Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(5): 1085 – 1094 (2025)
www.ejabf.journals.ekb.eg

Exploring the Synergistic Effect of Feeding Rates and Feeding Frequency on the Population Growth of *Brachionus plicatilis*

Rizal B. Ismail[1], Sitti R. M. Shaleh[1]*, Norfazreena M. Faudzi[1], Faihanna C. Abdullah[1], Najamuddin A. Basri[1], Kenneth Tze Kin Teo[2]

[1] Higher Institution Center of Excellence (HICoE), Borneo Marine Research Institute, University Malaysia Sabah, 88400, Kota Kinabalu, Sabah, Malaysia

[2]Department of Electrical and Electronic, Faculty of Engineering, University Malaysia Sabah, 88400, Kota Kinabalu, Sabah, Malaysia

*Corresponding Author: sittirae@ums.edu.my

ARTICLE INFO
Article History:
Received: July 2, 2024
Accepted: Sep. 10, 2025
Online: Sep. 22, 2025

Keywords:
Hatchery,
Aquaculture,
Rotifer,
Live feed,
Population growth

ABSTRACT

Rotifers play a crucial role in the aquaculture industry, particularly in the early stages of marine fish and crustacean larval rearing. This study aimed to identify the optimal feeding rates and frequencies for rotifers (*Brachionus plicatilis*), which are essential live feed in aquaculture. *B. plicatilis* (L- strain, mean lorica length 195.5 µm) was used in this experiment. The experiment consisted of 9 different combination treatments of feeding rates and feeding frequencies. 4- liter plastic jars were used for a 5-day experiment. An automated feeder was used to regulate the feeding rates and frequencies. Water temperature was set at 30°C using aquarium heaters. Commercial concentrated *Nannochloropsis* sp. was used as feed throughout the experiment. The highest rotifer density, 378±12 rotifers/mL, was observed with a feeding rate of 200,000 cells/rotifer and a feeding frequency of 6 times per day (GR=1.18±0.006 day^{-1}). This was followed by a rotifer density of 292±9.54 rotifers/mL at a feeding rate of 100,000 cells/rotifer and the same feeding frequency (GR=1.13±0.007 day^{-1}). In contrast, the lowest density, 129±4.73 rotifers/mL, occurred with a feeding rate of 200,000 cells/rotifer and a feeding frequency of 2 times per day (GR=0.96±0.008 day^{-1}). The highest egg percentage (EP), 32.24±1.43%, was observed with a feeding rate of 200,000 cells/mL and a feeding frequency of 6 times per day, compared to the other treatments. It is deduced that both feeding rate and frequency have a significant effect on the population growth and the production of rotifers for aquaculture.

INTRODUCTION

Aquaculture has become a significant and rapidly growing sector contributing to global food security by providing high quality protein source. The growth of this sector is driven by the increasing demand for seafood, and the depletion of wild fish stocks due to overfishing. In 2020, global aquaculture production reached 87.5 million tonnes, marking a 20% increase over a decade **(FAO, 2022)**. This skyward trend is expected to continue, driven by increased yields in both freshwater and marine fish. To

sustain this growth, several critical factors must be addressed, particularly the success of hatchery operations in producing both the quantity and quality of larvae. The production of healthy larvae is determined by key factors, including the supply of live feeds. Rotifers, specifically *Brachionus plicatilis* are essential live feed for larviculture of marine finfish such as groupers and snappers. Their leisurely swimming, appropriate size, ability to tolerate salinity changes, and nutrient-rich composition make them excellent live feed for early larval development in aquaculture industries **(Kailasam *et al.*, 2015)**.

Efficient production methods and maintenance of live feeds in fish hatcheries are crucial for a stable, sufficient and consistent supply of rotifers. Rotifers need to be nutritionally enriched, and microalgae are primary feed source for rotifers as they provide nutrients, especially fatty acids, proteins and vitamins, for growth and reproduction. By feeding rotifers with microalgae, the rotifers can enrich the larval fish with essential nutrients for the development of the immune system, brain and growth. Feeding regime is one of the main factors that has a direct effect toward the population growth of rotifers. The amount, frequency and feed type are directly affecting their reproduction rate and overall health. Providing sufficient feed to meet the metabolic needs of rotifers without overfeeding is vital as overfeeding can result in water deterioration and poor growth. Inconsistent feeding can lead to starvation, slowing down reproduction and probable decline of the population. Thus, frequent feeding is vital to ensure that rotifers have a continuous food supply to maintaining high population growth rates.

In today's aquaculture landscape, where efficiency and sustainability of rotifer production are paramount, investigating the influence of various parameters—particularly feeding rates and frequency—holds significant importance. By elucidating optimal feeding regimes, we can significantly maximize biomass yield, improve nutritional quality, and enhance overall rotifer production, which is beneficial for fish fry development. This study aimed to identify the most effective feeding rates and frequencies for *Brachionus plicatilis* culture in an aquaculture system.

MATERIALS AND METHODS

1. Rotifer stock culture

Rotifer (*Brachionus plicatilis*, L-strain, with a mean lorica length of 195.5 µm) was obtained from the University Malaysia Sabah Crustacean Hatchery. The rotifers were cultured in a 100-liter conical tank at a temperature of 28°C. Initially, the density of the stock culture was 10 rotifers/mL. The culture was subsequently maintained and monitored until it reached a density in the range of 100 to 150 rotifers/mL. The salinity of the culture was set to 30 PSU. Concentrated microalgae, specifically Nano3600 (*Nannochloropsis* spp.), were used as the primary feed for the rotifer culture, which was fed twice daily at a rate of 10.0×10^6 cells/mL. Rotifers were cultivated in a batch

culture system. Water parameters such as temperature and dissolved oxygen (DO) were measured daily during the cultivation of the stock culture.

Experimental design

Nine different combinations of feeding rates and feeding frequencies were tested using a factorial design. The initial stocking density for each experimental tank was 10 rotifer/ml. The feeding rates were assessed at 1.0 x 10^5, 1.5 x 10^5, and 2.0 x 10^5 cells/rotifer while the feeding frequencies were 2, 4, 6 times/day. The experimental tank was equipped with a filter and an aeration which were regulated to 200mL/ min. Water bath with submersible heaters were used to control the desired temperature, 30℃. Batch culture was applied in this experiment for 5 days. The water temperature (°C), dissolved oxygen (mg/L), pH and salinity (ppt) of each tank was measured and recorded daily using BLE-9100 dissolved oxygen analyzer. To maintain the water quality, filter was cleaned every 2 days to remove any debris and waste.

2. Rotifer diet preparation

The concentrated micro-algae paste was diluted and stored in a 1L plastic jar. The algae were vigorously mixed using SOBO Aquarium Wave Maker which automatically turned on every feeding time. Mixing is important as the algae tends to settle down to the bottom of the plastic jar. The plastic jars were kept in a Styrofoam box containing ice pack. The feeds were administered automatically according to each treatment (feeding rates × feeding frequencies) using JEBAO Automatic Feeder Doser 2.4.

3. Rotifer counting

One milliliter (1mL) samples of rotifers were taken from each experimental tank daily at 0800h for counting. Lugol's solution was used to immobilize the rotifers prior to counting using a Sedgwick-Rafter chamber. Empty loricae belonging to dead rotifers were not counted. Each sample was counted three times, and the mean value was recorded. The population growth rate of *Brachionus plicatilis* was determined using the formula: $G = {}^{1}/_{T} \ln(N_T - N_0)$, where T is the period of culture days, N_0 is the initial number of rotifer; N_T is the total number of rotifer after T days of culture **(Radhakrishnan *et al.*, 2017)**.

RESULTS

The population growth curves of rotifers under different combinations of feeding rates and feeding frequencies are presented in Fig. (1). Both feeding rate (*P*= 0.002) and feeding frequency (*P*= 0.001) had significant effects on the growth rate (GR). Feeding rate also significantly influenced egg production (EP) and final population density (FPD), with *P*= 0.002 and 0.001, respectively.

The highest FPD was observed in the treatment with 2.0 × 10^5 cells/mL, fed 6 times/day, with an average of 378 ± 11.72 rotifers/mL. This was followed by the treatment 1.5 × 10^5 cells/mL, fed 6 times/day, with an average FPD of 292 ± 10.97

rotifers/mL. The lowest FPD was recorded in the treatment with 1.0×10^5 cells/mL, fed 2 times/day, averaging 126 ± 3.21 rotifers/ mL.

When analyzing feeding frequency regardless of feeding rate, rotifers fed 6 times/day exhibited a significantly higher mean GR compared to those fed 2 or 4 times/day. However, no significant difference in GR was observed between the 4 times/day and 6 times/day feeding frequencies.

Rotifers fed 2.0×10^5 cells/mL had significantly higher EP than other groups. Additionally, those fed 1.5×10^5 cells/mL had significantly greater EP than those fed 1.0×10^5 cells/mL. However, there was no significant difference in FPD between the 1.5×10^5 and 1.0×10^5 cells/mL groups.

FPD significantly increased with increasing feeding frequency. The interaction between feeding rate and feeding frequency had a significant effect on both GR and FPD (P= 0.001), but no significant interaction effect was observed for EP (P= 0.967).

Overall, GR, FPD, and EP increased with increasing feeding rate and frequency. The highest values for GR, FPD, and EP were obtained in the treatment fed 2.0×10^5 cells/mL, 6 times/day. Conversely, the lowest values for these parameters were observed in the treatment fed 1.0×10^5 cells/mL, 2 times/day (Table 1).

Based on these results, the optimum feeding rate and frequency for rotifer (*B. plicatilis*) culture is 2.0×10^5 cells/mL at 6 times/day.

DISCUSSION

Bielańska-Grajner and Cieplok (2017) stated that rotifers vigorously graze the water column, feeding on particles ranging from 1 to 10µm in size. The highest rotifer production was observed at a concentration of 2.0×10^5 cells/mL when *Nannochloropsis* spp. was used as the rotifer diet, with production increasing as feeding frequency increased. Based on previous observations, **Ferreira *et al.* (2018)** suggested that using a feeding rate of 75,000 cells/rotifer with *Nannochloropsis* algal paste as the diet, rotifer production can reach up to 1,500 rotifers/mL in a semi-continuous culture system.

In Japan, a *Chlorella*-based diet enriched with vitamin B_{12} is commonly used for mass rotifer culture **(Hagiwara *et al.*, 2017)**. The condensed freshwater *Chlorella* product has a cell density of approximately 20×10^9 cells/mL, compared to 65×10^9 cells/mL for *Nannochloropsis* paste. This *Chlorella* product has been used to maintain *Brachionus rotundiformis* culture densities between 3,000 and 6,000 rotifers/mL in a 100-L continuous culture system over 110 days **(Lubzens *et al.*, 2020)**. Furthermore, when feeding with freshwater *Chlorella* paste and starting with a rotifer density of 20,000/mL, a density of more than 1.6×10^5 rotifers/mL was achieved in a 4-day batch culture **(Lubzens *et al.*, 2020)**.

In another study, **Loka *et al.* (2016)** reported that supplying 1g of bread yeast to 10^6 S-type and L-type rotifers, with feeding administered twice daily, resulted in an increase from 20 rotifers/mL to approximately 100 rotifers/mL within a 7–10 day period.

.

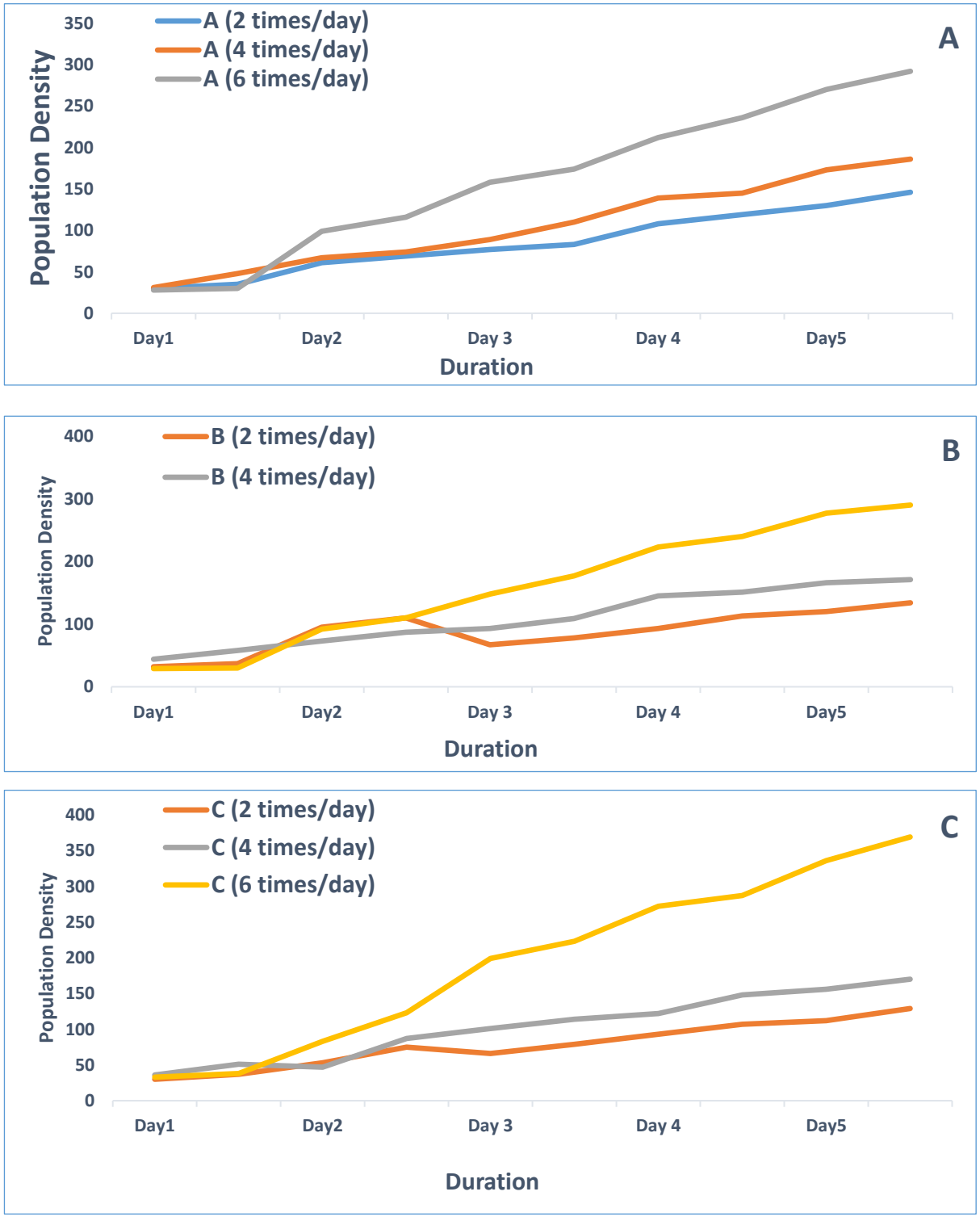

Fig. 1. Population growth of rotifer *Brachionus plicatilis* under different feeding frequencies (2,4 and 6 times/day) and feeding rates 100,000 cells/rotifer (A), 150,000 cells/rotifer (B) and 200,000 cells/rotifer (C). The population growth represents an average of 3 replicates for each treatment

Table 1. Growth parameters (FPD=Final Population Density; EP= Egg Production; GR= Growth Rate) of *B. plicatilis* for 5 days cultivation at different feeding rates (FR, cells/mL) and feeding frequencies (FF, times/day)

Items	FR	FF		
		2/day	4/day	6/days
FPD (rotifer/mL)	1.0 x 10^5	146±3.21^{a}	186±7.64^{b}	292±9.54^{c}
	1.5 x 10^5	134±8.08^{d}	171±2.0^{e}	290±10.97^{c}
	2.0 x 10^5	129±4.73^{d}	170±15.50^{e}	378±11.72^{f}
EP (%)	1.0 x 10^5	19.79±2.87^{b}	18.51±3.04^{b}	25.19±1.76^{a}
	1.5 x 10^5	25.43±9.28^{a}	23.39±0.31^{d}	27.60±1.99^{cd}
	2.0 x 10^5	28.05±4.12^{cd}	26.12±1.60^{cd}	32.24±1.43^{f}
GR (r)	1.0 x 10^5	0.98±0.005^{a}	0.92±0.013^{a}	1.13±0.007^{b}
	1.5 x 10^5	0.96±0.013^{c}	0.92±0.003^{c}	1.13±0.007^{b}
	2.0 x 10^5	0.96±0.008^{cd}	0.93±0.149^{cd}	1.18±0.006^{cd}

Table 2. Water quality parameter of *B. plicatilis* culture during 5 days cultivation at different feeding rates (FR, cells/mL) and feeding frequencies (FF, times/day)

Parameter	FR	FF		
		2/day	4/day	6/days
Temperature(°C)	1.0 x 10^5	30.13±0.057^{a}	30.06±0.57^{a}	30.03±0.57^{a}
	1.5 x 10^5	30.20±0.15^{a}	30.07±0.23^{a}	30.03±0.57^{a}
	2.0 x 10^5	30.10±0.17^{a}	30.03±0.52^{a}	30.03±0.50^{a}
Dissolve Oxygen (mg/l)	1.0 x 10^5	7.86±0.14^{a}	7.82±0.22^{a}	7.55±0.13^{a}
	1.5 x 10^5	8.67±0.10^{b}	7.08±0.13^{a}	7.59±0.15^{a}
	2.0 x 10^5	8.64±0.18^{b}	7.40±0.12^{a}	7.75±0.17^{a}
Salinity (psu)	1.0 x 10^5	30.10±0.007^{a}	30.3±0.03^{a}	30.2±0.02^{a}
	1.5 x 10^5	30.10±0.032^{a}	30.10±0.06^{a}	30.1±0.05^{a}
	2.0 x 10^5	30.20±0.071^{a}	30.50±0.10^{a}	30.4±0.03^{a}

Oda *et al.* (2015) reported that optimum rotifer productivity was achieved by feeding *Nannochloropsis oculata* at 250,000 cells/rotifer/day, administered twice daily over a four-day culture period, during which rotifer density increased from 20 to 158 rotifers/mL. In comparison, the present study recorded a higher rotifer density of 378 ± 11.72 individuals/mL within five days at a feeding rate of 2.0×10^5 cells/mL with a feeding frequency of six times per day. **Hepburn (2015)** noted that the average ingestion rate for rotifers ranges between 100,000 and 150,000 cells/individual/day.

Using a feeding rate of 2.0×10^5 cells/mL is sufficient to meet the rotifer's daily intake. Moreover, the availability of surplus food benefits newly hatched rotifers, enabling greater reproductive success due to constant food availability. Combining this with a feeding frequency of six times per day allows for controlled feed administration, minimizing overfeeding and reducing waste accumulation. This also helps maintain better water quality and provides optimal culture conditions for rotifers.

Hepburn (2015) also highlighted that the filtration rate of rotifers fed with *Nannochloropsis* spp. increased with algal cell density at low concentrations, but decreased at higher concentrations—except when fed *Dunaliella tertiolecta*. In their study, a peak ingestion rate was achieved at a *Nannochloropsis* concentration of 360×10^5 cells/mL, beyond which ingestion declined.

Das *et al.* (2014) observed population growth rates ranging from 0.02 to 0.28/day. In the present study, significantly higher growth rates were recorded at a feeding frequency of two times per day, with growth rates of 0.92 for 1.0×10^5 cells/mL, 0.92 for 1.5×10^5 cells/mL, and 0.93 for 2.0×10^5 cells/mL. Increasing feeding frequency consistently enhanced rotifer production, even at constant feeding rates.

Oda *et al.* (2015) also studied the effect of yeast concentration on rotifer yield. The highest density (200 rotifers/mL) was observed at 650mg/ L of yeast, followed by 150 rotifers/mL at 1000mg/ L, although a lower feed quality was used to produce one million rotifers. In another study, the density of *Brachionus calyciflorus* significantly increased with higher feeding frequency—130 rotifers/mL at three times/day compared to 110 rotifers/mL at two times/day**.**

Maintaining sterile water conditions and high-water quality standards is essential for sustaining high-density rotifer cultures over extended periods. One major challenge in rotifer mass production is water quality deterioration due to waste accumulation, including feces, urine, and uneaten feed **(Radhakrishnan *et al.*, 2017)**, which can negatively impact rotifer density.

Salinity also plays a crucial role in rotifer culture. **Lawrence *et al.* (2012)** found that *B. plicatilis* exhibited optimal density at salinities ranging between 5–15 ppt, which is higher than those used in the present study.

Ogata (2017) observed consistent temperature trends in rotifer cultures fed different algal diets. A positive correlation was noted between temperature and rotifer lifespan; however, a decline was observed when temperatures exceeded the optimal range of 25°C **(Lubzens *et al.*, 2020)**. Maintaining optimal temperature not only enhances reproduction but also minimizes the bacterial and protozoan load, helping to

prevent sudden culture collapse. While rotifers can consume bacteria and protozoans **(Ward, 2018)**, this alone is insufficient to support sustained growth.

In the present study, a temperature range of 28– 30°C was found to be optimal for mass rotifer production.

High dissolved oxygen (DO) levels are also essential for robust rotifer culture. However, excessive aeration should be avoided to prevent mechanical damage to *B. plicatilis* **(Cebreros *et al.*, 2017)**. Although rotifers can survive at DO levels as low as 1mg/ L, significantly greater growth is observed at around 8mg/ L **(Lawrence *et al.*, 2012; Sterzelecki *et al.*, 2020)**. DO levels can be influenced by various factors such as temperature, salinity, population density, and feed type **(Vaidya, 2017)**.

CONCLUSION

Implementing efficient methods in rotifer production by optimizing feeding rate and feeding frequency can significantly reduce the overall cost of producing rotifers in aquaculture industry. Precise feed delivery will improve feed conversion efficiency where rotifers are fully utilizing the feed they consume resulting in high population and reduced costs per individual rotifers produced. By using accurate feed amounts and optimal feeding time, hatcheries can reduce the amounts of expensive microalgae required, minimize feed waste and improve water quality. Eventually, efficient rotifer production leads to a more cost-effective and sustainable operation in providing high quality live feed for larval fish production.

ACKNOWLEDGEMENT

This study was funded by Universiti Malaysia Sabah under Flagship Research Grant (DFM 2101). The author would like to thank all staff and research assistant of Borneo Marine Institutes for their technical assistance throughout the time frame of study.

REFERENCES

Abaho, I.; Bwanika, G.; Walekhwa, P.; Arinaitwe, A. V. I. and Kwetegyeka, J. (2016). Fatty acid profiles and growth of African catfish (*Clarias gariepinus*, Burchell, 1822) larvae fed on freshwater rotifer (*Brachionus calyciflorus*) and Artemia as live starter feeds. Int. J. Fisheries & Aquatic Studies, 4(1), 189-196.

Abd Rahman, A. R.; Cob, Z. C.; Jamari, Z.; Mohamed, A. M.; Toda, T. and 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.

Bielańska-Grajner, I.; Mieczan, T. and Cieplok, A. (2017). Ecology of moss-dwelling rotifers in a raised bog: Differentiation of rotifer communities in microhabitats. *Biologia*, *72*(2), 175-183.

Cebreros, A. H. R.; Castro, L. I.; Carlock, E. G.; Sánchez, J. L. and Lajonchère, L. A. (2017). Pilot-scale production of the rotifer *Brachionus sp.* under different culture systems. Revista de biología marina y oceanografía, 52(3), 539-549.

Das, P.; Mandal, S. C.; Bhagabati, S. K.; Akhtar, M. S. and Singh, S. K. (2012). Important live food organisms and their role in aquaculture. Frontiers in aquaculture, 5(4), 69-86.

FAO (2022). The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO.https://doi.org/ 10.4060/cc0461en

Ferreira, M.; Cortina-Burgueño, Á.; Freire, I. and Otero, A. (2018). Effect of nutritional status and concentration of *Nannochloropsis gaditana* as enrichment diet for the marine rotifer *Brachionus sp.* Aquaculture, 491, 351-357.

Hagiwara, A.; Kim, H. J. and Marcial, H. (2017). Mass culture and preservation of *Brachionus plicatilis sp.* complex. Rotifers: Aquaculture, Ecology, Gerontology, and Ecotoxicology, 35-45.

Hepburn, J. (2015). Taking aquaculture seriously. Organic farming, winter soil association. International Journal of Research in Fisheries and Aquaculture, 63, 74-83.

Lawrence, C.; Sanders, E. and Henry, E. (2012). Methods for culturing saltwater rotifers (*Brachionus plicatilis*) for rearing larval zebrafish. *Zebrafish*, 9(3), 140-146.

Loka, J.; Philipose, K. K. and Sonali, S. M. (2016). Effect of microalgal diets on filtration and ingestion rates of the rotifer *Brachionus plicatilis*. Indian Journal of Fisheries, 61(3), 76-80.

Lubzens, E.; Hamo, R.; Blais, I.; Jeries, S.; Almog-Gabai, O. and Assaraf, Y. G. (2020). Rotifer resting eggs: An alternative for rotifer mass cultures in fish farms–Lessons from a comprehensive study on production, storage and hatching of resting eggs. Aquaculture, 528, 735505.

Odo, G. E.; Agwu, J. E.; Iyaji, F. O.; Madu, J. C.; Ossai, N. I. and Allison, L. N. (2015). Mass production of rotifer (*Branchionus calyciflorus*) for aquaculture in south-eastern Nigeria. International Journal of Fisheries and Aquaculture, 7(9), 151-159.

Ogata, Y. (2017). Use of freshwater Brachionus for aquaculture. Rotifers: Aquaculture, ecology, gerontology, and ecotoxicology, 75-85.

Radhakrishnan, K.; Aanand, S.; Rameshkumar, S. and Divya, F. (2017). Effect of feeding rate and feeding frequency in mass culture of *Brachionus plicatilis* in semi-continuous method with a yeast-based diet. Journal of Fisheries and Life Sciences, 2, 40-44.

Sterzelecki, F. C.; dos Santos Cipriano, F.; Vasconcelos, V. R.; Sugai, J. K.; Mattos, J. J.; Derner, R. B. and Cerqueira, V. R. (2021). Minimum rotifer density for best growth, survival and nutritional status of Brazilian sardine larvae, S*ardinella brasiliensis*. Aquaculture, 534, 736264.

Vaidya, S. R. (2017). Role of zooplankton in aquaculture. International Journal of Advanced Research and Development, 2(5), 951-954.

Ward, L. T. (2018). Effects of extended-rotifers inclusion and live food-enrichment with probiotics on the survival, metamorphosis, development time and growth of mud crab, *Scylla paramamosain* (Estampador) larvae.