Hindawi
Aquaculture Research
Volume 2024, Article ID 8837330, 10 pages
https://doi.org/10.1155/2024/8837330

Research Article

Effects of Different Carbon Sources on the Growth and Production of Rotifer (*Brachionus plicatilis*) in a Zero-Water Exchange Biofloc Culture System

Md. Eilious Hosain,[1,2] **S. M. Nurul Amin**,[1,3] **Mohd Salleh Kamarudin**,[1,4] **Aziz Arshad**,[1,4] **Murni Karim**,[1,4] **Md. Niamul Naser**,[2] **and Ravi Fotedar**[3]

[1]*Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia*
[2]*Department of Zoology, Faculty of Biological Sciences, University of Dhaka, Dhaka 1000, Bangladesh*
[3]*Curtin Aquatic Research Laboratories, School of Molecular and Life Science, Faculty of Science and Engineering, Curtin University, Bentley, Perth 6102, Western Australia, Australia*
[4]*International Institute of Aquaculture and Aquatic Sciences, Universiti Putra Malaysia, Batu 7, Jalan Kemang 6, Teluk Kemang, Si Rusa, Port Dickson 71050, Negeri Sembilan, Malaysia*

Correspondence should be addressed to Md. Eilious Hosain; mehosain83@gmail.com

Received 24 January 2024; Accepted 21 February 2024; Published 4 March 2024

Academic Editor: Lee Seong Wei

Brachionus plicatilis is considered an indispensable first live feed for many fish and crustacean larvae; the demand for the species has increased globally. The mass production of the rotifer involves quality microalga and a standard diet; this culture is expensive and needs a skilled workforce. The hatchery's incubators are likely to have limited resources leading to sudden rotifer culture crashes that ultimately disrupt the larvae production. More recently, improved sustainable rotifer production has been achieved through biofloc technology (BFT) that uses fish wastes and wheat flour. However, various carbon sources, which are typically used in BFT-based systems need to be explored and tested for their efficacies. A 4-day rotifer, *B. plicatilis* batch culture, was conducted in BFT systems by adding four carbon sources: molasses, rice bran, maize starch, and palm kernel expeller versus a control (without any carbon source). Fifteen 125 L containing polyethylene tanks with a water volume of 100 L were used for this experiment, and each tank was stocked with 5×10^6 rotifer (50 rotifers mL^{-1}). Different carbon sources in triplicates including a control were tested as treatments. The carbon : nitrogen ratio in the study was maintained at 10 : 1. The rotifers were fed with Baker's yeast at 1.0, 0.50, and 0.25 g $million^{-1}$ rotifers for the first, second, and third day and continued after that. Total ammonia–nitrogen (TAN) and pH values were found to be significantly ($p < 0.05$) lower in all four treatments of the BFT system than in the control. Significantly higher ($p < 0.05$) settleable solids were obtained in the molasses and rice bran treatments than those in the maize starch or palm kernel expeller. Likewise, the significantly ($p < 0.05$) higher density of *B. plicatilis* and their specific growth rate were obtained in the molasses and rice bran-adding treatments, followed by those in palm kernel expeller, maize starch, and the control. This study indicates that molasses and rice bran as carbon sources when added to BFT-based systems enhance *B. plicatilis* production.

1. Introduction

Rotifera is one of the smallest metazoa, comprising over 2,300 species that consist of a head, including a corona, a trunk that contains the organs, and the foot [1, 2]. They are planktonic organisms, typically free-swimming (but sedentary and colonial forms are also known) and wheel-bearing animalcules, with from 50 to 2,000 μm; ubiquitously distributed throughout the world [2, 3]. The rotifers are unsegmented, pseudocoelomate, bilaterally symmetrical invertebrates, most of which are found in freshwaters; some of these do inhabit brackish and marine waters, and limnoterrestrial habitats, for example, mosses, lichens, liverworts [1–3]. They are filter feeders, eating various foods, including dead organic material, algae, and other microscopic living organisms, multiplying by parthenogenesis and sexual reproduction under distress conditions [1, 2].

A production system using biofloc technology (BFT) is environmental friendly as it maintains good water quality by

removing nitrogenous metabolites and provides an additional food source in the form of an added organic carbon source [4–8]. The BFT-based systems are often zero-exchange systems, which congregate potentially consumable "biofloc," composed of various microorganisms, such as bacteria, fungi, microalgae, and zooplankton [5–7]. Various zooplankton communities, such as ciliates, flagellates, rotifers, copepods, and nematodes, naturally occur in a BFT system. The rotifers are second dominant species after ciliates, including a variety of genera, for example, *Brachionus, Euchlanis, Lecane, Colurella, Anuropsis, Gastropus, Habrotrocha*, and *Philodina*, that are usually preyed upon by the cultured larvae [5–7]. The rotifers also contain an essential portion of microorganism biomass in a BFT system or an unique ecosystem [2, 9, 10]. The biochemical composition of bioflocs includes protein, lipids, carbohydrates, amino acids, fatty acids, and antioxidants reflected by the presence of various microorganisms [4, 11, 12]. The biofloc composition is known to result in higher growth, production, survival, and spawning rates, besides increasing the immunity of the cultural species [7, 8].

To create a BFT system, the suitable carbon source with an optimal nitrogen ratio is one of the prerequisites (C : N 10–20 : 1). For instance, the different simple and complex carbon sources have been used in BFTs for many species of fish, prawns, shrimps, crayfish, and live feeds. Various carbon sources, such as acetate, corn starch, glycerol, molasses, etc., have typically been added [5, 13]. These simple carbon sources facilitate the removal of nitrogenous toxicants faster than complex ones. At the same time, some of them for example, glucose and molasses, are known to suppress *Vibrio*'s, known to increase the total bacteria [13, 14]. Among the complex carbon sources, rice bran is more widely administered than others in microbial-based systems, the addition of 24 hr of fermented rice bran is more effective to create high-quality bioflocs and it leads to a higher animal production [5, 12, 13, 15].

There are several species of freshwater (*Brachionus calyciflorus*) [16–18], brackish, and marine (*B. plicatilis, B. rotundiformis*, and *Proales similis*) [1, 19–21] rotifers. The euryhaline *B. plicatilis* species has size variations 100–400 μm and is an excellent live feed for many species of marine fish, shrimp, and crab larvae [3, 22–24]. This species has been widely used as an indispensable source of first live feed in freshwater to marine larviculture due to its suitable size, ease of culture, high growth rate, slow mobility, and ease of enriching with various nutritional elements [9, 25–29]. The mass production of *B. plicatilis* in hatcheries uses batch, semicontinuous, and continuous culture systems [25, 30].

The mass rotifer culture depends on supplementing high-quality unicellular microalgae and/dried algae, yeast, enriched diets [31–33]. For example, a defatted microalgal meal (*Haematococcus pluvialis*) has significantly enhanced the growth and reproduction performance of *B. plicatilis* and its total carotenoid and astaxanthin contents [34]. Furthermore, adding high-quality unicellular microalgae in the rotifer production system has enhanced rotifer population density, along with fatty acid contents, including EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) [33, 35]. On the other hand, dried algae are seldom used to rotifer production systems in hatcheries due to their commercial unavailability and being expensive [1] and resulting in reduced population growth [36–38]. The supplementation of commercially available yeasts can maintain good growth of *B. plicatilis*, which is also considered cheaper; however, yeast-fed rotifers are often deficient in highly unsaturated fatty acids (HUFA) [35]. Meanwhile, HUFA is essential for marine fish and crustacean larval nutrition, growth, survival, and overall health status [26, 39]. High-quality *B. plicatilis* mass production can be achieved using a recirculation aquaculture system (RAS) to supplement highly mechanized artificial diets requiring a skilled workforce [40].

A laboratory-grown bacterial supplementation including *Pseudomonas, Moraxella, Micrococcus, Alteromonas*, unidentified gram-negative strain-B3 to the axenic culture of *B. plicatilis* has increased its reproduction, leading to increasing rotifer numbers and resting eggs [41–43]. Similarly, the addition of probiotic bacteria with microalgae (*Chlorella*/*Nannochloropsis oculata*) alone, a combination of microalgae and yeast, and or an artificial diet with yeast have improved *B. plicatilis* production [44–46] and suppressed *Vibrio*'s [45, 47]. Additionally, the waste-grown phototrophic bacteria were shown to increase rotifer growth and biomass [48, 49]. However, despite this progress, each supplementation can have different drawbacks, including disrupting rotifer production in hatcheries and a high demand on resources. Moreover, continuous rotifer mass culture requires a vast amount of high-quality microalgae, a heavy burden for many hatcheries [1, 36]. A microbial or BFT-based system could be an alternative technology as it proliferates microorganisms that are composed of natural probiotic bacteria, flagellates, ciliates, etc., which are likely ingested by *B. plicatilis* further leading to higher rotifer production [15, 50].

Several studies have examined the potential application of the BFT to rotifer culture. For example, tilapia BFT system grown floc associated bacteria was sieved using 20 μm net and fed to *B. angularis*, in which the rotifer densities were 18.07 and 15.97 Ind. mL^{-1} for 21 and 25°C on day 60, respectively [50]. Adding fish waste and wheat flour has significantly enhanced *B. rotundiformis* density (1,188 Ind. mL^{-1}, on day 13) than those fed fish wastes or microalgae *C. vulgaris* only [51]. Panigrahi et al. [15] cultured *B. plicatilis* using BFT based on the aquamimicry and added microalgae *Nannochloropsis sp*. and filtered fermented juice when rotifers reached a minimum density of 150 Ind. mL^{-1}. However, no studies have assessed the effect of different carbon sources on the growth and production of a rotifer culture in a BFT system. Thus, this study aimed to evaluate the effect of four different carbon sources such as molasses (MO), rice bran (RB), maize starch (MS), and palm kernel expeller (PKE) on *B. plicatilis* mass production in a zero-water exchange BFT system.

2. Materials and Methods

2.1. Ethics Statement. The institutional concern authority has reviewed this study ethically in accordance with the journal's author guidelines page. This committee also approved and granted this study with an Aquaculture Research Permit.

Table 1: Media composed of the fertilizers and chemicals was administered to an unicellular marine microalgae *Nannochloropsis oculata* culture tank; this microalgae was added daily during *B. plicatilis* stock culture [11].

Fertilizers/chemicals	Quantity
Distilled water	1 L
Ammonium sulfate	40 mg
Ferrous sulfate	10 g
Urea	20 g
Triple super phosphate (TSP)	10 g
Ethylenediaminetetraacetic acid (EDTA)	4 g

2.2. Stock B. plicatilis Culture. This experiment was carried out at the International Institute of Aquaculture and Aquatic Sciences (I-AQUAS), Universiti Putra Malaysia (UPM), Port Dickson, Negeri Sembilan, Malaysia. The stock 25‰ saline water was prepared in a 2.5-tonne fiberglass tank by adding 5 ppm chlorine, and then, vigorous aeration was used to bubble off the chlorine before use [4]. Approximately 25 million rotifers, *B. plicatilis*, were acquired from the I-AQUAS hatchery. These rotifers were stocked at 50 Ind. mL^{-1} in the 1-tonne tank with a water volume of 500 L. The rotifers were fed with Baker's yeast at 1.0, 0.50, and 0.25 g $million^{-1}$ rotifers for the first, second, and third days, respectively [38;, 52]; these were added twice a day at 9:00 and 18:00 hr. On day 4, the rotifers were harvested for experimental use. Additionally, 50 L of *N. oculata* (approximately 2×10^6 cell mL^{-1}) was added daily. The microalgae were cultured with fertilizers at a rate of 1 mL L^{-1} daily (Table 1), using 30 L containers with 25 L water volume, aerated using two airstones (size: 15 mm × 42 mm).

2.3. Preparation of Biofloc Inoculum and Study Protocols. The biofloc inoculum was prepared by modifying the methodology of Hapsari [53], and Hosain et al. [5]. To prepare four carbon-sourced biofloc inoculums, four 7.5 L containing cylindrical polyethylene buckets were used. Each bucket was filled with 5 L treated saline water at 25%. Each inoculum was added with 0.1 g yeast, 50 mg ammonium sulfate, 2.1 g carbon source, and 0.1 g commercial probiotics (total bacteria $\geq 1.0 \times 10^9$ CFU g^{-1}, PondPlus®, Novozymes). The mixture of these inoculums was vigorously aerated for 24 hr using an airstone. Then, ammonia–N was measured, and it was not detected. After that, each inoculum (1 L) was added to respective carbon source treatment tanks only once at the beginning of this study.

This study was conducted in an enclosed greenhouse room at I-AQUAS, UPM, for a 4-day mass rotifer culture. There was a total of five treatments: control (transparent water system and no addition of carbon source) and four different tested carbon sources; MO, RB, MS, and PKE. Livestock feed-grade MO was dried at 60°C in an oven before the using it in the experimental. Commercially available MS (Cap Bintang corn starch, Korea) was added. Raw RB and PKE were purchased from a livestock feed ingredient store in Serdang, Selangor; these were then warmed up for an hour at 50°C using an oven before grinding. After that, these were hammer milled to a fine powder and passed through a sieve of 200 μm mesh size. Before rotifer culture, 15 tanks were filled with 100 L treated saline water at 25%. The experimental tanks were set up with a completely randomized design for five treatments, each of which was triplicated. Each experimental tank was stocked at approximately 5 million rotifers (50 Ind. mL^{-1}). The average length (±SE) of each rotifer was 167.40 ± 2.12 μm.

The rotifers were fed with Baker's yeast at 1.00, 0.50, and 0.25 g $million^{-1}$ for the first, second, and third day and continued after that [38, 52]. Carbon sources were added daily to maintain the carbon-to-nitrogen ratio at 10 : 1 [54]. Schryver et al. [55] calculated the daily additional quantity of carbon sources, where approximately 50% of the most organic carbon sources on a dry matter basis was carbon. The daily amount of nitrogen was determined based on the daily calculation of yeast adding and its total protein level (yeast contained 40% protein, USDA Nutrient Database). The daily yeast whole protein nitrogen level was calculated [56], where approximately 16% of the protein was nitrogen. According to the aforementioned empirical methodology, the daily carbon sources from each carbon source tank were adjusted and weighted. Each estimated carbon source and yeast were mixed with 200 mL water using an airstone in a 250 mL glass beaker. These mixtures were added to each carbon source tank at 10:00 and 18:00 hr. This was done to help ensure that none of the carbon sources and yeast would clump in the culture tanks.

2.4. Water Quality Variables, Settleable Solids, Rotifer Populations, and Size. Water quality parameters such as temperature, pH, salinity, and dissolved oxygen (DO) were determined daily at 09:00 hr in each tank with a multiparameter meter (YSI Model 556, YSI Incorporated, Yellow Springs, Ohio, USA). Total ammonia–nitrogen (TAN), nitrite–nitrogen (NO_2–N), and nitrate–nitrogen (NO_3–N) were measured using API® commercial test kits (API® Aquarium Pharmaceuticals, North America) daily. The settleable solids (mL L^{-1}) were estimated with an Imhoff cone daily, according to Romano et al. [57]. The Imhoff cone was filled with 1 L of culture water and allowed 30 min to settle the solids; after that, the volume was recorded as settleable solids. A 50 mL rotifer sample was collected from each tank daily. The daily rotifer population abundance was calculated using Sedgwick Rafter and profile projector with a 1-mL sample from each tank in triplicate. Based on this calculation, the quantity of daily yeast and carbon sources was estimated, as described previously. On day 4, the average size of rotifers was measured from each tank, and 30 rotifers were randomly measured using a compound microscope (Olympus Model BX41-CCD) and a USB digital microscope camera facility with a set magnification (10x). The specific growth rate of rotifer culture was estimated following the equation used by Suantika et al. [40]:

$$\text{Specific growth rate (SGR)} : \frac{(\ln N_t - \ln N_0)}{t}, \quad (1)$$

where N_t = rotifer density after a culture period t (individuals mL^{-1}), N_0 = initial rotifer density (individuals mL^{-1}), and t = culture period (day).

Table 2: Water quality parameters in control and the various carbon sources using biofloc treatments during a 4-day rotifer, *B. plicatilis* culture.

Variables	Control	Carbon sources			
		MO	RB	MS	PKE
Temperature (°C)	27.07 ± 0.02^{a}	28.08 ± 0.03^{a}	28.18 ± 0.04^{a}	28.11 ± 0.03^{a}	28.11 ± 0.01^{a}
pH	7.37 ± 0.02^{a}	7.06 ± 0.04^{b}	7.09 ± 0.04^{b}	7.11 ± 0.04^{b}	7.08 ± 0.04^{b}
DO ($mg\,L^{-1}$)	7.55 ± 0.06^{a}	6.70 ± 0.29^{ab}	6.91 ± 0.21^{ab}	6.76 ± 0.13^{ab}	6.66 ± 0.27^{b}
TAN ($mg\,L^{-1}$)	3.33 ± 0.92^{a}	0.43 ± 0.09^{b}	0.58 ± 0.16^{b}	0.79 ± 0.19^{b}	0.52 ± 0.11^{b}
NO_2–N ($mg\,L^{-1}$)	0.18 ± 0.06^{a}	0.33 ± 0.06^{a}	0.35 ± 0.06^{a}	0.37 ± 0.06^{a}	0.33 ± 0.06^{a}
NO_3–N ($mg\,L^{-1}$)	1.25 ± 0.65^{b}	8.75 ± 2.22^{a}	5.83 ± 1.20^{ab}	5.83 ± 1.20^{ab}	5.41 ± 1.29^{ab}
SS ($mL\,L^{-1}$)	0.58 ± 0.31^{b}	7.16 ± 1.84^{a}	7.25 ± 1.88^{a}	1.83 ± 0.63^{b}	2.41 ± 0.82^{ab}

Mean values with the same superscript letters in the same row were not significantly different ($p > 0.05$), while different letters did significantly differ ($p < 0.05$). CON: control; MO: molasses; RB: rice bran; MS: maize starch; and PKE: palm kernel expeller.

2.5. Statistical Analysis. Statistical analysis was done using the computerized SPSS version 25. Before analysis, data were checked for normality and homogeneity of variances using Levene's test. Differences in water quality parameters and the rotifer population density, SGR, and rotifer length data of five treatments were performed using analysis of variance (ANOVA). When significant differences were detected ($p < 0.05$), Tukey's multicomparison test was used for post hoc compare the mean among different treatment groups.

3. Results

3.1. Water Quality Variables and Settleable Solids. Temperature did not differ significantly among the five treatments during 4 days of this study (Table 2 and Figure 1(a)). pH values in the four other carbon source biofloc groups were significantly ($p < 0.05$) decreased from day 2 to day 4 than control group (Table 2 and Figure 1(b)). Similarly, DO significantly ($p < 0.05$) decreased at day 2 to day 4 in the four different carbon sources than the control (Table 2 and Figure 1(c)). TAN concentration was significantly ($p < 0.05$) increased over the period in the control, while this was not substantially increased during the study among four carbon source biofloc groups (Table 2 and Figure 1(d)). Nitrite–N concentrations was similar among the five different treatments (Table 2 and Figure 1(e)). Significantly ($p < 0.05$) higher Nitrate–N level was detected in the MO biofloc group than the control, which was similar to other carbon sourced bioflocs groups (Table 2 and Figure 1(f)). Settleable solids was significantly higher in the MO and RB biofloc groups than those in the control and MS biofloc group (Table 1 and Figure 2).

3.2. Rotifer B. plicatilis Density, Specific Growth Rate and Size. Significantly ($p > 0.05$) higher *B. plicatilis* density and specific growth rate were in the MO and RB biofloc treatments, followed by PKE, MS, and control, respectively (Table 3 and Figure 3). Moreover, *B. plicatilis* density was significantly higher in the MO, RB, and PKE adding biofloc groups at the day 2, 3, and 4 when compared to control and MS biofloc group (Figure 3). In terms of *B. plicatilis* length, it was significantly more prominent in the control and MS adding biofloc group than those of RB and palm kernel adding bioflocs treatments (Table 3).

4. Discussion

Developing a zero-exchange system with BFT provides microbial feeds associated with small particles and ensures good water quality conditions for cultured animals [5, 8, 13]. A BFT-based system can remove nitrogenous toxicants and improve the overall health status of cultured animals [13, 58]. For instance, in this study, four carbon source-based BFT systems have ensured a better water quality status than control over the entire experimental period.

In this study, TAN concentration was within suitable limits in the four carbon sources-based biofloc systems, which were more than four times lower than the control. Furthermore, the TAN concentration in the control group gradually increased over the period; in contrast, the TAN spikes decreased on days 3 and 4 in all four carbon-sourced biofloc systems than on day 2. These indicate that heterotrophic bacteria require time to establish. However, during the 4-day study, TAN increased in the control group, and inadequate heterotrophic bacterial numbers could not remove TAN due to the absence of any carbon source. On the contrary, there was two times higher nitrite–N and more than four times higher nitrate–N concentrations in the four groups of the biofloc system than in the control. These indicate that adding four carbon sources inoculums has rapidly facilitated the establishment of heterotrophic bacteria, which has contributed to removing TAN and nitrite–N and accumulating nitrate–N as an end-product [4, 5, 59].

In a biofloc system, the key strategy is the proliferation of microbial aggregation "biofloc" [7]. This biofloc includes bacteria, plankton, and even inert or dead particles, estimated as settleable or total suspended solids. In this study, the settleable solids were significantly higher in the RB and MO carbon source BFT groups over the periods than in the PKE, MS, or control group. This higher settleable solid in the RB and MO carbon source BFT groups are likely supported to produce the higher number of rotifer *B. plicatilis* grown in those treatments, indicating that MO and RB suit to *B. plicatilis* production in biofloc systems.

Furthermore, these carbon sources have provided a favorable culture environment that might include rotifers, feeds, or other facilities. For example, Pekkoh et al. [60] stated that the addition of MO and RB mixture in a BFT system had

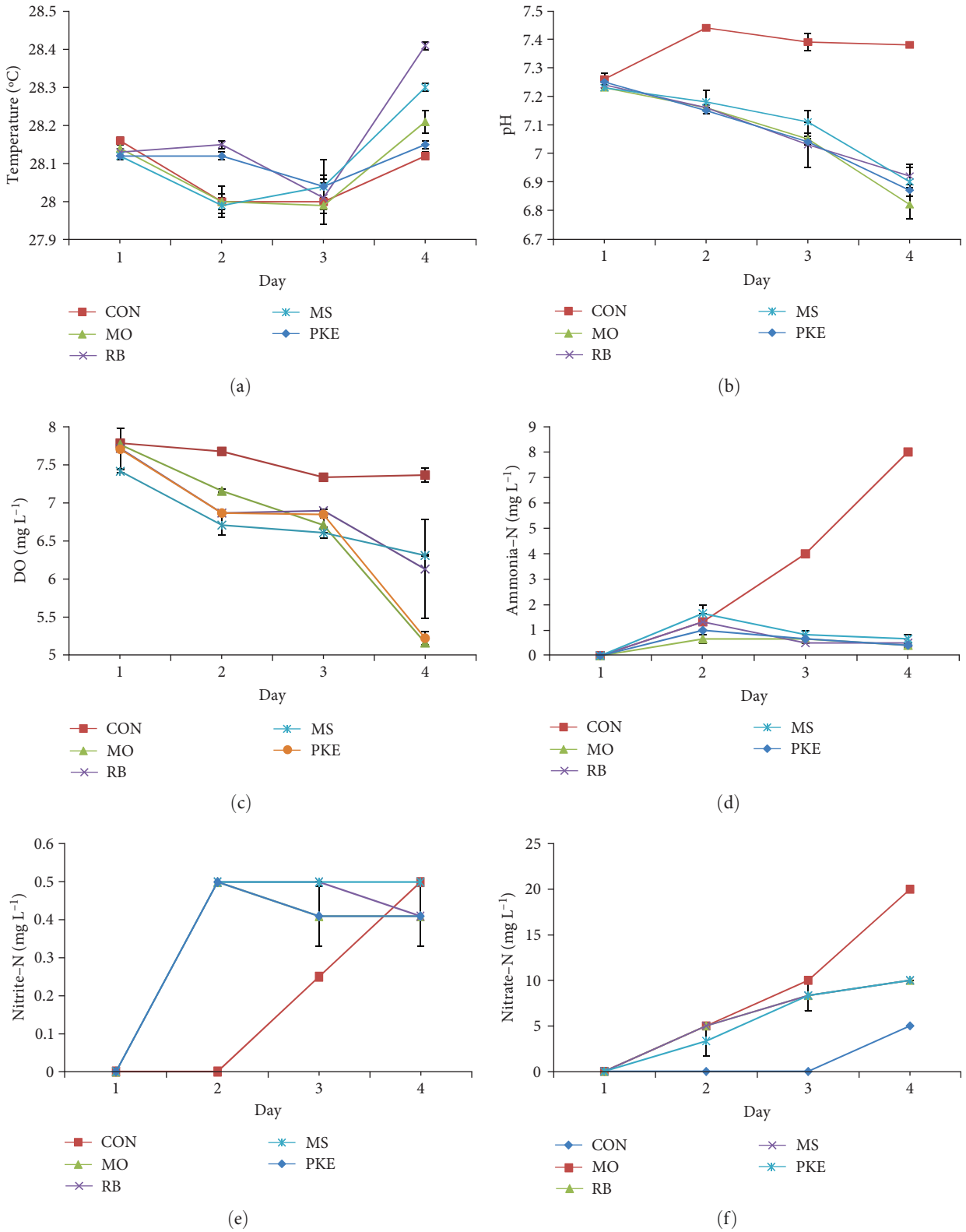

FIGURE 1: Daily mean (±SE): (a) temperature (°C), (b) pH, (c) dissolved oxygen (DO; mg L^{-1}), (d) ammonia–nitrogen (mg L^{-1}), (e) nitrite–nitrogen (mg L^{-1}), and (f) nitrate–nitrogen (mg L^{-1}) in control and four different carbon sources using biofloc systems for the culture of Brachionus plicatilis. CON: control; MO: molasses; RB: rice bran; MS: maize starch; and PKE: palm kernel expeller.

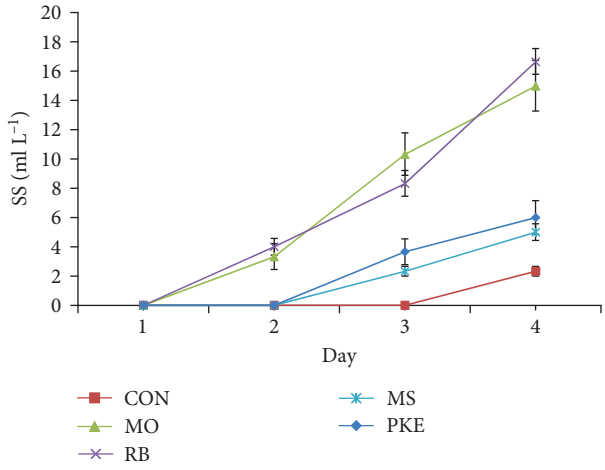

FIGURE 2: Daily mean (±SE) settleable solids (SS; mL L^{-1}) in control and four different carbon sources using biofloc systems for the culture of *Brachionus plicatilis*. CON: control; MO: molasses; RB: rice bran; MS: maize starch; and PKE: palm kernel expeller.

TABLE 3: The rotifer *B. plicatilis* density, specific growth rate (SGR), and length in control and various carbon sources using biofloc treatments after 4 days of culture.

Variables	Control	Carbon sources			
		MO	RB	MS	PKE
Density (Ind. ml^{-1})	95.0 ± 4.16^{c}	1058.67 ± 42.74^{a}	886.34 ± 86.36^{a}	156.0 ± 24.95^{c}	584.0 ± 21.45^{b}
SGR (r)	0.16 ± 0.01^{d}	0.76 ± 0.01^{a}	0.72 ± 0.02^{a}	0.28 ± 0.03^{c}	0.61 ± 0.01^{b}
Length (μm)	171.97 ± 1.33^{a}	169.42 ± 1.37^{ab}	165.98 ± 1.25^{b}	172.07 ± 1.48^{a}	166.37 ± 1.36^{b}

Mean values with the same superscript letters in the same row were not significantly different ($p > 0.05$), while different letters did significantly differ ($p < 0.05$). CON: control; MO: molasses; RB: rice bran; MS: maize starch; and PKE: palm kernel expeller.

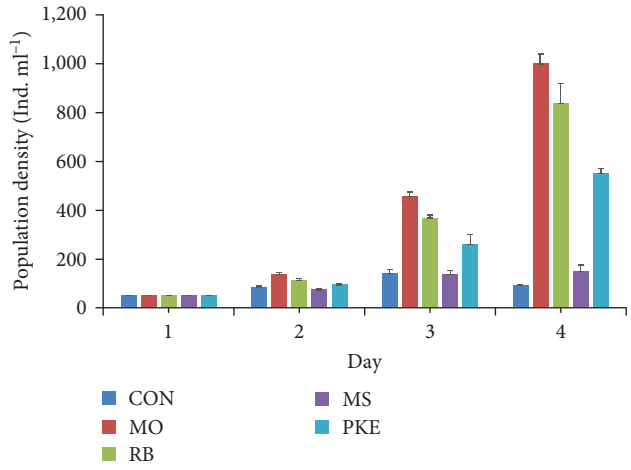

FIGURE 3: Daily mean (±SE) population density (Ind. mL^{-1}) in control and different carbon sources using biofloc systems for the culture of *Brachionus plicatilis*. CON: control; MO: molasses; RB: rice bran; MS: maize starch; and PKE: palm kernel expeller.

enhanced the bacterial–algal performance that provides the most compact biofloc structure and better settleable solids, including larger biofloc particle/substrates. For instance, in this study, the addition of MO and RB with yeast and commercial probiotics facilitated in increasing biofloc volume/settleable solids or provided the excellent consumable bacteria-algae-yeast substrates that have been grazing by rotifers and resulted in the higher number of *B. plicatilis* in these two treatments. The rotifer *B. plicatilis* is notably known to feed on bacteria, algae, yeast, and even smaller sizes of inert particles; these contribute to the higher production of rotifers in an optimal culture condition.

In this study, the higher *B. plicatilis* densities of 1,058.67, 886.34, and 584.0 in Ind. mL^{-1} were found after the 4-day culture in the MO, RB, and PKE carbon sourced biofloc systems, respectively. The specific growth rates of 0.76, 0.72, and 0.61 were obtained for MO, RB, and PKE carbon sourced biofloc system. Compared to well-established rotifer culture studies, these three carbon sources can be considered a viable *B. plicatilis* batch production technique. For example, the maximal density of *B. plicatilis* was found to be 1,162 Ind. mL^{-1} on day 5 when rotifers were fed with frozen *Nannochloropsis* (Japanese) at the rate of 1.75 g (dry weight) to 10^{6} rotifers per day [61]. Green algae (*Chlorella saccharophila*) fed *B. plicatilis* showed a higher density and specific growth rate of 324 Ind. mL^{-1} and 0.19, respectively. It decreased in the order of *Isochrysis galbana* (191 Ind. mL^{-1}, 0.14) > *Tetraselmis suecica* (168 Ind. mL^{-1}, 0.14) > *Saccharomyces cerevisiae* (150 Ind. mL^{-1}, 0.13) > *Thalassiosira pseudonana* (104 Ind. mL^{-1}, 0.11) [62]. Density and specific growth rate (257.6 Ind. mL^{-1} and 0.29) of *B. plicatilis* were maintained in a semicontinuous rotifer mass culture, in which rotifers were fed with Selco Sparkles (INVE S.A., INVE, Ghent, Belgium) using an automatic feeder [15]. More recently, Bhosale and Mugale [63] have maintained a

rotifer (*B. calyciflorus*) density at 381 Ind. mL^{-1} in the RAS, wherein the rotifers were fed with *C. vulgaris*, and 30% of them were harvested daily. Lubzens et al. [30] reported in a review that supplemental feeding of Baker's yeast to rotifer culture could be considered a feasible and easy technique, in which rotifer densities can be reached at 150 Ind. mL^{-1}, but under specific culture conditions, it could go up to 1,500–2,000 Ind. mL^{-1}.

Typically, the total length of *B. plicatilis* complex fluctuates between 100 and 400 μm [64], while neonate females do increase their length from birth to adulthood [65, 66]. In this study, the average length of *B. plicatilis* was 172.07 μm that was found in the maize starch that was approximately 3.53% longer than the 165.98 μm in rice bran biofloc group. The study exhibited that the increase in rotifer density was likely related to decrease in length in the RB, PKE, and MO biofloc system than the control and MS groups. It appears that the small length of *B. plicatilis* at day 4 in the RB, PKE, and MO was due to the abundance of neonate females that doubled within a day, and its density was 11 and 9 times higher in the MO and RB than the control. Various sizes of rotifers *Brachionus* sp. were used in incubators for fish and crustacean larvae culture, 130–340 μm and 150–250 μm were in *Chlorella* using hatcheries, 150–350 μm in Baker's yeast using hatcheries [25]; approximate body size 150–250 μm was in *N. oculata* in hatcheries [67]. For instance, in this study, *B. plicatilis* cultivated in the MO (147–193 μm), RB (148–193 μm), and PKE (146–193 μm) would be an excellent live feed for farmed marine fish such as neon goby *Elacatinus figaro*, torktail blenny *Meiacanthus atrodorsalis*, false clownfish *Amphiprion ocellaris*, and spotted seahorse *Hippocampus kuda* with their suit mouth gape around 350, 307, 300, and 260 μm, respectively [68]. On the other hand, enriched rotifers are often used in hatcheries according to the nutritional requirements of larvae [25–28],. However, more studies should be warranted using biofloc-based systems produced by rotifers with enrichments of larval nutritional requirements and their subsequent effects on larval zootechnical performance and overall health status.

Currently, the high density of rotifer culture strategy has been practiced field to meet the higher demand at fish, shrimp, and crab hatcheries in developed countries such as Japan, Canada, Taiwan, Turkey, and USA [32, 69–72]. These high-density or ultrahigh-density rotifer mass culture systems are supplemented with the concentrated microalgal paste (*Chlorella* and *Nannochloropsis*) and artificial rotifer diets [35, 72, 73]. These rotifer mass culture systems are usually shown to have increased nitrogenous toxicants and decreased pH values [35, 73]. In a higher density rotifer mass production in a batch culture or recirculation aquaculture system, *B. plicatilis* was stocked at 250 Ind. mL^{-1} [73]. The authors recorded the maximum rotifer density and growth rate of 860 Ind. mL^{-1} and 0.31 for a batch culture after 4 days, 8,000 Ind. mL^{-1}, and 0.35 for an RAS with a 500% daily recirculation rate after 8 days. From this standpoint, mass culture of rotifer in a biofloc system could be an excellent option because in the current study, the growth rate of *B. plicatilis* was two times higher at 0.76 and 0.72 in the MO and RB carbon source using biofloc system after the 4 days of culture. However, more studies are warranted to optimize the batch and semicontinuous high or ultrahigh-density mass culture of *B. plicatilis*. Additionally, these studies should examine the optimal carbon-to-nitrogen ratio, the nutritional values of *B. plicatilis* cultured in a BFT system, and their subsequent effect on fish, shrimp, and crab larvae or post larvae.

5. Conclusion

The results obtained from the biofloc system could be considered an improved mass-production technology of rotifer (*B. plicatilis*) using the readily available and cheaper carbon sources of MO or RB. However, further research is recommended to optimize the rotifer production in biofloc systems and their subsequent effect on fish, shrimp, crab larvae, post larvae, or as an inoculum in a biofloc-based system.

Data Availability

The data that supports the findings of this study are available in the supplementary material of this article.

Additional Points

Highlights. (1) Carbon sources from molasses and rice bran were optimal for enhancing the *B. plicatilis* growth and production in the biofloc system. (2) Biofloc volume was higher in the molasses and rice bran-adding systems due to a more remarkable occurrence of *B. plicatilis*. (3) Biofloc system had produced desirable *B. plicatilis* and the rotifer-dominated biofloc could be an excellent inoculum in a biofloc of shrimp and fish nursery phase. (4) The biofloc system could be excellent for mass production of rotifer, *B. plicatilis* in hatcheries.

Disclosure

The paper was presented in "WORLD AQUACULTURE 2023" held in Darwin, Northern Territory, Australia between May 29 and June 1, 2023.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Md. Eilious Hosain participated in the experiment design, conducted this experiment, and wrote the manuscript. S M Nurul Amin, Mohd Salleh Kamarudin, Murni Karim, and Aziz Arshad participated in the experimental design, supervised, and edited the manuscript. Md. Niamul Naser and Ravi Fotedar provided critical insights and edits throughout.

Acknowledgments

The first author thanks the International Institute of Aquaculture and Aquatic Sciences (I-AQUAS) of UPM, Port Dickson, for providing laboratory facilities. We thank

Mr Nurisham Bin Yahaya, Research Assistant, I-AQUAS, for assisting with the experimental setup.

Supplementary Materials

Supplementary 1. Daily water quality parameters including temperature, pH, DO, total ammonia–nitrogen (TAN), nitrite–N, nitrate–N and settleable solids (SS) in the control and the various carbon sources using biofloc treatments (MO: molasses; PKE: palm kernel expeller; RB: rice bran; and MS: maize starch) during a 4-day culture of the rotifer, *Brachionus plicatilis*.

Supplementary 2. Daily rotifer, *Brachionus plicatilis* number (Ind. mL^{-1}) in the control and the various carbon sources using biofloc treatments (MO: molasses; PKE: palm kernel expeller; RB: rice bran; and MS: maize starch) during a 4-day culture.

Supplementary 3. Rotifer, *Brachionus plicatilis* number (Ind. mL^{-1}) and specific growth rate (SGR) in the control and the various carbon sources using biofloc treatments (MO: molasses; PKE: palm kernel expeller; RB: rice bran; and MS: maize starch) at day-4.

Supplementary 4. Rotifer, *Brachionus plicatilis* length (μm) in the control and the various carbon sources using biofloc treatments (MO: molasses; PKE: palm kernel expeller; RB: rice bran; and MS: maize starch) at day-4.

References

[1] A. Hagiwara and T. Yoshinaga, *Rotifers: Aquaculture, Ecology, Gerontology, and Ecotoxicology*, Springer, Singapore, 2017.

[2] R. L. Wallace and T. W. Snell, "Chapter 8 rotifera," in *Ecology and Classification of North American Freshwater Invertebrates*, pp. 173–236, Academic Press, 3rd edition, 2010.

[3] E. Lubzens and O. Zmora, "Production and nutritional value of rotifers," in *Live Feeds in Marine Aquaculture*, J. G. Støttrup and L. A. McEvoy, Eds., p. 313, Blackwell Science Ltd., 2003.

[4] M. E. Hosain, S. M. N. Amin, M. S. Kamarudin, A. Arshad, and N. Romano, "Effects of C—N ratio on growth, survival and proximate composition of *Macrobrachium rosenbergii* post larvae reared under a corn starch based zero- exchange brackish water biofloc system," *Aquaculture Research*, vol. 52, no. 7, pp. 3015–3025, 2021.

[5] M. E. Hosain, S. M. N. Amin, A. Arshad, M. S. Kamarudin, and M. Karim, "Effects of carbon sources on the culture of giant river prawn in biofloc system during nursery phase," *Aquaculture Reports*, vol. 19, Article ID 100607, 2021.

[6] M. E. Hosain, S. M. N. Amin, M. S. Kamarudin, A. Arshad, M. Karim, and N. Romano, "Effect of salinity on growth, survival, and proximate composition of *Macrobrachium rosenbergii* post larvae as well as zooplankton composition reared in a maize starch based biofloc system," *Aquaculture*, vol. 533, Article ID 736235, 2021.

[7] M. H. Khanjani, A. Mohammadi, and M. G. C. Emerenciano, "Microorganisms in biofloc aquaculture system," *Aquaculture Reports*, vol. 26, Article ID 101300, 2022.

[8] A. B. Dauda, "Biofloc technology: a review on the microbial interactions, operational parameters and implications to disease and health management of cultured aquatic animals," *Reviews in Aquaculture*, vol. 12, no. 2, pp. 1193–1210, 2019.

[9] D. A. Silva, P. C. M. de Lima, A. E. M. da Silva et al., "Effects of adding rotifers on the water quality, plankton composition and growth of Pacific white shrimp, *Litopenaeus vannamei* juvenile, when cultured with biofloc technology," *Aquaculture Research*, vol. 52, no. 9, pp. 4380–4393, 2021.

[10] L. O. Brito, I. G. S. dos Santos, J. L. de Abreu, M. T. de Araújo, W. Severi, and A. O. Gàlvez, "Effect of the addition of diatoms (*Navicula* spp.) and rotifers (*Brachionus plicatilis*) on water quality and growth of the *Litopenaeus vannamei* postlarvae reared in a biofloc system," *Aquaculture Research*, vol. 47, no. 12, pp. 3990–3997, 2016.

[11] M. E. Hosain, S. M. N. Amin, A. Arshad et al., "Effect of copepod (*Apocyclops dengizicus*) addition on growth, survival and proximate composition of *Macrobrachium rosenbergii* postlarvae cultured in brackish water biofloc system," *North American Journal of Aquaculture*, 2023.

[12] M. H. Khanjani, L. O. B. da Silva, G. K. Fóes et al., "Synbiotics and aquamimicry as alternative microbial-based approaches in intensive shrimp farming and biofloc: novel disruptive techniques or complementary management tools ? A scientific-based overview," *Aquaculture*, vol. 567, Article ID 739273, 2023.

[13] G. Abakari, G. Luo, E. O. Kombat, and E. H. Alhassan, "Supplemental carbon sources applied in biofloc technology aquaculture systems: types, effects and future research," *Reviews in Aquaculture*, vol. 13, no. 3, pp. 1193–1222, 2021.

[14] A. Panigrahi, M. Sundaram, C. Saranya, S. Swain, R. R. Dash, and J. S. Dayal, "Carbohydrate sources deferentially influence growth performances, microbial dynamics and immunomodulation in Pacific white shrimp (*Litopenaeus vannamei*) under biofloc system," *Fish and Shellfish Immunology*, vol. 86, pp. 1207–1216, 2019.

[15] A. Panigrahi, R. R. Das, S. Sarkar, R. Aravind, P. N. Rekha, and K. P. Sandeep, "Biofloc-based farming of Indian white shrimp, *Penaeus indicus*, in recirculating aquaculture system (RAS) enriched with rotifers as feed supplement," *Aquaculture International*, vol. 31, no. 2, pp. 657–680, 2023.

[16] T. D. Mau and D. Quang-Hung, "Effects of cultural conditions on life history characteristics of the freshwater rotifer *Brachionus calyciflorus*," *International Journal of Aquatic Biology*, vol. 11, no. 2, pp. 104–114, 2023.

[17] A. Rajendiran and P. Subramanian, "Mass production of freshwater rotifer, *Brachionus calyciflorus*, under different diets and regimes," *Journal of Applied Aquaculture*, vol. 19, no. 3, pp. 101–111, 2007.

[18] H. G. Park, K. W. Lee, S. H. Cho, H. S. Kim, M. Jung, and H. Kim, "High density culture of the freshwater rotifer, *Brachionus calyciflorus*," *Hydrobiologia*, vol. 446, no. 447, pp. 369–374, 2001.

[19] H. Ge Xing, Q. Ni, J. Li, Z. Chen, and F. Zhao, "Integration of white shrimp (*Litopenaeus vannamei*) and green seaweed (*Ulva prolifera*) in minimum-water exchange aquaculture system," *Journal of Applied Phycology*, vol. 31, no. 2, pp. 1425–1432, 2019.

[20] U. A. Rebolledo, N. Sarma, G. A. R. de Oca et al., "The potential use of the euryhaline rotifer *Proales similis* for larval rearing of the freshwater pike silverside *Chirostoma estor estor*," *Aquaculture*, vol. 534, Article ID 736246, 2021.

[21] K. Yoshimura, K. Tanaka, and T. Yoshimatsu, "A novel culture system for the ultra-high-density production of the rotifer, *Brachionus rotundiformis*—a preliminary report," *Aquaculture*, vol. 227, no. 1–4, pp. 165–172, 2003.

[22] H. Jamali, A. Imani, D. Abdollahi, R. Roozbehfar, and A. Isari, "Use of probiotic *Bacillus* spp. in Rotifer (*Brachionus plicatilis*)

and Artemia (*Artemia urmiana*) enrichment: effects on growth and survival of Pacific white shrimp, *Litopenaeus vannamei*," *Larvae, Probiotics and Antimicrobial Proteins*, vol. 7, no. 2, pp. 118–125, 2015.

[23] M. Guarizo, T. M. Costa, and M. Z. Marochi, "Effect of diet during larval development of *Menippe nodifrons* Stimpson, 1859 and *Callinectes danae* Smith, 1869," *Aquaculture International*, vol. 28, no. 5, pp. 1969–1980, 2020.

[24] A. Hagiwara and H. S. Marcial, "The use of non- *Brachionus plicatilis* species complex rotifer in larviculture," *Hydrobiologia*, vol. 844, no. 1, pp. 163–172, 2019.

[25] K. Hamre, "Nutrient profiles of rotifers (*Brachionus* sp.) and rotifer diets from four different marine fish hatcheries," *Aquaculture*, vol. 450, pp. 136–142, 2016.

[26] N. A. Samat, F. M. Yusoff, N. W. Rasdi, and M. Karim, "Enhancement of live food nutritional status with essential nutrients for improving aquatic animal health: a review," *Animals*, vol. 10, no. 12, pp. 1–27, 2020.

[27] D. K. Radhakrishnan, I. AkbarAli, B. V. Schmidt, E. M. John, S. Sivanpillai, and S. T. Vasunambesan, "Improvement of nutritional quality of live feed for aquaculture: an overview," *Aquaculture Research*, vol. 51, no. 1, pp. 1–17, 2020.

[28] T. Kotani, "Enrichment of rotifers and its effect on the growth and survival of fish larvae," in *Rotifers: Aquaculture, Ecology, Gerontology, and Ecotoxicology*, pp. 47–62, Springer, Singapore, 2017.

[29] C. Lawrence, E. Sanders, and E. Henry, "Methods for culturing saltwater rotifers (*Brachionus plicatilis*) for rearing larval zebrafish," *Zebrafish*, vol. 9, no. 3, pp. 140–146, 2012.

[30] E. Lubzens, O. Zmora, and Y. Barr, "Biotechnology and aquaculture of rotifers," *Hydrobiologia*, vol. 446, no. 447, pp. 337–353, 2001.

[31] H. J. Kim, C. Sawada, J. S. Rhee, J. S. Lee, K. Suga, and A. Hagiwara, "Nutritional effects on the visual system of the rotifer *Brachionus plicatilis* sensu stricto (Rotifera: Monogononta)," *Journal of Experimental Marine Biology and Ecology*, vol. 460, pp. 177–183, 2014.

[32] K. M. Eryalçın, "Nutritional value and production performance of the rotifer *Brachionus plicatilis* Müller, 1786 cultured with different feeds at commercial scale," *Aquaculture International*, vol. 27, no. 3, pp. 875–890, 2019.

[33] A. Hagiwara, H. J. Kim, and H. Marcial, "Mass culture and preservation of *Brachionus plicatilis* sp. Complex," in *Rotifers: Aquaculture, Ecology, Gerontology, and Ecotoxicology*, A. Hagiwara and T. Yoshinaga, Eds., Rotifers. Fisheries Science Series, pp. 56–71, Springer, Singapore, 2017.

[34] H. Li and J. Liu, "Effects of defatted *Haematococcus pluvialis* meal (DHPM) supplementation on the growth performance, and the carotenoid content and composition in the rotifer (*Brachionus plicatilis*)," *Aquaculture*, vol. 505, pp. 34–40, 2019.

[35] P. Dhert, G. Rombaut, G. Suantika, and P. Sorgeloos, "Advancement of rotifer culture and manipulation techniques in Europe," *Aquaculture*, vol. 200, no. 1-2, pp. 129–146, 2001.

[36] X. W. Yin, W. W. Min, H. J. Lin, and W. Chen, "Population dynamics, protein content, and lipid composition of *Brachionus plicatilis* fed artificial macroalgal detritus and *Nannochloropsis* sp. diets," *Aquaculture*, vol. 380–383, pp. 62–69, 2013.

[37] A. M. M. Heneash, M. Ashour, and M. Matar, "Effect of un-live microalgal diet, *Nannochloropsis oculata* and *Arthrospira (Spirulina) platensis*, comparing to yeast on population of rotifer, *Brachionus plicatilis*," *Mediterranean Aquaculture Journal*, vol. 2015, no. 7, pp. 48–54, 2015.

[38] K. M. Eryalcin, "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*, vol. 18, pp. 81–90, 2018.

[39] A.-F. M. El-Sayed and M. Izquierdo, "The importance of vitamin E for farmed fish—a review," *Reviews in Aquaculture*, vol. 14, no. 2, pp. 688–703, 2022.

[40] G. Suantika, P. Dhert, E. Sweetman, E. O'Brien, and P. Sorgeloos, "Technical and economical feasibility of a rotifer recirculation system," *Aquaculture*, vol. 227, no. 1–4, pp. 173–189, 2003.

[41] A. Hagiwara, K. Hamada, S. Hori, and K. Hirayama, "Increased sexual reproduction in *Brachionus plicatilis* (rotifera) with the addition of bacteria and rotifer extracts," *Journal of Experimental Marine Biology and Ecology*, vol. 181, no. 1, pp. 1–8, 1994.

[42] G. Rombaut, P. Dhert, J. Vandenberghe, L. Verschuere, P. Sorgeloos, and W. Verstraete, "Selection of bacteria enhancing the growth rate of axenically hatched rotifers (*Brachionus plicatilis*)," *Aquaculture*, vol. 176, no. 3-4, pp. 195–207, 1999.

[43] P. Douillet, "Bacterial additives that consistently enhance rotifer growth under synxenic culture conditions," *Aquaculture*, vol. 182, no. 3-4, pp. 249–260, 2000.

[44] T. Abu-Rezq and C. M. James, "Application of probiotics in rotifer production systems for marine fish hatcheries," *Aquaculture Asia*, vol. X, no. 3, pp. 27–29, 2005.

[45] A. R. S. Harzevili, H. Van Duffel, P. Dhert, J. Swings, and P. Sorgeloos, "Use of a potential probiotic *Lactococcus lactis* AR21 strain for the enhancement of growth in the rotifer *Brachionus plicatilis* (Muller)," *Aquaculture Research*, vol. 29, no. 6, pp. 411–417, 1998.

[46] R. A. Contreras-Tapia, M. E. Castellanos-Páez, S. Nandini, M. I. Benítez-Díaz-Mirón, and G. Garza-Mouriño, "Enhancing rotifer *Brachionus calyciflorus* population growth with commercial probiotics," *Aquaculture Research*, vol. 51, no. 5, pp. 2102–2111, 2020.

[47] J. Sahandi, P. Sorgeloos, H. Xiao et al., "Possible effects of probiotic strains on suppression of *Vibrio* and enhancement of growth in rotifer, *Brachionus plicatilis*," *Iranian Journal of Fisheries Sciences*, vol. 19, no. 6, pp. 3018–3033, 2020.

[48] P. L. Loo, V. C. Chong, S. Vikineswary, and S. Ibrahim, "Waste-grown phototrophic bacterium supports culture of the rotifer, *Brachionus rotundiformis*," *Aquaculture Research*, vol. 47, no. 10, pp. 3029–3041, 2016.

[49] P. L. Loo, S. Vikineswary, and V. C. Chong, "Nutritional value and production of three species of purple non-sulphur bacteria grown in palm oil mill effluent and their application in rotifer culture," *Aquaculture Nutrition*, vol. 19, no. 6, pp. 895–907.

[50] L. N. Matus, R. V. Rodríguez, L. A. Jiménez et al., "Population density comparison of *Brachionus angularis* (Gosse, 1851) cultured in laboratory at 19°, 21° and 25°C, fed with bacteria produced in Biofloc system," *International Journal of Fisheries and Aquatic Studies*, vol. 5, no. 1, pp. 13–17, 2017.

[51] E. O. Ogello, S. Wullur, Y. Sakakura, and A. Hagiwara, "Composting fishwastes as low-cost and stable diet for culturing *Brachionus rotundiformis* Tschugunoff (rotifera): influence on water quality and microbiota," *Aquaculture*, vol. 486, pp. 232–239, 2018.

[52] V. Kostopoulou, M. Vasilakis, and P. Divanach, "Semi-continuous mass culture of rotifers (*Brachionus plicatilis*) using an automatic feeder," *Aquaculture Research*, vol. 43, no. 1, pp. 91–98, 2012.

[53] F. Hapsari, "The effect of fermented and non fermented biofloc inoculated with bacterium bacillus cereus for catfish (*Clarias gariepinus*) juveniles," *AACL Bioflux*, vol. 9, pp. 334–339, 2016.
[54] Y. Avnimelech, "Carbon/nitrogen ratio as a control element in aquaculture systems," *Aquaculture*, vol. 176, pp. 227–235, 1999.
[55] P. De Schryver, R. Crab, T. Defoirdt, N. Boon, and W. Verstraete, "The basics of bio-flocs technology: The added value for aquaculture," *Aquaculture*, vol. 277, pp. 125–137, 2008.
[56] S. Craig, D. Kuhn, and M. Schwarz, "Understanding fish nutrition, feeds, and feeding," *Virginia Cooperative Extention*, pp. 1–6, 2017.
[57] N. Romano, A. B. Dauda, N. Ikhsan, M. Karim, and M. S. Kamarudin, "Fermenting rice bran as a carbon source for biofloc technology improved the water quality, growth, feeding efficiencies, and biochemical composition of African catfish *Clarias gariepinus* juveniles," *Aquaculture Research*, vol. 49, no. 12, pp. 3691–3701, 2018.
[58] G. R. Robles-Porchas, T. Gollas-Galván, M. Martínez-Porchas, L. R. Martínez-Cordova, A. Miranda-Baeza, and F. Vargas-Albores, "The nitrification process for nitrogen removal in biofloc system aquaculture," *Reviews in Aquaculture*, vol. 12, no. 4, pp. 2228–2249, 2020.
[59] J. M. Ebeling, M. B. Timmons, and J. J. Bisogni, "Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems," *Aquaculture*, vol. 257, pp. 346–358, 2006.
[60] J. Pekkoh, C. Chaichana, T. Thurakit et al., "Dual-bioaugmentation strategy to enhance the formation of algal-bacteria symbiosis biofloc in aquaculture wastewater supplemented with agricultural wastes as an alternative nutrient sources and biomass support materials," *Bioresource Technology*, vol. 359, no. 3, Article ID 127469, 2022.
[61] E. Lubzens, O. Gibson, O. Zmora, and A. Sukenik, "Potential advantages of frozen algae (*Nannochloropsis* sp.) for rotifer (*Brachionus plicatilis*) culture," *Aquaculture*, vol. 133, no. 3-4, pp. 295–309, 1995.
[62] W. D. Nagata and J. N. C. Whyte, "Effects of yeast and algal diets on the growth and biochemical composition of the rotifer *Brachionus plicatilis* (Müller) in culture," *Aquaculture and Fisheries Management*, vol. 23, no. 1, pp. 13–21, 1992.
[63] M. M. Bhosale and R. R. Mugale, "Recirculation aquaculture system for rotifer *Brachionus calyciflorus* production: construction and operation," *North American Journal of Aquaculture*, vol. 84, no. 4, pp. 447–453, 2022.
[64] A. R. A. Razak, C. C. Zaidi, J. Zainoddin, A. M. Mohamed, T. Toda, and O. H. Ross, "The effects of microalgae as live food for *Brachionus plicatilis* (rotifer) in intensive culture system," *Tropical Life Sciences Research*, vol. 29, no. 1, pp. 127–138, 2018.
[65] T. W. Snell, R. K. Johnston, and A. B. Matthews, "Utilizing *Brachionus* biodiversity in marine finfish larviculture," *Hydrobiologia*, vol. 844, no. 1, pp. 149–162, 2019.
[66] T. W. Snell and K. Carrillo, "Body size variation among strains of the rotifer *Brachionus plicatilis*," *Aquaculture*, vol. 37, no. 4, pp. 359–367, 1984.
[67] R. J. V. de Andrade, E. P. dos Santos, G. K. de Almeida Costa et al., "Effect of different frequencies of the addition of *Brachionus plicatilis* on the performance of *Litopenaeus vannamei* in a nursery biofloc system with rice bran (anaerobic and aerobic) as an organic carbon source," *Aquaculture*, vol. 540, 2021.
[68] M. Hill, A. Pernetta, and N. Crooks, "Size matters: a review of live feeds used in the culture of marine ornamental fish," *Asian Fisheries Science*, vol. 33, no. 2, pp. 161–174, 2020.
[69] C. Y. Kuo, C. C. Chang, C. I. Biao, C. Yang, and T. Chen, "Automated system of tubular filtration for rotifer production," *Journal of the World Aquaculture Society*, vol. 53, no. 6, pp. 1145–1154, 2022.
[70] I. C. Zink, P. A. Douillet, and D. D. Benetti, "Improvement of rotifer *Brachionus plicatilis* population growth dynamics with inclusion of *Bacillus* spp. probiotics," *Aquaculture Research*, vol. 44, no. 2, pp. 200–211, 2013.
[71] R. Haché, S. Plante, B. S. Forward, and F. Pernet, "Cod larviculture using high-density rotifer production with different enrichments," *Journal of the World Aquaculture Society*, vol. 48, no. 1, pp. 57–69, 2017.
[72] T. Yoshimatsu and M. A. Hossain, "Recent advances in the high-density rotifer culture in Japan," *Aquaculture International*, vol. 22, no. 5, pp. 1587–1603, 2014.
[73] G. Suantika, P. Dhert, M. Nurhudah, and P. Sorgeloos, "High-density production of the rotifer *Brachionus plicatilis* in a recirculation system: consideration of water quality, zootechnical and nutritional aspects," *Aquacultural Engineering*, vol. 21, no. 3, pp. 201–213, 2000.