Survival rate of brown-marbled grouper *Epinephelus fuscoguttatus* cultured with seaweed *Gracilaria changii* in multitrophic microcosm models

I Yasir¹,³,⁴, J Tresnati²,³,⁴, R Aprianto³ and A Tuwo¹,³,⁴

¹Marine Science Department, Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar, Indonesia
²Fisheries Department, Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar, Indonesia
³Multitrophic Research Group, Faculty of Marine Science and Fisheries, Universitas Hasanuddin, Makassar, Indonesia
⁴Center of Excellence for Development and Utilization of Seaweed, Universitas Hasanuddin, Kampus Tamalanrea KM. 10, Makassar 90245, Sulawesi Selatan, Indonesia

Email: ambotuwo62@gmail.com

**Abstract.** Groupers (*Epinephelus*) are economically important fish. *Gracilaria changii* is a red alga with a high economic value that is widely cultivated in brackish water ponds. In this study, *Epinephelus fuscoguttatus* and *G. changii* were polycultured in a multitrophic microcosm model. This study aimed to evaluate the possibility of these two species being cultured multitrophically. In this study we analysed the survival rate of groupers under two seaweed cultivation models (a bottom method and floating method). A control microcosm had no seaweed (grouper monoculture). Fish weight used in the treatment was 135.94 to 359.36 g. The study lasts 47 days at the controlled indoor tanks. The survival rate is lower than the results in previous studies. The condition of water quality parameters was quite good during the treatment, except for ammonia and phosphate. The high ammonia and phosphate content is thought to be the cause of the low survival rate. The survival rate ranges from 47-87%.

1. **Introduction**

Brown-marbled grouper *Epinephelus fuscoguttatus* is a superior species in the development of marine culture in Indonesia. Grouper is an export commodity that has a selling value nearly five times greater than other frozen fish species [1]. Grouper is traded alive. These live fish are mostly caught in nature. The capture of groupers from nature continues to increase as market demand increases. This increasing demand causes the catch in nature to be unable to meet the increasing market demand.

One of the efforts to meet the needs of live groupers is cultivation. Currently, cultivation development is generally carried out in the sea in the form of monoculture floating net cages[2, 3] and polyculture [4]. In addition, grouper cultivation in ponds [5, 6] and controlled media have also begun to develop [7].

Currently grouper cultivation is mostly maintained in the sea in floating net cages [8]. The floating net method, besides having advantages, also has disadvantages, including hydrodynamic conditions which are very volatile in certain seasons [8] making it difficult to do throughout the year. Likewise with aquaculture in ponds which often have problems with water quality. This study aims to test...
whether the multitrophic culture model between Brown-marbled grouper and Gracilaria changii seaweed can be an alternative model in grouper cultivation. 

*G. changii* is a seaweed that is widely cultivated in ponds [9]. *G. changii* was chosen because it has been shown to be able to live in a very fluctuating salinity range in the pond. In addition, *G. changii* has also been proven to be a bioremediator agent [10] which is resistant to variations in water physics and chemistry factors [11].

2. Materials and Methods

The research took place during one seaweed rearing cycle (47 days). In this preliminary study, multitrophic cultivation was used with a model of three series of tanks each connected to one another [12]. Each tank with a volume of one ton measuring 1 x 2 x 0.5 m filled with water as high as 0.4 m or about 800 liters. The three tanks are placed side by side at different levels so that water can flow continuously from the highest tank (tank 1) to the middle (tank 2) and to the lowest tank (tank 3) (Figure 1a). To flow water from tank 3 to tank 1, a water pump with a pump capacity of 800 liters per hour is used (Figure 1b). In tank 2 for each circuit there are two pumps with a power of 800 liters per hour to rotate the water and supply oxygen (Figure 1c). In addition, for all tanks 2 containing five Brown Marble Groupers, two aerators of 3.5 liters/minute each provided for extra aeration (Figure 1d). For tank 1 and all tanks filled with seaweed, 2 of 27 Watts LED lamps were added with light intensity between 5400-6300 lux (5706 ± 514) (Figure 1a, d).

In this study, three treatments were used, namely: (1) fish and seaweed using bottom and hanging methods; (2) fish and seaweed using bottom method; and (3) fish, without seaweed as control (Figures 1 e, f). Each treatment has three replications. The method used was a completely randomized design, with details of the results of the randomization described in Table 1. Tanks containing seaweed (both hanging and bottom methods) were given seaweed weighing 500 g.

![Figure 1. Multitrophic cultivation facilities used during treatment](image-url)

Before the research began, the Brown-Marbled Grouper was adapted to the environmental conditions of the research tank. During this adaptation process, the fish are given natural food in the form of fresh fish meat at 2% of their body weight per day. Because there is always leftover feed, during the study, the Brown-Marbled Grouper was only fed 1.5-1.9% of the average weight. Before feeding, the remaining food is removed from the tank to prevent water quality from deteriorating. Water quality parameters measured were temperature, salinity, pH, nitrate, nitrite, ammonia, and phosphate. The treatment parameter observed was the number of fish that survived. At the beginning of the treatment, tank 2 for each treatment and replication was filled with 5 Brown-Marbled Groupers.
Survival rate (SR) was determined based on the equation $SR = \frac{N_f}{N_0} \times 100\%$. $N_0$: number of grouper when the experiment started, $N_f$: number of grouper at the end of the experiment.

Table 1. Treatment matrix of Brown-Marbled Grouper *Epinephelus fuscoguttatus* cultured with seaweed *Gracilaria changii* in multitrophic microcosm models

| Treatment | Tank 1 (seaweed) | Tank 2 (Fish) | Tank 3 (seaweed) |
|-----------|------------------|---------------|------------------|
| 1.1       | Off Bottom Method| 5             | Floating Method  |
| 1.2       | Off Bottom Method| 5             | Floating Method  |
| 1.3       | Off Bottom Method| 5             | Floating Method  |
| 2.1       | Off Bottom Method| 5             | None             |
| 2.2       | Off Bottom Method| 5             | None             |
| 2.3       | Off Bottom Method| 5             | None             |
| 3.1       | None             | 5             | None             |
| 3.2       | None             | 5             | None             |
| 3.3       | None             | 5             | None             |

Figure 2. The placement of research tanks based on randomization results

3. Results

3.1 Size and Survival Rate

Although the fish weight that used in the experiment varied from 118.34 to 394.93g (with the average of 252.68g±59.45) (Table 2), there was no significant different found (P> 0.05) between treatments.

Table 2. The weight of Brown-Marbled Grouper *Epinephelus fuscoguttatus* in each treatment

| Treatment | The weight of Brown-Marbled Grouper *Epinephelus fuscoguttatus* (g) | mean ± STD |
|-----------|---------------------------------------------------------------------|------------|
|           | 1                      | 2          | 3          | 4          | 5          |
| 1.1       | 176.12                 | 359.36     | 394.93     | 285.01     | 278.99     | 298.88±84.47 |
| 1.2       | 305.65                 | 230.23     | 287.09     | 226.04     | 210.24     | 251.85±41.84 |
| 1.3       | 299.62                 | 264.49     | 206.64     | 176.89     | 257.96     | 241.12±48.90 |
| 2.1       | 310.07                 | 288.43     | 205.55     | 167.69     | 148.90     | 224.13±71.96 |
| 2.2       | 167.66                 | 353.54     | 319.67     | 235.82     | 297.47     | 274.83±73.70 |
| 2.3       | 246.41                 | 354.37     | 135.94     | 279.20     | 251.73     | 253.53±78.60 |
| 3.1       | 118.34                 | 188.61     | 200.29     | 192.98     | 274.07     | 194.86±55.23 |
| 3.2       | 293.27                 | 293.27     | 301.10     | 261.65     | 245.52     | 194.86±55.23 |
| 3.3       | 261.27                 | 266.82     | 255.57     | 288.50     | 267.49     | 267.93±12.47 |
At the end of the experiment, the survival rate varied between 0 to 100% (Table 3). The average test results showed that the survival rate was not significantly different (P> 0.05) for all treatments.

**Table 3. Survival rate Brown-Marbled Grouper *Epinephelus fuscoguttatus* cultured with seaweed *Gracilaria changii* in multitrophic microcosm model.**

| Treatment | N₀ | Nₜ | Survival Rate (%) | Range | Mean | STD |
|-----------|----|----|-------------------|-------|------|-----|
| 1.1       | 5  | 0  | 0-100             | 46.67 | 50.33|
| 1.2       | 5  | 5  | 0-100             | 66.67 | 57.74|
| 1.3       | 5  | 2  | 0-100             | 86.67 | 11.55|
| 2.1       | 5  | 5  | 0-100             | 46.67 | 50.33|
| 2.2       | 5  | 4  | 80-100            | 86.67 | 11.55|
| 3.1       | 5  | 4  | 80-100            | 86.67 | 11.55|
| 3.2       | 5  | 5  | 80-100            | 86.67 | 11.55|
| 3.3       | 5  | 5  | 80-100            | 86.67 | 11.55|

Notes.: N₀: number of fish when the experiment started, Nₜ: number of fish left at the end of the experiment

3.2. Feed

The weight of the feed given varied between 1.57-190g, while the consumption varied between 0.37 to 1.90 g (Table 4). The average test results showed that the percentage of feed given and eaten was not significantly different (P> 0.05) for all treatments.

**Table 4. Feed and food consumption of Brown-Marbled Grouper *Epinephelus fuscoguttatus***

| Treatment | Feed Eaten (% of total body weight) | 1   | 2   | 3   | 4   | 5   | Mean±STD |
|-----------|-----------------------------------|-----|-----|-----|-----|-----|---------|
| 1.1       |                                   | 1.89| 1.63| 1.82| 1.77| 1.75| 1.77±0.10|
| 1.2       |                                   | 1.88| 1.90| 1.83| 1.76| 1.75| 1.82±0.07|
| 1.3       |                                   | 1.86| 1.90| 1.77| 1.77| 1.75| 1.81±0.07|
| 2.1       |                                   | 1.87| 1.75| 1.83| 1.75| 1.74| 1.79±0.06|
| 2.2       |                                   | 1.81| 1.72| 1.82| 1.80| 1.71| 1.77±0.05|
| 2.3       |                                   | 1.84| 1.57| 1.78| 1.74| 1.76| 1.74±0.10|
| 3.1       |                                   | 1.85| 1.86| 1.85| 1.72| 1.73| 1.80±0.07|
| 3.2       |                                   | 1.86| 1.85| 1.81| 1.73| 1.74| 1.80±0.06|
| 3.3       |                                   | 1.84| 1.79| 1.80| 1.71| 1.71| 1.77±0.06|

| Treatment | Feed Eaten (% of total body weight) | 1   | 2   | 3   | 4   | 5   | Mean±STD |
|-----------|-----------------------------------|-----|-----|-----|-----|-----|---------|
| 1.1       |                                   | 0.74| 1.56| 1.65| 0.95| 0.57| 1.09±0.49|
| 1.2       |                                   | 1.88| 1.90| 1.83| 1.76| 1.21| 1.72±0.29|
| 1.3       |                                   | 1.54| 1.90| 1.77| 1.77| 0.84| 1.56±0.42|
| 2.1       |                                   | 1.87| 1.75| 1.83| 1.75| 1.74| 1.79±0.06|
| 2.2       |                                   | 1.81| 1.72| 1.82| 1.80| 1.71| 1.77±0.05|
| 2.3       |                                   | 1.25| 0.95| 0.95| 1.34| 1.08| 1.11±0.17|
| 3.1       |                                   | 1.85| 1.86| 1.85| 0.87| 0.83| 1.45±0.55|
| 3.2       |                                   | 1.86| 1.85| 1.81| 0.71| 1.52| 1.55±0.49|
| 3.3       |                                   | 1.14| 1.66| 1.80| 0.97| 0.37| 1.19±0.57|

3.3. Nitrate

The nitrate content at the beginning of the study ranged from 0.796 to 2.008 (mean 1.407 ± 0.423), and at the end of the study ranged from 1.270 to 1,950 (mean 1,652 ± 0.256) (Table 5). All treatments showed an increase in nitrate content at the end of the treatment. The average test results showed that the increase in nitrate content was not significantly different (P> 0.05) for all treatments.
Table 5. Nitrate content in the water at the beginning (Day 0) and at the end (Day 47) of the experiment

| Treatment | Nitrat-NO\textsubscript{3} (ppm) | Day 0 | Range | Mean±STD | Day 47 | Range | Mean±STD |
|-----------|-------------------------------|-------|-------|---------|-------|-------|---------|
| 1.1       | 0.796                         | 0.796-1.700 | 1.181±0.467 | 1.370   | 1.370-1.931 | 1.675±0.284 |
| 1.2       | 1.048                         | 0.796-1.700 | 1.724 | 1.931   | 1.370-1.931 | 1.675±0.284 |
| 1.3       | 1.700                         | 1.513-1.700 | 1.735 | 1.532   | 1.532-1.905 | 1.724±0.187 |
| 2.1       | 1.513                         | 1.513-1.700 | 1.597±0.080 | 1.905   | 1.532-1.905 | 1.724±0.187 |
| 2.2       | 1.673                         | 1.513-1.700 | 1.597±0.080 | 1.905   | 1.532-1.905 | 1.724±0.187 |
| 2.3       | 1.604                         | 2.008-2.008 | 1.442±0.600 | 1.447   | 1.270-1.950 | 1.556±0.353 |
| 3.1       | 0.813                         | 0.813-2.008 | 1.673 | 1.370   | 1.370-1.931 | 1.675±0.284 |
| 3.2       | 1.506                         | 0.813-2.008 | 1.673 | 1.370   | 1.370-1.931 | 1.675±0.284 |
| 3.3       | 0.813                         | 0.813-2.008 | 1.673 | 1.370   | 1.370-1.931 | 1.675±0.284 |

3.4. Ammonia

The ammonia content in the water at the start of the study was undetectable, and at the end of the study ranged from 0.323 to 1.255 (mean 0.581 ± 0.280) (Table 6). All treatments showed an increase in nitrate content at the end of the treatment. The average test results showed that the increase in nitrate content was not significantly different (P> 0.05) for all treatments.

Table 6. Ammonia content in the water at the beginning (Day 0) and at the end (Day 47) of the experiment

| Treatment | Ammonia-NH\textsubscript{3} (ppm) | Day 0 | Day 47 | Range | Mean±STD |
|-----------|---------------------------------|-------|-------|-------|---------|
| 1.1       | 0.000                           | 0.702 |
| 1.2       | 0.000                           | 0.455 | 0.455-0.702 | 0.554±0.130 |
| 1.3       | 0.000                           | 0.506 |
| 2.1       | 0.000                           | 0.323 |
| 2.2       | 0.000                           | 0.612 | 0.323-0.612 | 0.502±0.157 |
| 2.3       | 0.000                           | 0.572 |
| 3.1       | 0.000                           | 0.455 |
| 3.2       | 0.000                           | 0.350 | 0.350-1.255 | 0.687±0.495 |
| 3.3       | 0.000                           | 1.255 |

3.5. Phosphate

The phosphate content at the start of the study ranged from 6,410 to 10,540 (mean of 7,398 ± 1,273), and at the end of the study ranged from 5,563 to 8,762 (mean of 6,824 ± 0.984) (Table 7). There was an increase of average phosphate content in the water for treatment 1. In contrary, a decrease was observed in treatment 2 and 3. The average test results showed that the increase or decrease in the phosphate content was not significantly different (P> 0.05) for all treatments. Freshwater phosphate content is 25.64 ppm.

Table 7. The phosphate content at the beginning (Day 0) and at the end of the experiment (Day 47).

| Treatment | Phosphate-PO\textsubscript{4} (ppm) | Day 0 | Range | Mean±Std | Day 47 | Range | Mean±Std |
|-----------|---------------------------------|-------|-------|---------|-------|-------|---------|
| 1.1       | 7,422                           | 7,422 | 6.410-7.422 | 6.747±0.584 | 5.563 | 5.563-8.762 | 7.386±1.646 |
| 1.2       | 6,410                           | 6.410-7.422 | 6.747±0.584 | 5.563 | 5.563-8.762 | 7.386±1.646 |
| 1.3       | 6,410                           | 6.410-7.422 | 6.747±0.584 | 5.563 | 5.563-8.762 | 7.386±1.646 |
| 2.1       | 7,532                           | 7,532 | 6.793-10.540 | 8.288±1.985 | 6.793 | 6.657-7.122 | 6.857±0.239 |
| 2.2       | 10,540                          | 10,540 | 6.793-10.540 | 8.288±1.985 | 6.793 | 6.657-7.122 | 6.857±0.239 |
| 3.1       | 7,751                           | 7,751 | 6.684-7.751 | 7.158±0.543 | 6.547 | 5.918-6.547 | 6.228±0.315 |
| 3.2       | 7,039                           | 7,039 | 6.684-7.751 | 7.158±0.543 | 6.547 | 5.918-6.547 | 6.228±0.315 |
| 3.3       | 6,684                           | 6,684 | 6.684-7.751 | 7.158±0.543 | 6.547 | 5.918-6.547 | 6.228±0.315 |
3.6. **Dissolved Oxygen**
The dissolved oxygen of the culture media at eight measurement periods in 24 hours varied from 4.38 to 5.89 (Table 8). The average test results showed that dissolved oxygen content was not significantly different (P > 0.05) for all treatments.

| Treatment | Time (Hour) | 09.00 | 12.00 | 15.00 | 18.00 | 21.00 | 00.00 | 03.00 | 06.00 |
|-----------|-------------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1.1       |             | 5.40  | 5.80  | 6.00  | 5.87  | 6.73  | 5.03  | 5.50  | 5.17  |
| 1.2       |             | 6.10  | 5.33  | 4.87  | 4.80  | 4.33  | 4.80  | 4.97  | 4.10  |
| 1.3       |             | 5.90  | 4.47  | 5.10  | 4.23  | 5.23  | 5.20  | 4.67  | 3.87  |
| Average± STD |         | 5.80±0.36 | 5.20±0.68 | 5.32±0.60 | 4.97±0.83 | 5.43±1.21 | 5.01±0.20 | 5.04±0.42 | 4.38±0.69 |

3.7. **pH**
The pH of the water at eight measurement periods in 24 hours varied from 6.86 to 7.31 for Treatment 1, from 7.22 to 7.42 for Treatment 2, and from 6.99 to 7.43 for Treatment 3 (Table 9). The average test results showed that the pH of the water was significantly different (P < 0.05) for all treatments.

| Treatment | Time (Hour) | 09.00 | 12.00 | 15.00 | 18.00 | 21.00 | 00.00 | 03.00 | 06.00 |
|-----------|-------------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1.1       |             | 6.99  | 7.14  | 7.36  | 7.26  | 6.86  | 6.95  | 6.95  | 7.01  |
| 1.2       |             | 7.19  | 7.22  | 7.19  | 7.31  | 7.05  | 7.14  | 7.15  | 7.12  |
| 1.3       |             | 7.16  | 7.07  | 7.25  | 7.25  | 7.01  | 7.05  | 7.09  | 7.29  |
| Mean± STD |             | 7.11±0.11 | 7.14±0.08 | 7.27±0.09 | 7.27±0.03 | 6.97±0.10 | 7.05±0.10 | 7.06±0.10 | 7.14±0.14 |
| 2.1       |             | 7.25  | 7.36  | 7.27  | 7.39  | 7.26  | 7.28  | 7.28  | 7.22  |
| 2.2       |             | 7.30  | 7.42  | 7.42  | 7.42  | 7.29  | 7.33  | 7.34  | 7.24  |
| 2.3       |             | 7.29  | 7.38  | 7.31  | 7.40  | 7.27  | 7.28  | 7.30  | 7.22  |
| Mean± STD |             | 7.28±0.02 | 7.39±0.03 | 7.33±0.08 | 7.40±0.02 | 7.27±0.02 | 7.30±0.03 | 7.31±0.03 | 7.23±0.02 |
| 3.1       |             | 7.29  | 7.28  | 7.43  | 7.34  | 7.15  | 7.19  | 7.22  | 7.16  |
| 3.2       |             | 7.19  | 7.20  | 7.36  | 7.19  | 7.12  | 7.10  | 7.22  | 7.10  |
| 3.3       |             | 7.13  | 6.99  | 7.29  | 7.28  | 7.28  | 7.02  | 7.06  | 7.09  |
| Mean± STD |             | 7.20±0.08 | 7.16±0.15 | 7.36±0.07 | 7.27±0.08 | 7.18±0.08 | 7.10±0.08 | 7.17±0.09 | 7.11±0.04 |

3.8. **Salinity**
The water salinity at the eight measurement periods in 24 hours varied from 30.0 to 33.5 in Treatment 1, from 30.9 to 33.4 in Treatment 2, and from 32.4 to 33.6 in Treatment 3 (Table 10). The mean test results showed that the salinity in treatment 1 and 3 was not significantly different (P > 0.05) for all treatments, while the other pairs were significantly different (P < 0.05).

3.9. **Temperature**
The water temperature at eight measuring periods in 24 hours varied between treatments (Table 11). The average test results showed there was no significant different in temperature between treatment 2 and 3 (P > 0.05), however the other pairs were significantly different (P < 0.05).
Table 10. The water salinity at the eight measurement periods in 24 hours.

| Treatment | Time (Hour)      |
|-----------|-----------------|
|           | 09.00 | 12.00 | 15.00 | 18.00 | 21.00 | 00.00 | 03.00 | 06.00 |
| 1.1       | 33.0  | 32.8  | 32.8  | 33.2  | 33.2  | 33.2  | 33.1  | 33.5  |
| 1.2       | 33.2  | 33.3  | 33.0  | 33.3  | 33.3  | 33.2  | 33.4  | 33.4  |
| 1.3       | 30.1  | 33.2  | 30.0  | 33.3  | 33.3  | 33.2  | 33.2  | 33.5  |
| Mean± STD | 32.1±1.7 | 33.1±0.3 | 31.9±1.7 | 33.3±0.1 | 33.4±0.1 | 33.2±0.0 | 33.3±0.2 | 33.5±0.1 |

2.1 31.1 31.0 30.9 30.9 31.0 31.0 31.0 32.5
| 2.2  | 33.4  | 32.6  | 33.2  | 32.6  | 32.6  | 32.6  | 32.6  | 32.7  |
| 2.3  | 31.8  | 32.3  | 31.6  | 32.3  | 32.3  | 32.4  | 32.4  | 31.1  |
| Mean± STD | 32.1±1.2 | 31.9±0.9 | 31.9±1.2 | 31.9±0.9 | 32.0±0.9 | 32.0±0.9 | 32.0±0.9 | 32.1±0.9 |

3.1 33.3 33.2 33.1 32.4 33.2 33.2 33.2 33.3
3.2 32.8 32.9 32.7 32.9 33.0 32.8 32.9 32.4
3.3 33.6 32.9 32.9 32.8 32.9 32.2 33.1 33.4
| Mean± STD | 33.2±0.4 | 33.0±0.1 | 32.9±0.2 | 32.8±0.4 | 33.1±0.1 | 33.0±0.2 | 33.1±0.1 | 33.0±0.6 |

Table 11. The temperature at the eight measurement periods in 24 hours

| Treatment | Time (Hour)      |
|-----------|-----------------|
|           | 09.00 | 12.00 | 15.00 | 18.00 | 21.00 | 00.00 | 03.00 | 06.00 |
| 1.1       | 24.8  | 26.2  | 25.9  | 25.4  | 25.4  | 25.5  | 25.5  | 25.4  |
| 1.2       | 24.8  | 26.2  | 25.4  | 25.3  | 25.3  | 25.4  | 25.3  | 25.3  |
| 1.3       | 24.1  | 26.0  | 25.3  | 25.2  | 25.1  | 25.3  | 25.1  | 25.2  |
| Mean± STD | 24.8±0.4 | 26.1±0.1 | 25.5±0.3 | 25.3±0.1 | 25.3±0.1 | 25.4±0.1 | 25.3±0.2 | 25.3±0.1 |

2.1 24.3 26.0 25.5 25.2 25.1 25.2 25.2 25.1
| 2.2  | 24.0  | 26.0  | 25.2  | 25.1  | 25.1  | 25.2  | 25.1  | 25.1  |
| 2.3  | 24.1  | 26.0  | 25.6  | 25.2  | 25.2  | 25.2  | 25.1  | 25.0  |
| Mean± STD | 24.1±0.1 | 26.0±0.0 | 25.4±0.2 | 25.2±0.0 | 25.1±0.0 | 25.2±0.0 | 25.1±0.0 | 25.0±0.1 |

3.1 24.5 26.1 25.2 25.3 25.2 25.3 25.2 25.3
3.2 24.5 26.0 25.2 25.2 25.2 25.3 25.2 25.2
3.3 24.3 26.0 25.3 25.2 25.3 25.2 25.3 25.2
| Mean± STD | 24.4±0.1 | 26.1±0.0 | 25.2±0.1 | 25.2±0.1 | 25.2±0.0 | 25.3±0.0 | 25.2±0.1 | 25.2±0.1 |

4. Discussion

4.1. Fish Size and Survival Rate
The size of the fish used in this study is bigger than the size of the grouper seeds that are usually kept in the sea, which is around 60 g [4]. The survival rate in this study is much smaller than that of grouper fish cultivation in the sea which can reach 100% [4]. Previous studies have shown that the Brown-Marbled Grouper culture fed pellets produced the lowest survival rate (76.67%), and those fed trash fish produced the highest survival rate (96.66%). The survival rate in this study was lower than the survival rate for the Brown-Marbled Grouper who was fed pellets [13]. This indicates that there were other causes of death besides food.

4.2. Feed
The assumption that fish farming is the cause of eutrophication is confirmed by the results of this study. In this study it is known that the Brown-Marbled Grouper consumes less than 2% of the total body weight, while in grouper cultivation so far using a feeding ratio of 10-15% [4]. There is over feeding of 8-13% of fish weight which can have serious impacts on the waters if grouper farming is carried out intensively and massively.
4.3. Nitrate
Nitrate is an important source of nitrogen for aquaculture, it is very soluble and stable in water. Nitrate is the main nutrient for plankton growth [14] and algae [7]. Nitrate are produced from the complete oxidation of nitrogen compounds in the water [15]. Nitrate is also used to classify the level of fertility in waters. Oligotrophic waters contain 0 to 1 ppm of nitrate, 1 to 5 ppm of mesotrophic waters, and 5 to 50 ppm of eutrophic waters [15].

The nitrate content during the study was quite low. Nitrate levels in water that are harmful to fish and invertebrates range from 1,000 to 3,000 ppm. Therefore, nitrate poisoning in aquatic animals is very rare [16]. However, for grouper maintenance in ponds, the nitrate concentration should not exceed 10 ppm [17]. Nitrate content in waters depends on several factors such as water movement, oxidation, reduction, assimilation, and decomposition of organic matter [18]. The ideal nitrate content for the growth of organisms ranges 2 to 3.5 ppm [15]. Nitrate content above 3.5 ppm can harm the waters [19].

In the sea where Grouper is kept, nitrate levels on the surface of the waters range from 0.015-0.026 ppm with an average value of 0.020±0.003 ppm, while nitrate levels near the bottom range from 0.024 to 0.035 ppm with an average value of 0.030±0.003 ppm [20]. The average difference in nitrate levels between the two depth layers is quite large (0.011 ppm). The average nitrate concentration for the surface area is lower because the nitrate on the surface is widely used by phytoplankton. In addition, the production of nitrate from the biodegradation of organic materials into ammonia which is subsequently oxidized to nitrate occurs in the sediment [21] and this also increases nitrate levels near the bottom. Nitrate content in marine waters generally ranges from 0.00014 to 0.70000 ppm [22].

4.4. Ammonia
Ammonia in waters comes from the process of decomposing organic materials. Ammonia usually comes from the feces of organisms and the activity of microorganisms in the process of decomposition of nitrogen-rich organic matter. In an uncontaminated environment, ammonia content is lower than nitrate [15]. In ponds, the ammonia content will increase in line with decomposition process and increasing water temperatures. The ammonia content in the water should not be more than 1 ppm [23].

In the cultivation of Brown-Marbled Grouper in ponds, the water ammonia content ranges from 0.0013 to 0.0695 ppm [7]. This content is much lower than the ammonia content at the end of this study. These results indicate that the levels of ammonia in all treatments do not support the growth and life of the grouper. This is thought to be the cause of the low survival rate in all treatments.

4.5. Phosphate
Although the phosphate content at the beginning and end of the treatment is quite high, it is relatively stable. The addition of fresh water which is used to maintain the salinity of the culture medium due to the evaporation process, also adds phosphate to the culture medium because of its high concentration (25.64 ppm). Although the addition of fresh water also means that it also increases the levels of phosphate in the culture medium, the stable phosphate content indicates that the two multitrophic culture models developed are quite good at reducing the relatively large phosphate content. The phosphate content which far exceeds the normal threshold of 0.015 ppm [24] is thought to be the indirect cause of the low survival rate. Even so, it is suspected that the effect of phosphate in this study is related to water quality parameters or other factors that have not been revealed in this preliminary study.

In marine waters where groupers are cultured, the phosphate content ranges from 0.005 to 0.015 ppm with an average of 0.009 ppm. Phosphate levels in normal marine waters, range from 0.00031-0.12400 mg/l [22]. The upper limit of phosphate content in uncontaminated waters is 0.087 ppm, while the upper limit for marine life is 0.015 ppm 0.015 ppm [25]. These results indicated that the phosphate levels in all treatments were less supportive for grouper growth and life. This is thought to be the cause of the low survival rate in all treatments.
4.6. **Dissolve Oxygen**

The oxygen content in normal marine waters ranges from 5.7 to 8.5 ppm [26]. The range of dissolved oxygen content during the study was quite low when compared to oxygen content for grouper aquaculture in the sea, namely 4.64 to 5.85 [20], 5.9-7.7 ppm [4] or 6.6-7.6 ppm [8]. Even so, the dissolved oxygen content in this study (4.38 to 5.89 ppm) was still in the optimal range for aquaculture in ponds whose values were between 3.5 to 8.0 ppm [17].

Low dissolved oxygen content in water can be caused by the breakdown of organic matter which requires oxygen. Dissolved oxygen levels in water will decrease as a result of inhibited decomposition of organic matter, respiration and aeration [27]. The value of dissolved oxygen in the mass of water is relative, usually in the range of 6-14 ppm [28]. In areas of fertile coral waters, the oxygen content ranges from 4.27 to 7.14 ppm [20].

In general, an oxygen content of 5 ppm is still good for fish life [29], even in waters that are not contaminated with toxic compounds, an oxygen content of 2 ppm is sufficient to support aquatic organisms [30]. The ideal dissolved oxygen content for the growth of Brown-Marbled Grouper seeds is above 5 ppm [31]. The daily fluctuation of dissolved oxygen will be very dangerous for grouper life if it exceeds the tolerance limit (3.4-6.5 ppm) for a long time [32].

4.7. **pH**

The process of respiration and decomposition will affect the pH of the water. The pH value of water can decrease if there is a process of respiration and decomposition of organic substances. The decrease in the pH of the culture media can cause a decrease in fish blood pH (acidosis) which will inhibit the function of blood to carry oxygen. The pH during maintenance is still good, even though it is below the pH range for grouper culture in the sea, range between 7.5 to 8.3 [8] or 7.7 to 8.3 [4]. Low pH can make fish more susceptible to disease and is usually followed by a high mortality rate [7]. Groupers can tolerate pH range of water between 6.5 and 9.0 [14, 33], but groupers live better if they are kept in seawater with a pH 8.0 – 8.2 [34].

4.8. **Salinity**

Salinity is a measure of the concentration of ions dissolved in water which is expressed in grams per liter (g / l) or parts per thousand (ppt) [35]. Salinity is one aspect of water quality that plays an important role because it affects the osmotic pressure of the water. The higher the salinity of the water, the higher the osmotic pressure [36]. The effect of salinity is related to energy transformation and osmoregulation processes in an effort to maintain the balance of body fluid pressure and its environment.

Grouper favors water with salinity between 25 to 35 ppt, however for certain species, such as humpback grouper, can live and grow well at a salinity of 5 to 35 ppt [17]. Although the grouper is a marine animal that can tolerate low salinity, if it is to be cultivated in a lower salinity media, the acclimation should be performed first [7].

The optimal salinity for grouper cultivation in ponds is in the range of 25 to 35 ppt [17, 31]. The salinity of the cultivation media during the experiment was in a range for grouper cultivation (30-34 ppt).

4.9. **Temperature**

Water temperature can affect survival, growth, morphology, reproduction, behavior, fish metabolism, solubility of gases, reaction speed of elements and compounds contained in water [7]. The high mortality is thought to be related to lower temperatures (< 26.1°C) compared to the lowest temperature of grouper culture in the sea which is 29.5°C [4], while the optimal temperature for grouper cultivation in ponds is around 25 to 30.2°C [7, 17].

5. **Conclusion**

The survival rate is lower than that which has been reported in previous studies. The condition of water quality parameters was quite good during the treatment, except for ammonia and phosphate. The high ammonia and phosphate content is thought to be the cause of the low survival rate.
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