Polyculture of sea cucumber (*Holothuria scabra*) and milkfish (*Chanos chanos*) in controlled tanks

Ni Ketut Maha Setiawati1,*, Sari Budi Moria Sembiring1, Sudewi Sudewi1, and I.N.A. Giri1

1Research Center for Fishery, National Research and Innovation Agency (BRIN), Cibinong, Bogor, Indonesia

**Abstract.** Milkfish (*Chanos chanos*) and sea cucumbers (*Holothuria scabra*) are fishery commodities that can grow well in ponds. The commodities have different feeding habits. Polyculture trials in controlled tanks are possible. This study aimed to determine the growth and survival of sea cucumbers reared in polyculture in tanks and to know whether sea cucumbers can use milkfish feces as feed. This study used 12 PE tanks of 500 L to ascertain that no other food sources were available in the tanks. The initial size of sea cucumber seeds was 3.4 – 5.4 cm and milkfish 19.0 – 22.5 cm. The treatments designed were (A) monoculture of sea cucumber (28.6 g/m²), (B) polyculture of sea cucumbers (28.6 g/m²) and milkfish (0.28 g/L), (C) polyculture of sea cucumbers (28.6 g/m²) and milkfish (0.56 g/L), and (D) monoculture of milkfish (0.56 g/L). Results showed that both monoculture and polyculture of sea cucumbers and milkfish resulted in high survival rates (100%). Although the polyculture of sea cucumbers showed no significant difference in growth, polyculture with milkfish (C) tended to have a higher final weight of sea cucumbers than the other treatments. Sea cucumbers could utilize milkfish feces and increase the growth of sea cucumbers.

1 Introduction

Milkfish in Indonesia is a featured product of pond culture besides seaweed, grouper, and Asian sea bass. However, pond waste, such as uneaten feed containing nutrients N and P, is released into the environment, causing water pollutant loads [1]. The increasing scale of milkfish culture causes environmental pollution problems, which is very prominent [1]. Effective control of milkfish culture in sustainable aquaculture and achieving clean production has become a problem that needs to be solved through scientific research [1].

Sea cucumbers are typical sea creature that filters sediments and feeds on considerable amounts of bacteria, debris particles, organic matter, and dead organisms [2]. Sea cucumbers have been known to have a control function and environmental regulation [3]. A previous study reported that sea cucumbers ingested and utilized food remains in the sediment of shrimp culture with an assimilation efficiency of organic matter 42.06% [4]. This finding indicated that sea cucumbers can reduce organic matter pollution in the aquatic environment.

* Corresponding author: mahasetiawati@yahoo.com

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Research showed that the sediment of milkfish culture contains several nutrients, such as N and P [1], which can be a good feed source for sea cucumbers. Polyculture of sea cucumber with other aquaculture commodities provides not only an environmental advantage but also, at the same time, significant economic benefits [4]. There is no available literature on the symbiotic mechanism of mutualism between sea cucumbers and milkfish. Therefore, this is the research gap that needs to be investigated.

Some of the previous research on the polyculture of sea cucumbers had been done between *Holothuria scabra* and red tilapia [5], *Holothuria scabra* with red seaweed *Kappaphycus striatum* [6], *Holothuria scabra* with *Litopenaeus vannamei* [4], *Holothuria scabra* with *Haliotis squamata* [7]. However, no reports on the polyculture of Holothuria scabra with milkfish are available. This study has an idea of managing the milkfish culture environment with *Holothuria scabra* through polyculture.

One of the problems in a polyculture system is determining the most effective combination of fish species in utilizing the natural food available in the culture environment. In addition, the combination of the fish species must be able to live together without causing competition for food or space. The present study assumed that sea cucumbers can utilize waste products from milkfish. Further, the polyculture of sea cucumbers and milkfish offers the efficient use of water space. Sea cucumbers inhabit the bottom of tanks, whereas milkfish are in the water column. The carrying capacity of sea cucumbers *Holothuria scabra* was reported to vary widely, from 100 to 692 g/m² [8]. This study investigated the growth and survival of sea cucumbers, *Holothuria scabra*, and milkfish reared in a polyculture system in controlled tanks.

### 2 Materials and methods

The experiment was carried out at the Institute for Mariculture Research and Fisheries Extension (IMRAFE), Gondol-Bali, using 12 PE tanks of 500 L. Rearing of sea cucumbers and milkfish indoors in a flow-through water system. Sea cucumbers and milkfish seeds used in this study were derived from the hatchery of IMRAFE in an indoor area. The initial size of sea cucumber seeds was 3.4 – 5.4 cm in total length and 3 - 4.9 g in weight. At the same time, the initial full length and weight of milkfish were 19.0-22.5 cm and 56 - 91 g, respectively. This weight range was wide, but the milkfish were the same age.

This experiment assessed different densities of milkfish in the polyculture of sea cucumbers and milkfish in the controlled tanks. Treatments in this study were culture methods of (A) monoculture of sea cucumber (28.6 g/m²), (B) polyculture of sea cucumbers (28.6 g/m²) and milkfish (0.28 g/L), (C) polyculture of sea cucumbers (28.6 g/m²) and milkfish (0.56 g/L), and (D) monoculture of milkfish (0.56 g/L). Earlier studies reported that the carrying capacity of sea cucumbers *Holothuria scabra* is around 100 to 692 g/m² [8]. The study was designed with a completely randomized design with three replications.

The sea cucumbers in treatment A were fed pellets produced at IMRAFE. The pelleted feed was formulated according to [9]. The protein content of the sea cucumber feed was 14%. The milkfish in treatment B, C, and D were fed a commercial pellet diet at 2.5-3 % of the biomass. This commercial pellet is specific for milkfish, which contains a protein of 26 %. Whereas the sea cucumbers in treatments B and C were unfed.

The experiment was performed for one month. The parameters measured in this study were the growth of the sea cucumbers and the milkfish with sampling methods (weight and total length). The length measurement was done using a ruler with an accuracy of 1 mm, and weight using a digital scale with an accuracy of 0.1 g. The survival rate was calculated at the end of the study. Specific growth rate (SGR) calculated using the equation: Ln Wt-LnWo)/t x 100%, where: Wt = average weight of sea cucumber at the end of rearing (g), W0 = average weight of sea cucumber at the beginning of rearing (g), t = rearing period (day). Total
nitrogen and phosphorus in the feces of each treatment and the milkfish and sea cucumber feeds were analyzed. Total nitrogen and phosphor were analyzed using the standard methods of H$_2$S destruction and distillation and destructing HNO$_3$ [10]. Water quality measurements included temperature, pH, salinity, ammonia, and light intensity. Feces collection was done daily from all the replicated tanks for each treatment. First, the feces was precipitated and filtered using a plankton net of 80-micron. Then, it was placed in a chamois to reduce the water content. Weighing of feces was done every day for 20 days. Data were analyzed by ANOVA test at a 95% confidence level using the SPSS program.

3 Results and discussion

3.1 Survival rates

Survival rates of sea cucumbers and milkfish after 30 days of experiment achieved 100%. This survival implied that sea cucumbers and milkfish could be farmed in a polyculture system. In this system, the sea cucumbers utilize uneaten feed and milkfish feces, which in turn support the growth of the sea cucumbers.

3.2 Body weight and total length

At the end of the experiment, sea cucumber reared with milkfish (0.56 g/L) (in treatment C) was seen to have a higher final body weight (6.55±2.36 g) than in treatments A and B, 3.78±0.59 g and 3.43±0.38 g, respectively (Figure 1). However, statistically, this was not significantly different (p>0.05). The monoculture of sea cucumbers (treatment A) and the polyculture of sea cucumbers with milkfish (0.28 g/L) (treatment B) also had similar final body weights (Figure 1). The total length of the sea cucumbers in monoculture (treatment A) and polyculture with milkfish was equal, 3.78±0.59 cm, 3.43±0.38 cm, and 4.03±0.64, respectively (Figure 2).

**Fig. 1.** Body weight (g) of the sea cucumber at the initial and termination of the experiment. (A) monoculture of sea cucumbers (28.6 g/m$^2$), (B) polyculture of sea cucumbers (28.6 g/m$^2$) with milkfish (0.28 g/L), (C) polyculture of sea cucumbers (28.6 g/m$^2$) with milkfish (0.56 g/L).
Fig. 2. Total length (cm) of the sea cucumber at the initial and termination of the experiment. (A) monoculture of sea cucumbers (28.6 g/m²), (B) polyculture of sea cucumbers (28.6 g/m²) with milkfish (0.28 g/L), (C) polyculture of sea cucumbers (28.6 g/m²) with milkfish (0.56 g/L).

The final weight of milkfish in the polyculture of sea cucumbers with 0.56 g/L milkfish/tank (treatment C) also tended to be higher than in the polyculture of sea cucumbers in treatment B, and monoculture of milkfish (D), 100±19.19 g compared with 92.18±17.70 g and 93.4±23.16, respectively (Figure 3). However, this value was not different among the treatments (p>0.05). Therefore, it can be stated that the polyculture of sea cucumbers with milkfish (treatments B and C) and monoculture of milkfish (D) resulted in a similar final weight of milkfish.

Fig. 3. Body weight (g) of the milkfish at the initial and termination of the experiment. (B) polyculture of sea cucumbers (28.6 g/m²) with milkfish (0.28 g/L), (C) polyculture of sea cucumbers (28.6 g/m²) with milkfish (0.56 g/L), (D) monoculture of milkfish (0.56 g/L).

In terms of the final total length of the milkfish, the polyculture of sea cucumbers with milkfish (treatment B and C) and monoculture of milkfish (D) resulted in a similar final total length, 21.65±1.53 cm; 22.04±1.44 cm and 21.86±1.87 cm (Figure 4).
Fig. 2. Total length (cm) of the sea cucumber at the initial and termination of the experiment. (A) monoculture of sea cucumbers (28.6 g/m$^2$), (B) polyculture of sea cucumbers (28.6 g/m$^2$) with milkfish (0.28 g/L), (C) polyculture of sea cucumbers (28.6 g/m$^2$) with milkfish (0.56 g/L).

**3.3 Specific Growth Rate (SGR)**

The specific growth rate of the sea cucumbers tends to be higher in polyculture with milkfish (treatment C), 1.99±0.74 %/day in comparison with only 0.26±1.58 %/day in treatment B and 0.50±0.64 %/day in monoculture of sea cucumbers (treatment A) (Figure 5). However, this specific growth rate was not different among treatments (p>0.05).

Fig. 5. The specific growth rate of sea cucumbers in monoculture (28.6 g/m$^2$) (A), (B) polyculture of sea cucumbers (28.6 g/m$^2$) with milkfish (0.28 g/L), (C) polyculture of sea cucumbers (28.6 g/m$^2$) with milkfish (0.56 g/L).

**3.4 Amount of feces in each treatment**

In this experiment, feces in polyculture treatments had not been separated between the feces of the sea cucumbers and the milkfish. Data shows that polyculture of sea cucumbers with milkfish (C) resulted in the highest amount of feces, while monoculture of milkfish (D)
showed the lowest amount (Figure 6). Although the number of milkfish in D and C was the same, in D, no sea cucumbers could utilize the feces, and therefore, it tended to dilute in the water column. As a consequence, its number was low. Whereas in B and C, the feces of the milkfish had been utilized by the sea cucumbers as their feed. Therefore, it could be that the high number of feces in C was mostly derived from the sea cucumbers. This statement could be supported by the number of feces in C, similar to that in A, where only sea cucumbers were in the tanks. When comparing the number of feces in B and C, it is seen that B had a lower number of feces. This could be due to the lower number of milkfish stocked in tank B, considering that the number of sea cucumbers in tanks B and C was the same.

Fig. 6. Amount of feces (g) in each treatment. Feces in B and C were a mixture of sea cucumbers and milkfish feces. (A) monoculture of sea cucumbers (28.6 g/m²), (B) polyculture of sea cucumbers (28.6 g/m²) with milkfish (0.28 g/L), (C) polyculture of sea cucumbers (28.6 g/m²) with milkfish (0.56 g/L), and (D) monoculture of milkfish (0.56 g/L).

3.5 Total Nitrogen (N) and total Phosphor (P) in the feces and the feeds

Results of total N analysis indicated that monoculture of milkfish (D) resulted in the highest content of total N in the feces (1.0998 %) than the other treatments. In the meantime, feces in the monoculture of sea cucumbers (A) contained a total N of 0.6099 %. Polyculture of sea cucumbers with milkfish (C and D) had similar total N content of 0.3806 % and 0.3304 %. In polyculture, the N content of feces was lower than in monoculture. The data show that polyculture could decrease the total N in the feces (Table 1). At the same time, the total P in the feces in polyculture tends to be higher than in both monocultures of sea cucumbers and milkfish (Table 1). Total P in the feces of the polyculture treatments was also higher than in the milkfish and sea cucumber feeds. This was probably because, in polycultures, the feces of the sea cucumber were mixed with the feces of the milkfish, which increased its total P content. The mixing of milkfish and sea cucumber feces occurred during the polyculture treatment on the N measurements.
Table 1. Total Nitrogen (N) and Phosphorus (P) in the feces in each treatment and the milkfish and sea cucumber feeds.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
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<th>B</th>
<th>C</th>
<th>D</th>
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</thead>
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<tr>
<td>Total N (%)</td>
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<td>0.6099</td>
<td>0.3806</td>
<td>0.3304</td>
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</tr>
<tr>
<td>Total P (%)</td>
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3.6 Water quality

Data on water quality are presented in Table 2. Temperatures and salinity ranges were the same between treatments, 28°C, and 33-34 ppt, respectively. Ranges of pH were similar among the treatments. Whereas ammonia was higher in the polyculture of sea cucumbers with milkfish (C) and in the monoculture of milkfish (D). Ammonia in the monoculture of sea cucumbers (A) and polyculture of sea cucumbers with milkfish (B) tended to be lower. The ammonia content in water increased with more milkfish in the tanks.

This study was conducted indoors, and regarding the light intensity, it is seen that the monoculture of milkfish (D) and polyculture of sea cucumbers (C) had lower light intensity compared to the monoculture of sea cucumbers (A) and the polyculture of sea cucumber in treatment B. This case was observed at 10:00 am and 1:00 pm measurements (Table 2).

Table 2. Water qualities in the rearing media during the experiment. (A) monoculture of sea cucumbers (28.6 g/m²), (B) polyculture of sea cucumbers (28.6 g/m²) with milkfish (0.28 g/L), (C) polyculture of sea cucumbers (28.6 g/m²) with milkfish (0.56 g/L), and (D) monoculture of milkfish (0.56 g/L).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatment</th>
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<th>B</th>
<th>C</th>
<th>D</th>
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<tr>
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<td>7.57-8.01</td>
<td>7.32-8.01</td>
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<tr>
<td>Salinity (ppt)</td>
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<td>33-34</td>
<td>33-34</td>
<td>33-34</td>
</tr>
<tr>
<td>Ammonia (mg/L)</td>
<td></td>
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<td>0.065-0.304</td>
<td>0.111-0.882</td>
<td>0.109-0.425</td>
</tr>
<tr>
<td>Light intensity (lux)</td>
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<td>300</td>
<td>300</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1:00 pm</td>
<td>1200</td>
<td>1200</td>
<td>800</td>
</tr>
</tbody>
</table>

Survival rates for sea cucumbers and milkfish for 30 rearing days were 100%. This implied that the polyculture system did not have adverse effects on both the animals, assuming that the density of the animals met the carrying capacity. Survival of sea cucumbers in this study was much higher than in the study by [7]; they found only around 7-9% survival. Their study used a 1 m³ tank with 100 sea cucumbers and 100 abalone /tank. In addition, probably that the differences in the light intensity affected survival rates. The light intensity in their study was around 400-550 lux. While in the present study, the light intensity achieved 800-1200 lux at 1:00 p.m.
Although no sea cucumber feed was given to tanks B and C, the sea cucumber grew well (Figure 1). This data represents that the sea cucumbers could utilize the uneaten feed and the feces of the milkfish. The sea cucumber in treatment B had similar growth to that in A but much lower growth than in C. This was due to the number of milkfish in tank B being only 0.28 g/L, whereas in tanks C was 0.56 g/L. This different density of milkfish produced a different number of feces. The higher density of milkfish in C produced higher feces, which the sea cucumbers can utilize. As a result, the sea cucumber in tank C had higher growth than in B, though this was not different from the other treatments (p>0.05).

The present study showed that unfed sea cucumbers in tanks B and C grew well. They fed on the uneaten feed of milkfish as well as other waste materials. This result aligns with the finding of [7], sea cucumber (*Holothuria scabra*) utilizing feces of abalone in a polyculture system. Proximate analysis indicated that the feces of abalone contained a protein of 9.99%, which could support the growth of the sea cucumber. No comparative analysis was done in the present study. However, total N showed that polyculture decreased the total N in the feces (Table 1). The decrease in total N was likely due to the sea cucumbers reared in the polyculture system utilizing nitrogen compounds available in the rearing media. This finding demonstrated sea cucumbers' functional role and potential to improve some adverse effects of organic matter enrichment in coastal ecosystems. Total N in the milkfish feed was higher (1.557 %) than in the feces of all the treatments. This was probably because the milkfish used the nitrogen content in the feed, and that contained in the feces of the milkfish was used by the sea cucumbers in the polyculture system. The total N in the milkfish feed was also higher than in the sea cucumber feed. Total N in the feces of sea cucumbers in A was slightly lower than in the sea cucumbers’ feed. This was likely because the sea cucumbers could only utilize a small number of nutrients containing nitrogen from the feed given.

Some studies also found that sea cucumbers are capable of utilizing waste materials. According to [11], sea cucumbers can utilize foods with low protein content, such as detritus and seaweed, and decomposing bacteria found in organic matter as food. Furthermore, the results of [12], regarding the polyculture of *Parastichopus californicus* with sablefish (*Anoplopoma fimbria*) showed that sea cucumbers consume organic matter derived from sablefish waste which was suitable food for sea cucumbers, stimulates the growth of these sea cucumbers. Several studies also prove that sea cucumbers can consume waste from polycultures such as green mussels, *Perna canaliculus* [13], shrimp [14], and oysters, *Crassostrea gigas* [15].

The growth of milkfish in the polyculture (B and C) and monoculture of milkfish (D) was not different (p>0.05). These results implied that culture systems of monoculture and polyculture of sea cucumber and milkfish did not affect the growth of the milkfish. In the present study, the protein content of the formulated diet was 26 %. A previous study found that a protein content of 25 % resulted in good growth of juvenile milkfish [16].

It has been known that appropriate light intensity is important to provide better growth of larval and juvenile sea cucumbers. Juvenile sea cucumber requires an optimal light intensity of 1,000 lux [17]. According to [18], the light intensity required for the growth of sand sea cucumbers is in the range of 800-1,500 lux, and [19] also reported the effect of the photoperiod on sea cucumber *Apostichopus japonicus*, where low light intensity during the rearing period significantly affected movement and also reduced the feeding time of sea cucumbers when compared to sea cucumbers reared in the natural light cycle. Light intensity has also been associated with the growth of diatoms or epiphytes as a natural food source for the sea cucumber culture. Low light intensity will also inhibit the growth of these diatoms and microalgae [20]. In the present study, the lower light intensity in tank C (800 lux) likely did not affect the growth of the sea cucumbers, as their growth was similar to the other treatments. This light intensity was still in the optimal range for sea cucumber culture.
Water quality observations, including temperature, pH, salinity, and ammonia levels, are shown in Table 2. Based on the results of water quality measurements during maintenance, it can be maintained at an optimum level for the growth of juvenile sea cucumbers. This range of water quality parameters corresponds to the optimum environmental conditions reported from existing research results [21].

The present study suggested that the polyculture of sea cucumbers and milkfish provides benefits to the growth of the sea cucumbers without any drawbacks to the growth of the milkfish. In addition, this polyculture does not require providing feed to the sea cucumbers, which provides economic benefits. In this polyculture, it was assumed that the sea cucumbers utilized uneaten feed and the feces of the milkfish, meaning that it lessens waste from the milkfish. However, this requires further research to confirm the existing uneaten feed and milkfish feces in the digestive tract of sea cucumbers.

4 Conclusion
Polyculture of sea cucumbers and milkfish resulted in the same survival (100%) with either monoculture of sea cucumber or monoculture of milkfish. The polyculture of sea cucumbers showed similar growth, though polyculture with milkfish (0.56 g/L) tended to have a higher final weight of sea cucumbers as well as milkfish than the other treatments.

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