Original Research Article

Effect of different diets on the growth rate of the rotifer, *Brachionus plicatilis* under 40 ppt salinity stress.

ABSTRACT

Aquaculture is important business some fish farms use underground water that may has high salinity. The aim of this study is to evaluate the effect of eight different types of diets on enhancing the growth rate of the rotifer, Brachionus plicatilislives in 40 ppt saline water. The diets used in the study are T1 (Nannochloropsis oculata), T2 (Tetraselmis suecica), T3 (N. oculata + T. suecica), T4 (N. oculata + yeast; Saccharomyces cerevisiae), T5 (T. suecica + yeast), T6 (S. parkle), T7 (S. Parkle + yeast) and T8 (yeast). The results revealed that the rotifer fed for S. parkle showed the highest growth population (818 Indv. /mL) with a highly significant difference ($p < 0.05$). On the other hand, rotifers fed the yeast (S. cerevisiae) showed the lowest population growth (355 Indv. /mL). The outcome of the current study indicates that S. parkle commercial diet is very appropriate for the stock culture of B. plicatilis due to its ability to increase the growth rate of the rotifer in a short time under the current conditions.

Keywords: S.parkle, Yeast, Tetraselmis suecica, Nannochloropsis oculata, Zooplankton, Fish food

1. INTRODUCTION

Zooplankton is the first food for many fish crops due to its effectiveness in increasing the growth rate of fish larvae. It is also considered as an easily digestible protein for fish and shellfish (EL-Kassas *et al.,* 2015). Rotifers as a zooplankter is used as natural food for larvae (Ichthyoplankton) in hatcheries as they are the favorite prey of most fish larvae (Pfejffer and Ludwig, 2007).

One of the vital difficulties faced production of fish fry in hatcheries is the lack of live food for the nutrition of early stages of fish larvae. Therefore, there is no alternative food that could replace the using of rotifers as preliminary food for larvae until now (Hagiwara *et al*., 2001; Yoshimatsu and Hossain 2014). Moreover, the rotifer, *Brachionus plicatilis* is an essential

food type especially in the early larval stages of many fish species due to its small size and its ability to propagate in captivity at high density and reproductive rate (Alam and Shah, 2004). Additionally, it has the ability to stand wide ranges of salinity (Abd Rahman *et al*, 2018).

Furthermore, rotifers need different types of food to survive and be active in the hatcheries. Various species of microalgae have been used as food for rotifers including; *Tetraselmis* spp., *Nannochloropsis* spp., *Chaetoceros* spp., *Rhodomonas* spp. and *Isochrysis* spp. (Dhert *et al*. 2001; Hoff and Snell 2001; and Wikforsand Ohno, 2001). In contrast, baker's yeast, *Saccharomyces cerevisiae* is immediately available compared to the microalgae (fresh, frozen or dried) which are laborious, time-consuming and expensive. Additionally, the yeast, *S. cerevisiae* has an important role to give a high population growth rate for rotifers (Heneash *et al*, 2015). However, *Nannochloropsis* sp. is widely used as live food for rotifers as it provides the predicted yield on time with high density because of its ease of cultivation (Abd Rahman *et al*, 2018).

In Egypt, El-Wafa fish farm is located in the Ismailia governorate next to the Suez Canal and the groundwater well is the source of aquaculture water, the well's salinity is 40 ppt. The farm produces *Dicentrarchus labrax* and *Sparus aurata*. Consequently, during the feeding of the grown fish with natural food (zooplankton), species of zooplankton that tolerate salinities above normal sea water (35-37 ppt). Hence, the present work aims to study the effects of 40 ppt salinity on the rotifer (*B. plicatilis*) growth on different feeding diets and the effect of an artificial food type and initial stocking density on the population growth of rotifers to induce high productivity of *B. plicatilis* to be used as fish food.

2. MATERIAL AND METHODS

The experiment was conducted in El-Wafa fish farm, Ismailia, Egypt. Brachionus plicatilis specimens were obtained from the marine hatchery of the National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt. The rotifers were divided into eight groups each one has three replicates. Then, they were stocked in twenty-four 9- liters plastic jars supplied with continuous aeration. Water parameters were measured daily including water temperature (digital thermometer), water acidity using Jenway pH meter, (Model 350) and salinity using digital salinometer. Two marine green microalgae were tested; *Nannochloropsis oculata* and *Tetraselmis suecica.* Ecch algal species were then combined with the baker's yeast (*Saccharomyces cerevisiae*) and Selco S. parkle; which is yeast-based ready commercial diet for rotifers manufactured by INVE.

Dietary treatments:

Eight diets are (T1-T8), where T1 (*N. oculata*) was considered as a control, T2 (*T. suecica)*, T3 (*N. oculata* + *T. suecica*), T4 (*N. oculata* + yeast; *S. cerevisiae*), T5 (*T. suecica* + yeast), T6 (S.parkle), T7 (S.parkle + yeast) and T8 (yeast).

Comment [mg1]: Why is N. Oculata a witness environment?

Experimental procedure

The experiment started with two litters initial volume containing 150 indv./L initial rotifer density for each replicate in each treatment. They were supplied with the indicated diet (Table 1) twice daily at 09.00 AM and 14.00 PM in 1000 mL saline water (underground water) for 8 days.

Comment [mg2]: 8 days for growth experimentation isn't it a little? why not at least 24 or 30 days?

Table 1**:** The eight diets of feeding diets and their feeding rates/jar/day.

Treatment	Feeding diets	Initial volume of water/Jar 1st day	Feeding rate/Jar/day
T1	*Nannochloropsis oculata*		1000 mL
T2	*Tetraselmis suecica*		1000 mL

T3	*N. oculata* + *T. suecica*	1000 mL*	1000 mL
T4	*N. oculata* + Yeast (*Saccharomyces cerevisiae*)		1000 mL+ 0.1 gm/l
T5	*T. suecica* + Yeast (*S. cerevisiae*)		1000 mL+ 0.1 gm/l
T6	S.parkle		0.1 gm/l * 50 mL
T7	S.parkle + Yeast (*S. cerevisiae*)		0.1 gm/l * 50 mL
T8	Yeast (*S. cerevisiae*)		0.1 gm/l * 50 mL

* Each one mL contains 150 indv. /l

Sampling and counting of *B. plicatilis*

Each zooplankton density in each treatment zooplankterwas determined using a binocular microscope (40X). The density of rotifer organisms was calculated as their total number per cubic meter. The concentrated rotifers were subsampled using a Stempel pipette (Heneash, 2015; El-Damhoghy *et al*., 2016; Aboul Ezz *et al*., 2014; El-Kassas *et al*., 2015).

Population (R)

Along the eight experimental days, the population of rotifer was calculated as the increase in number every 24 hours by counting the individuals (three times) in a 1-mL glass pipette. These concentrated rotifers were sampled with a Pasteur pipette and were counted under the dissecting microscope (Braley, 1994).

Comment [mg3]: Zoolanctonic count was done every 24 hours. Why? Knowing that good zooplanktonic mutilation or reproduction is at least 72H

Population Growth Rate (R^r)

The population growth rate of rotifers (R^r) was calculated according to Okauchi and Fukusho (1984) using the following equation:

$$R^r = \frac{(IN_t - IN_0)}{t}$$

where R^r = the instantaneous growth rate; N_0 = the initial number of rotifers; N_t = the final number of rotifers after t days; t = culture days.

Statistical analysis

Data were analyzed using analysis of variance test (One-way ANOVA) to determine the significant differences among rotifer populations between treatments. Also, Duncan's multiple range test was used to provide more information of ranges of all the pairs of treatments using IBM SPSS Statistics (v22).

Comment [mg4]: Why didn't you apply the homogeneity test before the ANOVA application?

3. RESULTS

Water quality

During the experiment period, the pH value was 8.0 ± 0.2. Water temperature and salinity were 28.0 ± 2.0°C, 40 ± 1.0 ppt, respectively.

Effect of different diets on the growth rates of *B. plicatilis* during the experiment period.

During the eight experiment days, T6 (S.parkle) was the most effective diet which gave the maximum average density of *B. plicatilis*, (818 Indv. /mL). On the other hand, T8 was the least effective diet which contributed the minimum average density represented only by (355 Indv. /mL). Figure (1) shows the impact of the eight experimental diets studied during the experiment period, indicating that the highest average value listed in the ninth day was (935 Indv./mL), while the lowest average value recorded in the first day was (265 Indv./mL). Besides, the aforementioned figure revealed that T6 was the most effective diet which gave a distinct increase in the growth rate of *B. plicatilis.*

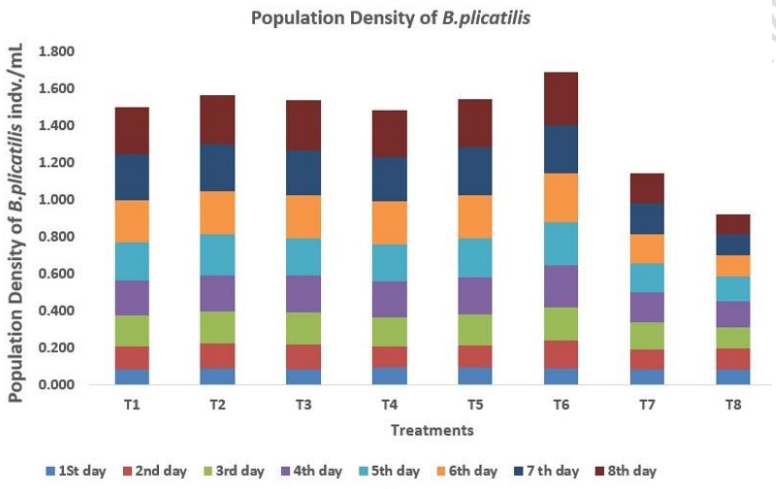

Figure1: Effect of the eight experimental diets during the experiment period.

Furthermore, Table (2) revealed that there were highly significant differences among the eight experimental diets where the significance level of ($F = 2.665$, $p = 0.019$) is less than the threshold value of ($p< 0.05$). In conclusion, not all the group means were equal which revealed that the effects of treatments were not similar in the present experiment.

This leads to the fact that at least one of the groups tested differs from the other. In addition, Duncan's multiple range test was used to determine the critical values for comparisons among means. The test revealed that the treatment pairs T1 and T3, T1 and T4, T1 and T5, T1 and T6, T1 and T7, T1 and T8, T2 and T5, T2 and T6, T2 and T7, T2 and T8, T3 and T6, T3 and T7, T3 and T8, T4 and T7, T4 and T8, T5 and T7, T5 and T8 had mean differences with significant value ($p< 0.05$). The rest pairs of treatments showed a significance level greater than (0.05) which means there were no statistically significant differences between them.

Comment [mg5]: this figure must be accompanied by the anova table

Comment [mg6]: Why don't you just do this text in the table. transforming the text into the table will facilitate the understanding of the work

Table 2: Results of the one-way ANOVA test for the population density of rotifer for the eight experimental diets.

Sources of Variation	Sum of Squares	df	Mean Square	F	*p*-value
Between Groups	0.057	7	0.008	2.665	0.019
Within Groups	0.170	56	0.003		
Total	0.227	63			

The pairwise comparison among treatments showed a highly significant difference in T6 (S.parkle) and T8 (*S. cerevisiae*) where *p*-value= 0.0015 (Figure. 2).

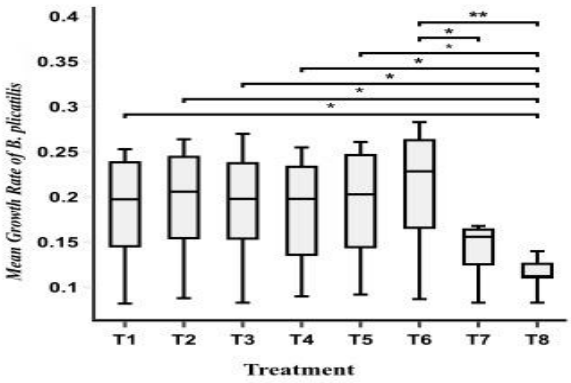

Figure 2: Boxplot provides a pairwise comparison between the experimental treatments (Duncan's multiple range test), the number of asterisks depends upon the *p*-value of particular pair of samples; * for $p < 0.05$ and ** for $p < 0.01$.

Gravid females of *B. plicatilis*

The gravid females of *B. plicatilis* (Indv. /mL) during the Experiment period was shown in Figure (3). Treatment 4 displayed the greatest average number of gravid females, (144 Indv. /mL)which began with (96 Indv. /mL) on the first day of the experiment. In contrast, the lowest average number of the gravid females of the studied rotifers was for T7 (47 Indv. /mL) which started by (43 Indv. /mL). for T6, the average number of gravid females was (57.1875 indv. /mL) which was (46 indv. /mL) at the first day of the experiment.

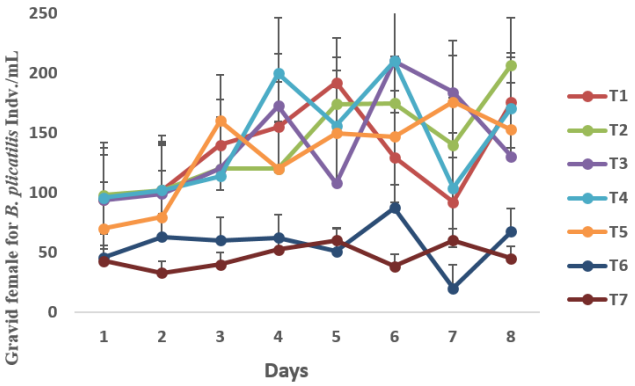

Figure 3: The gravid females of *B. plicatilis* (Indv. /mL) during the experiment period.

Comment [mg7]: why don't you do the same thing for density

Population Density and Egg ratio of *B. plicatilis*

Figure 4. Illustrated the relationship between the population density of the rotifer *B. plicatilis* and the egg ratio in the present study. The population density of the studied rotifers increased exponentially with time for all the treatments and reached a noticeable level after eight days (end of the experiment). The egg ratio decreased gradually over time during the different growth levels.

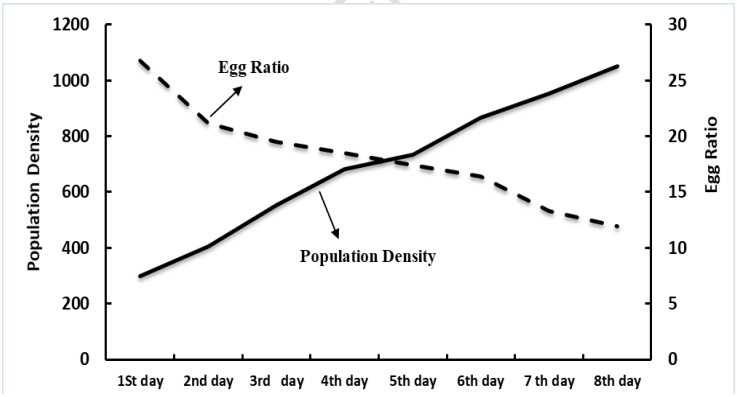

Figure 4:Schematic representation of changes in population density and egg ratio of *B. plicatilis* in relationship to time in the mean diet treatments.

4. DISCUSSION

Comment [mg8]: the discussion of a scientific article does not follow the comparison of these results with other results of literature. It must be noted that the reason (the cause) of this difference should be noted. This brings new information to science in general and biology in particular. It is therefore important to review the discussion in depth in order to enhance these results

Rotifers are cosmopolitan organisms as they are osmoconformers and can be adapted to salinities ranging from 1‰ to 97‰, however, the changes in salinity may cause shock for rotifers affects their production rate (Epp and Winston,1977 and Walker,1981). Increased

salinity may shorten the lifetime and decrease the fecundity of female and growth rate in some rotifer species (Serra *et al*., 1994). where Oltra and Todoli (1997) found negative effect for decreasing salinity on growth rate of the marine rotifer, *Synchaeta cecilia valentine*. Numerous studies established the effect of salinity on growth rate of rotifer, *B. plicatilis* (Bosque *et al*., 2001).; where it can tolerate wide range of salinities (Bayly, 1972) but it has the highest reproductive rate at salinities ranging from 10- 20‰ (Miracle and Serra, 1989); They addedthat the ability of *B. plicatilis* to tolerate wide salinity range is due to its colony origen, where rotifers taken from the coastal marshes stand high salinities more than those taken from brackish water lagoons.

Comment [mg9]: Does the marine rotifers behave in the same way as freshwater?

Comment [mg10]: take off a point

In the present study, the statistical analysis revealed that the eight diets showed different effects through the eight days of the experiment. The commercial diet T6 (S.parkle) Caused gradual increase in rotifer density from the third; where Lind (2014) and Eryalcin, (2018) recorded the highest growth rate mean for S.parkle comparing with the other rotifer diets used in his study. Furthermore, Kostopoulou *et al.* (2012) found positive effect on the growth rate of the rotifer*B. plicatilis*with the dilution rate of S.parkle .

In contrast to the present and aforementioned findings, Qi *et al*. (2009) found an increased growth rate of rotifer cultures fed on *Nannochloropsis* algae than S.Parkle.More importantly, Reitan and Olsen (1994) found that S.parkle diet had promoted more bacteria than the algal diets; they found that yeast diet did not support growth rate of rotefers where the Yeast *S. cerevisiae* was the less effective diet in the current study; this was confirmed by the results of Planas and Estevez (1989). In contrast, (Khatun *et al*, 2014) reported that yeast is more effective than *Chlorella* powder as combined food with fresh *Chlorella*. Sarma *et al*. (2001) stated that the yeast was not appropriate for the rotifer cultivation but the mixture of yeast and fresh *Chlorella* could be more effective on the culture of the rotifer *B. calyciflorus* and better than the other mixtures (Ashraf *et al*., 2010; Pena-Aguado *et al*., 2005).

The egg ratio was determined in the present study in order to estimate the growth rate of*B. plicatilis*. Edmondson (1965) and Paloheimo (1974) reported that the egg ratio was used to evaluate the fecundity and the mortality rates for deriving the population growth rates. Ohman *et al*. (1996) and Razlutskij (2000) had introduced a very useful bases for constant improvement in the use of the egg ratio method for zooplankton. There is usually an inverse, linear or curvilinear relationship between the density of population and the egg ratio. Duncan (1984), Sarma and Rao (1991) recorded that the planktonic rotifer populations continue to produce between 3 and 5 eggs per female per day at low population densities when there are plenty of resources.

5. CONCLUSION

The present study concluded that the commercial diet S.parkle was the most effective diet among the experimented diets in the current study, because it showed the highest growth rate compared with the other rotifer diets.

REFERENCES

1. Aboul Ezz, Sawsan M., Heneash, Ahmed M.M., Gharib, Samiha M. Variability of spatial and temporal distribution of zooplankton communities at Matrouh beaches, south-eastern Mediterranean Sea, Egypt. *Egyptian Journal of Aquatic Research, V. 40, Issue 3, P. 283–290,2014.*
2. Alam, M.J. and Shah, M.M.R. Growth and reproductive performance of locally isolated brackish water rotifer (*Brachionus plicatilis*) feeding different micro algae. *Bangladesh, Fish. Res., 8(2), 127-133, 2004.*
3. Ashraf, M., Ullah, S., Rashid, T., Ayub, M., Bhatti, E.M., Naqvi, S.A. and Javaid, M. Optimization of Indoor Production of Fresh Water Rotifer, *Brachionus calyciflorus*, b: Feeding Studies. *Pakistan Journal of Nutrition, 9 (6): 582-588,2010.*
4. Bayly, I.A.E. Salinity tolerance and osmotic behavior of animals in athalassic saline and marine waters.*Annu.Rev. Ecol. Syst. 3, 233–268,1972.*
5. Bosque, T., Hernández, R. Pérez, R., Oltra, Todolı´, R.Effects of salinity, temperature and food level on the demographic characteristics of the seawater rotifer *Synchaeta littoralis* Rousselet.*Journal of Experimental Marine Biology and Ecology,2001, 258. 55–64.*
6. Duncan, A. Factors influencing the composition, body size and turnover rate of zooplankton in Parakarama Samudra, an irrigation reservoir in Sri Lanka. *Hydrobiologia 113: 210–215,1984.*
7. Edmondson, W. T. Reproductive rate of planktonic rotifers as related to food and temperature. *Ecological Monographs 35: 61–111,1965.*
8. El-Damhoghy, K. A.; Heneash, A. M. M.; Nasef, A. and Khater, M. M.Diversity and distribution of *Brachionus* community (Rotifera: Brachionidae) at lake Maryout, Alexandria, Egypt. *International Journal of Fisheries and Aquatic Studies 4(5) 500-506,2016.*
9. El-Kassas, H. Y., Heneash, A. M.M. and Hussein, N. R. Cultivation of *Arthrospira (Spirulina) platensis* using confectionary wastes for aquaculture feeding. *Journal of Genetic Engineering and Biotechnology,2015 (ISSN1687-157X) 13, 145–155.*
10. Epp, R. W., Winston, P. W.Osmotic Regulation in the Brackish-Water Rotifer *Brachionus Plicatilis* (Muller).*Journal of Experimental Biology 68: 151-156,1977.*
11. Eryalcin, Kamil Mert. Effects of Different Commercial Feeds and Enrichments on Biochemical Composition and Fatty Acid Profile of Rotifer (*Brachionus Plicatilis*, Müller, 1786 and *Artemia Franciscana.Turkish Journal of Fisheries and Aquatic Sciences 18: 81-90, 2018.*
12. Hagiwara A, Gallardo W G, Assavaaree M, Kotani T and de Araujo A B.Live food production in Japan: recent progress and future aspects. *Aquaculture 200 (2-1): 117-127.117–127. 00696-2,*2001.

13. Heneash, A. M.M., Ashour, M. and Matar, M. Effect of un-live microalgal diet, *Nannochloropsis oculata* and *Arthrospira (Spirulina) platensis*, comparing to yeast, on Population and Growth rate of *Brachionus plicatilis*. *Mediterranean Aquaculture Journal (ISSN: 1687- 7617) 2015, Vol. 7; 63 – 70.*
14. Khatun, B., Rahman, R. and Rahman, M. S. Evaluation of Yeast *Saccharomyces cerevisiae* and Algae *Chlorella vulgaris* as Diet for Rotifer *Brachionus calyciflorus.A Scientific Journal of Krishi Foundation, The Agriculturists 12(1): 1-9, 2104.*
15. Kostopoulou, Venetia, Vasilakis, Manolis and Divanach, Pascal. Semi-continuous mass culture of rotifers (*Brachionus plicatilis*) using an automatic feeder. *Aquaculture Research, 43, 91–98,2012.*
16. Lind, Ryan D.Effects of Selected Commercial Diets and Yeast Substitution on the Growth and Associated Microbiota of Rotifer (*Brachionus plicatilis*), 2014.
17. Miracle, M.R., Serra, M.Salinity and temperature influence in rotifer life history characteristics.*Hydrobiologia 186r187, 81–102,1989.*
18. Ohman, M. D., D. L. Aksnes and J. A. Runge. The interrelationship of copepod fecundity and mortality. Limnology and Oceanography 41: 1470–1477,1996.
19. Ohse, Silvana, Derner, Roberto Bianchini, Ozório, Renata Ávila, Corrêa, Rafaela Gordo, Furlong, Eliana Badiale, Cunha, Paulo Cesar Roberto. Lipid content and fatty acid profiles in ten species of microalgae. *V. 33, Nº 1. Páginas 93-101 IDESIA (Chile) 2015 / Enero-Febrero.*
20. Okauchi M. and Fukusho K. Environmental conditions and medium required for mass culture of a minute algae,*Tetraselmis tetrathele* (Prasiniphyceae). *Bulletin of the National Resource Institute of Aquaculture 5,1-11,*1984.
21. Oltra, R., Todoli´, R.Effects of temperature, salinity and food level on the life history traits of the marine rotifer *Synchaeta ceciliaValentina*, n. *subsp. J. Plankton Res. 19, 693–702, 1997.*
22. Paloheimo, J. Calculation of instantaneous birth rate. *Limnology and Oceanography 19: 692–694*, 1974.
23. Pena-Aguado, F., Nandini, S. and Sarma, S.S.S. Differences in population growth of rotifers and Cladoceras raised on algal diets supplemented with yeast. *Limnologica-Ecology and Management of inland Waters, 35(4): 298-303, 2005.*
24. Pfejffer, T. J. and Ludwig, G. M. Small-Scale System for the Mass Production of Rotifers Using Algal Paste. *North American Journal of Aquaculture 69:239-243, 2007, Anrerican Fisheries Socie*l*).*
25. Planas, M. and Estevez, A.Effects of diet on population development of the rotifer *Brachionus plicatilis* in culture. *Helgolander Meeresunters. 43, 171-181,*1989.
26. Qi, Z., Dierckens, K., Defoirdt, T., Sorgeloos, P., Boon, N., Bao, Z., and Bossier, P.Effects of feeding regime and probionts on the diverting microbial communities in rotifer *Brachionus* culture. *Aquaculture International, 17(4), 303-315, 2009.*

27. Rahman, ARA, Cob, ZC, Jamari, Z, Mohamed, AM, Toda T, Ross OH.The Effects of Microalgae as Live Food for *Brachionus plicatilis* (Rotifer) in Intensive Culture System. *Tropical Life Sciences Research, 29(1), 127–138, 2018.*
28. Razlutskij, V. I.Estimating cladoceran birth rate: use of the egg age distribution to estimate mortality of ovigerous females and eggs. *Hydrobiologia 428: 135–144, 2000.*
29. Reitan, K., Olsen, Y. Comparison of rotifer culture quality with yeast plus oil and algal-based cultivation diets. *Aquaculture International 2, no. 4: 225–238,*1994
30. Sarma, S. S. S. and Rao, T. R. The combined effects of food and temperature on the life history parameters of *Brachionus patulus* Muller (Rotifera). *Internationale Revue der gesamten Hydrobiologie 76: 225–239,1991.*
31. Sarma, S.S.S., Jurado, P.S.L. and Nandini, S., 2001. Effect of three food types on the population growth of *Brachionus calyciflorus* and *Brachionus patulus* (Rotifera: Brachionidae). *Revista de Biología Tropical, 49(1): 77-84.*
32. Serra, M., Carmona, M.J., Miracle, M.R.Survival analysis of three clones of *Brachionus plicatilis* (Rotifera). *Hydrobiologia 227, 97–105,*1994.
33. Walker, K.F.A synopsis of ecological information on the saline lake rotifer *Brachionus plicatilis* Müller 1786. *Hydrobiologia 81, 159–167,*1981.
34. Yoshimatsu, T and Hossain M A. Recent advances in high-density rotifer culture in Japan. *Aquaculture International 22(5):1587–1603. s10499-014-9767-5,*2014.