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Original Article

Bioremediation efficiency of sea cucumber *Holothuria scabra* (Jaeger, 1833) on the quality of water and sediment of shrimp *Penaeus monodon* (Fabricius, 1798) pond culture

Jomar F. Besoña*[1], Elgen M. Arriesgado[1], Rey Y. Capangpangan[2], Dan M. Arriesgado[1], Fiona L. Pedroso[1]

[1]Department of Fisheries Science, School of Marine Fisheries and Technology, Mindanao State University at Naawan, 9023 Naawan, Misamis Oriental, Philippines.
[2]Department of Physical Sciences and Mathematics, College of Marine and Allied Sciences, Mindanao State University at Naawan, 9023 Naawan, Misamis Oriental, Philippines.

Abstract: The bioremediation efficiency of the sea cucumber, *Holothuria scabra,* held at different stocking densities as an eco-based waste treatment technology on the quality of water and sediment of the *Penaeus monodon* pond culture, was evaluated in this study. Two separate experiments were simultaneously conducted indoors for five days using 100-L capacity aquaria. The treatments for the pond water experiment were: (T1) control, 10 L pond water only, (T2) 10 L pond water + one sea cucumber, and (T3) 10 L pond water + two sea cucumbers. For the pond sediment experiment, the conditions of the three treatments were the same except that pond sediments were added in each aquarium. The results showed that T2 had a significant reduction of ammonia (28%), and nitrite (84%) in pond water, and nitrogen (88%) and phosphorus (9%) in pond sediment. It also efficiently improved the management of the microbial load with a significant decrease, particularly in heterotrophs (39%) in pond water compared to that in the control treatment. The results showed that the presence and activity of *H. scabra* have a positive bioremediation effect on waste as demonstrated by reduced ammonia, nitrite, nitrogen, and phosphorus levels and microbial load from the *P. monodon* pond water and sediment. Furthermore, the stocking density of one sea cucumber is more efficient in reducing these wastes compared to the two sea cucumbers stocking density. The result suggested that *H. scabra* can be a potentially efficient bioremediator of waste in shrimp ponds.

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Introduction

Aquaculture is undoubtedly a rapidly growing food-production technology that addresses the declining fish and seafood supply from traditional capture-based methods (Anderson et al., 2017). Outperforming wild-stock landings as a vital food source, aquaculture has brought about numerous benefits, including increased food production, income generation, and improved quality of life for many individuals. However, as this industry expands, it raises various environmental challenges that demand urgent attention. The accumulation of organic and inorganic waste near fish farms due to fecal matter, excretions, and uneaten feeds is one of the concerning issues in aquaculture. Such waste can harm the bacterial community, causing benthic hypoxia, anoxia, and hydrogen sulfide accumulation (Fodelianakis et al., 2015). Additionally, fish's excretion of ammonia and phosphates further contributes to environmental degradation and eutrophication, particularly in low-flushing areas. Addressing these problems is crucial to ensure the well-being of the cultured species, protect the aquatic environment, and maintain the profitability of the aquaculture industry. Therefore, implementing appropriate waste treatment processes is imperative for aquaculture's sustainable development (Wang, 2017).

To combat pollution from aquaculture activities, various strategies like Integrated Multi-Trophic Aquaculture (IMTA), Recirculating Aquaculture System (RAS), and green water aquaculture have been advocated (Israel et al., 2019). Nevertheless, information on species with bioremediation potential to enhance these technologies and address waste management issues remains limited. Sea cucumbers are promising bioremediators that have high economic

*Correspondence: Jomar F. Besoña
E-mail: jomar.besona@msunaawan.edu.ph

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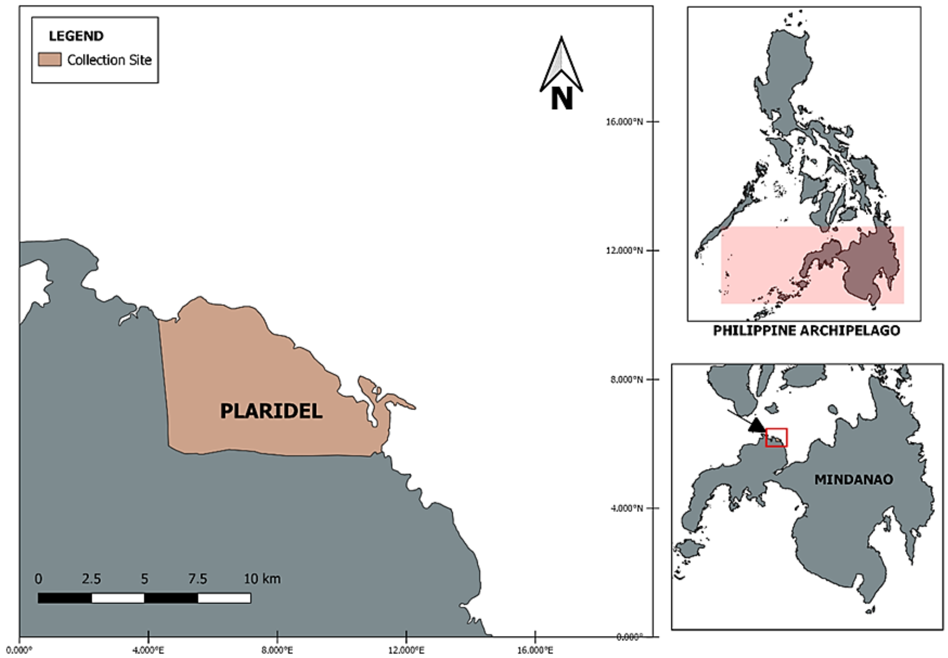

Figure 1. The map of the collection site of the experimental organism in Plaridel, Misamis Occidental.

value in Asian markets. In the marine ecosystem, sea cucumbers are vital as bioturbators and recyclers, making them excellent candidates for integrated systems (Yuval et al., 2014). In particular, *Holothuria scabra* (Jaeger 1833) has earned attention due to its high value and declining wild stocks, leading to aquaculture research and development efforts. Several studies have demonstrated the inclusion of sea cucumbers, especially *H. scabra*, in IMTA systems in marine open waters (Senff et al., 2020). Their ability to act as bioremediators, facilitating nutrient removal from sediments, has been well recognized, making them valuable in such integrated systems (Slater and Chen, 2015). Studies have shown that the incorporation of sea cucumbers in aquaculture systems can reduce nitrogen and total carbon content, leading to improved water quality (MacDonald et al., 2013; Robinson et al., 2019). Moreover, using sea cucumbers to consume waste from species like *Penaeus monodon* has shown promising results in mitigating organic and inorganic loads in ponds (Watanabe et al., 2012).

Notably, utilizing sea cucumbers in treatment systems offers various advantages, including cost-effectiveness, organic processes, waste-to-biomass conversion, and enhanced growth of cultivated species. Their deposit-feeding behavior ensures a thorough bioturbation of sediments, similar to their natural habitat (Manship, 1995). Despite the promising potential of *H. scabra* in waste management for aquaculture, there is a lack of documented studies on its inclusion as an extractor and its efficiency in reducing microbial load and waste from *P. monodon* aquaculture, particularly in the Philippines. Therefore, this study was undertaken to fill this crucial information gap, aiming to contribute to the revival and sustainability of *P. monodon* aquaculture in the Philippines. Information on the bioremediation capabilities of sea cucumbers can take a significant step towards achieving a more sustainable and environmentally friendly approach to meet the world's demand for *P. monodon* and *H. scabra*.

Materials and Methods

Experimental organism, collection, and transport:

The sandfish, *H. scabra*, had been chosen as an experimental organism because of its available hatchery and culture technology. It is also one of the commercially important sea cucumber species in Mindanao (Arriesgado et al., 2022b). Fifty-four individuals of adult sandfish with an average body

Table 1. The different treatments in the two experiments with their description.

Treatment	Description
Experiment 1: Pond water set-up	
T1	10 L pond water only (Control)
T2	10 L pond water + one sea cucumber*
T3	10 L pond water + two sea cucumbers*
Experiment 2: Pond sediment set up	
T1	95 g pond sediment + 10 L pond water (Control)
T2	95 g pond sediment + 10 L pond water + one sea cucumber*
T3	95 g pond sediment + 10 L pond water + two sea cucumbers*

* The stocking density was adopted from MacDonald et al. (2013)

weight of 143.7±2.05 g from a size range of 100-200 grams were randomly handpicked from the seagrass beds of Plaridel, Misamis Occidental, Philippines (8°59'47.8"N,123°74'34.7"E) (Fig. 1), where the species is naturally available and abundant. The collected samples were packed in ambient seawater, diffused with oxygen, and transported to the Mindanao State University at Naawan (MSUN), Sea Cucumber Research and Development Center (SCRDC) Hatchery for the experiments.

Acclimatization of the collected experimental organisms: Upon arrival, *H. scabra* specimens were acclimatized to a 1.5-ton capacity tank providing the optimum water condition. The salinity levels ranging from 20 to 25‰ were maintained to mimic the existing level in the ponds. Any fecal matter remaining in the tank was siphoned out and discarded. The acclimatization was done for 48 hrs.

Experimental set-up: Two experiments were conducted simultaneously to assess the bioremediation efficiency of *H. scabra* as an eco-based waste treatment technology on the quality of water and sediment during the *P. monodon* culture in ponds. The experiments were conducted indoors at the MSUN-SCRDC hatchery. The first experiment utilized the pond water from the *P. monodon* pond culture and the second experiment used the pond sediment to determine the bioremediation efficiency of *H. scabra* in reducing microbial load and waste. Both experiments were conducted simultaneously and lasted for five days.

***Experiment 1: Pond water set-up*:** For the first experiment, nine 100-L aquaria were used with three replicated treatments (Table 1) arranged in a completely randomized design (CRD). The water samples used were sourced from the *P. monodon* pond managed by MSUN. Each water sample was collected by dipping a 10 L-capacity carboy in the water column from one of the four corners of the pond, then the carboy was sealed, and transported to the study site for set-up. Sea cucumbers were randomly placed in each aquarium with the number based on the assigned treatment. Tanks were provided with aeration. The water temperature was kept at 28-30°C, and the water salinity at 27‰. The set-up was subjected to a natural light regime.

***Experiment 2: Pond sediment set-up*:** Nine 100-L aquaria were used for the second experiment with three replicated treatments and arranged in a completely randomized design (Table 1). The sediments used in the treatments were collected from the *P. monodon* pond at Simanoc, Naawan, Misamis Oriental. The sediments were collected from the different sides of the pond by scraping at least 150-300 mm of the topmost layer of the pond bottom. These were mixed homogeneously and used as the substrate of sea cucumber during the study. Each aquarium was supplied with 95 g of sediment and 10 L of pond water. Aeration was interrupted for approximately 15 minutes to allow the waste to settle to the bottom of the tank. Sea cucumbers were randomly selected and placed in each aquarium with the number based on the assigned treatment. The sea cucumbers were provided with ambient aeration, a constant temperature (28-30°C), salinity (27‰), and under the natural light regime.

Data collection

***Water and sediment quality parameter analyses*:** Data from the two experiments were collected on Day 0 (Initial) and Day 5 (final). For the pond water

Table 2. Waste concentration of ammonia, nitrite, and phosphate and the percent decrease for the pondwater experiment.

Waste Type	Treatment	Stocking density of Sea cucumber (individual/ 10 Liter)	Mean±SE Waste Concentration* (mg/L)			Change±SE (%increase or decrease)
			Initial	Final	Difference (Initial-Final)	
Ammonia	1	0	0.421±0.053	0.634±0.056	-0.213	+50±0.693[a]
	2	1	0.428±0.035	0.307±0.003	0.121	-28±0.614[b]
	3	2	0.419±0.069	0.305±0.005	0.114	-27±1.129[b]
Nitrite	1	0	0.122±0.012	0.038±0.005	0.084	-68±0.676[a]
	2	1	0.149±0.012	0.023±0.001	0.126	-84±0.565[b]
	3	2	0.097±0.003	0.033±0.002	0.064	-66±1.25[a]
Phosphate	1	0	0.455±0.079	0.408±0.033	0.047	-10±1.996[a]
	2	1	0.349±0.012	0.174±0.023	0.175	-50±0.837[a]
	3	2	0.353±0.023	0.276±0.018	0.077	-22±0.716[a]

Note: Different superscripts on change within waste type indicate a significant difference among means ($P<0.05$). + percentage value indicates an increase in aquaculture waste products; - percentage value indicates a decrease in aquaculture waste products

experiment, the measured parameters were ammonia, nitrite, and phosphate, following the methods of the American Public Health Organization (APHA, 1989) and the Association of Official Analytical Chemists (AOAC, 1990). The spectrophotometric method analyses of ammonia (NH_3), nitrite (NO_2), and phosphates (PO_4) were conducted at the MSUN Institute of Fisheries Research and Development (IFRD) Laboratory. Other analyses, such as salinity, temperature, and dissolved oxygen, were conducted *in situ* using a multi-water parameters apparatus (Hanna Instrument HI9829).

For the pond sediment experiment, the levels of organic matter, nitrogen, and phosphorus were measured and analyzed following the methods of APHA (1989) and AOAC (1990). The spectrophotometric method analyses of the organic matter, total nitrogen, and total phosphorus were also conducted in the MSUN-IFRD Laboratory.

***Microbial analyses*:** Data collection from the two experiments was done and analyzed on Day 0 (Initial) and Day 5 (final). The preparation of the sample dilutions and bacteriological assays of the water and soil for the two experiments were performed following APHA (2005). Water samples were collected using 500 mL sterile glass bottles immersed halfway (40 cm) deep from surface water and 0.4 cm above the sediment (bottom water). The samples were homogenized, and a sub-sample of 500 mL of water was utilized for the analysis. On the other hand, sediment samples (10 cm depth in soil) were collected in each treatment in the same location as the water samples and placed in sterile glass bottles. The water and soil samples were placed in ice chests and were immediately transported to the accredited microbiological laboratory near MSUN. The time from collections and analysis was less than an hour. The different microbial analyses were further subjected to biochemical tests for confirmation.

Statistical analysis: The statistical software, Past v.4.03 was used for all statistical analyses. Below is the computation using the formula of Mandario et al. (2019) of the percent decrease of ammonia, nitrite, phosphate, organic matter, nitrogen, and phosphorus. The percent decrease in bacterial load after five days from water and sediment was also analyzed using the formula of % decrease = (initial concentration-final concentration) / (initial concentration) x 100.

All percentage data were arcsine-square root transformed before the statistical analysis. Homogeneity of variances and normality among mean values were assessed using Levene's test for equality of variance errors and the Shapiro-Wilk test. A one-way analysis of variance was done to evaluate the effect of sea cucumbers in pond water and sediment on the percent decrease of ammonia, nitrite, phosphate, organic matter, and nitrogen. The percent decrease in bacterial load from water and sediment was also analyzed. The mean differences of each treatment were compared using Tukey HSD and

Table 3. Waste concentration of organic matter, nitrogen, and phosphorus and the percent decrease for the pond sediment experiment.

Waste Type	Treatment	Stocking density of Sea cucumber (individual/ 10 Liter)	Mean±SE Waste Concentration*(mg/L)			Change±SE (%increase or decrease)
			Initial	Final	Difference (Initial-Final)	
Organic Matter	1	0	4.29±0.304	2.95±0.496	1.34	-31±0.633[a]
	2	1	5.13±0.162	2.67±0.068	2.46	-48±0.288[a]
	3	2	4.97±0.049	2.85±0.097	2.12	-43±0.2206[a]
Nitrogen	1	0	0.19±0.005	0.17±0.006	0.02	-9±0.380[a]
	2	1	0.18±0.004	0.02±0.006	0.16	-88±0.337[b]
	3	2	0.19±0.004	0.03±0.006	0.16	-84±0.635[b]
Phosphorus	1	0	0.43±0.005	0.46±0.006	-0.03	+7±0.367[a]
	2	1	0.44±0.004	0.40±0.006	0.04	-9±0.406[a]
	3	2	0.45±0.004	0.44±0.0062	0.01	-2±0.538[a]

Note: Different superscripts on change within waste type indicate a significant difference among means (P<0.05)
+ percentage value indicates an increase in aquaculture waste products; - percentage value indicates a decrease in aquaculture waste products

Scheffe tests at a 95% confidence interval.

Results

Changes of wastes

Experiment 1: Pond water setup**:** For the pond water experiment, the two treatments with sea cucumbers (T2 and T3) showed the highest significant (P<0.05) change (% decrease) of ammonia (28 and 27%) compared to that of T1 (control) (Table 2). An elevated ammonia level was observed in T1 (50% increase). For the nitrite waste concentration, all treatments showed a decrease in waste concentration at the end of the experiment and T2 (stocking density of one sea cucumber) had the significantly highest decrease (84%, P<0.05) compared to the other two treatments. Similarly, all treatments showed a decrease in phosphate level during the experiment, however, no significant differences were detected between treatments.

Experiment 2: Pond sediment setup**:** For experiment 2, pond sediment set-up, the sediments' organic matter and nitrogen concentration were decreased in all the treatments at the end of the experiment (Table 3). In particular, nitrogen concentration decreased in all treatments same as in the first experiment, but the decrease in T2 and T3 characterizing the presence of sea cucumber was significantly higher than T1. The phosphorus waste concentration increased in T1 but decreased in T2 and T3. However, there were no significant differences in the decrease of phosphorus concentration between all treatments.

Changes in bacterial load

Experiment 1: Pond water setup**:** The changes in bacterial load of the treatments in the two experiments are shown in Table 4. T1 showed an increase in the total heterotrophic bacteria after five days. T2 and T3 showed a significant decrease in the bacterial load. A decline in the total *Vibrio* population was observed in all treatments. Meanwhile, an increase in *Pseudomonas* count was recorded in the three treatments after the five-day study period.

Experiment 2: Pond sediment set-up**:** For experiment 2, the results showed an increase in the total heterotrophs in all treatments (Table 4). Treatment 1 (38% increase) had significantly higher total heterotroph counts than those of the two treatments, but the latter two treatments showed no significant differences. All treatments showed a decrease in the total *Vibrio* count but the decrease rates were significantly similar in all treatments. On *Pseudomonas*, all treatments showed an increase in the *Pseudomonas* concentration. However, T1 had a slight increase (7%) and the rate of increase was significantly lower compared to those of the two treatments which were not significantly different from each other.

Discussions

Bioremediation efficiency of *H. scabra* to reduce microbial load and waste in the pond water with

Table 4. Total heterotrophs, *Vibrio* and *Pseudomonas* counts and the percent decrease for the two experiments.

Bacteria (Total)	Treatment	Stocking density of sea cucumber (individual/ 10 Liter)	Mean±SE Waste Concentration* (cfu/ml)			Change±SE (% increase or decrease)
			Initial	Final	Difference (Initial-Final)	
Experiment 1: Pond water set-up						
Heterotrophs	1	0	906±1.523	1363±3.163	-457	+50±1.192[a]
	2	1	707±2.088	429±1.712	278	-39±0.787[b]
	3	2	639±9.963	513±1.117	126	-19±0.287[b]
Vibrio	1	0	1560±2.274	973±2.469	587	-38±2.200[a]
	2	1	2723±4.605	880±6.609	1843	-68±0.338[a]
	3	2	2520±1.610	1227±2.846	1293	-51±0.365[a]
Pseudomonas	1	0	350±9.404	365±2.093	-15	+4±0.978[a]
	2	1	140±9.404	210±8.504	-70	+50±0.532[a]
	3	2	80±9.404	97±1.333	-17	+21±1.667[a]
Experiment 2: Pond sediment set up						
Heterotrophs	1	0	3573.33±3.70	148000±1.44	-144426.67	+38±0.831[a]
	2	1	3346.67±2.19	49866.67±2.15	-46520	+13±0.635[b]
	3	2	4520±1.42	39200±5.32	-34680	+8±0.465[b]
Vibrio	1	0	10166.67±6.53	7000±1.900	3166.67	-31±0.554[a]
	2	1	14666.67±9.82	8533.33±6.75	6133.34	-42±0.173[a]
	3	2	10533.33±3.89	7066.67±3.15	3466.66	-33±0.104[a]
Pseudomonas	1	0	6133.33±5.33	8000±4.25	-1866.67	+30±1.136[a]
	2	1	3666.67±1.49	5028.33±3.13	-1361.66	+37±0.367[a]
	3	2	6019±3.19	6466.67±8.50	-447.67	+7±0.682[b]

Note: Different superscripts on change within bacterial count indicate a significant difference among means ($P<0.05$)

varying stocking density: The main drivers of eutrophication are the intensification of the culture system through the increased use of pelleted feeds, the high stocking density of cultured organisms, and the extension of production areas. This study demonstrated the bioremediation efficiency of *H. scabra* to reduce the waste from the *P. monodon* pond water conducted under controlled laboratory conditions. The efficiency of bioremediation is assessed based on the bioturbating and recycling abilities of sea cucumbers, which make them highly suitable candidates for integrated systems. Bioremediation involves utilizing living organisms to consume or break down environmental pollutants. In this study, the key metric for evaluating bioremediation efficiency is the percentage reduction in waste products. A decrease in waste products signifies effective bioremediation, whereas a lack of decrease indicates less efficient bioremediation. Therefore, the percentage change in waste products serves as the basis for determining the efficiency of bioremediation in this context. The result of the study showed that the presence and activity of *H. scabra* have a positive bioremediation effect on waste in the form of reduced ammonia, nitrite, and phosphate levels in the *P. monodon* pond water. Furthermore, the stocking density of one sea cucumber per 10 L pond water was more efficient in reducing these wastes compared to the two sea cucumber stocking densities.

Although ammonia, nitrite, and phosphate had not exceeded the ideal limits, these levels collected from the *P. monodon* pond were generally high, as manifested in initial readings. They will negatively affect the health of the cultured organism and the quality of the culture environment. Ammonia is known as one of the toxicants in aquaculture systems (Wang et al., 2017). In shrimp aquaculture, this can be elevated by the direct excretion of shrimp and released from the degradation of their feces. According to Wang et al. (2017), dependent on the farming method, there is a chance for the non-lethal ammonia levels to exceed the optimum level; thus, negatively affecting the aquaculture production of several shrimp species, including *P. monodon*, *P. chinensis*, and *Litopenaeus* *vannamei*. Adverse effects on ammonia have been observed by Qiu et al. (2018) in shrimp and include direct damage to tissues such as gills and hepatopancreas when ammonia levels in the water exceed their tolerance limit. Shrimp also suffer adverse effects on their growth and survival, which cause economic losses (Arriesgado et al., 2022a).

In this study, a reduction in ammonia levels was observed in treatments stocked with sea cucumbers, whereas in the treatment without sea cucumbers, a significant increase in ammonia levels was observed. Although various factors can modify the ammonia level in the water, the only difference between treatments was the presence or absence and the number of sea cucumbers. Treatments with sea cucumbers had reduced the ammonia levels. The result of the study is in agreement with the findings of Purcell (2004), who found that sea cucumbers bioturbated sediment by burying and feeding on wastes may reduce waste such as ammonia.

Nitrite can cause various physiological changes in marine species. Aquaculture pond effluent is a major anthropogenic source of nutrient pollution in coastal areas (Lacerda et al., 2008). In the present study, nitrite levels decreased in all treatments during the experimental period. However, the reduction of nitrite levels was evident in treatments with sea cucumbers. The reduction can be attributed to the indirect interaction of bacterial mineralization which conforms to the study conducted by Sadeghi-Nassaj et al. (2018). Moreover, they reported that the concentration of different parameters, including nitrate in the effluent tank with holothurians was lower than those of tanks without holothurians. Although sea cucumber could help to break down nitrite from the environment, it also might be a contributor of nitrite in the form of waste, thus, appropriate stocking density should be considered.

Preventing shrimp and fish diseases in aquaculture requires understanding the appropriate levels of nutrients, including phosphate, in its rearing environment for effective water quality management (Yang et al., 2017). The phosphate levels in the study have decreased throughout the experimental periods, both with and without sea cucumber. However, the presence of sea cucumbers hastens the process of reducing the phosphate, especially at one sea cucumber per 10 L of pond water. According to Burford (2003), high nutrient concentrations facilitated the proliferation of bacteria, phytoplankton (mainly autotrophic flagellates), and protozoa. On the contrary, the study's results revealed that sea cucumbers were more readily capable of breaking down phosphate than phosphorus in the sediment, demonstrating the best bioremediation efficiency on the phosphate in the water experiment. In chemistry, inorganic phosphorus is readily soluble phosphate, hence bioavailable to animals. The stocking density of *H. scabra* in the system should be considered according to Namukose et al. (2016) because they also affect the system's total organic matter and total organic carbon budgets. Their findings showed that the increased stocking density of *H. scabra* in the culture system could influence the buildup of organic debris.

Changes in bacterial load from water experiment: The high proliferation of the total heterotrophs was observed in groups without sea cucumber and a reduction in T2. The increased level of nutrients such as nitrate and phosphate in the control treatment can influence the growth of total heterotrophic bacteria. In contrast, in treatments with sea cucumber, these nutrients were reduced. The increase in total heterotrophic count in the water from the control treatment can be attributed to the presence of more nutrients due to the absence of animals that can reduce nutrient load, thereby regulating the growth of bacterial biomass in the culture system. Heterotrophic bacterial communities exist in all marine environments; however, several factors limit it. Maintaining the heterotrophic bacterial load would control the pathogenic bacteria load (Panigrahi, 2015). Due to high stocking densities, large amounts of organic material from feces and unconsumed feed can accumulate with low water exchange rates. Consequently, the load of heterotrophic bacteria in water can be very high in shrimp and might increase over time (Bauer et al., 2020). Our findings revealed a reduction in total heterotrophs during experiments in treatments with *H. scabra*. Furthermore, sea cucumbers can directly consume bacteria (Slater and Carton, 2009), which probably influences the reduction in the population of heterotrophs in the treatments with sea cucumbers.

Vibrio is a common bacteria found in brackish and salt water, making up much to 40% of the bacterial community (Buller, 2014). This study showed that *P. monodon* pond water exhibited a considerable amount of *Vibrio* sp. which is initially equal in all treatments, followed by a steep decline with the presence of sea cucumbers. The study's findings support the idea that *Vibrio* sp. populations in culture ponds should be decreased because, although several *Vibrio* species are naturally occurring in fish and shellfish (Buller, 2014), some pathogenic species can be dangerous to humans and cultured shrimp.

The increase in total *Pseudomonas* count in all treatments agreed with the results of Sombatjinda et al. (2011) that it is a dominant microorganism in aquaculture systems. Moreover, in this study, the change in bacterial count can be observed in the treatment with sea cucumbers. The promotion of bacteria is designed to increase the breakdown of organic matter and plays a role in heterotrophic nitrification activity. However, more research is required to verify this theory to determine which species of *Pseudomonas* group performs the heterotrophic nitrification function and the relationship between these groups and environmental variables. The dynamics of environmental conditions may be reflected in the difference in species composition (Trung et al. 2019).

Bioremediation efficiency of *H. scabra* to reduce microbial load and waste in the pond sediment with varying stocking density: Disease outbreaks frequently result when the environment in shrimp ponds deteriorates because of an increase of organic matter on the bottom sediment from shrimp feces and leftover feed. Aquaculture waste pollution, including nitrogen compounds and phosphorus, can pollute the receiving water bodies and lead to eutrophication (San Diego-McGlone et al., 2008). In mitigating pollution within the cultural environment, the efficiency of reducing this eutrophication-causing waste must be considered. The results of this study of adding sea cucumber in the pond sediment have been supported by the findings of Heilskov et al. (2006), that the activities of burrowing marine organisms to rework and ventilate the sediments improved the condition of the environment and hence, prevents the formation of toxic gases. Further, recent studies have demonstrated that sea cucumbers that graze or feed on sediments reduce the nutrient load in enriched systems, recycle nutrients in natural systems, and have a significant impact on the inorganic carbon content of sediments and seawater. In their natural habitat, deposit-feeding sea cucumbers like *Holothuria forskali* consume organic-rich sediments at a rate that bioturbates surface sediment (Manship, 1995).

Compared to the other species of holothurians, Slater and Carton (2009) reported that grazing of *A. mollis* beneath mussel farms significantly decreased the level of organic carbon. Further, *H. atra* has been shown to hold bacterial production, consuming as much as 10-40% of bacterial carbon produced in sediment diets daily. Kristensen and Kostka (2005) found that the macrofaunal burrow structures have a significant role in the waste cycles by facilitating solute transport and continually recycling electron acceptors crucial to the oxidation of organic matter and coupled nitrification-denitrification. Accordingly, the ability of sea cucumbers to reduce the effects of eutrophication depends critically on understanding the balance of nutrients.

Changes in bacterial load from the pond sediment experiment: Changes in the bacterial load from the pond sediment appeared to be consistent with changes in the bacterial load in the water experiment. No significant difference was seen between T2 and T3, indicating that relevant reductions in the total heterotrophic count and *Vibrio* count could be independent of the stocking densities. Macdonald et al. (2013) assessed the consumption of *H. forskali* in seabass waste, bacterial consumption, and detrital assimilation were essential mechanisms in reducing nutrients. Although there are still various factors to consider, the reduction of relevant bacteria shows that most sea cucumbers are omnivorous deposit-feeders that directly consume benthic bacteria (Hillerislambers et al., 2006).

The process of bioturbation, which occurs when an organism alters the distribution of pollutants between dissolved and particulate forms, causes the sediment to often release various contaminants into the water column (Belzunce-Segarra et al., 2015). Maintaining pollutants in oxidized forms inside surface sediment layers and burrow walls increases the contact of sediments with the surrounding water. Settlement ponds were built in Australia to catch nutrient discharges from ponds before releasing them. This technique reduced total suspended particles by as much as 60% (Preston et al., 2000). The integration of *H. scabra* with appropriate stocking density to reduce the concentrations of particulate organic matter and transform it into forms that another organism can utilize more effectively can be a successful strategy.

This study showed the influence of *H. scabra* on reducing the waste from the pond water and sediment and thus suggested the efficiency of this organism on its extent to bioremediate, wherein nutrient concentrations in the pond water and sediment could be altered through excretion (Uthicke, 2001). Even though more precise information is required, the sequence of studies in this study suggests that *H. scabra,* with considerable stocking density, as demonstrated in this study, could bioremediate eutrophication and enhance water and sediment quality in a shrimp pond. Henceforth, this study can offer a solution for a possible cost-effective and sustainable treatment of aquaculture waste and eventually could increase aquaculture production to solve challenges to global food security.

This study also demonstrated the bioremediation efficiency of *H. scabra* to reduce microbial load and waste from *P. monodon* pond water and sediment under laboratory conditions. The presence of one *H. scabra* per 10 L pond water exhibited most of the waste reduction. In addition, the reduction of microbial heterotrophs from the pond water and sediment experiment can be observed with one *H. scabra* per 10 L pond water. It is recommended that one *H. scabra* per 10 L pond water can be integrated into the waste treatment system. This eco-based waste treatment technology can help mitigate the introduction of organic enrichment from aquaculture-derived pollutants into the natural body of water. At the same time, wastes can be utilized by sea cucumbers to be converted into biomass, suggesting that the *H. scabra* can be integrated as an aquaculture commodity in ponds.

Future research should examine the efficacy of using deposit-feeder effluent treatment systems in conjunction with currently practiced land-based aquaculture to treat effluent from *P. monodon* aquaculture systems. Additionally, a study that mimics IMTA using sea cucumbers in a laboratory setting is needed to determine how they can use waste to produce valuable biomass for aquaculture. Furthermore, it is advised to test various *H. scabra* sizes and stages and other sea cucumber species and the treatment method on other aquaculture commodities. Upgrading the treatment systems would be the next step to assess cost-effective production and broaden the use of the systems. Hence, sustainable production of shrimp pond aquaculture will be attained.

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