Predators effects on mortality of sandfish *Holothuria scabra* cultured in multitrophic system

J Tresnati¹,²,³, I Yasir¹,²,³, Syafuddin¹,²,³, R Aprianto¹,³, Mutmainnah¹, A Yanti¹,³, A D Bestari¹,³ and A Tuwo²,³

¹ Fisheries Department, Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar, Indonesia
² Marine Science Department, Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar, Indonesia
³ Multitrophic Research Group, Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar, Indonesia
⁴ Center of Excellence for Development and Utilization of Seaweed, Universitas Hasanuddin, Makassar, Indonesia

Email: ambotuw62@gmail.com

Abstract. Sandfish *Holothuria scabra* is a species of high economic value that causes sandfish to be the most captured sea cucumber species, and is an important source of income for fishermen in coastal villages. Sandfish capture causes already over fishing. If the wild population of sandfish is not managed properly, over fishing could cause sandfish extinction. This reason, an alternative efforts are needed to increase sandfish production, such as cultivation and restocking. Sandfish was a species that can live in the estuary region, so that it has the potential to be maintained in brackish water ponds. One of the problems of Sandfish cultivation in the brackish water ponds was the predators attack. This study aims to analyze the predator attack on sandfish. The study was carried out on a microcosm scale by using the tanks. The organism used was Sandfish *H. scabra*, Nile Tilapia *Oreochromis niloticus*, and seaweed *Gracilaria* sp. Mangrove Snail attack can cause death if the sandfish has already eviscerated. The healing process takes one to several weeks, depending on the size of the wound. Nile Tilapia attacks were generally not deadly. Death only occurs if the wound is too deep and causes internal organs to come out, but this was very rare. During the study, only one case occurred in which the sandfish died due to the attack of Nile Tilapia. The sandfish that not injured in the ventral side, and does not suffer from evisceration, the mortality was zero. Sandfish wounds heal within one to three weeks, depending on the surface area of the tegument eaten by Nile Tilapia. Healed wounds appear darker in color.

1. Introduction

There are several important economic values of sea cucumbers in Indonesia, such as *Holothuria scabra*, *H. fuscogilva*, *Stichopus chloronotus*, *S. variegatus*, and *Thelenota ananas* [1-3]. *H. scabra* or sandfish was the most captured species, and is an important source of income for fishermen in coastal villages in Southeast Asian countries [4]. This capture causes sandfish already over fishing [5]. If the wild population of sandfish is not managed properly, over fishing could cause sandfish extinction [6].
By this reason, alternative efforts are needed to increase sandfish production, such as cultivation and restocking. Sandfish production has the potential to be increased through marine cultivation [7] and restocking [8, 9].

Sandfish was a species that can live in the estuary region, so that it has the potential to be maintained in brackish water ponds. Sandfish is a deposit feeder that takes muddy particles mostly with particle size of 125-250, this indicate that Sandfish prefers muddy substratum than the sandy bottom [10]. This food habit makes Sandfish a cultured organism that can reduce excess organic matter in fish and shrimp farming ponds [11], so that it is potential to be used as aquaculture object for multitrophic system in brackish water ponds.

One of the problems of Sandfish cultivation in the brackish water ponds was the predators attack [12]. Another problem is the use of chemicals products. Intensive use of chemicals products in brackish water ponds [13] can have a negative impact on Sandfish. Chlorine was a chemical product that was widely used in shrimp culture at brackish water ponds [14]. Chlorine can cause irritation to the skin of the sea cucumber which ends in death. This study aims to analyze the predator attack on sandfish \( H. \) \( scabra \) which was cultured with Nile Tilapia \( Oreochromis \) \( niloticus \) and seaweed \( Gracilaria \) \( sp \) without using chemical products. At present, cultivation of sandfish, Nile Tilapia, and seaweed continues to be developed to meet the growing market needs [1, 4, 5, 15, 16]. This research was expected to contribute to the development of the next multitrophic system that biologically and environmentally safe, especially when the world's attention food bio-safety as well as the conservation of the environment continue to increase [17].

2. Materials and Methods

The study was conducted in 2016 for Nile Tilapia attack and 2018 for Mangrove Snail attack at the Multitrophic Laboratory, Hasanuddin University. The study was carried out on a microcosm scale. The equipments and materials used were nine culture tanks, sized 1 x 2 x 0.9 m, aerator, circulation pump, water quality measurement tool, artificial feed, and three experimental animals, i.e. Sandfish, Nile Tilapia, and seaweed. Each tank was filled with 0.6 m of filtered sea water. The numbers of experimental animals used were 20 Sandfish, 30 Nile Tilapia, and 600 gram seaweed. Each tank was aerated by using air pump and aerated circulating water pump.

The shell height and width of Mangrove Snail \( Cerithidea \) \( sp \) were measured with accuracy of 0,000 mm, and the weight was weighed with accuracy of 0.00000 g. Sandfish was fed with artificial food and dry spirulina; and Nile Tilapia was fed with pellets. The water quality parameters measured were \( NH_3 \), \( NO_2 \) and \( NO_3 \), Temperature, Salinity, \( pH \), and \( O_2 \).

Predator attack observation was done visually, and Mortality (Zt) [18] was calculated based on equations:

\[
Z_t = \left( \frac{N_t}{N_0} \right) \times 100, 
\]

where \( N_0 \) was the initial number of Sandfish or Nile Tilapia, \( N_t \) was the number of Sandfish or Nile Tilapia at time \( t \).

3. Results

3.1. Mangrove Snail

The symptom of a sandfish that was attacked by predators was doing a rolling motion in the tanks. This Sandfish were taken and observed in the laboratory, by this observation, it was found many Mangrove Snail attached to the tegument of Sandfish. The sizes of Mangrove Snails that attack Sandfish were very small. The shell height were from 1.00 to 5.00 mm, shell width from 0.10 to 1.40 and total weight from 0.0002 to 0.0060 grams (Table 1). The average number of Mangrove Snails that attack sandfish were 4 to 20, with minimum number of attacks was one and maximum was 59 (Table 2). Mangrove Snail attacks 100 percent of Sandfish by attaching and entering into tegument (Figure 1).

Of the 328 Sandfish observed, 100% were attacked, of which 169 Sandfish (51.52%) had injuries with a total of one to two injuries, but some had up to 23 injuries. This attack causes small to large sores on Sandfish which end in death (Figure 2).
Percentage of Sandfish being infected by Mangrove Snail were 17 to 43% (Table 3), with the number of Sandfish wounds or lesions caused by a Mangrove Snail were 6-51 (Table 4), with an average number of Sandfish wounds or lesions caused by a Mangrove Snail were 2.16; and the percentage of Sandfish wounds or lesions caused by a Mangrove Snail were 29 to 72% (Table 5).

| Table 1. The size of Mangrove Snail *Cerithidea* sp in each observation (date) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|------------------|
| **Width (mm)**                  | **Observation** |
| **Date**                        | **27-5-18**     | **02-6-18**     | **11-6-18**     | **18-6-18**     | **7-7-18**       |
| **Minimum**                     | 0.100           | 0.440           | 0.540           | 0.320           | 0.280            |
| **Maximum**                     | 2.390           | 1.240           | 1.040           | 1.620           | 1.100            |
| **Average**                     | 0.768           | 0.875           | 0.766           | 0.808           | 0.665            |
| **STD**                         | 0.251           | 0.173           | 0.128           | 0.248           | 0.255            |
| **Number**                      | 142             | 95              | 36              | 93              | 20               |
| **Height (mm)**                 | **Observation** |
| **Date**                        | **27-5-18**     | **02-6-18**     | **11-6-18**     | **18-6-18**     | **7-7-18**       |
| **Minimum**                     | 1.200           | 1.030           | 1.380           | 0.490           | 0.740            |
| **Maximum**                     | 3.790           | 4.080           | 3.060           | 3.890           | 3.540            |
| **Average**                     | 2.427           | 2.935           | 2.094           | 2.037           | 3.540            |
| **STD**                         | 0.482           | 0.636           | 0.356           | 0.744           | 0.794            |
| **Number**                      | 142             | 95              | 36              | 93              | 20               |
| **Weight (g)**                  | **Observation** |
| **Date**                        | **27-5-18**     | **02-6-18**     | **11-6-18**     | **18-6-18**     | **7-7-18**       |
| **Minimum**                     | 0.00010         | 0.00010         | 0.00010         | 0.00010         | 0.00010          |
| **Maximum**                     | 0.00290         | 0.00370         | 0.00110         | 0.00170         | 0.00130          |
| **Average**                     | 0.00053         | 0.00073         | 0.00042         | 0.00037         | 0.00130          |
| **STD**                         | 0.00051         | 0.000076        | 0.000025        | 0.00033         | 0.00043          |
| **Number**                      | 142             | 95              | 36              | 93              | 20               |

| Table 2. The number of Mangrove Snail *Cerithidea* sp in each tank |
|-------------------------|-----------------|-----------------|-----------------|-----------------|------------------|
| **Date**                | **Observation** | **Average**     |
|                        | **Tank 1**      | **Tank 2**      | **Tank 3**      | **Tank 4**      | **Tank 5**       | **Tank 6**       | **Tank 7**       | **Tank 8**       | **Tank 9**       | **2**            |
| May, 27 2018            | 12              | 18              | 27              | 10              | 13              | 21              | 35              | 20              |
| June, 02 2018           | 10              | 4               | 22              | 12              | 11              | 10              | 11              | 15              | 8               | 11               |
| June, 11 2018           | 8               | 18              | 23              | 5               | 10              | 5               | 59              | 14              | 7               | 17               |
| June, 18 2018           | 4               | 5               | 12              | 6               | 8               | 3               | 30              | 13              | 15              | 11               |
| July, 07 2018           | 6               | 1               | 5               | 5               | 1               | 4               | 1               | 4               | 7               | 4                |

| Table 3. Percentage of Sandfish *Holothuria scabra* being infected by a Mangrove Snail *Cerithidea* sp. |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Date**                                        | **Tanks**       | **Average**     |
| **1**                                           | **2**           | **3**           | **4**           | **5**           | **6**           | **7**           | **8**           | **9**           |
| May, 27 2018                                    | 36.84           | 42.11           | 57.89           | 42.11           | 42.11           | 36.84           | 47.37           | 31.58           | 55.00           | 43.54           |
| June, 02 2018                                   | 47.37           | 21.05           | 52.63           | 21.05           | 36.84           | 31.58           | 36.84           | 10.53           | 30.00           | 31.99           |
| June, 11 2018                                   | 33.33           | 52.94           | 73.68           | 21.05           | 42.11           | 21.05           | 63.16           | 47.37           | 36.84           | 43.50           |
| June, 18 2018                                   | 20.00           | 26.67           | 36.84           | 22.22           | 33.33           | 10.53           | 73.68           | 44.44           | 55.56           | 35.92           |
| July, 07 2018                                   | 33.33           | 6.67            | 21.05           | 23.53           | 5.88            | 15.79           | 5.26            | 22.22           | 22.22           | 17.33           |
Figure 1. Juvenile of Mangrove Snail *Cerithidea sp* attacks on Sandfish *Holothuria scabra*

Figure 2. Effect of Mangrove Snail attacks on Sandfish *Holothuria scabra*

Table 4. Number of Sandfish *Holothuria scabra* wounds or lesions caused by a Mangrove Snail *Cerithidea sp.*

| Date       | Tanks 1 | Tanks 2 | Tanks 3 | Tanks 4 | Tanks 5 | Tanks 6 | Tanks 7 | Tanks 8 | Tanks 9 | Average |
|------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| May, 27 2018 | 20      | 90      | 31      | 27      | 51      | 5       | 62      | 101     | 75      | 51.33   |
| June, 02 2018 | 10      | 6       | 35      | 28      | 42      | 5       | 28      | 7       | 26      | 20.78   |
| June, 11 2018 | 14      | 25      | 50      | 57      | 28      | 20      | 47      | 12      | 30      | 31.44   |
| June, 18 2018 | 21      | 24      | 34      | 39      | 17      | 4       | 52      | 10      | 20      | 24.56   |
| July, 07 2018 | 3       | 2       | 14      | 6       | 7       | 2       | 12      | 2       | 8       | 6.22    |
Table 5. Percentage of Sandfish *Holothuria scabra* wounds or lesions caused by a Mangrove Snail *Cerithidea sp.*

| Date       | Tanks 1 | Tanks 2 | Tanks 3 | Tanks 4 | Tanks 5 | Tanks 6 | Tanks 7 | Tanks 8 | Tanks 9 | Average |
|------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| May, 27 2018 | 52.94   | 94.12   | 64.71   | 64.71   | 64.71   | 64.71   | 88.23   | 75.00   | 82.35   | 72.39   |
| June, 02 2018 | 57.89   | 30.00   | 63.16   | 47.37   | 52.63   | 52.63   | 78.94   | 72.22   | 80.00   | 59.43   |
| June, 11 2018 | 31.58   | 82.35   | 89.47   | 68.42   | 47.36   | 55.56   | 84.21   | 68.42   | 78.94   | 67.37   |
| June, 18 2018 | 40.00   | 62.50   | 57.89   | 83.33   | 42.10   | 31.58   | 84.21   | 52.53   | 78.94   | 59.23   |
| July, 07 2018 | 40.00   | 13.33   | 52.63   | 40.00   | 22.22   | 21.05   | 26.31   | 27.78   | 22.22   | 29.50   |

Wounds caused by Mangrove Snail can heal with a long healing time depending on the size of the wound (Figure 3), the small wounds or lesions could heal in one week, while larger wounds or lesions took longer, 2 or 3 weeks.

Figure 3. Wound conditions that have healed in the dorsal and ventral side of Sandfish *Holothuria scabra*

Mangrove Snail attacks has caused the death of 17 sea cucumbers (5.18%). This study shows that attack of Mangrove Snail could cause the death at Sandfish only if the Sandfish have eviscerated or without digestive tract (Figure 4), because without the digestive tract, the sandfish was unable to meet the cumulative energy demands for regenerating internal organs and repairing wounds or lesions caused by the Mangrove Snail.

Figure 4. Dorsal and ventral conditions of the wound Sandfish *Holothuria scabra* that has been eviscerated

3.2. Nile Tilapia

The average length of sandfish in each tank was between 8.10 and 12.02, and the average weight was 22.49 and 77.07. The average length of Nile Tilapia (Figure 5) in each tank was between 5.87 and 9.38 and 12.02, and the average weight ranges between 3.70 and 13.40 (Table 6).

During the study, Sandfish were attacked by Nile Tilapia in 7 of 10 tanks or 30%. Sandfish are not attacked by Nile Tilapia at the Tank 2, 3 and 6 (Table 7). Sandfish suffered injuries on his dorsal part due to the bite of Nile Tilapia (Figure 6). Sandfish mortality was not affected by mortality of Nile
Tilapia (0.161). The Sandfish mortality was also not significantly affected by the weight of Seaweed growth (0.069), but the Sandfish mortality was significantly affected by the length of tilapia (0.049). The bigger the size of tilapia, the more significant the effect (0.026).

Figure 5. Nile Tilapia *Oreochromis niloticus*

Table 6. Length and weight parameters of Sandfish *Holothuria scabra*, Nile Tilapia *Oreochromis niloticus* and Seaweed weight

| Tank | Sandfish |  | Nile Tilapia |  | Seaweed weight |
|------|---------|---------|-------------|---------|----------------|
|      | N₀ | L (cm) | W (g) | N₀ | L (cm) | W (g) | Beginning | End | Δ     |
| 1    | 20 | 8.72 | 36.71 | 30 | 8.44 | 10.76 | 600 | 480.63 | 80.11 |
| 2    | 20 | 8.22 | 22.49 | 30 | 5.87 | 3.70 | 600 | 397.86 | 66.31 |
| 3    | 20 | 10.84 | 77.07 | 30 | 7.68 | 7.96 | 600 | 907.88 | 151.31 |
| 4    | 20 | 10.37 | 49.40 | 30 | 7.46 | 7.96 | 600 | 387.73 | 64.62 |
| 5    | 20 | 12.02 | 58.85 | 30 | 7.36 | 6.71 | 600 | 565.59 | 94.27 |
| 6    | 20 | 8.10 | 27.12 | 30 | 9.28 | 13.40 | 600 | 835.4 | 139.23 |
| 7    | 20 | 11.56 | 35.62 | 30 | 7.92 | 8.48 | 600 | 596.9 | 99.48 |
| 8    | 20 | 8.08 | 29.06 | 30 | 7.21 | 6.82 | 600 | 857.65 | 142.94 |
| 9    | 20 | 8.93 | 41.03 | 30 | 7.78 | 8.13 | 600 | 668.44 | 111.41 |
| 10   | 20 | 8.32 | 34.46 | 30 | 7.76 | 8.28 | 600 | 558.41 | 93.07 |

Table 7. Conditions of Sandfish, Seaweed, Nile Tilapia at the end of observation

| Tanks | N₀ | Dorsal Wounds | Ventral Wounds & Evicered | Nᵢ | Z Sandfish (%) | N₀ | Nᵢ | Z Nila (%) |
|-------|----|---------------|----------------------------|----|----------------|----|----|-----------|
| 1     | 20 | 7 (36.84%)   | 0 (0%)                    | 19 | 5.00          | 30 | 27 | 90.00     |
| 2     | 20 | 0 (0%)       | 20 (100%)                 | 0  | 100.00        | 30 | 19 | 63.33     |
| 3     | 20 | 0 (0%)       | 4 (25%)                   | 16 | 20.00         | 30 | 25 | 83.33     |
| 4     | 20 | 4 (21.05%)   | 1 (5.26%)                 | 19 | 5.00          | 30 | 29 | 96.67     |
| 5     | 20 | 1 (5.00%)    | 0 (0%)                    | 20 | 0.00          | 30 | 30 | 100.00    |
| 6     | 20 | 0 (0%)       | 8 (66.67%)                | 12 | 40.00         | 30 | 13 | 43.33     |
| 7     | 20 | 9 (45.00%)   | 0 (0%)                    | 20 | 0.00          | 30 | 20 | 66.67     |
| 8     | 20 | 6 (37.50%)   | 4 (25.00%)                | 16 | 20.00         | 30 | 30 | 100.00    |
| 9     | 20 | 4 (21.05%)   | 1 (5.26%)                 | 19 | 5.00          | 30 | 28 | 93.33     |
| 10    | 20 | 5 (29.41%)   | 3 (17.65%)                | 17 | 15.00         | 30 | 29 | 96.67     |
Figure 6. Dorsal sores suffered of Sandfish *Holothuria scabra* due to Nile Tilapia *Oreochromis niloticus* attack.

Nile Tilapia attacks can cause the death if the sandfish has already eviscerated before or after attack (Figure 7), but the sandfish that still has internal organs, the wound can heal totally (Figure 8).

Figure 7. Sandfish *Holothuria scabra* without internal organs that die after being attacked by Nile Tilapia *Oreochromis niloticus*.

4. Discussion

4.1. Mangrove Snail

Snail is a natural predator of sea cucumber, such as *Charonia saulias* and *C. tritonis* [19]. Sandfish were thought to have been attacked by Mangrove Snail since they were in their natural habitat, but were not detected because the size of Mangrove Snails were still too small when the sandfish were captured. The probability of sandfish being attacked by Mangrove Snail through sea water was low
because the sea water has been filtered several times through physical filters and UV sterilization. To control the attack of this predator, Mangrove Snail was mechanically removed from tegument by using small needles.

During the study, several cleaning or removal of Mangrove Snail has been carried out, because not all juveniles can be detected in one observation. Juveniles that were very small, it was very difficult to see, and also very difficult to remove from sandfish tegument because they are already deeply embedded in tegument. If the Mangrove Snail has been stuck deep and not visible, it can only be removed at the next observation when the wound has enlarged and the Mangrove Snail juveniles come out to attack the other parts of the tegument that produce new wounds.

Figure 8. Sandfish *Holothuria scabra* that alive and have healed their wound after being attacked by Nile Tilapia *Oreochromis niloticus*.

Generally Sandfish can recover from wounds within one week for small wounds, while larger wounds need 2 to 3 weeks. The sandfish that have wounds too large and severe usually die. Sandfish that were seriously injured and then die were generally the sandfish that do not have internal organs. During the study, it found only one sandfish dead that has internal organs, the other dead does not have internal organs.

Sandfish that were reared in controlled tanks as in this study, will be more susceptible to Mangroves Snail, because in the tanks without substrate, sandfish cannot immerse themselves in substrate. The behavior of sandfish to immerse themselves in the substrate was thought to be the effort sandfish to clean the dorsal part of the predator. Substrate use was usually avoided in controlled systems to keep good sea water quality. During this study, by using the tanks without a substrate, the sea water quality parameters can be keep in the optimal range for the life of sandfish and Nile Tilapia.

4.2. Nile Tilapia

Previous researchers have categorized Nile Tilapia fish as herbivorous fish [20, 21] who ate Cyanophyta, Chlorophyta, Chrysophyta, and Pyrophyta [22]. But there were also previous researchers who classified Nile Tilapia as omonivores with food compositions that differed according to the size
of fish. Nile Tilapia measuring less than 20 cm has a food composition dominated by green algae, while fish above 20 cm have a food composition dominated by green algae, rotifers, copepod and other in the form of unidentified green spheres (40%), diatoms (10%), and detritus (50%) [23]. Such food habits allow Nile Tilapia to eat whatever it finds, including eating the dorsal tegument of sandfish.

The attack of Nile Tilapia on sea cucumber has never been reported before. Sea cucumber was rarely eaten by fish [19]. It seems like the sandfish avoids the attack of predators by immersing themselves into the substrate, so that it was not visible to fish [24, 25], sea-stars and crabs [25-27] trigger fish, emperor fish, and breams [28].

Tanks without substrate cause the sandfish cannot immerse themselves, so that they were easily seen by Nile Tilapia, especially during the daytime. Seaweed in the tanks was not enough to be used as a shelter from Nile Tilapia attacks; even though during the daytime the sandfish were under a pile of seaweed, but seaweed has not been able to disguise sandfish from the Nile Tilapia view. This shows that multitrophic system with a single tank and without substrate does not meet the requirements for cultivating sandfish with Nile Tilapia.

The high mortality in three tanks (Tank 2, 3, and 6) that were not attacked by Nile Tilapia showed that the death of sandfish was not directly related to the attack of Nile Tilapia. In all three tanks, ventral and evisceration injuries were quite severe. This shows that another factor that causes death was evisceration.

Nile Tilapia attacks were generally not deadly. Death only occurs if the wound is too deep and causes internal organs to come out, but this was very rare. During the study, only one case occurred in which the sandfish died due to the attack of Nile Tilapia (Tank 1). In Tanks 5 and 7 where sandfish were not injured in the ventral side, and does not suffer from evisceration, the mortality was zero. Sandfish wounds heal within one to three weeks, depending on the surface area of the tegument eaten by Nile Tilapia. Healed wounds appear darker in color.

5. Conclusion
The study reveals that Mangrove Snail attack occur when sandfish was still in their natural habitat. Snail juveniles attack can cause death if the sandfish has already eviscerated. The healing process takes one to several weeks, depending on the size of the wound. The tank without substrate makes the sandfish unable to immerse itself so that it can be seen easily and can be attacked by Nile Tilapia at any time. Therefore, for the cultivation of sandfish with multitrophic systems without substrates, it is recommended to use double or triple tanks that are interconnected for the cultivation of sandfish and Nile Tilapia in separate tanks but ecologically connected.

Acknowledgment
We would like to thank Kemenristekdikti for providing research funding (contracts numbers 123/SP2H/PTNBH/DPRM/2018 and 1740/UN4.21/PL.01.10/2019), and Australia Awards Fellowship under Grant R170570.

References
[1] Tuwo, A. and C. Conand. 1996. Commercial holothurians in southwest Sulawesi (preliminary observations). Torani Bulletin Ilmu Kelautan. 6(2): p. 129-134.
[2] Yusuf, S., et al. 2017. Teripang fishing activities at Barang Lompo Island, Sulawesi, Indonesia: An update 20 years after a visit in 1996. SPC Beche-de-mer Information Bulletin, p. 99-102.
[3] Tuwo, A. 1992. Developments in beche-de-mer production in Indonesia during the last decade. SPC Beche-de-mer Information Bulletin, 1992. 4: p. 2-3.
[4] Tuwo, A. and J. Tresnati. 2015. Sea Cucumber Farming in Southeast Asia (Malaysia, Philippines, Indonesia, Vietnam). Echinoderm Aquaculture, 2015: p. 331-352.
[5] Tuwo, A. 2005. Status of sea cucumber fisheries and farming in Indonesia. FAO fisheries Technical Paper, 2005: p. 49-56.
[6] Massin, C., et al. 2009. *Taxonomy of the heavily exploited Indo-Pacific sandfish complex (Echinodermata: Holothuriidae).* Zoological Journal of the Linnean Society. \textbf{155}(1): p. 40-59.

[7] Duy, N.D. 2012. *Large-scale sandfish production from pond culture in Vietnam.* Asia–Pacific Tropical Sea Cucumber Aquaculture. ACIAR Proceedings, 2012. \textbf{136}: p. 34-39.

[8] Purcell, S.W. and D.S. Kirby. 2006. *Restocking the sea cucumber Holothuria scabra: Sizing no-take zones through individual-based movement modelling.* Fisheries Research. \textbf{80}(1): p. 53-61.

[9] Purcell, S.W., B.F. Blockmans, and N.N. Agudo. 2006. *Transportation methods for restocking of juvenile sea cucumber, Holothuria scabra.* Aquaculture. \textbf{251}(2-4): p. 238-244.

[10] Baskar, B. 1994. *Systematics, biology, ecology And zoogeography of holothurians: Some observations on the biology of the holothurian Holothuria (metriatyla) scabra (Jaeger).* CMFRI Bulletin. \textbf{46}: p. 39-43.

[11] Funge-Smith, S.J. and M.R. Briggs. 1998. *Nutrient budgets in intensive shrimp ponds: implications for sustainability.* Aquaculture. \textbf{164}(1-4): p. 117-133.

[12] Bachère, E. 2000. *Shrimp immunity and disease control.* Aquaculture. \textbf{191}(1-3): p. 3-11.

[13] Nga, B., et al. 2005. *Chemical and physical effects of crowding on growth and survival of Peneaus monodon Fabricius post-larvae.* Aquaculture. \textbf{246}(1-4): p. 455-465.

[14] Boyd, C.E. and L. Massaut. 1999. *Risks associated with the use of chemicals in pond aquaculture.* Aquacultural Engineering. \textbf{20}(2): p. 113-132.

[15] Syamsuddin, R., A. Tuwo, and N. Aswar. 2019. *Weight gain and carrageenan content of Kappaphycus alvarezii (Rhodophyta, Solierisceae) polycultured with Sargassum polycystum (Paeophyta, Sargassaceae).* in *IOP Conference Series: Earth and Environmental Science.* IOP Publishing. 7 p.

[16] Mulyani, S., et al. 2018. *Effect of seaweed Kappaphycus alvarezii aquaculture on growth and survival of coral Acropora maricata.* Aquaculture, Aquarium, Conservation & Legislation. \textbf{11}(6): p. 1792-1798.

[17] Yaqin, K., et al. 2014. *The use of Byssogenesis of green mussel, Perna viridis, as a biomarker in laboratory study.* Current Nutrition & Food Science. \textbf{10}(2): p. 100-106.

[18] Pauly, D., *Some simple methods for the assessment of tropical fish stocks.* 1983: FAO Fish.Tech.Pap. (234): 52 p.

[19] Yanagisawa, T. 1998. *Aspects of the biology and culture of the sea cucumber, in Tropical mariculture.* Elsevier. p. 291-308.

[20] Getachew, T. and C. Fernando. 1989. *The food habits of an herbivorous fish (Oreochromis niloticus Linn.) in Lake Awasa, Ethiopia.* Hydrobiologia. \textbf{174}(3): p. 195-200.

[21] Teferra, G. and C. Fernando. 1989. *The food habits of an herbivorous fish (Oreochromis niloticus Linn.) in Lake Awassa, Ethiopia.* Hydrobiologia. \textbf{174}: p. 195-200.

[22] Getachew, T. 1987. *A study on an herbivorous fish, Oreochromis niloticus L., diet and its quality in two Ethiopian Rift Valley lakes, Awasa and Zwai.* Journal of Fish Biology. \textbf{30}(4): p. 439-449.

[23] Bwanika, G., et al. 2004. *Observations on the biology of Nile tilapia, Oreochromis niloticus L., in two Ugandan crater lakes.* African Journal of Ecology. \textbf{42}: p. 93-101.

[24] Hamel, J.-F., et al. 2001. *The sea cucumber Holothuria scabra (Holothuroidea: Echinodermata): its biology and exploitation as beche-de-mer.* Academic Press. 41: p. 129-223

[25] Pitt, R. and N.D.Q. Duy. 2005. *Breeding and rearing of the sea cucumber Holothuria scabra in Viet Nam.* FAO Fisheries Technical Paper. p. 333-346.

[26] Lavitra, T., et al. 2009. *Problems related to the farming of Holothuria scabra (Jaeger, 1833).* SPC Beche-de-mer Information Bulletin. \textbf{29}: p. 20-30.

[27] Purcell, S.W. and M. Simutoga. 2008. *Spatio-temporal and size-dependent variation in the success of releasing cultured sea cucumbers in the wild.* Reviews in Fisheries Science. \textbf{16}(1-3): p. 204-214.

[28] Pitt, R. 2001. *Review of sandfish breeding and rearing methods.* Beche-de-mer information bulletin. \textbf{14}: p. 14-21.