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MORPHOMETRIC, HEMATOLOGICAL INDICES AND HEAVY METALS DETECTION IN *OREOCHROMIS NILOTICUS* SAMPLED FROM SELECTED FISH FARMS

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ABSTRACT

This research was conducted for the morphometric characters, hematology and heavy metals detection in Nile tilapia fishes from different sites of Punjab. Three sites (River Ravi, Madina fish hatchery Kasur, Head Baloki) were selected to collect fishes. About 20 fishes from Mari Patan, 5 from Madina fish hatchery and 4 from Head Baloki were collected with nets. The morphometry was noted. Heavy metal detection and hematology was performed. The morphometric parameters show length of pectoral fins with average of 3.81±0.92 cm, anal fins 3.70±0.62 cm, dorsal fins 7.00±0.66 cm, pelvic fins 3.71±0.57 cm, and caudal fins 3.47±0.53 cm. The anal fins and pelvic fins show significant differences. The fish weight was 5.78±1.623 g. The length with tail was 13.56±1.31 cm. The focal length was 11.99±1.65 cm. The fish width with anal fin was 5.78±1.623 cm. The weight shows significant while others show non-significant results. Hematology includes Erythrocytes containing 1.60±0.42, Leukocytes with 66.53±21.23, platelets with 92.67±23.08 and Hemoglobin 6.3±0.52. Mean cellular volume, mean cellular hemoglobin concentration with 29.36±8.23, 179.09±7.38, 43.43±14.81 and 24.83±8.46 respectively. Hematology shows non-significant results. Different metals Nickel, Chromium, and Lead shows different concentrations. The order of nickel concentration is muscle>gills>liver, chromium is muscle>gills>liver and of lead is muscle>gills>liver. It is concluded that the morphometric measurements show significant differences in anal and pelvic fins. Hematology shows non-significant differences. Heavy metals concentration showed random variations. The concentration of nickel was more in muscles then gills, chromium was more in muscles then gills, and lead was more in muscles then gills.

KEYWORDS: Tilapia, Morphometry, Hematology, Heavy metals, Lead, Chromium, Nickle.

INTRODUCTION

Tilapia (Oreochromis sp.) are freshwater fishes belongs to Cichlidae family, found in Africa and Middle East areas,[1] and third largest family of fish having bones. Tilapia is a globally significant profitable freshwater fish and plays an important role in aquaculture.[2] This is suggested as a farmed fish species by the Food and Agriculture Organization of the UN, as it can contribute to increasing the production of protein worldwide.[3] Growing tilapia is characterized by its high production rate, high fertility, easy for management, better purchasing, capability to breed in suboptimal nutritional conditions, and hostile environments such as low O2 and high NH3 levels.

Previous research on tilapia has focused almost exclusively on its suitability for aquaculture.[4] Adaptation of seawater habits.[5] and meat quality.[6] Morphometric measurements are associated with the study of change and shape, that is, the size and shape of living organisms.[7] Morphometric studies in fish show relationships between body parts such as head length, nose, eyes, body, fins and tail. This technique is very simple method of non-animal species identification.[8]

Fish hematology studies can be drawn back to 1943.[9] Since, the studies on various techniques for analyzing fishes blood cells has increased significantly, and our awareness of analyzing fish blood cells has also increased significantly.[10-13] Hematology is a significant technique for studying physical and pathological

variations, and employed by fish researchers in different countries of world.[14] In addition, qualitative and quantitative variations in hematology are important outcomes from a diagnostic point of view.[15] Standard ranges for different hematological parameters in fishes had recognized by various researchers in fish structure and pathology.[16] Blood parameters are difficult to understand as they vary with internal and external features like sex, size, supplying density and environmental influences.

Heavy metals free into the atmosphere from a diversity of organic and human sources. Transportation is one of the most important human resources of metals like chromium, zinc, cadmium and lead.[17] Evaluation of metals growth in fishes is essential from individuals health perspective for human consumption.[18] Cadmium and lead are naturally non-essential metals. Cadmium is a poisonous heavy metal.[19] Work in China showed on large mature inhabitants observed relation among blood lead levels and enlarged incidence of cardiovascular disease (CVD).[20] Heavy metals affect not only fish users, but also exposed fishes. Fish exposure to heavy metals interferes with reproductive hormone secretion and causes pathological variations.[21] Exposure to sub lethal levels of lead has been described to have potential immunosuppressive effects in tilapia fish.[22] The purpose of the current study was to examine the morphometric characters, hematological parameters and heavy metals detection in Nile tilapia fishes obtained from different sites of Punjab region.

MATERIAL AND METHODS

Study sites

Three sites were selected to collect fishes for morphology and heavy metals analysis. This study was conducted from River Ravi (Mari Patan Bridge), Madina fish hatchery Qasoor and Head Baloki near Pattoki of Punjab, Pakistan. About 20 tilapia fishes were collected from Mari Patan, 5 fishes were collected from Madina fish hatchery Qasoor and 4 fishes from Head Baloki near Pattoki. The fish samples collected from the river Ravi were of variable size. Fishes were collected from the river with the help of nets by fisher mans. The morphometric parameters i.e., length and weight and other parameters of 29 fishes were noted. Heavy metal detection was also performed on 4 fishes from each site. The study was conducted after the approval from ethical committee of university.

Morphometric measurements

The morphometric parameters were done by measuring the distance of fish from head to tail and without tail, length of the anal fin with tail fin and without tail fin, length of the pectoral fin, pelvic fin length, length of the dorsal fin. The total rays were counted of the anal fin, caudal fin, pectoral fin, pelvic fin and the dorsal fin. The width of the tail fin, dorsal fin, pectoral fin was also measured. The diameter of the eye was also measured. The distance between the nostrils of the fish was also measured. The total weight of each taken fish was calculated by an electronic digital balance. After every 10 days the round to river was made and fishes were sampled from the river for the purpose of morphometric parameters and for heavy metals analysis. About 3 months was taken to do this research.

The length, weight relationship of a fish is generally spoken by equation W = a Lb (Ricker, 1973). Where, W is weight of body measured in (g), L is total length measured in (cm), a is interrupt and b is slope (the fish growth rate). The index of body parts will be projected by the formula: Index of body part = weight of body parts (g) ⁄ wet body weight (g) × 100. Conditions factor will be projected by the technique of.[23,24]

$K = W / L3 \times 100$

Hematological indices

Blood collected from the sampled fishes through the caudal aorta with syringe. Blood parameters analyzed were red blood cell count (RBCs), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH), hematocrit (Hct) and total white blood cell count (WBCs). The hematocrit was determined according to the method described by.[25] and the RBC, WBC and MCV values were obtained by employing a "Sysmex CC-120 Microcell Counter". The Hb was determined by using the cyanmet-hemoglobin method. The MCH was calculated in picograms/cell = Hb/RBC X10 and the MCHC as the Hb in 100 mL blood/Hct X 100.[26] The significance level was taken as $P < 0.05$.

Heavy Metals Detection

After conveyance to the lab, fish specimens were permitted to place at room temperature and non-digestible parts was detached with blade. Digestible parts were washed with distilled water and dissect it in parts (2–3 cm) with blade and edible portion of the fish specimens was then wash away with distilled water and clean polyethylene sheets. Specimens place to dry in air to eliminate extra water. Tissue of muscles was dry in incubator to get constant weight of fish specimen. The dehydrated specimens was ground in glass or piston mortar, separate 1 mm mesh and store in airtight plastic containers or vessels.[27] Digestion of fish samples were performed according to the method used by.[28]

Statistical analysis

All specimens were examined and the identical tests was statistically similar is paired-samples t-test, at 95% significance. The calculation was performed by Microsoft Excel 2010.[27]

RESULTS

This study was conducted from River Ravi (Mari Patan Bridge), Madina fish hatchery Qasoor and Head Baloki near Patoki of Punjab, Pakistan. About 20 tilapia fishes were collected from Mari Patan, 5 fishes were collected

from Madina fish hatchery Qasoor and 4 fishes from Head Baloki near Patoki. The morphometric parameters i.e length and weight and other parameters of 29 fishes were noted.

Morphometric parameters

The fishes also have several fins with variable shapes and length. The length of the pectoral fin was in the average of 3.81±0.92 cm, anal fin was in the average of 3.70±0.62 cm, dorsal fin was in the average of 7.00±0.66 cm, pelvic fin was in the average of 3.71±0.57 cm, and caudal fin was in the average of 3.47±0.53 cm (Table 1). The standard deviation and mean, the P value and T value of this fish is given in the table. If the P value is greater than 0.05, then this is known as a non-significant value. If the P value is less than 0.05, then this is known as a significant value. If the P value is less than 0.01, then this is known as a highly significant value. This table consists of only measurements of fin. The measurements including pectoral fins, dorsal fins, and caudal fins were non-significant while anal fins and pelvic fins show significant results.

Table 1: Measurements of fins of *O. niloticus.*

Measurements (n=15)	Mean± SD	T-value	P-value
Pectoral fin (cm)	3.81±0.92	0.87	0.09
Anal fin (cm)	3.70±0.62	0.74	0.006
Dorsal fin (cm)	7.00±0.66	0.92	0.36
Pelvic fin (cm)	3.71±0.57	0.75	0.02
Caudal fin (cm)	3.47±0.53	0.49	0.25

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = highly significant (P<0.01) SE = Standard error

The weight of fishes was 5.78±1.623 g. The total length with the tail was 13.56±1.31 cm. The focal length was 11.99±1.65 cm. The girth/width of fish with anal fin was 5.78±1.623 cm (Table 2). The weight shows highly significant results while the total length, focal length and width shows non-significant results. The length and weight relationship of the fish is shown in figure 1.

Table 2: Length and weight of different body parts of *O. niloticus.*

Measurements (n=15)	Mean± SD	T-value	P-value
Weight (g)	5.78±1.623	0.95	0.001
Total-length (cm)	13.56±1.31	0.98	0.73
Focal length (cm)	11.99±1.65	0.98	0.71
Width (cm)	5.78±1.623	0.88	0.05

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = highly significant (P<0.01) SE = Standard error

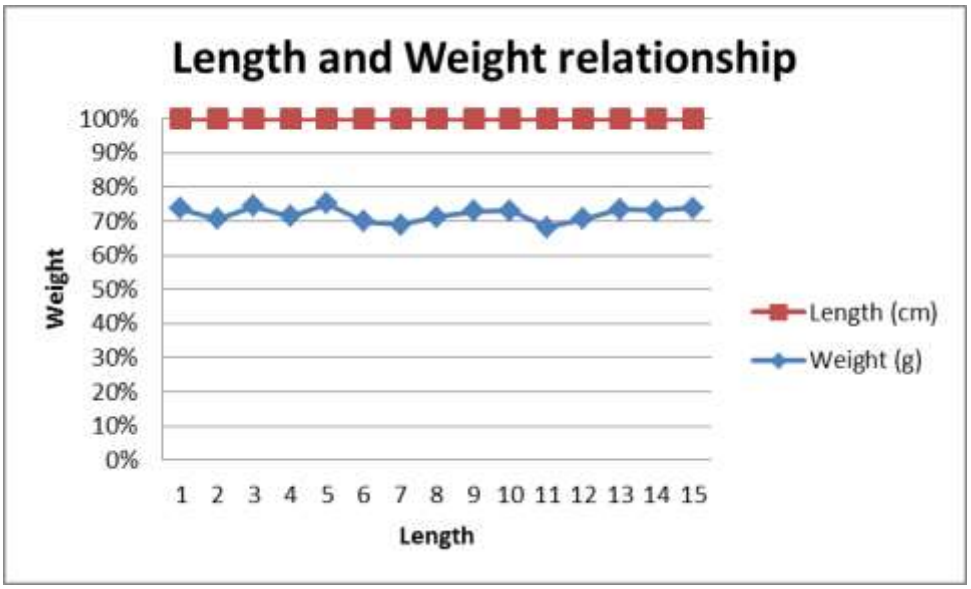

Figure 1: Weight length relationship of *O. niloticus.*

Hematological indices

The blood parameters of *Oreochromis niloticus* are given in the table 3. In it, the Mean and Standard deviation of Erythrocytes (red blood cells) containing 1.60±0.42, Leukocytes (white blood cells) containing 66.53±21.23, platelets containing the value of 92.67±23.08, Hemoglobin (Hb) contains 6.3±0.52. The other parameters of fish blood samples were, mean cellular

volume, mean cellular hemoglobin, mean cellular hemoglobin concentration reports. The values of these parameters 29.36±8.23, 179.09±7.38, 43.43±14.81 and 24.83±8.46 were respectively. All the blood parameters show non-significant results (i.e., p>0.05).

Table 3: Shows the hematological indices of *O. niloticus* fish.

Blood Parameters	Normal Value	Mean ± SD	T-value	P-value
Hb (g/dl)	13.0-17.0	6.3±0.52	0.93	0.92
RBC (x10^12/I)	4.5-5.5	1.60±0.42	0.98	0.92
HCT (%)	40.0-50.0	29.36±8.23	0.99	0.99
MCV (Fl)	80.0-100.0	179.09±7.38	0.98	0.97
MCH (Pg)	27.0-32.0	43.43±14.81	0.97	0.97
MCHC (g/dl)	31.5-34.5	24.83±8.46	0.97	0.97
Platelet count (x10^9/I)	150.0-450.0	92.67±23.08	1	1
WBC Count (TLC) (x10^9/I)	4.0-11.0	66.53±21.23	0.99	0.99

Heavy Metals Concentration

Concentration of different metals of Oreochromis niloticus fish muscles, liver, and gills is given in the table 4. Different metals include Nickel (Ni), Chromium (Cr) and Lead (Pb) shows different concentrations in the given factors. The average and standard deviation of Ni in muscles was 90.70±114.52, in liver was 30.23±60.45 and in gills was 46.10±61.45. The concentration of Cr in muscle was 444.13±222.46, in liver was 51.18±102.35 and in gills was 116.77±206.26. The concentration of Pb in muscle was 150.79±124.06, in liver was 74.50±149.00 and in gills was 130.30±192.77. The concentration of nickel was more in muscles then gills. The order of heavy metal concentration of nickel is muscle>gills>liver. The concentration of chromium was more in muscles then gills. The order of heavy metal concentration of chromium is muscle>gills>liver. The concentration of lead was more in muscles then gills. The order of heavy metal concentration of lead is muscle>gills>liver. The concentration of Nickle, chromium and lead in muscles, liver and in the gills of fish is shown in figure 2.

Table 4: Shows the concentration of different metals in fish liver, fin, muscle and gills.

Metals	Organs		
	Muscle	Liver	Gills
Nickel (Ni)ug/kg	90.70±114.52	30.23±60.45	46.10±61.45
Chromium (Cr)ug/kg	444.13±222.46	51.18±102.35	116.77±206.26
Lead (Pb)ug/kg	150.79±124.06	74.50±149.00	130.30±192.77

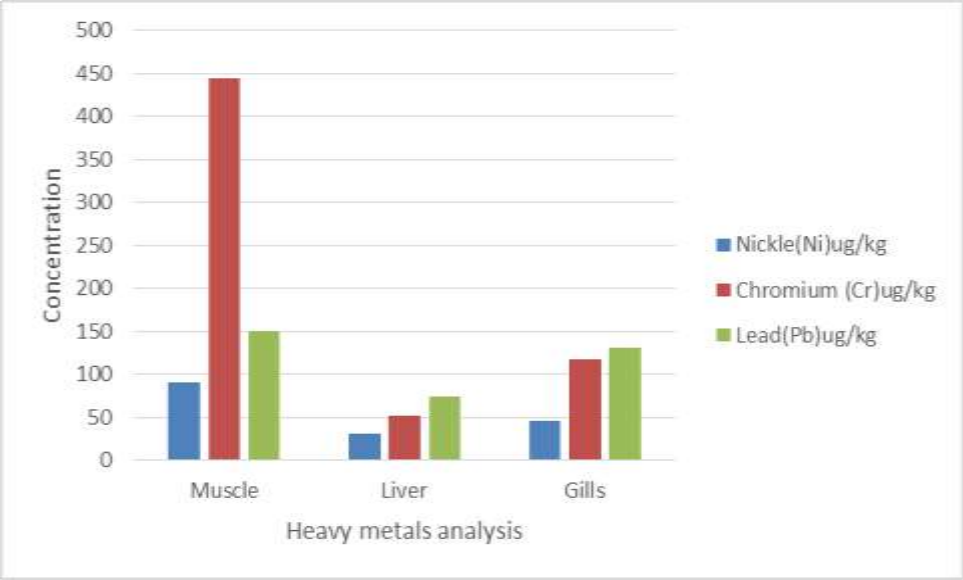

Figure 2: Shows the concentration of heavy metals in muscles, liver and gills of fish.

Eight morphometric parameters were calculated to adjacent 0.01-mm by a numerical caliper.[29] The parameters includes length of body (BL), standard length (SL), length of head (HL), length of tail (TL), length of trunk (RL), depth of body (BD), thickness of body (BT), and thickness of head (HT).[30] They calculated the morphometry of 300 specimens of Nile tilapia from

uninhabited and cultivated environments. They found significant difference in stock (i.e., $P < 0.05$).[30]

DISCUSSION

During this study, nine morphometric parameters were studied including pectoral fin, anal fin, dorsal fin, pelvic fin, caudal fin, weight, total length, focal length and width of the fish. The length of the pectoral fin was in the average of 3.81±0.92 cm, anal fin was in the average of 3.70±0.62 cm, dorsal fin was in the average of 7.00±0.66 cm, pelvic fin was in the average of 3.71±0.57cm, and caudal fin was in the average of 3.47±0.53 cm. In the current study, the morphometry of Nile Tilapia was intended to find a relation, with total length showing linear association. This was described by many researchers. Among eighteen morphometric parameters, some shows the high values of r, means that were highly related with total length of fish i.e., standard length (r^2 = 0.9605), body depth (r^2 = 0.9319), head length of fish (r^2 = 0.9449), fins of fish include dorsal fins (r^2 = 0.9633), pectoral fins (r^2 = 0.8274) anal fins (r^2 = 0.8361), caudal fins (r^2 = 0.8117). As a result, the increase of total length coordinated with diverse degree of the increase to body parts under study. This was same as early studies shows. The low values of r also means a low association among total length of fish and parameters under study i.e., eye diameter of fish (r2 = 0.3388), snout length of fish (r2 = 0.5202), upper jaw length of fish (r2 = 0.6703), and lower jaw length of fish (r2 = 0.6310), which may be owing to the less growth variations in parameters over fish size.[31,32] The difference of morphometry among populations may appears because of either heritable difference or surroundings.[33] In this study, the measurements including pectoral fins, dorsal fins, and caudal fins were non-significant while anal fins and pelvic fins show significant results.

Many investigators have described the length-weight relationships of Tilapia in various sites and times. They observed a similar range as the old studies showed.[31,34] In this study, the weight of fishes was 5.78±1.623 g. The total length with the tail was 13.56±1.31 cm. The focal length was 11.99±1.65 cm. The girth/width of fish with anal fin was 5.78±1.623 cm. They estimated the parameters were found to be 3.026, within the range for fish. The results were observed to be satisfactory for the calculation of length and weight relationships.[35] During this study, the weight shows highly significant results while the total length, focal length and width shows non-significant results.

They evaluated the hematological analysis of fishes to determine the red blood cells count (RBC), hematocrit (PCV), hemoglobin concentration (Hb), erythrocyte indexes (MCV, MCH, MCHC), total leukocytes count (WBC) and thrombocytes count.[36] To find the disease in fishes hematology is a less expensive and fast method.[37] During this study, the hematology analysis of the blood sample of nine fishes was also calculated. In it, the Mean and Standard deviation of RBCs containing 1.60±0.42, WBCs containing 66.53±21.23, platelets containing the value of 92.67±23.08, Hemoglobin (Hb) contains 6.3±0.52. Other parameters were HCT, MCV, MCH, and MCHC reports. The values of these parameters 29.36±8.23, 179.09±7.38, 43.43±14.81 and 24.83±8.46 were respectively. The hematology shows non-significant differences ($P>0.05$) among the diseased and unaffected fishes. There was no leukocytosis and anemia, or any other abnormality of red blood cells. This was summarized because of the low levels of parasitemia that also results in the lack of clinical symptoms.[38] During this study, All the blood parameters shows non-significant results (i.e., $p>0.05$).

They evaluated the heavy metals by atomic absorption method. They found cyclic variations in the accumulation of metals. In cold days, minimum absorption of lead, copper and nickel was documented while the peak level was noted in hot days. The order of accumulation of lead and nickel in organs was like liver > kidney > muscle > gills and liver > gills > kidney > muscle in cold days and hot days, respectively. The accumulation direction of copper as kidney > liver > gills > muscle and gills > kidney > liver > muscle in cold days and hot days, respectively. The heavy metal absorption was crossing the limits recommended by World health organization. Histopathology study of fish kidney shows overcrowding, expansion in bowman capsule space, necrosis. Fish liver showed cytoplasmic vacuolation, necrosis, sinusoid dilation.[39] In this study, the concentration of various metals of *Oreochromis niloticus* fish muscles, liver, and gills was also calculated. The average and standard deviation of Ni in muscles was 90.70±114.52, in liver was 30.23±60.45 and in gills was 46.10±61.45. The concentration of Cr in muscle was 444.13±222.46, in liver was 51.18±102.35 and in gills was 116.77±206.26. The concentration of Pb in muscle was 150.79±124.06, in liver was 74.50±149.00 and in gills was 130.30±192.77. The order of heavy metal concentration of nickel was muscle>gills>liver, chromium was muscle>gills>liver and lead were muscle>gills>liver.

CONCLUSIONS

It is concluded that the morphometric measurements show different variations in the collected fishes and shows significant differences in anal fins and pelvic fins then other parameters. The growth speed of body weight and morphometric measurements also varied. The hematology showed non-significant differences. The heavy metals concentration shows random variations. The concentration of nickel was more in muscles then gills, chromium was more in muscles then gills, and lead was more in muscles then gills. The heavy metals concentration was compared with the previous work and shows that the high levels of metals causes serious hazards to human health.

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Acronyms
Hb, RBCs, WBCs, PCV, MCV, MCH, MCHC.

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Authors Contribution
All authors contributed equally.

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