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Research Article

Effect of C/N Ratio Levels and Stocking Density of Catla Spawn (*Gibelion catla*) on Water Quality, Growth Performance, and Biofloc Nutritional Composition in an Indoor Biofloc System

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A 20-day 3 $*$ 3 factorial experiment was conducted in 100 L HDPE experimental tanks to investigate the effect of the C/N ratio (10, 15, and 20) and stocking density (3, 4, and 5 spawn L^{-1}) on *Gibelion catla* spawn nursery rearing in the indoor biofloc system. Rice bran was used as the carbon source for manipulating C/N ratios. Each treatment was stocked with catla spawn of average length (6.7 ± 0.4 mm) and average weight (1.6 ± 0.2 mg). Water parameters showed that increasing the C/N ratio from 10 to 20 significantly ($p < 0.05$) reduced total ammonia nitrogen (TAN) and nitrite nitrogen (NO_2-N) and increased nitrate nitrogen (NO_3-N) in the water. The insignificant difference ($p > 0.05$) and lowest final average length, average length gain, average weight gain, and specific growth rate (SGR) were recorded in C/N ratios of 10 and 15 compared to a C/N ratio of 20. A significant difference ($p < 0.05$) in survival was observed with the increasing C/N ratio. Increasing the fish stocking density resulted in higher mortality. However, a higher amount of fry produced was observed in the treatments with 3 and 4 spawn L^{-1}. Crude protein content increased significantly ($p < 0.05$) with the increasing C/N ratio with higher content in C/N 20. No significant difference ($p > 0.05$) in proximate composition of biofloc was observed in different stocking density groups. In conclusion, the application of the biofloc technology with a C/N ratio of 20 at a stocking density of 4 spawn L^{-1} could be recommended to increase the production of catla fry in the indoor biofloc system.

1. Introduction

The success of the aquaculture industry relies on a steady supply of high-quality seed as one of its key inputs, accounting for 5–10% of aquaculture production costs [1, 2]. In India, Indian major carps account for 87 percent of the country's total freshwater aquaculture production [3]. Hatchery-produced seed meets demand, but hatcheries are more concerned with quantity than quality, and the spawn-to-fry yield ratio of these species is 3 : 1 in earthen ponds, with catla fry performance always being lower in terms of growth and survival [4–6]. Fish in the nursery stage are also often subjected to intensification which generally increases stress and subsequently susceptibility to infectious disease, hence requiring proper management to obtain optimum growth and survival [7, 8]. Biofloc systems (BFTs) are a potential alternative strategy for the intensification of fish larviculture which enhances immunity of the cultures.

Biofloc technology is studied for its application in nurseries as an additional feed source for postlarvae, to provide extra essential nutrients [9–11] and improve water quality to support better larval survival and growth [12, 13]. BFT has been applied successfully in the nursery phase in different fish and shrimp species such as *Oreochromis niloticus* [12], *Rhamdia quelen* [13], *Clarias gariepinus* [14], *Labeo rohita* [15–17], *Carassius auratus* [8], *Farfantepenaeus brasiliensis* [18], and *Penaeus vannamei* [19].

Maintenance of appropriate carbon to nitrogen (C/N) is of prime importance for optimal performance of culture species in the biofloc system [20]. The addition of the external carbon source manipulates the carbon-to-nitrogen ratio for uptake of nitrogen elements and subsequent conversion to microbial proteins in the BFT system [21–23]. The carbon source and the C/N ratio influence the nutritional content and quality of biofloc [24–26]. An increase in the C/N ratio induces shifting of the biofloc community to a heterotrophic system that effectively maintains TAN and NO_2-N at lower concentrations even at higher stocking densities in the culture water [27–30].

Freshwater fish production under the BFT system is still new, and studies on fish larval development are even rarer [8, 12, 13]. Therefore, the objectives of this study were to evaluate its use in nursery rearing of the *Gibelion catla* at varying C/N ratios and stocking densities in the indoor biofloc system.

2. Materials and Methods

2.1. Experimental Design, Biofloc Preparation, Fish Stocking, and Management. Gibelion catla spawn ($n = 25000$) procured from CIFA, Bhubneshwar, Orissa, India, were transported to Wet Laboratory, Department of Aquaculture, College of Fisheries, Ratnagiri, Maharashtra, India, and the same were acclimatized for 3 days in two-1000 L FRP (fibreglass reinforced plastic) tanks fed with GNOC two times a day and 2 L of green water every day in morning hours. A factorial design (3 × 3) was performed with three C/N ratios (10, 15, and 20) [21] as the first factor and three-level stocking densities, viz., 3, 4, and 5spawn L^{-1} [15] as the second factor in a completely randomized design with triplicates. An experiment was conducted for 20 days in 100 L capacity HDPE (high-density polyethylene) tanks filled with up to 80 L working volume.

Biofloc inoculum was prepared by mixing 5 g L^{-1} pond soil, 10 mg L^{-1} ammonium sulphate, and 200 mg L^{-1} carbon source (rice bran) [27] in a 500 L FRP tank filled with ground water up to 300 L. When biofloc concentration reached 20 ml L^{-1}, it was used for inoculation in the already prepared experimental tanks at a rate of 5 L of inoculum to 75 L of freshwater. The rice bran (33.33% carbon) was added once in a day based on the calculation described by De Schryver *et al.* [21] to maintain the desired C : N ratio in the BFT treatment during the experiment. An air pump (HAILEA, Model: HAP-60) having a capacity of about 60 W was used for aeration to meet oxygen demand of the fishes and keep the flocs in continuous suspension.

For the experiment, 8640 spawn (*Gibelion catla*) were used and stocked at a rate of 3, 4, and 5 spawn L^{-1}, respectively, after acclimatization. A subsample of spawn was taken from the stock and weighed using an electronic digital balance (Himedia; precision: 1 mg) to determine the initial live body weight which was average (1.6 ± 0.2 mg). Locally available GNOC and rice bran powdered, sieved, and mixed in a 1 : 1 ratio were used as a feed source for spawn. The fishes were fed in the morning at 10:00 am and evening 4:00 pm at a rate of 400% body weight per day for first five days and 800% body weight per day for next fifteen days [15]. In biofloc tanks, no regular water exchange was carried out except the addition of water to compensate the evaporation loss.

2.2. Physicochemical Parameters of Water. Water quality parameters such as temperature, pH, dissolved oxygen (DO), alkalinity, total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), and nitrate nitrogen (NO_3-N) were monitored every fifth day during the experimental period. pH and temperature of the water were estimated using a universal indicator and a mercury thermometer, respectively. Dissolved oxygen and alkalinity of water were estimated as per the standard procedures [31]. Total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), and nitrate nitrogen (NO_3-N) were determined with API® commercial test kits (API® freshwater master test kit). For total suspended solid (TSS) estimation, 100 ml of the water sample was collected from each replicate and filtered through a predried and weighed glass fibre (GF/C) filter paper using the micropore vacuum filter. Floc volume was measured by allowing the floc to settle down in the Imhoff cone for 20 minutes without disturbance [32].

2.3. Growth Analysis. Initial weight and length of spawn were taken. All surviving fry were counted at the end of the experiment. The average weight gain, percent weight gain, average length gain, percent length gain, specific growth rate (SGR), and survival were calculated using the following equations:

$$
\begin{aligned}
\text{average weight gain (mg)} &= \text{final weight (mg)} - \text{initial weight (mg)},\\
\text{weight gain (\%)} &= \left[\frac{(\text{final weight} - \text{initial weight})}{\text{initial weight}}\right] \times 100,\\
\text{average length gain (mm)} &= \text{final length (mm)} - \text{initial length (mm)},\\
\text{length gain (\%)} &= \left[\frac{(\text{final weight} - \text{Initial weight})}{\text{initial weight}}\right] \times 100,\\
\text{SGR}\left(\frac{\%}{\text{da y}}\right) &= \left[\frac{(\ln \text{finalweight} - \ln \text{initial weight})}{\text{number of da ys}}\right] \times 100,\\
\text{survival (\%)} &= \left(\frac{\text{total number of survived fish}}{\text{total number of fish stocked}}\right) \times 100,\\
\text{apparent feed conversion ratio (AFCR)} &= \frac{\text{total feed intake}}{\text{weight gain}}.
\end{aligned}
\tag{1}
$$

2.4. Proximate Composition of Biofloc. At the end of the experiment, concentrated floc samples were collected from each tank using a 100 μm mesh and dried in an oven at 60°C and then preserved in a refrigerator till the proximate analysis was performed [33]. The nutritional contents of biofloc such as crude protein (Kjeldahl method), crude lipids [34], ash content [35], and moisture [35] were analysed.

2.5. Statistical Analysis. The experimental data such as length gain, weight gain, SGR, and survival percentage were analysed by two-way analysis of variance. Differences were considered significant at $p < 0.05$. If difference was found significant, the means were compared by Tukey's test. The statistical analysis was performed by using SPSS 16.0.

3. Results

3.1. Water Quality Parameters. The mean values of water quality parameters and outcomes of two-way ANOVA are presented in Table 1. There was a significant ($p < 0.05$) effect of the C/N ratio and stocking density on the water quality in biofloc treatments. Furthermore, there was no significant ($p > 0.05$) interaction effect of the C/N ratio and stocking density on the water quality of different treatments. Increasing stocking densities significantly ($p < 0.05$) reduced DO content in water. pH was significantly different ($p < 0.05$) among different C/N ratios with reduced pH at a higher C/N ratio, but no significant difference ($p > 0.05$) was observed in stocking densities. Total alkalinity reduced significantly ($p < 0.05$) with an increase in the C/N ratio, but no significant difference ($p > 0.05$) was observed in C/N 15 and 20. A higher stocking density showed a significantly lower ($p < 0.05$) value of total alkalinity. The increase in the C/N ratio significantly ($p < 0.05$) reduced TAN and NO_2-N. However, the level of TAN did not vary significantly ($p > 0.05$) in stocking densities. Levels of NO_2-N increased significantly ($p < 0.05$) with an increase in the stocking density from SD3 to SD5. The NO_3-N level was significantly ($p < 0.05$) lower in C/N 10, but no significant difference ($p > 0.05$) was observed in C/N 15 and 20. A significant ($p < 0.05$) increase in the level of NO_3-N was observed with increasing stocking densities of catla spawn. TSS was significantly ($p < 0.05$) lower in C/N 10, and no significant difference ($p > 0.05$) was observed in C/N 15 and 20. However, TSS increased significantly ($p < 0.05$) with an increase in the stocking density. Floc volume increased significantly ($p < 0.05$) with an increase in both the C/N ratio and stocking density.

3.2. Effect on Survival and Growth Parameters of Gibelion catla Fry. Growth performance of catla spawn in the biofloc system after 20 days of the rearing period is presented in Table 2. Based on two-way ANOVA, there was a significant ($p < 0.05$) effect of the C/N ratio and stocking density on growth performance and survival of spawn. But there was no significant ($p > 0.05$) interaction between C/N ratios and stocking densities on the growth performance and survival of spawn in the biofloc treatments. The final average body weight, weight gain, percent weight gain, final length, length gain, percent length gain, and specific growth rate (SGR) were significantly ($p < 0.05$) higher, and AFCR was significantly the lowest ($p < 0.05$) in C/N 20. No significant difference ($p > 0.05$) was observed in growth performance, SGR, and AFCR of spawn at C/N 10 and C/N 15. An increase in the C/N ratio significantly ($p < 0.05$) increased the survival rate. The stocking density influenced the growth parameters differently. The highest growth performance in terms of average final weight, weight gain, percent weight gain, and specific growth rate (SGR) was obtained in the lower stocking density of SD3 except for SD4 which did not show a significant difference ($p > 0.05$) with the spawn of the former group. AFCR of fish was significantly ($p < 0.05$) lower at a lower stocking density of SD3. Significantly ($p < 0.05$) higher final length, length gain, and percent length

Table 1: Water quality parameters recorded from different experimental groups during the experimental period based on two-way ANOVA.

Water parameters	C/N ratio			Stocking density (no.'s L^{-1})			Two-way ANOVA		Interaction
	10	15	20	3	4	5	C/N	SD	C/N × SD
Temperature (°C)	26.79 (25.1–28.2)	26.76 (25.0–28.1)	26.81 (25.0–28.1)	26.74 (25.0–28.0)	26.80 (25.1–28.2)	26.82 (25.1–28.1)	NS	NS	NS
DO (mg L^{-1})	5.9^{a} (5.4–6.2)	5.8^{a} (4.6–6.2)	5.7^{a} (4.6–6.2)	5.9^{A} (4.6–6.2)	5.8^{AB} (4.8–6.2)	5.7^{B} (4.6–6.2)	NS	**	NS
pH	7.70^{a} (7.0–8.5)	7.70^{a} (7.0–8.5)	7.40^{b} (6.5–8.5)	7.70^{A} (7–8.5)	7.50^{A} (6.5–8.5)	7.60^{A} (6.5–8.5)	**	NS	NS
Total alkalinity (mg L^{-1})	42.67^{a} (28–56)	39.06^{b} (26–52)	37.17^{b} (22–52)	41.22^{A} (24–56)	40.67^{A} (26–54)	37.00^{B} (22–54)	**	**	NS
TAN (mg L^{-1})	0.26^{a} (0.091–0.560)	0.24^{ab} (0.085–0.559)	0.22^{b} (0.050–0.754)	0.24^{A} (0.05–0.48)	0.25^{A} (0.073–0.472)	0.26^{A} (0.068–0.754)	**	NS	NS
Nitrite-N (mg L^{-1})	0.030^{a} (0.012–0.065)	0.025^{b} (0.010–0.057)	0.022^{c} (0.01–0.042)	0.019^{C} (0.01–0.031)	0.023^{B} (0.01–0.038)	0.035^{A} (0.011–0.065)	**	**	NS
Nitrate-N (mg L^{-1})	12.27^{b} (2.27-29.76)	13.76^{a} (2.34-28.98)	14.45^{a} (3.64-29.75)	11.27^{C} (2.29-26.64)	13.40^{B} (3.19-28.74)	15.81^{A} (2.27-29.76)	**	**	NS
TSS (mg L^{-1})	98.35^{b} (28.2–204.49)	124.29^{a} (28.4–240.19)	125.40^{a} (39.1–250.98)	93.55^{C} (28.2–198.23)	113.80^{B} (43.92–235.89)	140.68^{A} (52.89–250.98)	**	**	NS
Floc volume (ml L^{-1})	3.09^{c} (0.5–9.0)	4.31^{b} (0.5–12)	5.49^{a} (1–15)	2.33^{C} (0.5–6)	4.56^{B} (0.5–9)	6.01^{A} (0.75–15)	**	**	NS

The mean values followed by the different superscript letter in each factor indicate significance at $p < 0.05$. If the effects were significant, ANOVA was followed by Tukey's test. **Indicates a significant difference at $p < 0.05$. NS, not significant. ^{a,b,c}Values of water parameters of C/N ratio groups in a row with different superscripts differ significantly ($p < 0.05$). ^{A,B,C}Values of water parameters of stocking density groups in a row with different superscripts differ significantly ($p < 0.05$).

Table 2: Nursery production performance of *Gibelion catla* cultured in biofloc systems with different C/N ratios (10, 15, and 20 : 1) and stocking density (3, 4, and 5 no.'s L^{-1}) for 20 days based on two-way ANOVA.

Growth performance	C/N ratio			Stocking density (no.'s L^{-1})			Two-way ANOVA		Interaction
	10	15	20	3	4	5	C/N	SD	C/N *x* SD
Average initial length (mm)	6.7 ± 0.4^{a}	6.7 ± 0.4^{a}	6.7 ± 0.4^{a}	6.7 ± 0.4^{A}	6.7 ± 0.4^{A}	6.7 ± 0.4^{A}	NS	NS	NS
Average final length (mm)	18.34 ± 0.83^{b}	18.77 ± 1.21^{b}	19.76 ± 0.89^{a}	19.86 ± 0.93^{A}	18.91 ± 0.94^{B}	18.10 ± 0.80^{B}	**	**	NS
Average length gain (mm)	11.68^{b}	12.09^{b}	13.08^{a}	13.19^{A}	12.23^{B}	11.42^{B}	**	**	NS
Percent length gain (%)	174.73^{b}	181.19^{b}	195.99^{a}	197.47^{A}	183.3^{B}	171.14^{B}	**	**	NS
Average initial weight (mg)	1.6 ± 0.2^{a}	1.6 ± 0.2^{a}	1.6 ± 0.2^{a}	1.6 ± 0.2^{A}	1.6 ± 0.2^{A}	1.6 ± 0.2^{A}	NS	NS	NS
Average final weight (mg)	56.09 ± 9.83^{b}	61.02 ± 14.90^{b}	76.69 ± 15.38^{a}	75.17 ± 16.22^{A}	64.01 ± 15.83^{AB}	54.62 ± 7.98^{B}	**	**	NS
Weight gain (mg)	54.49^{b}	59.42^{b}	75.09^{a}	73.57^{A}	62.41^{AB}	53.02^{B}	**	**	NS
Percent weight gain (%)	3405.50^{b}	3713.60^{b}	4693.10^{a}	4598.10^{A}	3900.70^{AB}	3313.50^{B}	**	**	NS
SGR (%/day)	7.33^{b}	7.48^{b}	7.96^{a}	7.92^{A}	7.58^{AB}	7.29^{B}	**	**	NS
AFCR	4.21^{a}	3.94^{a}	3.10^{b}	3.18^{B}	3.78^{AB}	4.29^{A}	**	**	NS
Survival (%)	66.85^{c}	70.12^{b}	74.68^{a}	73.43^{A}	71.01^{A}	67.22^{B}	**	**	NS

The mean values followed by the different superscript letters in each factor indicate significance at ($p < 0.05$). If the effects were significant, ANOVA was followed by Tukey's test. **Indicates a significant difference at $p < 0.05$. NS, not significant. ^{a,b,c}Values of nursery production performance of *Gibelion catla* cultured in biofloc systems of C/N ratio groups in a row with different superscripts differ significantly ($p < 0.05$). ^{A,B,C}Values of nursery production performance of *Gibelion catla* cultured in biofloc systems in stocking density groups in a row with different superscripts differ significantly ($p < 0.05$).

gain were observed in a lower stocking density of SD3. An increase in the stocking density significantly ($p < 0.05$) reduced the survival percent of spawn. However, no significant difference ($p > 0.05$) in survival was observed for SD3 and SD4 for catla fry production. The interaction of the C/N ratio and stocking density was not significant ($p > 0.05$) for all the growth parameters.

3.3. Proximate Composition of Biofloc. The results of proximate compositions of biofloc are presented in Table 3. Based on two-way ANOVA, there is a significant effect of the C/N ratio on the proximate composition of biofloc with crude protein content increased significantly ($p < 0.05$) with the increasing C/N ratio, and the ash content was significantly ($p < 0.05$) lower in the higher C/N ratio group. The C/N 15 group did not show any significant difference for crude protein and ash content with C/N 10 and 20 groups. No significant difference ($p > 0.05$) in moisture and crude lipid content was observed for various C/N groups. There is no significant ($p > 0.05$) effect of the stocking density, C/N ratio, and stocking density interaction on the biofloc composition.

4. Discussion

4.1. Water Quality. Water quality is strongly influenced by the stocking density of the cultured animal, environmental parameters, species combination, and quality and quantity of nutritional input added to the system [25, 36]. In the present study, temperature remained within the range (23.0–28°C) required for culture of catla spawn [37, 38]. Fluctuations in pH, DO, and nitrogenous waste concentrations are usual features of biofloc systems [33, 39]. Increasing weight of culture species and boosted bacterial population often decreases DO of the BFT system, as observed in the present study at a higher stocking density [40, 41]. A higher DO level reduces larger and compact floc sizes into smaller ones allowing fish to easily consume the floc and hence enhances fish growth [42, 43]. The higher DO content in biofloc treatment with lower stocking (SD3) might have resulted in the reduction of floc size and have provided better opportunity for spawn to consume floc and enhance growth. An increase in the nitrification process and respiration rate of heterotrophic microorganisms reduces pH and alkalinity of the culture system, which was reflected in this study with the increased C/N ratio [13, 25, 28, 44, 45]. pH below 7 negatively affects the nitrification rate and the growth of the cultured species [46, 47]. The average pH was in the suitable range (7.40–7.70) as reported in earlier studies for nursery rearing of spawn in indoor systems [6] except for reduced pH and alkalinity in a higher stocking density, which might have affected the survival and growth of spawn in the experimental units due to acid stress as the pH effect is related to age and development, and larval stages are most sensitive to pH changes [48, 49]. The increase in heterotrophic bacteria increases acid production through the nitrification process and consumes alkalinity in the intensive biofloc system which reduced total alkalinity with the increased C/N ratio and stocking density [50]. The TAN and NO_2-N levels were within the safe range for catla spawn [51]. Furthermore, reduction of TAN with the increasing C/N ratio shows that the TAN-N levels were influenced by varying C/N ratios as during microbial assimilation, certain microbes present in the biofloc has potential to assimilate TAN into microbial biomass, and that higher carbon inputs support faster production of heterotrophic bacteria, thus converting dissolved nitrogen into bioflocs [28, 52, 53]. Although there was no significant difference in TAN-N concentration in different stocking densities, a higher mean concentration of nitrogenous compounds in a higher stocking density could be the result of higher biomass in the system and retarded development of nitrifying bacteria before biofloc formation [50, 54, 55]. The presence of more nitrifying bacteria converts ammonia to nitrite and then nitrite to nitrate which might have resulted in increased nitrate-N concentration at a higher stocking density in BFT [28, 56]. Becerril–Cortés et al. [57] recorded similar nitrate-N concentration for tilapia fry.

The C/N ratio and stocking density have a significant effect on the TSS and floc volume. In zero water exchange tanks, TSS tends to increase over time primarily due to decreased water exchange, a high amount of organic substances, and an increase in microbial biomass [58]. Excessive TSS levels can become detrimental, particularly with some fish; however, the ideal TSS concentration for fish in biofloc is not determined [56, 59]. The TSS concentration up to 300 mg/L showed no negative effect on growth performance of goldfish larvae [8]. An increase in the TSS concentration affects the growth performance of fish larvae; however, lower TSS levels provide a more nutritious food source for the larvae [13]. The increase in the C/N ratio and stocking density increased TSS and floc volumes which might have reduced the survival of spawn at a higher stocking density. Hosain et al. [56] observed lower survival of *Macrobranchium rosenbergii* postlarvae at a higher floc volume of up to 10 ml/L when molasses and wheat bran were used as a carbon source. Similarly, in the present study, floc volume increased over time, which might have led to reduced survival of catla spawn at higher stocking densities.

4.2. Effect on Survival and Growth Parameters of Gibelion catla Fry. *Gibelion catla* is a surface feeder and can also explore the middle and bottom layers of water [60, 61]. Alikunhi [62] designated species as a surface and midwater feeder. Catla spawn starts feeding on plankton (mainly zooplankton) from the third day after hatching. Defining the biofloc, it is the heterotrophic conglomeric aggregation of microbial communities, such as phytoplankton, bacteria, and living and dead particulate organic matter, and the presence of zooplankton species such as rotifers, moina, daphnia, copepods, and freshwater infusoria makes the BFT system prosper for rearing of spawn [17, 63].

The present study is the first to analyse the nursery rearing of catla spawn in the BFT system. Different C/N ratios significantly affect the growth of catla spawn, and the performance was better in the C/N 20 treatment. As bacteria

Table 3: Proximate composition of biofloc (% dry weight) collected from *Gibelion catla* based on the biofloc technology (BFT) system after 20 days of the experimental period.

Biofloc proximate (%)	C/N ratio			Stocking density (no.'s/L)			Two-way ANOVA		Interaction
	10	15	20	3	4 (A)	5 (A)	C/N	SD	C/N × SD
Moisture	86.7^{a}	87.1^{a}	87.4^{a}	87.3^{A}	86.1	87.8	NS	NS	NS
Crude protein	22.66^{b}	24.26^{ab}	26.53^{a}	24.60^{A}	24.32	24.52	**	NS	NS
Crude lipid	1.8^{a}	2.1^{a}	2.2^{a}	2.0^{A}	2.0	2.1	NS	NS	NS
Ash	11.4^{a}	10.8^{ab}	10.5^{b}	10.9^{A}	11.1	10.7	**	NS	NS

The mean values followed by the different superscript letters in each factor indicate significance at $p < 0.05$. If the effects were significant, ANOVA was followed by Tukey's test. **Indicates a significant difference at $p < 0.05$. NS, not significant. ^{a,b,c}Values of the proximate composition of biofloc of C/N ratio groups in a row with different superscripts differ significantly ($p < 0.05$). ^{A,B,C}Values of the proximate composition of biofloc of stocking density groups in a row with different superscripts differ significantly ($p < 0.05$).

need about 20 units of carbon per unit of nitrogen assimilated [27], in this study, it is confirmed that a C/N ratio of 20 : 1 favored biofloc promotion which acts as a supplemental food source available 24 hrs [33, 64–66]. The improvement in growth of catla spawn at C/N 20 could be associated with the presence of adequate natural protein-lipid source and other nutrients at any time as observed for *Carassius auratus* larvae at C/N ratios of 20 and 25 : 1 and *Labeo rohita* spawn at C/N 15 and 20 [17], whereas the insignificant difference in the C/N 10 and 15 groups might indicate the limited number of prey production as reduction in organic carbon addition can slow down the formation of floc as observed with lower floc volume at a lower C/N ratio in the present study [29, 67], or consumption of biofloc does not contribute to growth of the fish [12]. Biofloc is known to improve feed utilisation of fish by supplementing essential amino acids, vitamins, lipids, and minerals and stimulates digestive enzyme activity resulting in improved digestion of nutrients in the fish gut [68]. Lower AFCR at a higher C/N ratio of 20 in the present study confirms biofloc contribution as complementary natural feed and suggests for improved feed utilisation in the fish reared in the biofloc system [25]. The stocking density is one of the important factors determining the survival, growth, and final biomass of the culture [69]. In the present study, a lower stocking density of SD3 resulted in improved growth performance as compared to other groups. The results agree with the findings of Dey *et al.* [17] who observed enhanced growth of *Labeo rohita* spawn in terms of final length, final weight, and SGR at a lower stocking density (1, 2, and 3 spawn L^{-1}) than at a higher stocking density (4 and 5 spawn L^{-1}) in biofloc systems. Impaired growth of goldfish larvae with an increase in the stocking density in the BFT system was recorded by Besen *et al.* [8]. The average total length and weight of fry after 20 days of rearing were in the range of 18.10–19.86 mm and 54.62–76.69 mg, respectively (Table 2). The growth performance was better with higher length and weight gain obtained in short duration than fry rearing under normal indoor conditions; hence, reducing the rearing period in indoor can increase production. The higher performance of the spawn in the BFT system indicates that bioflocs were consumed by the spawn and optimized growth. In this study, AFCR is influenced by the stocking density. AFCR significantly increased in a high stocking density of the SD5 group, which might indicate a reduction or decrease in efficiency of the fish to graze microbial community at higher density [70, 71]. No significant difference in AFCR and growth performance of spawn in the SD4 group with SD3 might indicate biofloc consumption as supplemental feed, and favorable effects of biofloc such as better water quality, "antistress" probiotic effect, and presence of exogenous microbial enzymes and endogenous digestive enzymes may promote digestion of food and improve the performance of fish in the biofloc system as reported in earlier studies [68, 72].

Survival is considered the most important parameter for culturing success during the larval and nursery phases. Higher survival at C/N 20 compared to a lower C/N ratio could be due to better water quality and greater availability of prey in the BFT system [8, 56, 73]. The consumption of microbial floc enhances larvae tolerance to environmental stress, hence, improving survival [73, 74]. Fry survival showed an inverse relationship with the stocking density. A higher stocking density affects the growth performance of spawn mainly due to physiological stress caused due to overcrowding which leads to competition for food and space and poor water quality [4, 37, 75]. A similar decreasing trend in survival was also obtained with catla and rohu fry stocked at a rate of 100, 125, and 150 larvae/15-L aquarium, respectively, in the recirculating system [76]. In the present study, SD4 showed an insignificant difference with SD3; similarly, survival was unaffected by increasing stocking densities of goldfish larvae from 10 larvae L^{-1} to 30 larvae L^{-1} in the BFT system, suggesting adoption of a higher stocking density for larger quantity of larvae production [8]. Also, indoor rearing allows for intensive seed rearing as it prevents higher mortality due to predation and ecological problems [4].

4.3. Proximate Composition of Biofloc.

The proximate composition of microbial flocs varies according to the carbon source, proximal feed composition, environmental conditions, culture time, and other factors [77, 78]. In this study, biofloc collected from different treatments showed nutritional values with 22.66 to 26.53% crude protein, 1.83 to 2.17% crude lipid, and 10.5 to 11.4% ash. The significant difference in biofloc nutritional composition in different C/N ratio treatments of the present study indicates that the amount of carbon source affects the nutritional composition of microbial floc. The stocking density in the biofloc system did not influence the biofloc nutritional composition. The C/N 20 treatment protein and ash content were similar with those of Romano et al. [79] when using raw rice bran as a carbon source for the culture of African catfish, and C/N 10 and C/N 15 showed similar content with that of Megahed and Mohamed [80] when shrimp feed with 25% protein feed and C/N ratio of 12.1. Higher crude protein in the C/N20 treatment could be due to the richness of microorganism in the treatment [56], but differences in nutritional compositions among different treatments might be due to difference in microbial community [81]. In the present study, the crude lipid and ash content were in the range as observed by Becerril–Cortés *et al.* [57] in rice bran-developed biofloc except for lower crude protein. Lipid is the important source of metabolic energy for growth of fish. Lower lipid with higher floc volume can cause decrease in survival as observed in this study [56], though higher values of crude lipid in a higher C/N ratio might have attributed to higher growth and survival of spawn compared to other treatments.

5. Conclusion

The present study demonstrates that increasing the C/N ratio to 20 : 1 can improve the nursery rearing of *Gibelion catla* in the biofloc system at a stocking density of 3 spawn L^{-1}. Biofloc conditions support the microbial protein content which is known to be highly nutritious for the spawn during their nursery rearing. It is likely that catla spawn were able to obtain this nutrition for their growth. The best growth results are obtained with a stocking density of 3 spawn L^{-1}. However, an increase in a stocking density of 4 spawn L^{-1} can be supported with the increased survival percentage of fry.

Data Availability

The data supporting the findings of the study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest in the study.

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