Optimization of stocking density of milkfish (*Chanos chanos*) in polyculture system with seaweed (*Gracilaria sp.*) on the traditional pond

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Abstract. To determine the optimal stocking in the traditional pond is the objective of this study. The study was carried out from March until June 2019 in Karawang, West Java. The milkfish stocking density is the treatment A: 3 fish/container (2,500 fish/ha); B: 6 fish/container (5,000 fish/ha) and C: 9 fish/container (7,500 fish/ha). The weight of the seaweed planted is 900 g (1 ton/ha) for each treatment. Net as a research container measuring 3 x 3 meters was placed in the middle of the pond. The main variables analyzed were growth rate, absolute weight, daily growth rate, survival, and final weight of milkfish. For seaweed, weight and length gain, absolute weight, and daily growth rate. The observed water quality includes temperature, pH, DO, salinity, and brightness. The results showed that the milkfish density of 3 fish/container gave the highest (p<0.05) of absolute weight 25.43 (±1.75) g, survival rate 88.9 (±1.27) %, and ADG (p>0.05). The lowest weight of seaweed occurred in the treatment of 9 fish/container which is 74.67 g. The optimal number of milkfish stocking in a polyculture system with seaweed is 3 fish/container (2,500 fish/ha).

Keywords: milkfish; polyculture; seaweed; stocking density

1. Introduction
Milkfish (*Chanos chanos*) culture in the traditional pond is expected to provide more productivity through polyculture with seaweed (*Gracilaria sp.*). Thus two commodities have high economic value. The application of ecological space in the polyculture system improved value to the two commodities cultivated [1]. Seaweed helps as a producer of oxygen as well as a shelter for milkfish from the sunlight. [2]. This system can also improve land-use efficiency and water quality. Nevertheless, information about the implementation of milkfish and seaweed polyculture in ponds is still not widely known.

In the polyculture system, milkfish as a consumer of natural food consists of a benthic complex of blue-green algae, protozoa, diatoms, bacteria, and detritus and epiphyte [3]. Despite this fact, milkfish waste will be used as nutrients and fertilizer by seaweed. The moving of milkfish to the bottom of the waters column to find food helps to stimulate the growth of seaweed thallus which is dense with algae and plankton to avoid blooming.

The most important species of seaweed that are cultivated in Indonesia are *Gracilaria* sp. and *Eucheuma* sp.. Indonesia is in second place after the Philippines for seaweed production and export. The potential for seaweed development in Indonesia reaches 1.11 million ha with production estimated at...
167,937 MT per year. The Ministry of Marine Affairs and Fisheries reported that seaweed cultivation produces around 99.73% of the total seaweed production [4].

Until now the problem in polyculture of milkfish and seaweed not yet optimal for a stocking density of milkfish so that the objectives of the polyculture system are not achieved. High stocking density of milkfish correlates stunting seaweed to grow that causes thallus of seaweed grassing by milkfish. The culture of milkfish in a traditional pond takes 6–8 months by relying on natural food (klekap) which is not adequate to support food during cultivation time. While low stocking density causes the seaweed to be filled with moss. In realistic farmer applied low stocking density of milkfish [5] and may cause the presence of epiphyte in the ponds that will decrease the seaweed quality. Research is needed to determine an optimal comparison between milkfish and seaweed in a polyculture system.

The objective of this study was to determine the optimal stocking density of milkfish in a polyculture system with seaweed in the traditional pond to increase the production of both commodities gain the added value of the environment.

2. Materials and methods
This research was conducted in March–June 2018 in Karawang, West Java. The main materials consist of milkfish fry and seaweed seed. Completely randomized design (CRD) with three treatments and 3 replications respectively. The milkfish stocking density is the treatment, namely: A = 3 fish/container (2,500 fish/ha); B = 6 fish/container (5,000 fish/ha); and C = 9 fish/container (7,500 fish/ha). The weight of seaweed that planted was the same in all treatment, namely 900 gram/container of seaweed (1 ton of seaweed/ha).

2.1. Pond preparation and research container setting
The traditional pond was used measuring 0.6 ha with rectangle form. Selection of location and compartments based on technical feasibility, namely sandy mud of the bottom pond, has a source of seawater and a source of freshwater, with water flow accessibility and strong main embankment with a height of 30 cm above the highest tide. The location safety factor also needs to be considered. The pond preparation includes sunlight drying for 2 days until the soil looks dry and cracked. Afterward, the pond is filled with water that flows through the river upstream. The process of liming and seawater filling as follows. The water level in the pond is maintained in range 50–70 cm depth. No pond fertilizer was added during this experiment.

The research container was a water net. Nine water nets with a size of 3 x 3 meters are equipped with a bamboo frame and were placed in the middle of the pond and coded according to the treatment. Net is also weighted with an anchor to avoid shifting during the experiment. The placement of the net container and the arrangement in the pond shows in figure 1 below.

![Figure 1. The placement of net container and the arrangement in the pond](image-url)
2.2. Milkfish fry and seaweed seed
Milkfish fry is obtained from local farmers with a range of weight 15.3–20 g each when stocked. Acclimatization process for milkfish in about 30 minutes. The stocking density of milkfish in research containers is different according to treatment. No artificial feed (pellets) are given during the experiment and only rely on natural food and algae feed. At the end of the research, weight measurements were taken and the number of fish harvested was calculated to determine the survival rate.

*Gracilaria* sp. seeds used are 15–20 days old with selected criteria namely fresh, has many branches, not slimy, has elastic thallus, not easily broken. Preparation for planting includes cleaning of dirt, sticky mud, and weighing. The stocking of seeds carried out after the water pond dept has reached 50 cm. The bottom stocking method was used for seaweed cultivation. For sampling purposes, seaweed is grown specifically using plastic baskets with an initial weight of 50 g. A sampling of weight and length of seaweed was carried out once a week. Sampling was taken from each research container with three different sampling points, two at the edge, and one in the middle of the plot.

2.3. Water quality parameters
Measurement of water quality parameters is carried out in-situ and was taken at three different points from the entire research area. Sampling time was done once a week. The water quality parameters measured included temperature, dissolved oxygen, pH, salinity, and transparency. The tools are an alcohol thermometer, pH paper, DO test kit, refractometer, and Secchi disk.

2.4. Data analysis
Data sorting and tabulation was done for collected data. The biological aspect of milkfish was analyzed for absolute weight, daily average weight gain (ADG), survival rate (SR), and biomass weight. Absolute growth is calculated using the formula by [6]:

\[
\Delta W = W_t - W_0
\]

Where \(\Delta W\) is absolute growth in grams, \(W_t\) is the weight of t-day (g) and \(W_0\) is the initial weight (g).

The average daily growth rate (ADG) is calculated by:

\[
ADG = \frac{\text{end weight (g)} - \text{initial weight (g)}}{\text{Total day of culture}}
\]

Survival rate (%) is calculated by the number of the sample that lives at the end divided by the number of animals at the beginning and then multiplied by 100.

Data were analyzed statistically. To determine the effect of treatment on the response of the measured parameters the analysis of variance or the F test is used. Data of seaweed were processed for weight gain rate, absolute weight, ADG, and average length growth. Water quality parameter data is processed and presented in the range of each sampling time. The results of data analysis are presented quantitatively and descriptively.

3. Results

3.1. Absolute weight, average daily growth rate (ADG) and survival of milkfish (*Chanos chanos*)
The results of milkfish measurements of weight gain, absolute weight, daily growth rate (ADG), and survival rate (SR) during this study are shown in table 1 below.

| Treatment | Absolute weight (g) | ADG (g)  | SR (%)  |
|-----------|---------------------|----------|---------|
| A         | 25.43 ± 1.75b       | 0.46 ± 0.32a | 88.9 ± 1.27c |
| B         | 17.71 ± 4.86a       | 0.32 ± 0.02a | 72.2 ± 0.90b  |
| C         | 10.70 ± 2.51a       | 0.19 ± 0.39a | 66.7 ± 0.50a  |

\(a,b,c\) the results of the F test
The difference in absolute weight between treatments was relatively significant. Treatment A was significantly different (p<0.05) from B and C. While treatment B was not significantly different from C (p>0.05). The stocking density of 9 milkfish/containers has resulted as the lowest for the absolute weight (10.70 ± 2.51g), ADG (0.19 ± 0.39g), and also SR (66.7 ± 0.50%). The highest average daily growth rate (ADG) of milkfish during this study are (0.46 ± 0.32g) found at treatment A (3 milkfish/container) although there were no significantly different (p>0.05) for all treatments. The highest survival rate (88.9 ± 1.2%) was also recorded in the treatment with 3 milkfish/containers and significantly different (p<0.05) among the treatments.

The result of the absolute weight and survival rate turn affects biomass weight yield. The yields from treatment 3, 6, and 9 milkfish/container were 126.2 ± 1.8g, 98.95 ± 4.6g, and 92.1 ±1.3g respectively (figure 2).

![Figure 2. The yield of milkfish in this study.](image)

The yield shows that treatment A has the best on biomass weight and was significantly different (p<0.05) with treatment B and C. Based on this result can be concluded that the optimal stocking density in this polyculture system is 3 milkfish/container or 2,500 milkfish/ha.

3.2. Seaweed (Gracilaria sp.) weight and length growth pattern
The weight and growth patterns of seaweed during the culture period are presented in figures 3 and 4 as below.

![Figure 3. Seaweed (Gracilaria sp.) weight growth pattern.](image)
All the treatments of the weight growth showed increased following by the time of the culture period. Seaweed at treatment A and B were the same patterns but on day 28 seaweed at treatment B was the highest and treatment C was the lowest (figure 3). The length growth of seaweed showed relative constant until at the end of the experiment and treatment A has the lowest growth (figure 4).

The absolute weight and daily growth rate of *Gracilaria* sp. in this study can be seen in table 2. All treatment are significantly different (p<0.05) for the absolute weight while not significantly different (p>0.05) for ADG.

**Table 2.** The absolute weight and ADG of *Gracilaria* sp..

| Treatment | Absolute weight (g)       | ADG (g)   |
|-----------|---------------------------|-----------|
| A         | 664.6 ± 3.23<sup>b</sup>  | 0.012 ± 0.04<sup>a</sup> |
| B         | 727.7 ± 5.58<sup>c</sup>  | 0.012 ± 0.07<sup>a</sup> |
| C         | 157.6 ± 1.69<sup>a</sup>  | 0.003 ± 0.04<sup>a</sup> |

<sup>a,b,c</sup> the results of the F test

### 3.3. Water quality

The water quality parameters measurement was done to determine the condition of waters for the growth of milkfish and seaweed. The water quality parameters at the research-pond shown in table 3.

**Table 3.** The water quality parameters at the research pond.

| Parameters  | Day     | Day     | Day     | Day     | Day     | Day     | Day     |
|-------------|---------|---------|---------|---------|---------|---------|---------|
|             | 0       | 7       | 14      | 21      | 28      | 35      | 42      | 49      |
| Temperature (°C) | 25–28  | 24–27   | 22–28   | 25–30   | 30–32   | 29–32   | 31–34   | 28–33   |
| DO (mg/l)   | 8       | 5–6     | 8       | 5       | 8       | 8       | 8       | 5       |
| pH          | 6–7     | 6–7     | 6       | 6       | 6       | 7       | 6       | 6       |
| Salinity (g/l) | 4–5    | 5–6     | 6       | 5–6     | 7–8     | 7–8     | 8       | 10      |
| Transparency (cm) | 18–20 | 37–43   | 42–43   | 38–47   | 30–36   | 60–61   | 55–62   | 53–60   |
All water quality parameters showed suitable for both milkfish and seaweed cultivation. The water temperature, salinity, and water transparency increased by the time of the culture period. The dissolved oxygen was a little bit fluctuated. pH was relatively constant from day 0 to days 49 (table 3).

4. Discussion

Treatment C which is the highest stocking density in this study has the lowest absolute weight because milkfish only eat natural food in the form of plankton that grows by utilized nutrients from the decomposition of organic material at the bottom of the pond. The high growth of the absolute weight of milkfish in treatment A besides getting natural food, milkfish also eat epiphytic organisms in thallus 

Gracilaria sp. while the fish population is lower than other treatments. The growth of milkfish influences also the ability to used feed, in case of polyculture system the higher stocking density the feed utilization decreases. The fish weight growth of this study is different from [7] the average weight of milkfish did not differ between the 3 treatments of stocking density in polyculture with seaweed.

The fish growth rate depends on the amount of feed consumed and the protein content in the feed. Meanwhile, other factors such as heredity, age, endurance, and ability of these fish to utilize feed will affect their growth [8]. Optimization of the density of the two commodities in the polyculture system is expected to be used for the effective utilization of the pond water column, especially space and feed. Utilization of the water column is ecologically advantageous to increase pond productivity as much as possible. Rearing milkfish in traditional pond normally feeds plankton and kelp (compost of microorganisms that live on the surface of pond bottom such as green algae Chaetomorpha sp., Enteromorpha sp. [9]. Its supported by the results of Deswati and Luhur [10] that analysis of the intestines of the milkfish found a lot of basic microorganisms.

The problem in seaweed farming (monoculture system) is the growth of other types of algae that can interfere with optimal seaweed growth. Whenever the algae thrive tend to be a pest to seaweed. The polyculture system with milkfish could be a solution to this problem because milkfish’s food is natural food (plankton, algae, and epiphytic organisms) that grow in cultivation pond. The application of the polyculture system between milkfish and seaweed can meet the ecological needs of the pond. Figure 2 shows the growth rate of seaweed during this study. The growth has increased from the beginning to the end of the study. From the beginning until 3 weeks of cultivation, the range of weight gain in each treatment was 7–8 grams/week. Seaweed can absorb significant amounts of milkfish waste as nutrients and fertilizer [1], controlling eutrophication and stability of the pond ecosystem [11]. After 4 week (day 28), the growth pattern was slowdown in all treatment. Its supposed due to the bottom method of seaweed cultivation in this study. According to Arbit et al. [12] the stocking method of Gracilaria sp. cultivated in pond allows the thalli to touch and overlap one another and affect the growth and shapes of thalli attempt to avoid each other to seek the sunlight.

The lowest weight of seaweed occurred in treatment with 9 milkfish/containers, namely 74.67 g. This study carried out that more milkfish stocked has provided a lot of waste as nutrients and natural fertilizers to accelerate the growth of seaweed but the length and weight growths are the lowest. Its seen from the graph that seaweed reduction in the average length and weight at some point of sampling and C treatment (figures 2 and 3). The large growth on stem diameter of thallus faster than its growth length thereby increasing the weight of the seaweed. The presence of milkfish also makes it possible to eat young thallus, so those growth seaweed was more on weight gain. The swimming activity of milkfish causes water movement which can increase oxygen diffusion, thereby increasing the concentration of dissolved oxygen in the water. This water movement affects the speed of change in the suspension of organic matter into nutrients needed by Gracilaria sp. [1]. Furthermore will accelerate the growth of the seaweed. This is supported by the opinion of Neori et al. [11], polyculture helpful in water use and provides nutrients inorganic for seaweed to grow.

The present study suggesting that the activity of milkfish with high stocking density may affect the distribution of particles such as suspended solid where the particles has covered the leaf of seaweed that a lot of stomata. This condition can inhibit the respiration process such as photosynthesis. Stocking density of 9 milkfish/container occurs the lowest absolute weight of Gracilarisa sp. The absolute weight
of seaweed was not linear with milkfish stocking density. The higher stocking density for milkfish did not result in more seaweed production. According to Herliany et al. [13] the water depth is one factor that affects the success of seaweed cultivation. Gracilaria sp gave the highest on absolute growth and biomass in cultivation at 30 cm of depth.

The water level in the pond during research is maintained in range 50–70 cm depth so not optimal to support the growth of seaweed. The depth of cultivation seaweed affects the absorption of light and so related to the process of photosynthesis that produces food for its growth [14]. Gracilaria sp. can produce high biomass [15] also widely cultivated in Indonesia and other countries. This seaweed is the potential for a polyculture system with milkfish (Chanos chanos), tiger shrimp (Penaeus monodon) [16], white shrimp (Litopenaeus vannamei) [17, 18], and crab (Scylla serrata) [16]. The use of Gracilaria sp. to increase the traditional production is greatly recommended for the establishment of a polyculture system and the improvement of pond management. Based on the observations during the study, the temperature ranged from 22–34 °C. The optimum temperature for seaweed cultivation is 25–30 °C [19] and Anggadiredja et al. [20] said the optimal water temperature 26–30 °C. Gracilaria sp. is one type of seaweed with wide tolerance on water temperature [15]. Water temperature is one of the water quality parameters that can affect some physiological functions of seaweeds such as photosynthesis, respiration, metabolism, growth, and reproduction. The range values were also within good tolerance limits to support the growth of milkfish.

Dissolved oxygen (DO) measurements ranged 5.0–8.0 mg/l which appropriate for milkfish and Gracilaria sp. in this polyculture system. Milkfish culture suitable to DO 3.0–8.0 mg/l. Polyculture system is based on the principle of natural balance, where seaweed functions as an oxygen producer and milkfish can also absorb CO2 which is derived from fish respiration so found no problem of oxygen deficiency in this study.

The water pH ranged from 6–7 maintenance the growth and survival of the milkfish and seaweed. According to the [21], the optimum pH for seaweeds cultivation range from 6–9, and pH 6.5–8.5 is the optimal pH for Gracilaria sp. According to Odum [22], pH values are influenced by several factors such as temperature, photosynthesis, respiration of fish, and the presence of ions in pond water. Salinity value during the study recorded 4–10 g/l. More similar results report by Rohman et al. [23], salinity concentration in the seaweed ponds around Bekasi 10–11 g/l. Optimum salinity range for Gracilaria sp. 15-30 g/l [23], Gracilaria sp. is a type of seaweed that has a wide range of salinity or a euryhaline seaweed. Milkfish is also a euryhaline fish so salinity has greatly affect on metabolic and growth process. The salinity concentration during the study is a low category but still within the limiting factor for Gracilaria sp. as well as for milkfish.

The level of water transparency range from 18–62 cm. This transparency is very good to support the maximum photosynthesis process [24]. Sunlight penetration reaches the bottom of the pond and ideal for seaweed to grow. Mubarak et al. [25] stated that water brightness should range between 80–100% for good seaweed cultivation.

Conclusion
The results of this study conclude to optimize biomass both of fish and milkfish production, the high stocking density of milkfish is 3 milkfish/container or applicable for 2,500 milkfish/ha.

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