A Microcosm Multitrophic Aquaculture System

A Tuwo1,3, I Yasir1,3, J Tresnati2,3, Mutmainnah3, R Aprianto2,3, A Y anti, A D Bestari2,3 and M Nakajima4

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
4School of Integrative and Global Majors, Life Science Innovation Program, University of Tsukuba, Tsukuba, Japan.

Email: ambotuwo62@gmail.com

Abstract. Environmental degradation has continued to increase in the last decade, requiring us to put increasing efforts into environmental mitigation, including in terms of food production. Future food production must be able to minimize the use of fresh water, fertilizers and pesticides, and reduce soil erosion. The Multitrophic Aquaculture System (MAS) approach could be one positive solution to this problem, by cultivating multitrophic organisms in the same system. This study aims to present details of the processes involved in implementing MAS on a microcosm scale that is currently underway. In the future, this system will be scaled up to in-situ level using brackish water ponds. In the present study, red seaweed Gracilaria changii was used as an autotrophic organism while the grouper Epinephelus fuscoguttatus as a heterotrophic organism. There are two treatments and one control, each with three replicates. This study shows that the system developed works well enough for seaweed. Seaweed seems to grow well, even though the growth rate is still low. Groupers, which are in the same system, are also in quite good condition, although some groupers showed low appetite, most likely due to low oxygen supply and excessive nutrition in the water. These challenges may be overcome by using higher seaweed stocking density. The system can maintain several water quality parameters, such as pH, at optimal level.

1. Introduction
The pace of environmental degradation, that continues to increase, degrade the environmental quality [1] and the health of fish communities [2]. This condition requires an effort to mitigate the environment, especially in terms of food production and renewable energy [3]. Future food production must be able to minimize the use of fresh water [4], fertilizers and pesticides [5, 6], and simultaneously reduce the soil erosion [7, 8]. These four aspects require the process of future food production to be moved gradually from the land to coastal and marine areas. This shift requires an innovative food production system and technologies.

The Multitrophic Aquaculture System (MAS) approach is an option which could contribute to addressing these challenges [9-12]. A MAS involved cultivation of several trophic levels (producers and consumers) in the same system (e.g. seaweed, fish, bivalves, molluscs, sea cucumbers). The goal
of developing MAS system is to increase the quantity and quality of food products through a sustainable production and energy efficient process. This multitrophic aquaculture model was developed to address four main problems in pond aquaculture: (1) the low carrying capacity of ponds due to environmental degradation [13]; (2) the perception of pond aquaculture as unsustainable and less environmental friendly; (3) required high investment with large production facilities & electric energy [14]; and (4) high risk (in monoculture, fish farmers have no alternative product in case of production or market failure). This study aims to introduce the MAS concept through presenting the details of the processes involved in implementing MAS on a microcosm scale that is currently underway. In the future, this system will be scaled up to in-situ level using brackish water ponds.

2. Materials and Methods
The MAS system comprises three compartments (reservoir, autotrophic and heterotrophic cultivation compartments), connected through a re-circulation system. In this trial, the microcosmic model used one ton tanks, with three tanks/unit, connected via a recirculation system driven by a submerged pump with a capacity of 1000 l/hour (Figure 1).

![Figure 1. The microcosmic model using three connected 1 ton tanks, with circulation driven by a submerged pump](image1)

The lighting system consists of two 27 Watts LED lights per unit. The lights were situated above the surface of the autotrophic tanks (Figure 2).

![Figure 2. The lighting system: two 27 Watt LED lights suspended above the surface of the tank(s) where autotrophic organisms (e.g. algae) are cultivated](image2)
Two pumps, each with capacity of 800 l/h, were placed in the tanks where heterotrophic organisms (e.g. fishes) were to be cultivated. These pumps were placed in diagonally opposed corners of the tank to provide oxygen and create current (Figure 3).

**Figure 3.** Heterotrophic culture tanks with two pumps (800 l/h capacity) in opposite corners to provide oxygen and create current

This trial used a red seaweed (*Gracilaria changii*) as an autotrophic organism [15] and the grouper *Epinephelus fuscoguttatus* as a heterotrophic organism [14] (Figure 4). With two treatments and a control and 3 replications of each treatment, there were a total of 9 experimental units.

**Figure 4.** The grouper *Epinephelus fuscoguttatus* as heterotrophic organism (left and centre) and the red seaweed *Gracilaria changii* as autotrophic organism (right)

The first treatment (S1) used two types of seaweed cultivation: an off-bottom system (first tank), and a floating system (third tank) (Figure 5), with groupers placed in the second tank. The second treatment (S2) used only the off-bottom seaweed cultivation method (first tank); the groupers were in the second tank and the third tank was empty. For the control (S3), there was no seaweed in the system, and the groupers were placed in the second tank. Grouper mortality was observed during two experimental stages. During the first stage (days 0-37) aeration was limited to that provided by the circulation pumps, while during the second stage (days 37-51) the grouper tanks were aerated.
Figure 5. Two types of seaweed cultivation, off-bottom system and floating system

Parameters measured for the autotrophic organisms (algae) included weight, ‘branch’ length and thallus diameter, while for heterotrophic organism (groupers) total length and weight were measured. All measurements were carried out every 10 days. Water quality parameters measured for each tank were salinity, temperature, pH, dissolved oxygen, nitrate, phosphate and ammonia concentrations.

3. Results

In S1 and S2, the relative daily growth of the seaweed varied greatly between thalli but was similar between treatments. The range in S1 was 0.028-0.123 g (mean 0.081±0.039 g), and in S2 -0.023-0.150 (mean 0.077±0.078 g). The seaweed relative growth on day 31 was significantly compared to days 11, 21 and 41 but not to day 51. The total fish mortality over 37 days was 31% (14 fishes, Table 1). No fish died in the control system (S3).

Table 1. Fish mortality from Day 0 to Day 37 in the multitrophic aquaculture systems

| Multitrophic Systema | Replicate | Live fish in unit | Mortality % | Mortality Day and number of fish |
|----------------------|-----------|------------------|-------------|--------------------------------|
|                      |           | Day 0 | Day 37 |               |                                |
| S1                   | 1         | 5     | 1     | 80           | Day 18: 2 fish; Day 20: 2 fish |
|                      | 2         | 5     | 5     | 0            |                                |
|                      | 3         | 5     | 0     | 100          | Day 19: 2 fish; Day 21: 2 fish; day 22: 1 fish |
|                      | Average   |       |       | 60           |                                |
|                      | SD        |       |       | 53           |                                |
| S2                   | 1         | 5     | 5     | 0            | Day 16: 4 fish; Day 22: 1 fish |
|                      | 2         | 5     | 0     | 100          |                                |
|                      | 3         | 5     | 5     | 0            |                                |
|                      | Average   |       |       | 33           |                                |
|                      | SD        |       |       | 58           |                                |
| S3                   | 1         | 5     | 5     | 0            |                                |
|                      | 2         | 5     | 5     | 0            |                                |
|                      | 3         | 5     | 5     | 0            |                                |

a S1: Off Bottom and Floating System; S2: Off Bottom system; and S3: No Seaweed

The first fish died on day 16 in S2 treatment (4 fishes). Subsequent deaths occurred under the S1 treatment almost every day. During the first stage of treatment (day 0 to day 37), fish appeared passive with very little movement. The fish became less active, lost their appetite, and some eventually died. The amount of feed eaten was small, and the fish tended to congregate near the circulating water pump that created oxygen bubbles. This behaviour usually occurs when the need for oxygen is not met. By
day 22, two units (one replicate each from the S1 and S2 treatments) had experienced 100% mortality, with 80% mortality in another S1 replicate. Due to this mortality there were replicates with no or only one fish, so on the 29th day of observation, the fish were redistributed with 3-4 fish per replicate. After redistribution, the fish activity remained low, and centred around the circulating water pumps; therefore, on day 37 it was decided to install aerators to increase the supply of oxygen (Figure 6).

Noticeable improvements in fish condition occurred rapidly once the aerators were installed. The fish were more active, more agile and their appetite increased. The increase in food consumption after the aerator installation was statistically significant (p < 0.05), with average daily feed per fish (mean ± SD) rising from 6.23±0.81 g to 8.16±0.95g. The fish were no longer observed gathering near the water circulating pump.

This improved condition lasted until the end of the experiment on day 51. This 2nd stage lasted for 14 days, with mortality limited to the death of two fish, both in the same tank (Table 2). These deaths were most likely related to disease since both fish had an abnormal appearance with swollen eyes and lesions on several areas of the skin.
Table 2. Fish mortality from Day 37 to Day 51 in the multitrophic aquaculture system

| Multitrophic System | Tanks       | Live fish in unit (Day 37) | Mortality % Mortality Day and number of fish |
|---------------------|-------------|---------------------------|------------------------------------------|
| S1                  | Tank 1      | 4                         | 0                                       |
|                     | Tank 3      | 4                         | 0                                       |
|                     | Tank 5      | 4                         | 0                                       |
| S2                  | Tank 7      | 3                         | 0                                       |
|                     | Tank 8      | 3                         | 0                                       |
|                     | Tank 9      | 4                         | 0                                       |
| S3                  | Tank 2      | 3                         | 0                                       |
|                     | Tank 4      | 3                         | 1                                       |
|                     | Tank 6      | 3                         | 0                                       |
| Average             |             |                           | 22                                      |
| SD                  |             |                           | 38                                      |

*S1: Off Bottom and Floating System; S2: Off Bottom; and S3: No Seaweed

The water quality varied both on a daily (24-hour) cycle and between days (Table 3). Over the 24-hour daily cycle the between treatment differences were significant for most water quality parameters; however based on the daily measurements over the study period, only pH differed significantly between treatments (Table 4).

Table 3. Mean values (± standard deviation) of water quality parameters measured during the study

| Observed System (Sn) | 24 hour cycle ranges | Daily measurement ranges |
|----------------------|-----------------------|--------------------------|
|                      | Salinity ppt | Temperature ºC | DO mg/l | pH | Salinity ppt | Temperature ºC | DO mg/l | pH |
| S1                   | 33.28±0.08 | 25.51±0.27 | 4.10±0.69 | 7.24±0.17 | 32.55±1.15 | 25.45±0.16 | 4.66±0.68 | 7.23±0.07 |
| S2                   | 32.03±0.09 | 25.29±0.24 | 3.63±3.93 | 7.45±0.16 | 32.76±0.56 | 25.35±0.08 | 4.43±0.55 | 7.29±0.07 |
| S3                   | 32.89±0.32 | 25.42±0.25 | 3.70±0.68 | 7.30±0.16 | 32.88±0.35 | 25.42±0.14 | 4.63±0.21 | 7.44±0.01 |

*S1: Off Bottom and Floating System; S2: Off Bottom; and S3: No Seaweed

Table 4. Significance levela of differences in mean values of water quality parameters (paired T-test)

| Observed System (Sn)b | 24 hour cycle | Daily measurements |
|-----------------------|---------------|--------------------|
|                       | Salinity | Temperature | DO | pH | Salinity | Temperature | DO | pH | Ammonia |
| S1 and S2             | 0.000*   | 0.001*       | 0.003* | 0.000* | 0.691 | 0.335 | 0.775 | 0.026* | 0.487 |
| S1 and S3             | 0.090    | 0.000*       | 0.009* | 0.050 | 0.613 | 0.642 | 0.956 | 0.035* | 0.506 |
| S2 and S3             | 0.000*   | 0.000*       | 0.026* | 0.000* | 0.423 | 0.244 | 0.460 | 0.062* | 0.560 |

*a *= statistically significant (p < 0.05)
b*S1: Off Bottom and Floating System; S2: Off Bottom; and S3: No Seaweed

The nutrient content in the water also fluctuated (Table 5). Nitrate and ammonia concentrations did not vary significantly between observations, while orthophosphate was significantly different between day 0 and day 11, and between day 11 and day 21 (Table 6)
Table 5. Nitrate, orthophosphate and ammonia concentrations (mean ± SD) measured on three days during the study period

| Time of Observation | Nitrate mg/l | Orthophosphate mg/l | Ammonia mg/l |
|---------------------|--------------|---------------------|--------------|
| Day 0               | 0.662±0.314  | 4.888±0.083         | 0.760±0.469  |
| Day 11              | 0.209±0.097  | 1.482±0.112         | 0.476±0.114  |
| Day 21              | 0.368±0.182  | 4.688±0.379         | 0.507±0.091  |

Table 6. Paired T-test results: significance* of between-day differences in nitrate, orthophosphate and ammonia concentrations ($\alpha = 0.05$)

| Time of Observation | Nitrate | Orthophosphate | Ammonia |
|---------------------|---------|----------------|---------|
| Day 0 - Day 11      | 0.187   | 0.010*         | 0.487   |
| Day 0 - Day 21      | 0.412   | 0.379          | 0.506   |
| Day 11 - Day 21     | 0.160   | 0.004*         | 0.560   |

* = statistically significant ($p < 0.05$)

4. Discussion

Aquaculture and the surrounding environment are two things that cannot be separated [16]. Aquaculture can have a negative impact on the environment and vice-versa, so that management of environmental factors is a key concern in aquaculture. The MAS concept is one aquaculture model that can accommodate aquaculture interests and environmental mitigation. A MAS is an aquaponic system that converts toxic components usable by plants [17] so as to optimize production capacity [18]. In the natural ecosystem, seaweeds can be a competitor of coral animals, with a negative impact on the coral ecosystem [19,20]. However, in artificial ecosystems, such as MAS, seaweed is a nutrient bio-circulator and biofilter [21], making it an asset in multitrophic and polyculture cultivation systems [22]. The implementation of MAS is expected to increase environmentally friendly fish and seaweed production. MAS is also expected to strengthen Indonesia's position as the largest seaweed producer in the World [23].

Seaweed and grouper are commodities with several competitive advantages. Firstly, seaweed can grow rapidly, with efficient cultivation systems, simple cultivation techniques, and low production costs [24]. Secondly, demand for seaweed is high, because it is widely used in many industries including the food, cosmetics, and pharmaceutical industries [25-27]. Thirdly, groupers are high-value food fish that can be cultivated in ponds [14].

The water temperature remained above 25 °C throughout the MAS trial. Although the closed room situation caused lower temperatures in the microcosms compared to aquaculture in ponds that were directly exposed to sunlight, this is still within the range (25-30 °C) recommended for tropical fish and seaweed cultivation [28].

The pH of the culture media in the systems remained between 7 and 8 during the MAS trial, a range which is suitable for both seaweed and groupers. According to [29], pH between 7 and 8 is the optimal range for fish or shrimp survival and growth. The salinity range (32 and 33 ppt) was in the recommended range for both seaweeds and groupers. This salinity range is quite high compared to that preferred by shrimp and other fish commonly raised in ponds, most of which prefer lower salinity, in the 10-25 ppt range [30].

The dissolved oxygen concentrations in the MAS systems (between 3 and 5 mg/l) was quite low, and most likely a major causal factor in the high grouper mortality in the MAS systems before the installation of aerators to supplement the limited oxygenation effect of the circulation pump. Concentrations between 5-7 mg/l are generally regarded as appropriate for the growth of aquatic biota [31]. This consideration was addressed through the installation of aerators on day 37.
Orthophosphate content during the study ranged from 1 to 5 mg/l, higher than orthophosphate level recommended for fish cultivation by [32]. Ammonia also exceeded the recommended limits, reaching 0.93-1.54 mg/l. Ammonia content ranged between 0.3 and 1.3 mg/l, a range considered high for fish, but good for seaweed growth. The concentration considered safe for many aquatic organisms is less than 0.1 mg/l [13]. This excess of nutrients in the MAS system could probably be overcome by increasing the stocking density of the seaweed.

The nitrate content during the study ranged from 0.1 to 1.0 mg/l. This range is within that considered appropriate for both seaweed and groupers. According to [33], algal growth tends to be optimal at nitrate concentrations of 0.09-3.5 mg/l, while at concentrations below 0.01 mg/l or above 4.5 mg/l, nitrate can be a limiting factor.

5. Conclusion

The microcosm MAS system developed is already functioning quite well for the autotrophic organism (Gracilaria changii). The seaweed appeared healthy throughout the trial although growth was rather slow. Initially, there was high mortality of the heterotrophic organisms (Epinephelus fuscoguttatus). Grouper appetite and activity were lower than normal, with excessively high nutrient levels and low dissolved oxygen, thought to have been the main cause of the observed mortality. After the installation of aerators, grouper condition improved markedly. The system treatments were able to maintain several water quality parameters, e.g. pH, within the optimal range for the cultured organisms. The build-up of excess nutrients could probably be controlled by increasing seaweed stocking density.

Acknowledgment

We thank the Ministry of Research, Technology and Education of the Republic of Indonesia and Universitas Hasanuddin for providing research funding (contract number 1740/UN4.21/PL.01.10/2019 dated April 11th, 2019).

References

[1] Warner R F 1991 Impacts of environmental degradation on rivers, with some examples from the Hawkesbury-Nepean system The Australian Geographer 22 1-13
[2] Fausch K D, Lyons J, Karr J R and Angermeier P L 1990 Fish communities as indicators of environmental degradation. In: American fisheries society symposium: Bethesda) pp 123-44
[3] Fischer C and Newell R G 2008 Environmental and technology policies for climate mitigation Journal of environmental economics and management 55 142-62
[4] Bouwer H 2000 Integrated water management: emerging issues and challenges Agricultural water management 45 217-28
[5] Bryant J E and Haggstrom J 2012 An environmental solution to help reduce freshwater demands and minimize chemical use. In: From Potential to Production: SPE/EAGE European Unconventional Resources Conference & Exhibition. ISBN 2214-4609
[6] Sharpley A N, Daniel T, Gibson G, Bundy L, Cabrera M, Sims T, Stevens R, Lemunyon J, Kleinman P and Parry R 2006 Best management practices to minimize agricultural phosphorus impacts on water quality. In: ARS-163. USDA-ARS, (Washington, DC: Agricultural Research Service Publication) p 52
[7] Morgan R P C 2009 Soil erosion and conservation (Australia: Blackwell Publishing)
[8] Montgomery D R 2007 Soil erosion and agricultural sustainability Proceedings of the National Academy of Sciences 104 13268-72
[9] Chopin T, Buschmann A H, Halling C, Troell M, Kautsky N, Neori A, Kraemer G P, Zertuche-González J A, Yarish C and Neefus C 2001 Integrating seaweeds into marine aquaculture systems: a key toward sustainability Journal of Phycology 37 975-86
[10] Murtiati E Y, Murtiana T and Sunarma A 2010 Perekayasaan teknik perbaikan kualitas air dan kesehatan ikan pada sistem resirkulasi.
[11] Tyson R V, Simonne E H, White J M and Lamb E M 2004 Reconciling water quality
parameters impacting nitrification in aquaponics: the pH levels. In: *Proceedings of the Florida State Horticultural Society*, pp 79-83

[12] Tyson R V 2007 Reconciling pH for ammonia biofiltration in a cucumber/tilapia aquaponics system using a perlite medium. University of Florida

[13] Suparjo M N 2008 Daya Dukung Lingkungan Perairan Tambak Desa Mororejo Kabupaten Kendal *Saintek Perikanan: Indonesian Journal of Fisheries Science and Technology* 4 50-5

[14] Supratno T 2006 Evaluasi lahan tambak wilayah pesisir Jepara untuk pemanfaatan budidaya ikan kerapu. Program Pasca Sarjana Universitas Diponegoro

[15] Arbit N I S, Omar S B A, Tuwo A and Soekendarsi E 2018 Effect of Global Warming Scenarios on Carotenoid Pigments Gracilaria changii *International Journal of Environment, Agriculture and Biotechnology* 3 2039-42

[16] Pillay T 2004 Aquaculture and the environment. Former Programmed. Fishing News Books, Blackwell Publishing, Ltd)

[17] Nelson R L 2008 Aquaponic equipment: the biofilter *Aquaponic J* 48

[18] Nugroho R A, Pambudi L T, Chilmawati D and Haditomo A H C 2012 Aplikasi Teknologi Aquaponic pada budidaya ikan air tawar untuk optimalisasi kapasitas produksi *Saintek Perikanan: Indonesian Journal of Fisheries Science and Technology* 8 46-51

[19] Mulyani S, Tuwo A, Syamsuddin R and Jompa J 2018 Effect of seaweed *Kappaphycus alvarezii* aquaculture on growth and survival of coral *Acropora muricata* *Aquaculture, Aquarium, Conservation & Legislation* 11 1792-8

[20] Sawall Y, Jompa J, Litaay M, Maddusila A and Richter C 2013 Coral recruitment and potential recovery of eutrophied and blast fishing impacted reefs in Spermonde Archipelago, Indonesia *Mar. Pollut. Bull.* 74 374–82

[21] Syamsuddin R, Tuwo A and Aswar N 2019 Weight gain and carrageenan content of *Kappaphycus alvarezii* (Rhodophyta, Solieriscea) polycultured with *Sargassum polycystum* (Paeophyta, Sargassaceae). In: *IOP Conference Series: Earth and Environmental Science: IOP Publishing*) pp 1792-8

[22] Aprilillatu P D 2016 Indonesia Dinilai jadi Penghasil Rumput Laut Terbesar di Dunia. https://merdeka.com.

[23] Kim J K, Yarish C, Hwang E K, Park M and Kim Y 2017 Seaweed aquaculture: cultivation technologies, challenges and its ecosystem services *Algae* 32 1-13

[24] Freile-Pelegrín Y and Murano E 2005 Agars from three species of Gracilaria (Rhodophyta) from Yucatán Peninsula *Bioresource Technology* 96 295-302

[25] [Ma Z, Khalid N, Shu G, Zhao Y, Kobayashi I, Neves M A, Tuwo A and Nakajima M 2019 Fucoxanthin-Loaded Oil-in-Water Emulsion-Based Delivery Systems: Effects of Natural Emulsifiers on the Formulation, Stability, and Bioaccessibility *ACS Omega* 4 10502-9

[26] Melanie H, Taarji N, Zhao Y, Khalid N, Neves M A, Kobayashi I, Tuwo A and Nakajima M 2019 Formulation and characterisation of O/W emulsions stabilised with modified seaweed polysaccharides *International Journal of Food Science & Technology* *in press* 10 p

[27] Buwono I D 1993 *Tambak udang windu: sistem pengelolaan herpola intensif*: Penerbit Kanisius

[28] Cheng W, Chen S-M, Wang F-I, Hsu P-I, Liu C-H and Chen J-C 2003 Effects of temperature, pH, salinity and ammonia on the phagocytic activity and clearance efficiency of giant freshwater prawn *Macrobrachium rosenbergii* to *Lactococcus garvieae* *Aquaculture* 219 111-21

[29] Utaminingsih and Hermiyaningsih 1985 Persyaratan tanah dan air. In: *Pedoman Budidaya Tambak*. (Jakarta: Ditjen Perikanan, Departemen Pertanian)

[30] Kordi M and Tancung A B 2007 Pengelolaan kualitas air dalam budidaya perairan *Rineka*
[32] Astuti M Y, Damai A A and Supono S 2017 Evaluasi Kesesuaian Perairan Untuk Budidaya Ikan Nila (Oreochromis niloticus) Di Kawasan Pesisir Desa Kandang Besi Kecamatan Kota Agung Barat Kabupaten Tanggamus e-Jurnal Rekayasa dan Teknologi Budidaya Perairan 5 621-30

[33] Resti M 2002 Pemetaan Sebaran Klorofil–a Terhadap sebaran kandungan Nitrat dan Fosfat di Perairan Kebupaten Brebes. [Skripsi] Fakultas Perikanan dan Ilmu Kelautan, Universitas Diponegoro, Semarang 71