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Domestication Process Influencing the Growth, Gonadal Development and Haematology of the Endangered Spiny Eel (*Mastacembelus armatus*)

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ABSTRACT

A study was conducted to determine the suitable rearing technique for the endangered *Mastacembelus armatus*, also known as spiny eel, in captive conditions. Three different domestication methods were used: T_1 (control), T_2 (muddy bed) and T_3 (biofloc). A total of 90 adult *M. armatus* fish were obtained from the Narashunda River in the Kishorganj area of Bangladesh. After 90 days, all treatments showed a decrease in growth tendency, but T_3 recorded higher growth performance than the other two treatments. Moreover, gonadal development progress was higher in T_3, as indicated by the gonado-somatic index (GSI) and histology study. However, muddy bed conditions in T_2 led to fish disease infestation, which was observed through liver histology. Among several hematological parameters, glucose was significantly lower in T_2 than in the other two treatments, while cholesterol significantly decreased in both T_2 and T_3 compared to T_1. However, hemoglobin did not significantly vary among all treatments. Higher RBC and lower WBC values in T_3 also indicate comparatively suitable rearing conditions than the other treatments. In conclusion, the results of the study suggest that T_3 (biofloc) treatment can be a better option for *M. armatus* domestication. This method can be used by farmers and breeders to rear *M. armatus* in captive conditions, which can ultimately be helpful for the conservation of this species.

INTRODUCTION

Freshwater eels are highly valued as a food fish worldwide including Bangladesh; they have been extensively used in experimental research in various fields of fish physiology. Due to their lucrative size and high protein content, the *M. armatus*, also known as the spiny eel and locally referred to as Shalbaim, is highly preferred by many people in Bangladesh as a table fish. Eel flesh is also reported to have a high caloric value of up to 303Kcal 100g-1 **(Jahan *et al*., 2020)**.

This nocturnal species thrives in various aquatic environments, such as highland streams, lowlands, wetlands, still waters, coastal marshes, and rivers with sandy or rocky riverbeds and heavy vegetation **(Mollah *et al*., 2013)**. They tend to reside in canals, lakes, and other floodplain areas during the flood season. The freshwater spiny eel is commonly found throughout Bangladesh, especially in mud holes in shallow beels and boro-paddy fields in Sylhet, Mymensingh, and Tangail districts **(Rahman, 1989; Chakraborty *et al*., 2019)**. As nocturnal carnivores, they feed on benthic insect larvae, earthworms, blackworms, small- sized teleosts and molluscs and some submerged plant materials **(Nasar, 1997; Gupta & Banerjee, 2016; Absar *et al*., 2020)**. It was also reported that small dead fish and dead small shrimps are suitable food items for freshwater eels **(Narejo, 2003; Miah *et al*., 2013)**. Freshwater spiny eels can play a significant role in the socio-economic welfare of the area, and urban fisheries can be developed to leverage this potential. The species commands a good market value when sold alive as food and ornamental fish in domestic and international markets including the Bangladesh market. Therefore, it has a high production potential for aquaculturists.

However, in recent years, anthropogenic activities such as habitat modification, dam construction, introduction of invasive species, and overexploitation have had a profound impact on the population dynamics of freshwater fishes worldwide including the spiny eel **(Maitland, 1995; Suresh *et al*., 2006; Abujam & Biswas, 2011)**. It is crucial to take timely action to mitigate the man-made adversities that affect these ichthyofaunal resources and prevent further decline in their natural stocks. Therefore, *M. armatus* has been categorized as an endangered fish species, highlighting the need for immediate conservation measures **(IUCN, 2015)**.

In this regard, aquaculture which is the fastest growing production globally represents a viable alternative to boost the productivity of fish **(FAO, 2020)**. To protect the endangered *M. armatus* species from extinction is crucial, and developing its culture technique can aid in its conservation. Culture is dependent on the seed supply, which relies only on the natural sources for this species, and that is not enough. Therefore, the collection of fry and fingerlings from hatchery sources could be potential for pond aquaculture which is still unexplored. Previously, **Narejo *et al*. (2002)** and **Rahman *et al*. (2004)** investigated the reproductive biology of *M. armatus* under laboratory conditions. **Mollah *et al*. (2013)** conducted a preliminary artificial breeding trial but did not observe any hatching of eggs despite successful fertilization. Unfortunately, no significant progress has been made in Bangladesh to bring this species to aquaculture or restore its natural habitat for conservation. While, some entrepreneurs recognize the tremendous potential of this species for aquaculture, the lack of basic scientific information on its biology has hindered progress. To develop culture or breeding techniques, the first step should be domesticating the fish in a captive condition, which will open the door to sustainable fish production. However, previous findings have shown that traditional

rearing in the captive condition was not satisfactory, and in some cases, it led to decreased growth and feeding cessation. Therefore, this study aimed to identify a suitable domestication process for *M. armatus* through three different methods in the captive condition.

MATERIALS AND METHODS

1. Experimental site and study period

A total of 90 adult *M. armatus* fish were collected from Narashunda River, Kishoreganj, Mymensingh, Bangladesh through local fishermen. The fish were transported to the experimental site of the department of the Genetics and Fish Breeding Laboratory, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh with proper care. They were stocked in tanks (500L) for domestication. Fish were disinfected using potassium per manganate before releasing to the tank. Fish were acclimatized for 15 days before starting the experiment.

2. Design of experiment

Fish were reared for 90 days from the 14th of May to the 13th of August 2021 and subjected to three different treatments, with three replications for each. Fish specimens were kept in tanks with fresh water as control (T_1), in freshwater with muddy bottom (T_2), and in biofloc media (T_3), where carbon nitrogen ratio of 15 was maintained. The size of the experimental plastic circular tanks (500L) was 3.14×0.38m×0.38m× 0.71m/ each. Fish of all treatments were fed with commercial feed and live tubificid worm, at a ratio of 1:2, respectively, as *M. armatus* do not take much commercial feed in captive condition. Tubificid worms were selected due to their carnivorous nature and dependency on insects **(Gupta & Banerjee, 2016)**. Each tank was designed with good inlet and outlet system for facilitating at least 50% of water exchange weekly. Shades were provided to prevent excess heat of sunlight. Special shelter of 0.91m long PVC pipe having a diameter of 10cm for hiding was also provided in the tanks. Stocking density was maintained at 10-fishes/tank by random selection. As the fishes are nocturnal in habit **(Mollah *et al*., 2013)**, feeds were supplied in the early morning and in the evening. (Fig. 1).

2.1. Tubificid worm development

Tubificid worms were cultured in a recirculatory system to ensure the continuous water supply and to avoid the contamination alongside the experimental setup for feeding the fish. It ensured the regular supply of feed to the fishes. As experimental fish are carnivorous, tubificid worms could be the ideal live feed for them. At first, tubificid worms were collected from local drains of BSMRAU and cleaned by water flow and held in a water flow-through-system for 24hrs. The worms were cultured in rectangular plastic bowls with continuous water flow, and adjusted through the plastic pipes. Adapted from the research conducted by **Alam *et al*. (2021)**, the media contained 5kg of rice bran + 5

kg of cow dung + 100g of yeast powder for the primary growth. The ingredients were mixed with water and kept for 4 days to enhance decomposition. After 15 days of primary culture worms were transferred to the bowls with clear water, where semi-solid commercial fish feed were provided for their nutrition. Bowls were set in the multistoried iron frame to save the space.

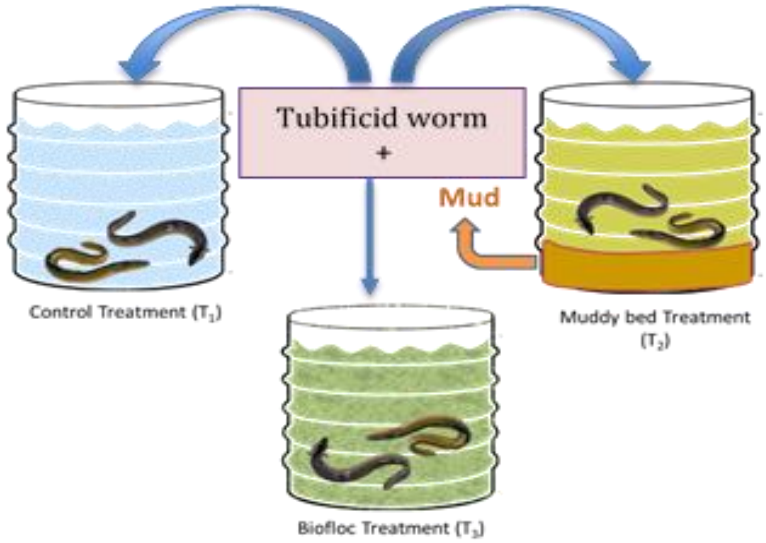

Fig. 1. Experimental design for the rearing of *M armatus* under three different treatments.

3. Monitoring physico-chemical parameters

The physico-chemical parameters such as water temperature (°C) was monitored using a digital thermometer. A digital DO meter (Hach Co., Colorado, USA) was used to determine the dissolved oxygen content of water. Whereas, pH was measured by using a digital pH meter (Hach Co., Colorado, USA). Furthermore, ammonia (mg/l) was determined by HANNA instrument Test Kit.

4. Growth performance

The total length (snout to caudal tip) of each fish sample was measured by a simple measuring scale. All the length data were recorded in cm. The weight of the fish was recorded by the digital weight balance in every sampling date. Average weight gain and specific growth rate were calculated with the following formulae:

Average weight gain (AWG) = Mean final body weight – Mean initial body weight

Specific growth rate (SGR %) = (Ln W2 (g) – Ln W1 (g)) / (T2 - T1) × 100

Where,

W1 = Initial live body weight of fish (g) at time T1 (day)

W2 = Final live body weight (g) of fish at time T2 (day)

T2 - T1 = No. of days of the experiment

5. Collection of gonad and gonado-somatic index (GSI)

The ventral side of samples was cut and opened from the anus towards the lower jaw by using scissors carefully. Then, the muscle of the abdomen was vertically cut from the anus towards the vertebral column. Muscle, fat tissue, digestive organs and blood vessels

were properly removed. After that, the gonad was taken out by forceps. The weights of the gonads were measured carefully with the help of a sensitive portable electronic balance "OHAUS Scout pro" in gram. Then, gonad samples were cut into small pieces using scissors. Finally, the samples were taken in vials filled with 10% buffered formalin and kept at room temperature for preservation. GSI is frequently applied to determine the reproductive cycle of a fish species over the year at monthly or less intervals. The value of GSI in the percentage was calculated using the following formula:

$$\text{GSI} = \frac{Gonad\ weight(GW)}{Body\ weight(BW)} \times 100$$

6. Histological analysis

The gonad and liver of spiny eel were removed and preserved in 10 % buffered formalin for histological analyses. Histological samples measuring about 10-12 mm in length were taken out in a perforated plastic holder, which was covered by perforated steel plate. Dehydration, clearing and infiltration were carried out in an automatic tissue processor (Leica, Model-TP1020) using a series of alcohol of increasing concentrations, three changes of xylene and finally through molten paraffin wax (two series). The samples were then embedded in melted paraffin wax. Yolk-laden tissues were, however, very brittle and difficult to section when routinely embedded. This drawback was due to the difficulty in getting the wax penetrated into the yolky region of the oocytes. It was found that yolky oocytes required a very specific timing for dehydration and wax infiltration. Paraffin wax embedded tissue blocks were sectioned by microtome knife at 10-12 µm thickness, and the sections were kept in a water bath at a temperature of 40ºC for stretching so that they can easily be placed on glass slide. Slides were then kept overnight on a slide drier at a temperature of 38ºC. The sections were attached to slide using Mayer's egg albumin. A small drop of Mayer's egg albumin was smeared over the surface of the slide with the finger, and the excess amount was rubbed off. Then, the sections were routinely stained with hematoxylin and eosin according to **Humason (1972)**.

6.1. Microscopic observation of the gonadal tissue section

The stained sections were mounted on the glass slide with DPX mountant and covered by coverslips and studied under a compound microscope (SWIFT M 4000-D). The photographic records were done simultaneously for future documents of the study.

7. Analysis of blood parameters

7.1. Blood collection

The *M. armatus* were anesthetized with clove oil and the length and weight were measured. Then, blood samples were taken from the tail vein with a syringe containing 1% heparin (Sigma, USA) and stored in a 2mL test tube containing K3 EDTA solution, which was used as an anticoagulant. The blood samples were rapidly collected to avoid blood's rapid coagulation. The blood is used to measure glucose, hemoglobin, cholesterol, RBC and WBC.

7.2. Estimation of blood parameters

Total red blood cells (tRBCs) were counted using an improved Neubaur hemocytometer **(Shah & Altindağ, 2004)**. Blood was diluted at a ratio of 1:200 with Hayem's fluid **(Mishra *et al*., 1977)**. Erythrocytes were counted in the loaded hemocytometer chamber, and total numbers were reported as 106mm-^{3} **(Wintrobe, 1967)**.

Total white blood cells (WBC) were counted using an improved Neubaur hemocytometer **(Mgbenka *et al*., 2003; Shah & Altindağ 2005)**. Blood was diluted (1:20) with Turk's diluting fluid and placed in a hemocytometer. 4 large (1 sq mm) corner squares of the hemocytometer were counted under the microscope (Olympus) at 640 X. The total number of WBC was calculated in mm^{3} x10^{3} **(Wintrobe, 1967)**.

Glucose, hemoglobin and cholesterol were determined with an EasyTouch GCHb Blood Glucose/Cholesterol/Hemoglobin Multi-Function Test Kit (3 in 1) Type ET-301.

8. Data interpretation

During the experimental period, all the data were collected, recorded and preserved on a computer spreadsheet. The mean value and standard deviation were calculated using MS Excel. The data were statistically analyzed via one-way ANOVA, using statistical software Statistix 10. All the data were compiled, compared and analyzed for constructive interpretation.

RESULTS

1. Water quality parameters

The average mean values of each water quality parameter during the experimental period under different treatments are presented in Table (1). There was no significant difference among different treatments for the values of physico-chemical parameters such as temperature, pH, dissolved oxygen except ammonia of water in the experimental tank reared *M. armatus.* Temperature ranged between 26 to 29 ºC, pH ranged from 7.5 to 8.3 and DO ranged from 9.5 to 10.5ppm, as observed in three different treatments. For ammonia wide range value was observed, where the highest value was found (0.4 ppm) in T_3 and the lowest was in T_1, but all values were in the suitable ranges.

Table 1. Mean values of water quality parameters during the experimental period of 90 days

Average water quality parameters				
Treatment	**Temperature (°C)**	**pH**	**DO (ppm)**	**Ammonia (ppm)**
T_1	26.92 ± 1.51	8.1 ± 0.51	10.19 ± 0.5	0.15^{a}
T_2	26.88 ± 3.47	7.92 ± 0.09	10.25 ± 0.3	0.24^{b}
T_3	26.77 ± 2.77	8.16 ± 0.05	10.18 ± 0.51	0.4^{c}

Mean values with different superscript letters in the same row were significantly different (P< 0.05).

2. Growth performance

The growth rates of the three treatments varied significantly from one another. In case of biofloc treatment (T_3), on day 15, the growth increased. After a modest decline in growth rate after 45 days, the growth curve remained almost unchanged until day 90. The situation was different in the case of the muddy bed treatment (T_2) from the biofloc treatment. The growth rate quickened around day 15. Following that, growth slowed slightly for the next 60 days. But then, there was a drastic fall of growth till the 90th day. However, the growth rate in control treatment (T_1) decreased from the beginning of the experiment. The growth rate significantly decreased after 30 days, with the least growth compared to other treatments (Fig. 2). Although negative SGR values were found in all treatments, comparatively better SGR was found in T_3. Similar result was also observed for AWG (Table 2).

Table 2. Comparative specific growth rate and average weight gain of spiny eel in three different treatments

Treatment	SGR	AWG
T_1	-0.16	-20.5
T_2	-0.12	-16.75
T_3	-0.03	-4.83

*SGR= specific growth rate; AWG= average weight gain.

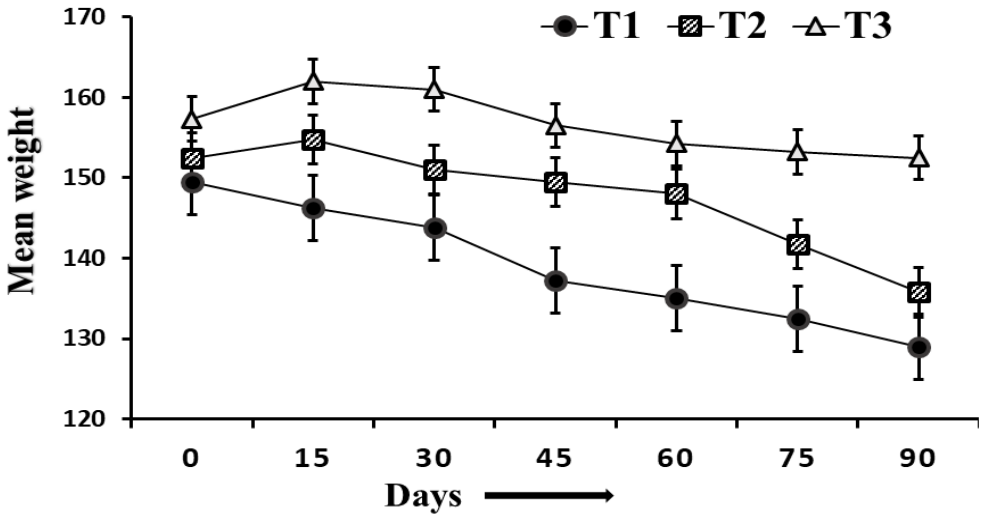

Fig. 2. Mean weight (g) of *M. armatus* under different treatments during the experimental period. (T1= control; T2= muddy bed; T3= biofloc)

2.1. Integrated effects of tubificid worm and biofloc

Tubificid worms were provided as their daily meal along with the formulated feed. Interestingly, after 15 days, the presence of tubificid worm was observed in T_3 treatment water among the three treatments (Fig. 3B). In addition, tubificid worm was attached to the uneaten pellet feed (Fig. 3B, right bottom corner), which was not found in other T_1 and T_2 treatments (Fig. 3A).

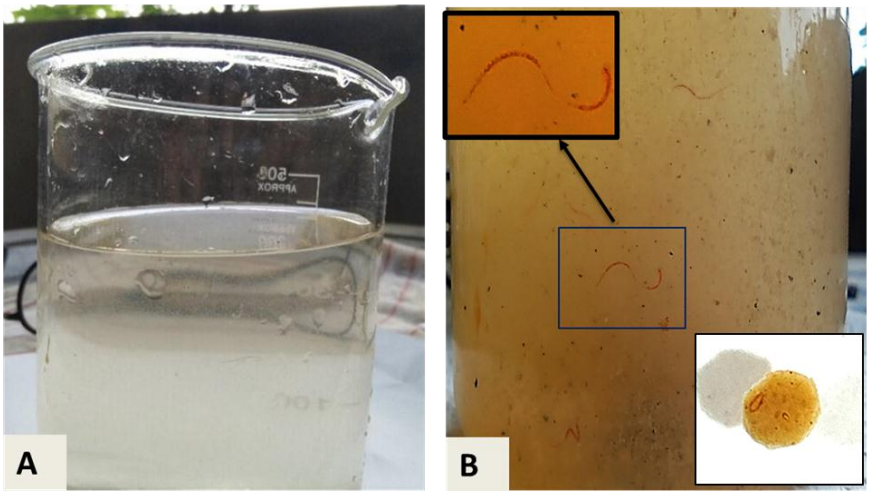

Fig. 3. **(A)** Control treatment, no tubificid worm found; **(B)** Biofloc treatment with tubificid worms and attachment of tubificid worm with pellet feed

3. Gonadal development of *M. armatus*

3.1. The morphology of the ovaries

Ovaries, the female reproductive organ were paired, elongated with various widths of frilled ribbon-like structure lying ventral to the body cavity and were of unequal length, with the right ovary commencing further forward than the left ovary, anterior to the gall bladder (Fig. 4). The left ovary extended posteriorly beyond the right ovary. The two lobes were connected along the dorsal surface by mesenteries, from which they were suspended in the posterior ends into a short oviduct, which fused and led to the urino-genital pore. The color of ovary varied from reddish-brown to light yellowish.

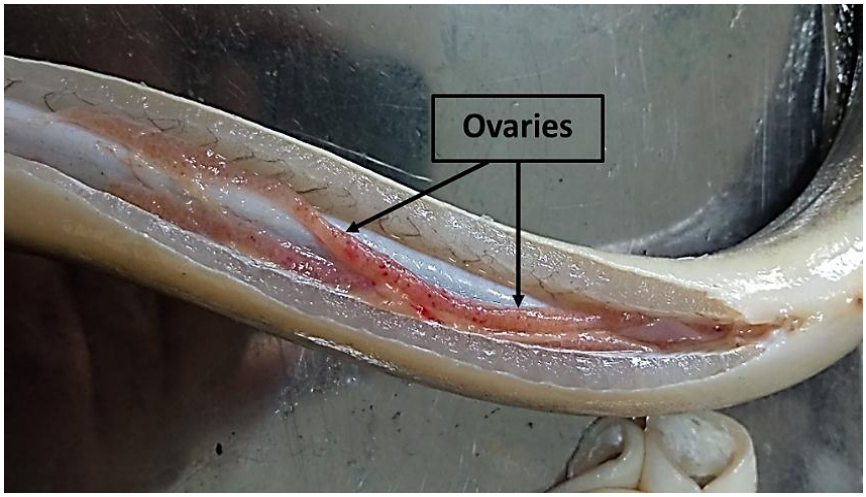

Fig. 4. Location and morphology of ovaries of *M. armatus* after discectomy of intestinal cavity

3.2. Gonado-somatic index (GSI) of M. armatus

The GSI of the fish of *M. armatus* (female) increased as the fish reached maturity and declined with the start of spawning activities. During the present study, higher values of GSI (0.46) were observed in T_3, which was significantly different from other treatments. And the lowest value was found in the T_1, which was 0.0.32 but T_2 value was slightly higher (0.38). The means of GSI value of *M. armatus* are presented in Fig. (5).

3.3. The histology of the ovaries

From the histological study of *M. armatus* ovaries of different treatments it was observed that, oocytes did not synchronously develop, and oocytes at various maturation stages were observed in paired ovaries. In control treatment (T_1), the gonad was very thin. After the histology of the gonad, it was observed that the gonads were mostly immature, and oocytes stages were limited at the primary and secondary stages (Fig. 6A). Gonads of fish reared in T_2 treatment showed asynchronous natur,e where yolk vesicle stage oocytes were found along with primary and secondary stages oocytes although the number of maturing oocytes were relatively low (Fig. 6B). However, the gonad histology scenario in T_3 was different compared to the other two treatments. Large number of maturing and mature stage oocytes were found in gonads of fish reared in T_3 although some primary and secondary stage oocytes were also present (Fig. 6C).

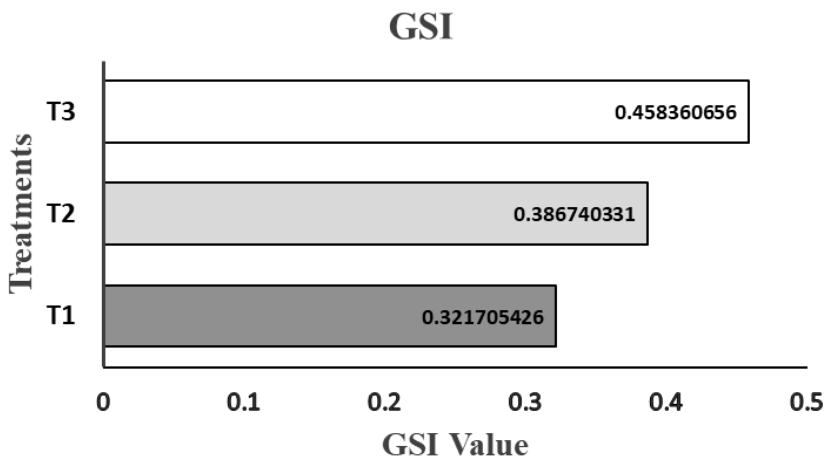

Fig. 5. Comparison of mean % of gonado-somatic index (GSI) of *M. armatus* among three different treatments

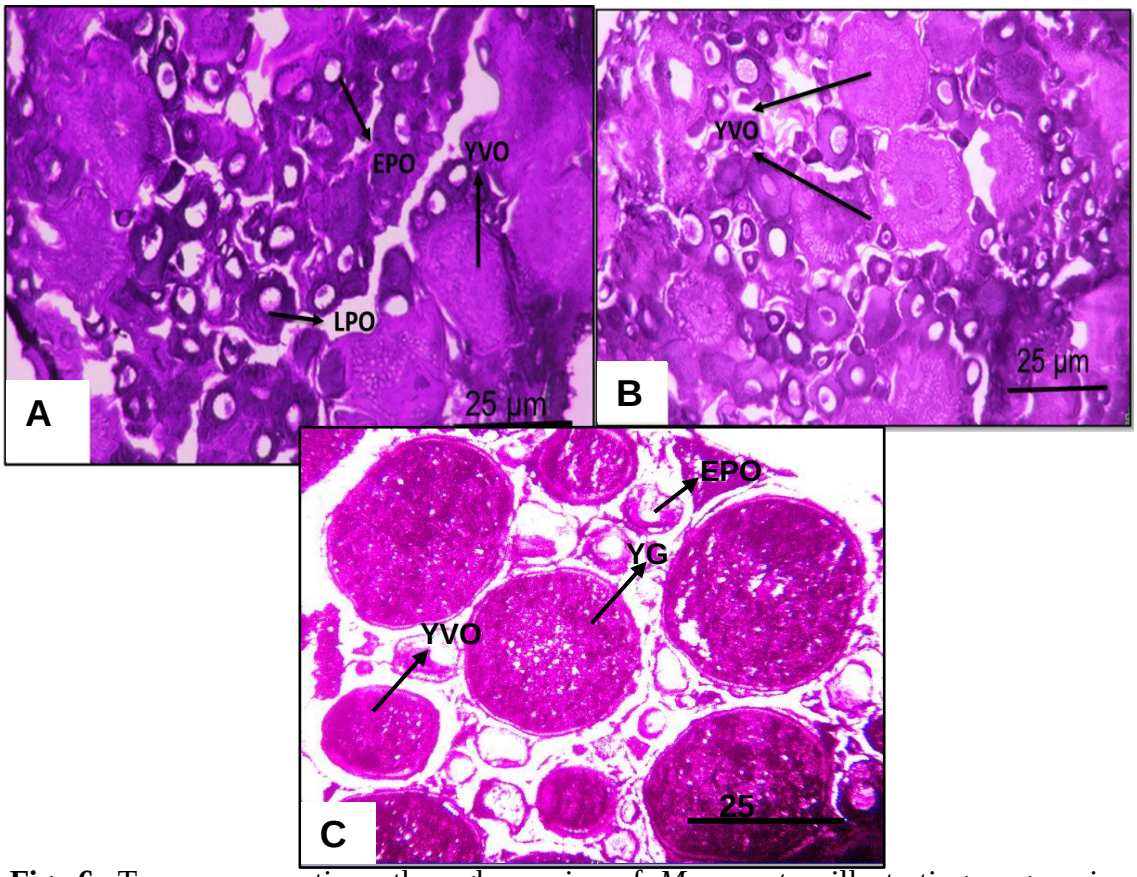

Fig. 6. Transverse sections through ovaries of *M. armatus* illustrating oogenesis of: Control treatment, T_1 **(A)**; Muddy bed treatment, T_2 **(B)**, and Biofloc treatment, T_3 **(C)**. EPO: Early perinuclear oocyte; LPO: Late perinuclear oocyte; YVO: Yolk vesicle oocyte; YGO: Yolk granule oocyte (Sainted with H&E, scale bar = 25 µm).

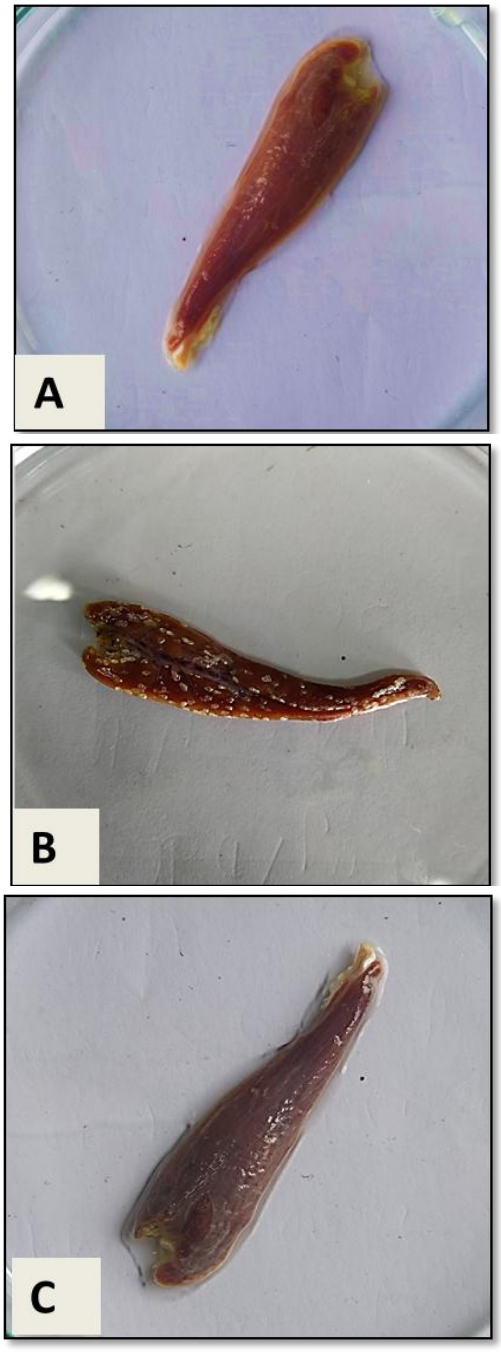

Fig. 7. Visual observation of liver A. Control treatment; B. Muddy bed treatment; C. Biofloc treatment

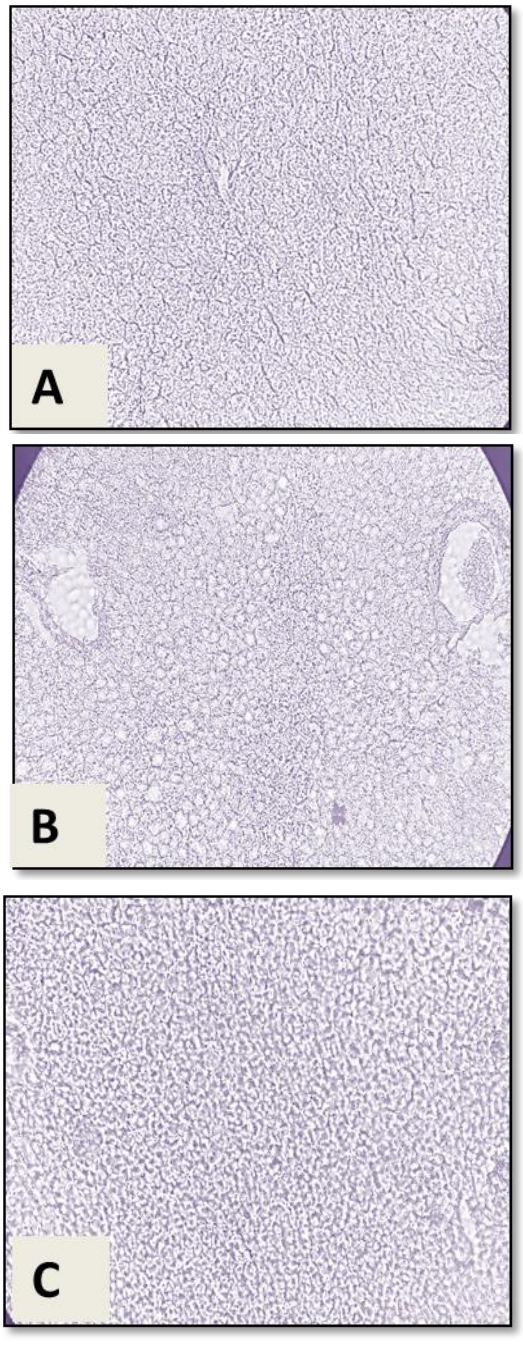

Fig. 8. Representative photomicrographs from the histological slide of liver: A. T_1; B. T_2; C. T_3

4. Observation of *M. armatus* liver

4.1. Visual observation of liver

After dissecting the *M. armatus* of the three treatments, there was a difference in the appearance of liver, which was identified with the naked eye. Although the uniform structure of liver was found in all treatments, comparatively light-colored liver was observed in T_1 (Fig. 7A). Interestingly, white- colored spore like structures were observed in the liver of the fish reared in T_2 (Fig. 7B), whereas normal colored and structured liver was found in T_3 fish (Fig. 7C).

4.2. Histological analysis of liver

Microscopic examination of liver tissue revealed different histopathological changes in *M. armatus*. Liver histology of T_1 fish and T_3 fish exhibited normal structural features. The hepatocytes are arranged in branching and anastomosing cords interrupted by hepatic sinusoids (Fig. 8A, C). While, in case of T_2 treatment, the histology of liver tissues showed several necrosis (Fig. 8B).

5. Hematological analysis of *M. armatus*

Among the three treatments, the highest and similar glucose level was observed both in T_1 and T_3, compared to T_2 where it was significantly lower (Fig. 9A).

In the present study, the highest cholesterol level was observed in the control treatment, which was 310 mg/dl, compared to other two treatments (T_2 and T_3) with their significantly lower level of cholesterol (Fig. 9B). However, there was no significant differences between T_2 and T_3 treatments (Fig. 9B). The Hb concentration of fish reared in T_1 was comparatively higher than the other two treatments, although there was no significant difference among the three treatments (Fig. 10).

Hematological parameters observed in the blood of experimental fish collected from all treatments are presented in Table (3). In the present study, it was observed that T_1 had a lower RBC value, and T_3 treatment showed significantly higher value (2.81×10^6 mm^3). Unlikely, lower amount of WBC was found both in the T_1 and T_3 compared to T_2 where significantly higher number of WBC was found (51.50×10^3 mm^3). In case of packed cell volume (PCV), T_1 treatment showed a higher percentage (33.15%) compared to both T_2 (25.95%) and T_3 (26.58%) treatments. There were significant differences among the mean corpuscular volume (MCV) values of the three treatments. The highest MCV value was found in T_1 (205.9 μm^3), and the lowest value was found in T_3 (94.5 μm^3). Although MCH (mean corpuscular hemoglobin) value was significantly higher in T_1 (68.63 pg) compared to other two treatments (T_2 and T_3), there were no significant differences of MCHC (Mean cell haemoglobin concentration) values among three different treatments.

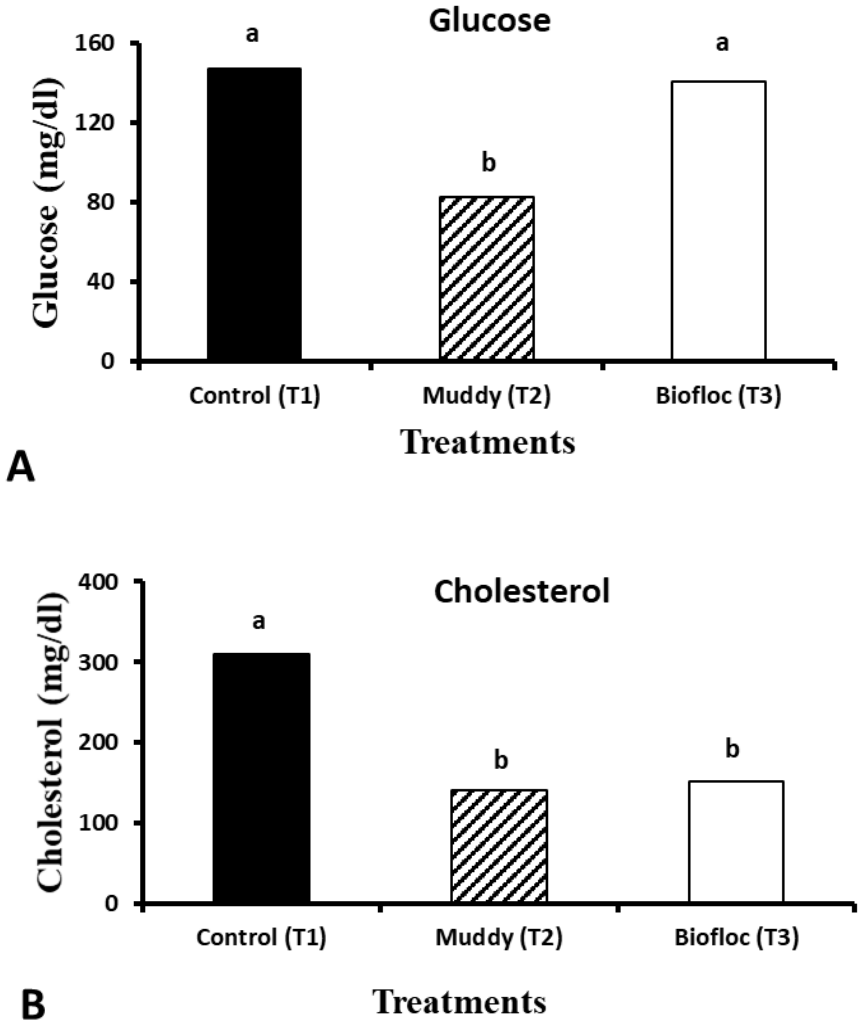

Fig. 9. (A) Glucose concentration; **(B)** Cholesterol concentration of spiny eel, *M. armatus* in different treatments

Table 3. Hematological parameters of freshwater spiny eel, *M. armatus* in different treatments

Treatment	RBC (10^6/mm^3)	WBC (10^3/mm^3)	PCV %	MCV (µm^3)	MCH pg	MCHC g/dl
T_1	1.61 ± 35.3^a	31.5 ± 6.02 ^{a}	33.15 ^{a}	205.9 ^{a}	68.63 ^{a}	33.33 ^{a}
T_2	2.06 ± 46.7^a	51.5 ± 5.63 ^{b}	25.95 ^{b}	125.66 ^{b}	41.88 ^{b}	33.32 ^{a}
T_3	2.81 ± 64.0^b	38.33 ± 5.39 ^{a}	26.58 ^{b}	94.5 ^{b}	31.5 ^{b}	33.33 ^{a}

X±SD: Mean Value ± Standart Deviation: $p<0.05$ significant level. RBC = Red blood cell; WBC = White blood cell; PCV = Packed cell volume; MCV = Mean cell volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean cell haemoglobin concentration

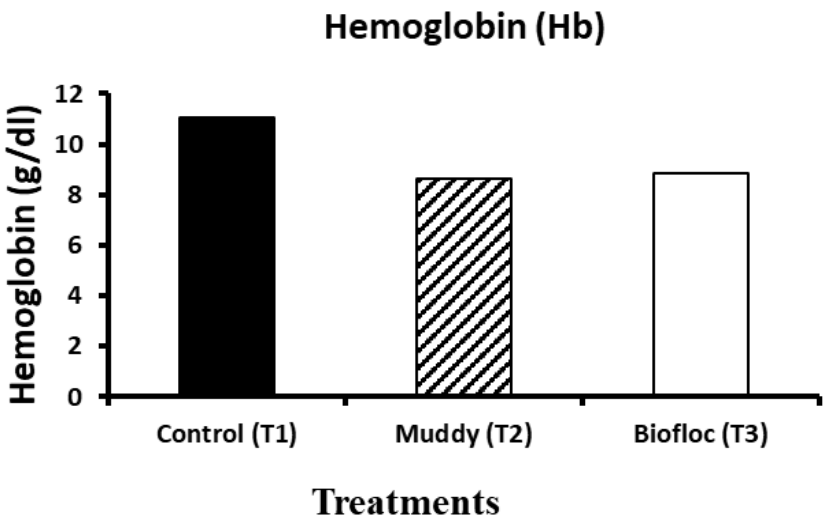

Fig. 10. Haemoglobin (Hb) concentration of spiny eel, *M. armatus* in different treatments

DISCUSSION

1. Water quality parameters

Water quality is one of the important factors determining the growth and survival of fish in the aquatic environment. The physico-chemical conditions such as temperature, pH, dissolved oxygen and ammonia of water in the experimental tank under different treatments of *M. armatus* were in suitable range. Previously, the water quality parameters were also recorded throughout the study period and was within the suitable ranges that agree with other findings **(Narejo *et al.*, 2005)**. **Sukardi *et al.*, (2018)** studied the effect of stocking density on the performance of glass eels, *Anguilla bicolor* in the biofloc system maintaining average water temperature at 27.1°C, pH 7.6 and DO 7.2 $mg.L^{-1}$. They mentioned that the water parameters did not significantly affect the final findings of the experiment. But, in case of ammonia, the biofloc showed a higher value compared to the control treatment. Ammonia accumulates in most intensive biofloc systems because of the cumulative feed loading to the system. It can be tempered by dilution through water exchange, but this defeats the purpose of intensive water use and reduces the beneficial bacteria load in the system **(Hargreaves, 2013)**. In the current study, a similar incidence occurred. Excess amount of feed accumulation in the bottom along with no water exchange facility increased the ammonia value in biofloc treatment. **Putra *et al.* (2020)** observed that, the ammonia concentration dropped during the first week of the experiment, became stagnant during the second and third weeks, and increased again on day 32 of the experiment. These results are comparable to those of our investigation.

2. Growth performance

The findings of the present study indicate a clear and highly significant negative correlation among the treatments, suggesting that the spiny eel experienced weight loss. In contrast to the other treatments, T_3 showed better performance, as evidenced by a significantly lower SGR value. The higher SGR and AWG values observed in the other treatments indicated rapid weight loss, particularly in the control treatment.

Proper nutrition is crucial for the healthy growth and maturation of fish gonads. According to **Abujam and Banerjee (2010)**, spiny eels are zooplankton feeders at their early stages and in their later develop insectivorous feeding habits. As adults, they primarily consume earthworms, insects, microcrustaceans, and larvae of other aquatic invertebrates. Similarly, **Alam *et al*. (2013)** found that *M. armatus* preferentially feeds on aquatic insects, crustaceans, molluscs and annelids. In our study, we provided tubificid worms along with formulated feed as the daily meal for the fish. Surprisingly, we observed the presence of tubificid worms in the biofloc treatment (T3) water after a few days, which was not the case in the other treatments (Fig. 3B). Additionally, tubificid worms were found attached to the uneaten pellet feed in the biofloc treatment (Fig. 3B), indicating that the biofloc and waste feed acted as a growing medium for tubificid worms. As these worms can grow and survive for an extended period, the fish could easily consume them whenever they wanted. This approach not only makes it easier for the fish to find food at any time, but it also reduces feeding costs and time. Consequently, there was no shortage of food in the biofloc treatment.

According to **Sharma *et al*. (2015)**, the biofloc system reduced the reliance on artificial feed and improved the utilization of bioflocs as feed by up to 50%. Similarly, the growth of *Oreochromis* sp. increased by 35% in the biofloc treatment, and the survival of the Indian white shrimp *Penaeus indicus* improved **(Panigrahi *et al*., 2020)**. Another study showed a 45% higher production of tilapia in biofloc tanks than in control tanks, confirming the utilization of biofloc by fish as food **(Azim & Little, 2008; Ekasari *et al*., 2015)**. However, it remains unclear whether *M. armatus* feeds on biofloc. Additionally, tubificid worms could multiply in the biofloc system as the nutritional value of the biofloc helps in their culture.

In this study, although formulated feed was provided as part of the fish's diet, the fish mainly consumed live food. Similar results were found by **Mollah *et al*. (2013)**, who observed that fish preferred trash fish and chicken viscera to formulated feed. In contrast, **Suloma *et al*. (2021)** found that the European eel in a heterotrophic biofloc system successfully consumed dry diet pellets after the first month of the experiment. However, the study disagree with previous findings in freshwater eels and other eel fish. In the present study, the formulated feed primarily served as a nutrient source for the plankton and tubificid worms within the biofloc system, which were eventually consumed by the fish. Interestingly, tubificid worms were observed to attach to unused formulated feed in

the biofloc system, suggesting a potential connection between the worms and the biofloc culture technique that requires further investigation.

On the other hand, the weight loss observed in the control treatment could be attributed to the unsuitable rearing conditions provided in the plastic tank, which was very different from their natural habitat, causing them to consume less food. Freshwater eels, like *M. armatus*, have a burrowing habit and often spend their day hiding under crevices, stones and mud. In this experiment, PVC pipes were used as shelter to improve the growth of *M. armatus*, and previous studies have shown that water hyacinth and PVC pipes are suitable shelters for this species. However, in the muddy bed treatment, weight loss was observed after 60 days due to difficulty in maintaining water quality and turbid water hindering feeding monitoring, making the fish more susceptible to disease. This problem could be minimized by using larger tanks for *M. armatus* culture, where decomposition of waste particles is not a problem like in natural water bodies. Weight loss tendency in all treatments might be due to insufficient tank area, fewer hiding places, lack of natural environment or suitable feed. Therefore, the use of the biofloc system could be a more suitable technique for *M. armatus* domestication, considering the circumstances mentioned above.

3. Gonadal development of *M. armatus*

The quality of the feed used can significantly impact egg quality. In line with the improved fish development performance in the biofloc tank, the gonado-somatic index (GSI) value was also higher in this treatment compared to others. According to **Ali *et al*. (2013)**, the highest GSI of female *M. armatus* was 14.40±1.48 in June, and the lowest was 0.44±0.06 in September. In the present study, GSI was calculated in August, which matches with the findings of previous authors. The results of this study closely resemble those obtained by **Uthayakumar *et al*. (2013)** and are also consistent with observations reported by **Abujam and Biswas (2020)** in freshwater spiny eel, *Macrognathus pancalus*.

3.1. Histological analysis of gonad and liver of M. armatus

It was observed that fish in the muddy bed treatment exhibited immature stages of maturation, specifically early and late perinucleolar stage oocytes, while the condition in the control treatment was much poorer. However, in the biofloc treatment, the results were more promising, with yolk vesicle stage oocytes and early yolk granule stage oocytes present. These findings were collected in August-September and are consistent with **Rahman's (2007)** experiment on the gonadal cyclic development of spiny eel and mud eel. The occurrence of two types of yolk inclusions, yolk vesicles and yolk granules, is a common finding in many teleosts **(Malone and Hisaoka, 1963; Kennedy, 2002)**. **Rahmatullah *et al*. (2005)** reported that in *M. armatus*, the ovaries had distinct yolk vesicles stage and yolk granules stages in August. In September, the follicular cells of the oogonia ruptured and shrunk, forming irregularly shaped structures in October. Previous research has shown that the progression of gonad development and differentiation is

related to the attained body size **(Bieniarz *et al.*, 1981; Durif *et al.*, 2009)**. As the biofloc treatment showed a positive growth rate, it would be natural to expect better gonadal development in this treatment.

There is a lack of direct observations on eel social behavior in both wild and farmed environments. However, in laboratory settings, eels have shown aggressive behavior and established social hierarchies **(Hirt-Chabbert *et al.*, 2014)**. These social stressors can cause physiological and morphological changes that activate the hypothalamic-hypophysial-interrenal system **(Peters *et al.*, 1980; Stewart *et al.*, 2014)**, which could potentially hinder gonadal development in certain treatment conditions. While, it is likely that social stress and its effects impact gonadal development,but this hypothesis still requires further research to confirm.

In this study, the liver histology of T_1 and T_3 fish showed a normal appearance, but T_2 treatment fish exhibited signs of infection. One of the primary reasons for this problem could be the attack of pathogens. The histological examination of the liver provides direct evidence of the effect on fish health and helps to determine the possible impact of parasitism on the functions of different body systems **(Kaur *et al.*, 2012)**. Parasite distribution within the fish liver is variable, and their attachment can cause liver damage. **Devi and Pinky (2014)** also studied the effect of helminth parasites in *M. armatus*, focusing on the hepato-somatic index. Furthermore, infected liver may appear smaller in size, with few encysted parasites visible on the external surface **(Devi & Pinky, 2014)**, supporting our findings. Another possible reason for the infection could be the environmental conditions in the muddy bed treatment tank. In the control treatment, excess feed particles and feces were removed by siphoning, ensuring good water quality. Similarly, in the biofloc treatment, denitrifying bacteria maintained water quality by removing ammonia and other toxic gases. Unfortunately, these techniques were not present in the muddy bed tank, which was full of waste feed particles and fish feces. This could have deteriorated the water quality and provided a breeding ground for many pathogens and bacteria, which eventually attacked the fish. Eels tend to stay hidden in the bottom, coming into direct contact with these pathogens. Therefore, our future research will investigate how to mitigate disease infestations in the rearing system.

4. Hematological analysis of *M. armatus*

Hematology plays a crucial role, particularly in the surveillance and prevention of fish diseases. Furthermore, the examination of hematological parameters serves as a significant tool in assessing the physiological condition of fish and other aspects related to industrial aquaculture **(Fazio, 2019)**.

In Figure 9A, the lowest glucose value was observed in T_2 among the three treatments, while the other two treatments showed relatively similar values. Various authors have reported a wide range of variations in plasma glucose in fishes. Nevertheless, blood glucose has been identified as a sensitive indicator of environmental stress caused by pollution **(Johansson *et al.*, 1972; Fırat *et al.*, 2011)**. The decreased glucose level in the

T_2 treatment, which was infected due to poor water quality, could be one of the reasons. However, it is worth noting that, blood glucose levels in vertebrates are influenced by several factors, including analytical techniques, strain, sex, age, nutritional status, environmental conditions, anesthesia and methods of handling animals **(Young, 1974)**.

Several teleosts have much higher values of total plasma cholesterol (300~700mg %) **(Shibata *et al.*, 1974)**. In our present study, the control treatment exhibited the highest cholesterol value of 310 mg/dl, whereas no significant differences were observed in the other two treatments. The increased blood lipid (cholesterol) content, combined with a higher body fat content, may represent an adjustment of the eel to its new stage of life. This elevated cholesterol level is in line with studies in maturing salmonids, where increased levels of cholesterol in plasma and gonads during the spawning season were documented **(Balm *et al.*, 2007)**. It is possible that the high tendency of blood lipids deposition in the fish of the control treatment may occur due to a lack of natural conditions, which may not be present in the other two treatments. According to **Van Ginneken *et al.* (2016)**, a redistribution of cholesterol occurs from other tissues to the gonads in silver eel.

Establishing normal hematological values in fish is crucial for aiding in disease diagnosis and assessing the effects of pollution on fish health **(Ahmed *et al.*, 2020)**. The hematological findings observed in our study are consistent with the range found for other fish species. While, the Hb concentration in the control treatment was comparatively higher than that recored in the other two treatments; no significant differences were found among them. Environmental conditions strongly influence the functional properties of fish hemoglobin **(Johansen & Weber, 2013)**. Hemoglobin is typically composed of globin with hematin, with each molecule of hematin containing one iron atom. This iron atom enables hemoglobin to carry large amounts of oxygen **(Dhanya & Sushama, 2006)**. Therefore, the higher Hb concentrations in the control treatment may be due to the higher oxygen level of the water body.

From the present study, we observed that T_3 fish contain higher RBC value (2.81×10^6 mm^3), compared to other two treatments. Previously, **Narejo *et al.* (2002)** counted erythrocyte of *M. armatus* ranging from 1.30 to 2.7 $\times10^6$ mm^3, with a mean of 1.97 $\times10^6$ mm^3. This result is similar to the findings of **Prasad *et al.* (1977)** who found the erythrocyte count of 1.78 $\times10^6$ mm^3 in *M. aculeatum* that also support the present findings. High levels of RBC in blood show the high air-breathing characteristics through carrying oxygen into the blood.

However, WBC was significantly higher in T_2 (51.5×10^3 mm^3) than other treatments. The increases in WBC in the blood were accepted as a response of the cellular immune system to pollution. It can be noted from the studies of **Palikova and Navratil (2001)**, **Şahan and Cengizler (2002)** and **Huntingford *et al.* (2006)** that immune system of fish creates similar responses to unfavorable conditions. According to **Narejo *et al.* (2002)**, the WBC counts ranged from 30.60 to 60.3 $\times10^3$ mm^3 in *M. armatus*. **Kharat and**

Kothawad (2012) found 59.4 to 94×10^3 mm^3 in *C. batrachus*. **Tavares- Dias and Moraes (2007)** found 41.5×10^3 mm^3 as an average WBC count of *C. punctatus*. Whereas, **Mahajan and Dheer (1979)** reported an average for the WBC count of the same species with a valu of 60.4 ×10^3 mm^3. These findings are similar to the results of the present study.

In case of packed cell volume (PCV), the control treatment showed a higher value (33.15, and muddy bed treatment showed a lower value (25.95%). The decrease in the PCV value clearly indicates the anemic conditions in the fish. **Narejo *et al*. (2002)** observed PCV that ranged from 26 to 33.5% in *M. armatus* which accorded with that of 25 to 50% in *Labeo rohita* **(Zutshi *et al*., 2010)**.

There was a significance difference between the mean corpuscular volume (MCV) values of three treatments. The highest MCV value was found in control treatment (205.9 μm^3), and the lowest value was found in biofloc treatment (94.5 μm^3). A decrease in MCV in freshwater fishes is due to abnormal stress conditions, particularly seasonal, environmental and toxic substances **(Ahmed *et al*., 2020)**. Chronic fungal, bacterial, viral infection causes a significant decrease in MCV values in fish species like *Saccobranchus fossilis*, whose condition is called normochromic microcytic anaemia **(Verma *et al*., 1979; Koteshwar *et al*., 2015)**. Similar result was found in the case of mean corpuscular haemoglobin (MCH). The value was higher in control (68.63pg) and lower in biofloc treatment (31.5 pg). **Saleheen and Mandal (2013)** detected MCH in cuchia from Mymensingh (48.00 pg), which is not significantly different from our present study.

Fig. 11. Schematic diagram of interrelation of biofloc-tubificid worm-feed-fish

CONCLUSION AND RECOMMENDATION

Freshwater eels are highly valued as food fish in many countries, but their populations have been declining due to human activities. To develop effective culture techniques, domestication of these fish in a captive environment is essential. In this study, three different rearing methods were compared to determine their feasibility for the domestication of *M. armatus*. Based on the results of growth parameters, histological analysis, and hematological parameters, biofloc treatment was found to be a promising option for domestication. This treatment reduced feeding rates and created an interrelationship among feed, live food, fish and biofloc. However, further research is needed to clarify the relationship between fish-biofloc and tubificid worm-biofloc. Rearing *M. armatus* in large tanks could improve growth performance in all treatments, especially when using biofloc.

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REFERENCES

Alam, A.; Gopinath, V.; Jha, D.N.; Joshi, K.D.; Kumar, J.; Das, S.C.S.; ... and Das, B.K. (2020). Food and Feeding Biology of Commercially Important Freshwater Eel, *Mastacembelus Armatus* (LACEPÈDE, 1800) from the Ganga River, India. Oceanogr. and Fish. Open Access J., 11(4): 555819.

Abujam, S. and Biswas, P. (2010). Notes on the biology and rearing feasibity of Spiny Eel. Sibcoltejo., 5: 87-91.

Abujam, S. and Biswas, P. (2011). Studies on the reproductive biology of spiny eel, *Macrognathus aral* from upper Assam. J. Environ. Biol., 32(5): 635.

Abujam, S.K. and Biswas, S.P. (2020). Reproductive biology of the spiny eel *Macrognathus pancalus* Hamilton, 1822 from upper Assam, India. Indian J. Fish., 67(1): 36-46.

Ahmed, I.; Reshi, Q.M. and Fazio, F. (2020). The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. Aquac. Int., 28(3): 869-899.

Alam, M.A.; Khan, M.A.; Sarower-E-Mahfuj, M.D.; Ara, Y.; Parvez, I. and Amin, M.N. (2021). A model for tubificid worm (Tubifex tubifex) production and its effect on growth of three selected ornamental fish. Bangladesh J. Fish., 33(2): 205-214.

Alam, M.M.; Flowra, F.A. and Hussain, M.A. (2013). Diet composition and feeding intensity of wild zigzag eel, *Mastacembelus armatus*. Trends Fish. Res., 2; 24-26.

Ali, M.R.; Mollah, M.F.A. and Sarder, M.R.I. (2013). Fecundity and gonado-somatic index of wild freshwater spiny eel *Mastacembelus armatus* (Lacepede) from Kishoreganj region of Bangladesh. J. Bangladesh Agric. Univ., 11(2): 365-372.

Azim, M.E. : Little, D.C. (2008). The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia *(Oreochromis niloticus).* Aquaculture, 283(1-4): 29-35.

Balm, S.P.; Durif, C.; van Ginneken, V.; Antonissen, E.; Boot, R.; van Den Thillart, G. and Verstegen, M. (2007). Silvering of European eel (*Anguilla anguilla* L.): seasonal changes of morphological and metabolic parameters. Anim. Biol., 57(1): 63-77.

Bieniarz, K.; Epler, P.; Malczewski, B. and Passakas, T. (1981). Development of European eel (*Anguilla anguilla L*.) gonads in artificial conditions. Aquaculture (Netherlands).

Chakraborty, B.K.; Shahroz, M.H. and Salma, A. S. (2019). A Sustainable Control Natural Breeding Technology of Mud Eel, *Monopterus Cuchia* (Hamilton, 1822) in Bangladesh. Int. j. oceanogr. aquac., 3(2): 000165.

Devi, H. and Pinky, K. (2014). Effect of helminth parasites in *Mastacembalus armatus* with special reference to hepato-somatic index. Int. J. Recent Sci. Res., 5: 992-995.

Dhanya, P.Y. and Sushama, S. (2006). Effect of a pesticide lambda cyhalothrin on haematological changes in the fresh water fish, *Labeo rohita* under short and long term exposure periods. Labeo, 7: 4.

Durif, C.M.; van Ginneken, V.; Dufour, S.; Müller, T. and Elie, P. (2009). Seasonal evolution and individual differences in silvering eels from different locations. In Spawning migration of the European eel (pp. 13-38). Springer, Dordrecht.

Ekasari, J. Rivandi, D.R.; Firdausi, A.P.; Surawidjaja, E.H.; Zairin Jr, M.; Bossier, P. and De Schryver, P. (2015). Biofloc technology positively affects Nile tilapia (*Oreochromis niloticus*) larvae performance. Aquaculture, 441: 72-77.

FAO. (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome

Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: a review. Aquaculture, 500: 237-242.

Fırat, Ö.; Cogun, H.Y.; Yüzereroğlu, T.A.; Gök, G.; Fırat, Ö.; Kargin, F. and Kötemen, Y. (2011). A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Oreochromis niloticus*. Fish Physiol. Biochem., 37(3): 657-666.

Gupta, S. and Banerjee, S. (2016). Food, feeding habit and reproductive biology of tire-track spiny eel (*Mastacembelus armatus*): A review. J. Aquac. Res. Dev., 7(5): 429.

Hargreaves, J.A. (2013). Biofloc production systems for aquaculture (Vol. 4503, pp. 1-11). Stoneville, MS: Southern Regional Aquaculture Center.

Hirt-Chabbert, J.A.; Sabetian, A. and Young, O. A. (2014). Effect of size grading on the growth performance of shortfin eel (*Anguilla australis*) during its yellow stage. N. Z. J. Mar. Freshw. Res., 48(3): 385-393.

Huntingford, F.A.; Adams, C.; Braithwaite, V.A.; Kadri, S.; Pottinger, T.G.; Sandøe, P. and Turnbull, J.F. (2006). Current issues in fish welfare. J. fish biol., 68(2): 332-372.

Humason, G.L. (1972). Animal tissue techniques. W. H. Freeman and Co., San Francisco, CA.

IUCN Bangladesh (2015). Red List of Bangladesh Volume 5: Freshwater Fishes. IUCN International Union for Conservation of Nature, Bangladesh Country Office, Dhaka, Bangladesh, pp. 360

Jahan, N.; Salam, M.A.; Ahsan, A.; Rahman, M.L.; Paul, S.I.; Das, S.K. and Mazumder, S.K. (2020). Domestication Technique of Commercially Important Freshwater Mud Eel, *Monopterus cuchia* (Hamilton, 1822). Malaysian Appl. Biol., 49(1): 95-104.

Johansen, KJ.E.L.L. and Weber, R.E. (2013). On the adaptability of haemoglobin function to environmental conditions. Persp. in expt. Biol., 1: 219-34.

Johansson, N.; Larsson, A. and Lewander, K. (1972). Metabolic effects of PCB (polychlorinated biphenyls) on the brown trout (*Salmo trutta*). Comp. Gen. Pharmacol, 3(11): 310-314.

Kaur, P.; Qureshi, T.A.; Shrivastav, R.; Manohar, S. and Bilal, A. (2012). Histopathological and haematological investigations on *Nandus nandus* (Ham.) parasitized by metacercariae of *Clinostomum complanatum* (Rudolphi, 1819). Int. J. Environ. Sci., 2(3): 1324.

Kennedy, A.M. (2002). Reproduction of striped bass *Morone saxatilis*; a structural, biochemical and functional characterization of atresia.

Kharat, S. and Kothawad, S. (2012). Haematological study of Clarias batrachus with reference to trypanosomiasis. Trends Fish. Res., 1(1): 6-9.

Koteshwar, R.; Podeti and Benarjee, G. (2015). Haematological Changes In Fresh Water Fish, *Channa striatus* Diagnised with the Epizootic Ulcerative Syndrome (EUS). Int. J. Adv. Biotec. Res., 6(2): 238-244.

Mahajan, C.L. and Dheer, J.S. (1979). Seasonal variations in the blood constituents of an air- breathing fish, *Channa punctatus* Bloch. J. Fish Biol., 14(4): 413-417.

Maitland, P.S. (1995). The conservation of freshwater fish: past and present experience. Biol. Conserv., 72(2): 259-270.

Malone, T.E. and Hisaoka, K.K. (1963). A histochemical study of the formation of deuto- plasmic components in developing oocytes of the zebrafish, *Brachydanio rerio*. J. Morphol., 112(1): 61-75.

Mgbenka, B. O.; Oluah, N.S. and Umeike, I. (2003). Effect of Gammalin 20 (lindane) on differential white blood cell counts of the African catfish, *Clarias albopunctatus*. Bull. Environ. Contamin. and toxicol., 71(2): 0248-0254.

Miah, M.F.; Naser, M.N. and Ahmed, M. K. (2015). The freshwater mud eel, *Monopterus cuchia*-a review. J. Glob. Biosc., 4(3): 1780-1794.

Mishra, N. Pandey, P.K.; Datta Munshi, J.S. and Singh, B. R. (1977). Haematological parameters of an air- breathing mud eel, *Amphipnous cuchia* (Ham.)(Amphipnoidae; Pisces). J. Fish Biol., 10(6): 567-573.

Mollah M.F.A.; Ali, M. R. and Taslima, K. (2013). Domestication and observation on induced breeding of spiny eel *Mastacembelus armatus*. American J. Food Sci. and Technol., 1(4): 82-86.

Narejo, N.T. (2003). Comparative studies on the biology and culture of *Monopterus cuchia* and *Mastacembelus armatus* of Mymensingh region (Doctoral dissertation, Ph. D. Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh).

Narejo, N.T.; Mamnur Rashid, M. and Rahmatullah, S. M. (2002a). Seasonal and sex related haematological variations of the freshwater spiny eel, *Mastacembelus armatus* reared in the cemented cisterns. Bangladesh J Fish. Res., 6(2): 141-147.

Narejo, N.T.; Rahmatullah, S.M. and Rashid, M.M. (2002b). Studies on the reproductive biology of freshwater spiny eel, *Mastacembelus armatus* (Lacepede) reared in the cemented cisterns of BAU, Mymensingh, Bangladesh. Pakistan J. Biol. Sci., 5(7): 809-811.

Narejo, N.T.; Salam, M.A.; Sabur, M.A. and Rahmatullah, S.M. (2005). Effect of stocking density on growth and survival of indigenous catfish, *Heteropneustes fossilis* (Bloch) reared in cemented cistern fed on formulated feed. Pakistan J. Zool., 37(4): 49.

Nasar, S.S.T. (1997). Backyard eel culture: International Institute of Rural Reconstruction, Silag, Cavity, Philippines, 88pp.

Palikova, M. and Navratil, S. (2001). Occurrence of *Anguillicola crassus* in the Water Reservoir Koryčany (Czech Republic) and its Influence on the health condition and haematological indices of eels. Acta Vet. Brno, 70(4): 443-449.

Panigrahi, A.; Das, R.R.; Sivakumar, M.R.; Saravanan, A.; Saranya, C.; Sudheer, N. S.; ... and Gopikrishna, G. (2020). Bio-augmentation of heterotrophic bacteria in biofloc system improves growth, survival, and immunity of Indian white shrimp *Penaeus indicus*. Fish & shell. Immunol., 98: 477-487.

Peters, G.; Delventhal, H.A.R.T.M.U.T. and Klinger, H.E.I.N.E.R. (1980). Physiological and morphological effects of social stress on the eel, *Anguilla anguilla* L. In Fish Diseases (pp. 225-227). Springer, Berlin, Heidelberg.

Prasad, S.; Pandey, B.N.; Sinha, D.P. and Chanchal, A.K. (1977)**.** Haematological Studies on the Indian Fresh- water Mud- eel, *Macrognathus aculeatum*. Zeitschrift für Tierphysiologie Tierernährung und Futtermittelkunde, 40(1- 6): 295-301.

Putra, I.; Effendi, I.; Lukistyowati, I.; Tang, U.M.; Fauzi, M.; Suharman, I. and Muchlisin, Z.A. (2020). Effect of different biofloc starters on ammonia, nitrate, and nitrite concentrations in the cultured tilapia *Oreochromis niloticus* system. F1000Research, 293.

Rahman, A.A. (1989). Freshwater fishes of Bangladesh. Zoological Society of Bangladesh.

Rahman, M.M. (2007)**.** Reproductive biology and fry rearing of freshwater eels *Monopterus cuchia* (Ham.) and *Mastacembelus armatus* (Lacep.) of Mymensingh, Bangladesh (Doctoral dissertation, PhD thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh).

Rahman, M.M.; Ahmed, G.U. and Rahmatullah, S.M. (2004). Changes in gonad of wild freshwater spiny eel *M. armatus* (Lacepede) during early spent period collected from a river of Netrokona district, Bangladesh. Prog. Agric, 15, 115-120.

Rahmatullah, S.M.; Rahman, M.M. and Ahmed, G.U. (2005). Reproductive biology, artificial propagation and larval rearing of two freshwater eels, *Monopterus cuchia* and *Mastacembelus armatus.* Bangladesh J. Fish. Res., 9(1): 13-14.

Şahan, A. and Cengizler, İ. (2002). Determination of Some Haematological Parameters in Spotted Barb (*Capoeta barroisi*, Lortet, 1894) and Roach (*Rutilus rutilus*, Linnaeus, 1758) Living in Seyhan River (Adana City Region). Turkish J. Vet. & Ani. Sci., 26(4): 849-858.

Salehin, A.N. and Mandal, S.C. (2013). Haematology of air breathing mud eel, *Monopterus cuchia* (Hamilton, 1822) of Mymensingh and Kishoreganj districts of Bangladesh. Dhaka Uni. J. Biol. Sci., 22(2): 127-134.

Shah, S. L. and Altindag, A. (2004). Hematological parameters of tench (*Tinca tinca* L.) after acute and chronic exposure to lethal and sublethal mercury treatments. Bull. Environ. Contam. Toxicol., 73(5): 911-918.

Shah, S. L. and Altindağ, A. (2005). Alterations in the immunological parameters of Tench (*Tinca tinca* L. 1758) after acute and chronic exposure to lethal and sublethal treatments with mercury, cadmium and lead. Turk. J. Vet. Anim. Sci., 29(5): 1163-1168.

Sharma, D.A.; Sharma, K. and Sangotra, R.A.S.H.M.I. (2015). Biofloc culture and its utilisation as feed in limited water exchange system for the culture of *Labeo rohita*. Int. Multidiscip. Res. J J. Int. Academ. Res. Multidiscip., 2320: 1-9.

Shibata, N.; Kinumaki, T. and Ichimura, H. (1974). Triglyceride, cholesterol, free fatty acid, glucose and protein contents in plasma of cultured rainbow trout. Bulletin of Tokai Regional Fisheries Research Laboratory. 77-87

Stewart, A.M.; Braubach, O.; Spitsbergen, J.; Gerlai, R. and Kalueff, A. V. (2014). Zebrafish models for translational neuroscience research: from tank to bedside. Trends neurosci., 37(5): 264-278.

Sukardi, P.; Prayogo, N.A.; Winanto, T.; Siregar, A.S., and Harisam, T. (2018). Nursery I: The effect of stocking density on the performance of glass eels, *Anguilla bicolor* in the biofloc system. In E3S Web of Conferences, 47: 02009. EDP Sciences.

Suloma, A.; Gomaa, A.H.; Abo-Taleb, M.A.; Mola, H.R.; Khattab, M.S., and Mabroke, R.S. (2021). Heterotrophic biofloc as a promising system to enhance nutrients waste recycling, dry diet acceptance and intestinal health status of European eel (*Anguilla anguilla).* AACL Bioflux, 14(2): 1021-1035.

Suresh, V.R.; Biswas, B.K.,: Vinci, G.K.; Mitra, K. and Mukherjee, A. (2006). Biology and fishery of barred spiny eel, *Macrognathus pancalus* Hamilton. Acta Ichthyol. Piscat. 36(1): 31-37.

Tavares- Dias, M. and Moraes, F.R. (2007). Haematological and biochemical reference intervals for farmed channel catfish. J. Fish Biol., 71(2): 383-388.

Uthayakumar, V.; Sreedevi, P.R.; Senthilkumar, D.; Munirasu, S.; Kiruba, A. and Ramasubramanian, V. (2013). Impact of seasonal variation and feeding on reproductive behavior of fresh water spiny eel *Mastacembelus armatus* from Cauvery River. Asian Pacific Journal of Reproduction, 2(3), 189-195.

Van Ginneken, V. J.; Saddozai, S.; Achakzai, W.M.; Jamil, N.; Rafique, N.; Razzaq, W., ... and Verstegen, M. (2016). Precocious Silvering of Farmed Eels in Relation to Their Physiological Aspects. J. Appl. Environ. Biol. Sci, 6(7): 30-37.

Verma, S.R.; Bansal, S.K.; Gupta, A.K., and Dalela, R. C. (1979**).** Pesticide induced haematological alterations in a fresh water fish *Saccobranchus fossilis*. Bull. Environ. Contam. Toxicol., 2(1): 467-474.

Wintrobe, M.M. (1967). Clinical hematology. Lea and Febiger, Philadelphia. In Library of Congress, Print USA.

Young, J.E. (1974). Blood carbohydrates: Vertebrates other than man. In "Biology Data Book" edited by Philip L. Altman and Dorothy S. Dittmer. Vol.III: 1821-1830, Federation of American Societies for Experimental Biology, Bethesda, Maryland, U.S.A.

Zutshi, B.; Prasad, S.R. and Nagaraja, R. (2010). Alteration in hematology of *Labeo rohita* under stress of pollution from Lakes of Bangalore, Karnataka, India. Environ. Monit. Assess., 168: 11-19.