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Filtering Activity and Nutrient Release by the Keratose Sponge *Sarcotragus spinosulus* Schmidt, 1862 (Porifera, Demospongiae) at the Laboratory Scale

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Abstract: Sponges are an important constituent of filter-feeder benthic communities, characterized by high ecological plasticity and abundance. Free bacteria constitute an important quota of their diet, making them excellent candidates in aquaculture microbial bioremediation, where bacteria can be a serious problem. Although there are studies on this topic, certain promising species are still under investigation. Here we report applied microbiological research on the filtering activity of *Sarcotragus spinosulus* on two different concentrations of the pathogenic bacterium *Vibrio parahaemolyticus* in a laboratory experiment. To evaluate the effects of the filtration on the surrounding nutrient load, the release of ammonium, nitrate, and phosphate was also measured. The results obtained showed the efficient filtration capability of *S. spinosulus* as able to reduce the *Vibrio* load with a maximum retention efficiency of 99.72% and 99.35% at higher and lower *Vibrio* concentrations, respectively, and remarkable values of clearance rates (average maximum value 45.0 ± 4.1 mL h^{-1} g DW^{-1}) at the highest *Vibrio* concentration tested. The nutrient release measured showed low values for each considered nutrient category at less than 1 mg L^{-1} for ammonium and phosphate and less than 5 mg L^{-1} for nitrate. The filtering activity and nutrient release by *S. spinosulus* suggest that this species represents a promising candidate in microbial bioremediation, showing an efficient capability in removing *V. parahaemolyticus* from seawater with a contribution to the nutrient load.

Keywords: sponges; clearance rate; retention efficiency; excretion rate; nutrient release; *Vibrio*

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1. Introduction

Marine sponges (Phylum Porifera) are ancient metazoans that dominate many of the hard-bottom benthic habitats around the world along a wide geographical distribution and depth range [1,2]. These sessile organisms are benthic filter-feeders with a high capability to filter huge amounts of water (0.002–0.84 mL s^{-1} cm^3 of sponge tissue) through their aquiferous system [3–5] and to retain a wide range of 0.1–50 µm organic particles, including phytoplankton, heterotrophic eukaryotes, bacteria, and viruses with a retention efficiency of up to 99% for nano and picoplankton [6–12]. In addition, sponges play a relevant role in benthic–pelagic coupling [13,14], serve as mediators of the biogeochemical flow by respiring organic matter and facilitating the consumption and release of nutrients, such as ammonium, nitrate, and phosphate [15].

The importance of free bacteria in the diet of sponges [16] and the ability to concentrate and digest large numbers of microorganisms suggested that sponges could be effective in reducing bacterial abundance, including microbial pollution, caused by sewage in coastal areas [17], such as near mariculture facilities where bacteria, including potentially pathogenic species, are often abundant [18–30].

In highly anthropized marine environments, such as intensive or confined mariculture systems, the excessive release of excreta from farmed species and organic matter from uneaten feed create favorable conditions for pathogenic bacteria growth, especially *Vibrio*, responsible for diseases and high mortality in target species, with consequent economic losses [31–37]. To overcome this problem, the use of antibiotics has spread despite the increase in production costs and the negative consequences on farmed species and the surrounding environment. Indeed, antibiotic residues can remain in products for human consumption and antibiotics released into the environment can induce the development and spread of antibiotic-resistant bacteria in the food chain [38].

Laboratory and in situ studies have demonstrated excellent microbial and chemical bioremediation performance by different sponge species. In these studies, the high capabilities to remove organic carbon, accumulate and digest different bacterial species, and degrade organic pollutants (e.g., lindane) were thoroughly demonstrated [19–21,26,39–44]. In addition, sponges, serving as "biofilters", have been shown to have the ability to bioremediate seawater in integrated aquaculture systems [19,21,23,24,45].

The co-cultivation of sponges in association with mariculture plants may be considered an eco-friendly alternative to prevent and control the growth and spread of bacteria, pathogenic and non-pathogenic, in aquaculture waste [19,21,24,26,40]. sponge cultivation may be suitable for the eco-sustainable supply chain of biomass for certain target species [27,41,46,47]. The sponge biomass obtained in polyculture systems has considerable potential from a commercial point of view, having good appeal for hobbyists as well as cosmetic and natural bioactive compound companies (e.g., [25]).

Zoo-remediation is a poorly considered approach to reduce aquatic pollution, primarily due to ethical reasons. In the case of invertebrate animal species, while overcoming ethical issues, further criticism, such as the availability of the appropriate amount of biomass to obtain a valuable result, management of the zoo-remediator biomasses, survival skills in critical conditions, and excessive collection efforts on wild populations, needed to be addressed [17,48]. To find sustainable solutions to these issues, recently an in situ innovative integrated multitrophic aquaculture (IMTA) system in a Mediterranean fish farm, in which explants of the keratose sponge *Sarcotragus spinosulus* Schmidt 1862 (Porifera, Demospongiae) were co-cultured, showed promising survival and growth performances with a doubling of the sponge biomass after one year of rearing [49]. To date, no studies are available on the filtering performance and nutrient release [15,25], despite representing a deeply studied species in the research of basic and applied biology (e.g., microbiology, mariculture and the extraction of bioactive compounds) [50–52]. Conversely, the natural products that can be extracted from this species are well-known (e.g., polyprenylhydroquinones) [53] and have drawn particular attention due to the wide spectrum of their antibacterial, antiviral, anti-inflammatory, and cytotoxic activities [52].

In this paper, the filter-feeding activity of *S. spinosulus* on the bacterial load was investigated in laboratory conditions by estimating the clearance rate and retention efficiency versus the Gram-negative halophilic bacterium *Vibrio parahaemolyticus* (family Vibrionaceae). Data were also related to the release of nutrients (NH_4^+, NO_3^-, and PO_4^{3-}). Thus, the present study represents a contribution to the knowledge of the filtering activity and nutrient release of *S. spinosulus*, which can permit a better focus on its suitability as a microbial bioremediator within sustainable mariculture facilities.

2. Materials and Methods

2.1. Studied Species

Sarcotragus spinosulus Schmidt 1862 (Porifera, Demospongiae, Keratosa, Dictyoceratida, Irciniidae) is a massive horny sponge, common in Mediterranean coastal environments, occurring in shallow waters and also just below the tide line [54–56]. Among the Mediterranean demosponges, this species can be considered one of the most light-tolerant, being screened by a thick layer of superficial pigmented tissue (pinacoderm) made by a large number of melanocytes and a dense bacterial simbiocortex [55]. The species is

considered of high ecological plasticity, being able to live both in high-energy vertical cliffs and in low-energy semi-enclosed bays with a high sedimentation regime [47,55,57].

2.2. Sponge Sampling

Sponge specimens of *S. spinosulus* were randomly collected from Mar Grande of Taranto (40°21′ N–17°18′ E) by scuba diving at a depth of 5–10 m (T = 20 °C) in January 2019. The samples were carefully detached from the substratum, immediately transported to the laboratory within cooled bags, then cleaned of sediment and macrofouling organisms with seawater, and kept in an aquarium containing 100 L of artificial filtered seawater (AFSW) (0.22 µm pore size filters, Millipore). Samples underwent acclimatization for 2 days before testing in a temperature-controlled room (20 °C), and the water was substituted twice with new AFSW to avoid water contamination by bacteria and particulate matter (starved sponge specimens).

2.3. Experimental Procedures

The laboratory experiment aimed to investigate the filtering activity of the demosponge *Sarcotragus spinosulus* at two different *Vibrio parahaemolyticus* concentrations and to evaluate its nutrient release (NH_4^+, NO_3^-, and PO_4^{3-}).

The CIRPS 4253 *V. parahaemolyticus* strain, part of our laboratory collection [58], was used to prepare AFSW with 10^4 CFU mL^{-1} (concentration C1) and 10^6 CFU mL^{-1} (concentration C2). CIRPS 4253 was grown in 3% NaCl Luria–Bertani (LB, OXOID, Milan, Italy) broth and incubated overnight (O/N) at 37 °C. The concentration of viable bacteria (CFU mL^{-1}) in the O/N culture was calculated using a standard viable count assay. Briefly, 0.1 mL of the serially diluted bacterial culture was plated on 3% NaCl LB, and the plates were incubated O/N at 37 °C. The bacterial colonies formed on each plate were counted, and the CFU mL^{-1} was calculated with respect to the dilution factor and the volume plated. The test to evaluate the CFU mL^{-1} of the O/N culture was performed in triplicate.

The filtering experiment was performed in triplicate and consisted of 30 tanks (10 tanks per experiment) placed in a temperature-controlled room at 20 °C (the average seasonal seawater temperature during the sponge harvest period) with continuous airing and artificial lighting (16:8 light/dark, light intensity 250 µmol m^{-2} s^{-1}) and each filled with 3 L of AFSW. The tanks were kept under the same experimental conditions during the three experimental procedures.

A total of 10 tanks per experiment were set up. Six tanks contained one starved sponge specimen each, two of which were aseptically inoculated with an aliquot of the CIRPS 4253 O/N culture to reach the concentration C1 (treatment tanks, T_C1); similarly, two tanks were inoculated to reach the concentration C2 (treatment tanks, T_C2); two tanks were not inoculated and used as a control to test the possible release of bacteria belonging to the family Vibrionaceae due to the sponge (sponge control tanks, SC). Four tanks did not contain sponges: two of which were considered as negative control tanks containing only AFSW (NC), and the last two tanks were considered as positive controls inoculated with the CIRPS 4253 O/N culture at the final concentrations C1 and C2 (PC_C1 and PC_C2, respectively).

The bacterial viable count and content of the nutrients were evaluated in each tank at five sampling times (0, 2, 4, 24, and 48 h). The viable bacterial count was performed at each sampling time by spreading 0.1 mL of serially diluted seawater samples onto thiosulfate-citrate-bile salt-sucrose agar (TCBS, OXOID, Milan, Italy) with 2% NaCl, a selective and differential medium for halophilic *Vibrio*. The plates were incubated at room temperature (22 ± 2 °C) for 24–48 h. Colonies with a yellow color (considered to be *V. parahaemolyticus*) formed in each plate were counted, and the CFU mL^{-1} was calculated with respect to the dilution factor and the volume plated. The data were reported as the mean value ± the standard error (SE) of each experimental tank set.

The well-being of the sponge specimens was monitored throughout the experiment by observing the sponge surface and the osculum openings. At the end of the experiment,

the volume of each specimen was measured by means of a graduated beaker (125 ± 28 mL, mean value), then the sponges were dried in preweighed aluminum foil at 100 °C for 24 h and weighed to determine the dry weight (DW, mean value 44.8 ± 10 g).

2.4. Filtering Activity Assessment

At each sampling time, the retention efficiency (R) was calculated as a percentage for the difference in bacterial concentrations by the following equation:

$$R\ (\%) = 100 * \left[\frac{(C_{t0} - C_{tx})}{C_{t0}}\right] \quad (1)$$

where C_{t0} is the initial bacterial concentration and C_{tx} is the bacterial concentration at each successive sampling time [42].

The clearance rate (CR) was estimated following the equation given by Coughlan [59], which measures the bacterial removal from the seawater as a function of time T, volume V of water used in the filtering experiment, and sponge size W:

$$CR = \frac{ln\left(\frac{C_{t0}}{C_{tx}}\right) V}{TW} \quad (2)$$

The data were reported as weight-specific clearance rates and expressed in milliliters per hour per gram of dry sponge tissue (mL h^{-1} g DW^{-1}).

2.5. Nutrient Analysis

To evaluate the contribution of *S. spinosulus* in terms of the dissolved inorganic nutrients in the surrounding environment, the release of ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{-3}) in the seawater at each sampling time during the experiment was measured.

The ammonium content was monitored with a pH meter (HI 5222, Hanna Instruments, Woonsocket, RI, USA) equipped with an ammonium ion-selective electrode (HI 4101, Hanna Instruments, Woonsocket, RI, USA), calibrated in the range 0–10 mg L^{-1} according to the manufacturer's instructions and expressed as mg NH_3 L^{-1} [60,61].

The nitrate content was determined spectrophotometrically with a Beckman DU 6400 spectrophotometer according to [62] with minor modifications. Seawater samples (1.25 mL) were treated with 0.025 mL HCl and the specific absorbance, measured at 220 nm, was adjusted by subtracting the nonspecific absorbance at 275 nm due to the interference of organic compounds. The nitrate concentration was determined by referring to a standard curve with a range of 0–10 mg L^{-1} and expressed as mg NO_3^- L^{-1}.

The phosphate content was determined according to Strickland and Parsons [63] by the spectrophotometric determination of a blue phosphomolybdic complex that specifically absorbs at 882 nm. The phosphate concentration was determined by referring to a standard curve with a range of 0–5 mg PO_4^{3-} L^{-1}.

Finally, the sponge excretion rate (E) was calculated by multiplying each nutrient concentration value for the water volume in a tank, dividing for the dry weight (DW) biomass per unit time (h). Consequently, the nutrient excretion rates were expressed as micromoles N or P per gram of dry weight per hour (μmol g DW^{-1} h^{-1}) according to [64]:

$$E = \frac{(Nc_{tx} - Nc_{t0})\ V}{g\ DW\ h} \quad (3)$$

where Nc_{t0} and Nc_{tx} were the nutrient (NH_4^+, NO_3^-, and PO_4^{3-}) contents in seawater at the initial time t_0 and at each sampling time (t_1–t_4), respectively.

2.6. Statistical Analysis

All experimental data were computed as dependent variables using PERMANOVA as an approach similar to parametric ANOVA. Univariate PERMANOVA tests were run on Bray–Curtis similarity matrices with 9999 permutations [65]. The bacterial concentration (C, 3 levels) and time (t, 5 levels) factors were used to detect differences in the CFU, retention efficiency (*R*), clearance rate (*CR*), nutrient concentrations (NH_4^+, NO_3^- and PO_4^{3-}) and excretion rate (*E*) in t × C interactions. Each interaction was individually analyzed using Univariate PERMANOVA tests with the same experimental design. If necessary, transformed data in a Bray–Curtis similarity matrix with 9999 permutations was used to perform the analyses [65]. If it was impossible to obtain enough permutations for PERMANOVA analysis, the reference p was obtained using a permutation simulation test (Monte Carlo test). The pairwise test was applied to discover statistically significant differences in each pair of factor levels based on the significance value of PERMANOVA/Monte Carlo tests. All analyses were conducted using Primer v6+ PERMANOVA software [66].

3. Results

3.1. Filtering Capability

The sponge health status was assessed visually during both the starvation period (2 days) and the 48 h experiment showing no signs of stress neither to the external surface nor to changes on oscula openings.

The trend of bacterial concentrations (CFU mL^{-1} ± SE) for both experimental *Vibrio parahaemolyticus* concentrations (C1 = 1.0×10^4 and C2 = 1.0×10^6) in the treatment (with sponge, T_C1, T_C2, and SC) and in control tanks (without sponge, PC_C1, PC_C2, and NC) are shown in Figure 1. During the experiment, the CFU trend was affected by the initial concentration of bacteria (C), the time (t), and their interaction (t × C) (univariate PERMANOVA, pseudo-F = 4.5983, df = 8, $p = 0.001$). Two hours after the beginning of the experiment (t1), the *V. parahemolyticus* concentration in the treatment tanks was significantly lower than that found in the corresponding control tanks (T_C1 = $6.7 \pm 1.4 \times 10^3$, T_C2 = $3.2 \pm 0.6 \times 10^5$ and PC_C1 = $1.2 \pm 0.1 \times 10^4$, PC_C2 = $1.0 \pm 0.1 \times 10^6$) (pairwise test, PC_C1 > T_C1; PC_C2> T_C2).

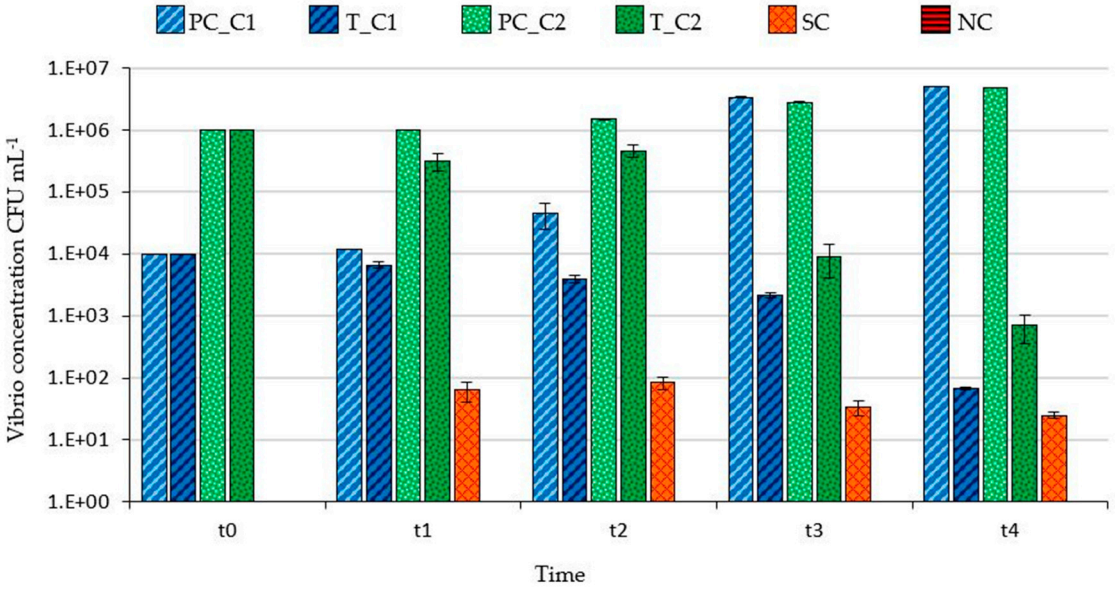
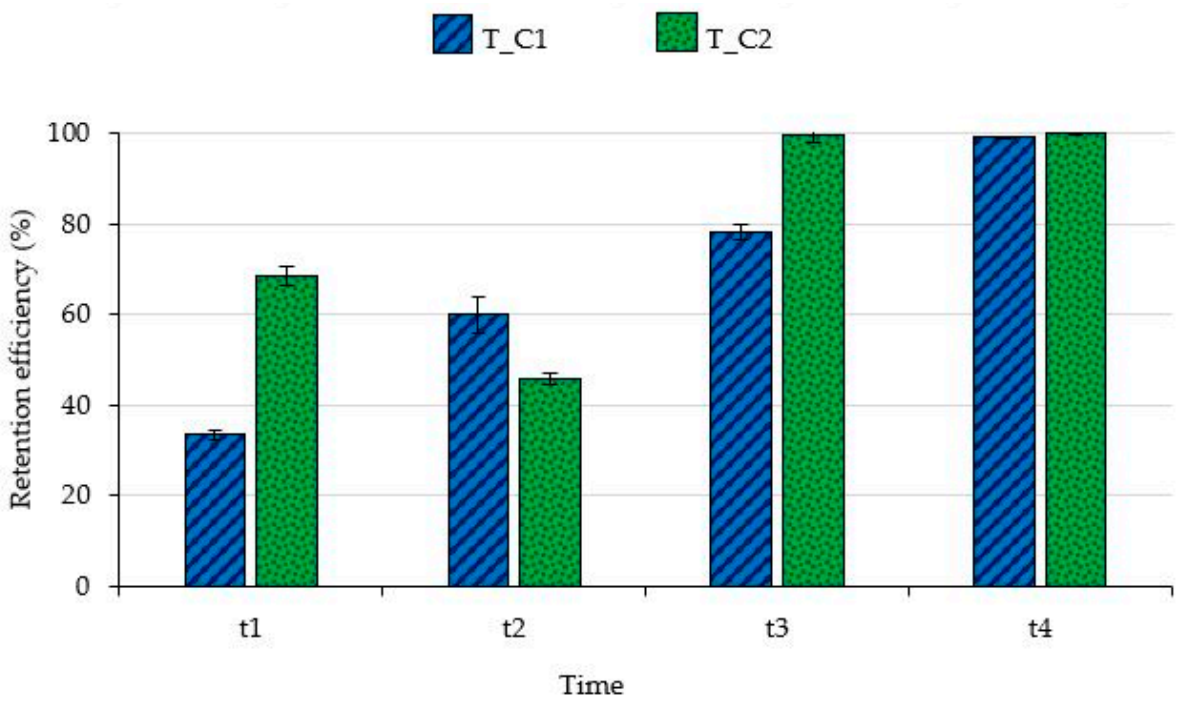


Figure 1. *Vibrio parhaemolyticus* concentrations (mean value ± standard error (SE)) in seawater calculated in the control and treatment tanks at the tested concentrations C1 and C2. PC_C1 = positive control at C1; T_C1 = treatment at C1; PC_C2 = positive control at C2; T_C2 = treatment at C2; SC = sponge control; NC = negative control.

This relationship was maintained throughout the experiment and the differences among treatments and controls continually increased. The pairwise comparison as a function of the *Vibrio* concentration highlighted a significant decrease over time in both treatments (Pairwise test, TC_C1: t0 > t1 > t2 > t3 >> t4 and TC_C2: t0 >> t1 = t2 >> t3 >> t4). At the end of the experiment, after 48 h (t4), the *Vibrio* concentrations showed the highest decrease in the treatment tanks, reaching values that were three (T_C1 = 67 ± 7) and four (T_C2 = 383 ± 41) orders of magnitude lower than the initial concentrations.

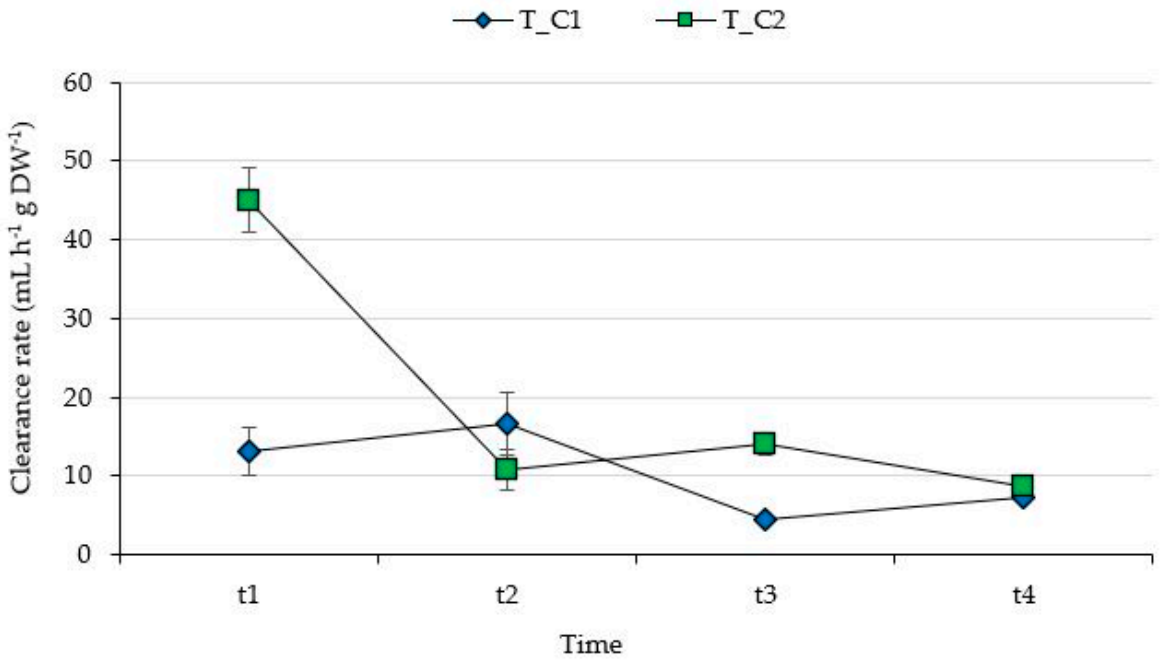
The *Vibrio* concentration increased in the control tanks (PC_C1 and PC_C2), reaching, at the end of the experiment, the same mean value ($4.9 \pm 0.1 \times 10^6$). In the negative control (NC), no bacterial colonies were registered during the experiment. As for the SC control tanks, the appearance of bacteria was observed starting from t1. The bacterial concentration in these tanks remained at lower levels (from 63 ± 23 at t1 to 25 ± 2.89 at t4) than each other tanks (five orders of magnitude lower than the PCs), without subsequent increases.

The retention efficiency of *S. spinosulus* in removing bacteria, calculated as a percentage change in the bacterial concentration between two successive times (retention efficiency = R), was affected by the initial concentration of bacteria (C), the time (t), and their interaction (t × C) (univariate PERMANOVA, df = 3, pseudo-F = 7.3315, p = 0.01). Significant differences were highlighted both between the two *Vibrio* concentrations and over time. At t1 (two hours from the beginning), R showed lower values in the T_C1 tanks compared to the T_C2 ones (t1:33.3% and 68.5%, respectively; pairwise test, T_C2 >> T_C1) (Figure 2a), and reached values close to 100% at t4 (48 h) (99.35% and 99.72% at C1 and C2, respectively). This latter value was reached for the T_C2 samples as early as t3 (24 h) (pairwise test t3: T_C1 << T_C2; t4: T_C1 = T_C2).



(**a**)

Figure 2. *Cont.*



(**b**)

Figure 2. Time course of (**a**) retention efficiency and (**b**) clearance rate of *Sarcotragus spinosulus* registered at both *V. parahaemolyticus* concentrations tested (C1 and C2). Error bars indicate standard errors.

The clearance rate was calculated considering the volume of water processed by the sponge for a certain time (CR: mL h^{-1} g DW^{-1} ± SE), as shown in Figure 2b. The maximum values of the clearance rates were recorded at t2 for T_C1 (16.6 ± 0.9) and at t1 for T_C2 (45.0 ± 4.1) (Pairwise test, t × C, on factor t: T_C1: t1 = t2 >> t3 < t4; T_C2: t1 >> t2 = t3 < t4). The mean value over a 48 h trial (t4) was 7.4 ± 0.2 at the lower *V. parahaemolyticus* concentration tested (T_C1) and 8.7 ± 0.9 at the higher concentration tested (T_C2). The relationship between the treatments (T_C1 and T_C2) is superimposable to that of retention efficiency. Statistical analysis revealed a significant relationship as a function of time (t) and of the interaction between time and the initial bacterial concentration (C) (univariate PERMANOVA, df = 3, pseudo-F = 3.5463, $p = 0.009$).

3.2. Nutrient Release

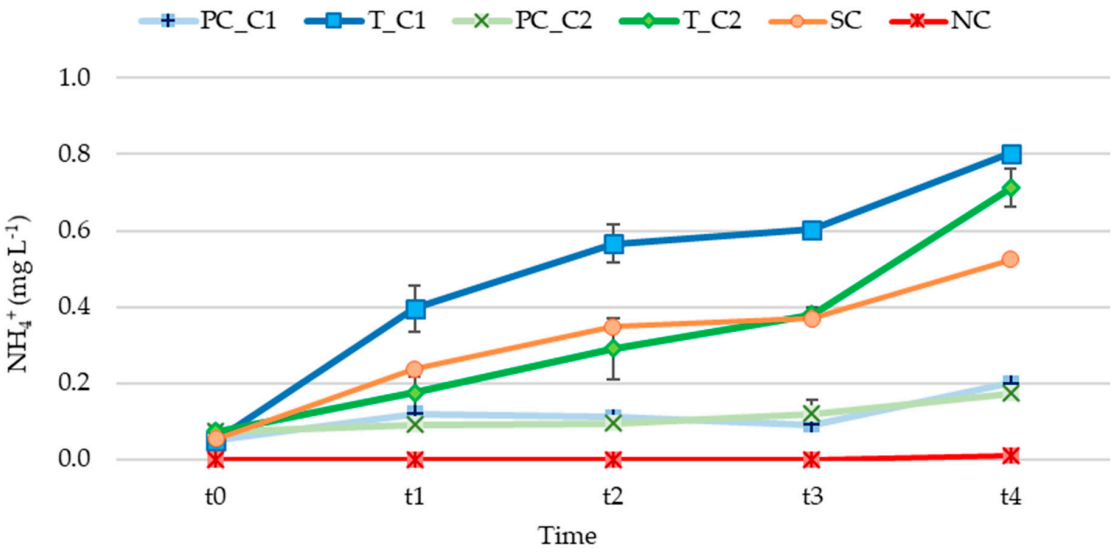
The nutrient release (mg L^{-1} ± SE) from *S. spinosulus* during the laboratory experiments showed low values for each considered nutrient category (NH_4^+, NO_3^-, and PO_4^{3-}), which were less than 1 mg L^{-1} for ammonium and phosphate and less than 5 mg L^{-1} for nitrate (Figure 4). The highest nutrient concentrations were found in each tank containing sponges (T_C1, T_C2, and SC), while, in each control tank (PC_C1, PC_C2, and NC), the nutrient values remained very low.

Regarding the ammonium, a considerable increase was recorded in the tanks containing the sponges (T_C1, T_C2, and SC), with final values (t4) higher than 0.5 mg L^{-1} (Figure 4a). Statistical analysis showed a significant relationship of NH_4^+ concentration for the factor time (t), concentration (C), and their interaction (t × C). The a posteriori test at t4 highlighted significant differences between all tanks containing sponges (Pairwise test: T_C1> T_C2 >> SC) and no difference between the positive controls (Pairwise test: PC_C1 = PC_C2). The highest ammonium concentrations were recorded in the T_C1 and T_C2 treatments (0.80 ± 0.01 and 0.71 ± 0.38, respectively) with T_C1 > T_C2. In the negative control (NC) samples, the NH_4^+ remained zero during the whole experiment.

The nitrates also showed a continuous increase over time in the tanks with sponges (T_C1, T_C2, and SC) while each other tank showed almost constant values, or a slight

decrease (Figure 4b). The NO_3^- concentration was affected by the initial concentration (C), time (t), and the interaction between the two factors (C × t) (univariate PERMANOVA, df = 8, pseudo-F = 10.094, $p = 0.001$). At the end of the experiment, the highest values were recorded in T_C1 (4.24 ± 0.24), greater than SC and T_C2 and the other tanks (pairwise test, t4: T_C1> SC> T_C2> NC = PC_C2 = PC_C1).

The phosphates showed a general increase over time (Figure 4c), albeit with some slight variations between times t2 and t3, reaching a maximum value near 0.20 mg L^{-1}. PERMANOVA highlighted significant differences as a function of time and in the interaction between the time and concentration (C × t) (univariate PERMANOVA, df = 8, pseudo-F = 11.367, $p = 0.0001$). The highest values at the end of the experiment (t4) were recorded in the SC samples (0.21 ± 0.01), followed by the two treatments T_C1 (0.18 ± 0.01) and T_C2 (0.15 ± 0.01), the negative control NC (0.09 ± 0.001), and the two positive controls PC_C2 and PC_C1 (pairwise test, t4: SC> T_C1> T_C2 >> NC >> PC_C2 = PC_C1).



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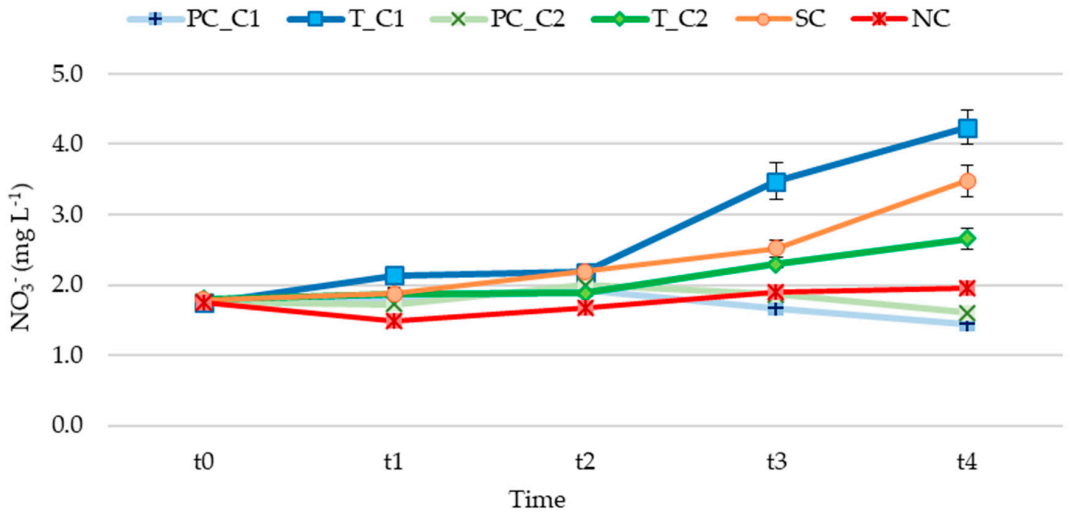

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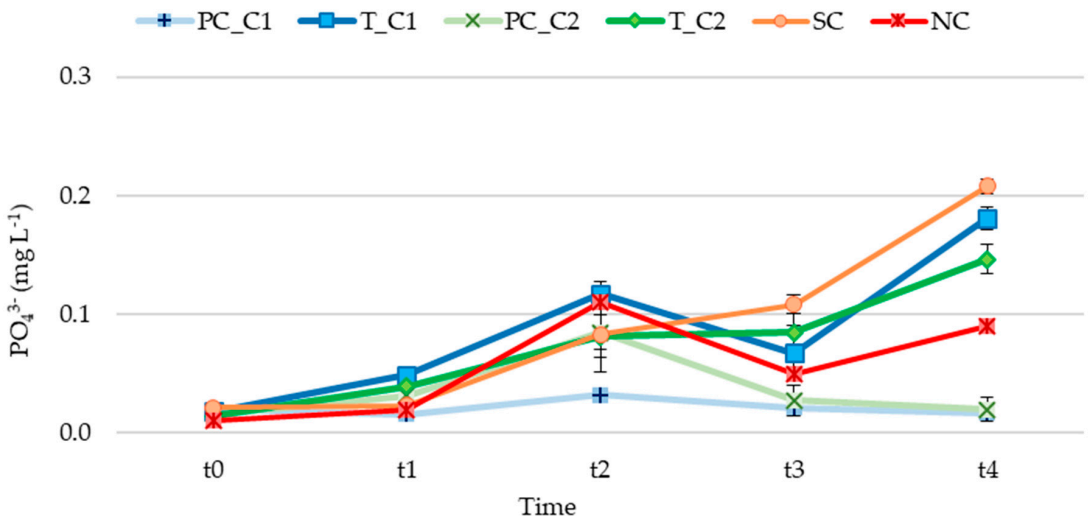
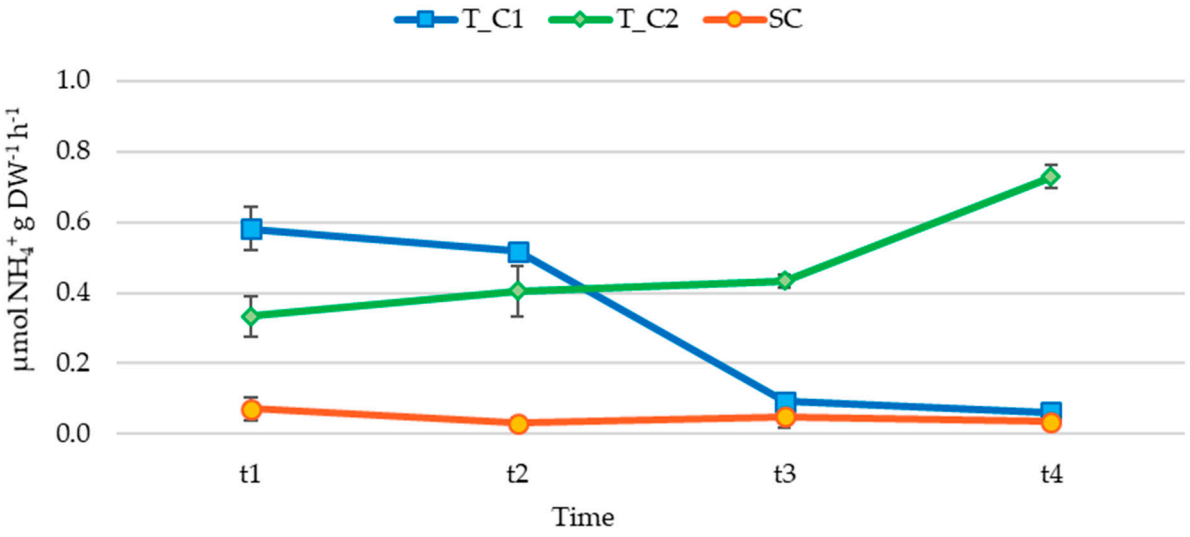
Figure 3. *Cont.*



(**c**)

Figure 3. Changes of nutrient concentrations: ammonium (**a**), nitrate (**b**), and phosphate (**c**) found in the seawater of each tank during the laboratory experiment at each sampling time (t0, t1, t2, t3, and t4), at different *V. parahaemolyticus* concentrations (C1 and C2) and in absence of *Vibrio*. PC_C1 = positive control at C1; T_C1 = treatment at C1; PC_C2 = positive control at C2; T_C2 = treatment at C2; SC = sponge control; NC = negative control.

The excretion rate (E, µmol g DW^{-1} h^{-1}) of nutrients by *S. spinosulus*—calculated by relating the concentration of nutrients, the sponge biomass, and the processed water—is given in Figure 4. The highest values were recorded for ammonium (0.73 at t4), followed by nitrates (0.23 at t1) and phosphates (0.02 at t2). A decrease in E was observed for the ammonium and nitrates during the experiment, except for ammonium in T_C2, which contrarily showed an increase (Figure 4).



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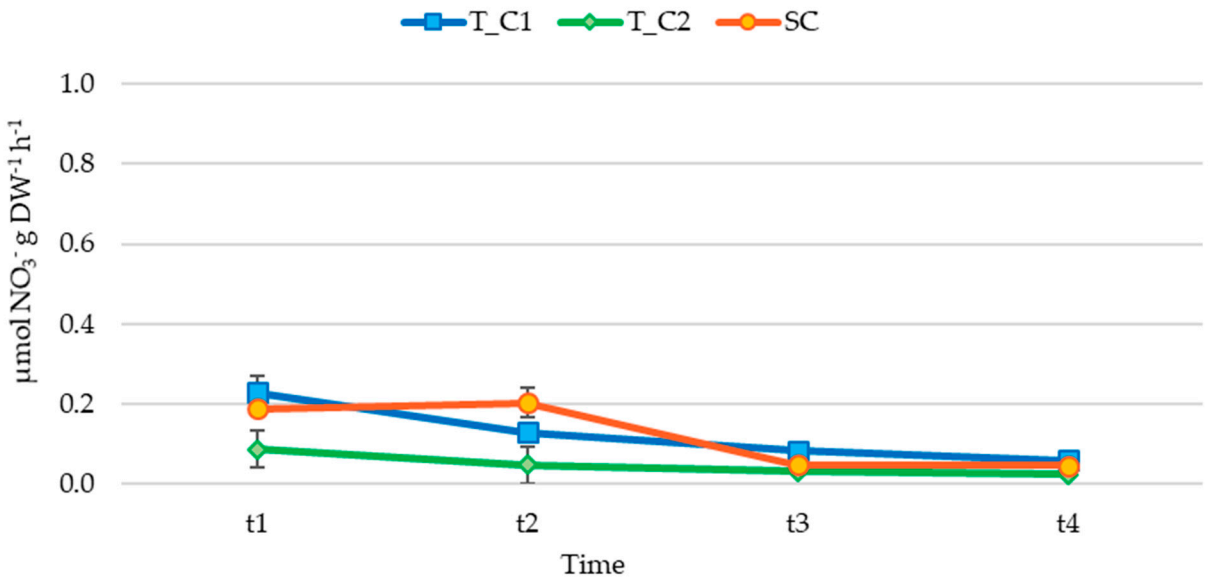

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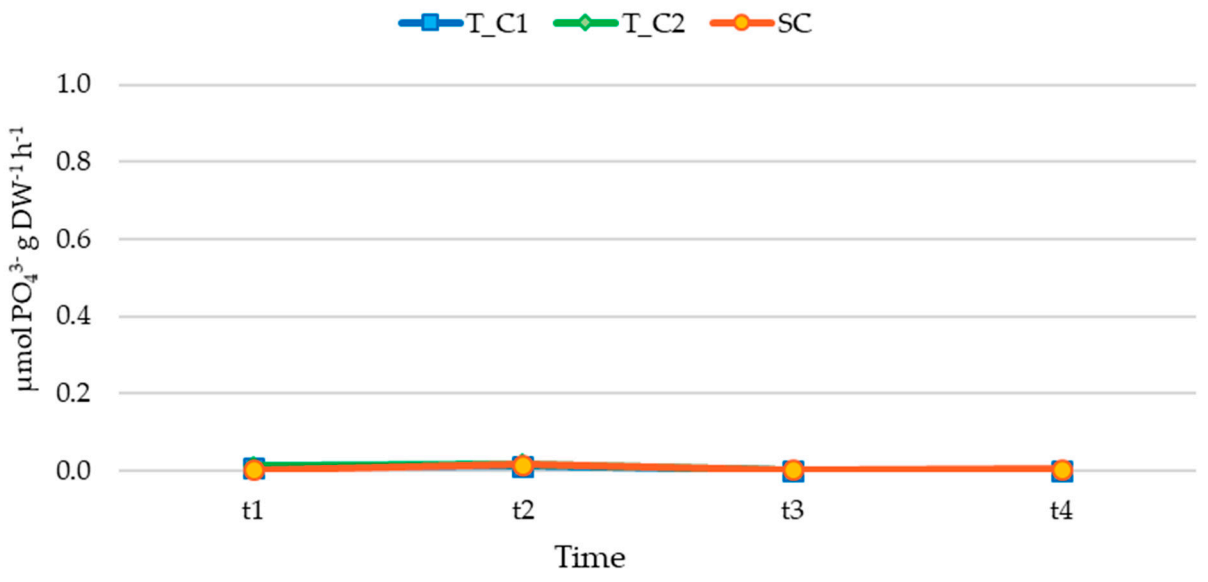

(**c**)

Figure 4. Excretion rate for ammonium (**a**), nitrate (**b**), and phosphate (**c**) in the tanks containing sponges (mean value ± SE) during the laboratory experiment at both *V. parahaemolyticus* concentrations tested (C1 and C2).

4. Discussion and Conclusions

The polyculture of fish with organisms at different food web levels has considerable environmental and economic potential, particularly if edible and/or non-edible species with a potentially high commercial value are co-cultured. Among the non-edible organisms, Porifera (sponges) represents a valuable candidate due to its key role in organic matter recycling and sustainable and commercially appealing biomass production [45].

The filtering activity and nutrient release by *S. spinosulus* obtained in the present study demonstrate that this species represents a valuable candidate in microbial bioremediation, showing the efficient capability in removing *V. parahaemolyticus* from seawater in the laboratory experiment. The results obtained showed that *S. spinosulus* effectively controlled

the growth of *V. parahaemolyticus* in the laboratory experiment. This finding is rather appealing due to the high pathogenicity of *Vibrio* spp. for both humans and aquaculture animals. *V. parahaemolyticus,* indeed, is a human bacterial pathogen widely occurring in marine environments, frequently isolated from a variety of seafood, including bivalves, crustaceans, and fish [67]. Vibriosis is currently responsible for most disease outbreaks in aquaculture [31–34,68].

Although the release of Vibrionaceae found during our experiment in sponge control tanks could question their potential application for bioremediation by appearing to be a problem rather than a solution, as questioned by some authors [69], the recorded concentrations in the sponge control tanks are negligible compared to the treatments (four/six orders of magnitude lower), and are likely related to the response of the specimens to the experimental conditions. We emphasized, however, that these bacteria appeared after two hours from the beginning of the experiment and remained at low concentrations up to the end. The final balance between the bacteria removed and those that appeared is strongly in favor of removal.

The retention efficiency (R) values found in the present experiment represent a further encouragement to the use of this sponge in aquaculture. Our findings showed R values up to 99.72%, in line with further studies that reported retention efficiencies ranging from 70% to 99% for small suspended particles such as *Vibrio* spp. [4,11,42,70–72]. Our experiment showed that the retention efficiency increased gradually, reaching the maximum value after 48 h at C1 and after 24 h at C2. This latter evidence indicates that the retention of *Vibrio* cells by *S. spinosulus* is positively related to their greater availability in the experimental tanks.

The clearance rate (CR), indicating a measure of the food depletion as a function of time, the cleared water volume, and the sponge size [10,59], estimated at both *Vibrio* concentrations tested, demonstrated the worthy filtering performances of *S. spinosulus.* The highest CR value was registered at the highest *Vibrio* concentration (C2), with values ranging between 8.7 and 45.0 mL g DW^{-1} h^{-1}. The highest value was quickly observable two hours after the start of the experiment highlighting that, as for R, the increased availability of *Vibrio* positively affected the rate of bacterial concentration change for this sponge. *S. spinosulus,* at its maximum filtering activity, was able to clean up a water volume of 17 times its volume in 1 h.

Although the comparison of the CR between different sponge species is challenging due to the intraspecies variability, the effect of sponge size, the morpho-physiological features, and the different units in which CR are expressed [4,25,73], our results are comparable with those reported for other Mediterranean species (Supplementary Table S1). A careful analysis of the results requires further considerations related to the characteristics of the tested sponge. *S. spinosulus* is attributed to high microbial abundance sponge species (HMA), hosting an abundant and diversified microbial community (two/four magnitude orders of bacteria per gram of sponge tissue higher than the surrounding seawater) and lower pumping rate than low microbial abundant (LMA) species [5,74].

The CR found here is of the same order of magnitude reported for Mediterranean HMA sponge species at a comparable investigation time and sponge size (Supplementary Table S1) [5,25]. Although the specimens of *S. spinosulus* used in our experiment were larger than the closely related Mediterranean species compared, such as *Ircinia variabilis* and *Spongia officinalis,* the CR here found is in line with or greater than that measured at a comparable time evaluation (Supplementary Table S1).

In the present study, the nutrient release (ammonium—NH_4^+, nitrate—NO_3^-, and phosphate—PO_4^{-3}) from *S. spinosulus* was measured for its contribution to the nutrient overload in the surrounding seawater. Our results are in line with those reported in the literature, confirming, as for other Mediterranean demosponges, the behavior of the studied HMA sponge *S. spinosulus* as a nutrient source (Supplementary Table S2) [15,75].

The major contribution is due to the release of ammonium with a positive relationship with the availability of bacteria in the tanks. For nitrogen, the final ammonium release (t4) was about eight times higher than the initial one, while the nitrates showed only a doubling

of values. At the low bacterial experimental concentration (C1), a higher ammonium and nitrate release was observed with respect to the treatment C2 and SC (sponge control), likely linked to the decrease of bacteria in the water due to the sponge filtering activity. The contribution of the sponge in the release of phosphates in any experimental condition was negligible.

Metabolic processes in sponges occur at the cellular level and cannot be neglected in the role of the associated microbial community in the sponge metabolic balance. Large metabolic differences (filtration rates, nutrient flow, etc.) between LMA and HMA sponges were documented [15]. Sponges feed on both particulate organic matter (POM) and dissolved organic matter (DOM). Some sponge species have high value for use in IMTA due to their ability to convert DOM into POM, making it available for other suspension feeders and detritivores [45,76]. Among POM can be counted several types of planktonic cells: primarily picoplankton (mainly bacterioplankton and phytoplankton) and partly nanoplankton (e.g., diatoms), but also non-living particles (i.e., debris).

As for DOM, the ability of sponges to remove or release dissolved organic or inorganic compounds depends on photoautotrophic and chemotrophic processes mediated by the associated microbial community (such as archaea, bacteria, cyanobacteria, yeasts, and also diatoms) [15]. The POM utilization produces dissolved organic compounds, ammonium, and phosphate, which can be released directly into the water but can be transformed in the processes of nitrification, photoautotrophy, denitrification, and/or anammox (oxidation of ammonia in the absence of O2) by the associated microbiomes particularly in HMA species [15,77–80].

In addition, the sponge microbiota can influence whether the holobiont acts as a net source or sink of bioavailable nitrogen [81,82] and can be capable of releasing nitrogen at ecologically relevant values in oligotrophic marine environments [83,84]. Regarding the phosphorus flow, sponges are considered as sources of PO_3^+ independently of whether they are HMA or LMA species; moreover, research demonstrated the ability to store intracellular polyphosphate granules in three reef species mediated through symbiotic microorganisms [75,85]. Therefore, sponges with their microbiota can affect both the quantity and speciation of inorganic nutrients, making them available to nearby primary producers [77].

Our findings showed that *S. spinosulus* (attributed to HMA) acts as a source of inorganic nitrogen since, due to its microbiota, the ammonium produced in the metabolic processes is nitrified to NOx [51]. This nitrogen availability could facilitate the growth of primary producers, such as phytoplankton, which can be exploited by further filter feeders, such as bivalves or could be utilized by seaweed, thus, underlining its utility in IMTA systems.

The sponges grown in IMTA carry out their filtration activity by removing both the organic matter from the water and harmful particles (bacteria, viruses, and also fecal pellets) and converting these into food for other invertebrates, operating an important bypass from DOM to POM. Some of these bacteria could be pathogenic for both fish and humans; thus, their removal could represent a useful tool for reducing the use of antibiotics in aquaculture, acting in parallel for good environmental quality, on the organoleptic qualities of the farmed products and on the problem of antibiotic resistance. Considering the rapid expansion of the aquaculture sector, combining complementary filter-feeder macroinvertebrates, such as sponges with traditional mariculture, could allow aquaculture to reach environmental sustainability of mariculture, minimizing the microbial impacts.

In conclusion, we provided evidence that *S. spinosulus* is able to remove the inoculated *V. parahaemolythicus* from seawater in test tanks at different concentrations, showing better performance at the higher concentration, with a contribution to the nutrient load. The promising survival and growth performance already obtained by this species in a Mediterranean IMTA system [49] highlights the ability of this sponge species to withstand the environmental conditions of an aquaculture facility. In addition, the biomass obtained [49] appears to be sufficient to implement the rearing system over time, thus avoid-

ing ethical problems due to the depletion of wild stocks. *S. spinosulus* represents an effective mediator and bioremediator in integrated multitrophic aquaculture systems.

Supplementary Materials: The following are available online at https://www.mdpi.com/2077-1312/9/2/178/s1, Table S1: Clearance rate (CR, mean ± SE) of Mediterranean sponges in different experimental conditions, Table S2: Excretion rate by Mediterranean sponge species measured under ex situ conditions.

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References

1. Bell, J.J.; Mcgrath, E.; Biggerstaff, A.; Bates, T.; Cárdenas, C.A.; Bennett, H. Global conservation status of sponges. *Conserv. Biol.* **2015**, *29*, 42–53. [CrossRef]
2. Van Soest, R.W.M.; Boury-Esnault, N.; Vacelet, J.; Dohrmann, M.; Erpenbeck, D.; de Voogd, N.J.; Santodomingo, N.; Vanhoorne, B.; Kelly, M.; Hooper, J.N.A. Global diversity of sponges (Porifera). *PLoS ONE* **2012**, *7*, e35105. [CrossRef] [PubMed]
3. Reiswig, H.M. *In Situ Feeding in Two Shallow Water Hexactinellid Sponges*; Rützler, K., Ed.; Smithsonian Institution Press: Washington, DC, USA, 1990.
4. Ribes, M.; Coma, R.; Gili, J.M. Natural diet and grazing rate of the temperate sponge Dysidea avara (Demospongiae, Dendroceratida) throughout an annual cycle. *Mar. Ecol. Prog. Ser.* **1999**, *176*, 179–190. [CrossRef]
5. Weisz, J.B.; Lindquist, N.; Martens, C.S. Do associated microbial abundances impact marine demosponge pumping rates and tissue densities? *Oecologia* **2008**, *155*, 367–376. [CrossRef] [PubMed]
6. Reiswig, H.M. Bacteria as food for temperate-water marine sponges. *Can. J. Zool.* **1975**, *53*, 582–589. [CrossRef]
7. Wilkinson, C.R. Microbial associations in sponges. I. Ecology, physiology and microbial populations of coral reef sponges. *Mar. Biol.* **1978**, *49*, 161–167. [CrossRef]
8. Simpson, T.L. *The Cell Biology of Sponges*; Springer: New York, NY, USA, 1984.
9. Larsen, P.S.; Riisgård, H.U. The sponge pump. *J. Theor. Biol.* **1994**, *168*, 53–63. [CrossRef]
10. Riisgård, H.; Larsen, P. Filter-feeding in marine macro-invertebrates: Pump characteristics, modelling and energy cost. *Biol. Rev.* **1995**, *70*, 67–106. [CrossRef]
11. Pile, A.J.; Patterson, M.R.; Witman, J.D. In situ grazing on plankton <10 µm by the boreal sponge. *Mar. Ecol. Prog. Ser.* **1996**, *141*, 95–102. [CrossRef]
12. Hadas, E.; Marie, D.; Shpigel, M.; Ilan, M. Virus predation by sponges is a new nutrient-flow pathway in coral reef food webs. *Limnol. Oceanogr.* **2006**, *51*, 1548–1550. [CrossRef]
13. Ribes, M.; Coma, R.; Atkinson, M.J.; Kinzie, R.A. Sponges and ascidians control removal of particulate organic nitrogen from coral reef water. *Limnol. Oceanogr.* **2005**, *50*, 1480–1489. [CrossRef]
14. de Goeij, J.M.; van Oevelen, D.; Vermeij, M.J.A.; Osinga, R.; Middelburg, J.J.; de Goeij, A.F.P.M.; Admiraal, W. Surviving in a marine desert: The sponge loop retains resources within coral reefs. *Science* **2013**, *342*, 108–110. [CrossRef]
15. Maldonado, M.; Ribes, M.; van Duyl, F.C. *Nutrient Fluxes Through Sponges. Biology, Budgets, and Ecological Implications*, 1st ed.; Elsevier Ltd.: Amsterdam, The Netherlands, 2012; Volume 62, ISBN 9780123942838.
16. Van de Vyver, G.; Vray, B.; Belaouane, S.; Touussaint, D. *Efficiency and Selectivity of Microorganism Retention by Ephydatia fluviatilis*; New Perspe; Rutzer, K., Ed.; Smithsonian Institution Press: Washington, DC, USA, 1990.
17. Gifford, S.; Dunstan, R.H.; O'Connor, W.; Koller, C.E.; MacFarlane, G.R. Aquatic zooremediation: Deploying animals to remediate contaminated aquatic environments. *Trends Biotechnol.* **2007**, *25*, 60–65. [CrossRef]
18. Pronzato, R.; Bavestrello, G.; Cerrano, C. Morpho-functional adaptations of three species of Spongia (Porifera, Demospongiae) from a Mediterranean vertical cliff. *Bull. Mar. Sci.* **1998**, *63*, 317–328.
19. Milanese, M.; Chelossi, E.; Manconi, R.; Sarà, A.; Sidri, M.; Pronzato, R. The marine sponge Chondrilla nucula Schmidt, 1862 as an elective candidate for bioremediation in integrated aquaculture. *Biomol. Eng.* **2003**, *20*, 363–368. [CrossRef]
20. Fu, W.; Sun, L.; Zhang, X.; Zhang, W. Potential of the marine sponge Hymeniacidon perleve as a bioremediator of pathogenic bacteria in integrated aquaculture ecosystems. *Biotechnol. Bioeng.* **2006**, *93*, 1112–1122. [CrossRef]

21. Fu, W.; Wu, Y.; Sun, L.; Zhang, W. Efficient bioremediation of total organic carbon (TOC) in integrated aquaculture system by marine sponge Hymeniacidon perleve. *Biotechnol. Bioeng.* **2007**, *97*, 1387–1397. [CrossRef] [PubMed]
22. Osinga, R.; Sidri, M.; Cerig, E.; Gokalp, S.Z.; Gokalp, M. Sponge aquaculture trials in the East-Mediterranean Sea: New approaches to earlier ideas. *Open Mar. Biol. J.* **2010**, *4*, 74–81. [CrossRef]
23. Zhang, X.; Zhang, W.; Xue, L.; Zhang, B.; Jin, M.; Fu, W. Bioremediation of bacteria pollution using the marine sponge Hymeniacidon perlevis in the intensive mariculture water system of turbot Scophthalmus maximus. *Biotechnol. Bioeng.* **2010**, *105*, 59–68. [CrossRef]
24. Ledda, F.D.; Pronzato, R.; Manconi, R. Mariculture for bacterial and organic waste removal: A field study of sponge filtering activity in experimental farming. *Aquac. Res.* **2014**, *45*, 1389–1401. [CrossRef]
25. Gökalp, M.; Kooistra, T.; Rocha, M.S.; Silva, T.H.; Osinga, R.; Murk, A.J.; Wijgerde, T. The Effect of Depth on the Morphology, Bacterial Clearance, and Respiration of the Mediterranean Sponge Chondrosia reniformis (Nardo, 1847). *Mar. Drugs* **2020**, *18*, 358. [CrossRef] [PubMed]
26. Stabili, L.; Licciano, M.; Longo, C.; Corriero, G.; Mercurio, M. Evaluation of microbiological accumulation capability of the commercial sponge Spongia officinalis var. adriatica (Schmidt) (Porifera, Demospongiae). *Water Res.* **2008**, *42*, 2499–2506. [CrossRef] [PubMed]
27. Pronzato, R.; Bavestrello, G.; Cerrano, C.; Magnino, G.; Manconi, R.; Pantelis, J.; Sarà, A.; Sidri, M. Sponge farming in the Mediterranean Sea: New perspectives. *Mem. Qld. Mus.* **1999**, *44*, 485–491.
28. Corsi, I.; Iacocca, A.; Mercurio, M.; Longo, C.; Giangrande, A.; Pierri, C.; Lembo, G.; Spedicato, M.; Focardi, S. Promising extractive species for integrated mariculture system: Preliminary results on resistance to organophosphate insecticides. *Eur. Aquac. Soc.* **2004**, *34*, 247–248.
29. Pronzato, R. Sponge-fishing, disease and farming in the Mediterranean Sea. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **1999**, *9*, 485–493. [CrossRef]
30. Nemoy, P.; Ehud, S.D.A. Sustainable cultivation of sponges in the Eastern Mediterranean Sea: Integrated aquaculture with fish farms. In Proceedings of the 10th World Sponge Conference, Book of Abstract. Galway, Ireland, 25–30 June 2017; p. 89.
31. Benediktsdóttir, E.; Helgason, S.; Sigurjónsdóttir, H. Vibrio spp. isolated from salmonids with shallow skin lesions and reared at low temperature. *J. Fish. Dis.* **1998**, *21*, 19–28. [CrossRef]
32. Hann, P.J.; Altmann, K.; Chen, D.; Smith, A.; Cosic, S.; Moon, P.; Hammond, L.S. Development of monoclonal antibodies for the rapid identification of epizootic Vibrio species. *J. Fish. Dis.* **1992**, *15*, 63–69. [CrossRef]
33. Silva-Aciares, F.; Moraga, D.; Auffret, M.; Tanguy, A.; Riquelme, C. Transcriptomic and cellular response to bacterial challenge (pathogenic Vibrio parahaemolyticus) in farmed juvenile Haliotis rufescens fed with or without probiotic diet. *J. Invertebr. Pathol.* **2013**, *113*, 163–176. [CrossRef]
34. Sung, H.H.; Li, H.C.; Tsai, F.M.; Ting, Y.Y.; Chao, W.L. Changes in the composition of Vibrio communities in pond water during tiger shrimp (Penaeus monodon) cultivation and in the hepatopancreas of healthy and diseased shrimp. *J. Exp. Mar. Bio. Ecol.* **1999**, *236*, 261–271. [CrossRef]
35. Almeida, A.; Cunha, Â.; Gomes, N.C.M.; Alves, E.; Costa, L.; Faustino, M.A.F. Phage therapy and photodynamic therapy: Low environmental impact approaches to inactivate microorganisms in fish farming plants. *Mar. Drugs* **2009**, *7*, 268–313. [CrossRef] [PubMed]
36. Noya, M.; Magariños, B.; Lamas, J. Interactions between peritoneal exudate cells (PECs) of gilthead seabream (Sparus aurata) and Pasteurella piscicida. A morphological study. *Aquaculture* **1995**, *131*, 11–21. [CrossRef]
37. Toranzo, A.E.; Barreiro, S.; Casal, J.F.; Figueras, A.; Magarin˜os, B.; Barja, J.L. Pasteurellosis in cultured gilthead seabream (Sparus aurata): First report in Spain. *Aquaculture* **1991**, *99*, 1–15. [CrossRef]
38. Reilly, A.; Käferstein, F. Food safety hazards and the application of the principles of the hazard analysis and critical control point (HACCP) system for their control in aquaculture production. *Aquac. Res.* **1997**, *28*, 735–752. [CrossRef]
39. Aresta, A.; Marzano, C.N.; Lopane, C.; Corriero, G.; Longo, C.; Zambonin, C.; Stabili, L. Analytical investigations on the lindane bioremediation capability of the demosponge Hymeniacidon perlevis. *Mar. Pollut. Bull.* **2015**, *90*, 143–149. [CrossRef]
40. Longo, C.; Corriero, G.; Licciano, M.; Stabili, L. Bacterial accumulation by the Demospongiae Hymeniacidon perlevis: A tool for the bioremediation of polluted seawater. *Mar. Pollut. Bull.* **2010**, *60*, 1182–1187. [CrossRef] [PubMed]
41. Longo, C.; Cardone, F.; Corriero, G.; Licciano, M.; Pierri, C.; Stabili, L. The co-occurrence of the demosponge Hymeniacidon perlevis and the edible mussel Mytilus galloprovincialis as a new tool for bacterial load mitigation in aquaculture. *Environ. Sci. Pollut. Res.* **2015**, *23*, 3736–3746. [CrossRef]
42. Stabili, L.; Licciano, M.; Giangrande, A.; Longo, C.; Mercurio, M.; Marzano, C.N.; Corriero, G. Filtering activity of Spongia officinalis var. adriatica (Schmidt) (Porifera, Demospongiae) on bacterioplankton: Implications for bioremediation of polluted seawater. *Water Res.* **2006**, *40*, 3083–3090. [CrossRef] [PubMed]
43. Ledda, F.D.; Manconi, R.; Pronzato, R. Retention rates on bacteria and organic matter by Ircinia variabilis (Demospongiae, Dictyoceratida) in experimental sponge farming for bioremediation. *Biol. Mar. Mediterr.* **2008**, *15*, 164–165.
44. Madri, P.P.; Claus, G.; Kunen, S.M.; Moss, E.E. Preliminary studies on the Escherichia coli uptake of the redbeard sponge Microciona prolifera (Verrill). *Life Sci.* **1967**, *6*, 889–894. [CrossRef]
45. Gökalp, M.; Mes, D.; Nederloff, M.; Zhao, H.; de Goeij, J.M.; Osinga, R. The potential roles of sponges in integrated mariculture. *Rev. Aquac.* **2020**. [CrossRef]

46. Pronzato, R.; Manconi, R.; Corriero, G. Tipologie di Impianto Modulare per la Spongicoltura Subacquea Anche in Policoltura USAMA (Underwater Sponge Aquacolture Modular System). Italian Ministry Patent 0001334230, 2006.
47. Corriero, G.; Longo, C.; Mercurio, M.; Marzano, C.N.; Lembo, G.; Spedicato, M.T. Rearing performance of Spongia officinalis on suspended ropes off the Southern Italian Coast (Central Mediterranean Sea). *Aquaculture* **2004**, *238*, 195–205. [CrossRef]
48. Gifford, S.; Dunstan, R.H.; O'Connor, W.; Roberts, T.; Toia, R. Pearl aquaculture-profitable environmental remediation? *Sci. Total Environ.* **2004**, *319*, 27–37. [CrossRef]
49. Giangrande, A.; Pierri, C.; Arduini, D.; Borghese, J.; Licciano, M.; Trani, R.; Corriero, G.; Basile, G.; Cecere, E.; Petrocelli, A.; et al. An innovative IMTA system: Polychaetes, sponges and macroalgae co-cultured in a Southern Italian in-shore mariculture plant (Ionian Sea). *J. Mar. Sci. Eng.* **2020**, *8*, 733. [CrossRef]
50. Pérez-López, P.; Ledda, F.D.; Bisio, A.; Feijoo, G.; Perino, E.; Pronzato, R.; Manconi, R.; Moreira, M.T. Life cycle assessment of in situ mariculture in the Mediterranean Sea for the production of bioactive compounds from the sponge Sarcotragus spinosulus. *J. Clean. Prod.* **2017**, *142*, 4356–4368. [CrossRef]
51. Hardoim, C.C.P.; Costa, R. Temporal dynamics of prokaryotic communities in the marine sponge Sarcotragus spinosulus. *Mol. Ecol.* **2014**, *23*, 3097–3112. [CrossRef] [PubMed]
52. Abed, C.; Legrave, N.; Dufies, M.; Robert, G.; Guérineau, V.; Vacelet, J.; Auberger, P.; Amade, P.; Mehiri, M. A new hydroxylated nonaprenylhydroquinone from the mediterranean marine sponge Sarcotragus spinosulus. *Mar. Drugs* **2011**, *9*, 1210. [CrossRef] [PubMed]
53. Cimino, G.; De Stefano, S.; Minale, L.; Fattorusso, E. Ircinin-1 and -2, linear sesterterpenes from the marine sponge Ircinia oros. *Tetrahedron* **1972**, *28*, 333–341. [CrossRef]
54. Mercurio, M.; Scalera Liaci, L.; Corriero, G. La fauna a poriferi del bacino della Strea di Porto Cesareo (LE). *Biol. Mar. Mediterr.* **2001**, *8*, 403–412.
55. Corriero, G. Distribuzione ed Ecologia dei Poriferi in Ambienti 'Confinati Mediterranei'. Ph.D. Thesis, University of Genova, Genova, Italy, 1990.
56. Corriero, G.; Gherardi, M.; Giangrande, A.; Longo, C.; Mercurio, M.; Musco, L.; Marzano, C.N. Inventory and distribution of hard bottom fauna from the marine protected area of porto cesareo (ionian sea): Porifera and polychaeta. *Ital. J. Zool.* **2004**, *71*, 237–245. [CrossRef]
57. Mercurio, M.; Corriero, G.; Gherardi, M.; Baldacconi, R.; Elda, G. Sexual reproduction in Sarcotragus spinosulus from two different shallow environments. *Mar. Ecol.* **2013**, *34*, 394–408. [CrossRef]
58. Ottaviani, D.; Leoni, F.; Rocchegiani, E.; Santarelli, S.; Masini, L.; D'Annibale, M.L.; Pianetti, A.; Carraturo, A. A severe case of aeromonas veronii biovar sobria travellers' diarrhoea characterized by vibrio parahaemolyticus co-isolation. *J. Med. Microbiol.* **2013**, *62*, 161–164. [CrossRef] [PubMed]
59. Coughlan, J. The estimation of filtering rate from the clearance of suspensions. *Mar. Biol.* **1969**, *2*, 356–358. [CrossRef]
60. Neori, A.; Shpigel, M.; Ben-Ezra, D. A sustainable integrated system for culture of fish, seaweed and abalone. *Aquaculture* **2000**, *186*, 279–291. [CrossRef]
61. Sanz-Lázaro, C.; Marin, A. Benthic recovery during open sea fish farming abatement in Western Mediterranean, Spain. *Mar. Environ. Res.* **2006**, *62*, 374–387. [CrossRef]
62. Carvalho, A.P.; Meireles, L.A.; Malcata, F.X. Rapid spectrophotometric determination of nitrates and nitrites in marine aqueous culture media. *Analysis* **1998**, *26*, 347–351. [CrossRef]
63. Strickland, J.D.H.; Parsons, T.R. *A Practical Handbook of Seawater Analysis*; the Alger Press L.T.D: Ottawa, ON, Canada, 1972.
64. De Goeij, J.M.; De Kluijver, A.; Van Duyl, F.C.; Vacelet, J.; Wijffels, R.H.; De Goeij, A.F.R.M.; Cleutjens, J.P.M.; Schutte, B. Cell kinetics of the marine sponge Halisarca caerulea reveal rapid cell turnover and shedding. *J. Exp. Biol.* **2009**, *212*, 3892–3900. [CrossRef]
65. Anderson, M.J. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* **2001**, *26*, 32–46. [CrossRef]
66. Anderson, M.J.; Gorley, R.N.; Clarke, K.R. *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*; PRIMER-E Ltd.: Plymouth, UK, 2008.
67. Su, Y.C.; Liu, C. Vibrio parahaemolyticus: A concern of seafood safety. *Food Microbiol.* **2007**, *24*, 549–558. [CrossRef]
68. Colwell, R.R.; Grimes, D.J. Vibrio diseases of marine fish populations. *Helgol. Mar. Res.* **1984**, *37*, 265–287. [CrossRef]
69. Maldonado, M.; Zhang, X.; Cao, X.; Xue, L.; Cao, H.; Zhang, W. Selective feeding by sponges on pathogenic microbes: A reassessment of potential for abatement of microbial pollution. *Mar. Ecol. Prog. Ser.* **2010**, *403*, 75–89. [CrossRef]
70. Reiswig, H.M. Particle Feeding in Natural Populations of Three Marine Demosponges. *Biol. Bull.* **1971**, *141*, 568–591. [CrossRef]
71. Topçu, N.E.; Pérez, T.; Grégori, G.; Harmelin-Vivien, M. In situ investigation of Spongia officinalis (Demospongiae) particle feeding: Coupling flow cytometry and stable isotope analysis. *J. Exp. Mar. Bio. Ecol.* **2010**, *389*, 61–69. [CrossRef]
72. Duckworth, A.R.; Brück, W.M.; Janda, K.E.; Pitts, T.P.; McCarthy, P.J. Retention efficiencies of the coral reef sponges Aplysina lacunosa, Callyspongia vaginalis and Niphates digitalis determined by Coulter counter and plate culture analysis. *Mar. Biol. Res.* **2006**, *2*, 243–248. [CrossRef]
73. Reiswig, H.M. Water transport, respiration and energetics of three tropical marine sponges. *J. Exp. Mar. Bio. Ecol.* **1974**, *14*, 231–249. [CrossRef]
74. Hardoim, C.C.P.; Esteves, A.I.S.; Pires, F.R.; Gonçalves, J.M.S.; Cox, C.J.; Xavier, J.R.; Costa, R. Phylogenetically and Spatially Close Marine Sponges Harbour Divergent Bacterial Communities. *PLoS ONE* **2012**, *7*, e53029. [CrossRef]

75. Jiménez, E.; Ribes, M. Sponges as a source of dissolved inorganic nitrogen: Nitrification mediated by temperate sponges. *Limnol. Oceanogr.* **2007**, *52*, 948–958. [CrossRef]
76. Rix, L.; De Goeij, J.M.; Van Oevelen, D.; Struck, U.; Al-Horani, F.A.; Wild, C.; Naumann, M.S. Reef sponges facilitate the transfer of coral-derived organic matter to their associated fauna via the sponge loop. *Mar. Ecol. Prog. Ser.* **2018**, *589*, 85–96. [CrossRef]
77. Pita, L.; Rix, L.; Slaby, B.M.; Franke, A.; Hentschel, U. The sponge holobiont in a changing ocean: From microbes to ecosystems. *Microbiome* **2018**, *6*, 46. [CrossRef]
78. Hoffmann, F.; Radax, R.; Woebken, D.; Holtappels, M.; Lavik, G.; Rapp, H.T.; Schläppy, M.L.; Schleper, C.; Kuypers, M.M.M. Complex nitrogen cycling in the sponge Geodia barretti. *Environ. Microbiol.* **2009**, *11*, 2228–2243. [CrossRef]
79. Fiore, C.L.; Labrie, M.; Jarett, J.K.; Lesser, M.P. Transcriptional activity of the giant barrel sponge, Xestospongia muta Holobiont: Molecular evidence for metabolic interchange. *Front. Microbiol.* **2015**, *6*, 364. [CrossRef]
80. Schläppy, M.L.; Schöttner, S.I.; Lavik, G.; Kuypers, M.M.M.; de Beer, D.; Hoffmann, F. Evidence of nitrification and denitrification in high and low microbial abundance sponges. *Mar. Biol.* **2010**, *157*, 593–602. [CrossRef] [PubMed]
81. Fiore, C.L.; Baker, D.M.; Lesser, M.P. Nitrogen Biogeochemistry in the Caribbean Sponge, Xestospongia muta: A Source or Sink of Dissolved Inorganic Nitrogen? *PLoS ONE* **2013**, *8*, e72961. [CrossRef]
82. Bayer, K.; Schmitt, S.; Hentschel, U. Physiology, phylogeny and in situ evidence for bacterial and archaeal nitrifiers in the marine sponge Aplysina aerophoba. *Environ. Microbiol.* **2008**, *10*, 2942–2955. [CrossRef] [PubMed]
83. Southwell, M.W.; Weisz, J.B.; Martens, C.S.; Lindquist, N. In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. *Limnol. Oceanogr.* **2008**, *53*, 986–996. [CrossRef]
84. Richter, C.; Wunsch, M.; Rasheed, M.; Kötter, I.; Badran, M.I. Endoscopic exploration of Red Sea coral reefs reveals dense populations of cavity-dwelling sponges. *Nature* **2001**, *413*, 726–730. [CrossRef]
85. Zhang, F.; Blasiak, L.C.; Karolin, J.O.; Powell, R.J.; Geddes, C.D.; Hill, R.T.; Karl, D.M. Phosphorus sequestration in the form of polyphosphate by microbial symbionts in marine sponges. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 4381–4386. [CrossRef]