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Sexual Dimorphism in Clinical Chemistry and Profile of Hybrid Catfish (*Heterobranchus longifilis*)

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ABSTRACT

Sex has been reported to influence the clinical chemistry of several species of fish. Whether sex impacts serum biochemistry composition and electrolyte profile of *Heterobranchus longifilis* is not well captured in the literature. This study aimed to evaluate the impact of sex on the clinical chemistry composition and electrolyte profile of hybrid catfish, *Heterobranchus longifilis*. Blood samples were collected and biochemically analyzed. The analytes analyzed included alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, creatinine, total bilirubin, conjugated bilirubin, unconjugated bilirubin, serum protein, albumin, total cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride, and urea. Uric acid, bicarbonate, Chloride, Sodium, and Potassium from 40 healthy *Heterobranchus longifilis* (20 males and 20 females). The males and females were not reproductively active at the time of sampling (None of the females had eggs). Most clinical chemistry analytes and electrolyte profiles of *Heterobranchus longifilis* showed differences between male and female values even though only a few (alanine aminotransferase, aspartate aminotransferase, creatinine, triglyceride, and uric acid) were statistically significant ($p < 0.05$). Based on the findings in this study, we suggest that sexual differences affect the clinical chemistry and electrolyte profile of Heterobranchus longifilis. Hence, sexual differences should be taken into consideration during sampling in both natural and experimental studies in Heterobranchus longifilis.

Keywords

Electrolytes, Heterobranchus longifilis, clinical chemistry, Sex

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Abbreviations

ALP: Alkaline phosphatase
AST: Aspartate aminotransferase
ALT: Alanine aminotransferase –
HDL: High-density lipoprotein – HDL
LDL: Low-density lipoprotein
CSB: Conjugated serum bilirubin
UCSB: Unconjugated serum bilirubin

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Introduction

Fishes are increasingly getting more recognition as an economic and reliable source of quality protein of animal origin owing to their rich nutritional values [1]. Aquaculture is presently one of the fastest-growing sectors of the food production industry globally, taking about 50 % of the overall food supply [2]. Aquaculture is known to be one of the most efficient means of food production [3, 1]. Fish is widely consumed by a range of people, notwithstanding their age, level of income, or health status [4, 2]. *Heterobranchus longifilis*, on the other hand, has been documented to have the capacity for an efficient feed conversion rate [5]. Several criteria made *H. longifilis* suitable for aquaculture, and these qualities include its capacity for high yield potential, fast growth rate, high fecundity, hardiness, and palatability [6]. Serum biochemistry analysis could help in identifying target organs of toxicity in addition to unraveling the general health status of fish [7, 8]. Several scholars have reported that the biochemical parameters of fish have become useful tools for the determination of physiological and pathological changes in diverse fish species [9, 10, 11]. This is because these biochemical indices offer valuable information on the responses of fish to diverse ecological and physiological changes [5, 12, 8]. Additionally, several pathological changes are reflectedin serum chemistry long before the manifestation of clinical diseases [13, 8].

The values of biochemical parameters are substantially influenced by several physiological factors, including the sex of the fish [14]. A study assessing serum chemistry parameters discovered that sex can induc some level of influence on some parameters of C. gariepinus [15]. Other scholars have repeatedly reported that sex, age, diet, fish species and strains, nutritional state, geographical location, disease, feeding regime, sexual maturity cycle, and seasonal variations in temperature and salinity, can strongly influence values of biochemical parameters and health status of fish [4, 5, 1, 11]. While several studies have evaluated the serum biochemistry of different species of fish, there is limited information on variations in serum chemistry and electrolyte profile of *H. longifilis* concerning sex. The influence of sexual differences on serum chemistry analytes and electrolyte profiles in H. longifilis may offer valuable baseline information that could enhance further studies on mechanisms associated with the influence of sexual differences on the biochemical parameters of fish. This study aimed to evaluate the impact of sexual dimorphism on the serum biochemical and electrolyte profile of hybrid catfish (*Heterobranchus longifilis*).

Result

Mean Serum enzymes

Analysis of the serum enzymes results showed mean serum Aspartate Aminotransferase (AST) level of male *H. longifilis* was higher with a mean value of 114.6 U/L compared to the females with mean serum AST value of 111.2 U/L while the mean AST value of both males and females combined was 112.9 U/L (Fig. 1). For ALP on the other hand, the mean serum Alkaline Phosphatase level of the male H. longifilis is not significantly different ($p > 0.05$), with a mean value of 37.8 U/L compared to the female with mean serum Alkaline Phosphatase value of 40.1 U/L while the mean Alanine Aminotransferase value of both male and female combined was 39.0 U/L. Even though there was no statistically significant difference between sex, the ALP value of the females unlike AST, was slightly higher compared to that of the males. The mean serum alanine Aminotransferase (ALT) level of the male H. longifilis was however, significantly higher ($p < 0.05$) with a mean value of 28.5 U/L compared to the females with a mean value of 18.5 U/L while the mean Alanine Aminotransferase value of both male and female combined was 23.5 U/L.

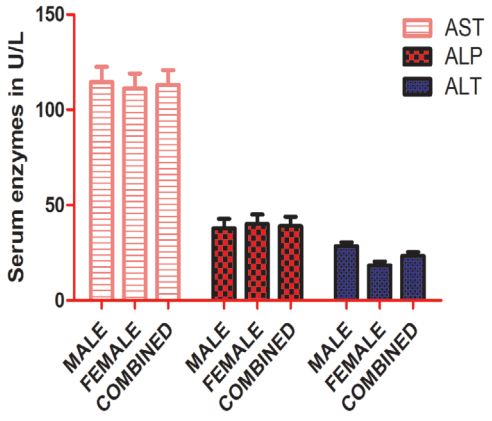

Figure 1.
Mean Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), and Alanine Aminotransferase (ALT) from male and female H. longifilis and combined values, all in mg/dL

Mean serum Creatinine level

Analysis of the serum creatinine level showed serum creatinine level of male *H. longifilis* was significantly higher ($p < 0.05$) compared to the females with mean serum creatinine values of 50.7 µmol/L as against 44.5 µmol/L while the mean creatinine value of both male and female combined was 47.6 µmol/L (Fig. 2).

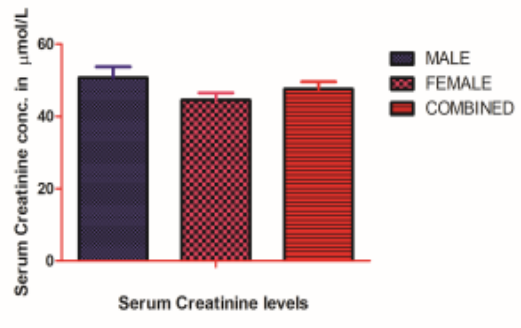

Figure 2.
Mean serum Creatinine concentration from male and female *H. longifilis* and combined mean serum Creatinine value

Serum Bilirubin levels

Analysis of the results showed there was no statistically significant difference between male and female *H. longifilis* in mean total serum bilirubin levels. The male and female mean total serum bilirubin values were 14.0 mg/dL and 13.9 mg/dL respectively while the mean value of both males and females combined was 14.0 mg/dL (Fig. 3). There was also no statistically significant difference between male and female H. longifilis concerning conjugated serum bilirubin (CSB) level, even though the males had substantially higher values compared to the females. The male and female mean CSB values were 8.23 mg/dL and 6.12 mg/dL respectively while the mean value of both males and females combined was 7.18 mg/dL. Statistical analysis of the unconjugated bilirubin results also showed there was similarly no statistically significant difference ($p > 0.05$) between male and female *H. longifilis*, even though the value was higher in females compared to males. The male, female, and combined mean UCSB values were 5.77 mg/dL, 7.78 mg/dL, and 6.77 mg/dL respectively.

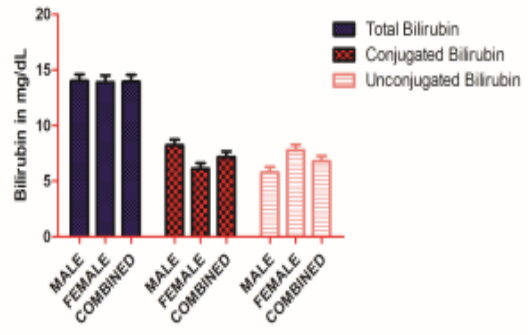

Figure 3.
Mean values of total, conjugated and unconjugated Bilirubin concentrations from male, and female *H. longifilis*, and their combined mean values

Serum Protein and Albumin Levels

Statistical analysis of protein and albumin results showed there was no statistically significant difference ($p > 0.05$) between male and female *H. longifilis* with regards to mean total serum protein level (Fig. 4), even though the value was substantially higher in females compared to males. The male and female mean total serum protein values were 53.0 g/dL and 61.0 g/dL respectively, while the mean value of both male and female combined is 57.0 g/dL. Analysis of the albumin results showed no statistically significant difference between male and female *H. longifilis*, even though the value was higher in males compared to females. The male, female, and combined mean serum albumin values were 24.5 g/dL, 26.2 g/dL, and 25.4 g/dL respectively.

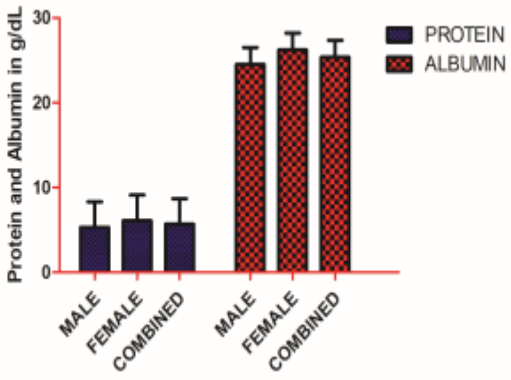

Figure 4.
Mean serum total Protein and Albumin concentrations from male and female *H. longifilis* and their combined mean values in g/dL.

Total serum Cholesterol level

Results of serum cholesterol, as observed in this study, are depicted in Fig. 5. Analysis of the results showed there was no statistically significant difference ($p > 0.05$) between male and female *H. longifilis*, even though the value was slightly higher in females compared to males. The male and female mean total serum cholesterol and their combined values were 2.42 g/dL, 2.62 g/dL, and 2.52 g/dL, respectively.

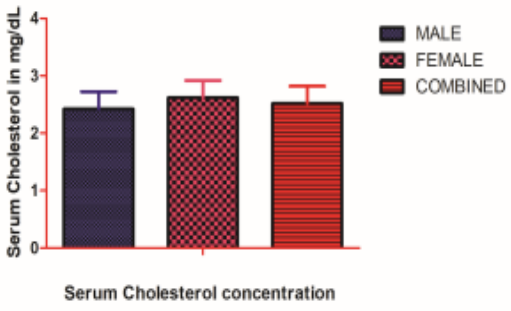

Figure 5.
Mean serum Cholesterol concentration from male and female H. longifilis and combined mean serum Cholesterol value

Mean Serum High-Density Lipoproteins and Low-Density Lipoproteins Levels

Statistical analysis of lipoproteins results showed there was no statistically significant difference ($p > 0.05$) between male and female *H. longifilis* in serum HDL levels (Fig. 6), even though the value was substantially higher in females compared to males. The males and females had mean serum HDL values of 1.35 mg/dL and 1.63 mg/dL, respectively while the mean value of both males and females combined was 1.49 mg/dL. Analysis of serum LDL results, on the other hand, showed that there was no statistically significant difference between male and female *H. longifilis,* even though the value was slightly higher in females compared to males. The male and female mean LDL values and the combined values were 1.20 mg/dL, 1.35 mg/dL, and 1.28 mg/dL, respectively.

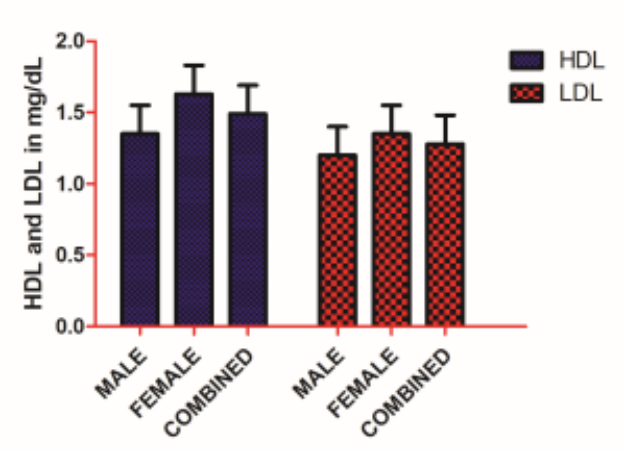

Figure 6.
Mean High Density and Low-Density Lipoprotein levels from male and female *H. longifilis* and their combined mean values

Mean Serum Triglyceride level

The results of serum triglyceride levels in males, and females and their combined value are depicted in Fig. 7. Analysis of the results showed there was a statistically significant difference ($p < 0.05$) between male and female *H. longifilis*, with the females having higher value (1.65 mg/dL) compared to the male with serum triglyceride values of 1.39 mg/dL, while the mean value of both male and female combined is 1.52 mg/dL.

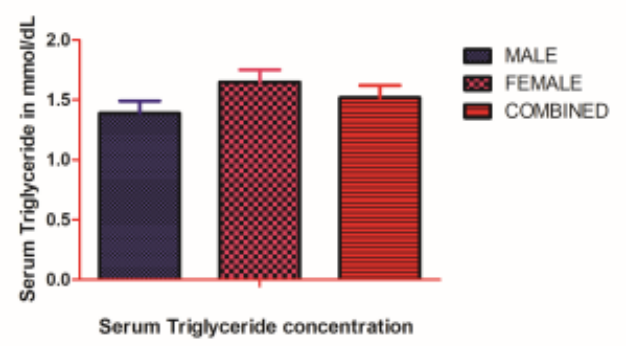

Figure 7.
Mean Triglyceride concentration from male and female *H. longifilis* and combined mean Triglyceride value

Mean Serum Urea and Uric acid Concentrations

Mean serum urea and uric acid concentrations for males, females, and their combined value are shown in Fig. 8. Analysis of the results showed there was no statistically significant difference ($p > 0.05$) between male and female *H. longifilis* in serum urea concentration, even though males had slightly higher serum urea concentration compared to the females. The male and female serum Urea concentrations were 3.20 mmol/L and 2.90 mmol/L respectively while the mean value of both male and female combined is 3.05 mmol/L. On the other hand, results showed a statistically significant difference ($p < 0.05$) between the concentration of serum uric acid in male and female *H. longifilis*. The male, female, and combined serum uric acid concentrations were 65.45 µmol/L, 71.87 µmol/L, and 68.66 µmol/L respectively.

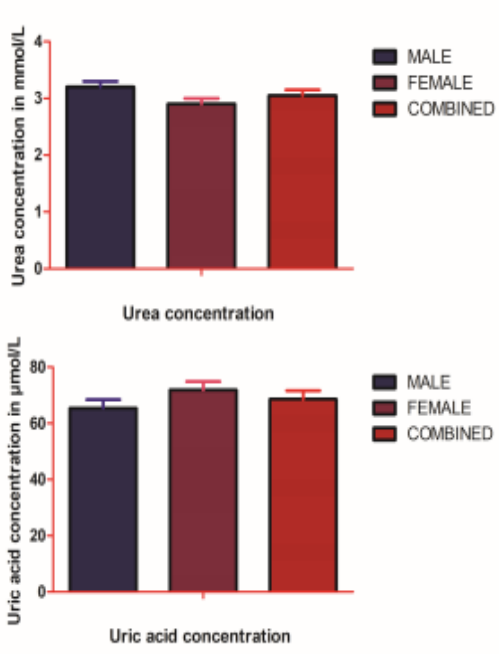

Figure 8.
Mean Urea and Uric acid concentrations from male and female *H. longifilis* and their combined mean values

Mean Serum Sodium and Chloride Concentration

Sodium and chloride concentrations for males, females and combined values are presented in Fig. 9. Statistical analysis of the results showed no statistical-

ly significant difference ($p > 0.05$) between male and female *H. longifilis* in serum Sodium concentration. The male and female serum sodium concentrations were 145.44 mmol/L and 139.12 mmol/L, respectively, while the mean value of both males and females combined was 142.28 mmol/L. Even though there was no statistically significant difference in Sodium concentrations between males and females, the mean sodium concentration of the males was slightly higher than that of the females. chloride results showed no statistically significant difference between male and female *H. longifilis*. The male, female and combined serum Chloride concentrations were 109.27 mmol/L, 102.0 mmol/L, and 105.64 mmol/L respectively. However, the mean Chloride concentration of the males was substantially higher when compared to that of the females.

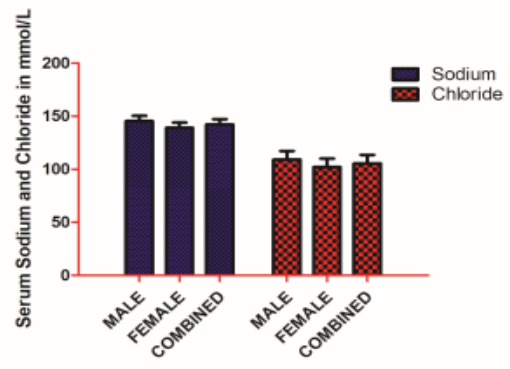

Figure 9.
Mean serum Sodium and Chloride concentrations from male and female *H. longifilis* and their combined mean values

Mean Serum Bicarbonate and Potassium Concentrations

Bicarbonate and potassium concentrations observed in this study for males, females and combined values are shown in Fig. 10. Statistical analysis of serum bicarbonate concentration results showed there was no statistically significant difference between male and female *H. longifilis*. The male and female serum bicarbonate concentrations were 27.55 mmol/L and 25.97 mmol/L respectively while the mean value of both male and female combined is 26.76 mmol/L. Although there was no statistically significant difference between serum bicarbonate concentrations of males and females, the mean serum Bicarbonate concentrations of males were slightly higher compared to that of the females. For serum potassium concentration, on the other hand, there was no statistically significant difference between male and female *H. longifilis*. The male, female, and combined serum potassium concentrations were 6.25 mmol/L, 5.10 mmol/L, and 5.68 mmol/L respectively. The mean potassium concentration of the males was higher compared to that of the females.

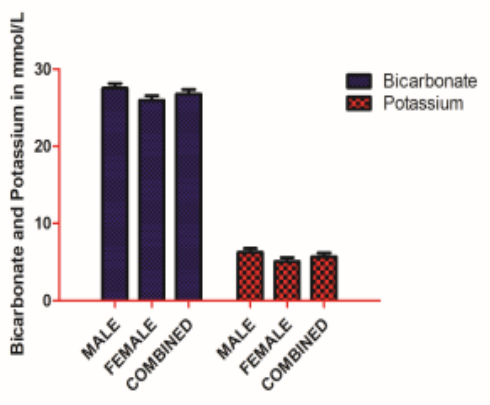

Figure 10.
Mean serum Bicarbonate and Potassium concentrations from male and female *H. longifilis* and their combined mean values

Discussion

Fish are the largest and most extensively diversified species of aquatic organisms. Owing to their proximity to the aquatic environment, alterations in their environment are rapidly expressed in their blood [16, 11]. Diverse scholars are increasingly interested in the study of clinical chemistry characteristics of fish as they reflect the overall health status of fish. These indices offer reliable insight into metabolic disorders, chronic conditions, and deficiencies, before clinical manifestations [12, 16]. Several pathologic changes have been reported in serum chemistry long before the manifestation of any clinical disease [12, 16]. In this study, the mean serum AST and ALT of the males were significantly higher compared to those of the females, while ALP was higher in females even though insignificantly compared to the males. The observed differences in serum enzymes between males and females are in tandem with the findings in other recent related studies, where different species of fish were found to have differences in levels of these serum enzymes and attributed the difference to sex-linked physiological processes in the fish [5, 14, 12]. However, while few scholars [17, 13] found that AST and ALT were higher in females than males, the contrary was our findings in this study. The differences may not be unconnected with several physiological factors and environmental conditions and many other factors such as temperature and quality of the water management practices at the time of such experiments, since fish are known to be in close proximity to their environment [11].

Creatinine is excreted through the kidney, hence

an increase in the level of serum creatinine in fish reflects severe kidney injury [14]. In this study, the mean serum creatinine levelwas higher in males than females, even though the differences were not statistically significant. These findings agreed with the results of earlier studies [17, 14], where male rainbow trout and Notopterus notopterus were reported to have higher creatinine levels than females. The overall creatinine levels in both males and females were not high, which indicates the normal function of the kidney in both males and females in this experiment. Bilirubin is a bile pigment synthesized endogenously, and its accumulation in the body could be toxic. An increase in the conjugated bilirubin concentration of serum reflects a distorted balance between the rate of haem conversion to bilirubin and the capacity of the liver to produce conjugated bilirubin [18, 19]. In this study, total bilirubin and conjugated bilirubin in males were slightly (statistically insignificant) higher than in females, while unconjugated bilirubin in females was significantly higher than in males. This study reported a higher level of unconjugated bilirubin in females, which is consistent with previous studies that female *H. longifilis* had higher bilirubin levels than males [20, 5].

Changes in total serum proteins and albumin are known to be clinically relevant in establishing the health status of fish [21]. The total serum protein level is a critical and reliable indicator to evaluate the physiological status, nutritional state, stress, and general well-being of fish [22]. The higher level of total serum protein observed in females compared to males confirms the findings reported by Sharma et al. [21] and Jan and Ahmed [10], who found higher total serum protein in snow salmon and Schizothorax labiatus and Barilius bendelisis, respectively. Similar to the findings of this study, other studies [23, 21] have reported higher albumin levels in Salmo trutta fario and beluga whale (Huso huso) males compared to females. However, the differences in albumin levels between males and females were not statistically significant, and this has also been similarly reported in a related study [10]. These differences in both total serum protein and serum albumin have been attributed to feed intake, starvation, growth rate, andfeed conversion rate [24, 25]. Total serum protein varies seasonally between sexes [10].

Generally, cholesterol is vital for appropriate body function as it serves as a substrate for the synthesis of several crucial and active biological constituents, including sex hormones [13, 21]. Several scholars have reported that seasons and rate of growth/stage influence cholesterol levels [5, 10]. In this study, there was no significant difference in cholesterol levels between males and females, even though that of the females was slightly higher compared to males. These results confirm the findings reported in related studies, where cholesterol levels did not significantly differ between males and females. [5, 21]. In fish, lipid storage serves as the primary energy reserve, and fluctuations in serum lipid levels in various fish species have been recorded [26, 21]. High-density lipoprotein cholesterol could be determined directly from the serum by enzymatic techniques, using cholesteryl esterase and cholesterol oxidase methods [27]. In this study, there was no significant difference in HDL between males and females, even though the HDL in females was slightly higher compared to females. In a related study, Sharma *et al.* [21] reported similar findings in male and female Barilius bendelisis from Central Himalaya, India. On the other hand, low-density lipoproteins are recognized as mediators of cholesterol and cholesterol ester absorption in several fish tissues [14]. The higher LDL levels in females compared to males, as observed in this study, were also reported in an earlier study, and the differences were attributed to reproduction, maturation, and metabolic rate of the fish [21].

The level of triglyceride, in concurrence with other lipids, is valuable in the diagnosis of several conditions, such as triglyceridemia, dyslipidemia and hyperlipoproteinemia [28]. The observed higher levels of triglyceride in females compared to males in this study are similar to the findings reported in an earlier related study [21], where female Tench (Tinca tinca) had higher levels of triglyceride compared to males. The variations in the triglyceride between males and females were attributed to different metabolic rates, feeding intensity, and seasons [29, 21]. The concentration of urea in the blood is a reflector of protein metabolism in the system [16]. In this study, the level of urea was higher in males compared to females, even though the difference was not statistically significant. Several researchers have also documented higher urea concentrations in males compared to females [14, 10]. Uric acid, on the other hand, constitutes a major water-soluble antioxidant in fish blood [30]. In this study, uric acid level was significantly higher in females compared to males. This study appears to be the first one that evaluates uric acid in male and female catfish, and the differences in uric acid levels between males and females could only be speculatively attributed to differences in the rate of excretion, mainly via the kidney, as well as from overproduction of uric acid owing to excess purine precursors synthesis, turnover of cells [31].

Blood electrolytes such as Sodium (Na^+), Potassium (K^+), Chloride (Cl^-), and Phosphorous (*P*) are common parameters employed in the determination of physiological states, toxicity, and health status of

fishes, and their levels reflect operations of diverse homeostatic mechanisms in fish [14]. In this study, Sodium, Chloride, Bicarbonate, and Potassium levels were higher in males than females, even though the differences were not statistically significant. In a similar study, Kulkarni [14] found that male Notopterus notopterus had lower levels of these electrolytes compared to males. The differences in the electrolyte levels were attributed to differences in sensitivity to environmental changes and strength [32, 14].

Conclusion

This study revealed for the first time the effects of sexual dimorphism on the serum chemistry and electrolyte profile of male and female H. longifilis. While there is no significant difference in the majority of analytes, there were few significant differences between males and females. Based on the findings of this study, it is recommended that sex be considered in both natural and experimental investigations of serum chemical analytes and electrolytes in catfish.

Materials and Methods

Experimental catfish

The 40 apparently healthy adult male and female catfish (20 males and 20 females) weighing 1 kg to 1.3 kilograms used for the study were procured from a reputable catfish farm within the Jos metropolis with GPS Coordinates 9.851095 (N905113.9416411) and 8.923327 (E8055125.2188411) at altitude 1275 meters above sea level. The catfish had no physical deformity and were acclimatized for one week in a section of the farm in a 2000-liter plastic water tank before the onset of the study. The fish were exposed to natural light day and night without any artificial light. The fish were fed commercial pelleted diets (Coppens) daily in the morning and evening at 10% of their body weight. The water in the tanks was changed every three days through partial draining to ensure that clean water was maintained during the acclimatization. For sampling, a handheld net was used to catch the fish from the tank without completely draining the water from the tank, and a soft, clean towel was used to hold the catfish in place during blood collection.

Experimental design

The 40 catfish were male and female. Following acclimatization, blood samples were collected from 20 male and 20 female catfish. The water used before and during the study was borehole water used in keeping the fish on the farm. The study was conducted in June 2022, and the water temperature during the study period ranged from 20°C to 24°C, pH ranged from 7.0 to 7.1, while the average environmental temperature was 26°C during the day and 18°C at night.

Separation of serum from the blood and analysis

Blood samples collected from the caudal vein into non-heparinized tubes were immediately transported to the Microbiology and pathology laboratory of the Faculty of Veterinary Medicine, University of Jos, Plateau State, where they were centrifuged and the sera were harvested after centrifugation for serum chemistry analysis using an automated serum chemistry analyzer, Cobas C111 (Roche Diagnostics GmbH, Indianapolis, IN, USA).

Statistical analysis

Data analysis was done using JMP statistical software, version 10. All data were found to have a normal distribution. Since two groups were compared, the student t-test was used to identify statistically significant differences between males and females. Differences between sexes were considered statistically significant at $p < 0.05$. Results are expressed as the mean ± standard error (SE).

Authors' Contributions

P.N.T., and G.B. conceived and planned the experiments. P.N.T., G.B., and M.M.S. carried out the experiments. P.N.T., G.B., and M.M.S. contributed to sample preparation. P.N.T., G.B. and M.M.S. contributed to the interpretation of the results. P.N.T. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Competing Interests

The authors have nothing to disclose.

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