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Water quality, bacterial load and hematological investigations of three over-wintering catfishes in Mymensingh District of Bangladesh

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ABSTRACT

Studies were conducted to determine the water quality parameters, bacterial load in water and hematology of *Pangasianodon hypophthalmus*, *Heteropneoustes fossilis* and *Ompok pabda* reared in 15 over-wintering catfish ponds located at Mymensingh district of Bangladesh. Participatory rural appraisal (PRA) technique tools such as personal interview, focus group discussion (FGD) and key informant interview with fish farmers were done to collect preliminary data. Fish and water samples were collected from catfish ponds and were analyzed in the Fish Disease Laboratory of Bangladesh Agricultural University. The microbial loads of pond water (CFU mL^{-1}) were determined by ten-fold serial dilution using Tryptone Soya Agar (TSA) after incubation at 25 °C for 48 h. Hematological analysis of blood samples were done to determine RBC, WBC, hemoglobin, blood glucose level and Mean Corpuscular Hemoglobin (MCH) of the over-wintered catfish. It was found that the water temperature, pH, dissolve oxygen, total alkalinity and ammonia ranged from 20.5 to 27°C, 7.0 to 8.2, 5.0 to 7.0 ppm, 120 to 190 ppm, 0.02 to 0.04 ppm, respectively during the study period. Among the 15 ponds, the highest and lowest temperature was recorded in *P. hypophthalmus* ponds. The highest alkalinity was observed in *P. hypophthalmus* ponds and minimum was found in *O. pabda* ponds. The ammonia concentration was more or less similar (0.03 ppm) in 15 ponds. Average bacterial load of rearing water was ranged from the highest $2.6 \pm 1.60 \times 10^5$ CFU mL^{-1} to lowest $2.3 \pm 1.93 \times 10^3$ CFU mL^{-1} in *H. fossilis* ponds. The study revealed maximum RBC, WBC contents and the highest glucose level in *O. pabda* and minimum in *H. fossilis* and *P. hypophthalmus*. However, MCH and hemoglobin levels were found higher in *P. hypophthalmus* and lower in *O. pabda*. The study provided data regarding over-wintering farm condition, bacterial loads of over-winter water, hematology and water quality parameters of three over-wintering catfish which also indicated the unplanned management of over-wintering ponds that sometimes affects water quality and hematology of catfish. The findings may contribute for the proper health management of fish reared under over-wintering condition.

Keywords: Bacterial load, catfish, hematology, over-wintering, water quality

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1 Introduction

Fish is a principal source of animal protein for over half of the global population (FAO, 2018). Fisheries sector is contributing significantly in food security through providing safe and quality animal protein; almost 60% of animal protein comes from fish. Bangladesh is one of the world's leading fish producing countries with a total production of 4.27 million MT in the fiscal year 2017-18, where aquaculture production contributes 56.24% of the total fish production (DoF, 2018). Average growth performance of aquaculture sector is 5.26% for last 10 years. Government is trying to sustain this growth performance, which eventually ensures to achieve the projected production target of 4.55 million MT by 2020-21 (DoF, 2018). Among the farmed fish species, catfish is an important group, which is successfully being cultured in many countries of South Asia and South East Asia. In Bangladesh, farming of freshwater catfish in pond and lake has begun for the last few decades. Aquaculture production in Mymensingh and adjacent districts has been increased progressively and some catfish species *viz.*, pangas (*Pangasianodon hypophthalmus*), shingi (*Heteropneoustes fossilis*), pabda (*Ompok pabda*) are considered as successful monoculture species in this region due to suitability in culture, taste, high-market demand and suitability to local climate conditions. However, spawning season of these popular aquaculture species begins in April and seeds are not normally available until June-July which ultimately reduces the duration of the production season (Hill and Hill, 1994; Motin et al., 2013; Saha et al., 2014). For this reason, fry/ fingerlings produced in a season are reared at a higher density over the winter period and stocked into the grow-out ponds at lower densities in the next year at the onset of the warmer period (late February to early March). By this means the fish may get a longer warm period and grow to a larger size at harvest. Generally, these types of fish fry/ fingerlings are known as over-wintering fish which are kept in a higher stoking density using lower amount of food. Under these circumstances, over-wintering of late spawned catfish fry is the only way to continue the supply of fingerlings at early period of the following grow-out season.

The major concerns of the fish culturist should deal with the aspects of water quality, which may cause poor growth or death of fish (Boyd, 1978; Piper et al., 1982). Water quality parameters, which are of prime importance, are mainly temperature, turbidity, dissolved oxygen, carbon dioxide, ammonia, pH, alkalinity etc. Any change in the water quality has direct influence on biotic communities where different species of flora and fauna exhibit great variations in their responses to the altered environment (Abalaka, 2013; Watson and Lawrence, 2003). Strong relationship between the bacterial contamination of healthy freshwater fish and its surrounding environment have already been revealed where high bacterial loads ultimately influenced greater harbour of enteropathogenic bacteria in fish but the immunological properties of fish thought to have influences against pathogens (Roy and Barat, 2011). The bacteriological quality of water also plays a vital role in the disease spreading in farmed fish. The flesh of fish can be infected with a wide range of microorganisms present in the water body (Pyatkin and Krivoshein, 1986), hence the bacterial flora of the fish depicts the level of bacteria of the water environment (Torimiro et al., 2014) and become pathogenic when conditions such as temperature changes, dietary, hormonal stresses and other physico-chemical parameters are favorable for the development of pathogenesis (Willoughby, 1976; Svobodova et al., 1993). In addition, substantial knowledge on the hematological parameters is an important tool that can be used as an effective index to monitor the physiological and pathological changes in fishes (Rambhaskar and Rao, 1987; Zhou et al., 2008). These indices have been proven to be a valuable approach for analyzing the health of the fish (de Pedro et al., 2005) or any other animals and also provided reliable information on metabolic disorders, deficiencies and chronic stress status (Bahmani et al., 2001; Cnaani et al., 2004). When environmental condition does not maintain in optimal range for normal fish growth, the fish culture could be affected.

Generally, there is a big potential for pond catfish farming in the Mymensingh aquaculture zone. High disease resistances, higher stocking density with greater production rates make some catfish species ideal for increasing the aquaculture production. The spawning season of most of the aquaculture species in Bangladesh begins in April. That is why, seeds are not normally available until June-July which results in the reduced duration of the production season. In this case, over-wintering of catfish fry is the best way to get fry/ fingerlings at the right time of culture period. However, information regarding the status over-wintering catfish ponds, physiological status of these catfish as well as water quality of the over-wintering catfish rearing ponds is rear. Thus, the present study was planned to determine the water quality parameters, bacteriological loads of rearing water and hematology of some over-wintering farmed catfish *viz.*, pangas (*P. hypophthalmus*), shingi (*H. fossilis*) and pabda (*O. pabda*) in Mymensingh, Bangladesh.

2 Materials and Methods

2.1 Selection of farms and ponds

Although, a considerable number of fish farms are now-a-days engaged in catfish farming and sporadically distributed in different areas adjacent to

Bangladesh Agricultural University, Mymensingh, all of them do not stock the over-wintering fry/ fingerlings. Information of the catfish farms, those have the records regarding the continuous stocking of over-wintering fry/ fingerlings for at least several years, were taken from the Upazila Fisheries Office after the successful discussions with the Upazila Fisheries Officers (UFOs). Fifteen over-wintering catfish farms were selected from different upazila *viz.*, Tarakanda, Fulbaria, and Mymensingh Sadar located under Mymensingh district where 5 farms were decided for *P. hypophthalmus*, 5 for *H. fossilis* and rest 5 farms for *O. pabda*. From the each category of farm (i.e., *P. hypophthalmus*, *H. fossilis* and *O. pabda* farm), one over-wintering pond was selected. Thus, 5 over-wintering ponds were selected for *P. hypophthalmus*, 5 ponds for *H. fossilis* and remaining 5 ponds for *O. pabda*.

2.2 Data collection

Field data on the status of over-wintering catfish farms were collected for a period of 3 months from January to March, 2019 using a questionnaire survey from the selected 15 farms. A set of questions was organized in a sequential and required logical format to collect the data. Participatory rural appraisal (PRA) tool including focus group discussion (FGD) was conducted with fish farmers. Prior to field survey, background information on the number, location and distribution of over-wintering catfish farms and aquaculture activities were collected. Data collection method was divided into three steps i.e., (i) Questionnaire interviews, (ii) Focus group discussion and (iii) Observation and photography.

2.3 Fish and water sampling

Fish were sampled during data collection and caught by the hand held scoop net. During every sampling water quality parameters e.g., dissolved oxygen (DO) (mg L^{-1}), water temperature (°C), pH and free ammonia (NH_3) contents were monitored on the pond spots to get the instant data without any time laps. The dissolved oxygen values were recorded using a dissolved oxygen test kit "Aqua D.O." Advance Pharma Company, Bangkok, denoted as milligram per liter (mg L^{-1}). Water pH of individual pond was recorded using a portable pH test kit (Advance Pharma Company, Bangkok). Also, ammonia content in rearing water was determined using Ammonium test kit, "VET-AMMONIUM" Vet Superior Aquaculture Company, Bangkok and total alkalinity measured by using alkalinity test kit "AQUA BASE" Advance Pharma Company, Bangkok. Water temperature was measured by hand thermometer and denoted as °C. Collected water samples were analyzed and the data were recorded.

2.4 Bacterial load analysis

Samples for pond water bacterial load examination were collected from 15 over-wintering catfish ponds located in Mymensingh district. Water was sampled from the mid-level of the ponds. About 200 mL water was collected in sterile plastic bottle, transported to Fish Disease Laboratory, BAU, Mymensingh serially diluted using 0.85% physiological saline and plated on Tryptone Soya Agar (TSA). The plates will be incubated at 25°C for 48 h. The colonies were counted and expressed in colony forming unit per mL (CFU mL^{-1}).

2.5 Hematological examination

2.5.1 Blood sampling

Blood was collected from the caudal vein using tips following the technique of Raihan (2018). Whole blood withdrawal process took less than 1 min per fish which was considered important to avoid stress effects in order to minimize any error in normal blood values. Blood samples were mixed gently with respective fluid and were discarded when difficulty in taking them or clots seen in the vial during examination at the laboratory.

2.5.2 Hematological analysis

Total RBCs count and WBCs count were determined by using Improved Neubauer hemocytometer (Hesser, 1960), Hemoglobin (Hb) concentration was estimated by cyanmethemoglobin (Blaxhall and Daisley, 1973), Blood glucose levels (mg dL^{-1}) were measured using glucose strips in a digital Easy-Mate® GHb and WBC, RBC and Mean Corpuscular Hemoglobin (MCH) was calculated using the formulae mentioned by Dacie and Lewis (2001).

$$WBC = \frac{N_l \times D_F}{V_F} \tag{1}$$

where, WBC = WBC count, N_l = number of large square cell (1), D_F = dilution factor (40), and V_F = volume factor (0.1).

$$RBC = \frac{N_l \times D_F \times C_F}{N_s} \tag{2}$$

where, RBC = RBC count, N_l = number of large square cell (5), D_F = dilution factor (200), C_F = counting factor (4000), and N_s = number of small squares counted (80).

$$MCH = \frac{Hb \times 10}{RBC} \tag{3}$$

where, MCH = Mean Corpuscular Hemoglobin (ρg)

2.6 Data analysis

Collected data were analyzed using "Microsoft Excel 2010". The results were shown in descriptive tabular and graphical presentation. One-way ANOVA followed by Tukey's multiple comparisons test was performed using GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA.

3 Results

3.1 General information the ponds

3.1.1 Biosecurity issues taken by the catfish ponds

From the study, it was observed that only few farms had boundary fences. About 60% of *P. hypophthalmus* and *O. pabda*, farms and only 40% of *H. fossilis* farms were surrounded by boundary. Most of the catfish farmers were well concerned about the protected dikes. Each of *P. hypophthalmus* and *H. fossilis* farmers had same ratio (80%) of protected dikes on the ponds and rest *O. pabda* farmers are mostly concerned about the dike protection. There were no driveways in the studied area. It was also found that there were no significant foot or tire bath facilities before entering into their farms except some *P. hypophthalmus* farms (20%) and cloth changing areas to change into a complete new set of freshly laundered clothing for their staffs or visitors. There were only less 20% *O. pabda* and *H. fossilis* farms having cloth changing and showers facility to make a complete head to toe shower (Fig. 1).

3.1.2 Water quality measurement

In the study area, most of the farmer have diminutive knowledge about water quality parameters of their ponds including temperature, dissolved oxygen, pH range, alkalinity, ammonia and others. Although every (100%) farmers measured only pH in the farms, 20% of them measured other water quality parameters like DO, Temperature, Alkalinity etc. respectively (Fig. 2).

3.1.3 Disinfection facilities

In the study area, almost all the farms of *P. hypophthalmus* and *O. pabda*, all farmers removed bottom waste and dried their over-wintering ponds after two or three cycle of production. In comparison to *H. fossilis* farmers, only 60% removed their pond waste. From the survey, it was found that 100% farmers of *H. fossilis* used different types of antibiotics and chemicals to control diseases. Only 60% of *O. pabda* farmers did not use any types of antibiotics and chemicals to control diseases (Fig. 3). Farmers used some common antibiotics like Bio-Oxy, Cepro plus, Aqua Magic, Ranaquin, Biotax etc. Application of lime and salt were the most commonly used chemicals for the treatment of diseases.

3.2 Water quality parameters

3.2.1 *P. hypophthalmus* ponds

The average water quality parameters (temperature, pH, dissolved oxygen, total alkalinity and ammonia) are presented in Table 1. Mean value of water temperature was found 23.84±2.90. The maximum value of water temperature was found in Solaiman Miah Fisheries, Mymensingh Sadar and minimum was found in Fahim Fisheries, Tarakhanda. The maximum value of pH (pH 8.2) was found in Nur Jahan Fisheries, Mymenshingh Sadar and Fahim Fisheries, Tarakhanda while minimum (pH 7.3) was found in Kader Fisheries, Dapunia. On the other hand, the highest dissolved oxygen was recorded in Sornalota Agro Fisheries Ltd., Fulbaria and Kader Fisheries, Dapunia) while lowest dissolved oxygen found was in Fahim Fisheries, Tarakhanda. In this experiment the highest total alkalinity 190 ppm was also recorded in Fahim Fisheries, Tarakhanda and the lowest was observed in Kader Fisheries, Dapunia. Ammonia concentrations were same in all the ponds (0.03 ppm).

3.2.2 *H. fossilis* ponds

The average water quality parameters are presented in Table 2. In this study the maximum water temperature (25°C) was found in Sagor Fish Farm, Tarakanda while minimum was observed in Sajeeb Mathsho Khamar, Tarakanda (23°C). The pH of the pond water was recorded as more or less similar at different ponds and maximum value was found in Sagor Fish Farm and Joynal Fisheries (Tarakanda) (pH 7.9) while minimum was revealed in Mosharaf Hossain Fisheries and Sajeeb Mathsho Khamar (Tarakanda) (pH 7.3). The highest dissolved oxygen was recorded in Joynal Fisheries, Tarakanda while lowest was found in Mosharaf Hossain Fisheries, Tarakanda). The highest total alkalinity 170 ppm was observed in Sagor Fish Farm, Tarakanda and the lowest was recorded in Nur Jahan Fisheries-2, Mymensingh Sadar). Ammonia concentrations were also same in the *H. fossilis* ponds (0.03).

3.2.3 *O. pabda* ponds

In the cases of *O. pabda* ponds, water temperature ranged from 22 to 27°C (Table 1) where the mean temperature was found 23.84±1.88. pH was varied from 7.0 to 7.9 where the mean pH was 7.66±0.39. The DO was ranged from 6.0 to 8.0 ppm in different ponds. The maximum value of DO was found in Sajeeb Fish Farm-2, Tarakanda (8.0 ppm) while the minimum was found in Anil Fisheries Enterprise

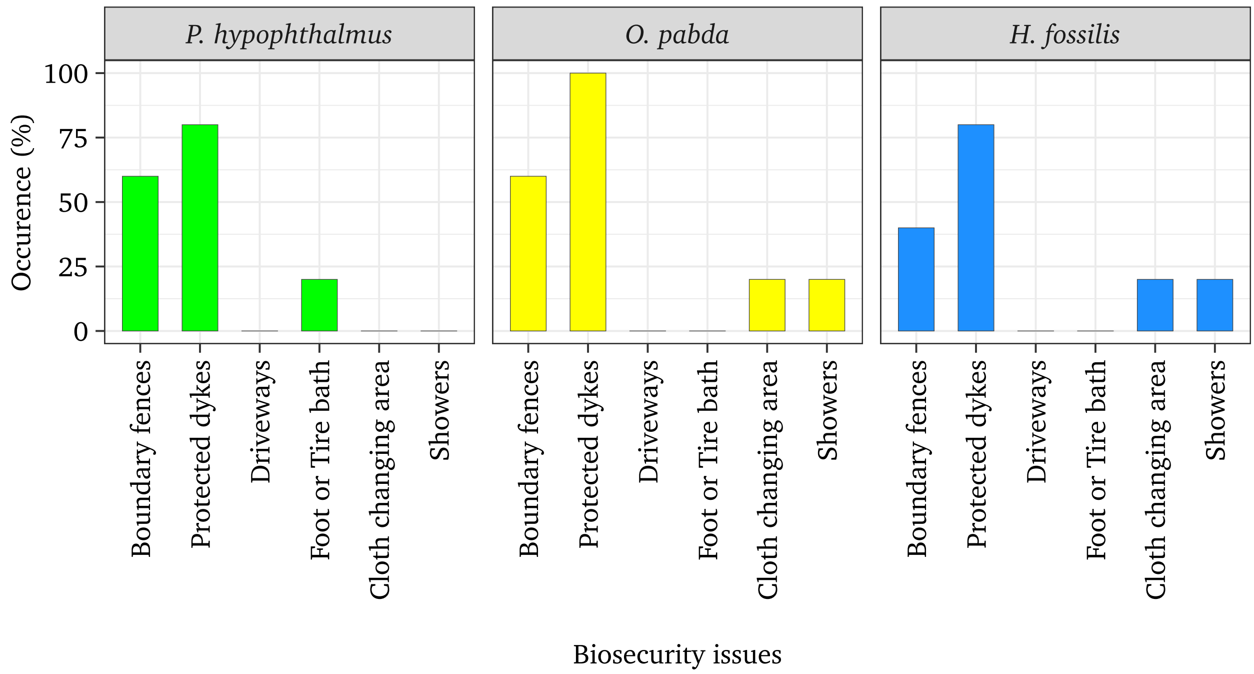

Figure 1. Biosecurity issues observed in the over-wintering catfish ponds of Mymensingh District of Bangladesh

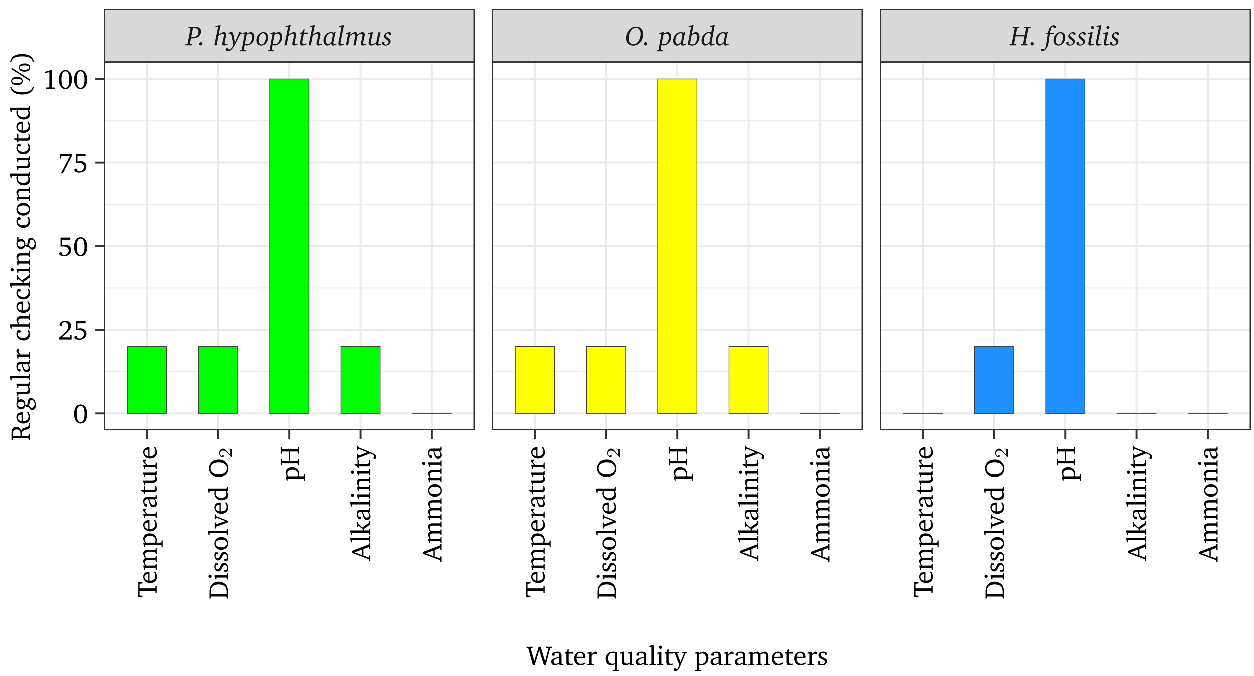

Figure 2. Regular checking of water quality parameters in over-wintering catfish ponds of Mymensingh District of Bangladesh

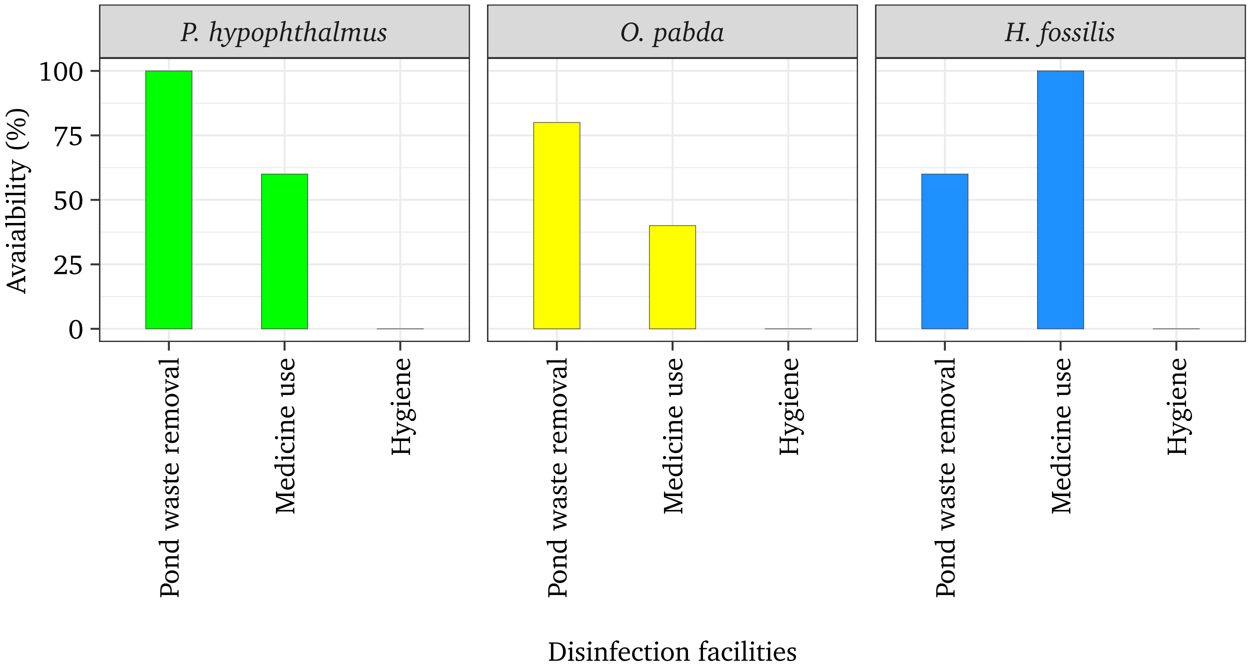

Figure 3. Disinfection facilities available in over-wintering catfish ponds of Mymensingh District of Bangladesh. Hygiene includes the availability disinfectant spray, disinfected boots and vehicles, and footbath facilities

and Setu Fish Farm (Tarakanda) (7.3 ppm). The highest total alkalinity 170 ppm was recorded in Sajeeb Fish Farm-2, Tarakanda and the lowest total alkalinity was observed in Dada Bhai Fish Farm, Tarakanda (120 ppm). Ammonia concentrations were same in five ponds (0.03 ppm). One-way ANOVA followed by Tukey's multiple comparisons test revealed that there was no significant variation in water temperatures (P = 0.5462), water pH (P = 0.5189), water DO (ppm) (P = 0.2915), and water total alkalinity (ppm) (P = 0.4044) among three different types of fish ponds. Moreover, the ammonia concentrations (ppm) of all the pond water were the same.

3.3 Bacterial load

The highest bacterial loads ($\sim 10^5$ CFU mL^{-1}) were observed in the case of 2 pangas farms from Mymenshingh Sadar (*viz.*, Solaiman Miah Fisheries and Kader Fisheries, Dapunia), a shigni farm from Tarakanda, Mymensingh (*viz.*, Sajeeb Mathsho Khamar) and 3 pabda farms from Tarakanda, Mymensingh (*viz.*, Anil Fisheries Enterprise, Sajeeb Fish Farm-2 and Setu Fish Farm),. On the other hand, lowest loads were reported from ($\sim 10^3$ CFU mL^{-1}) a pangas farm (*viz.*, Fahim Fisheries, Tarakhanda) and a shigni farm (*viz.*, Mosharaf Hossain Fisheries, Tarakanda). The farm-wise average bacterial loads estimated from the over-wintering catfish ponds were placed in Table 2. One-way ANOVA followed by Tukey's multiple comparisons test revealed no significant variation in average bacterial loads among three different types of fish ponds (P = 0.2599).

3.4 Hematological parameters

3.4.1 *P. hypophthalmus*

The erythrocyte counts (RBC) were ranged from 1.55±0.74 to 2.94±0.53 (Table 3). The maximum RBC ($\times 10^6$ mm^{-3}) was found in Kader Fisheries, Dapunia and minimum was observed in Fahim Fisheries, Tarakhanda. Number of WBC ($\times 10^3$ mm^{-3}) was ranged from 159.74±32.76 to 71.5±14.39. WBC value was the highest in Sornalota Agro Fisheries Ltd., Fulbaria and the lowest was recorded in Solaiman Miah Fisheries, Mymenshingh Sadar. The hemoglobin in the blood was tested and highest value (3.70±0.10 g dL^{-1}) was found in Kader Fisheries, Dapunia but the lowest reading was recorded as 2.1±0.14 (g $^{-1}$) in Sornalota Agro Fisheries Ltd., Fulbaria. The maximum blood glucose level (189±32.53 mg dL^{-1}) was found in Solaiman Miah Fisheries, Mymenshingh Sadar and minimum (131±2.83 mg dL^{-1}) was found in Fahim Fisheries, Tarakhanda. In addition, the value of mean corpuscular hemoglobin (MCH) was found to be varied between 17.54±5.69 to 7.94±1.33 ρg (Table 3) throughout the experiment. The highest MCH value was revealed from Fahim Fisheries, Tarakhanda (17.54±5.69 ρg) the lowest value was recorded from Kader Fisheries, Dapunia (7.94±1.33 ρg).

Table 1. Water quality parameters of over-wintering catfish ponds of recorded during the study period

Parameters	*P. hypophthalmus*		*H. fossilis*		*O. pabda*	
	Range	Average	Range	Average	Range	Average
Temperature (°C)	20.5–27.0	23.84±2.90	21.0–25.0	22.46±1.73	22–23.7	23.84±1.88
pH	7.3–8.2	7.86±0.40	7.3–7.9	22.46±1.73	7.0–7.9	7.66±0.39
DO (ppm)	5.0–6.0	6.2±0.84	5.0–6.0	6.0±0.71	6.0–8.0	6.8±0.84
Total Alkalinity (ppm)	140–190	158±19.24	140–170	156±11.40	120–170	144±19.49
Ammonia (ppm)	0.02–0.4	0.03±0.01	0.03–0.03	0.03±0.01	0.03–0.03	0.03±0.01

Values are average ± standard deviation

Table 2. Summary of average bacterial load estimated from over-wintering catfish ponds

Sl.	Name and location of the farms	Bacteria count (CFU mL^{-1}) †
	P. hypophthalmus	
1	Sornalota Agro Fisheries Ltd (Radhakanai, Fulbaria)	3.1 ± 0.26×10^4
2	Nur Jahan Fisheries (Madhabarera, Bypass road, Mymenshingh Sadar)	2.1 ±.0.83×10^4
3	Fahim Fisheries (Paschimtaldhigi, Tarakhanda)	2.9 ± 1.07×10^3
4	Solaiman Miah Fisheries (Bypassroad, Mymenshingh Sadar)	1.7 ± 0.77×10^5
5	Kader Fisheries (Majhihat, Santinagarbazar, Dapunia)	1.2 ± 0.81×10^5
	H. fossilis	
6	Sagor Fish Farm (Tengulia, Banihala, Tarakanda)	1.4 ± 0.79×10^4
7	Mosharaf Hossain Fisheries (Maskanda, Balikha, Tarakanda)	2.3 ± 1.93×10^3
8	Nur Jahan Fisheries-2 (Madhobarera, Bypass road, Mymensingh Sadar)	1.7 ± 0.93×10^4
9	Joynal Fisheries (Lautia, Balihala,Tarakanda)	8.9 ± 1.01×10^4
10	Sajeeb Mathsho Khamar (Madhupur, Uttarpara, Tarakanda)	2.6 ± 1.60×10^5
	O. pabda	
11	Anil Fisheries Enterprise (Kudaldhor Bazaar, Tarakanda)	1.4 ± 0.60×10^5
12	Dada Bhai Fish Farm (Majhiali, Balihala, Tarakanda)	8.8 ± 0.83×10^4
13	Sajeeb Fish Farm-2 (Madhupur, Tarakanda)	1.9 ± 0.97×10^5
14	Setu Fish Farm (Panguai, Tarakhanda)	2.3 ± 1.03×10^5
15	Catfish Agro Hatchery and Fisheries (Shamvugonj, Mymensingh Sadar)	9.3 ± 0.85×10^4

Values are average ± standard deviation; † Average heterotrophic bacteria count in TSA Agar

Table 3. Hematological parameters of *P. hypophthalmus*, *H. fossilis* and *O. pabda* reared in over-wintering ponds

Parameters	*P. hypophthalmus*		*H. fossilis*		*O. pabda*	
	Range	Average	Range	Average	Range	Average
RBC ($\times 10^6$ mm^{-3})	1.55 – 2.94	2.33±0.56	2.07 – 2.68	2.43±0.23	2.34 – 3.51	3.03±0.61
WBC ($\times 10^3$ mm^{-3})	71.5 – 159.74	123.18±33.42	68.16 – 158.7	142.43±40.70	136.65 – 242.1	198±42.04
Hemoglobin (g dL^{-1})	2.1 – 3.70	2.98±0.66	2.40 – 3.10	2.72±0.29	2.60– 3.40	3.04±0.36
Glucose (mg dL^{-1})	131 – 189	158±21.60	26 – 261	109.9±103.53	231 – 368.5	290.5±51.21
MCH (ρg)	7.94 – 17.54	13.59±3.76	11.38 – 11.97	11.42±0.62	9.43 – 11.44	10.39±0.94

Values are average ± standard deviation

3.4.2 *H. fossilis*

The results of RBC values ranged from 2.68±0.28 to 2.07±0.65 (cells $\times 10^6$ mm^{-3}). The highest RBC was counted from the collected fish of Mosharaf Hossain Fisheries, Tarakanda and the lowest from the Nur Jahan Fisheries-2, Mymensingh Sadar. Mean values were shown in Table 3. In this study, WBC ($\times$ 10^3 mm^{-3}) values were ranged from 68.16±18.01 to 158.7±31.04. WBC content was the highest in Sajeeb Mathsho Khamar, Tarakanda and the lowest was counted in Nur Jahan Fisheries-2, Mymensingh Sadar. The average hemoglobin levels (g dL^{-1}) were ranged from 3.15±0.07 to 2.40±0.14 and the highest was recorded from Mosharaf Hossain Fisheries, Tarakanda and the lowest from Nur Jahan Fisheries-2, Mymensingh Sadar. Range of blood glucose levels (mg dL^{-1}) were observed from 26±8.49 (mg dL^{-1}) to 261 ± 18.38. Blood glucose levels were found fluctuating during experiment and the highest average value were determined from Sajeeb Mathsho Khamar, Tarakanda whereas the lowest was recorded from Sagor Fish Farm, Tarakanda. In addition, the MCH value was the highest (17.97±2.40 ρg) in Nur Jahan Fisheries-2, Mymensingh Sadar and the lowest (10.39±3.46 ρg) in Sajeeb Mathsho Khamar, Tarakanda.

3.4.3 *O. pabda*

RBC values ($\times 10^6$ mm^{-3}) ranged from 2.34±0.62 to 3.51±0.71 (Table 3). The maximum RBC value was found in Sajeeb Fish Farm-2, Tarakanda and minimum was observed in Catfish Agro Hatchery and Fisheries, Mymensingh Sadar. Number of WBC ($\times 10^3$ mm^{-3}) ranged from 136.65±16.42 to 242.1±6.79 where the highest was recorded from the *O. pabda* sample of Catfish Agro Hatchery and Fisheries, Mymensingh Sadar and the lowest was counted from Dada Bhai Fish Farm, Tarakanda. Hemoglobin level was the highest in Sajeeb Fish Farm-2, Tarakanda and the lowest in Catfish Agro Hatchery and Fisheries, Mymensingh Sadar. The maximum blood glucose reading was found in in Sajeeb Fish Farm-2, Tarakanda (368.5±33.23 mg dL^{-1}) and the minimum was recorded in Anil Fisheries Enterprise, Tarakanda (231±39.60 mg dL^{-1}). MCH value varied between 9.43±1.70 to 11.44±0.77 (ρg). The maximum value was found in Catfish Agro Hatchery and Fisheries and minimum was observed in Sajeeb Fish Farm-2 (**??**). One-way ANOVA followed by Tukey's multiple comparisons test revealed that there was no significant variation in RBC (P = 0.0981), hemoglobin (P = 0.5295) and MCH (P = 0.1162) among the fish species. However, significant variations were found in fish RBC (P = 0.0140), glucose (P = 0.0034) among *P. hypophthalmus* and *O. pabda*, *H. fossilis* and *O. pabda*, but the difference was not significant among *P. hypophthalmus* and *H. fossilis*.

4 Discussion

Spawning season of most of the freshwater aquaculture species in Bangladesh begins in April and fries are not generally available before June-July, which ultimately reduce the duration of culture period. Fry/ fingerling produced in a late season are reared at higher densities with minimum feeding over the winter period and stocked into grow-out ponds at lower densities on the next year onset of the warmer period (late February to Early March). This technique of over-wintering the fish enables to get longer warmer period, grow faster and reach marketable or even larger size quickly. Over-wintering catfish has already become very important in the aquaculture industry of Bangladesh. In the present study, the over-wintering catfish farms in Mymensingh were investigated, relevant information were collected, water quality parameters of the ponds were measured, bacterial loads in pond water were determined as well as hematological parameters of fish were assessed.

4.1 General information about fish farms

In the study area, 40% of total *P. hypophthalmus* farms were used for the grow-out production purpose whereas, few farmers (>20%) of *H. fossilis* as well as *O. pabda* farms were engaged with the similar purpose during the over-wintering. Others were involved in hatchling rearing in their farms. Culture of these species upto harvestable size is profitable as these are commercially important for the aquaculture in Bangladesh. Ali et al. (2014) reported that most of the fish farmer (63%) in Barisal district, Bangladesh directly stocked the fish in grow-out ponds. On the other hand, 12% farmers stocked firstly at a nursery pond and 25% stocked in the hapa before releasing of the fry in the grow-out pond. Therefore, the present finding was supported by the findings of Ali et al. (2014).

It was also found that 60% of *P. hypophthalmus* farmers and 40% of *H. fossilis* as well as *O. pabda* farmers received training based on modern aquaculture practices from GOs and NGOs. The rests did not receive any training. Previously, 33% fish farmers of Trishal upazila, Mymensingh were reported to receive formal training from different organizations such as: DoF, BFRI and NGOs (Sheheli et al., 2013). This finding was quite relevant to the present study. However, the present findings revealed the stronger training background of the farmers than the output of Khatun et al. (2013) who observed that only 14% fish farmers received training on improved fish farming from upazila fisheries office with the help of Department of Fisheries of Bangladesh (DoF) and 10% from NGOs in Charbata, Noakhali, Bangladesh. It can be opined that many fish farmers under Mymensingh aquaculture zone are able to get training frequently which should include the over-wintering catfish management for sustainable fry/ fingerling supply. During over-wintering catfish pond management, most of the farmers (80%) of *P. hypophthalmus* and *O. pabda* dried their ponds in farms whereas removal of pond bottom wastes was done by less than 60% farms. In addition, almost all farmers of the study area were found regular in dike protection. These results were also confirmed by Sheheli et al. (2013) who found that almost all (86%) fish farmers in Trishal, Mymensingh dried their ponds.

Biosecurity plays an important role in every stage of the life cycle of a fish from hatching to harvesting and has become a set of necessary tools for ensuring healthy aquatic production. In the present study very few catfish farmers were conscious about the biosecurity issues. As a result, most of the farmers faced several problems like deterioration of water quality, bacterial infestation, reduction of fish growth etc. The uses of disinfected protective clothing, footbaths and hand sanitation are effective personnel biosecurity measures that considerably reduce the transmission of pathogens (Pollard et al., 2008). In the present study, it was also found that there were no significant foot or tire bath facilities before entering into their farms except in some *P. hypophthalmus* farms (20%). In addition, the farmers were very much aware about the water sources and most of the farmers used ground water by using deep tube well and shallow tube well during over-wintering period. However, biosecurity are maintined more in hatchery operation than grow-out farming operation. It was found that about 76.66% hatcheries in Mymensingh maintained the disinfecting method properly, 3.33% used foot bath and none of the hatcheries found use protective clothing for their own staff or visitor (Faruk, 2008).

Majority of the farmers (80%) collected fish fry from hatcheries for stocking in the ponds in their farms. Monitoring of water quality is of utmost important. In the study area, every farmer monitored water pH in their farms, although very few farmers (20%) measured other water quality parameters like DO, Temperature, Alkalinity etc. Poor monitoring of the water quality parameters might occur due to lack of the proper technical knowledge of the farmers. Ali et al. (2014) showed that only 3% of farmers measured pH, 2% of farmers measured temperature and 1% of farmers measured salinity. But no farmers measured ammonia and nitrate, some of these findings coincided with the present study. Almost all farmers in the study area preferred commercial feed and very few used farm made feed for fish in their farms. It was observed that 95% of the farmers applied supplementary feed such as rice bran, mustard oil cake and commercially manufactured feed for over-winter fish. Rest 5% of the farmers did not apply feed during over-winter and depended on the natural food of the pond. Alam (2006) also found that 80% of the farmers applied supplementary feed such as rice bran and mustard oil cake. Therefore, these findings also coincided with the present research outcome.

Lower percentages of farmers in the study area took some preventive measures like regular health checking, pond drying, application of lime, weeding of pond, addition of water etc., although around no farmers removed water turbidity as preventive measures from the ponds in the farms. On the contrary, Sheheli et al. (2013) reported that almost all fish farmers in Trishal upazila, Mymensingh dried their ponds and used lime. Similarly, majority of the fish farmers in rural area of Bangladesh took measures to prevent outbreak of diseases in their ponds such as pond drying, addition of water, use of lime before disease outbreak, removal of water etc. (Faruk et al., 2004). In addition, most of the farmers in Cumilla weeded the pond and half of the farmers dried their ponds, used lime as well as exchanged water for prevention of fish diseases (Hassan et al., 2019). All farmers of *H. fossilis* farms in the study area used different types of antibiotics and chemicals to control diseases of fish, whereas approximately half of the farmers in *P. hypophthalmus* and *O. pabda* farms applied antibiotics and disinfectants for disease treatment. Sheheli et al. (2013) observed that farmers in Trishal of Mymensingh used chemicals and toxic substances to control aquatic weeds, pests, predators and undesirable species and prevent different fish diseases. Among them 22% farmers used antibiotics. In cumilla, 66.67% of both selected and non-selected farmers used different types of chemotherapeutics like Timsen, Bio-Oxy, Cipro plus, Aqua Magic, Ranaquin, Biotax etc. (Hassan et al., 2019). On the other hand, few farmers in rural freshwater aquaculture of Bangladesh used antibiotics and vitamins (Faruk et al., 2004). In addition, most of the *P. hypophthalmus* farmers in Mymensingh used liming as the most common treatment followed by application of salt, potassium permanganate, antibiotics, pesticides and insecticides (Faruk, 2008). Application of different chemotherapeutic agents during winter season is a common practice in the freshwater aquaculture of Bangladesh. Approximately half of the farmers in the over-wintering catfish farms monitored fish health weekly or biweekly. Similarly, in Cumilla almost 50% selected farmers and 10% non-selected farmers regularly checked fish health for better production of aquaculture (Hassan et al., 2019).

Diseases of fish are one of the major constraints resulting from intensification of aquaculture and may eventually become a limiting factor to the economics of a successful and sustainable aquaculture industry. The most disease occurring season in the aquaculture of Bangladesh was reported as winter followed by rainy season (Faruk et al., 2004; Faruk, 2008). In the present study, about 40% farmers of all farms had to face bacterial and fungal disease problems whereas few farmers were concerned about parasitic diseases. Major bacterial diseases like motile aeromonas septicemia (MAS), dropsy, tail and fin rot diseases; fungal diseases like gill rot disease, sparolegniasis, epizootic ulcerative syndrome (EUS) and parasitic disease like argulosis were the most common diseases in the studied farms. Previously, 73% fish farmers of Trishal upazilla, Mymensingh reported diseases like tail and fin rot, EUS, oxygen deficiency disease, argulosis, saprolegniasis, gas bubble disease and nutritional deficiency (Sheheli et al., 2013). The present finding was also supported by the findings of Faruk (2008) who collected information from *P. hypophthalmus* culturists in Mymensingh and other report of Faruk et al. (2004) who gathered information from rural freshwater aquaculture area of Bangladesh.

4.2 Pond water quality parameters

The mean water temperature of the ponds were measured as 23.84±2.90, 22.46±1.73 and 23.84±1.88 °C in *P. hypophthalmus*, *H. fossilis* and *O. pabda*, respectively, which might fluctuate due to weather fluctuations, changes of sun brightness and/ or time of the day. Balla (2012) reported that temperature of water changes with respect to season and environmental conditions. Hossain (2002) observed the suitable temperature for aquatic production 22 to 32°C which was more or less similar to the present findings. The pH ranges of the overwintering pond water were found to be slightly alkaline (7.60 to 7.86). Azad et al. (2004) recorded pH ranging from 6.18 to 9.16 in polyculture ponds, which coincides with the present study. pH ranging from 6.5 to 9.0 is suitable for pond aquaculture (Swingle, 1967) whereas, slightly alkaline pH is beneficial for overall fish culture (Jhingran 1982). Thus, the pH of the pond ware observed during this study was within suitable ranges. Kohinoor et al. (2012) measured DO level from 4.23 to 5.32 mg L^{-1} in *H. fossilis* culture ponds, which was slightly lower than the present findings as the DO values of overwintering pond water were ranged from 6.0 to 6.8 mg L^{-1} during the study. Comparatively higher level of DO might be found due to the sampling time, because DO was monitored at about 11.00-12.00 am. Total alkalinity values depend upon the location, season, plankton population, nature of bottom deposits etc. In the present experiment, total alkalinity in all pond waters ranged from 144 to 158 ppm. Boyd (1982) opined that the total alkalinity should be more than 20 mg L^{-1} as production increases with the increase in total alkalinity. Moreover, the productive ranges of total alkalinity of water for aquaculture ponds (Wahab et al., 1995; Kohinoor et al., 1998) were corresponded to the present study. Boyd (1982) reported that the suitable range of ammonia-nitrogen (NH_4-N) in fish culture less than 0.1 mg L^{-1}. Kohinoor et al. (2001) also found that the ammonia-nitrogen ranged from 0.01-1.55 mg L^{-1} in monoculture ponds. The mean values of ammonia-nitrogen contents in the present study (0.03±0.01) were similar to all the over-wintering catfish ponds. Therefore, the present findings were entirely conformed the reference data

4.3 Total bacterial load

The overall bacterial load in *P. hypophthalmus*, *H. fossilis* and *O. pabda* pond water in the over-wintering catfish farms was found from lower range (2.3×10^3 CFU mL^{-1}) to higher (2.5×10^5 CFU mL^{-1}). The variation in bacterial load might be due to feed used in over-wintering pond, degree of water change or other environmental factors. Similar kind of observation was reported by Chowdhury et al. (1994) where the bacterial count ranged from 1.3×10^4 to 5.6×10^5 CFU mL^{-1} in catfish pond water. This is consistent with the study of Jun et al. (2000), who also reported microbial load of aerobic heterotrophic bacteria in the pond water which fluctuated between 0.01×10^5 CFU mL^{-1} and 8.7×10^5 in CFU mL^{-1}. The findings of these reports supported the present study.

4.4 Hematological parameters

Hematology is the study of the normal and pathologic aspects of blood and blood elements. Many biotic and abiotic factors may affect blood parameters in fish. Previous findings showed that physico-chemical differences in the habitats influenced the haematological parameters in fish which ultimately suggested that hematological parameters may be suitable for monitoring the effects of habitat changes on fish biology and fish culture practices (Fazio et al., 2012a,b). The present experiment revealed that the maximum number of erythrocytes (RBC) was observed in *O. pabda* and the minimum in *P. hypophthalmus*. The erythrocyte levels in fish determine the capacity to transport the oxygen (Nikinmaa, 1997). The differences in erythrocyte counts observed in the study could be either due to difference in fish species or the habitat they live along with their feeding habits. Duman and Şahan (2017) reported Erythrocyte content in *Garra rufa* 1.22×10^6 mm^{-3} to 1.97×10^6 mm^{-3} in winter, which supported the finding of the present study. White blood cells (WBCs) or leukocytes are cells that are directly associated with specific and nonspecific immunological responses (Iwama and Nakanishi, 1996). Leucocye levels in blood may vary according to the environmental quality (LeaMaster et al., 1990), nutritional state (Barros et al., 2002), the presence of infectious agents (Martins et al., 2008) and parasitism (Martins et al., 2004). In the present study, the lower level of WBC in *H. fossilis* was probably due to their lower innate immunity because of lower nutrient uptake in winter as increasing of WBC indicates increase of innate immunity (Rajikkannu et al., 2015). WBC counts in blood of fish varies between the seasons, species and sex (Sheikh and Ahmed, 2016), hence a greater variation of WBC values in the present trial were observed in different species at varied times.

Hemoglobin is the protein molecule in red blood cells that carries oxygen from lungs or gills to the body tissues where it releases the oxygen to burn nutrients to produce energy to power the functions of the organisms in metabolism process. Mohideen and Haniffa (2015) reported that higher hemoglobin levels resulted higher oxygen uptake from water. The present study observed the highest hemoglobin content in *P. hypophthalmus* and lowest in *H. fossilis* ponds. The Hb value in all the three species of fish was found variable which could be attributed to the fact that the oxygen carrying capacity of the fish was affected by different feeding habits, their habitat and metabolism (Southamani et al., 2015). Duman and Şahan (2017) observed Hb content in Garra rufa 5.58 to 7.83 g dL^{-1}, which was higher than the finding of the current study. Also, the lower HB value might be due to lower temperature and lower oxygen uptake by fish along with other intrinsic and extrinsic factors.

van de Nieuwegiessen et al. (2009) recorded that increased blood glucose levels let *Clarias gariepinus* respond to stress. In the present study, the highest blood glucose level (368.5±33.23 mg dL^{-1}) in over-wintering *O. pabda* was found might be due to quite stressful condition of pond water. This value exceeded the normal blood glucose level (80 to 100 mg dL^{-1}) and also was higher than the blood glucse level of *C. gariepinus* (75.33±4.70 mg dL^{-1}) cultivated with a density of 200 individuals/ m3 using a flowing water system (Hastuti and Subandiyono, 2013). Moreover, Vhatkar et al. (2016) mentioned that the rising of blood glucose levels due to mobilization which caused hyperglycemia. On the other hand, the Mean Corpuscular Hemoglobin (MCH) value in the present study ranged from 7.94±1.33 to 17.54±5.69 ρg, which was smaller than the MCH value of *Schizothorax plagiostomus* (25.76-28.45 ρg) reported by Sheikh and Ahmed (2016). This lower level of MCH in the study might be found due to higher percentage of RBC.

5 Conclusions

Some water quality parameters were found variable than the recommended values which turned the pond water sometimes stressful for over-wintering catfish species. Higher fish stocking density and improper management of water quality were probably responsible for higher bacterial loads. The hematological parameters provided preliminary information on the physiological condition of fish in rearing environment in winter/ later winter period. However, extreme stress can have alterations on hematology and internal physiology, and finally inability to adapt and leads to metabolic depression, thereby affecting growth. In conclusion, the results of this research provided a preliminary contribution probably for the first time regarding the studies in the overwintering farms, hematological condition and water quality parameters of three over-wintering catfish species. Further detailed investigation on immunological and physiological information of overwintering fish along with the stocking densities and rearing environment would provide advanced information for researchers which might help to improve management of over-wintering catfish species.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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