Nitrification Activity of the Sponge *Chondrosia reniformis* Under Elevated Concentrations of Ammonium

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This study examined the ability of a Mediterranean demosponge *Chondrosia reniformis* to oxidize exogenous ammonium, simulating N-rich conditions that occur near finfish farms. We hypothesized that as the concentration of ammonium increases in the surrounding seawater, nitrification mediated by microbes associated with *C. reniformis* will lead to enhancement of ammonium uptake, nitrate excretion and oxygen consumption by the sponge holobiont. To test this hypothesis, we conducted laboratory experiments with *C. reniformis* explants exposed to ammonium enrichments (300–6667 nM) and to ambient seawater (45–1511 nM ammonium). We analyzed inhaled (IN) and exhaled (EX) water samples for dissolved oxygen, ammonium, nitrates and retention of picoplankton cells. We observed ammonium uptake in nearly half the cases and excretion of nitrate in most experimental outcomes. Yet, the consumption of ammonium and oxygen, as well as the excretion of nitrate by *C. reniformis* were not related to the concentration of inhaled ammonium, which suggests that the nitrification activity of sponge-associated microbes is not necessarily related to the concentration of ammonium in the surrounding seawater. Further research is required to reveal the sources of nitrate released from sponges and the fate of this nitrate in natural and manipulated ecosystems.

Keywords: aquaculture, holobiont, marine ecology, nutrient fluxes, nitrification

INTRODUCTION

Marine sponges feed on microorganisms and host dense and diverse microbial communities in their bodies (Taylor et al., 2007; Fan, 2012; Webster and Taylor, 2012; Pita et al., 2018). Owing to the importance of microbe-mediated processes in the sponge's life, the term "holobiont," i.e., "whole organism," is used to emphasize that microbial consortia are integral parts of the sponge (Hentschel et al., 2003). Microbial associates contribute to sponge's metabolism and ecological functioning in various ways, such as the processing of dissolved inorganic nitrogen (DIN), which is the subject of the present study.

Sponges produce ammonium (NH_4^+) as a metabolic waste (endogenous ammonium), which makes the sponge body an attractive niche for microorganisms in nitrogen-limited marine environments (Han et al., 2013). Nitrification is an exclusively prokaryotic process whereby ammonium is oxidized to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-), and it is utilized to

obtain energy by different groups of bacteria and archaea associated with sponges (Kowalchuk and Stephen, 2001; Schläppy et al., 2010; Ribes et al., 2012; Pita et al., 2018). Several sponge species were found to consume exogenous ammonium while excreting nitrate, thereby resembling microbe-mediated nitrification (Corredor et al., 1988; Diaz and Ward, 1997; Jiménez and Ribes, 2007; Schläppy et al., 2010; Ribes et al., 2012).

The present study examines the potential of a Mediterranean high microbial abundance (HMA) sponge *Chondrosia reniformis* (Nardo, 1847) to act as a sink for ammonium under conditions of elevated concentrations of ammonium in seawater. Such conditions are expected near marine finfish farms, where the concentrations of ammonium are elevated because of fish metabolic wastes (Hargrave et al., 1993; Pitta et al., 2006; Korzen et al., 2016). By doing so, we tested the possible functioning of *C. reniformis* as an ammonium sink in Integrated Multi Trophic Aquaculture (IMTA), as suggested by Pronzato (1999), Chopin (2012), and Gökalp et al. (2019).

The working hypothesis of this study, hereafter referred to as the "ammonium-induced nitrification," predicted elevated uptake of ammonium, elevated excretion of nitrate and elevated consumption of oxygen by *C. reniformis* as the concentration of ammonium increases in seawater, due to the oxidation of ammonium to nitrate (nitrification) by the sponge-associated microbes. The "induced nitrification" hypothesis stems from the evidence of nitrification in *C. reniformis* obtained by Jiménez and Ribes (2007), Bayer et al. (2008), and Schläppy et al. (2010). Ammonia-oxidizing prokaryotes (bacteria and archaea) and functional genes for nitrification (ammonia monooxygenase, *amoA/amoB*) are usually found in HMA sponges including *C. reniformis* (Han et al., 2013; Bayer et al., 2014; Gantt et al., 2019). Hence, we chose to perform a practical analysis of nitrification in *C. reniformis*, especially regarding its possible role as an ammonium sink in human-impacted marine ecosystems such as aquaculture facilities.

Here we present simultaneous measurements of all nitrification components: ammonium, nitrite, nitrate, and oxygen by non-intrusive methods which require no physical contact with the sponge. We tested the "induced nitrification" hypothesis in laboratory aquaria enriched with ammonium, and the results were used to predict if *C. reniformis* would act as a sink for ammonium in integrated aquaculture.

MATERIALS AND METHODS

Sponge Collection and Handling

Live sponges were collected by SCUBA diving from 2 to 4 m depths at a rocky reef in the Eastern Mediterranean Sea (32°29′24.71″N, 34°53′9.29″E) in July 2014. Specimens covering roughly 10 cm^2 each were cut from different individuals, such that the larger part of the sponge was not removed to allow for its regeneration. The excised specimens, from here on referred to as "explants," were inserted underwater into sealed plastic bags and brought to the laboratory within 2 h after collection while constantly immersed in seawater to prevent possible damage to sponges from exposure to air.

In the laboratory, the explants were placed on porcelain tiles to provide them with substrate for attachment and kept in individual 6 L aquaria immersed in a sealed wooden table filled with seawater to maintain equal temperature among the aquaria. The aquaria were supplied with a constant flow of sand-filtered seawater that was pumped from 150 m off the coast at 2 m depth. Prior to initiation of experiments the sponges were checked for: (1) attachment to the substrate; (2) opening of osculae; (3) natural color; and (4) pumping of water by spreading fluorescein dye near the sponge and observing the efflux of the dye through the osculae.

The experiments were conducted in two seasons during 2014: summer (July–August) and autumn (November–December). The water temperature in experimental aquaria ranged between 27–28°C in the summer and between 18–21°C C in the autumn.

Experimental Design

To evaluate the influence of sponge filtration on the concentrations of ammonium, nitrite, and nitrate, we used direct sampling of water that is inhaled and exhaled by the sponge, IN-EX, *sensu* Yahel et al. (2005). We calculated the differences in concentrations of ammonium, nitrate and nitrite between the inhaled (IN) and the exhaled (EX) water samples. We combined the differences in concentrations of nitrite and nitrate as ΔNOx and addressed these as the difference in nitrate concentrations. To evaluate the oxygen consumption by sponge explants during the sampling, we measured the concentrations of dissolved oxygen at IN and EX sites by needle-type optode sensors (Fire-Sting®, Pyro-Science GmbH Germany, **Supplementary Figure 1**) and calculated the differences between the IN and EX oxygen concentrations as ΔO$_2$. The working hypothesis predicted that an increase in concentration of ammonium inhaled by the sponge (NH$_4$IN) would result in elevated uptake of ammonium (ΔNH$_4^+$), elevated excretion of nitrate (ΔNOx), and elevated oxygen consumption (ΔO$_2$).

We used a split-plot design to test the effect of inhaled ammonium concentration (NH$_4$IN) on three response variables: ΔNH$_4$, ΔNOx, and ΔO$_2$. We applied two treatments, "ambient" and "enrichment," to all explants as a whole-plot factor. For the ambient treatment, the concentration of ammonium in aquaria was not augmented artificially and thus represented natural concentrations. For the enrichment treatment, we added ammonium chloride (Sigma-Aldrich PN 254134) into a 10 L container with seawater, and from that container to an aquarium with a sponge in a flow-through manner. The ranges of ammonium concentrations were 0.045–1.511 μM and 0.344–6.667 μM for the ambient and the enrichment treatments, respectively. To account for the variance in responses to elevated ammonium concentration between the explants, we used each "explant" as a split-plot factor and applied both treatments to each explant alternately.

To verify that sponges were filtering the water, all IN-EX samples were examined by flow cytometry for retention of picoplankton. To control for the variability in analyte concentrations which are not caused by sponge activity, three pairs of samples from an aquarium without a sponge were

collected under two ambient and two enrichment treatments and analyzed in the same way as the samples obtained from sponges.

Sampling Procedure

The IN-EX samples were collected by siphoning through two identical Teflon hoses of 1 m length and internal diameter of 0.5 mm equipped with glass capillary tips of 300 μm internal diameter (**Supplementary Figure 1**). The samples of inhaled water (IN) were collected at a distance of 5 mm from the sponge's surface and at least 1 cm away from an osculum, and the samples of exhaled water (EX) were collected in front of the sponge's osculum by placing the sampling gear as close as possible to the osculum while not touching the sponge. The samples were collected into sterile test tubes and kept in a dark styrofoam box filled with ice in order to slow down the microbial transformations of DIN during the collection and handling. All sampling equipment was cleaned with a 10% HCl solution and rinsed with double-distilled water between the experiments.

Analytical Procedures

From each sample of an IN-EX pair (30 ml), 2 ml were analyzed for ammonium immediately after collection, 10 ml were filtered through 0.45 μm membranes (Millex PVDF, Millipore) and stored at 4°C until the analyses of nitrite and nitrate, and 1.5 ml were preserved with 10% glutaraldehyde (Sigma-Aldrich PN G7651) and stored at −80°C for flow cytometry analysis.

Ammonium

The concentration of ammonium (NH_4^+) was determined by the fluorometric method amended from Holmes et al. (1999), as described in Appendix S2 (**Supplementary Material**). Fluorescence was measured by a Trilogy fluorometer (Turner Designs, USA) with excitation at 350–380 nm and emission at 410–450 nm (ammonium kit 7200-041). The accuracy of ammonium assay was tested against a certified reference material (Fluka QC3179) and the precision between duplicate analyses of sample splits was <50 nM.

Nitrite and Nitrate

The analyses of nitrate (NO_3^-) and nitrite (NO_2^-) were conducted using a flow injection autoanalyzer (Lachat Instruments, QuikChem 8000) following the method of Hansen and Koroleff (1999). The analyses were fully automated and peak areas were calibrated using 0–5 μM standards prepared in nutrient-depleted filtered seawater (Meeder et al., 2012). The precision between duplicate analyses of sample splits was ±0.02 and ±0.05 μM for nitrite and nitrate, respectively.

Oxygen

Oxygen optodes underwent two-point calibration using 100% oxygen-saturated seawater and oxygen-depleted seawater made anoxic by addition of sodium sulfite. Dissolved oxygen readings were automatically adjusted to the water temperature that was recorded *in situ* by the instrument's temperature sensor (**Supplementary Figure 1**).

Flow Cytometry

Concentrations of picoplankton cells in the IN-EX samples were measured using an LSR II flow cytometer (Becton, Dickinson and Co., USA) and using the FCS Express v.5 software for data analysis. The specifications of the flow cytometer were adjusted to the concentrations of cells in the Eastern Mediterranean water and 1 μm beads (Invitrogen) were used for size reference (Appendix S3 in **Supplementary Material**).

Phytoplankton cells with red and orange fluorescence were classified as picoeukaryotes (Euk), prokaryotic *Prochlorococcus*-like (Pro) or *Synechococcus*-like (Syn) populations, and detected using the autofluorescence, size and shape characteristics, according to Marie et al. (2001). The non-photosynthetic heterotrophic bacteria (Het) were detected using SYBR Green (SG) staining for DNA as in Perea-Blázquez et al. (2012).

The efficiency of cell retention by the sponge (RE) was calculated as:

RE [%] = (([IN] − [EX])/[IN])*100%, where [IN], [EX] – are the concentrations of a given cell type (cells/ml) in the inhaled and the exhaled samples, respectively.

Negative retention rates resulting from cell concentrations in exhaled samples being higher than the concentrations in inhaled samples were interpreted as no retention of picoplankton and consequently changed to zero.
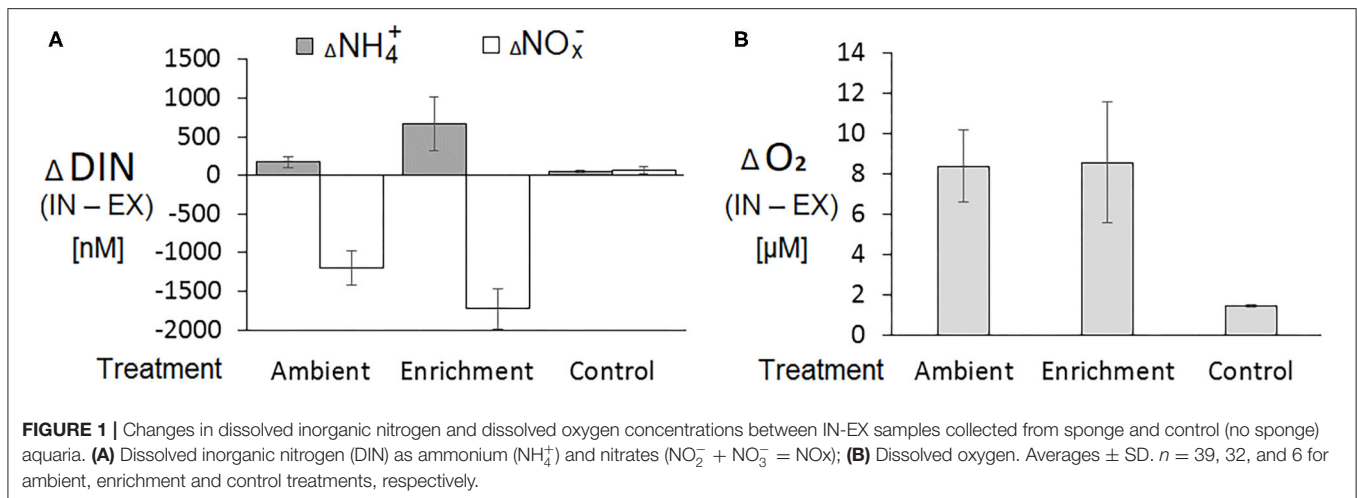
Statistical Analyses

We collected 87 IN-EX pairs of water samples from 10 *C. reniformis* explants. Nineteen IN-EX pairs were excluded from analyses due to missing results, low retention rates (<50% for all picoplankton groups) or suspicion of artifacts. The data selected for statistical analyses included 68 IN-EX pairs of DIN, oxygen, and picoplankton retention readings obtained from 10 different explants (Supplementary Material, **Data Sheet 1**).

The effects of inhaled ammonium concentration (NH_4IN) on the differences in ammonium, nitrites, and oxygen between the inhaled and the exhaled water (ΔNH_4^+, ΔNOx, and ΔO_2, respectively) were analyzed by the Generalized Linear Mixed Model (GLIMMIX) procedure of SAS version 9.4. We used *explant* as a random blocking variable and *treatment* (ambient vs. enrichment), *season* (summer vs. autumn), and *inhaled ammonium* (NH_4IN) as fixed effects. For each of the response variables (ΔNH_4, ΔNOx, and ΔO_2) a full-factorial model of fixed effects and their interactions was tested first, and after removing the non-significant interactions ($p \geq 0.05$) by backward elimination, the model was re-run.

RESULTS

During the experiments the explants maintained natural color, opened osculae, and pumped water (**Supplementary Figure 2**). Water pumping through the sponge was verified by spreading a small amount of fluorescein near sponge's surface and observing the efflux of fluorescein from the osculae.

The changes in DIN and oxygen concentrations between the inhaled (IN) and the exhaled (EX) water samples collected from sponge explants were considerably larger than the changes obtained from the control aquarium without a sponge (**Figure 1**). Positive differences between the inhaled and the exhaled concentrations of ammonium (ammonium uptake) were detected in 36 out of 68 IN-EX pairs. The differences in NOx concentrations between the inhaled and the exhaled samples

FIGURE 1 | Changes in dissolved inorganic nitrogen and dissolved oxygen concentrations between IN-EX samples collected from sponge and control (no sponge) aquaria. **(A)** Dissolved inorganic nitrogen (DIN) as ammonium (NH_4^+) and nitrates ($NO_2^- + NO_3^- =$ NOx); **(B)** Dissolved oxygen. Averages $\pm$ SD. $n =$ 39, 32, and 6 for ambient, enrichment and control treatments, respectively.

were negative in most cases (63 out of 68 IN-EX pairs), i.e., nitrates were excreted by the sponge (Supplementary Material, **Data Sheet 1**).

The absolute concentrations of nitrite did not exceed 20% of the total NOx ($NO_2 + NO_3$) and the changes in the concentration of nitrite between the IN and EX samples were much smaller than those of nitrate, usually by an order of magnitude (results not shown). Therefore, we combined the differences in concentrations of nitrite and nitrate as ΔNOx and addressed these as the difference in nitrate concentrations.

The effect of inhaled ammonium concentration (NH_4IN) alone on any of the response variables (ΔNH_4^+, ΔNOx, and ΔO_2) was not statistically significant. The effect of treatment was of borderline statistical significance on ΔNOx; and the effects of season and of the interaction NH_4IN*season on ΔO_2 were statistically significant (**Table 1**). The retention of picoplankton by *C. reniformis* was similar under both treatments, with highest retention for *Synechococcus*-like particles (**Table 2**).

DISCUSSION

The present study examined the effect of ammonium enrichment on the microbe-mediated nitrification in an HMA sponge *Chondrosia reniformis*. The augmentation of water with ammonium simulated the cultivation of *C. reniformis* next to fish farms where the water is enriched with ammonium. Based on the previous findings of Jiménez and Ribes (2007), Bayer et al. (2008), Schläppy et al. (2010), and Ribes et al. (2012), we assumed that the microbe-mediated nitrification in *C. reniformis* would be enhanced by elevating the concentrations of ammonium in the water inhaled by the sponge. Although we observed ammonium uptake in nearly half of experimental outcomes and excretion of nitrate in most IN-EX pairs, the oxidation of exogenous ammonium (the ammonium-induced nitrification) seems not to be the source for nitrate excretion, since ammonium and oxygen consumptions were not linked to nitrate excretion, as discussed below.

Nitrification and Feeding

The analysis of IN-EX results (**Table 1**) suggests that the fluxes of ammonium and NOx through *C. reniformis* are not related to the concentration of ammonium in the surrounding seawater. Fiore et al. (2013) observed in a field study both positive and negative fluxes of ammonium and NOx through the tropical HMA sponge *Xestospongia muta*. In a laboratory study of Bayer et al. (2008), the Mediterranean HMA sponge *Aplysina aerophoba* acted as a sink or a source of ammonium alternately, depending on the water temperature. In the present work, the flux of ammonium through *C. reniformis* was not affected by temperature: uptake and release of ammonium were observed at similar frequencies in summer and in autumn. Taken together, the findings of Bayer et al. (2008), Fiore et al. (2013), and the present work suggest that HMA sponges do not always act as a sink for ammonium.

It is noteworthy that while HMA sponges such as *C. reniformis* are usually positive for nitrate excretion (Jiménez and Ribes, 2007; Bayer et al., 2008; Hoffmann et al., 2009; Ribes et al., 2012; present study), sponges of the LMA group do not excrete nitrate or do so at much reduced rates (Jiménez and Ribes, 2007; Yahel et al., 2007; Ribes et al., 2012). Yet, there is evidence for aerobic nitrification and for anaerobic processes such as denitrification and anammox in both HMA and LMA sponges (Hoffmann et al., 2009; Mohamed et al., 2010; Schläppy et al., 2010). It is reasonable to assume that in the present study we observed the outcome of coexisting or even competing aerobic and anaerobic microbial processes co-occurring in the same sponge (*C. reniformis*), which might explain the variations in the excretion of nitrate. Further research is needed to decipher the specific contributions of co-occurring microbial processes to the net outcome of nitrite and nitrate excretion.

Besides nitrite and nitrate, nitrogen monoxide also known as nitric oxide (NO) is a DIN compound of considerable interest. NO is produced by the enzyme nitric oxide synthase (NOS), which converts arginine into NO and citrulline while consuming molecular oxygen (Alderton et al., 2001). NOS activity was described in diverse metazoan phyla: Cnidaria, Echinodermata, Annelida, Mollusca, Arthropoda, Urocordata, and Porifera

(Jacklet, 1997; Giovine et al., 2001; Palumbo, 2005; Ueda et al., 2016) and in microbial communities (Schreiber, 2006). Since NO is a signaling molecule rather than a primary metabolite, we

TABLE 1 | Results of GLIMMIX analysis of the effects of inhaled ammonium, treatment, and season on the outcomes of nitrification.

Response variable	Effect	df	F-value	p-value	Run
Type III tests of fixed effects					
ΔNH_4^+	NH_4IN	52	0.39	0.5333	1
	Treatment	52	0.38	0.5396	1
	Season	52	0.54	0.4661	1
	NH_4IN*treatment	52	0.08	0.7768	1
	NH_4IN*season	52	0.34	0.5640	1
	Treatment*season	52	0.86	0.3581	1
	NH_4IN*treatment*season	52	0.00	0.9706	1
	NH_4IN	56	0.07	0.7931	2
	Treatment	56	0.24	0.6258	2
	Season	56	0.12	0.7299	2
ΔNOx	NH_4IN	52	0.07	0.7936	1
	Treatment	52	0.02	0.8875	1
	Season	52	0.93	0.3384	1
	NH_4IN*treatment	52	0.06	0.8148	1
	NH_4IN*season	52	0.43	0.5132	1
	Treatment*season	52	0.15	0.6994	1
	NH_4IN*treatment*season	52	1.69	0.1999	1
	NH_4IN	57	3.23	0.0778	2
	Treatment	57	3.76	0.0575	2
	Season	58	0.91	0.3434	2
ΔO_2	NH_4IN	52	0.48	0.4900	1
	Treatment	52	0.24	0.6265	1
	Season	**52**	**12.39**	**0.0009**	**1**
	NH_4IN*treatment	52	0.00	0.9929	1
	NH_4IN*season	**52**	**11.20**	**0.0015**	**1**
	Treatment*season	52	1.58	0.2140	1
	NH_4IN*treatment*season	52	2.90	0.0947	1
	NH_4IN	54	0.85	0.3608	2
	Treatment	54	0.84	0.3630	2
	Season	**54**	**12.60**	**0.0008**	**2**
	NH_4IN*season	**54**	**8.94**	**0.0042**	**2**

df, degrees of freedom; run, model iteration after backward elimination of non-significant effects; significant effects at $p \leq 0.05$ are highlighted in bold.

did not measure its production by the *C. reniformis* holobiont in the present study. Nonetheless, in light of the evidence that their exposure to high concentrations of ammonia induces NOS activity in fish (Choudhury and Saha, 2012), further research is needed on NOS activity and NO concentrations in sponges exposed to high environmental concentrations of ammonia.

Sponges feed on picoplankton cells in the size range of 0.2–2 μm with almost 100% efficiency (Ribes et al., 1999; Topçu et al., 2010; Jimenez, 2011). In the present work, the efficiencies of picoplankton retention by *C. reniformis* (**Table 2**) were lower than those reported by Jimenez (2011) for the same species from the Western Mediterranean Sea. We observed 16% retention of *Prochlorococcus*-like population and 45% retention of heterotrophic bacteria, whereas Jimenez (2011) observed 93 and 98% retention of the corresponding populations. In our study, *Synechococcus*-like cells were retained with the highest efficiency (96%) as in the work of Jimenez (2011). Topçu et al. (2010) and Perea-Blázquez et al. (2012) also found that *Synechococcus*-like cells are the preferred food source for sponges, which suggests that in our study *C. reniformis* explants were feeding normally during the experiments.

Oxygen Consumption

The consumption of oxygen by *C. reniformis* explants was more affected by season than by the concentration of inhaled ammonium (**Table 1**). These results suggest that the contribution of microbe-mediated nitrification to the oxygen consumption by *C. reniformis* is minor. Moreover, although we verified that the sponges were feeding (**Table 2**), we did not account for possible changes in the sponge's other oxygen-consuming functions, such as water pumping. A sponge may use about 25% of its oxygen consumption to pump water (Hadas et al., 2008; Leys et al., 2011). Therefore, future studies of oxygen consumption and nutrient processing by sponges should account for seasonality and include measurements of pumping rates.

Conclusions and Suggestions for Further Research

Our findings suggest that we cannot generalize about HMA sponges serving as a source or a sink of ammonium. Particularly, this study shows that *C. reniformis* should not be considered as a sink for ammonium in models of integrated aquaculture.

Further research is needed into the possible effects of ammonium on the activity of nitric oxide synthase (NOS) enzymes in sponges. It is likely that elevated concentrations

TABLE 2 | Retention of picoplankton by *Chondrosia reniformis* explants.

Treatment	Retention efficiency (%) for different picoplankton groups (average, median, SD)			
	Synechococcus-like (*Syn*)	*Prochlorococcus*-like (*Pro*)	Pico-Eukaryotes	Heterotrophic Bacteria (*Het*)
Ambient	69, **95**, 35	16, **13**, 17	57, **69**, 32	41, **39**, 32
Enrichment	71, **97**, 38	14, **0**, 21	57, **71**, 31	49, **61**, 32

Bold values indicate median.

of exogenous ammonium may affect the arginine-NO-citrulline pathway, which is shared by the sponge host and the sponge-associated microbes (Song et al., 2020). We suggest further research into the roles of nitric oxide in the flux of DIN through the sponge and in regulating sponge's regeneration.

A recent study showed that the relative abundances of ammonia-oxidizing microbial taxa increase when sponges exhibit higher levels of ammonium consumption (Gantt et al., 2019). In order to elucidate the mechanisms underlying the variability in sponge-mediated DIN fluxes, we suggest combining physiological experiments as performed in the present work with sampling for microbial community composition as performed by Gantt et al. (2019). By knowing what the sponge-associated microbes are and what these microbes do in different conditions we can better predict the sponge-mediated DIN fluxes in natural and in human-impacted ecosystems and use these predictions in planning aquaculture or other applications.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

PN and DA contributed conception and design of the study. PN analyzed the data and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.607979/full#supplementary-material

Supplementary Figure 1 | Schematic sketch and photographs of the system used for water sampling and for measuring the concentration of dissolved oxygen. **(A)** Schematic sketch of the system, not to scale. 1 – Oxygen module with a PC (2) showing (3) the *in situ* oxygen readings by IN (green) and EX (red) optodes; 4 – temperature sensor; 5, 6 – water sampling sets and optodes; 7 – sponge explant attached to the substrate; 8 – tubes for collection of water samples; 9 – sub-samples for subsequent analyses. **(B)** Photograph of the sponge in aquarium with the sampling sets held by the GorillaPod manipulator, and the temperature sensor attached to the aquarium wall on the right; **(C)** close-up photograph of the sampling set, with an additional close-up **(D)** showing the tip of the oxygen sensor (top) and the tip of the water sampling capillary (bottom).

Supplementary Figure 2 | Photographs of the sponge *Chondrosia reniformis* during and after the experiment. **(A)** a sponge at the beginning of the experiment (July 2014), the arrow points to attachment of the sponge to a porcelain tile. **(B)** The same sponge after 4 months of handling in aquarium (October 2014), arrows point to the open osculae. **(C)** The same sponge after the experiment (May 2015). The scale bar under the sponge number is 1 cm.

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