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Growth response of rotifers on a bacterial-based diet made from fishwastes

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Abstract. Fishwastes are potential low-cost diet sources for replacing microalgae, which is the popular diet for rotifers. Growth responses of rotifer were tested under different weights of the diet (i.e. 0.05, 0.1, 0.2 and 0.4 g/L), different culture containers (i.e. using small tall (ST), small-short (SS), medium-tall (MT) and large-short (LS) containers), different initial densities of the rotifer (i.e. 10, 50 and 200 rotifers/mL) and mass cultured trials for 15 and 30 days. Population densities of rotifers increased significantly (ANOVA, $p=0.001$) when providing 0.1 to 0.4 gr/L than those fed 0.05 gr/L of the diet (Tukey HSD test, $p<0.05$). The densities also were significantly higher (ANOVA, $p=0.001$) when cultured using larger containers (LS and MT) than using smaller containers (ST, SS) (Tukey HSD test, $p<0.05$), but it was not significant when inoculated at different initial densities (ANOVA, $p>0.05$). The rotifers were successfully mass cultured for 30 days and were successfully harvested for 7 times at densities of about 2500 to 3300 ind./mL.

1. Introduction

Aquaculture growth is on the rise almost all over the world, and there is increased demand for live food biomass i.e. rotifers, as a suitable starter diet for larval fish rearing in hatcheries [1][2][3][4]. Indeed, studies have shown that much smaller rotifers are valuable in marine fish larviculture [5]. Despite the current success in ultrahigh-density cultures technique for rotifers [6][7][8], the technique still requires a continuous supply of high dense microalgae as food for the rotifers.

So far, microalgae are the first choice for feeding rotifers [9], thanks to their excellent influence on rotifer growth rate [7][8][10][11], rotifer nutrition [12][13] and for the nutrition of fish larvae that feeds on the rotifers [14]. However, culturing microalgae require high investment and running expenses [15], with difficulties in production [16][1], harvesting [17], and storage / preservation [18]. [19] introduced baker's yeast as a cost-effective method to replace microalgae for rotifer production. However, baker's yeast is deficient of essential nutrition [20][21], for proper growth and development of the rotifers and their predators [12][22]. Thus, requires further enrichment with expensive chemical



emulsions. On the other hand, there are evidences that rotifers, as top predators in the microbial web [23][24] can utilize soluble compounds of bacterial excrete that are found in the culture media as food [25]. Indeed, previous studies have reported that presence of bacteria in rotifer cultures provide better effects on growth and nutritional value of the rotifers and fish larvae that feeds on them [26][27]. Nonetheless, information on effects of pathogenic bacteria on rotifer growth is scanty, even though addition of lactic acid bacteria to rotifer cultures increases resistance of fish larvae against pathogenic bacteria e.g. of the vibrio genera [28].

In this study, we hypothesized that a fishwastes-based diet can provide an excellent substrate for proliferation of billions of bacterial cells, which are directly ingested by rotifers, as their food source. To date, the use of bacteria as food source for rotifers is limited to experimental scale and mainly added as supplementary to microalgae or baker's yeast [29]. In the present study, we investigated the possibility of culturing rotifers using the fishwastes in the same culture facility with the rotifers.

2. Materials and methods

2.1. Materials

Two strains of rotifer *Brachionus rotundiformis* Perth and Poigar strains were used in this study. The rotifer *B. rotundiformis* Perth strain was obtained from a clone culture at laboratory of Aquaculture Biology, Nagasaki University, while *B. rotundiformis* Poigar strain was isolated from estuary around Poigar, North Sulawesi, Indonesia. A diet based on fishwastes was used as dietary source for the rotifers for several generations. The fishery industrial waste was prepared following the protocols described in a patent registered in Indonesian with number: P00201609066 and was kept in freezer (-20°C) prior of using in this study.

2.2. Methods

Growth responses of the rotifers on the diet was investigated by culturing the rotifers at different diet weights, different culture containers and different initial density of rotifers using the diet as food source. Experiment on different weight of the diet (i.e. 0.05, 0.1, 0.2, 0.4 g/L in triplicate) was performed in 15 small-tall flasks (volume 0.075L: length: 65mm and width: 30mm). The flasks were filled with 75 mL filtered (Glass microfiber filters, Whatman) and sterilized (autoclaved at 121°C for 30 minute) seawater at salinity 22 ppt. The Perth strain rotifers were transferred into each flask at density of 10 ind./ml following the addition of respective treatments of diet weight in triplicates. The diet was added once at the beginning and no other addition afterwards till end of experiment. As control, microalgae *Nannochloropsis oculata* was prepared at density of 1.2×10^7 cell/mL. *N. oculata* density was maintained daily by adjusting the density similar to initial density. Observation was made once a day by counting the numbers of rotifer in 200 µl sample taken from three different layers (surface, middle and bottom) of each flask. A one-way ANOVA and Tukey-Kramer's test was performed to compare population densities of rotifer among the MRD weights.

Experiment on different culture containers was performed in four different shapes and volumes of culture containers in triplicate i.e. ST (small tall glass flasks: vol: 25 mL, length: 65mm and width: 30mm), SS (small-short glass beakers: vol: 75ml, length: 95mm and width: 55mm), MT, (medium-tall glass beakers, volume: 750ml, length: 181mm and width: 95mm) and LS (large-short polycarbonate tanks; volume: 30 l, length: 320cm and width: 350 cm). All flasks and tanks were firstly sterilized and were filled with sterilized seawater at salinity of 22 ppt. The Perth strain rotifers were introduced to each flasks and tanks at density of 10 ind./ml following the addition of the diet at 0.2 g/L. All flasks were placed in an incubator at constant temperature (28°C) while tanks were placed outdoor in a water bath at temperature of about 28°C. Observation on population density of rotifer was made once a day by counting the numbers of rotifer in 200 – 1000 µl sample taken from three different layers (surface, middle and bottom) of each container depends on volume of culture. A one-way ANOVA and Tukey-Kramer's test was performed to compare population densities of rotifer among the culture containers

Experiment on different initial rotifer density was performed in 30-L transparent polycarbonate cylindrical tanks. Three different initial densities of rotifer *B. rotundiformis* Perth strain at 10, 50 and 200 ind./mL were introduced to nine 30-L transparent polycarbonate cylindrical tanks. All tanks were filled with artificial seawater (Marine Art Hi, Tomita Pharmaceutical, Japan) at salinity 20-22 ppt and were placed inside water baths at constant temperature at 27-29°C. The diet (0.2 g/L) was added to the tanks soon after inoculating rotifers at respective densities. Observation on population density of rotifer was made once a day by counting the numbers of rotifers in 1000 µl sample taken from three different layers (surface, middle and bottom) of each tank. A one-way ANOVA and Tukey-Kramer's test was performed to compare population density of rotifer among the initial density treatments

Mass culture trials on the Poigar strain rotifer using the fishwastes as exclusive dietary source for the rotifer was conducted for 15 and 30 days. The rotifers were semi-continuously mass cultured in two fibre tanks (volume 750 L, length 125 cm, width 81 cm and height 75 cm). All tanks were filled with 750 litres of artificial seawater (salinity 20 ppt) and were placed outdoor. About 10 and 400 ind./ml of rotifers were initially introduced to each tanks following the addition of the diet at 0.27 gr/L. The cultures were harvested every 2 to 5 days depending on density of the rotifer following the addition of new diet to the cultures. No water exchange and no aeration were provided to the cultures during the experiment. Observation was made every 6 hours by counting numbers of rotifer in 1000 µl samples taken from three different layers (surface, middle, bottom) of culture.

3. Results and discussions

Population growth curves of rotifer S-type *B. rotundiformis* revealed that the fishwastes-based diet used in this study has significant effect on growth of rotifers. The rotifers are capable of utilizing organic materials produced by the microbial decomposing processes on the diet as their nutritional source for development and reproduction [30, 31]. The rotifer showed a significant response in growth when cultured under different weight of the diet (Anova test, $F = 8.13$, $P = 0.00$), culture duration ($F = 30.71$, $P = 0.00$) and the interaction between them ($F = 6.33$, $P = 0.00$) on rotifer population density (Table 1).

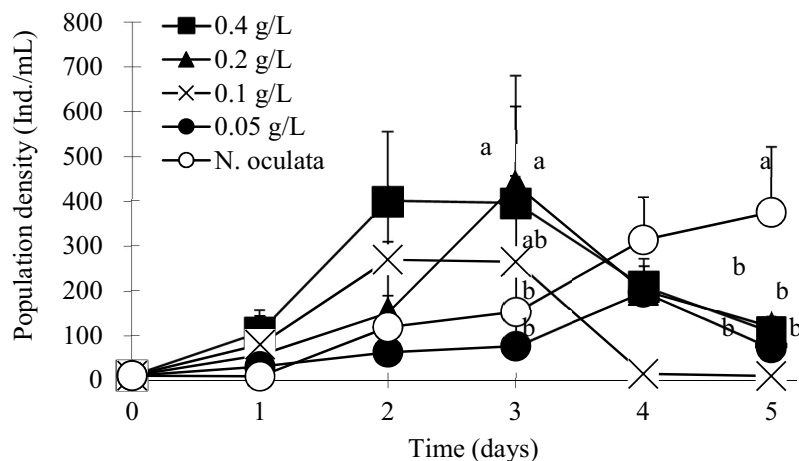


Figure 1. Population density curves of rotifer batch cultured under different MRD weight (0.4 (■), 0.2 (▲), 0.1 (×) and 0.05 g/L (●) and control (1.2×10^7 *Nannochloropsis oculata* cells/mL (○)). The values and error bars represent mean \pm SD of 3 replicates each. Different alphabetical letters indicate significant differences at $p < 0.05$, $a > b$

Higher population density was observed at diet weight 0.2 and 0.4 g/L compared to control experiment on day 3, before experiencing culture crash. The rotifer density in the control experiment

increased significantly on day 4 and 5 (Figure1). Population densities of rotifers cultured with 0.4 and 0.2 g/L of the diet were significantly higher than those cultured with 0.1 and 0.05 g/L (TukeyHSD test, $p < 0.05$). Mean maximum densities of 400 ± 155 and 438 ± 174 ind./mL were obtained at 0.4 and 0.2 g/L on 3, while 264 ± 193 ind./mL were obtained in the control. Results of the present study indicated that the organic materials produced from microbial decomposing processes on the diet could support nutritional requirement of rotifer for certain culture period, beyond this period, the system become exhausted and required a new diet resource. It is suggested that the diet weights between 0.2-0.4 g/L in this study supported nutritional requirement of rotifer until day 3. Unlike in the control treatment, population density of rotifer increased until the end of experiment due to daily addition of *N. oculata* to the rotifer.

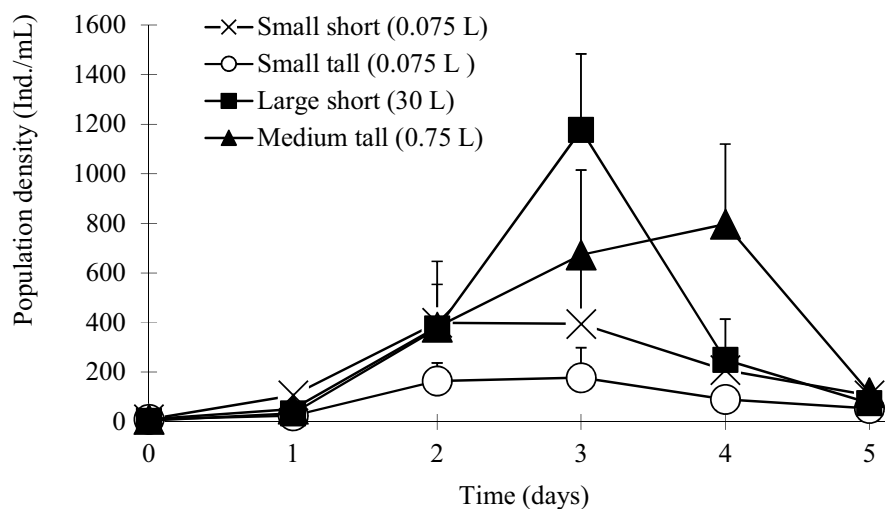


Figure 2. Population density curves of rotifer batch cultured under different volumes and shapes of culture containers i.e. ST ((O) small tall), SS ((X) small-short), MT ((▲) medium-tall) and LS (■) large-short polycarbonate tanks). The values and error bars represent mean \pm SD of 3 replicates each. Different alphabetical letters indicate significant differences at $p < 0.05$, $a > b$.

Growth response of rotifer on the fishwaste-based diet was significant to culture container ($F = 10.47$, $P = 0.00$), culture duration ($F = 26.89$, $P = 0.00$) and their interaction ($F = 5.59$, $P = 0.00$) (Table 1). Rotifer cultured using the diet showed higher densities in 30-L tanks and 750 mL flasks than those in 25 mL and 75 mL flasks on day 3 (Tukey's HSD test, $p < 0.05$). Maximum rotifer densities of 1170 ± 306 were obtained in 30-L, on day 3 (Figure 2). In general, rotifer cultured in small containers is mostly for maintaining rotifer stock, while those cultured in larger containers are for mass production. Result of the present study shows that the fishwastes-based diet can be used for maintaining stock and also for mass production purpose. High population density in the LS tanks (30L tanks) indicating a positive effect of the diet on growth of rotifer in larger containers. In regard to the current high-cost mass production of rotifer based on microalgae, result of the present study can be a breakthrough in mass production of rotifers without microalgae. The fishwastes-based diet is made from cheap sources thus offers opportunity to reduce the cost of rotifer production for profitable aquaculture production.

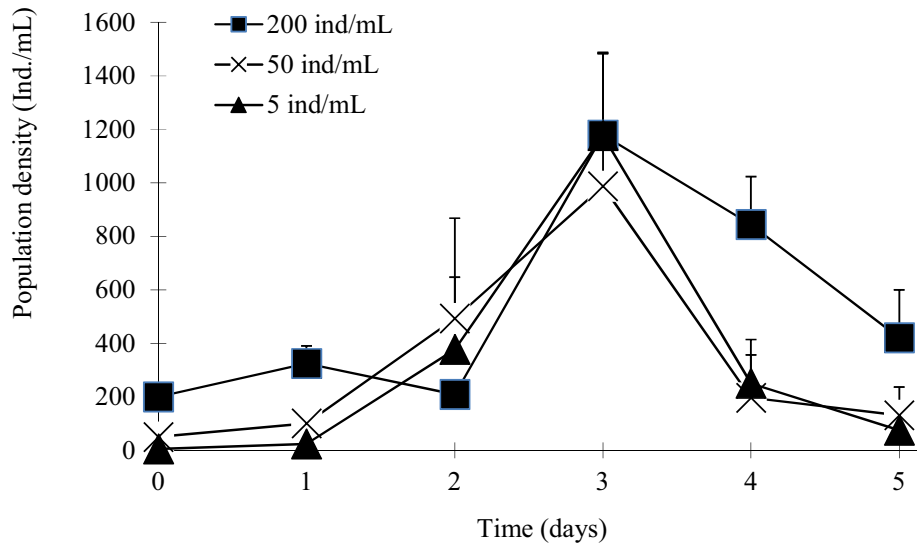


Figure 3. Population density curves of the rotifer batch cultured under different rotifer starter densities of 200 ind./mL (■), 50 ind./mL (X) and 5 ind./mL (▲). The values and error bars represent mean \pm SD of 3 replicates each. Different alphabetical letters indicate significant differences at $p < 0.05$, $a > b$

Initial density is closely related to competition between individual rotifer for food which, has strong influence on growth and reproduction performance of rotifers [32]. Response in growth of rotifers cultured using the fishwastes-based diet at different rotifer initial densities is shown in Figure 3. Initial density of rotifer had significant effect on population density of rotifer during 5 days culture (ANOVA test, $p = 0.01$). The density of rotifer at higher initial density treatment (200 ind./mL) was significantly higher than those at lower initial density (5 and 50 ind./mL) on day 4 and 5 of culture (TukeyHSD test, $p < 0.05$). Maximum densities of rotifer in all treatments were attained on day 3 at about 1170 ind./mL and the densities decreased sharply afterwards. The rotifers at initial densities of 200 ind./mL showed a little decrease on day 2 then sharply increased until day 3 of culture. It is suggested that until day 2, competition on food source had occurred on higher initial density treatment (200 ind./mL) indicated by decrease in population density, which was not seen in the lower initial density treatments (5 and 50 ind./mL). The fast growth pattern of rotifers till day 3 and crash afterwards, support our previous assumption that nutritious materials produced by microbial decomposing processes on the diet become exhausted for certain period. Lubzens reported a similar growth pattern by using the diet as food source for the rotifer [29].

Table 1. One-way ANOVA output of diet weight, culture container and rotifer starter density on population density of rotifer in all the treatments. df: degrees of freedom, SS: Sum of squares, MS: Mean square, *F*: F-ratio, *P*: level of significance (*denote significant difference at $p < 0.05$)

	df	SS	MS	<i>F</i>	<i>P</i>
<i>Diet weight</i>					
Day	5	16.38	3.28	30.71	0.00***
Diet weight	4	3.47	0.87	8.13	0.00***
Day × Diet weight	20	13.52	0.68	6.33	0.00***
Residuals	60	6.40	0.11		
<i>Culture container</i>					
Day	5	3141948	628390	26.89	0.00***
Culture container	3	733988	244663	10.47	0.00***
Day × culture container	15	1959188	130613	5.59	0.00***
Residuals	48	1121588	23366		
<i>Rotifer initial density</i>					
Day	5	6400008	1280002	30.38	0.00***
Rotifer starter density	2	516363	258182	6.13	0.00**
Day × rotifer starter density	10	870373	87037	2.07	0.05
Residuals	36	1516940	42137		

Unlike the batch culture system discussed above, semi-continuous culture system mainly performed for long term production to supply food requirement of fish larvae. In the present study, we successfully used the fishwastes-based diet to mass produce the rotifers using outdoor semi-continuous culture system without any addition of microalgae as the current mass production protocols.

In regards to the current problems on the cost of microalgae production for feeding rotifer in larviculture industry [30], the fishwastes-based diet can be a promising alternative for a profitable aquaculture production by reducing or eliminating the production cost of microalgae. Although the use of fishwastes-based diet for rotifer is just in the trial phase and still require optimizations, it had a strong positive influence on growth of rotifer in this culture system. The maximum density achieved (around 1357 – 3305 ind./mL), the long duration of culture (30 days culture) and the yield obtained (7 times harvest) during the trial can be a good starting point for future improvement.

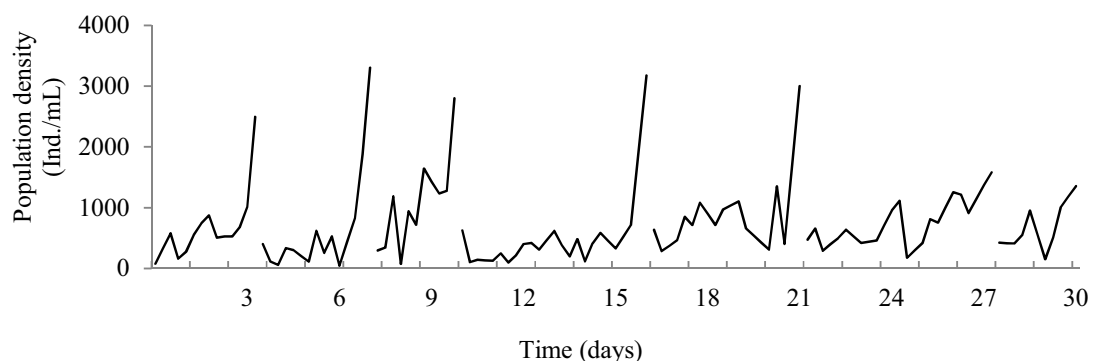


Figure 4. Population density curve of rotifer semi-continuously mass cultured in a 750 L outdoor culture tanks for 30 days.

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