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by Diana Chilmawati

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Effects of Aeration Flow Rate in the Culture Medium on the Growth Performance and Egg Production of Copepod *Oithona similis* Fed with Fermented Organic Diet

Diana Chilmawati[1,2,*] ,*Johannes* Hutabarat[2] ,*Sutrisno* Anggoro[3], and *Suminto* Suminto[2]

[1]Doctoral Program of Coastal Resources Management. Department of Aquatic Resources. Faculty of Fisheries and Marine Science.Diponegoro University. Jln. Prof Soedarto. SH.Tembalang. Semarang 50275. Indonesia
[2] Department of Aquaculture. Faculty of Fishery and Marine Science. Diponegoro University. Jln. Prof Soedarto. SH. Tembalang. Semarang 50275 Indonesia
[3]Department of Aquatic Resources Management. Faculty of Fishery and Marine Science.Diponegoro University, Indonesia.

Abstract. The availability of copepod *Oithona similis* as live food organism for shrimp and marine fish larvae is strongly influenced by the optimum feed and environment conditions. Optimization of dissolved oxygen (DO) for *O. similis* can be carried out by providing proper aeration in culture media. Feeding with fermented organic diet besides phytoplankton is expected to support the individual growth, metabolism, and reproduction of *O. similis*. The aim of this study was to examine the effect of different aeration flow rates on the growth performance and egg production of *O. similis* and to determine the optimum aeration flow rate. Completely Randomized Design Experiment used in this study with 4 treatments and 4 replications. The treatment was *O. similis* culture with different aeration flow rate of 0.00; 22.00; 45.67; 66.67 mL.second^{-1}. The results showed that the difference aeration flow rate significantly affected ($p < 0.05$) the growth performance and egg production of *O. similis*. The aeration flow rate of 45.67 mL.second^{-1} provided the best growth performance with total density of 81.25 ± 2.99 ind.mL^{-1}; population specific growth rate 0.220 ± 0.002 day^{-1}; and egg production 28.40 ± 0.48 eggs.ind^{-1}. Moreover, the optimum aeration flow rate culture medium for *O. similis* is 45.70 mL.second^{-1}.

1 Introductions

The utilization of copepods provided higher growth and survival of marine fish larvae than artificial feed [1]. Some research revealed the success of the use of copepods as live food for shrimp and marine fish larvae have been widely carried out, including [2]. To date, *Oithona* sp. is one of the Cyclopoid copepods that are abundant in Indonesian waters. Its potentially used as live food organisms, and more widely used in hatcheries of sea shrimp and other marine fish larvae than Artemia and Rotifer [1].

*Corresponding author: dianachilmawati@yahoo.com

Oithona sp. isolated from Indonesian coastal waters have been identified and classified as *Oithona similis*, which grow optimally at salinity of 19.4 ppt [3]. *O. similis* grow well by giving phytoplankton cells namely *Chaetoceros calcitrans*, and also grows very well when given 50% fermented organic feed [4].

The availability of copepods including *O. similis* as larval feed is affected by media quality and optimum environment condition [1]. Environmental factors, i.e. temperature and dissolved oxygen (DO) are essential for the production of nauplii copepods, individual growth rate, and metabolism [5]. In *O. similis* culture, DO is one of environmental factors which has a role in metabolism processes. Increase in DO in culture media might be done through aeration at proper flow rate in semi mass culture production. Optimization of DO for *O. similis* can be carried out by providing some amount of aeration flow rate into the culture media. Therefore, research about the effect of aeration flow rate in the culture media is very important to determine its optimum point for supporting *O. similis* growth at high density.

2 Material and Method

This study was conducted at the Coastal Area Development Laboratory, Faculty of Fisheries and Marine Science, Diponegoro University, Jepara Campus, Central Java, Indonesia from July to September 2018.

The temperature and pH of *O. similis* culture media were daily measured using Amtast EC910 Multiparameter. Meanwhile, Ammonia content was measured using Indophenol Spectrophotometer Method.

2.1 Preparation of fitoplankton culture and fermented organic feed

C. calcitrans was provided by the Life Feed Laboratory, Laboratory of Brackish Water Aquaculture Development Research Center (BWADRC) Jepara, Central Java, Indonesia. This phytoplankton was cultured at sterile sea water at 25-28°C, 28-30 ppt of salinity, and pH 8-9. The sea water was sterilized by adding 60 mg.L^{-1} of Sodium Hypochlorite (NaClO) for 10-30 minutes, and neutralized with 80 mg.L^{-1} of Sodium Thiosulphate (NaS_2O_3) for 24 hours with continuous aeration. The culture was carried out in 60 L plastic tube containing 40 L sterilized seawater using a modified Walne media at a dosage of 0.5 ml.L^{-1} with a 24 hours light photoperiod and at 1500 to 1800 lux and controlled aeration. The volume of inoculant of *C. calcitrans* was 10% from the total volume of medium culture (Lee *et al*., 1996). The density of *C. calcitrans* was observed every day by taking a sample of the microalgae and then counted under a microscope (Olympus CH20) 10x magnification with a hemocytometer (Improved Neubauer volume 0.0025 mm^3).

The fermented organic feed used the combination of organic materials of tofu flour, rice bran and fish flour in powder form with an average diameter range of 50-100 μm [4]. The content of fermented organic feed protein was 30%. The plastic tube was filled 10 mL of EM4 probiotic containing *Lactobacillus casei*, *Saccharomyces cerevisiae*, and *Bacillus* sp. Then, the mixture was filled with 200 mL sterilized water and 25 mL molasses for 1 kg of mixed organic feed. The incubation was conducted for 48 hours before being used.

2.2 Culture conditions of *Oithona similis*

O. similis was obtained from the culture collection of the Marine Culture Development Research Center MCDRC) in Lampung, Indonesia [3]. The *O. similis* culture was carried out in a 15 L of volume container with 8 L of sterile seawater at 19.44 ppt of salinity [3], at 27-28 °C, pH 8, with a innitial stocking density of 1 ind.mL^{-1} [8] for 20 days. The density of *C.*

calcitrans given to *O. similis* was 0.01 mg.ind^{-1} [8]. Based on [4] research, the dosage of fermented organic feed was 0.5 g.L^{-1} of culture media with the combinations of 50% phytoplankton cell and 50% of fermented organic feed [9]. Aeration flow rate treatments were established at 0.00; 22.00; 45.67; and 66.67 mL. second^{-1} by using flow meter (Matheson FM-1050-VIA Part No. 7642T-603). The population of *O. similis* at the nauplii, copepodite, and adult stages was observed under a microscope (Olympus CYK41). Each stadia is determined based on its morphology [10].

2.3 Design of experiment

A completely randomized design with 4 treatments and 4 replications was carried out in this study. The treatments were A (*O. similis* cultured with an aeration flow rate of 0.00 mL.second^{-1}) B (22.00 mL.second^{-1}), C (45.67 mL.second^{-1}), and D (66.67mL.second^{-1}). The density of *O. similis* was calculated every 4 days to obtain the total population, growth performance, and egg production. Calculation of total *O. similis* density consists of nauplii, copepodites, adult and female eggs laying densities. Calculation of the amount of *O. similis* was observed carefully using a microscope, magnifying glass, Petri dish, and dropper drops with adequate lighting. Samples were taken from 100 mL *O. similis* culture from each treatment. Egg production was calculated by comparing the abundance of eggs and the number of females laying eggs. Calculation of the number of eggs was done by randomly isolating *O. similis* to lay eggs from each treatment (n = 2). The egg-laying female was observed under a 10x - 40x magnification microscope. Egg abundance was calculated by multiplying the number of egg sacs by the average number of eggs per bag [11].

2.4 Data collection and statistical analysis

The density of *O. similis* was counted every 4 days during 20 days observations. Amount of 50 mL sub sample was taken from the culture medium to calculate the number of total *O. similis*, population specific growth rate (r) and egg production.
Growth performance was calculated using a formula by [12]:

$$r = \frac{\ln \ln Nt - \ln \ln No}{t} \quad (1)$$

Where r is population growth (day^{-1}), Nt is the final density of *O. similis*, No is an initial density of *O. similis* and t is days needed to achieve maximum growth.

Egg production is the number of eggs produced by the female *O. similis* on average during their lifetime according to [11]:

$$Egg\ production = \frac{\Sigma s \times e}{\Sigma n} \quad (2)$$

Where s is the amount of egg sac, e is the average amount of eggs per sac and n is the number of ovigerous females (ind).

Data were analyzed by One Way Analysis of Variance (ANOVA) to determine the effect of different aeration flow rate on growth performance and egg production of *O. similis*. Least Significant Different (LSD) test (α=0.05) by using SPSS 16 was conducted when the treatment had a significant effect. The optimum point of aeration rate flow is determined using polynomial orthogonal analysis with the MAPLE program 2016.

3 Result and Discussion

a. Result

3.1.1 Water quality in the difference aeration flow rate of O. similis culture medium

The treatment of different aeration flow rates of *O. similis* culture media provides different dissolved oxygen content (Figure 1). The measurement results of Dissolved Oxygen (DO) content in each treatment showed digression from the first day of culture to the last day of the observation (20th day). The treatment of 0.00 mL.second^{-1}aeration flow rate showed the lowest DO (2.50 mg.L^{-1}) compared to the other three treatments. The DO measurement results at the end of each observation were as follows: aeration flow rate of 66.67 mL.second^{-1} produced the highest DO (4.41 ± 0.06 mg.L^{-1}), 45.67mL.second^{-1} aeration flow rate produced DO of 4.18 mg.L^{-1}, and 22.00mL.second^{-1} aeration flow rate produced DO of 3.74 mg. L^{-1}.

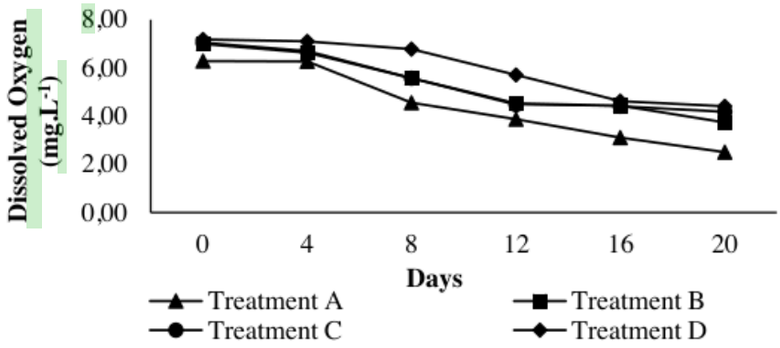

Fig. 1. Dissolved oxygen content at different aeration flow rate culture medium of *O. similis* during the observation

The relationship between DO and aeration flow rate (Figure 2) showed a simple linear relationship with a regression equation y = 0.0276x + 2.7799 with R^2 = 0.8658, which means that 86.58% DO of *O. similis* culture media was influenced by aeration flow rate factors and 13.42% was influenced by another factor.

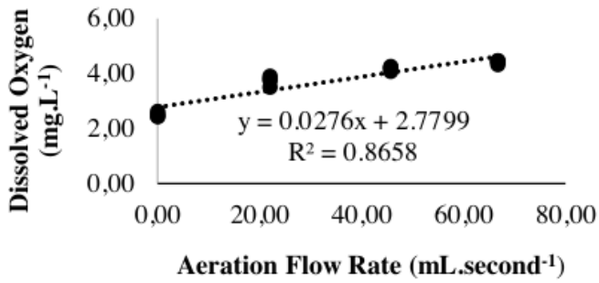

Fig. 2. Relationship between aeration flow rate and Dissolved Oxygen culture medium of *O. similis*

The average content of DO, pH, and Ammonia of *O. similis* culture media during the study (Table 1) showed a favourable levels for *O. similis*.

Tabel 1. Average content of DO, pH, and Ammonia of *O. similis* culture media during the study

Aeration flow rate (mL.second^{-1})	Temperature (°C)		pH	Ammonia (mg.L^{-1})
	Morning	Evening		
0.00	25.40±0.18	27.43±0.05	7.84±0.04	0.128±0.003

22.00	25.16±0.14	27.30±0.00	7.93±0.03	0.056±0.001
45.67	25.20±0.16	27.23±0.15	7.85±0.04	0.000±0.001
66.67	25.30±0.04	27.38±0.15	7.93±0.00	0.000±0.001

3.1.2 O. similis population

The difference in the aeration flow rate on culture media significantly affected ($p < 0.05$) on *O. similis* growth performance. Total density was the total number of nauplius, copepodites and adult copepods, including an adult with eggs. According to the results of each stage of *O. similis* on the last day of cultivation (Day 20), it was indicated that the lowest density was 45.67 mL.second^{-1}(Table 2). The results of the observations on day 20 showed that at the aeration flow rate of 45.67 and 66.67 mL.second^{-1} the population was dominated by *O. similis* stage copepodite. Whereas at an aeration flow rate of 0.00 and 22.00 mL.second^{-1} the population was dominated by *O. similis* stage copepodite.

Table 2. Numbers of nauplii, copepodite, adult and population of *O. similis* in different aeration flow rate culture media on the last day of cultivation (ind.mL^{-1})

Aeration flow rate (mL.second^{-1})	Stage			
	Nauplii	Copepodite	Adult	Population*
0.00	6.25±1.71	4.75±0.96	6.75±2.63	17.75±1.71^{d}
22.00	27.50±2.08	25.25±1.71	21.25±2.22	766.67±1.41^{c}
45.67	22.25±1.71	30.00±1.63	29.00±2.16	81.25±2.99^{a}
66.67	25.00±2.16	27.50±2.52	24.75±2.22	77.25±2.06^{b}

*Mean±SD. Different lowcase letter indicate a significant difference between treatment at $p<0.05$

The different aeration flow rates had a different effect on the population of *O. similis* for 20 days of observation (Figure 3). The 0.00 mL.second^{-1}aeration flow rate provides the lowest density compared to other aeration flow rates. 45.67 mL.second^{-1}aeration flow rate is proven to provide the highest density compared to aeration flow rates of 66.67 and 22.00 mL.second^{-1} *O. similis* density increased from the first observation, namely the 4th day, and continued to increase until the 20th day observation.

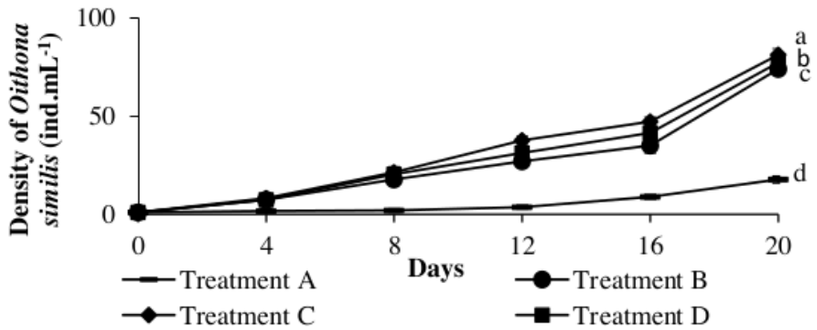

Figure 3. The density of *O. similis* at different aeration flow rate during 20 days culture period. Values not sharing the same letter are different significantly from one another ($p<0.05$) by Least Significant Difference Test

The correlation between aeration flow rate and total density shows a quadratic relationship patterned equation $y = -0.0307x^2 + 2.8801x + 19.752$ with $R^2 = 0.9668$, which means that 96.68% of total *O. similis* density is influenced by aeration flow rate factors and 3.32% is influenced by factors other. The *O. similis* total density response curve for the aeration flow rate (Figure 4) shows the optimum point of the aeration flow rate at 45.90mL.second^{-1}.

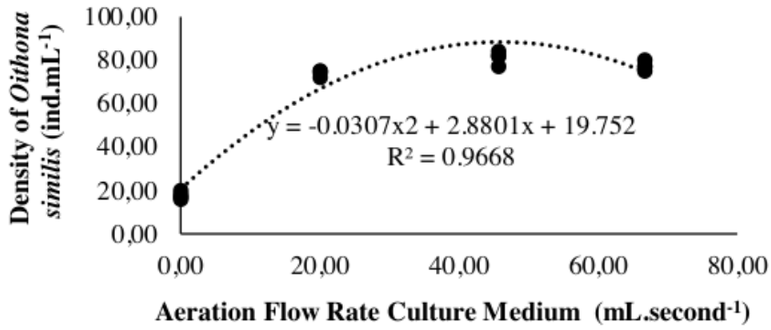

Figure 4. Corellation between aeration flow rate and density of *O. similis*

3.1.3 The population specific growth rate of O. similis.

The difference of aeration flow rates showed a significant effect ($p < 0.05$) on the specific growth rate of *O. similis* population (Figure 5). The highest value of population specific growth rate (r) (0.220 ± 0.002 day^{-1}) was shown in *O. similis* culture at giving an aeration flow rate of 45.67mL.second^{-1}. The aeration flow rates of 66.67 and 22.00mL.second^{-1}give r values that are not significantly different, each at 0.216 ± 0.001 and 0.215±0.001 day^{-1}. The lowest population specific growth rate (0.144 ± 0.005 day^{-1}) was shown in culture media with an aeration flow rate of 0.00 mL.second^{-1}.

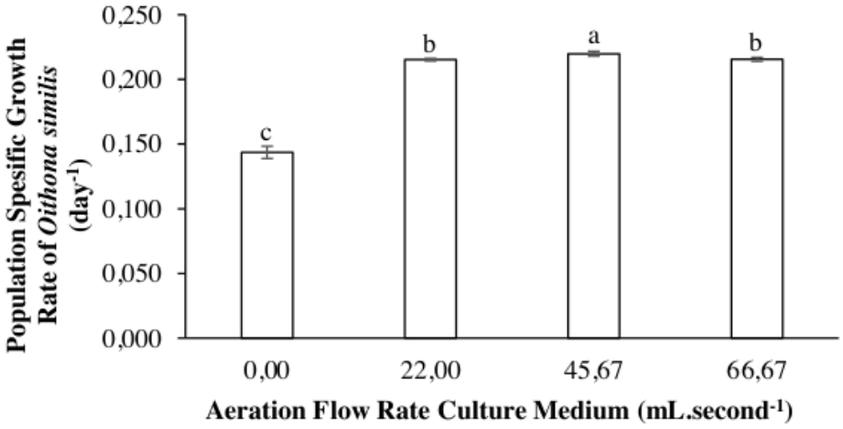

Figure 5. Histogram of the population specific growth rate of *O. similis* in different aeration flow rate culture media

The curve of *O. similis* specific population growth rate during the study with differences in aeration flow rate (Figure 6) showed there was quadratic pattern correlation relationship with the equation $y = -4E\text{-}05x^2 + 0.0036x + 0.1467$ with $R^2 = 0.9511$. This means 95.11% of the population specific growth rate was influenced by differences in the aeration flow rate of culture media and 4.89% influenced by other factors. The optimum point of aeration flow rate was *O. similis* culture medium at 45.00mL.second^{-1}.

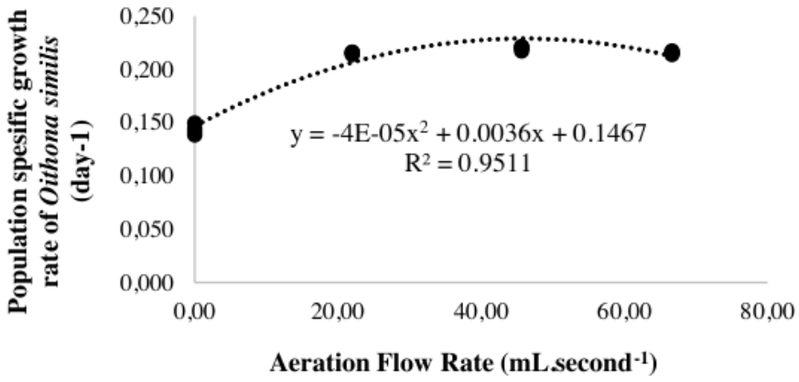

Figure 6. Correlation between aeration flow rate culture medium and the population specific growth rate of *O. similis*

3.1.4 Egg production of O. similis

The average histogram of *O. similis* egg production at the end of the observation (Figure 7) showed that culture medium with an aeration flow rate of 45.67 mL.second^{-1} gave the highest egg production (28.40 ± 0.48 egg.ind^{-1}) which was significantly different ($p < 0.05$) with all three other treatments. The aeration flow rates of 66.67 and 22.00 mL.second^{-1} generated egg production which was not significantly different ($p > 0.05$) but was significantly different from the aeration flow rate of 0.00 mL.second^{-1}(7.05 ± 1.84 egg.ind^{-1}).

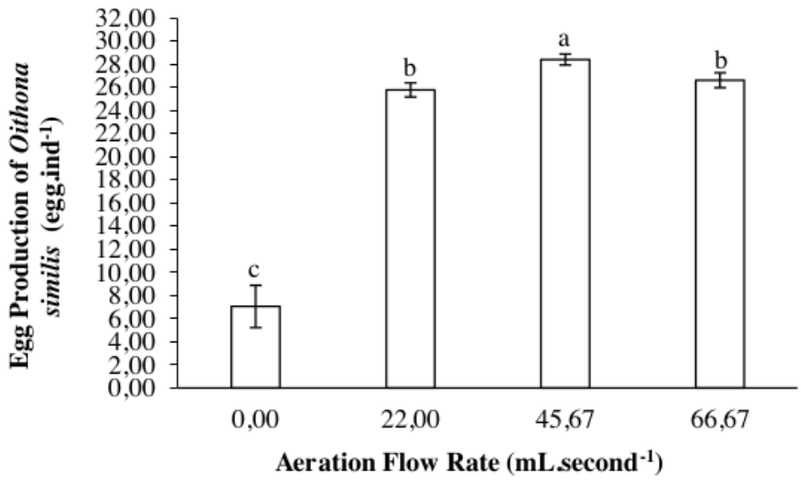

Figure 7. Egg production of *O. similis* in different aeration flow rate culture medium

Based on the orthogonal polynomial test of *O. similis* egg production on culture medium with different differences in aeration flow rate, quadratic patterned relationships (Figure 8) were obtained with the equation $y = -0.0105x^2 + 0.9723x + 7.6696$ with $R^2 = 0.9649$ and the optimum point of media aeration flow rate at 45.7mL.second^{-1}. This showed that *O. similis* egg production was influenced by the aeration flow rate factor of 96.49% and another factor of 3.51%.

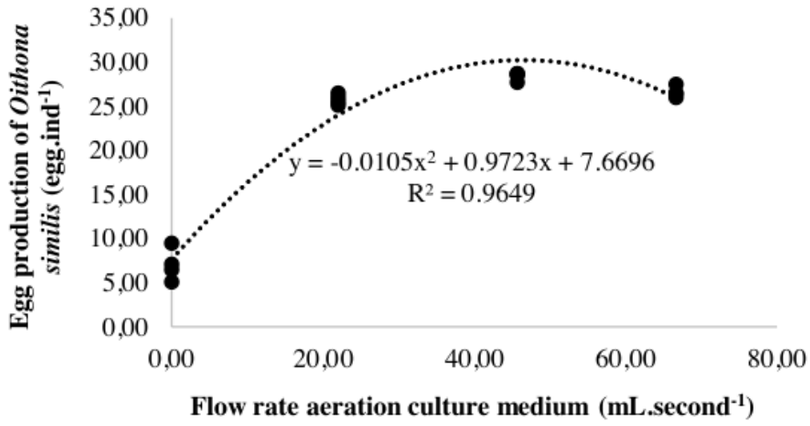

Figure 8. Correlation between aeration flow rate culture media and egg production of *O. similis*

b. Discussion

The results of water quality measurement from each different aeration flow rate show a range which is still suitable for *O. similis* life. The correlation of aeration flow rate and DO showed a simple linear correlation, where DO content will increase in line with the increase in aeration flow rate in *O. similis* culture media (Figure 2). This proves that there was a closeness in the relationship between the flow rate of aeration and DO.

The results showed that the difference in aeration flow rate has a significant effect on total density, specific population growth rate, and reproduction of *O. similis*. Treatment of C (aeration flow rate 45.67 mL.second^{-1}) with DO 4.18 mg.L^{-1} produced the highest of total density, population specific growth rate and egg production among the other treatments. It was suspected that the magnitude of the aeration flow rate has provided environmental conditions that in accordance with the needs and supports the growth and reproduction *of O. similis*. In addition, the movement of water generated by the aeration flow rate provided suitable conditions for the movement of *O. similis* in the water column. The environmental conditions of culture media that did not support will affect the growth and SR of *Oithona* sp. [13]. In nature, the factors that influence the population of copepoda (*Oithona* sp.) beside feed availability [14] are fish larvae (predators), other copepods (competitors), and abiotic factors, i.e. temperature, DO and salinity [15].

The observation during the study showed that the difference in the aeration flow rate had a different effect on the water movement in the *O. similis* culture media. Physically, treatment B showed a quieter water movement, but DO was lower than treatment C and D. This will relatively affect the growth and reproduction of *O. similis* because oxygen was also needed by phytoplankton *Chaetoceros calcitrans*. The presence of fermented organic food (feed organic fermented) added will also affect the DO content in the *O. similis* culture media. It was predicted that in treatment B the DO content was still insufficient for *O. similis* to grow and reproduce.

Treatment D, water movement was too strong that suspected as the stress factor for *O. similis*. These stress conditions will affect the use of a certain amount of energy to recover [16]. Copepode *O. similis* required sufficient energy to maintain its life so that the energy that should be allocated for growth was disrupted. This strong water movement causes

deposits of organic feed and other impurities at the bottom to stir up. The movement of *O. similis* to defend itself from the flow due to the movement of a large aeration flow rate also reduces the energy that is supposed to grow and metabolize.

Treatment A where culture media without aeration showed the lowest DO (2.50 mg.L^{-1}). The DO content was still feasible for the life of *O. similis* even though it provided the lowest growth and egg production performance. [17] stated that copepods could no longer tolerate DO <1.1 ppm as well as [18] stated that the minimum DO concentration that was still tolerated by copepod was 2 ppm. This is in accordance with the results of the research by [19] that copepods from the species *Calanipeda aqvaedulcis* and *Arctodiaptomussalinus* can grow well with a range of DO 1-8 ppm.

The values of temperature, pH and ammonia content in *O. similis* culture media (Table 1) are presumed to support the life of *O. similis* especially for its growth and egg production. The measurement results of temperature, pH and ammonia during the study of all treatments were still at a reasonable level for the life of *O. similis*. The optimum temperature range of copepod is 25ºC [15]; 25-30ºC [33]. Temperature plays an important role in the rate of metabolism and egg production [14]. Temperature affects the size of the female, which also affects egg production [20]. [22] reported copepod nauplii grow well at pH 7.7-8.5 and could tolerate ammonia ≤ 1.3 ppm. Low temperatures will increase total body length and prolong the growth and maturation of copepods [34]. In addition, temperature also affects the quality of copepods' nutrition. As an example, PUFA (Polyunsaturated Fatty Acid) content will increase at low temperature [33].

Based on the specific population growth rate, the optimum aeration flow rate for *O. similis* culture was 45.70mL.second^{-1}. In the optimum conditions will provide suitable and preferred environment to the high density, population specific growth rate and egg production of *O. similis*.

4 Conclusions

The difference in aeration flow rate have a significant effect ($p < 0.05$) on growth performance and egg production of *Oithona similis* where 45.67mL.second^{-1} aeration flow rate showed the best growth and egg production performance (total density of 81.25 ± 2.99 ind.mL^{-1}; specific population growth rate of 0.220 ± 0.002 day^{-1}, and egg production of 28.40 ± 0.48 egg.ind^{-1}. Based on the specific population growth rate, the optimum aeration flow rate for *O. similis* culture was 45.70mL.second^{-1}

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