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

Md. Ashrafuzzaman Alit, Mohammad Abdus Salam, Mohammad Shafiqul Alam, Sabuj Kanti Mazumder, Mohammad Lutfar Rahman *
Department of Genetics and Fish Breeding, Bangabandhu Sheik Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh
*Corresponding Author: mlrahman@bsmrau.edu.bd

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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<sub>1</sub> (control), T<sub>2</sub> (muddy bed) and T<sub>3</sub> (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<sub>3</sub> recorded higher growth performance than the other two treatments. Moreover, gonadal development progress was higher in T<sub>3</sub>, as indicated by the gonado-somatic index (GSI) and histology study. However, muddy bed conditions in T<sub>2</sub> led to fish disease infestation, which was observed through liver histology. Among several hematological parameters, glucose was significantly lower in T<sub>2</sub> than in the other two treatments, while cholesterol significantly decreased in both T<sub>2</sub> and T<sub>3</sub> compared to T<sub>1</sub>. However, hemoglobin did not significantly vary among all treatments. Higher RBC and lower WBC values in T<sub>3</sub> also indicate comparatively suitable rearing conditions than the other treatments. In conclusion, the results of the study suggest that T<sub>3</sub> (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 (T1), in freshwater with muddy bottom (T2), and in biofloc media (T3), 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 %) = \((\text{Ln } W2 \text{ (g)} - \text{Ln } W1 \text{ (g)}) / (T2 - T1) \times 100\)

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
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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:

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

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 40°C 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 38°C. 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 & Altındağ, 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 106 mm⁻³ (Wintrobe, 1967).

Total white blood cells (WBC) were counted using an improved Neubaur hemocytometer (Mgbenka et al., 2003; Shah & Altındağ 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 mm³ x 10³ (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 10.5 ppm, as observed in three different treatments. For ammonia wide range value was observed, where the highest value was found (0.4 ppm) in T₃ and the lowest was in T₁, but all values were in the suitable ranges.

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

<table>
<thead>
<tr>
<th>Average water quality parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>T₁</td>
</tr>
<tr>
<td>T₂</td>
</tr>
<tr>
<td>T₃</td>
</tr>
</tbody>
</table>

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 (T3), 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 (T2) 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 (T1) 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 T3. 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SGR</th>
<th>AWG</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>-0.16</td>
<td>-20.5</td>
</tr>
<tr>
<td>T2</td>
<td>-0.12</td>
<td>-16.75</td>
</tr>
<tr>
<td>T3</td>
<td>-0.03</td>
<td>-4.83</td>
</tr>
</tbody>
</table>

*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 T3 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 T1 and T2 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 urogenital pore. The color of ovary varied from reddish-brown to light yellowish.
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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 T₃, which was significantly different from other treatments. And the lowest value was found in the T₁, which was 0.0.32 but T₂ 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 (T₁), 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 T₂ treatment showed asynchronous nature 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 T₃ was different compared to the other two treatments. Large number of maturing and mature stage oocytes were found in gonads of fish reared in T₃ 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

![GSI Graph](image)

Fig. 6. Transverse sections through ovaries of *M. armatus* illustrating oogenesis of: Control treatment, T₁ (A); Muddy bed treatment, T₂ (B), and Biofloc treatment, T₃ (C). EPO: Early perinuclear oocyte; LPO: Late perinuclear oocyte; YVO: Yolk vesicle oocyte; YGO: Yolk granule oocyte (Stained 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. T₁; B. T₂; C. T₃
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 T₁ (Fig. 7A). Interestingly, white-colored spore-like structures were observed in the liver of the fish reared in T₂ (Fig. 7B), whereas normal colored and structured liver was found in T₃ fish (Fig. 7C).

4.2. Histological analysis of liver

Microscopic examination of liver tissue revealed different histopathological changes in *M. armatus*. Liver histology of T₁ fish and T₃ 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 T₂ 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 T₁ and T₃, compared to T₂ 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 (T₂ and T₃) with their significantly lower level of cholesterol (Fig. 9B). However, there was no significant differences between T₂ and T₃ treatments (Fig. 9B). The Hb concentration of fish reared in T₁ 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 T₁ had a lower RBC value, and T₃ treatment showed significantly higher value (2.81×10⁶ mm³). Unlikely, lower amount of WBC was found both in the T₁ and T₃ compared to T₂ where significantly higher number of WBC was found (51.50×10³ mm³). In case of packed cell volume (PCV), T₁ treatment showed a higher percentage (33.15%) compared to both T₂ (25.95%) and T₃ (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 T₁ (205.9 µm³), and the lowest value was found in T₃ (94.5 µm³). Although MCH (mean corpuscular hemoglobin) value was significantly higher in T₁ (68.63 pg) compared to other two treatments (T₂ and T₃), there were no significant differences of MCHC (Mean cell haemoglobin concentration) values among three different treatments.
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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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RBC (10⁶/mm³)</th>
<th>WBC (10³/mm³)</th>
<th>PCV %</th>
<th>MCV (µm³)</th>
<th>MCH pg</th>
<th>MCHC g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>1.61 ± 35.3ᵃ</td>
<td>31.5 ± 6.02ᵃ</td>
<td>33.15ᵃ</td>
<td>205.9ᵃ</td>
<td>68.63ᵃ</td>
<td>33.33ᵃ</td>
</tr>
<tr>
<td>T₂</td>
<td>2.06 ± 46.7ᵇ</td>
<td>51.5 ± 5.63ᵇ</td>
<td>25.95ᵇ</td>
<td>125.66ᵇ</td>
<td>41.88ᵇ</td>
<td>33.32ᵇ</td>
</tr>
<tr>
<td>T₃</td>
<td>2.81 ± 64.0ᵇ</td>
<td>38.33 ± 5.39ᵃ</td>
<td>26.58ᵇ</td>
<td>94.5ᵇ</td>
<td>31.5ᵇ</td>
<td>33.33ᵃ</td>
</tr>
</tbody>
</table>

X±SD: Mean Value ± Standard 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
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, T₃ 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 T1 and T3 fish showed a normal appearance, but T2 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 T2 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
T₂ 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 recorded 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 T₃ fish contain higher RBC value (2.81×10⁶ mm³), compared to other two treatments. Previously, Narejo et al. (2002) counted erythrocyte of M. armatus ranging from 1.30 to 2.7 ×10⁶ mm³, with a mean of 1.97 ×10⁶ mm³. This result is similar to the findings of Prasad et al. (1977) who found the erythrocyte count of 1.78 ×10⁶ mm³ 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 T₂ (51.5×10³ mm³) 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 ×10³ mm³ in M. armatus. Kharat and
Kothawad (2012) found 59.4 to $94 \times 10^3$ mm$^3$ in C. batrachus. Tavares- Dias and Moraes (2007) found $41.5 \times 10^3$ 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 $60.4 \times 10^3$ 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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