Contents lists available at ScienceDirect

Heliyon



journal homepage: www.cell.com/heliyon

Research article

5²CelPress

Comparative analysis of biometrical and reproductive indices, proximate composition, and hemato-biochemical variables of cuchia eel *Monopterus cuchia* (Hamilton, 1822) from six different localities of Bangladesh

Shishir Kumar Nandi ^a, Md Abdullah Al Mamun ^{b,***}, Afrina Yeasmin Suma ^a, Zulhisyam Abdul Kari ^{c,d,*}, Lee Seong Wei ^{c,d}, Albaris B. Tahiluddin ^{e,f}, Nesara Kadadakatte Manjappa ^g, Shamima Nasren ^h, Shuva Saha ^h, Guillermo Téllez-Isaías ⁱ, Walter G. Bottje ⁱ, Muhammad Anamul Kabir ^{a,d,**}

^a Department of Aquaculture, Sylhet Agricultural University, Sylhet-3100, Bangladesh

^b Department of Fish Health Management, Sylhet Agricultural University, Sylhet-3100, Bangladesh

^c Department of Agricultural Sciences, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia

^d Advanced Livestock and Aquaculture Research Group, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli,

Kelantan, Malaysia

^e College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography, Sanga-Sanga, Bongao, Tawi-Tawi 7500, Philippines

^f Department of Aquaculture, Institute of Science, Kastamonu University, Kastamonu 37200, Turkey

^g ACI Godrej Agrovet Pvt. Ltd., Rajshahi, Bangladesh

^h Department of Fish Biology and Genetics, Sylhet Agricultural University, Sylhet-3100, Bangladesh

ⁱ Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA

ARTICLE INFO

Keywords: Cuchia eel Proximate composition Hematology Plasma biochemistry

ABSTRACT

Cuchia eel (*Monopterus cuchia*) is among the most sought-after freshwater fish, owing to its exceptional nutritional profile and high consumer demand. The current research aimed to establish baseline data by comparing the proximate composition, hematological, and plasma biochemical indices of Cuchia eel populations across six different geographical locations in Bangladesh: Bogra, Haluaghat, Jamalpur, Moktagacha, Sylhet, and Tangail. By examining these parameters, we aim to gain valuable insights into the nutritional benefits, physiological responses, and potential adaptations of this species to varying environments. The statistical analysis revealed no significant (P > 0.05) variances in the whole-body proximate composition of the fish captured from distinct areas. However, it was observed that different geographical regions had remarkable impacts on the variations of the majority of the hematological parameters, except for some cases. Additionally, there was a notable (P < 0.05) increase or decrease in most of the serum biochemical contents in certain localities as compared to others in this study. Light microscopic examination of Cuchia eel blood smears exhibited lower numbers but larger sizes of RBCs. The

https://doi.org/10.1016/j.heliyon.2024.e25491

Available online 1 February 2024

^{*} Corresponding author. Department of Agricultural Sciences, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia.

^{**} Corresponding author. Department of Aquaculture, Sylhet Agricultural University, Sylhet-3100, Bangladesh.

^{***} Corresponding author. Department of Fish Health Management, Sylhet Agricultural University, Sylhet-3100, Bangladesh.

E-mail addresses: maamamun.fhm@sau.ac.bd (M.A. Al Mamun), zulhisyam.a@umk.edu.my (Z. Abdul Kari), anamul.aq@sau.ac.bd (M.A. Kabir).

Received 11 June 2023; Received in revised form 6 January 2024; Accepted 29 January 2024

^{2405-8440/© 2024} The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

findings of this study lead to the conclusion that different localities had significant impacts on the hematology and blood biochemical indices of Cuchia eel, even though the whole-body proximate composition showed no significant variations. This research contributes to a deeper understanding of the physiological aspects of Cuchia eel.

1. Introduction

Cuchia eel (*Monopterus cuchia*) is a highly significant air-breathing freshwater fish species within the Synbranchidae family and order Synbranchiformes. It is native to Bangladesh, India, Pakistan, Nepal, and Burma [1,2]. This species is commonly found in rice fields, ponds, beels, mud holes, and marshy lands in various districts of Bangladesh [3,4]. The Cuchia eel is carnivorous by nature, consuming small fish, earthworms, tubifex, frogs, mollusks, and detritus as its primary diet. It also holds great medicinal value. The tribal population conventionally employs this fish as a therapeutic agent, using it to treat several illnesses, particularly weakness, anemia, asthma, hemorrhoids, and diabetes [5]. Moreover, available literature suggests that direct intake of fresh Cuchia eel blood is believed to cure asthma, weakness, and anemia [6]. The flesh of Cuchia eel is considered a nutritionally balanced diet for humans due to its rich protein, amino acids, fatty acids, ash, and mineral contents [7,8]. The high nutritional profile of Cuchia eel makes it a unique and delicious food fish. Consequently, it has a high market demand in international trade. Live Cuchia eel is exported from Bangladesh to several South Asian and European countries [9]. Thus, the Cuchia eel seems to have multiple utilities linked with human health, food security, and livelihood as well.

However, there has been a decline in the annual catch of Cuchia from wild sources in recent years [10]. As a result, Cuchia has been classified as a vulnerable fish species in Bangladesh. The lack of appropriate culture systems, husbandry requirements, and artificial breeding techniques are the primary factors contributing to the risk of extinction for this fish. Additionally, numerous research studies have identified habitat degradation, overfishing, agricultural expansion, agrochemicals, pesticides, and fertilizers use, along with ecological variations, as other significant threats to the endangered status of this fish [11–13]. To overcome these issues, urgent research is needed to develop culture and breeding methods for Cuchia. Furthermore, the formulation and implementation of new policies and environmentally friendly harvesting practices are crucial steps towards achieving the seed production targets in the

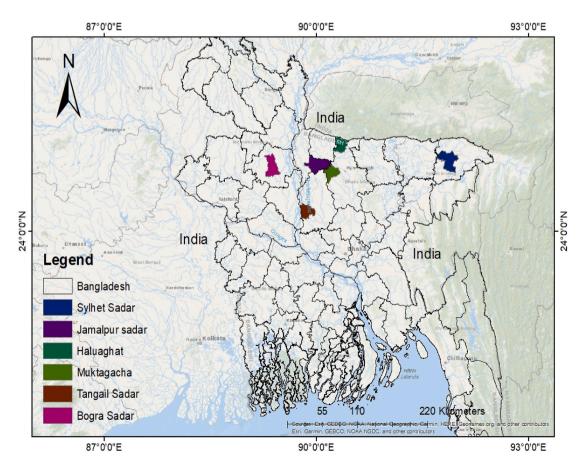


Fig. 1. Map showing sampling sites of Cuchia eel collected from different locations of Bangladesh.

aquaculture hatchery industry sector [5].

Very few reports have been published to evaluate the comparative analysis of the health status of Cuchia eel captured from various regions in Bangladesh. Salehin and Mandal [14] conducted research to investigate the hematological blood analysis of Cuchia eel between two distinct areas. Similarly, some related studies were performed to examine blood hematology [15,16], serum biochemistry [15,16], as well as blood cell morphology [17] of various fish species inhabiting different localities. However, the paucity of research on the geographical differences in the physiology and morphology of the Cuchia eel was addressed in the present study. In addition to the comparison at the population level, examining the differences in key physiological and morphological parameters will provide insights into the region-specific adaptations of the Cuchia eel in Bangladesh.

2. Materials and method

2.1. Study area

The sampling of Cuchia eel was conducted in six distinct geographical locations in Bangladesh, including Bogra Sadar (24°51′ 3.53″N and 89°22′ 15.89″E), Haluaghat (25°07′ 30.00″N and 90°20′ 60.00″E), Jamalpur Sadar (24°55′ 0.12″N and 89°57′ 29.88″E), Muktagacha (24°45′ 29.88″N and 90°16′ 0.12″E), Tangail Sadar (24°14′ 59.42″N and 89°54′ 59.58″E), and Sylhet Sadar (24°53′ 30.12″N and 91°52′ 59.88″E) as shown in Fig. 1. The precise position of each sampling site was determined using a global positioning system (GPS).

2.2. Fish collection and holding

A total of 120 healthy and lively Cuchia eel were captured from marshy lands in six different regions of Bangladesh: Bogra, Haluaghat, Jamalpur, Moktagacha, Sylhet, and Tangail. This collection, carried out in April 2022, involved capturing 20 fish from each area using bamboo traps. Subsequently, the harvested fish were transported in open tanks equipped with adequate aeration and were brought to the Laboratory of Fish Disease Diagnosis and Pharmacology, Department of Fish Health Management, Sylhet Agricultural University, Bangladesh. Upon arrival, the fish were stocked in separate tanks (capacity: 150 L/tank) for a 15-day acclimatization period before subsequent analysis. Throughout the acclimatization period, the fish were provided with small fish once daily in the evening, ensuring they were fully satiated. The tank's water quality parameters were carefully monitored and maintained at their optimum levels: temperature (28.01 \pm 0.03 °C), dissolved oxygen (5.09 \pm 0.03 mg/l), pH (7.49 \pm 0.12), and ammonia (0.11 \pm 0.02). Following this acclimatization phase, the process of sampling the fish for analysis commenced.

2.3. Biometrical and reproductive parameters

A total number of 10 fish from each group were selected and subjected to anesthesia with Tricaine Methanesulfonate (MS₂₂₂) at a dose of 0.1 g/L of water Kabir et al. [18]. After anesthetization, the length and weight of each fish were individually recorded. Dissection was performed to remove the gonad, viscera, and liver content, which were then weighed separately, following the ethical guidelines of the Animal Ethical Committee at Sylhet Agricultural University. The following formulas were utilized to calculate the gonadosomatic index (GSI), hepatosomatic index (HSI), and visceral somatic index (VSI), relative organ weight index (ROWI) as per Nandi et al. [19]:

```
GSI = 100 x (Gonad weight) / (Fish body weight)
```

HSI = 100 x (Liver weight) / (Fish body weight)

VSI = 100 x (Viscera weight) / (Fish body weight)

ROWI (%) = 100 x (Organ weight) / (Body weight + Body length)

2.4. Proximate composition analysis

The whole-body proximate composition analysis of the fish was performed following the standard methods proposed by AOAC [20], with samples being analyzed in triplicate. Shortly, the crude protein content of th test samples was estimated using the Kjeldahl method. Additionally, crude lipid was evaluated by using the Soxhlet apparatus, with n-hexane employed as an organic solvent. Ash content was calculated using a Muffle furnace, involving burning at 550 °C for 6 h. Moisture percentage was determined by using an oven set at 105 °C for 24 h. Fiber levels were evaluated employing a hot extraction unit.

2.5. Biochemical and hematological assays

The biochemical and hematological indices of fish were measured following the protocols outlined by Annino [21] and Schalm [22] with slight alterations. Briefly, three fish from each group were placed in separate tanks without feeding for approximately 5 h. Subsequently, the fish were anesthetized with MS_{222} at a concentration of 0.1 g/L of water to minimize any potential stress. Using a 1

mL heparinized and sterile syringe, approximately 150μ L of blood was collected from the caudal vein of each fish and then transferred into EDTA K₃ tubes. The hematological parameters were determined using an automatic hematology analyzer machine (Mythic 18 Vet, US).

Furthermore, the remaining blood samples were centrifuged at 5000 rpm for 10 min to separate the serum, which was subsequently stored in a freezer at -20 °C for later analyses. For biochemical assays, about 150 µL serum from each fish group was pipetted into cassettes containing the necessary reagents for determining each parameter (Brand name: IDEXX, USA). Each parameter was automatically evaluated employing a VetTest analyzer (Brand name: IDEXX, USA), with the exception of globulin. The globulin content was calculated by subtracting the values of albumin from total protein.

2.6. Blood cell morphology

The morphology of blood cells in Cuchia eel was performed according to Metin [23]. Briefly, thin blood smears were prepared from the blood samples collected previously and allowed to air-dry. Subsequently, the smears were fixed in absolute methanol for 1 min. Using Phosphate-buffered Geimsa, the slides were stained for 5 min and washed twice with dissolved water for 1 min each. Each slide was examined under the microscope, and images were captured at 100X magnification using a Leica DM LS2 digitalized camera. Blood cell morphology was then analyzed for identification.

2.7. Statistical analysis

All quantitative data, including biometrical and reproductive parameters, proximate composition, and hemato-biochemical indices, were analyzed using SPSS software version 26.0. Prior to the statistical analysis, all variables were tested employing a one-way analysis of variance (ANOVA), and Duncan's multiple range test was used to measure the significant differences (P < 0.05). The results obtained are presented as mean \pm standard deviation of means with three replicates each.

3. Results

3.1. Calculation of biometrical and reproductive parameters

The biometrical and reproductive indices of fish are documented in Table 1. Remarkable differences (P < 0.05) were observed in the biometrical and reproductive parameters (except for GSI, ROW, and ROWI) of Cuchia eel when captured from different regions at the same time. The highest mean body weight, total length, liver weight, HSI, and visceral weight were found in the Cuchia eel from the Jamalpur district, significantly (P < 0.05) higher than those from other localities. Gonad weight was notably greater (P < 0.05) in the fish caught in the Sylhet district. Additionally, the mean value of the VSI was observed to be the highest in Tangail, although no notable difference (P > 0.05) was observed in the VSI value of fish in the Moktagacha and Haluaghat regions. Nonetheless, the value of the GSI was not significantly (P > 0.05) altered among the various fish groups.

3.2. Whole-body proximate composition analysis

The whole-body proximate composition analysis of Cuchia eel is presented in Table 2. The analysis showed that the whole-body proximate composition, including crude protein, crude lipid, crude ash, moisture, crude fiber, calcium, and sand silica contents, exhibited no remarkable differences (P > 0.05) among the various fish groups collected from the six distinct regions. However, the overall mean protein content of all fish groups was notably higher (71.00 \pm 0.86 %), while the fiber content was found to be a lower

Table 1

The biometrical and reproductive indices of *Monopterus cuchia* collected from various regions (n = 10). Results are expressed as mean \pm standard deviation of means.

Parameters			Regions				
	Moktagacha	Bogra	Jamalpur	Haluaghat	Sylhet	Tangail	P value
BW (g)	237.32 ± 140.95^{b}	179.80 ± 51.34^b	394.83 ± 101.54^{a}	94.96 ± 8.49^{c}	214.38 ± 19.86^{b}	$70.55\pm18.45^{\rm c}$	0.00
TL (cm)	$59.69 \pm 10.64^{ m b}$	58.56 ± 4.62^{bc}	$74.83 \pm 5.71^{\mathrm{a}}$	53.26 ± 2.75^{c}	$60.61\pm2.12^{\rm b}$	$46.19 \pm 5.54^{\mathrm{d}}$	0.00
GW (g)	$0.89\pm0.46^{\rm b}$	$0.80\pm0.89^{\rm b}$	2.50 ± 1.34^{ab}	$0.86\pm0.12^{\rm b}$	3.51 ± 5.51^{a}	$1.06 \pm 1.10^{\rm b}$	0.02
GSI	0.52 ± 0.33	0.38 ± 0.35	0.59 ± 0.21	0.92 ± 0.16	1.64 ± 2.51	1.50 ± 1.79	0.38
LW (g)	$2.39 \pm 1.57^{\rm b}$	$1.57\pm0.60^{\rm c}$	$4.30\pm1.16^{\rm a}$	0.85 ± 0.19^{cd}	$1.50\pm0.67^{\rm c}$	$0.28\pm0.09^{\rm d}$	0.00
HSI	0.95 ± 0.20^{ab}	$0.87\pm0.18^{\rm b}$	$1.10\pm0.22^{\rm a}$	$0.89\pm0.17^{\rm b}$	$0.68\pm0.25^{\rm c}$	$0.41\pm0.12^{\rm d}$	0.01
VW (g)	$5.15\pm3.31^{\rm a}$	$2.57\pm0.55^{\rm b}$	$6.61\pm2.22^{\rm a}$	$2.02\pm0.10^{\rm b}$	$2.73\pm1.02^{\rm b}$	$1.81\pm0.84^{\rm b}$	0.03
VSI	2.25 ± 0.55^a	$1.49\pm0.29^{\rm c}$	$1.66\pm0.35^{\rm bc}$	$2.14\pm0.17^{\rm ab}$	$1.25\pm0.37^{\rm c}$	$2.56 \pm 1.17^{\rm a}$	0.02
ROW (%)	5.41 ± 4.96	2.97 ± 1.20	3.57 ± 1.02	3.95 ± 0.48	3.54 ± 1.17	5.21 ± 4.30	0.86
ROWI (%)	$\textbf{3.56} \pm \textbf{2.54}$	2.19 ± 0.77	$\textbf{2.97} \pm \textbf{0.76}$	2.52 ± 0.19	2.76 ± 0.95	3.02 ± 2.36	0.92

BW: Body weight; TL: Total length; GW: Gonad weight; GSI: Gonadosomatic index; LW: Liver weight; HSI: Hepatosomatic index; VW: Visceral weight; VSI: Visceral somatic index, ROW: Relative organ weight, ROWI: Relative organ weight index. Mean values with the different superscripts alphabet in each row are remarkably (P < 0.05) different.

Table 2

Whole-body proximate composition (dry matter basis) of *Monopterus cuchia* collected from 6 different areas of Bangladesh (n = 3). Results are expressed as mean \pm standard deviation of means.

Parameters			Regions				
	Moktagacha	Bogra	Jamalpur	Haluaghat	Sylhet	Tangail	P value
Crude protein %	72.33 ± 0.58	71.67 ± 0.58	72.00 ± 0.50	71.47 ± 0.90	71.60 ± 0.53	$\textbf{72.03} \pm \textbf{0.25}$	0.50
Crude lipid %	3.00 ± 0.01	3.06 ± 0.06	3.00 ± 0.00	3.06 ± 0.07	3.01 ± 0.02	3.03 ± 0.06	0.35
Crude ash %	18.33 ± 0.12	18.14 ± 0.12	18.31 ± 0.26	18.17 ± 0.28	18.29 ± 0.01	18.20 ± 0.26	0.77
Moisture %	5.45 ± 0.04	5.42 ± 0.02	5.42 ± 0.03	5.42 ± 0.03	5.42 ± 0.01	$\textbf{5.42} \pm \textbf{0.03}$	0.79
Crude fiber %	0.64 ± 0.01	0.64 ± 0.02	0.64 ± 0.02	0.64 ± 0.01	0.65 ± 0.05	0.64 ± 0.02	0.96
Calcium %	3.83 ± 0.12	3.87 ± 0.03	3.97 ± 0.06	3.92 ± 0.06	3.87 ± 0.02	3.91 ± 0.02	0.16
Sand silica %	1.06 ± 0.04	1.05 ± 0.03	1.07 ± 0.02	1.04 ± 0.03	1.07 ± 0.04	1.05 ± 0.04	0.83

Mean values with no superscripts letter in each row are not remarkably (P > 0.05) different.

level (0.64 \pm 0.01 %) compared to other parameters.

3.3. Blood hematological parameters

The blood hematological indices of Cuchia eel are summarized in Table 3. The findings of this study showed that the fish group from Haluaghat had significantly higher (P < 0.05) mean values of NEU, MCV, MCH, and MCHC compared to the fish from other localities. However, the lowest LYM count was observed in the fish captured from Moktagacha. The fish caught from Bogra had remarkably (P < 0.05) lower and higher mean values of MON and EOS, respectively. The significantly highest BAS content was recorded in the fish collected from the Jamalpur district, although no obvious remarkable difference (P > 0.05) was noted in the BAS levels in fish from the Bogra region. The fish group in Tangail displayed the lowest RBC, HGB, as well as HCT counts in comparison with other fish groups. However, no remarkable difference (P > 0.05) was noted in the WBC and RDW-CV values among the various fish groups. On the other hand, the RDW-SD was greatly affected by different localities, and the lowest value was observed in fish from Moktagacha. The mean values of PLT, MPV, and PCT were substantially greater (P < 0.05) in the fish obtained from the Tangail region. Nevertheless, the lowest PDW was documented in the fish from Moktagacha when compared to other regional fish.

3.4. Blood biochemical indices

Table 4 displays the serum biochemical parameters of Cuchia eel collected from six different regions of Bangladesh. The mean values of serum biochemical indices in terms of RBS (mg/dL), TP (g/dL), ALB (g/dL), GLOB (g/dL), CHOL (mg/dL), ALKP (u/L), and AG ratio, exhibited notable variations (P < 0.05) among the fish groups in various localities. Fish obtained from Bogra demonstrated

Table 3

Blood hematological variables of *Monopterus cuchia* obtained from six different areas of Bangladesh (n = 3). Results are expressed as mean \pm standard deviation of means.

Parameters			Regions			
	Moktagacha	Bogra	Jamalpur	Haluaghat	Sylhet	Tangail
WBC (10 ⁹ /L)	461.72 ± 0.08	461.71 ± 0.01	461.71 ± 0.05	$\textbf{461.68} \pm \textbf{0.11}$	$\textbf{461.70} \pm \textbf{0.05}$	461.68 ± 0.03
NEU (%)	0.64 ± 0.01^a	$0.62\pm0.01^{\rm b}$	$0.61\pm0.01^{\rm b}$	0.64 ± 0.01^a	0.64 ± 0.01^a	0.59 ± 0.01^{c}
LYM (%)	$0.01\pm0.00^{\rm d}$	$0.03\pm0.00^{\rm a}$	$0.03\pm0.00^{\rm a}$	$0.02\pm0.00^{\rm c}$	0.03 ± 0.01^{ab}	$0.02\pm0.01^{\rm bc}$
Mon (%)	$0.25\pm0.01^{\rm b}$	$0.23\pm0.01^{\rm c}$	0.27 ± 0.01^{a}	$0.24\pm0.01^{\rm bc}$	$0.25\pm0.01^{\rm b}$	$0.23\pm0.00^{\rm c}$
EOS (%)	$0.05\pm0.00^{\rm a}$	0.06 ± 0.01^{a}	$0.03\pm0.02^{\rm b}$	0.05 ± 0.01^{ab}	0.05 ± 0.01^{ab}	0.05 ± 0.00^{a}
BAS (%)	0.06 ± 0.01^{abc}	0.07 ± 0.00^{ab}	0.08 ± 0.00^{a}	$0.05\pm0.00^{\rm c}$	$0.06\pm0.02^{\rm bc}$	0.06 ± 0.02^{abc}
RBC(10 ¹² /L)	$1.63\pm0.02^{\rm a}$	$1.63\pm0.02^{\rm a}$	$1.43\pm0.20^{\rm a}$	$1.19\pm0.09^{\rm b}$	$1.43\pm0.20^{\rm a}$	$0.79\pm0.03^{\rm c}$
HGB (g/L)	$265.33 \pm 16.50^{\rm b}$	$282.67\pm2.08^{\rm a}$	215.00 ± 3.61^{d}	$250.33 \pm 6.11^{\rm c}$	$215.67 \pm 4.73^{ m d}$	$135.00 \pm 5.00^{\rm e}$
HCT (%)	$0.32\pm0.01^{\rm a}$	$0.34\pm0.02^{\rm a}$	$0.29\pm0.01^{\rm b}$	$0.29\pm0.01^{\rm b}$	$0.29\pm0.01^{\rm b}$	$0.18\pm0.02^{\rm c}$
MCV (fL)	$218.24\pm4.95^{\mathrm{b}}$	$206.94 \pm 1.53^{\rm c}$	214.78 ± 4.85^{bc}	249.96 ± 1.00^{a}	212.89 ± 6.25^{bc}	$217.7\pm4.88^{\rm b}$
MCH (pg)	$185.77 \pm 7.99^{\rm b}$	$170.67 \pm 0.72^{\rm c}$	$160.73\pm0.38^{\rm d}$	221.94 ± 2.34^a	$160.73\pm0.38^{\rm d}$	177.65 ± 7.15^{c}
MCHC (g/L)	$833.67 \pm 7.23^{\rm b}$	825.67 ± 4.16^{b}	$762.67 \pm 2.52^{\rm d}$	891.67 ± 9.45^a	830.00 ± 10.82^{b}	810.33 ± 9.61^{c}
RDW-CV (fL)	0.09 ± 0.01	0.09 ± 0.01	0.1 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.1 ± 0.01
RDW-SD (fL)	$83.00\pm2.00^{\rm b}$	84.73 ± 1.42^{ab}	$87.15 \pm 1.23^{\mathrm{a}}$	$87.11 \pm 1.62^{\rm a}$	$87.15 \pm 1.23^{\mathrm{a}}$	$85.97\pm0.77^{\mathrm{a}}$
PLT (10 ⁹ /L)	$34.67\pm1.53^{\rm bc}$	$29.00 \pm 1.00^{\rm d}$	$36.00\pm1.00^{\rm b}$	32.00 ± 0.00^{cd}	32.00 ± 2.65^{cd}	60.00 ± 2.65^a
MPV (fL)	7.97 ± 0.25^{ab}	8.33 ± 0.55^{ab}	8.23 ± 0.06^{ab}	$7.87\pm0.32^{\rm b}$	$7.87\pm0.06^{\rm b}$	8.60 ± 0.046^a
PDW (%)	$17.61\pm0.42^{\rm c}$	18.68 ± 0.46^{a}	18.14 ± 0.16^{abc}	18.41 ± 0.43^{ab}	17.97 ± 0.15^{bc}	18.27 ± 0.21^{ab}
PCT (mL/L)	$0.27\pm0.01^{\rm b}$	$0.27\pm0.02^{\rm b}$	$0.03\pm0.01^{\rm b}$	$0.23\pm0.07^{\rm b}$	$0.31\pm0.20^{\rm ab}$	$0.47\pm0.04^{\rm a}$

WBC: White blood cell; NEU: Neutrophil; LYM: Lymphocytosis; MON: Monocytes; EOS: Eosinophil; BAS: Basophil; RBC: Red blood cell; HGB: Haemoglobin; HCT: Hematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; RDW-CV: Red Cell Distribution Width-coefficient of variation; RDW-SD: Red Cell Distribution Width-standard Deviation; PLT: Platelet; MPV: Mean platelet volume; PDW: Platelet distribution width; PCT: Procalcitonin. Mean values with the different superscripts alphabet in each row are remarkably (P < 0.05) different.

Table 4

Blood biochemical indices of *M. cuchia* collected from different regions of Bangladesh (n = 3). Results are expressed as mean \pm standard deviation of means.

Parameters			Regions			
	Moktagacha	Bogra	Jamalpur	Haluaghat	Sylhet	Tangail
RBS (mg/dL)	81.00 ± 1.00^{cd}	$28.33\pm2.08^{\rm e}$	89.00 ± 3.46^{ab}	94.00 ± 2.65^a	86.67 ± 2.89^{bc}	76.67 ± 7.57^{d}
TP (g/dL)	$5.77\pm0.25^{\rm a}$	5.20 ± 0.61^{ab}	$4.73\pm0.32^{\rm b}$	$4.67\pm0.21^{\rm b}$	$4.93\pm0.55^{\rm b}$	5.17 ± 0.32^{ab}
ALB (g/dL)	$2.57\pm0.42^{\rm ab}$	$2.93\pm0.21^{\rm a}$	$2.04\pm0.15^{\rm a}$	$2.13\pm0.15^{\rm bc}$	$2.37\pm0.32^{\rm bc}$	2.30 ± 0.26^{bc}
GLOB (g/dL)	$3.2\pm0.17^{\rm a}$	$2.27\pm0.4^{\rm b}$	$2.69\pm0.17^{\rm ab}$	$2.54\pm0.06^{\rm b}$	$2.56\pm0.23^{\rm b}$	2.87 ± 0.58^{ab}
CHOL (mg/dL)	185.33 ± 4.51^{b}	$159.33\pm6.03^{\rm c}$	213.00 ± 11.27^{a}	207.00 ± 11.27^{a}	$189.33 \pm 6.66^{\rm b}$	$191.33 \pm 9.50^{\rm b}$
TRIG (mg/L)	$\textbf{27.67} \pm \textbf{0.58}$	27.33 ± 1.15	27.00 ± 1.73	27.67 ± 0.58	$\textbf{27.00} \pm \textbf{1.00}$	27.67 ± 1.53
SGPT (u/L)	12.00 ± 1.00	13.00 ± 1.73	13.17 ± 1.44	12.67 ± 2.02	11.10 ± 0.53	12.27 ± 0.64
SGOT (u/L)	13.33 ± 2.08	13.00 ± 1.00	12.00 ± 1.00	12.33 ± 0.58	12.33 ± 0.58	13.00 ± 1.00
ALKP (u/L)	$17.33\pm2.08^{\rm a}$	$8.50\pm0.50^{\rm c}$	$11.90\pm0.17^{\rm b}$	$8.80\pm0.72^{\rm c}$	$13.00\pm1.00^{\rm b}$	$13.83\pm0.76^{\rm b}$
AG ratio	$0.81\pm0.18^{\rm b}$	1.31 ± 0.14^{a}	$0.76\pm0.01^{\rm b}$	0.84 ± 0.04^{b}	0.92 ± 0.04^{b}	0.84 ± 0.27^{b}

RBS: Random blood sugar; TP: Total protein; ALB: Albumin; GLOB: Globulin; CHOL: Cholesterol; TRIG: Triglyceride; SGPT: Serum glutamic pyruvic transaminase; SGOT: Serum glutamic oxaloacetic transaminase; ALKP: Alkaline phosphatase; AG ratio: Albumin-globulin ratio. Mean values with the different superscripts alphabet in each row are remarkably (P < 0.05) different.

significantly lower (P < 0.05) levels of RBS (mg/dL), CHOL (mg/dL), and ALKP (u/L). Conversely, the highest levels of TP (g/dL) and ALKP (u/L) were found in the fish collected from Moktagacha. Although the fish from Bogra showed higher ALB (g/dL) content, no significant difference (P > 0.05) was seen in the ALB (g/dL) content between fish from Moktagacha and Jamalpur. However, other blood biochemical parameters such as TRIG (mg/L), SGPT (u/L), and SGOT (u/L) did not exhibit remarkable variations (P > 0.05) among the fish of different regions.

3.5. Blood cell morphology

Fig. 2 illustrates the light microscopic observation of blood smears from Cuchia eel, focusing on the cytoplasm and nucleus structure. The result indicated lower numbers of erythrocytes, but these were notably longer in the test fish. Additionally, it was observed that the erythrocytes exhibited an elliptical shape, with a centrally located purple nucleus. The cytoplasm was visible just outside the nucleus.

4. Discussion

This study aimed to investigate how the geographical areas influence the hemato-biochemical indices, proximate composition, and blood cell morphology of *M. cuchia*, providing a comprehensive understanding of this research. In this study, Cuchia eel captured from different regions exhibited significant differences in biometrical and reproductive parameters, with exceptions for GSI, ROW, and ROWI. Existing literature has noted the noteworthy impact of biometric parameters on the blood hematological indices of fish [24,25]. Additionally, we observed substantial variations in the serum biochemical data and whole-body nutritional content of Cuchia eel based on their biometric and reproductive parameters.

It is highly significant to examine the proximate composition of fish, as it reflects the nutritional quality of any fish used for food.

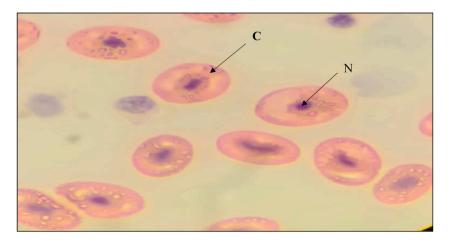


Fig. 2. Red blood cells (RBCs) of *Monopterus cuchia*. The microphotograph was demonstrated as cytoplasm (C) and nucleus (N). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

This current observation shows that the whole-body proximate composition of Cuchia eel remained consistent among the fish collected from various geographical regions. These results suggest a uniform nutritional profile of fish across the sampled regions. In addition, the protein content, at almost 72 %, was more dominant in fish compared to other nutritional contents. Mondal et al. [26] reported similar observations (dry matter basis) in indigenous Koi (*Anabas testudineus*) captured from various floodplains. Similarly, Azim et al. [27] documented corresponding results in Gangetic Sillago *Sillaginopsis panijus*, although the protein concentration varied among the different seasons. Suma et al. [28] stated that fish with a higher levels of protein and lower amounts of lipid are considered high-quality food. This study also revealed that reduced moisture content (<6 %) in all fish groups signifies an enhanced nutritional profile, as moisture is a non-nutrient component of fish. Moreover, this study demonstrated that Cuchia eel contained a very rich amount of calcium (Ca), an essential mineral for strengthening bones and nerve functions [27]. Overall, the current results stated that the adequate nutritional profile of Cuchia eel might make it an excellent source of amino acids, fatty acids, minerals, and vitamins.

It is important to note that hematological indices of blood are valuable tools for investigating the physiological aspects and health conditions of fish [16,29–33]. In addition, the hematological variables of fish may be affected by a variety of intrinsic features, including gender, stages of reproduction, size, age, and health [34–36], as well as external features, such as seasons, temperature, food, stress, and environment [35,37]. In this study, the consistent WBC count among the various groups of fish sourced from different areas might suggest an equivalent ability to phagocytize against foreign particles. Fazio et al. [15] reported a corresponding result when rainbow trout were sampled from two distinct farms. Significant changes in various leukocyte concentrations were observed among the various groups of fish collected from distinct areas, specifying considerable geographic effects on fish immunity. For instance, NEU combat infections, LYM are involved in antibody production, MON phagocytize pathogens, EOS target parasite and allergies, and BAS regulate allergic reactions, collectively contributing to fish blood's immune response. However, Meraj et al. [38] observed no substantial differences in these parameters when *Triplophysa marmorata* were sampled from three different streams. Another study by Salehin and Mandal [14] found similar mean values of NEU, LYM, MON, and EOS in *M. cuchia* captured from Mymensingh and Kishoreganj. The significantly lowest RBC was recorded in Cuchia eel harvested from Tangail compared to other regions. Similar variations were observed in *Oncorhynchus mykiss* [15] and *Mugil cephalus* [16]. Additionally, Salehin and Mandal [14] also found substantial variations in Cuchia eel RBC from the Mymensingh and Kishorgonj districts of Bangladesh. WBC and RBC are two vital parameters that reflect the immune status and oxygen-transferring capacity in fish, respectively [19,28,33].

In the present study, the HGB level showed significant variances in fish collected from different areas, with the highest value noted in the fish from the Bogra district. These results are consistent with previous reports [14,16]. HGB binds and transports oxygen from the gills to tissues, aiding respiration and metabolism. Geographical locations substantially influenced the values of HCT, MCV, MCH, as well as MCHC, in line with many previous reports [15,16,38]. Together, these parameters offer a comprehensive understanding of Cuchia eel's blood composition, aiding in assessing its physiological state and potential adaptations to its environment. Considerable variations in platelet counts were found among fish collected from various regions in the current research. In contrast, Salehin and Mandal [14] reported no significant changes in platelet concentration in Cuchia eel. The fish from the Bogra had the highest platelet count compared to other areas, indicating that this fish is much more capable of coagulating blood when injured. Additionally, other hematological parameters like RDW-SD, MPV, PDW, and PCT were significantly altered in this study. Overall, the changes in the majority of hematological parameters among various habitats indicate alterations in the health status and physiological condition of Cuchia eel.

Serum biochemical variables are also considered useful biomarkers for assessing fish health status [39]. The present research revealed considerable variations in RBS, TP, ALB, GLO, CHO, and AG ratio levels among the *M. cuchia* from various geographical locations, aligning with comparable findings in farmed rainbow trout from Italy and Turkey [15]. Francesco et al. [16] revealed corresponding results of TP, RBS, and CHO in Italian and Indian Grey mullet. RBS levels indicate the stress condition of fish. Kari et al. [33] and Nya and Austin [40] noted that ALB and GLO serve as plasma carriers and also reflect the healthy system. The TP in fish blood serves as an important indicator of various physiological processes like nutrition, immune response, and metabolic health. Meanwhile, CHO levels indicate lipid metabolism and cardiovascular health of fish. Conversely, the TRI concentrations in this study were not influenced by different areas, although Fazio et al. [15] observed opposite outcomes. TRI content reflects fat storage, energy utilization, and metabolic status of fish. The concentrations of SGPT and SGOT did not vary among the various groups of Cuchia eel fish. The identical results of these parameters reflect that differences in location, sex, and size had no discernible effects. However, no data were available regarding these parameters. Additionally, the ALP value in Cuchia eel harvested from six geographical areas was significantly different, with the lowest mean being detected in fish from Haluaghat. The present findings parallel the study by Fazio et al. [41], who noted significant variations of ALK between Indian and Turkish mullets.

The investigation of blood cell morphology aids in assessing fish health, detecting early pathology, and identifying disruptions in their aquatic environment Megarani et al. [42]. This study found that Cuchia eel blood smears contained fewer RBCs, despite their larger size. The present findings are corroborated by other fish species, such as *Carassius auratus* [43] and *Schizopyge niger* Ahmed and Sheikh [17]. Conversely, comparatively higher numbers and smaller erythrocyte sizes were observed in *M. cephalus* Parrino et al. [43] and *Schizopyge plagiostomus* Ahmed and Sheikh [17]. According to Wintrobe [44], erythrocyte size is a measure of a species' phylogenetic position in Vertebrata. It is worth noting that RBCs play a significant role in carrying oxygen into tissues.

5. Conclusions

Based on the serum biochemical and blood hematological data of Cuchia eel (*M. cuchia*), it can be summarized that different geographical locations had significant impacts on the serum biochemical and blood hematological parameters of Cuchia eel (with some exceptions), while the whole-body proximate composition of this species was not influenced by various localities of Bangladesh. This

study's findings will be useful as baseline information for future research. However, gaps in molecular analysis for genetic variations and habitat degradation effects require attention. Future research should focus on genetic diversity and habitat impacts to enhance conservation and management strategies for this valuable species.

Ethics approval and consent to participate

The codal formalities of Sylhet Agricultural University's Animal Ethical Committee were followed (SAU/291/083/23).

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding

Research was supported in part by funds provided by USDA-NIFA Sustainable Agriculture Systems, Grant No. 2019-69012-29905. Title of Project: Empowering US Broiler Production for Transformation and Sustainability USDA-NIFA (Sustainable Agriculture Systems): No. 2019-69012-29905.

CRediT authorship contribution statement

Shishir Kumar Nandi: Writing – original draft, Formal analysis. Md Abdullah Al Mamun: Supervision, Conceptualization. Afrina Yeasmin Suma: Writing – original draft. Zulhisyam Abdul Kari: Writing – review & editing, Supervision, Funding acquisition. Lee Seong Wei: Writing – review & editing. Albaris B. Tahiluddin: Writing – review & editing. Nesara Kadadakatte Manjappa: Formal analysis. Shamima Nasren: Writing – review & editing, Supervision. Shuva Saha: Visualization. Guillermo Téllez-Isaías: Writing – review & editing, Supervision, Funding acquisition. Walter G. Bottje: Visualization. Muhammad Anamul Kabir: Writing – original draft, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to the undergraduate students of the Faculty of Fisheries who collected Cuchia eel fish from different regions of Bangladesh. We are also thankful to the Department of Fish Health Management for providing laboratory facilities to conduct this research. The research article is from the collaboration between Universiti Malaysia Kelantan, Sylhet Agricultural University, Mindanao State University-Tawi-Tawi College of Technology and Oceanography and University of Arkansas. These collaborations are a part of the planning by the Advanced Livestock and Aquaculture Research Group - ALAReG under the Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus.

References

- [1] P.K. Talwar, A.G. Jhingran, Inland Fishes of India and Adjacent Countries, vol. 2, CRC press, Barrackpore, 1991, p. 1062.
- [2] M.F. Miah, H. Ali, E. Jannat, M.N. Naser, M.K. Ahmed, Rearing and production performance of freshwater mud eel, Monopterus cuchia in different culture regimes, Advances in Zoology and Botany (2015) 42–49.
- [3] M. Hussain, Freshwater Fishes of Bangladesh: Fisheries, Biodiversity and Habitat, Aquat Ecosyst Health Manag, 2010, pp. 85–93.
- [4] P.R. Neog, B.K. Konwar, The Distribution, Economic Aspects, Nutritional, and Therapeutic Potential of Swamp Eel Monopterus Cuchia: A Review, Fisheries Research, 2023 106635, https://doi.org/10.1016/j.fishres.2023.106635.
- [5] B. Chakraborty, Present status of mud eel, Monopterus Cuchia (Hamilton-Buchanan, 1822) in Bangladesh, Progress in Aqua farming and marine biology (2018) 180010.
- [6] L.N. Kakati, B. Ao, V. Doulo, Indigenous Knowledge of zootherapeutic use of vertebrate origin by the ao tribe of Nagaland, J. Hum. Ecol. (2006) 163–167, https://doi.org/10.1080/09709274.2006.11905874.
- [7] M.H. Faruque, R. Bhuiyan, K. Fatema, M.R. Hasan, Comparative evaluation of the nutritional quality of male versus female freshwater Mud Eel, Monopterus cuchia, Bioresearch Communications (BRC) (2022) 627–636.
- [8] M.A. Islam, M. Mohibbullah, S. Suraiya, M. Sarower-E-Mahfuj, S. Ahmed, M. Haq, Nutritional characterization of freshwater mud eel (Monopterus cuchia) muscle cooked by different thermal processes, Food Sci. Nutr. (2020) 6247–6258, https://doi.org/10.1002/fsn3.1920.
- [9] M.M. Hasan, B.S. Sarker, K.S. Nazrul, M.M. Rahman, A. Al-Mamun, Marketing channel and export potentiality of freshwater mud eel (Monopterus cuchia) of Noakhali region in Bangladesh, Int. J. Life Sci. Biotechnol. Pharma Res. (2012) 226–233.
- [10] M.F. Miah, M.N. Naser, M.K. Ahmed, The freshwater mud eel, Monopterus cuchia-a review, Journal of Global Biosciences (2015) 1780–1794.
- [11] B. Chakraborty, M. Mirza, Status of aquatic resources in someswari river in northern Bangladesh, Asian Fish Sci. (2010) 174–193.
- [12] B. Chakraborty, Status and position of aquatic biodiversity of four beels and its floodplain level of northern Bangladesh with a good practice of beel nurseries and community based co-management policy, Adv. Environ. Res. (2010) 121–164.
- [13] B. Chakraborty, Reproductive cycle of the mud eel, Monopterus cuchia (Hamilton-buchanan, 1822) in Bangladesh, International Journal of Oceanography and Aquaculture (2018) 000132.

- [14] A.N. Salehin, S.C. Mandal, Haematology of Air Breathing Mud Eel, Monopterus Cuchia (Hamilton, 1822) of Mymensingh and Kishoreganj Districts of Bangladesh, Dhaka University Journal of Biological Sciences, 2013, pp. 127–134.
- [15] F. Fazio, C. Saoca, G. Piccione, O.S. Kesbiç, Ü. Acar, Comparative study of some hematological and biochemical parameters of Italian and Turkish farmed rainbow trout Oncorhynchus mykiss (Walbaum, 1792), Turk. J. Fish. Aquat. Sci. (2016) 715–721.
- [16] F. Francesco, P. Satheeshkumar, D. Senthil Kumar, F. Caterina, P. Giuseppe, Comparative study of hematological and blood chemistry of Indian and Italian Grey Mullet (Mugil cephalus Linneaus 1758), HOAJ Biol. (2012) 1–5.
- [17] I. Ahmed, Z.A. Sheikh, Comparative study of hematological parameters of snow trout Schizopyge plagiostomus and Schizopyge Niger inhabiting two different habitats, The European Zoological Journal (2020) 12–19, https://doi.org/10.1080/24750263.2019.1705647.
- [18] M.A. Kabir, M.M. Iqbal, S.K. Nandi, M. Khanam, M.A.A. Sumon, A.B. Tahiluddin, Z.A. Kari, L.S. Wei, G. Téllez-Isaías, Comparative study of ovarian development in wild and captive-reared long-whiskered Sperata aor (Hamilton, 1822), BMC Zoology (2023) 10, https://doi.org/10.1186/s40850-023-00172-x.
- [19] S.K. Nandi, A.Y. Suma, A. Rashid, M.A. Kabir, K.W. Goh, Z. Abdul Kari, H. Van Doan, N.N.A. Zakaria, M.I. Khoo, L. Seong Wei, The Potential of Fermented Water Spinach Meal as a Fish Meal Replacement and the Impacts on Growth Performance, Reproduction, Blood Biochemistry and Gut Morphology of Female Stinging Catfish (Heteropneustes Fossilis), Life, 2023, p. 176.
- [20] M. Aoac, Association of official analytical chemists. Official methods of analysis. AOAC: Official Methods of Analysis (1990) 69-90.
- [21] J.S. Annino, Clinical Chemistry; Principles and Procedures, 3d ed ed., Boston: Little, Brown Boston, 1964.
- [22] O.W. Schalm, N.C. Jain, E.J. Carrol, Veterinary Haematology, third ed., Lea and Febiger Publication. 807, Philadelphia, 1975.
- [23] K. Metin, Y. Başimoğlu Koca, F. Kargin Kiral, S. Koca, o. Türkozan, Blood cell morphology and plasma biochemistry of captive mauremys caspica (gmelin, 1774) and mauremys rivulata (valenciennes, 1833), Acta Vet Brno (2008) 163–174, https://doi.org/10.2754/avb200877020163.
- [24] N. Adamu, R. Solomon, Effect of Weight and Length on Full Blood Count of Catfish (Clarias Gariapinus), Report and Opinion, 2015, pp. 72–87.
- [25] F. Fazio, C. Saoca, I. Vazzana, G. Piccione, Influence of body size on blood hemogram in rainbow trout Oncorhynchus mykiss (Walbaum, 1792), Vet Med Open J (2017) 91–94.
- [26] S. Mondal, R. Hasan, A.-A.U. Nur, Comparative study on the nutritional composition of native Koi and cultured Thai Koi (Anabas testudineus) in Bangladesh, International Journal of Fisheries and Aquatic Studies (2019) 251–254.
- [27] M. Azim, M. Islam, M.B. Hossain, M. Minar, Seasonal variations in the proximate composition of gangetic Sillago, Sillaginopsis panijus (perciformes: sillaginidae), Middle East J. Sci. Res. (2012) 559–562.
- [28] A.Y. Suma, S.K. Nandi, Z. Abdul Kari, K.W. Goh, L.S. Wei, A.B. Tahiluddin, P. Seguin, M. Herault, A. Al Mamun, G. Téllez-Isaías, M. Anamul Kabir, Beneficial effects of graded levels of fish protein hydrolysate (FPH) on the growth performance, blood biochemistry, liver and intestinal health, economics efficiency, and Disease resistance to aeromonas hydrophila of pabda (ompok pabda) fingerling, Fishes (2023) 147.
- [29] T.M. Clauss, A.D.M. Dove, J.E. Arnold, Hematologic Disorders of Fish, Veterinary Clinics of North America: Exotic Animal Practice, 2008, pp. 445–462, https:// doi.org/10.1016/j.cvex.2008.03.007.
- [30] O.K. Adeyemo, O. Okwilagwe, F. Ajani, Comparative assessment of sodium EDTA and heparin as anticoagulants for the evaluation of haematological parameters in cultured and feral african catfish (Clarias gariepinus), Brazilian Journal of Aquatic Science and Technology (2009) 19–24.
- [31] D. Padala, G.N. Marakini, A. Kokkam Valappil, P.L. Prabhakaran, M. Muhammad Abdullah Al, R. Kavalagiriyanahalli Srinivasiah, Effect of Dietary Peppermint (Mentha Piperita) on Growth, Survival, Disease Resistance and Haematology on Fingerlings of Rohu (Labeo Rohita), Aquaculture Research, 2021, pp. 2697–2705, https://doi.org/10.1111/are.15120.
- [32] Z. Abdul Kari, M.A. Kabir, K. Mat, N.D. Rusli, M.K.A.A. Razab, N.S.N.A. Ariff, H.A. Edinur, M.Z.A. Rahim, S. Pati, M.A.O. Dawood, L.S. Wei, The possibility of replacing fish meal with fermented soy pulp on the growth performance, blood biochemistry, liver, and intestinal morphology of African catfish (Clarias gariepinus), Aquaculture Reports (2021) 100815, https://doi.org/10.1016/j.aqrep.2021.100815.
- [33] Z.A. Kari, M.A. Kabir, M.A.O. Dawood, M.K.A.A. Razab, N.S.N.A. Ariff, T. Sarkar, S. Pati, H.A. Edinur, K. Mat, T.A. Ismail, L.S. Wei, Effect of Fish Meal Substitution with Fermented Soy Pulp on Growth Performance, Digestive Enzyme, Amino Acid Profile, and Immune-Related Gene Expression of African Catfish (Clarias gariepinus), Aquaculture, 2022 737418, https://doi.org/10.1016/j.aquaculture.2021.737418.
- [34] R.F. Nespolo, M. Rosenmann, Intraspecific allometry of haematological parameters in Basilichthys australis, J. Fish. Biol. (2002) 1358–1362, https://doi.org/ 10.1111/j.1095-8649.2002.tb01732.x.
- [35] J. Cazenave, D.A. Wunderlin, A.C. Hued, M.d. I.A. Bistoni, Haematological Parameters in a Neotropical Fish, Corydoras Paleatus (Jenyns, 1842) (Pisces, Callichthyidae), Captured from Pristine and Polluted Water, Hydrobiologia, 2005, pp. 25–33, https://doi.org/10.1007/s10750-004-1638-z.
- [36] K. Anjusha, M. Mamun, P. Dharmakar, N. Shamima, Effect of medicinal herbs on hematology of fishes, Int J Current Microbiol Appl Sci (2019) 2371–2376.
 [37] F.S. Rios, A.L. Kalinin, F.T. Rantin, The effects of long-term food deprivation on respiration and haematology of the neotropical fish Hoplias malabaricus, J. Fish.
- Biol. (2002) 85–95, https://doi.org/10.1111/j.1095-8649.2002.tb01738.x.
 [38] M. Meraj, A. Yousuf, F. Bhat, M. Ali, B. Ganai, N. Shahi, B. Habiba, Hematological profiling of Triplophysa marmorata (heckel 1838) from water bodies of
- kashmir HimalayaA perspective, J. Fish. Aquat. Sci. (2016) 296–303.
 [39] N. De Pedro, A.I. Guijarro, M.A. López-Patiño, R. Martínez-Álvarez, M.J. Delgado, Daily and Seasonal Variations in Haematological and Blood Biochemical Parameters in the Tench, Tinca tinca Linnaeus, 1758, Aquaculture Research, 2005, pp. 1185–1196, https://doi.org/10.1111/j.1365-2109.2005.01338.x.
- [40] E.J. Nya, B. Austin, Use of garlic, Allum sativum, to control Aeromonas hydrophila infection in rainbow trout, Oncorhynchus mykiss (Walbaum), J. Fish. Dis. (2009) 963–970, https://doi.org/10.1111/j.1365-2761.2009.01100.x.
- [41] F. Fazio, C. Saoca, A.C.A.R. Ü, R. Tezel, M. Celik, S. Yilmaz, O.S. Kesbic, F. Yalgin, M. YiĞİT, A comparative evaluation of hematological and biochemical parameters between theItalian mullet Mugil cephalus (Linnaeus 1758) and the Turkish mullet Chelon auratus (Risso 1810), Turk J Zool (2020) 22–30.
- [42] D.V. Megarani, A.B. Hardian, D. Arifianto, C.M. Santosa, S.I. Salasia, Comparative morphology and morphometry of blood cells in zebrafish (Danio rerio), common carp (Cyprinus carpio carpio), and tilapia (Oreochromis niloticus), JAALAS (2020) 673–680.
- [43] V. Parrino, T. Cappello, G. Costa, C. Cannavà, M. Sanfilippo, F. Fazio, S. Fasulo, Comparative study of haematology of two teleost fish (Mugil cephalus and Carassius auratus) from different environments and feeding habits, The European Zoological Journal (2018) 193–199, https://doi.org/10.1080/ 24750263.2018.1460694.
- [44] M.M. Wintrobe, Variations in the size and hemoglobin content of erythrocytes in the blood of various vertebrates, Folia Haematol. (1934) 32-49.