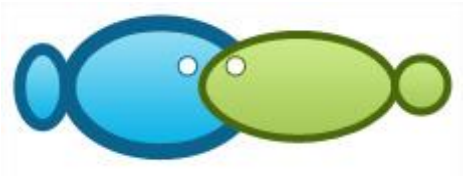

Enrichment of nutrition of *Brachionus* spp. in the tropical areas

Alfian F. Pratama, Endang D. Masithah, Widya P. Lokapirnasari

Department of Fisheries and Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Indonesia. Corresponding author: E. D. Masithah, endangdewimashitah@yahoo.com

Abstract. Larvae feeds incorporating *Brachionus* spp. play a great role in fish cultivation. However, the development and nutritional content of *Brachionus* spp. depends on the nutrient enrichment provided. This study aims to determine the best nutrient enrichment for *Brachionus* spp. cultivated in the tropical areas. This research applied an experimental method with a completely randomized design (CRD) with 5 treatments and 4 replications. The data was analyzed with ANOVA and Duncan's multiple range test. The five nutrient enrichments used are as follows: *Chaetoceros* spp., *Tetraselmis* spp., yeast, *Chaetoceros* spp. + yeast, *Tetraselmis* spp. + yeast. From the treatment groups, the nutrient enrichment using *Chaetoceros* spp. resulted in the highest population, producing 97 ind mL^{-1}. The treatment group with the nutrient enrichment using *Chaetoceros* spp. + yeast presents the highest protein content, of 14.52%. The best nutrition for *Brachionus* spp. cultivated in the tropics are feeds made of *Chaetoceros* spp., according to the results.
Key Words: *Brachionus* spp., *Chaetoceros* spp. *Tetraselmis* spp., yeast.

Introduction. Natural feed is a basic requirement in aquaculture of both fish and shrimp. The availability of seeds for cultivation is influenced by the availability of feed at the seed stage. However, in some cases, the natural feed is not sufficient for the initial stages of the fish larvae. This requires farmers to continuously find innovations, so that the availability of natural feed for seeds or substitutes becomes more adequate both in terms of quantity and in terms of quality.

Brachionus spp. is a natural feed from the rotifer group that is often used in aquaculture. *Brachionus* sp. has several advantages as a natural fish feed, including its small size, slow swimming, easy breeding, and high nutritional value, all helping the feeding of fish (Sartika et al 2013). However, the production of nutritious rotifers can depend on the production of microalgae or phytoplankton they feed on (Lubzens et al 2001).

Brachionus spp. has 26-30% crude protein and 9-28% crude fat (Lubzens & Zmora 2003). In an experiment conducted by Xu & Pan (2014) on white shrimp (*Litopenaeus vannamei*), it was found that the optimal protein diet rate for shrimp growth performance and cost efficiency was 32.9%. Zang et al (2013) showed that an optimum dietary fat content for *L. vannamei* is around 10-12% for optimum growth performances. This shows that *Brachionus* spp. requires feed enrichment to increase the nutritional content, especially protein.

Sometimes the availability of *Brachionus* spp. is in lower numbers, and it is necessary to find alternatives to natural feed. There are few alternative feeds that can replace *Brachionus* sp. as the initial feed for larvae (Hagiwara et al 2001; Yoshimatsu & Hossain 2014), since it is a great source of nutrients and improves growth (Andriyono et al 2015). In some cases, *Brachionus* spp. requires enrichment to increase its nutritional content for larvae feed. There are various kinds of emulsion used for *Brachionus* spp. enrichment, which generally contain fatty acids. Fat is a high-energy component in fish feeds, while protein is needed for growth. However, the energy for metabolic processes comes from fat and carbohydrates. Protein is an organic compound with a high molecular

weight. It is composed of C, H, O, and N, as well as other elements, such as P and S, forming amino acid units (Sorgeloos et al 2001).

The enrichment increases nutrient levels from natural feeds to approach or even reach the nutritional needs of aquaculture species. Enrichment with microalgae can increase the nutritional content of rotifers. Some types of microalgae are often used as food for *Brachionus* spp., such as *Tetraselmis* sp., *Nannochloropsis* sp., *Chaetoceros* sp., *Rhodomonas* sp., and *Isochrysis* sp. (Dhert et al 2001; Wikfors & Ohno 2001).

This rotifer can present better growth if administered the suitable feed for its development. Therefore, this research was conducted to determine a nutrition enrichment that can provide a higher protein content for the population growth of *Brachionus* spp. cultivated in the tropics.

Material and Method

Research design and samples. This research was conducted at the Brackish Water Aquaculture Center, Situbondo (BPAP Situbondo), East Java, Indonesia, from October to November 2017. The study was conducted using an experimental method to determine the effect of administrating *Tetraselmis* spp., *Chaetoceros* spp., yeast, yeast in combination with *Tetraselmis* spp., as well as yeast in combination with *Chaetoceros* spp. to a population of *Brachionus* spp. The research applied a completely randomized design (CRD), where all the experimental units were in the same conditions with different treatments (Montgomery 2001). Furthermore, the population density of *Brachionus* spp. was observed in each treatment.

The research materials used were yeast, seawater, chlorine, formalin, sodium thiosulfate, lights, Guillard fertilizer, Walne fertilizer, *Chaetoceros* spp., *Tetraselmis* spp. and *Brachionus* spp. This study used five treatments and four replications. *Brachionus* spp. seed starters were cultivated in containers with a volume of 300 mL with a density of 10 ind mL^{-1}.

Tetraselmis spp. was provided in a density of $1x10^6$ cells mL^{-1} and *Chaetoceros* spp. was provided in a density of $3x10^6$ cell mL^{-1} (Sutomo 2007). The dosage of yeast in the *Brachionus* spp. culture is equivalent to the administration of *Tetraselmis* spp. and *Chaetoceros* spp. The treatments applied in this study are:
Treatment A: *Chaetoceros* spp. with a density of $3x10^6$ cell mL^{-1};
Treatment B: *Tetraselmis* spp. with a density of $1x10^6$ cell mL^{-1};
Treatment C: yeast (0.002 g);
Treatment D: *Chaetoceros* spp. with a density of $1.5x10^6$ cell mL^{-1} and yeast (0.001 g);
Treatment E: *Tetraselmis* spp. with a density of $5x10^5$ cell mL^{-1} and yeast (0.001 g).

Experimental diagram. The placement pattern of the treatment containers was carried out randomly, as presented in Figure 1.

A1	B1	E4	C2	C4
D1	E1	C1	B4	A2
B2	D4	A3	E3	D2
C3	A4	B3	D3	E2

Figure 1. The placement of containers with different treatments.

Parameter tests. The main parameters monitored in this study are population growth and the protein content of *Brachionus* spp. The observations were conducted every day for 12 days. Population growth was calculated using a Sedgewick Rafter counting chamber with a microscope (100X), and a hand tally counter. Samples of *Brachionus* spp. were collected to analyze the protein levels using the Kjeldahl method at the Situbondo BPBAP Nutrition Laboratory.

The supporting parameters in this study are temperature, salinity, ammonia levels, and dissolved oxygen (DO). Temperature was measured using a thermometer, pH was measured using a pH meter, salinity was determined with a refractometer, the DO was measured using a DO meter, and ammonia levels were determined using an ammonia test kit. Temperature measurements were conducted twice a day, while salinity and pH measurements were carried out once a day. The measurements of DO and ammonia were carried out at the beginning and at the end of the experiment. Supporting parameters were used to complete the main parameter data.

Statistical analysis. Data from the results of this study were analyzed using ANOVA (Hestianah et al 2014). The data was analyzed through the SPSS version 16.0 software. When the results showed differences, further testing was conducted using Duncan's multiple range test to determine differences (Alamsjah 2010).

Results and Discussion

The growth of Brachionus spp. A population increase of *Brachionus* spp*.* was observed every day for 12 days. The results of the observations are presented in the form of densities of *Brachionus* spp*.* (Figure 2). The results of the ANOVA analysis are presented in Table 1. They show that different feeds significantly affected the population density of *Brachionus* spp. Each treatment had an influence on the growth of *Brachionus* spp.

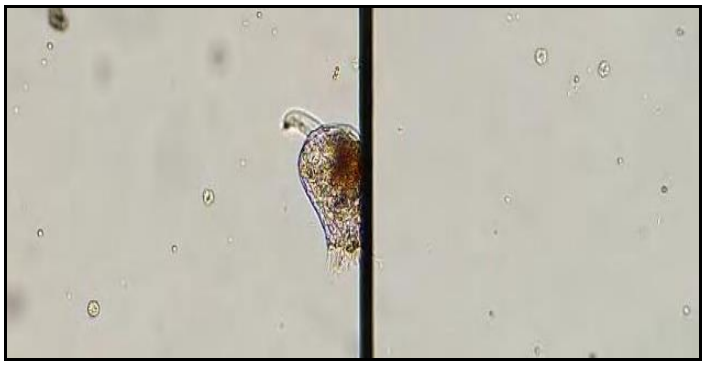

Figure 2. *Brachionus* spp (100X)*.*

Table 1

Brachionus spp*.* population growth results (ind mL^{-1})

Observation day	*Brachionus spp. (ind mL^{-1}) population numbers*				
	A	*B*	*C*	*D*	*E*
0	10.00	10.00	10.00	10.00	10.00
1	7.25^{b} ±1.89	12.50^{a}±2.38	7.50^{b}±0.57	12.25^{a}±1.70	9.75^{ab}±2.06
2	10.00^{a}±1.63	12.25^{a}±2.06	9.25^{a}±1.70	12.00^{a}±2.16	11.75^{a}±2.36
3	18.50^{a}±2.38	21.50^{a}±2.51	12.50^{b}±2.88	20.25^{a}±2.16	22.00^{a}±3.30
4	22.00^{ab}±3.46	23.75^{ab}±3.30	20.00^{c}±0.81	26.50^{a}±1.91	22.75ab±3.82
5	37.50^{a}±4.12	30.25^{b}±1.25	16.25^{d}±2.08	32.00^{b}±5.09	22.25^{c}±3.86
6	97.00^{a}±8.04	43.75^{c}±3.40	14.25^{d}±1.50	75.00^{b}±4.54	43.50^{c}±4.65
7	67.25^{a}±3.30	32.25^{c}±3.59	11.50^{d}±2.64	52.50^{b}±6.24	28.50^{c}±3.41
8	32.50^{a}±4.93	20.50^{c}±1.73	10.00^{d}±0.81	26.75^{b}±3.20	19.00^{c}±1.41
9	26.50^{a}±5.91	15.75^{b}±1.50	7.75^{c}±2.21	19.00^{b}±2.58	15.00^{b}±2.58
10	13.25^{a}±2.21	10.00^{b}±0.81	6.50^{c}±1.29	11.00^{b}±0.95	10.25^{b}±1.41
11	10.00^{a}±1.41	8.75^{b}±0.95	5.00^{c}±0.81	10.00^{b}±1.82	9.00^{b}±1.41
12	12.25^{a}±1.70	8.50^{b}±2.38	2.75^{c}±0.95	9.00^{c}±1.73	6.50^{b}±1.41

Note: Different superscript letters in the same column show significant differences ($P<0.05$). Treatment A - *Chaetoceros* spp. with a density of $3x10^{6}$ cell mL^{-1}; treatment B - *Tetraselmis* spp. with a density of $1x10^{6}$ cell mL^{-1}; treatment C - yeast (0.002 g); treatment D - *Chaetoceros* spp. with a density of $1.5x10^{6}$ cell mL^{-1} and yeast (0.001 g); treatment E - *Tetraselmis* spp. with a density of $5x10^{5}$ cell mL^{-1} and yeast (0.001 g).

Treatment A produces the highest population on day 6, with 97.00±8.04 ind mL^{-1}. Treatment B also produced the highest population on the 6th day, with 43.75±3.40 ind mL^{-1}. Treatment C presented the highest population on day 4, with 20.00±0.81 ind mL^{-1}, while treatments D and E presented highest densities in day 6, with 75.00±4.54 ind mL^{-1} and 43.50±4.65 ind mL^{-1}, respectively. The results from treatment A and D were significantly different from those of treatment C, but not significantly different from those of treatments B and E. The population growth from treatments B and E were not significantly different from that of treatment C.

The population growth patterns of *Brachionus* spp*.* (Table 1) presents an adaptation phase, a logarithmic growth phase and a death phase. The time needed to achieve the optimal rotifer population growth also varies for each type of feed used. In contrast to treatments A, B, D and E, which undergo an adaptation phase from the first day to the 4th day, C treatment undergoes an adaptation phase from the first day to the 3rd day. On the 4th day, treatment C experienced a growth peak phase, which decreased until the 12th day. Treatments A, B, D and E begin to enter the logarithmic growth phase on day 5 and reach the peak of the population on day 6, experiencing a phase of decline from day 7 to 12. The number of *Brachionus* spp*.* in each treatment increased until it reached its peak on the sixth day, excepting treatment C, which experienced a peak on the fourth day. This is presumably due to the inedible yeast C that caused a decline of environmental conditions and interfered with the maintenance process.

As in other studies, the increase in plankton population is visible in each day. *Brachionus* spp*.* fed with *Tetraselmis* spp. and *Chaetoceros* spp. developed proportional quality and an ever-increasing amount of nutrients (Ortega-Salas & Reyes-Bustamante 2013).

The highest average increase of *Brachionus* spp*.* population can be found in treatment A, with *Chaetoceros* spp., probably due to the fact that it is easily digestible. Biologically, *Chaetoceros* spp. is included in the class of diatoms that live in marine waters. Its exterior is covered by a shell from silicates with irregular geometric shapes (Hourmant et al 2009). Diatomic plankton is easily digested by zooplankton or fish (Sutomo 2007). *Chaetoceros* sp. is a diatomic plankton group containing β-carotene, thus being suitable for fish cultivation (Helm & Bourne 2004).

Treatment D shows an increase in the *Brachionus* spp*.* population that reached the peak of population on the sixth day, with a density of 75 ind mL^{-1}. The combination of microalgae and yeast can provide a sufficient population increase, good protein content, being also easily digested by the zooplankton group. These make *Chaetoceros* spp. a suitable feed for *Brachionus* spp*.* in combination with yeast. Combinations of microalgae and yeast have a positive effect on nutritional value, and can increase the growth and survival rate of rotifers (Sahandi & Jafaryan 2011). *Chaetoceros* spp. has good visibility, large size, and low ciliary contamination (Nhu 2004), being suitable as feed for rotifers.

The results of treatment B and treatment E indicate that significant differences are absent between the two treatments. The growth of *Brachionus* spp*.* with *Tetraselmis* spp. experienced a significant increase due to the density of food produced. This result is in line with the results of Rahman et al (2018), which measure rotifer growth rate (*Brachionus* spp.) fed different microalgae, such as *Nannochloris* sp., *Tetraselmis* sp., *Isochrysis* sp., *Chlorella* sp., and *Nannochloropsis* sp. in a density of 0.1×10^6 cells mL^{-1}). *Tetraselmis* sp. produced the highest growth rate value compared to other microalgae ($p < 0.05$), followed by *Tetraselmis* sp., *Isochrysis* sp., *Chlorella* sp., *Nannochloris* sp., and *Nannochloropsis* sp., with 1.40, 0.5, 0.24, and 0.1 cell mL^{-1}, respectively. However, their performance is still less productive compared to that of *Chaetoceros* spp. in regards to the cultivation of *Brachionus plicatilis* in the tropical areas.

Protein content of Brachionus spp. After finding the best nutrient enrichment that can increase the population of *Brachionus* spp. in a short period of time, the following step was to test the protein content of *Brachionus* spp. The crude protein content from *Brachionus* spp*.* is presented in Table 2.

The crude protein content of *Brachionus* spp*.* in treatment C is the lowest (5.13%) among the treatments. Thus, the sole administration of yeast did not work optimally in producing rotifer growth. The use of yeast in various densities results in similar

population growth. A combination of yeast and *Chlorella* sp. resulted in a maximum population increase of 25 ind mL^{-1}, which is considered a slow production (Khatun et al 2014). *Brachionus* spp. fed with yeast are unstable, have low nutritional value, and do not support high productions. Chilmawati & Suminto (2010) also state that yeast without the addition of supplements lacks the nutrients for the population growth of *B. plicatilis.*

Table 2

Crude protein levels of *Brachionus* spp. in different treatments

No	*Treatment*	*Crude protein content (%)*
1	A (*Chaetoceros* spp.)	11.15
2	B (*Tetraselmis* spp.)	5.81
3	C (*Saccharomyces* spp.)	5.13
4	D (*Chaetoceros* spp. + *Saccharomyces* spp.)	14.52
5	E (*Tetraselmis* spp. + *Saccharomyces* spp.)	10.29

Water quality. The water quality parameters are presented in Table 3. Water quality parameters during the study were still in a threshold suitable for the life of microalgae and *Brachionus* spp., except in treatment C, where ammonia was at the upper limit for *Brachionus* spp. life, which is 1 mg L^{-1}. Ammonia values during the study ranged from 0.003-1 mg L^{-1}. According to Fulks & Main (1991), the ammonia value in *Brachionus* spp culture should not exceed 1 mg L^{-1}. The high value of ammonia in treatment C is thought to be caused by dead and decayed yeast.

Table 3

Water quality during the eperiment

Water quality parameters	*Value range*				
	A	*B*	*C*	*D*	*E*
Temperature (^{O}C)	24.5-29.5	25.0-29.5	25-30	24.5-30	25.0-29.5
pH	7.4-8.2	7.5-8.2	7.5-8.2	7.5-8.2	7.5-8.2
Salinity (ppt)	29-32	28-32	28.0-30.0	29-32	28-32
Ammonia (mg L^{-1})	0.003-0.5	0.003-0.5	0.003-1.0	0.003-0.5	0.003-0.5
DO (ppm)	3.9-6.5	3.9-6.3	3.8-6.4	3.8-6.4	3.9-6.6

Note: DO - dissolved oxygen. Treatment A - *Chaetoceros* spp. with a density of 3x106 cell mL^{-1}; treatment B - *Tetraselmis* spp. with a density of 1x106 cell mL^{-1}; treatment C - yeast (0.002 g); treatment D - *Chaetoceros* spp. with a density of 1.5x106 cell mL^{-1} and yeast (0.001 g); treatment E - *Tetraselmis* spp. with a density of 5x105 cell mL^{-1} and yeast (0.001 g).

Water quality during the study in all treatments was between the limits suitable for the life of *Brachionus* spp. and microalgae. Water temperature values during the study ranged between 24.5-30^{O}C. According to Fukusho & Okauchi (1982), the optimum temperature for *Brachionus* spp. is between 25-35^{O}C. Values of pH during the study ranged from 7.5 to 8.5. According to Fulks & Main (1991), the pH values that can be tolerated by *Brachionus* spp. are between 5 and 9. Water salinity during the study ranged from 28-32 ppt. Dissolved oxygen content rangeed from 3.9 to 6.6 ppm. According to Effendi (2003), the DO level should be above 5 ppm.

Conclusions. The provision of different feeds and their combinations can increase population growth and crude protein content of *Brachionus* spp. For *Brachionus* spp. cultivated in the tropics, *Chaetoceros* spp. is the best feed out out the tested ingredients, because it contributes to the largest population growth, from a density of 10 ind mL^{-1} to 97 ind mL^{-1} or 970% on the sixth day. The highest crude protein content of *Brachionus* spp. (14.52%) was obtained in treatment D, with a combination of *Chaetoceros* spp. and yeast.

Acknowledgements. We are grateful to the Fisheries and Marine Faculty for providing facilities and support. We thank the staff members for the participation during the research at the Brackish Water Aquaculture Center, Situbondo (BPAP Situbondo), East Java.

References

Alamsjah M. A., 2010 Producing new variety of *Gracilaria* sp. through cross breeding. Research Journal of Fisheries and Hydrobiology 5(2):159-167.

Andriyono S., Masithah E., Winarni D., 2015 The study of sea cucumber (*Phyllophorus* sp.) gonads histology: thermal shock to the spawning process. Journal of Natural Sciences Research 5(11):101-105.

Chilmawati D., Suminto S., 2010 Effect of bakers yeast, vitamin B12, and vitamin C as nutritional improvement of food on the density production of *Brachionus plicatilis*. Saintek Perikanan: Indonesian Journal of Fisheries Science and Technology 5(2):47-53.

Dhert P., Rombaut G., Suantika G., Sorgeloos P., 2001 Advancement of rotifer culture and manipulation techniques in Europe. Aquaculture 200(1-2):129-146.

Effendi H., 2003 [Study of water quality for the management of resources and the aquatic environment]. Kanisius, Yogyakarta, 173 p. [In Indonesian].

Fukusho K., Okauchi M., 1982 Strain and size of the rotifer, *Brachionus plicatilis* being cultured in Southeast Asian countries. Bulletin of National Research Institute of Aquaculture 3:107-109.

Fulks W., Main K. L., 1991 Rotifer and microalgae cultur systems. Proceeding of a U.S.-Asia workshop. The oceanic institute, Honolulu, Hawaii, 364 p.

Hagiwara A., Gallardo W. G., Assavaaree M., Kotani T., de Araujo A. B., 2001 Live food production in Japan: recent progress and future aspects. Aquaculture 200(1-2):111-127.

Helm M. M., Bourne N., 2004 Hatchery culture of bivalves: a practical manual. Lovatelli A. (ed), FAO Fisheries Technical Paper 471, Rome, 177 p.

Hestianah E. P., Kusumawati I., Suwanti L. T., 2014 Toxic compounds of *Curcuma aeruginosa* causes necrosis of mice hepatocytes. Universa Medicina 33(2):118-125.

Hinkelmann K., Hinkelmann K., Kempthorne O., 2007 Completely Randomized Design. In: Design and Analysis of Experiments, Volume 1 - Introduction to Experimental Design. University of Virginia, pp. 153-212.

Hourmant A., Amara A., Pouline P., Durand G., Arzul G., Quiniou F., 2009 Effect of bentazon on growth and physiological responses of marine diatom: *Chaetoceros gracilis*. Toxicology Mechanisms and Methods 19(2):109-115.

Khatun B., Rahman R., Rahman M. S., 2014 Evaluation of yeast *Saccharomyces cerevisiae* and algae *Chlorella vulgaris* as diet for rotifer *Brachionus calyciflorus*. The Agriculturists 12(1):1-9.

Lubzens E., Zmora O., 2003 Production and nutritional value of rotifers. In: Live feeds in marine aquaculture. Stottrup J., McEvoy L. (eds), Blackwell Science, Oxford, pp. 300-303.

Lubzens E., Zmora O., Barr Y., 2001 Biotechnology and aquaculture of rotifers. Hydrobiologia 446:337-353.

Montgomery D. C., 2001 Design and analysis of experiments. 5th edition. John Wiley & Sons, New York, 684 p.

Nhu C. V., 2004 A comparison of yield and quality of the rotifer (*Brachionus plicatili*s - L. strain) fed different diets under aquaculture conditions, Vietnam. Asian Fisheries Science 17(2004):357-363.

Ortega-Salas A., Reyes-Bustamante H., 2013 Cultivation of the microalgae *Chaetoceros gracilis* to feed the rotifer *Brachionus Plicatilis*. UNED Research Journal 5(2):189-193.

Rahman A. R. A., Cob Z. C., Jamari Z., Mohamed A. M., Toda T., Ross O. H., 2018 The effects of microalgae as live food for Brachionus *plicatilis* (rotifer) in intensive culture system. Tropical Life Sciences Research 29(1):127-138.

Sahandi J., Jafaryan H., 2011 Rotifer (*Brachionus plicatilis*) culture in batch system with suspension of algae (*Nannochloropsis oculata*) and bakery yeast (*Saccharomyces cerevisiae*). AAVL Bioflux 4(4):526-529.
Sartika I. D., Mohaemin M., Maharani H. W., 2013 [The content of total protein (crude protein) *Brachionus plicatilis* with *Nannochloropsis* sp. in micro environmental stress condition]. e-Journal Rekayasa dan Teknologi Budidaya Perairan 2(1), 6 p. [In Indonesian].
Sorgeloos P., Dhert P., Candreva P., 2001 Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. Aquaculture 200(1–2):147-159.
Sutomo S., 2007 [Effect of microalgae feed on the growth of the harpacticoid kopepod population, *Tigriopus brevicornis* isolated from Marunda waters, North Jakarta]. Indonesian Journal of Marine Sciences 12(4):219-226. [In Indonesian].
Wikfors G. H., Ohno M., 2001 Impact of algal research in aquaculture. Journal of Phycology 37(6):968-974.
Xu W. J., Pan L. Q., 2014 Evaluation of dietary protein level on selected parameters of immune and antioxidant systems, and growth performance of juvenile *Litopenaeus vannamei* reared in zero-water exchange biofloc-based culture tanks. Aquaculture 426-427:181-188.
Yoshimatsu T, Hossain M. A., 2014 Recent advances in the high-density rotifer culture in Japan. Aquaculture International 22(5):1587-1603.
Zhang S. P., Li J. F., Wu X. C., Zhong W. J., Xian J. A., Liao S. A., Miao Y. T., Wang A. L., 2013 Effects of different dietary lipid level on the growth, survival and immune-relating genes expression in Pacific white shrimp, *Litopenaeus vannamei*. Fish & Shellfish Immunology 34(5):1131-1138.

Received: 29 August 2019. Accepted: 01 September 2019. Published online: 11 May 2020.
Authors:
Alfian Fajar Pratama, Department of Fisheries and Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Jl. Airlangga No. 4-6, 60115 Surabaya, East Java, Indonesia, e-mail: fajaralfian363@gmail.com
Endang Dewi Masithah, Department of Fisheries and Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Jl. Airlangga No. 4-6, 60115 Surabaya, East Java, Indonesia, e-mail: endangdewimashitah@yahoo.com
Widya Paramita Lokapirnasari, Department of Fisheries and Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Jl. Airlangga No. 4 - 6, 60115 Surabaya, East Java, Indonesia, e-mail: widyaparamitalokapirnasari@yahoo.com

How to cite this article:
Pratama A. F., Masithah E. D., Lokapirnasari W. P., 2020 Enrichment of nutrition of *Brachionus* spp. in the tropical areas. AACL Bioflux 13(3):1235-1241.