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Research Article

Enhancing of *Heteropneustes fossilis* (Bloch, 1794) Fry Nursery Rearing Through Biofloc Technology (BFT): A Sustainable Approach for Bangladesh's Aquaculture Sector

Shaila Akter[1], MD Zobayer Rahman[2*], Farzana Islam[1], Zamal Hussan[1], Rasel Mia[3], Kazi Rabeya Akther[1], Nirmal Chandra Roy[1]

[1]*Department of Fish Biology and Genetics, Sylhet Agricultural University, Sylhet-3100, Bangladesh.*
[2]*Department of Fish Health Management, Sylhet Agricultural University, Sylhet-3100, Bangladesh.*
[3]*Department of Aquatic Resource Management, Sylhet Agricultural University, Sylhet-3100, Bangladesh.*

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SA, FA, ZH: Investigation, methodology. SA, MZR, RM: Data curation. SA, MZR: Writing-original draft preparation. RM, MZR: Formal analysis. RM: Methodology. KRA, NCR: Supervision, fund management. NCR: Conceptualization, project administration, validation, review.

Keywords
Heteropneustes fossilis, Water quality, Biofloc Technology (BFT), Growth parameters, Nursery, Bangladesh

Abstract | Biofloc has recently gained popularity due to its profitable components, mostly focused on developing microorganisms that swiftly convert floc chemicals into feed for *H. fossilis*. The investigation focused on the nursery rearing of *H. fossilis* fry in non-biofloc (T_1) and biofloc (T_2, T_3 and T_4) reared systems for 60 days to examine growth without exchanging water. At the same time, water quality metrics were carefully monitored and maintained. The stocking density in all tanks was 800 fry of *H. fossilis* initial weight of (0.51±0.02 g)/1000 liters of water in an outdoor fish hatchery. During the trial, additional commercial floating powdered feed was administered. Finally, species obtained an average weight of 2.82±0.14 g, a final length of 9.0±0.40 cm, a mean final weight gain of 2.82±0.14 g, and a relative growth rate of 589.67±55.70%, lower FCR of 0.78±0.03 value with maximum growth. These changes took place during the T_2 trial, keeping the feed ratio at 75% feed and 25% floc. The survival rate, on the other hand, remained constant throughout all treatments. Only two parameters (specific growth rate and protein efficiency ratio) were discovered to have maximum output within the control. Throughout the trial, several water parameters were discovered to be optimal in each tank. During the experiment, BFT kept in T_2 (75% feed + 25% floc) trail optimized the most desirable traits of growth for *H. fossilis* fish fry.

Novelty Statement | This study introduces a novel approach for nursery rearing of *H. fossilis* fry in outdoor hatcheries, showing that a 75% feed and 25% floc mixture (T_2) significantly enhances growth metrics-such as weight gain, length, and feed conversion ratio-without water exchange. The findings demonstrate BFT's potential to improve feed efficiency, maintain water quality, and sustain high survival rates, offering a resource-efficient strategy to advance sustainable aquaculture practices.

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Corresponding Author: MD Zobayer Rahman
ronyrahaman320@gmail.com

Introduction

Aquaculture sector is a global option to battle hunger, alleviate poverty, and strengthen countries economic situations (FAO, 2023; Hambrey, 2017). With the world's population expected to reach 9.6 billion by 2050, it is critical to continue aquaculture methods (El-Sayed, 2021). Aquaculture, which currently employs 12% of the global population, is critical in meeting food demands (DoF, 2019). However, with shrinking landmass and waterbodies, extensive aquaculture operations are required to increase economically valuable species (Mugwanya *et al.*, 2021). Bangladesh, which is rated third in the world for fish production, has a favorable aquaculture terrain with plentiful water bodies, rivers, and diversified fisheries biodiversity (FAO, 2022). Small fish, also known as Small Indigenous Species (SIS), are popular in Asian countries due to its great taste, high protein content, and omega fatty acids, which contribute to better health (Puvaneswari *et al.*, 2009). Biofloc Technology (BFT) develops as an economically viable and environmentally sustainable alternative to fulfill rising food demand (Naylor *et al.*, 2000; Avnimelech and Kochba, 2009). BFT entails feeding aquatic organisms in a restricted environment, increasing nutrient recycling, and minimizing water exchange, hence promoting the growth of beneficial heterotrophic bacteria (Azim and Little, 2008; Avnimelech, 2007). This method, known for its high potential output and environmentally favorable structure, has been utilized for a variety of valuable species, including Tilapia, shrimp, and sturgeon (Serfling, 2006; Avnimelech, 2007; Taw, 2010). *Heteropneustes fossilis*, a rare Small Indigenous Species, is threatened by exploitation and environmental changes (Monir and Rahman, 2015; IUCN Bangladesh, 2000). This species has a high commercial value due to its air-breathing organ and nutrient-rich makeup. Recognizing the relevance of the critical larval rearing period in lowering production costs, boosting disease resistance, and improving fish survival quality, this study focuses on the *H. Fossilis* fry in a brief period of time. The use of BFT in nursery setups, which has been demonstrated to aid in the rapid development of shrimp species, is being studied to determine its impact on growth. The purpose of this project is to increase fish output in Bangladesh with BFT, which is in line with sustainable development goals. Despite the economic importance of *H. fossilis* fry, no previous research has explored the potential growth and stocking densities in Bangladesh using BFT. This study aims to fill that void by investigating the usage of BFT in *H. fossilis*. The impacts of *H. fossilis* nursery conditions on water quality markers are being investigated. The results are likely to aid Producers of *H. fossilis* fry are keen in promoting sustainable aquaculture practices in Bangladesh using BFT.

Materials and Methods

Experimental design

The entire experiment (four treatments with three replications) was given twelve concrete cells (5x4x3) ft^3 that were placed outside at the micro hatchery of the Department of Fish Biology and Genetics, Faculty of Fisheries, Sylhet Agricultural University, Sylhet. The experiment lasted 60 days (August to October, 2021). Each tank was cleaned, dried, and replenished with 1000 liters of clean water to a depth of 0.5 meters prior to the investigation. A comprehensive power supply was used to provide the perfect oxygenation system. To aerate the water, two massive air compressors were used.

Floc preparation

The floc was made with Everfresh-Pro Biofloc Exclusive (Blue Weight Biotech LLP, India), which contained 15 billion CFU/g of (*Bacillus subtilis, Bacillus licheniformis,* and *Bacillus pumilis*). An FCO (Fermented Carbon Organic) unit was established in a 200L plastic drum containing 10L water, 100g salt, 40g probiotics, and 400g molasses to induce floc production. After adding manufactured probiotics (Aqua Life-S and Everfresh-Pro Biofloc Exclusive), the floc appeared after 3-7 days. An Imhoff cone was used to determine floc density. Heterotrophic bacteria also absorb additional nutrients, which helps to improve the aquatic environment by lowering nitrogen levels and breaking down biological waste.

Experimental fish and diet supply

The experimental fish, *H. fossilis* fry, was cultivated for 60 days. 10,000 fries were taken from the renowned private fish hatchery of Sreemangal, Moulavibazar district and carried to the fish hatchery in an oxygenated plastic tank for 3 days of acclimation. Fish were fed 10% body weight commercial floating powder feed (crude protein 40%) twice a day (Table 1). The volume of feeding also depends on the feeding response; for example, feeding was halted when fish showed no reaction to feed. Unused feed was collected with a collecting net. Throughout the experiment, there was no water exchange. The control and BFT treatments were prepared (Table 2). The amount of molasses added each day is calculated in accordance to the study of Avnimelech (2011).

Table 1: Feed composition.

S. No.	Proximate composition	Percentage (%)
1	Crude protein	40.0
2	Lipid	5.0
3	Carbohydrates	38.0
4	Ash	5.0
5	Vitamin-Mineral premix	2.0
6	Moisture	10.0

Table 2: Setting up the experiment.

Group	Treatment	Replication	Feeding	Stocking density/tank
Control	Control group (T_1)	3	100% feeding rate	800
BFT	(T_2) BFT 75%	3	BFT + 75% feeding rate	800
	(T_3) BFT 50%	3	BFT + 50% feeding rate	800
	(T_4) BFT 25%	3	BFT + 25% feeding rate	800

Table 3: Growth parameters calculated using the following formulas.

Parameters	Calculation
Weight Gain (gm)	Final Weight (gm) – Initial Weight (gm)
Feed Conversion Ratio (FCR)	Total Feed Given (gm)/ Weight Gain (gm)
Specific Growth Rate (SGR) (% per day)	[{ln (Final Weight) – ln (Initial Weight) /days} × 100]
Relative Growth Rate (RGR) (%)	{(Final Weight – Initial Weight)/ Initial Weight} × 100
Protein Efficiency Ratio (PER)	Wet Weight Gain (gm)/ Protein Consumed (gm)
Survival Rate (%)	(Final Number of Fish)/ (Initial Number of Fish) × 100

Table 4: Water quality parameters (Mean ± SE) during the experimental period (60 days).

Parameters	T_1 (Control)	T_2 (BFT 75%)	T_3 (BFT 50%)	T_4 (BFT 25%)
Temperature (°C)	27.96±0.10	27.92±0.09	27.93±0.09	27.95±0.09
pH	7.34±0.03^b	7.21±0.02^a	7.22±0.03^a	7.22±0.02^a
TDS (ppm)	68.82±0.70^a	162.15±8.27^b	161.93±8.17^b	160.92±8.08^b
DO (ppm)	6.23±0.06^b	5.44±0.10^a	5.31±0.10^a	5.39±0.09^a
NH_3 (ppm)	0.026±0.003	0.025±0.003	0.030±0.003	0.026±0.003
Salinity (ppt)	0.00±.000^a	0.23±0.02^b	0.23±0.02^b	0.24±0.02^b
TAN	1.36±0.14	1.52±0.16	1.72±0.15	1.53±0.14
Floc volume (ppm)	0.00±0.00	10.57±0.46	12.25±0.57	15.79±0.80

Superscripts in an individual row indicate significant differences among the treatments at $P < 0.05$.

Water quality monitoring

Throughout the trial, water quality data were recorded on a regular basis before feed supply, between 7:00 and 8:00 a.m. According to the American Public Health Association (APHA, 2012), the YSI multimeter water quality parameter was employed.

Growth performances parameters

Fish were collected for sampling of various growth indicators, which was followed by (Sarker *et al*., 2016). By the end of the experiment, the total number of fish and all growth parameters had been counted and calculated to determine the growth parameters.

Statistical analysis

All experimental data were analyzed using the Microsoft Excel Analysis tools, IBM SPSS, and the Linear regression approach, and the coefficient of determination (R^2) was calculated to figure out how well the regression model suited the observed data.

Results and Discussion

Water quality

All the water quality parameters were recorded within a favorable range in both the control and treatment trials (Table 4).

The temperature across all treatments, including the control, remained consistent, ranging from 27.92 °C to 27.96 °C. The pH was slightly lower in BFT treatments (T_2-T_4) than the control, ranging between 7.21 and 7.34. TDS was significantly higher in BFT treatments (160.92-162.15 ppm) compared to the control (68.82 ppm). DO was highest in the control (6.23 ppm) and lowest in T_3 (5.31 ppm). Ammonia levels were low across treatments (0.025-0.030 ppm), and salinity was near zero, with minimal differences. TAN was lowest in the control (1.36 ppm) and slightly higher in T_3 (1.72 ppm). Floc volume was zero in the control and highest in T_4 (15.79 ppm).

The BFT treatments had considerably higher TDS values than the control, reflecting the increased biofloc material in the water. Dissolved oxygen was higher in control, with the BFT treatments exhibiting reduced levels, possibly due to increased organic activity in the biofloc systems. The control had no floc volume, while the BFT treatments showed a clear increase in floc volume, with T_4 having the highest volume. The control had lower TAN, while the BFT treatments had higher values, indicating that biofloc impacts the nitrogen levels in the water.

Finally, it can be state that, temperatures were consistent across all treatments, showing no effect from biofloc system. Ammonia (NH_3) levels remained steady, and salinity showed minimal variation among the BFT treatments.

Growth parameters

Biofloc had an impact on the growth of the nursery culture of the stinging catfish (Table 5). The final length was recorded highest in T_2 (BFT 75%) at 9.35±0.40 cm and lowest growth was observed in T_4 (BFT 25%) at 9.03±0. 46cm during the experiment (Figure 1A). The protein efficiency ratio was highest 2.98±0.12 in control T_1 and the lowest efficiency rate in T_2 was 2.05±0.17 (Figure 1B). The relative growth rate was higher in T_2 (BFT 75%) 589.67±55.70 than all treatment trials (Figure 1C). The specific growth rate was observed higher in T_1 (control) and lower in T_4 (Figure 1D). The mean final weight gain was highest in T_2 (BFT 75%) 2.82±0.14g and lowest in T_4 (BFT 25%) 2.32±0.06g (Figure 1F). Prior to the experimental fish had the same size in the initial period but after 35 days of the research, the average weight was highest in T_2 (BFT 75%) 3.25±0.15g and lowest in T_4 (BFT 75%) (2.32±0.12g) (Figure 1F-G). The best feed conversion ratio (FCR) with the lowest value (0.78±0.03) was found in T_2 (Figure 1H).

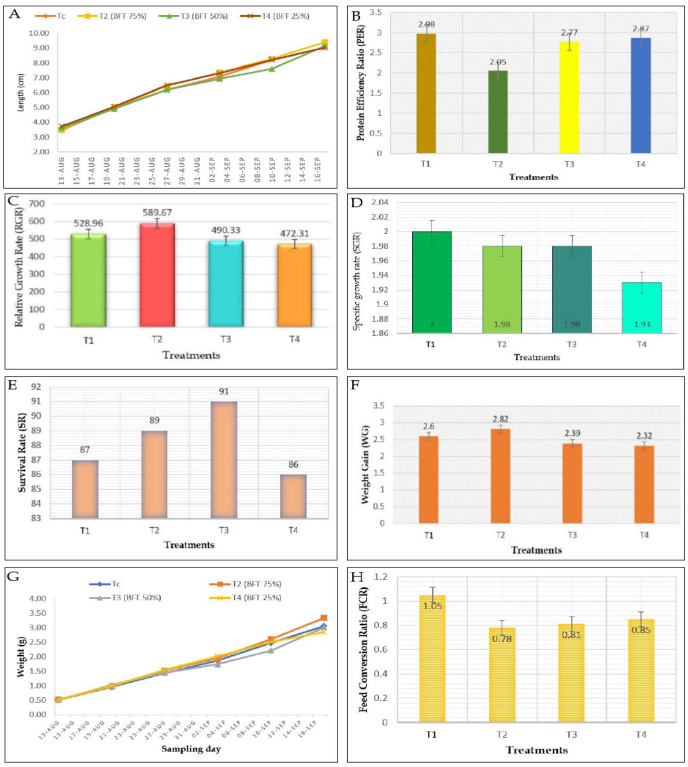

Figure 1: A, Average length (cm) of stinging catfish, *H. fossilis* over 5 weeks culture period and different biofloc treatment; B, Protein efficiency ration (PER); C, Relative growth rate (%); D, Specific growth rate (SGR)%; E, Weight gain (g); F, Average weight (g); G,

Feed conversion ratio (FCR).

The second-degree polynomial regression (Cohen, 1988) line shows the quadratic expression of the curve. To know the relation of the growth factor of biofloc with the regression line. Biofloc and growth factor had interlinkage which express through three varieties of second-degree polynomial regression including strong relation (0.8-0.9), moderate relation (0.5-0.6) and no relation (<0.5).

In this experiment, second-degree polynomial regression analysis had a strong positive relationship with between the specific growth rate, R^2 = 0.9084 (Figure 2C); and feed conversion ratio, R^2 = 0.9055 (Figure 2B). On the other hand, moderate relation was noticed amongrelative growth rate, R^2 = 0.6399 (Figure 2E); protein efficiency ratio, R^2 = 0.5143 (Figure 2D). Also, no relation was noticed at weight gain, R^2 = 0.6657 (Figure 2A). The regression analysis demonstrated that the growth and survival of stinging catfish were influenced by biofloc treatment.

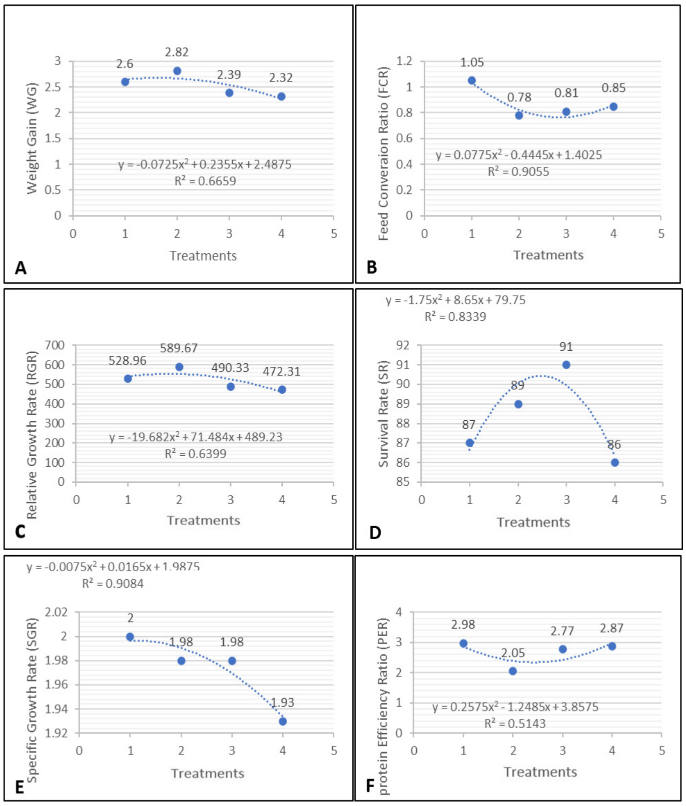

Figure 2: Second-degree polynomial regression between biofloc and weight gain (WG) (A); Feed conversion ratio (FCR) (B); Relative growth rate (RGR) (C); Survival rate (SR) (D); Specific growth rate (SGR) (E); Protein efficiency ratio (PER) (F). R^2 represents the coefficient of determination.

Biofloc technology (BFT) is a good optional operating system for modern aquafarming because it has reduced the amount of cost for supplementary feed, earlier it was assumed that 60% of the cost was needed to cover up the aquaculture production (Zimmermann *et al*., 2023; Lim *et al*., 2003). To keep the biofloc in a safe and good condition water quality maintenance is very crucial. All water

Table 5: Growth parameters of stinging catfish *(H. fossilis)* at fry stage under different treatments of biofloc technology after 60 days culture period.

Parameters	T_1(Control)	T_2(BFT 75%)	T_3(BFT 50%)	T_4(BFT 25%)
Initial weight (g)	0.51±0.02	0.52±0.03	0.51±0.03	0.53±0.03
Final weight (g)	3.11±0.10^{ab}	3.34±0.13^{ab}	2.91±0.07^{a}	2.85±0.06^{a}
Weight gain (g)	2.60±0.11^{ab}	2.82±0.14^{b}	2.39±0.08^{a}	2.32±0.06^{a}
SGR (%)	2.00±0.12	1.98±0.17	1.98±0.16	1.93±0.19
RGR (%)	528.96±37.04	589.67±55.70	490.33±40.96	472.31±41.81
PER (%)	2.98	2.05	2.77	2.87
FCR	1.05±0.05	0.78±0.03	0.81±0.04	0.85±0.04
Survival rate (%)	87.0	89.0	91.0	86.0

Superscripts in an individual row indicate significant differences among the treatments at $P < 0.05$.

quality parameters like Dissolved oxygen, temperature, pH, salinity, TDS, TAN, ammonia etc were found in the optimum range throughout the culture operation (Table 3) which agreed with the research of Boyd (1979). The temperature level monitored by (Keer *et al*., 2018) is similar to our research. The level of pH fluctuated during the period of nursing. Azim and Little (2008) observed the level of pH of *O. niloticus* which is very much suitable with our findings. Dissolved oxygen is a major parameter needed to be maintained for better growth of fish. Also, this DO observe in BFT for better survival for fish (Khanjani *et al*., 2024; Rahman, 2005; Mcgraw*et al*., 2001). Ammonia level is extremely maintained in our experiment because this ammonia residue is a very toxic compound which creates great havoc for fish, this ammonia and TAN components optimum level maintenance followed by the study of (Khanjani *et al*., 2024; Samocha *et al*., 2004). Ammonia concentration converted in the heterotrophic bacterial community in the indoor raceway system during the GIFT tilapia rearing (Felix *et al*., 2015). In commercial species cultures like marine shrimp, the water quality is also maintained in a tolerable range (Lara *et al*., 2017). The water temperature and DO of the hatchery were in an optimum condition of our experiment, relating to this pH sometimes got fluctuated with the water because of the related factor with photosynthesis (Ebeling *et al*., 2006). Continuous mixing of water and to avoid biofloc deposition enough air supply is necessary, excess feed and waste products increase the ammonia concentration (Chen *et al*., 2023; Hargreaves, 2013). Ammonia and other TAN compounds in the experiment were found higher in different treatments compared to the control and this scenario match the findings of (Luo *et al*., 2020; Ekasari, 2009).

Growth parameters of *H. fossils* are found best in BFT T_2 (75% feed + 25% floc) with the best output of FCR, as we know the very little amount of FCR value give the best output in the feed utilization. FCR count with the output of fish production got a brilliant observation with the report of (Xu and Pan, 2012). BFT technology is an additional supplement which develops the growth of desired fish (Wasielesky *et al*., 2006). Also, lowering the FCR value for biofloc systems leads to a positive rate of production (Zablon *et al*., 2022; Das and Ray, 1989). BFT is a very profitable culture system in which useful bacteria convert ammonia to a non-toxic material (e.g. nitrate) that promotes phytoplankton growth. Some growth parameters of GIFT tilapia under the AFM culture system were found to increase with the usage of floc (Long *et al*., 2015). In our experiment, weight gain was observed higher in BFT T_2 (75% feed + 25% floc), a similar observation made by (Gall and Bakar, 1999). Weight gain, FCR, and SGR were good in biofloc with red light treatment (Volpato *et al*., 2013). The common carp exhibits enhanced growth rates and gastrointestinal enzymatic action when bioflocs are included with the meal at BFT 75% (Najdegerami *et al*. 2016). In lowest stocking density rather than the higher stocking density had higher productivity and higher growth performance stated by (Malik *et al*., 2014). Yun *et al*. (2016) conducted that growth and productivity were higher in integrated biofloc culture systems. The result of the research showed the highest SGR value in the trial of T_1 (control) and lower in T_4, but on the other hand, (Oh *et al*., 2007) observed a different scenario compared with our studies, it could be because of the size of fishes or maybe the stocking densities. In biofloc tank addition of carbohydrates improved the growth and survivability but there was no significant difference in SGR, FCR so they thought that carbohydrates enriched the food acceptance by the fish (Avnimelech, 2007; Emerenciano *et al*., 2012; Gao *et al*., 2012). Several positive effects of BFT on various growth parameters were observed previously in a lot of experimental fishes like *O. mossambicus* (Avnimelech, 2007), *M. rosenbergii* (Asaduzzaman *et al*., 2008), *L. vannamei* (Burford *et al*., 2004) and *C. auratus* (Wang *et al*., 2015). During the experiment, the survival rate (%) (Figure 1E) was found similar to the analysis done by (Najdegerami *et al*., 2016). During the experiment zero water exchange was maintained according to the protocol followed by numerous researchers which has been reported in previous studies (Crab *et al*., 2009; Long *et al*., 2015). Additionally, BFT enhances biodiversity, resilience, and the establishment of elevated cultivation facilities without a water outflow during the whole farming season. More studies should be done to determine the best technique to control the BFT in aquaculture systems (Viau *et al*., 2013).

Xu and Pan (2012) demonstrated that shrimp reared in biofloc systems had better FCR compared to those in non-biofloc environments. Ekasari and Maryam (2012) found that *Oreochromis* sp. cultured in biofloc systems had lower mortality due to disease compared to those in conventional systems. A study by (Avnimelech and Kochba, 2009) showed that the additional nutrition provided by biofloc resulted in better growth rates of tilapia when compared to traditional aquaculture systems without biofloc. Crab *et al*. (2009) reported that biofloc technology effectively removed ammonia and nitrite from aquaculture water, resulting in significantly better survival rates of tilapia species.

This experiment suggests that the *H. fossilis* fry rearing in a nursery condition through biofloc technology could be possible and it could lead to a good quality of fish seed for the production system.

Conclusions and Recommendations

Based to the findings, BFT T_2 (75% feed + 25% floc) supplementation improved the growth and survival rate of *H. fossilis* fry reared in the biofloc culture method. BFT increased the specific growth rate, feed conversion ratio, and survival rate. This BFT method has the potential to promote sustainable aquaculture by improving water quality, reducing feed requirements, and increasing productivity to increase profit in shing aquaculture via a better fry supply for future production.

Declarations

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IRB approval

This research was approved, monitored and evaluated by Sylhet Agricultural University Research System (SAURES).

Conflict of interest

The authors have declared no conflict of interest.

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