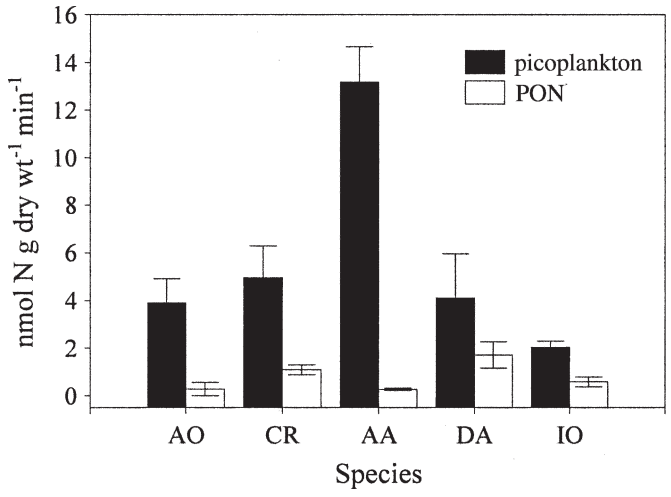

Fig. 4. Nitrogen ingestion for each sponge species calculated from picoplankton and from total PON.

natural range of temperature, oxygen, and food concentration at the site of collection during the study period (*see* Results). These experiments, performed with six ubiquitous temperate sponge species (Uriz et al. 1992; Ballesteros 2006), showed net accumulation or decrease of dissolved inorganic nitrogen (DIN) in the incubation aquaria. The fact that control aquaria did not exhibit the same pattern indicates that the release or consumption of DIN was from the sponge specimens.

We found differences in the types of compounds excreted by the different sponge species as well as in their excretion rates. *D. avara* is the only species that exhibited significant excretion of ammonium, with 100% of the nitrogen excreted in this form. *C. reniformis* and *A. aerophoba* were consuming ammonium with a net decrease in the aquaria with sponges. *A. oroides*, *C. reniformis*, *I. oros*, *A. aerophoba*, and *A. polypoides* were excreting all ingested nitrogen as nitrate. Bacteria and archaea ammonia oxidizers are the only organisms able to oxidize ammonium

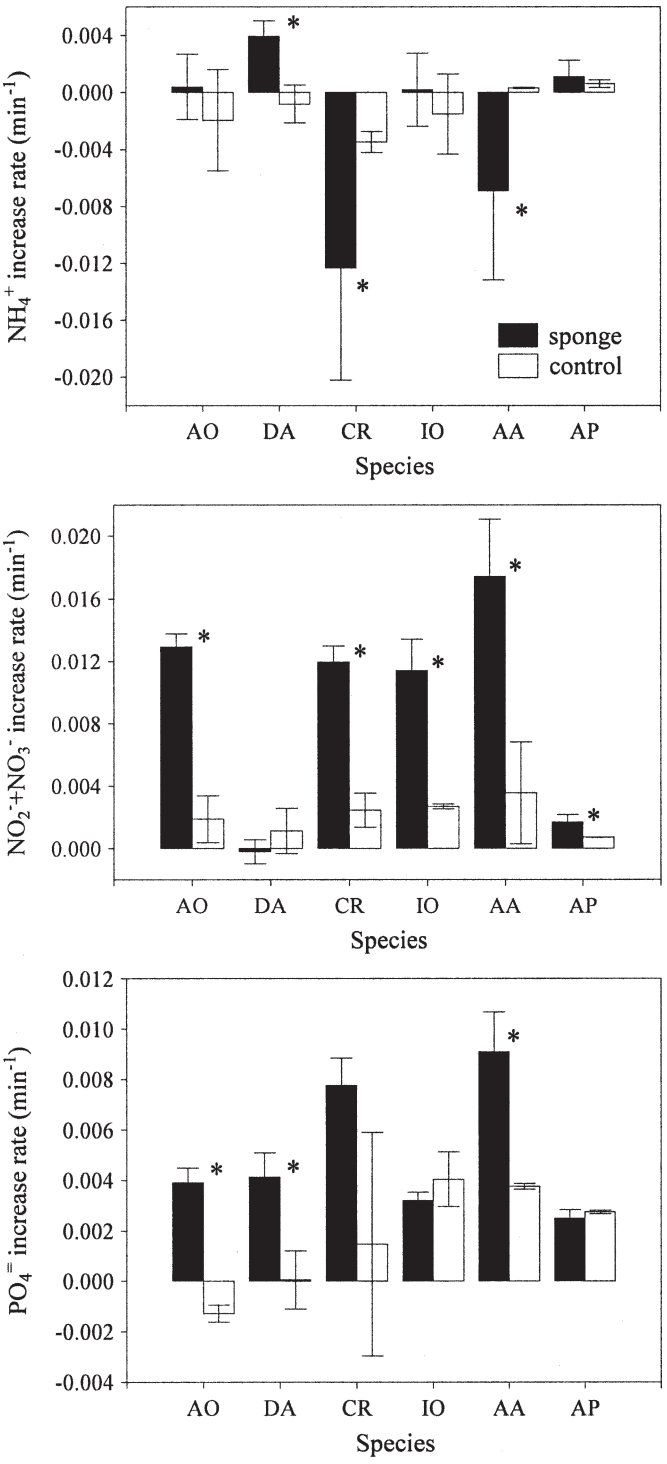

Fig. 5. Dissolved nutrient increase or decrease rates (mean ± SE) in the aquaria with sponges and controls for each sponge species. AO: *A. oroides*, DA: *D. avara*, CR: *C. reniformis*, IO: *I. oros*, AA: *A. aerophoba*. * indicates significant differences ($p < 0.05$) from two-tailed Wilcoxon test between sponge and control aquaria.

to nitrate (Ward 2000; Wuchter et al. 2006). The significant excretion of nitrate in most of the tested species appears to indicate high activity of microorganisms inside the sponge, which would produce nitrate from ammonium by microbial metabolism (*see* below). Therefore, an association between those microorganisms and the studied sponge species is expected given that all examined species were acting as nitrifiers. Although some data on bacteria–sponge associations have been reported for Mediterranean sponges (Vacelet and Donadey 1977), functions have been not studied for these associations. Ongoing research using molecular techniques focusing on the identification and quantification of ammonia oxidizers (bacteria and/or archaea) in Mediterranean sponges may contribute to understanding the differences in the types and rates of excreted compounds.

The fact that nitrification is tightly coupled with other important steps in the nitrogen cycle, such as nitrogen assimilation by phytoplankton or loss of fixed nitrogen by denitrification (Ward 2000), makes identification of nitrification sources especially relevant to the understanding of nutrient dynamics in marine systems. This work is the first description of nitrification mediated by sponges in coastal temperate systems, although nitrification mediated by sponges, also linked to the association between bacteria and sponge, was previously reported for tropical sponges (Diaz and Ward 1997). Sponges play an important role as a significant source of remineralized nitrogen through nitrification in coral reef communities (Diaz and Ward 1997; Ribes et al. 2005). We report nitrification rates for Mediterranean sponges of 3–13 nmol N g dry wt^{-1} min^{-1}. These values are at the low end of the range of nitrification rates reported for coral reef sponges (0.5–44 nmol N g dry wt^{-1} min^{-1}, Diaz and Ward 1997).

The few available data on sponge excretion show similarity in the rates of nitrogen excretion but differences in the types of excreted compounds between oceanic zones (Table 4). Tropical sponges have excretion rates between 0.5 nmol DIN g dry wt^{-1} min^{-1} and 44 nmol DIN g dry wt^{-1} min^{-1}, and nitrite and nitrate are the only DIN compounds excreted (Diaz and Ward 1997). In contrast, sponges from more productive zones such as Western Australia (Perth) had excretion rates between 8 nmol DIN g dry wt^{-1} min^{-1} and 11 nmol DIN g dry wt^{-1} min^{-1}, and ammonium was the only excretion compound detected (Hatcher 1994). Similarly sponges from the deep fjords of the Northeast Pacific showed excretion rates (assuming excurrent flow rate of 3 L min^{-1}) of 600–1,300 nmol N $sponge^{-1}$ min^{-1} (Yahel et al. 2007). The main excretion compound in the sponges from the Northwest Pacific was ammonium, and nitrification mediated by sponges was not found (Yahel et al. 2007). Our data from the Mediterranean Sea showed excretion rates of six sponge species of 3–13 nmol DIN g dry wt^{-1} min^{-1}, and in most cases, nitrate was the main form of DIN excreted. These observations pointed out that sponge excretion rates fit within a common range for species from different oceanic regions (but see *Chondrilla nucula*); however, the type of DIN excreted is different among sites (Table 4). This difference is probably related to the amount and type of associated bacteria, which in turn could be related to the productivity of the oceanic zone. Nitrification associated with sponges appears to be a general feature in oligotrophic zones, such as coral reefs and the northwestern Mediterranean, in contrast to

Table 3. Excretion rate ([E] mean±SD) of the inorganic nutrients excreted per biomass^{-1} or area^{-1} time^{-1}. Biomass in g dry weight and area in cm^2 sponge.*

E (nmol weight or area^{-1} min^{-1})	*D. avara*	*A. oroides*	*C. reniformis*	*I. oros*	*A. aerophoba*	*A. polypoides*
weight (g dry wt)						
$PO_4^{=}$	0.85±0.34	0.98±0.21	ns	ns	0.44±0.18	ns
NH_4^+	10.9±2.7	ns	−10.6±17.7	ns	−5.03±5.62	ns
NO_3^-+NO_2^-	ns	6.08±1.24	11.7±1.86	3.78±1.85	12.9±7.5	3.14±3.65
Total nitrogen	10.9±2.7	6.08±1.24	1.35±18.1	3.78±1.85	7.84±10.70	3.14±3.65
area (cm^2 sponge)						
$PO_4^{=}$	0.15±0.07	0.34±0.04	ns	ns	0.14±0.07	ns
NH_4^+	1.81±0.22	ns	−2.17±3.63	ns	−1.74±2.18	ns
NO_3^-+NO_2^-	ns	2.11±0.08	2.62±0.48	0.76±0.25	4.00±2.23	0.31±0.37
Total nitrogen	1.81±0.22	2.11±0.08	0.45±3.82	0.76±0.25	2.26±3.17	0.31±0.37

* ns, nonsignificant differences between aquaria with sponges and controls.

more productive zones where the lack of nitrification by sponges has been reported (Table 4). Further studies would be needed to corroborate this hypothesis.

Lack of balance between ingested and excreted nitrogen—We found a lack of balance between nitrogen release and nitrogen uptake from picoplankton, with higher release than uptake in most of the sponges studied. A potential underestimation of the nitrogen uptake could explain the observed lack of balance. In situ clearance and ingestion rates have previously been studied for one of the species (*D. avara*) for a year cycle. Our reported clearance rates of *D. avara* (15–30 cm^3 g dry wt^{-1} min^{-1}; Table 2) were similar to those previously documented in situ with incubation chambers (2–34 cm^3 g dry wt^{-1} min^{-1}; Ribes et al. 1999*a*). Sponges efficiently capture picoplankton cells (Pile et al. 1996; Ribes et al. 1999*a*; Yahel et al. 2007); but sponges can also feed on cells larger than 2 μm (Ribes et al. 1999*a*), although at a much lower efficiency. Therefore, we examined whether the availability of other particulate organic matter as food sources might explain the observed imbalance. Nanoplankton and microplankton can represent up to 25% of the *D. avara* diet, depending on the availability of potential food sources (Ribes et al. 1999*a*). However, even considering that nitrogen from picoplankton represented 75% of the diet and adding 25% from nanoplankton and microplankton, we would still have ingestion lower than excretion (ingestion/excretion rates for nitrogen of about 0.8 ± 0.6).

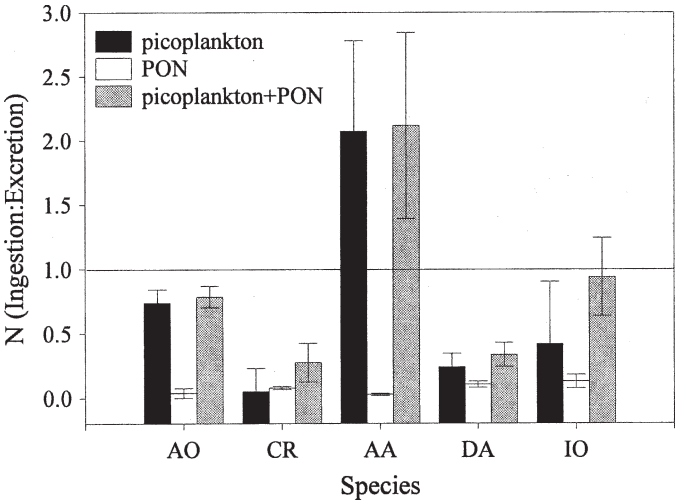

Fig. 6. Rates of nitrogen ingestion versus nitrogen excretion. Picoplankton: nitrogen ingestion rates from changes in picoplankton concentration, PON: nitrogen ingestion from total PON from GF/F filters, Picoplankton+PON: nitrogen ingestion determined as the sum of picoplankton ingestion and PON ingestion.

Because of the reported decrease in picoplankton concentration between the first sampling times (*see* above), nitrogen ingestion calculated from filters (total PON analysis after 180 min) cannot account for an integrated estimate of nitrogen consumption from all particle sizes. However, it may represent an estimate of the contribution of cells larger than 2 μm. The PON difference between the beginning and the end of the experiment, after removing the contribution of picoplankton to the initial and final PON concentration, showed about a 50% decrease after the 3-h incubation. This rate is consistent with previous field estimates of the consumption of particles >2 μm in *D. avara* (similar sponge size, 15% decrease per hour, Ribes et al. 1999*a*) and with the lower efficiency of sponges in capturing particles larger than 2 μm (Reiswig 1974; Frost 1987; Ribes et al. 1999*a*). These calculations showed an average value of the consumption of PON >2 μm of 0.147 nmols N g dry wt^{-1} min^{-1}, which ranged from 0.082 nmols N g dry wt^{-1} min^{-1} to 0.229 nmols N g dry wt^{-1} min^{-1} depending on species. However, estimates of PON capture should be considered as preliminary because of the lack of intermediate sampling during the incubations and to the underestimation of picoplankton related to filtering with GF/F filters (Gasol and Moran 1999). However, even if all nitrogen uptake from the filters is considered to be the amount of nitrogen uptake from particles larger than picoplankton, this would still account for only a small fraction of total nitrogen uptake and would not significantly affect the observed lack of balance between ingested and excreted nitrogen (Fig. 6).

Table 4. Excretion rates of dissolved inorganic nitrogen (DIN) by different sponge species.*

Species	Site	Size (g dry wt)	NH_4^+ (nmol DIN g dry wt^{-1} min^{-1})	$NO_3^-+NO_2^-$ (nmol DIN g dry wt^{-1} min^{-1})	Reference
Aplysina sp.	SE Indian Ocean	29±5	11	–	Hatcher 1994
Iotrochota baculifera	SE Indian Ocean	16±4	8	–	Hatcher 1994
Chondrilla nucula	Caribbean	3–26	–	17–44	Diaz and Ward 1997
Pseudaxinella zeai	Caribbean	4–167	–	0.5–17	Diaz and Ward 1997
Oligoceras violacea	Caribbean	2–20	–	7–10	Diaz and Ward 1997
Plakortis halichondroides	Caribbean	5–9	–	5	Diaz and Ward 1997
Aphrocallistes vastus	NE Pacific	Na	600*		Yahel et al. 2007
Rhabdocalyptus dawsoni	NE Pacific	Na	1,300*	–	Yahel et al. 2007
Dysidea avara	NW Mediterranean	9±4	11±3	–	This study
Agelas oroides	NW Mediterranean	31±6	–	6±1	This study
Chondrosi reniformis	NW Mediterranean	8±2	–	12±2	This study
Ircinia oros	NW Mediterranean	16±7	–	4±2	This study
Aplysina aerophoba	NW Mediterranean	21±8	–	13±7	This study
Axinella polypoides	NW Mediterranean	6±3	–	3±4	This study

* Values are in nmol DIN $sponge^{-1}$ min^{-1}.

It is well known that marine sponges harbor photosynthetic and nonphotosynthetic bacteria as well as dinoflagellates (Vacelet 1982; Hentschel et al. 2003 and references therein). Most of the bacteria living in association with sponges seem to be well established, growing and dividing extracellularly within the sponge mesohyl or inside the bacteriocyte cells (Imhoff and Stöhr 2003). Although not completely understood, several functions of microbial communities living in association with sponges have been identified (*e.g.*, Wilkinson 1984; Manz et al. 2000). These functions include the removal of waste products (Wilkinson 1978) and the use of bacteria as a source of food for the sponge (Vacelet and Donadey 1977). If we consider the consumption of the associated bacteria as a potential explanation of the observed lack of balance between ingested and excreted nitrogen, the number of associated bacteria that would need to be consumed to meet an ingestion/excretion ratio of about 1 can be calculated. If the nitrogen content of one bacterium is 1.8 fg, on average 4.33 $\pm$ 3.44 $\times$ 10^7 bacteria g dry wt^{-1} min^{-1} must be consumed by the cells of the sponges with a nitrogen imbalance (*D. avara*, *A. oroides*, and *C. reniformis*). We know that at least *A. oroides* and *C. reniformis* harbor nitrifier bacteria, so if we focus on this bacteria group and assume a rate of ammonium oxidation by nitrifier bacteria of 0.0625 pmol $cell^{-1}$ h^{-1} (Wafar et al. 1990), between 62% and 500% of the associated nitrifier bacteria must be consumed per minute by *C. reniformis* and *A. oroides* to result in an ingestion/excretion ratio of 1. Sponge microbial communities are very diverse, and the role of the potential consumption of associated bacteria based only on nitrifying bacteria would likely be an underestimation. Data on bacterial densities for our studied species are available only for *A. aerophoba* (Friedrich et al. 2001). If we consider the total bacteria density reported in *A. aerophoba* as a reference value for Mediterranean sponges, 1–10% of the associated bacteria would need to be consumed every minute to meet a ratio of nitrogen ingestion/excretion of 1. Therefore, although consumption of associated bacteria cannot be disregarded, our estimate suggests that consumption does not appear to be the most plausible mechanism to explain the observed lack of balance between ingested and excreted nitrogen.

Hatcher (1994) demonstrated that dissolved organic nitrogen uptake by *Aplysina* sp. and *Iotrochota baculifera* (sponges from Western Australia) accounted for 31–82% of the ammonium excreted by these species. Hatcher (1994) also showed that other benthic invertebrates, such chiton, abalone, gastropod, and ascidian species, did not uptake DON but did excrete DON. The imbalance between particulate carbon inputs and respired CO_2 was first reported by Reiswig (1974), and three decades later Yahel et al. (2003) demonstrated uptake of dissolved organic carbon by sponges. Therefore, the uptake of DON by sponge-associated microbes is intuitively the most probable explanation for the observed budget imbalance. Another possibility would be N_2 fixation by cyanobacteria associated with the sponges (Wilkinson 1979, 1999), however only *A. aerophoba*, among our studied species, harbors photosynthetic bacteria (Friedrich et al 2001). Therefore, although some consumption of associated bacteria by sponge cells cannot be disregarded, we suggest that the Mediterranean sponges are most probably using alternative organic nitrogen sources, such as DON, with uptake mediated by sponge-associated bacteria. Further research will be needed to elucidate the capability of Mediterranean sponges for DON uptake and their role in nutrient balances.

Carbon and nitrogen fluxes—The extrapolation of our results to the field revealed a relevant role of sponges in mediating nitrogen and carbon fluxes. Primary production in the northwestern Mediterranean is on the order of 0.22 g C m^{-2} d^{-1} (Estrada 1996). This production represents a nitrogen demand of 1.2 mmol N m^{-2} d^{-1}. Rocky bottom Mediterranean sponges seem able to remove most of the carbon and nitrogen fixed by planktonic primary producers (0.18–0.54 g C m^{-2} d^{-1} and 1.6–5 mmol N m^{-2} d^{-1}); however, the remineralization of particulate organic nitrogen and, probably, also dissolved organic nitrogen

would provide about 2–7 times the inorganic nitrogen needed for primary production.

The observed nitrification rates for the Mediterranean sponges (4,522–18,720 nmol N g dry wt^{-1} d^{-1}) are within the higher range of nitrification rates of bacteria associated with other benthic marine animals (Adriatic bivalve, gastropod, crustaceans and polychaetes , 71–22,868 nmol N g dry wt^{-1} d^{-1}, Welsh and Castadelli 2004). At community level, our estimation of the sponge nitrification rate (2.5–7.9 mmol N m^{-2} d^{-1}) was higher than values reported for the potential rate of animal-associated nitrification by Welsh and Castadelli (2004) on the mentioned benthic animals (0.7–3.1 N m^{-2} d^{-1}, in which densities for intensive commercial production of bivalves were included). Our estimation of the sponge nitrification rate also appears to be similar or even higher than that reported for sediments from coastal environments in productive waters (range 0.1–8; mean 2.7 $\pm$ 3 mmol m^{-2} d^{-1}, Herbert 1999). Planktonic nitrification rates at the northwestern Mediterranean have been documented to vary between 72 nmol of N L^{-1} d^{-1} and 144 nmol of N L^{-1} d^{-1} (Bianchi et al 1999). Therefore, considering an area of 1 m^2 to affect the cubic meter of water adjacent to the bottom, nitrification by the sponges will reach values from 2,500 mmol N L^{-1} d^{-1} to 7,900 mmol N L^{-1} d^{-1}, several orders of magnitude higher than the rates reported for Mediterranean plankton.

We can outline two main conclusions from this research. First, most of the Mediterranean sponges have the ability to nitrify. Nitrification rates of Mediterranean sponges are on the same order as those reported for tropical sponges, but they can nitrify at higher rates than planktonic communities and at similar or higher rates than sediments from coastal environments in productive waters. Second, the lack of balance between particulate nitrogen ingestion and DIN excretion by sponges leads us to suggest that Mediterranean sponges may be using alternative sources of nitrogen such as DON and/or consumption of associated bacteria by sponge cells. Using the estimated flux of nitrogen, we suggest that sponges may play a relevant role in recycling nutrients in oligotrophic coastal environments.

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