MAPPING OF CRITICAL PERIOD AND NATURAL FEED NEED BASED ON OXYGEN CONSUMPTION DATA ON MILKFISH LARVAE *CHANOS CHANOS* (FORSSKÅL, 1775) USING ARTIFICIAL INTELLIGENCE

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Abstract

This study aims to measure changes in oxygen consumption in relation to the amount of natural feed requirements based on artificial intelligence, analyze the relationship of oxygen consumption with the use of natural feeds monitored by artificial intelligence and calculate the appropriate natural feed density for *Brachionus plicatilis* in meeting daily natural feed requirements in rearing. milkfish larvae. This study used a completely randomized design (RBD) with three treatments and three replications. The treatments tried were *Brachionus plicatilis* densities of 25 ind/mL, 50 ind/mL, and 75 ind/mL. The number of hatching milkfish larvae used was 2880 larvae with an average body length of 0.376 mm ± 0.02 and reared for seven days. Oxygen consumption parameters were measured using the respirometer bottle method. The results showed that oxygen consumption tended to increase up to a density of 75 ind/mL and decrease again at a density of 50 ind/mL. The best density of *Brachionus plicatilis* in the study was 50 ind/mL. However, specifically the second to fifth days a density of 25 ind/mL is sufficient and the sixth and seventh days are given at a density of 50 ind/mL.

***Keywords**: Brachionus plicatilis, Oxygen Consumption, Milkfish Larvae, Artificial Intelligence, Environmental Monitoring, Growth, Survival*

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1. Introduction

Milkfish (*Chanos chanos*) is the only living species of the Chanidae family (Bhakta et al., 2021) which has fast growth, efficient use of natural food, herbivores, disease resistance and various ecological factors (Hussain et al., 2021).

In the larval period is one of the critical phases in the growth period, the larvae are very dependent on the availability of suitable feed (larval size or mouth opening), can be digested easily and can meet the nutritional needs that support good growth and survival (Pangkey et al., 2019) as well as the availability of the right amount of natural feed, especially at the early stages of larval development. The type of natural feed used in the maintenance of milkfish larvae is *Chlorella* sp. and *Brachionus plicatilis*.

Oxygen consumption in larvae is a physiological indicator that describes the metabolic status of larvae related to energy use. The level of oxygen consumption (TKO) in larvae is oxygen consumption carried out in units of mgO2/hour/g wet weight (Rudiansyah & Wahidin, 2021). By knowing the rhythm of oxygen consumption in the larvae, the natural food requirements for the larvae can be determined precisely.

In general, calculating the density of plankton is done by means of the subsample method. Phytoplankton calculations used a Haemocytometer (Taylor et al., 1997; Nasukha & Aslianti, 2019) and zooplankton used a Sedgewick Rafter Counting Chamber (APHA, 2005; Nasukha & Aslianti, 2019) which were observed under a microscope. Meanwhile, the measurement of water quality uses different tools and methods so it is less efficient for monitoring in real time. Based on this, new innovations are needed to overcome these problems by using artificial intelligence.

Artificial intelligence is a field of computer science to solve problems by interpreting intelligent tasks automatically (Mustapha et al., 2021) Artificial intelligence has been used in aquaculture activities. In addition, environmental monitoring is important because there is high variability that can pose a threat to aquaculture activities. In general, it is difficult and time consuming and can risk the species being cultivated (Mustapha et al., 2021).

The range of the amount of natural feed in rearing milkfish larvae is very important in order to minimize the shortage of using natural feed. However, information on daily natural food requirements, especially *Brachionus plicatilis* in milkfish larvae rearing, is still lacking, so this research needs to be conducted to analyze the appropriate daily natural food requirements for milkfish larvae based on oxygen consumption data to see energy consumption in larvae. In addition, real-time water quality measurements using artificial intelligence will be synergized and obtain big data to see the use of natural food in milkfish larvae.

2. Methods

This research was carried out from August to October 2022 at the Ecosystem Captive and Rehabilitation Laboratory, Faculty of Marine and Fisheries Sciences, Hasanuddin University, Makassar.

Test Animals

The test animal used was milkfish larvae from hatching eggs obtained from Hatchry PT. Esaputlii (Benur Kita) which is in Barru Regency, South Sulawesi. The number of hatched milkfish larvae used was 2880 larvae with an average body length of 0.376 mm ± 0.02.

Research Platform

The containers used in this study were 12 glass aquariums measuring 20 x 20 x 30 cm^3 with a volume of 10 L. The three sides of the container are covered with a yellow background to increase visibility and contrast of prey to make it easier for the larvae to catch natural food (Bera et al., 2019). In addition, the research containers were kept in glass aquariums measuring 60 x 30 x 30 cm^3 which were used as containers for storing research containers with 4 water bath systems. After that, a thermostat is given in the aquarium to maintain the temperature during maintenance.

Test Feed

The feed used in this study was natural feed of the type *Chlorella* sp., and *Brachionus plicatilis* which were given at different densities. *Chlorella* sp., given to D1 maintenance at a density of $5x10^4$ cells/mL. While D2 to D7 were given *Brachionus plicatilis*. Calculation of *Chlorella* sp., using Haemocytometer while *Brachionus plicatilis* using Sedgewick Rafter Counting. In addition, image J is also used for calculations.

Artificial Intelligence Design

Collection and utilization of natural food given daily will be monitored using the image J application (figure 1) and monitoring of the rearing media environment using artificial intelligence (figure 2).

Figure 1. Image J application

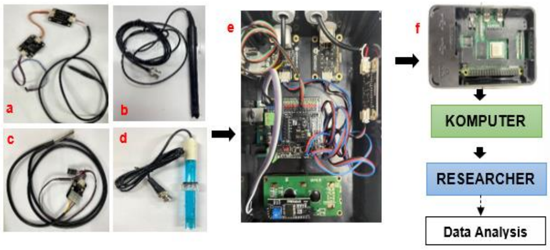


Figure 2. Artificial Intelligence Mechanism (Water Quality Digital): (a) TDS Sensor, (b) DO Sensor, (c) Temperature Sensor, (d) pH Sensor, (e) Arduino Uno, (f) Raspberry Pi

Experiment Design

The study consisted of three treatments and three replications each. This study consisted of 9 experimental units. The treatment tried was the difference in Density of *Brachionus plicatilis*, namely: 25 ind/mL, 50 ind/mL, 75 ind/mL.

Observed Parameters

Oxygen Consumption

Milkfish larvae oxygen consumption using the respiration bottle method. Measurements were taken in the morning, afternoon and evening. Oxygen consumption is calculated using the formula Djawad et al., (1996) as follows:

To find out the amount of dissolved oxygen without larvae (mg/hour):

$$X = (DO_{initial} - DO_{after}) \times \frac{V}{1000} : \frac{T}{60}$$

To determine the amount of dissolved oxygen with larvae (mg/hour):

$$Y = (DO_{initial} - DO_{after}) \times \frac{V}{1000} : \frac{T}{60}$$

Description: X is dissolved oxygen without larvae (mg/hour), Y is dissolved oxygen in the presence of larvae (mg/hour), V is volume of respiration bottle (ml), start/end DO is beginning/end oxygen solubility (mg/L) and T is time (minutes/hour).

To find out the actual amount of dissolved oxygen (mg/hour):

$$Z = Y - X$$

To find out the actual unit amount of dissolved oxygen into units of µmol O2/hour:

$$w = \frac{Z \times 1000}{\text{oxygen molecular weight}}$$

To convert the actual unit amount of dissolved oxygen into µL O2/hour:

$$U = W \times 22{,}41$$

Where it is assumed that 1 mole of O2 = 22.41

To determine the rate of oxygen consumption per wet body weight of larvae (µL $O_{2/}$mg BB/hour):

$$N = \frac{U}{\text{mg body weight}}$$

Daily Natural Feed Consumption

During maintenance, every day natural feed residue is checked in the maintenance aquarium container which is carried out before feeding to maintain the amount of density, by taking 1 mL on the maintenance media then taking pictures using a mobile camera calculated with the image J application.

$$F = Ft - F0$$

Note: F is Larvae Natural Feed Consumption (%), Ft is Final Total Natural Feed (ind), F0 is Initial Total Natural Feed (ind)

Growth

For growth measured absolute and specific growth. Growth observations were carried out by weighing the body weight of the larvae and measuring the length once a day during the study. The weighing of the larvae was observed using the sampling method, taking 10 individuals per aquarium at each replication for each treatment. Calculation of absolute growth in milkfish larvae is calculated using the Effendi formula (1997) as follows:

$$W = W_t - W_0$$

Description: W is the Growth of the absolute weight of the milkfish larva (gr), W t is the weight of the milkfish larva at the end of the study (gr), W0 is the weight of the milkfish larva at the beginning of the study (gr).

Effendi (1997) Effendi (1997)

$$SGR= (In\ W_t\text{-}In\ W_0)/T\ \ x\ 100\%$$

Description: SGR is the growth rate of specific weight (%/day), Wt is the average weight of larvae at the end of the study (gr), W0 is the average weight of larvae at the beginning of the study (gr), T is the length of maintenance (days)

Survival

Survival observations are carried out daily. To find out the survival data of test animals, the following formula is used:

$$SR = \left(\frac{Nti}{N0}\right) x\ 100$$

Description: SR is the Survival (%), Nti is the Number of larvae living at the end of rearing (tail), and N0 is the Number of larvae at the beginning of rearing (tail).

Water Quality

In this study, measurements of water quality parameters were used to support research data, including temperature, salinity, pH, dissolved oxygen and TDS. As for measuring water quality, the application of artificial intelligence is used, with several sensors that will be used in real time, namely temperature, pH, dissolved oxygen and TDS. The monitored parameters are sent to the remote control via the internet of things (IoT) and displayed on the computer. Each second will be detected by each and the sensors to Arduino and Arduino to Raspberry will collect data every 3 minutes.

Data Analysis

Data on changes in the rate of oxygen consumption of milkfish larvae were analyzed descriptively in graphical form. The relationship between the rate of oxygen consumption used by the larvae and the utilization of natural food using regression analysis. To determine the effect of density of *B. plicatilis* on larvae on the rate of oxygen consumption of milkfish larvae, growth and survival was carried out using data analysis using analysis of variance (ANOVA). If there is a significant effect then proceed with the W-Tuckey test. As a tool to carry out statistical tests, the SPSS version 23.0 program package is used. As for the water quality parameters of the maintenance media were analyzed descriptively.

3. Results and Discussion

Oxygen Consumption

Based on the results of the analysis of variance, it was shown that the density of *B. plicatilis* in rearing milkfish larvae showed a very significant effect on the results of the oxygen consumption rate. Furthermore, the results of the W-Tuckey test showed that the rate of oxygen consumption in treatment A density (25 ind/mL) was not significantly different from treatment C density (75 ind/mL) (P>0.05) but was very different from treatment B density (50 ind/mL) (P<0.01).

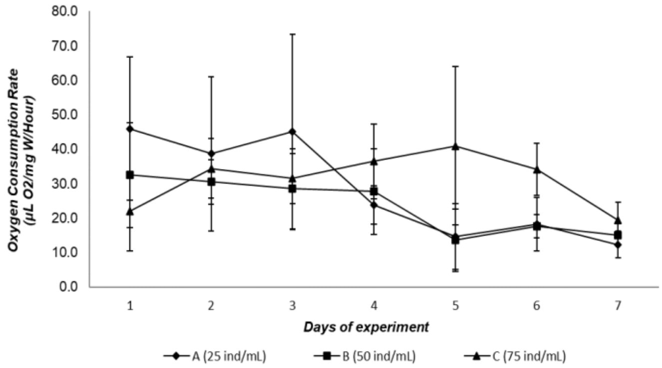
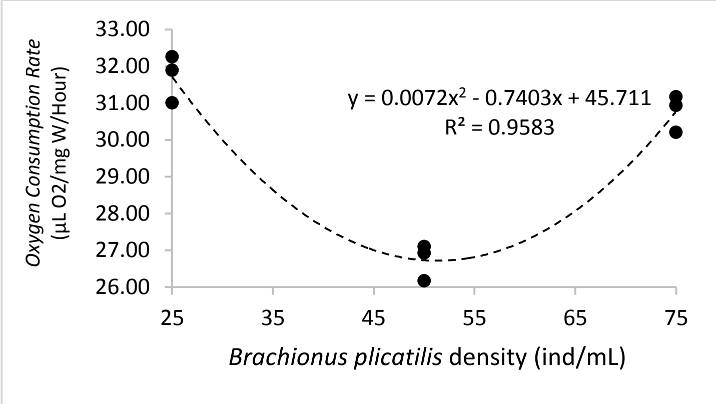


Figure 3. Average daily oxygen consumption rate of milkfish larvae (*C. chanos*) on each observation day



Graph 1. Regression analysis graph of oxygen consumption rate with different densities of *B. plicatilis*

Natural feed consumption

Based on the results of variety analysis showed that the density of *B. plicatilis* in the maintenance of milkfish larvae showed an intangible influence on natural feed consumption (P>0.05).

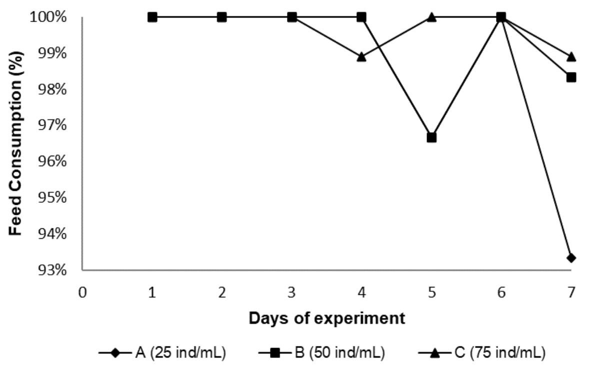


Figure 4. Percentage of daily feed consumption in milkfish larvae (*C. chanos)* on each observation day

Growth

The average growth of absolute and specific weights of milkfish larvae (*C. chanos*) given feed of *B. plicatilis* types with different densities can be seen in Table 1.

Table 1. Average growth of absolute and specific weights of milkfish larvae (*C. chanos*) at each treatment.

Density *of B. plicatilis* (ind/mL)	Absolute weight growth (mg) ± Stdv	Specific weight growth (%/day) ± Stdv
25 (A)	53,07 ± 4,90[a]	51,02 ± 5.19[a]
50 (B)	57,40 ± 16.80[a]	35,99 ± 2,56[b]
75 (C)	24,33 ± 8,62[b]	25,09 ± 6,23[b]

Description: The same letter in the same column indicates an unreal effect (p > 0.05).

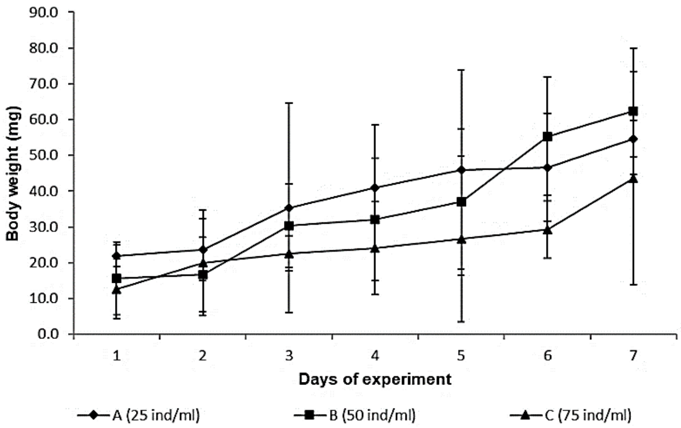


Figure 5. Growth of milkfish larvae (*C. chanos*) on each day of observation

Survival

Based on the results of variety analysis showed that the density of *B. plicatilis* showed an intangible influence on survival on milkfish larvae maintenance (P>0.05). The average survival of milkfish larvae in each treatment during maintenance can be seen in figure 6.

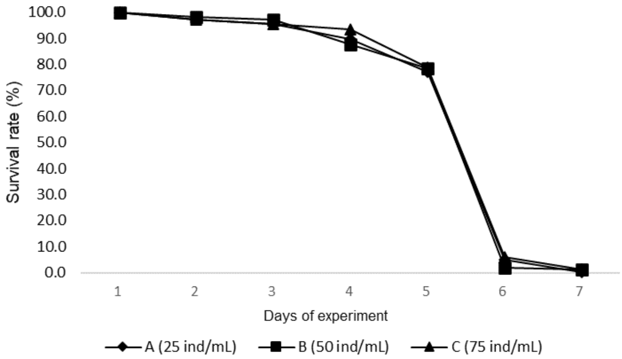


Figure 6. The average survival of milkfish larvae (*C. chanos*) given feed type *B. plicatilis* with different densities

Water Quality

Results During the study, several physical and chemical parameters of the environment were measured as supporting data which included dissolved oxygen, temperature, pH, salinity *and total dissolved solids* (TDS) in *real time*. Average water quality can be seen in Table 2.

Table 2. Average Water Quality of milkfish larvae (*C. chanos*) rearing media on each observation day

Day	**Water Quality**														
	DO (mg/L)			**Temperature (^{o}C)**			**pH**			**Salinity (ppt)**			**TDS (mg/L)**		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
D1	5.76	4.77	4.05	31.84	31.82	31.79	6	6	6	33	33	33	1165	1289	1256
D2	5.53	5.00	3.31	31.84	31.87	31.82	6	6	6	33	33	33	1164	1289	1261
D3	5.05	4.61	4.68	31.87	31.79	31.70	6	7	6	33	33	33	1169	1205	1261
D4	4.02	3.70	4.14	31.56	31.58	31.51	5	7	6	33	33	33	1180	1008	1261
D5	3.34	3.64	3.81	31.37	31.45	31.44	6	7	6	33	33	33	1179	1008	1261
D6	3.62	4.16	3.81	31.41	31.51	31.50	5	7	6	33	33	33	1174	1007	1259
D7	3.23	3.81	3.82	31.47	31.55	31.54	5	7	6	33	33	33	1170	1007	1258
Reference	>5 mg/L (Ganesh *et al.*, 2020)			20-43 ^{o}C (Ganesh *et al.*, 2020)			7,0-8,5 (SNI, 2013)			30-35 ppt (SNI, 2013)			1000 mg/L (PP No. 82 2001)		

The rate of oxygen consumption is a physiological indicator that can be used to describe the metabolic status of reared larvae. if you look at the results of dissolved oxygen measurements (Table 2) the average dissolved oxygen from the first day to the seventh day in treatment A (25 ind/mL) obtained an average dissolved oxygen of 4.36 mg/L, in treatment B (50 ind/mL) /mL) the average dissolved oxygen was 4.24 mg/L, in treatment C (75 ind/mL) the average dissolved oxygen was 3.95 mg/L. If it is associated with the results of the average oxygen consumption obtained (Figure 3), the fluctuations between dissolved oxygen and oxygen consumption for each treatment are almost the same. In treatment B (50 ind/mL) changes in dissolved oxygen and oxygen consumption in the larvae tended to be stable and in treatment A (25 ind/mL) the decrease in dissolved oxygen per day was not significant and was accompanied by high oxygen consumption as well. In treatment C (75 ind/mL) the average dissolved oxygen per day was low and supported by the use of high oxygen consumption, it was suspected that there was competition for oxygen between the larvae and natural food in the container. In general, the relationship between dissolved oxygen and oxygen consumption has a negative correlation where if high oxygen consumption means low dissolved oxygen and vice versa. This is because oxygen is the main ingredient or as a fuel in carrying out the process of breaking down energy (Rudiansyah & Wahidin, 2021).

Based on the results of research on natural feeding of *B. plicatilis* species with different densities, it showed a very significant effect on the rate of oxygen consumption in milkfish larvae ($p<0.01$). The relationship between the density of *B. plicatilis* and the rate of oxygen consumption in larvae from the results of the polynominal pattern regression analysis (Graph 1) provides information that the two variables have a strong relationship that has a positive correlation value (R2 = 0.9583) which shows that the higher and the low amount of natural feed density given, the oxygen consumption rate is also high. Based on the graph above it can be seen that at the point of 51.4 ind/mL a low oxygen consumption rate is obtained.

There was a significant decrease on the first day of larval rearing, it was suspected that the activity of the larvae increased in the process of transition to the exogenous feeding phase. According to Djawad et al (1996) activity is one of the factors that affect the metabolic rate of an organism, an organism that is active will have a high metabolic rate compared to a passive state. An increase in the oxygen consumption rate can be used as a sign that the larvae are in need of food and a low oxygen consumption rate indicates that the larvae are utilizing the food. In addition, there is fatigue and oxygen debt in the larvae at the end of rearing which causes a critical peak in the larvae and if it is associated with a graph the rate of oxygen consumption decreases which indicates a lack of movement or activity in the larvae. According to Djawad et al (1996) continuous swimming speed accompanied by an increase in oxygen consumption rate will cause fatigue and cause oxygen dept (oxygen debt). The decrease in oxygen content can also be explained by an increase in the rate of oxygen consumption due to a greater process of digestion/absorption of feed when the growth of fish increases (Fall et al., 2019).

Natural Feed Consumption

Based on Figure 4 it can be seen that the percentage of daily feed consumption in milkfish larvae tends to be stable until the fourth day (D4) and the sixth day (D6). However, on the seventh day (D7) each treatment decreased. Based on the results of the study, natural food for *B. plicatilis* species with different densities showed no significant effect on the consumption of natural food used during the study ($P> 0.05$). However, the results showed that the highest consumption of natural feed was in treatment C (70 ind/mL) namely 99.67%, then in treatment B (50 ind/mL) namely 99.33% and the lowest in treatment A (50 ind/mL) mL) 98.67%.

Natural food supply will optimize growth and weight gain in larvae, especially at the beginning of life. The larval stage is a stage with a high mortality rate, due to improper use and the type of food given (Araujo et al., 2022). Milkfish egg hatching occurs 12 to 17 hours after fertilization. The yolk sac will be absorbed during the first 24 hours. The mouth and digestive tract (the organ between the esophagus and rectrum) develop in two days. Therefore, the start of feeding occurs 45 to 50 hours after hatching (Araujo et al., 2022). Based on this, within two days the larvae were given natural food, such as *Chlorella* sp.

Growth

Growth is a change in size both in weight and length in a certain period (Sumiarsih et al., 2022). Growth will only occur if the energy content in the feed exceeds the energy needed to maintain the body and replace damaged cells (Sumiarsih et al., 2022)

In D1 the feed given is the type of *Chlorella* sp. Live food organisms contribute digestive enzymes that increase digestive activity to facilitate the digestive process until the larval digestive system is fully differentiated and developed (Dey et al., 2022). In D2, the larvae were given natural food, *B. plicatilis*. In addition, there is the highest distribution of energy utilization for somatic growth (weight and length gain) between the different treatments. In D3, changes in the distribution of energy utilization for somatic growth when compared to D2 suggest that the distribution of energy for somatic growth is not maximized simultaneously. At D5, the low weight gain and weight loss at this stage is thought to be due to the occurrence of a stress response and oxygen debt at D4 so that at D5 there is a decrease due to a change in energy to adapt. The stress response can result in decreased food intake, slower organ development and possible damage and even increased death rates (Qiang et al., 2019).

Survival

Survival is one of the benchmarks in cultivation. Survival is the percentage of the number of individuals that survive during rearing in a certain period of time or the ratio of the number of organisms reared at the end and the beginning of rearing. Survival during maintenance was low with a peak of critical mortality, namely the transition from the fifth day (D5) to the sixth day (D6).

Sumiarsih et al (2022) added that internal and external factors affect fish survival. Internal factors include disease resistance, feed, and age. External factors include stocking density, disease and water quality.

Based on Table 2 it can be seen that the pH and DO values during maintenance have an average value below the optimum. Based on this, a decrease in pH below the optimum is thought to be one of the triggers for a significant decrease in survival rate which makes the condition of the larvae partially stressful and unable to survive. The decrease in pH is suspected by feeding *B. plicatilis* every day so that some of the feed is not utilized or some of them die and no water changes are carried out. Whereas DO concentrations decrease with fish respiration and decomposition by living organisms (plankton) in the system (Chary et al., 2022).

Water Quality

Environmental parameters have an important role in waters and any environmental conditions that are not in accordance with the optimum tolerance can create stress conditions for aquatic organisms. In the early stages of larval growth and development, organ function is being formed and the process is very sensitive to changes in environmental factors (Qiang et al., 2019). In addition, the condition of the water will affect the diet of milkfish (Fakhriyah et al., 2022). The water quality of milkfish larvae rearing media measured was dissolved oxygen, temperature, pH, salinity, TDS (Table 2).

Dissolved oxygen (DO) is the main limiting factor for aquatic organisms (Wang et al., 2020). The dissolved oxygen during the study tended to fluctuate starting when the eggs were stocked until the end of rearing on the seventh day (D7). The dissolved oxygen (DO) value for each treatment during maintenance ranged from 3.23-5.76 mg/L. Based on Ganesh et al (2020) for milkfish the minimum lethal oxygen level is 0.1-0.3 mg/L. Meanwhile, DO levels >5 mg/L are very important to support good production (Ganesh et al., 2020).

Temperature is one of the most important abiotic factors because body temperature changes directly with environmental temperature (Wang et al., 2020). The average temperature in the maintenance media obtained at the beginning of maintenance D1 to D7 tends to be stable, ranging from 31^{o}C-32^{o}C. The optimum temperature for milkfish cultivation is 20-43 ^{o}C (Ganesh et al., 2020). Meanwhile, based on SNI (2013) the optimum temperature for the production of milkfish eggs and chicks is between 28°C - 32°C.

Water pH is critical for the survival and development of ectotherms (Wang et al., 2020). The average pH in the maintenance medium during maintenance ranges from 5-7. The pH range suitable for fish farming is between 6.7 and 9.5 and the ideal pH level is between 7.5 and 8.5 and above and below this stress the fish (Ganesh et al., 2020). The optimum water pH for milkfish egg and chick production is in the range of 7.00 – 8.5 (SNI, 2013).

Environmental salinity is an important factor affecting the survival of aquatic organisms and each fluctuation in water salinity can affect different physiological processes (Alam et al., 2020). The salinity used in the maintenance media is around 33 ppt. (Ayuzar et al., 2021). states that at a salinity of 15-35 ppt milkfish can grow well. Meanwhile, based on SNI (2013) the optimum salinity for milkfish egg and chick production is in the range of 30 ppt – 35 ppt.

The average TDS on the rearing medium obtained at the beginning of stocking up to D7 tended to decrease. The TDS obtained during the study ranged from 1007-1261 mg/L. Based on PP No. water quality standards. 82 (2001) (classes II and III), the range of TDS for fish farming is 1000 mg/L (Sukmawantara et al., 2021).

4. Conclusion

Based on the results of the study, it can be concluded that the *Brachionus plicatilis* density of 50 ind/mL had the lowest oxygen consumption rate and relatively good consumption of natural feed. More specifically, for the second day (D2) to the fifth day (D5) a density of 25 ind/mL can meet the daily natural food needs for milkfish larvae rearing and the sixth day (D6) and seventh day (D7) a density of 50 ind/mL is better.

Suggestion

Based on the results obtained from this study, it is suggested to give *Chlorella* sp. for the first day (D1) at a density of $5x10^4$ cells/mL, for the second day (D2) to the fifth day (D5) *Brachionus plicatilis* was given at a density of 25 ind/mL and then on the sixth day (D6) and seventh day (D7) it was increased to 50 ind/mL.

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