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Effect of parasites on the biochemical and haematological indices of some clariid (Siluriformes) catfishes from Anambra River, Nigeria

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Abstract
The present investigation was carried out to study the effect of parasite infestation on the liver function test of some clariid species of the Anambra River system. 360 fish species (231 *C. gariepinus* and 129 *C. anguilaris*) were examined for parasites. The parasites recovered were protozoans (*Trichodina acuta* and *Epistylis* sp.), cestodes (*Polyonchobothrium clarias* and *Monobothriode woodlandi*) and nematodes (*Rhabdochona congolensis* and *Procamallanus laeviconchus*) with a total prevalence of 42.1% and mean intensity of 4.15 ± 1.57. The present results showed that aspartate aminotransaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzyme activities as well as creatinine and urea values were elevated in the parasite infected fishes. The haematological manifestation of the infected fishes showed marked decrease in the content of haemoglobin concentration (Hb), packed cell volume (PCV) and red blood cells. However, the infected fishes had higher content of white blood cell (WBC) than the uninfected. Whereas, there was a significant ($p<0.05$) negative correlation between parasite intensity and condition factor, hepatosomatic index of the fishes increased with increase in parasite intensity. The biochemical and haematological alterations observed in the infected fishes reflect anemia and tissue damages caused by parasite invasion.

Keywords: Parasites, Enzymatic activities, Haematology, Hepatosomatic index, Clariidae and Anambra River.

Introduction
Parasites are a major concern to freshwater and marine fishes all over the world, and of particular importance in the tropics [1, 2, 3]. They constitute a major limiting factor to the growth of farmed fish in Nigeria [4]. The effects of parasites on fish include nutrient devaluation [5]; alteration of biology and behaviour [6]; lowering of immune capability, induction of blindness [7]; morbidity, mortality, growth and fecundity reduction [7]; and mechanical injuries depending on the parasite species and load [8, 9].

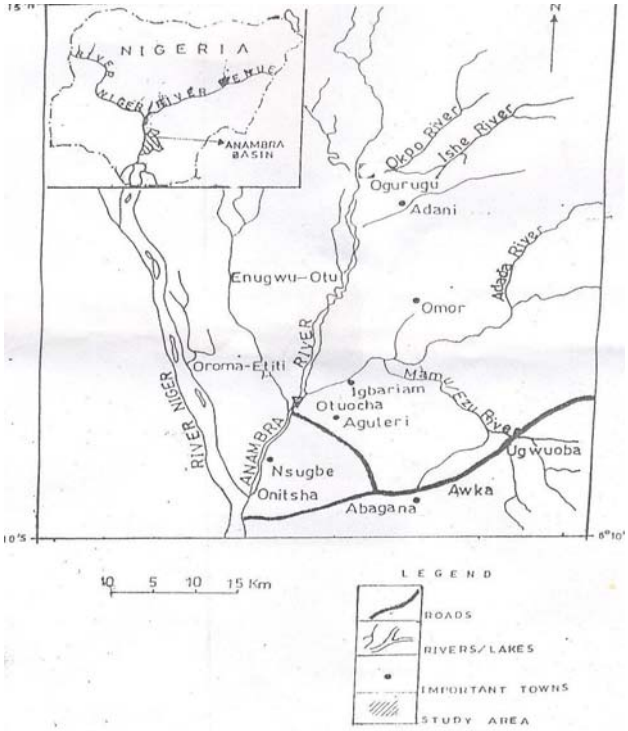
Haemato-biochemical indices have been employed in effectively monitoring the responses of organisms to stressors and thus its health status under such adverse conditions. Generally, haematological tests are used to establish normal health status and to diagnose diseases caused by various factors: viz heavy metals, environmental stress, parasitic infections, genotoxic effect of pollutants, nutrition, and pollution in human and veterinary science [10]. Haematological parameters act as physiological indicators to changing external environments [11] as a result of their relationship with energetic (metabolic levels), respiration (haemoglobin) and defence mechanisms (leukocyte levels) [12]. They also provide an integrated measure of the health status of an organism, which over time manifests in changes in weight [13]. Hence, the changes associated with Haemato-biochemical parameters due to various parasites establish a database, which could be used in diseases diagnosis and in guiding the implementation of the treatment or preventive measures [13].

Several researches on the biology, ecology and parasites of the fishes of the Anambra River have been documented [14, 15, 16, 17] among others. However, none of these studies have information on the physiological impact of parasites on the fishes of the River. This work therefore, aimed at investigating the impact of parasites on the haemato-biochemical characteristics of some clariid species of the river.

Materials and methods

Study area

Anambra River lies between latitudes $6^{0}10^{'}$ and $7^{0}40^{'}$ East of the Niger [14]. The river has its source in Ankpa highlands of Kogi State of Nigeria about 100 km North of Nsukka [14]. Essentially the river has a southward course crossing the Kogi / Enugu State boundary, it passed through Ogurugu to Otuocha from where it flows down to its confluence with the Niger at Onitsha. The main river channel, which has a total length of about 207.40 km [14], has its bank covered by such plants like *Echinoclae species, Salviniany mnellula, Ludiwigia decurrens, Imperita cylindirica, Andropogon spp, Jussiaea spp, Pennisetum spp and Cynodon* spp [18]. There is a rainy season (April – September / October) and a dry season (October / November - March). The mean annual rainfall is between 150 cm and 200 cm. From December to January / February, the basin is influenced by the harmattan, but their effect is not well marked. The water temperature and Secchi disc reading in the river range from 24ºC to 31ºC and 5 cm to 85 cm, respectively [11].



Source: [20].

Fig 1: Map of Anambra River

Fish collection, identification, morphometry and sex determination

A total of 360 clariid fish species comprising of *Clarias gariepinus* (N = 231) and *Clarias anguilaris* (N = 129) were obtained from fishers in the Otuocha river fish landing port along the Anambra River, Nigeria from April 2013 to May 2014. Fishes were identified to species level using keys and catalogues [21, 22]. The weight of the fish was taken to the nearest 0.1 g using a triple beam balance, while the total lengths were taken to the nearest 0.1 cm using a meter rule. The sexes of fishes were determined by both morphological examination and observation of the presence of testis and ovary using dissecting microscope upon dissection of the fish to expose the gonads [23].

Examination of Parasites

The external surfaces – fins, gills and skins were brushed into a petri-dish containing normal saline and examined with a hand lens for the presence of ectoparasites. Scrapings from the skin, fins and gills of each fish were taken and smeared on glass slides for examination of protozoan parasites and smaller metazoan parasites [24, 25, 3]. Fish gills were dissected out and each gill filament and arch were examined with a hand lens for the presence of monogeneans and myxosporidean cysts. The fishes were dissected to expose the viscera. The visceral cavities and organs were examined for cysts and larval endoparasites. The guts were removed and placed in petri dishes. The contents of the guts were flushed with normal saline into beakers and then shaken to loosen mucus and other intestinal debris. Parasites were recovered from the residue after centrifugation (1000 rpm) and decanting of supernatant. Recovered parasites were mounted on slides and viewed using Olympus microscope under high power magnification (×40) and identified to species level using appropriate keys [26, 24, 27]. All parasites recovered were recorded. Fish not examined were refrigerated (-4 ^{0}C) overnight and examined the following day for parasites.

Haemato-biochemical assay

Blood was collected from 360 live fishes from the caudal peduncle and heart using 2ml plastic syringe and needle treated with anti-coagulant as described by [28]. Blood samples for haematological studies were preserved in EDTA embedded bottles and that for enzymes {Aspartate aminotransaminase (AST), Alanine aminotransferase (ALT) and alkaline phosphatase (ALP), Creatinine and Urea} analysis in heparinised bottles. Each fish was then sacrificed by being given a blow on the head and the liver excised and weighed for organ indices assessment. Packed cell volume (PCV) was determined using Hawsley micropillary tubes and centrifuged for 5 min [29]. Red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb) and packed cell volume (PCV) were analysed according to the methods of [30]. The heparinised blood samples were centrifuged at 300 rpm for 10 minutes and the serum collected for analysis. AST and ALT were analysed according to the method of [30]; ALP according to [31]; urea according to [32] and creatinine according to the method of [31]. The organ indices were calculated according to the methods of [32]. Data obtained were subjected to statistical analysis using one way analysis of variance at 95% probability and means separated by Fishers Least Significant difference test at 95% probability.

Results

A total of 148 (41.1%) fish hosts were found to be infected with mean intensity of 4.15 ± 1.57, while 212 (58.89%) were uninfected. A total of 605 parasites were recovered, comprising Protozoan ciliates, *Tricodina acuta* and *Epistylis* sp.; Cestode, *Monobothroide woodlandi* (Caryophyllidae) and *Polyonchobothrium clarias* (Pseudophyllidae); Nematode, *Procamallanus laeviconchus* (Camallanidae), and *Rhabdochona congolensis* (Rhabdochanidae).

The protozoan parasite, *Trichodina acuta* (ciliate) was found on gills and skin of their fish hosts. They were large with disc shaped body. The adhesive disc is saucer shaped. The parasite is provided with several rows of cilia at the circular periphery and the inner circle of toothed dentricles. The macronucleus is horse shoe-shaped and the micronucleus is small and difficult to be seen in some specimens. *Epistylis sp.* was isolated from

the skin and gills of their fish hosts. It is a sessile contractile ciliate. It has a long and non-contractile stalk. It often formed branched colonies. The distal end of the organism is surrounded by rapidly moving cilia which appeared as a blur. The unsegmented cestode, *Monobothroide woodlandi* (Caryophyllidae) was found in the intestine of the fish hosts. The worm is white in colour and elongated. It has a large rounded or triangular scolex. The worm has only one bothrium at the anterior end. The segmented cestode, *Polyonchobothrium clarias* (Pseudophyllidae) was isolated from glandular stomach of their fish hosts. The scolex is elongated, triangular in shape and carries one row of hooks and bears laterally two shallow bothria. Segmentation began directly after the scolex with immature stages, then mature stages. The nematodes (round worms) were diverse in appearance in this study. Usually, they were elongated and cylindrical, tapering at both ends. In *P. laeviconchus* (Camallanidae), the nerve ring surrounding the oesophagus is located towards the anterior part of the oesophagus. The mouth is oval in shape with a blunt tail in the males and a tapering pointed tail in the females. *Rhabdochona congolensis* (Rhabdochanidae) another common nematode has the nerve ring on the intestine with a funnel-like mouth. The tail of both males and females are pointed.

The intensity of parasite was found to have highly significant influence (P<0.05) on the condition factor and hepatosomatic index of both *C. gariepinus* and *C. anguilaris.*

C. gariepinus recorded 0.611 and 0.977 correlation coefficient (r) for condition factor and hepatosomatic index respectively, while 0.548 and 0.965 were recorded by *C. anguilaris.*

Parasite intensity explained the 37.3% and 95.4% variation in the condition factor and hepatosomatic index of *C. gariepinus*. In the same vein, parasite intensity had 30.1% and 93.1% influence on the K-factor and HSI of *C. anguilaris* respectively (Fig. 2 - 5).

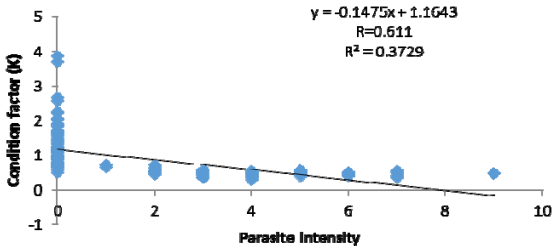


Fig. 2: Correlation graph of condition factor of *C. gariepinus* and parasite intensity

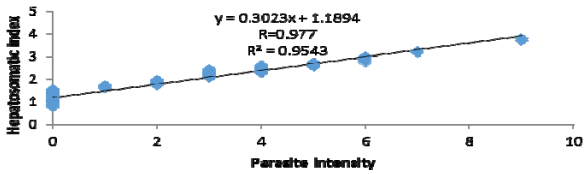


Fig 3: Correlation graph of hepatosomatic index of *C. gariepinus* and parasite intensity

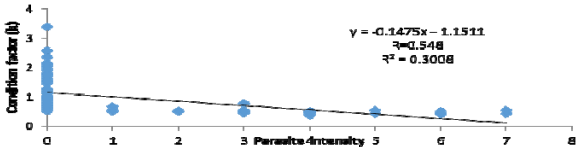


Fig. 4: Correlation graph of condition factor of *C. anguilaris* and parasite intensity

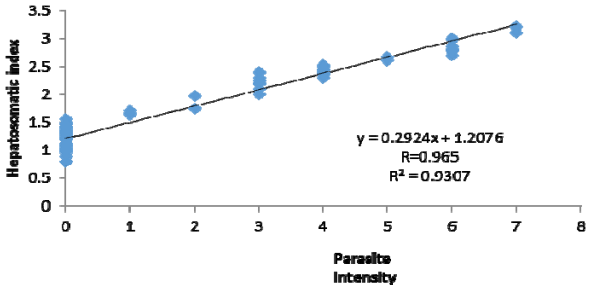


Fig 5: Correlation graph of hepatosomatic index of *C. anguilaris* and parasite intensity

The results of the haematological indices determined for both infected and uninfected species of *C. gariepinus* and *C. anguilaris* are presented in Table 1. The results indicated vividly that packed cell volume (PCV), Haemoglobin concentration (Hb) and Red blood cell count (RBC) were lower in the infected than in the uninfected fishes for both species studied. However, the WBC values were higher in the infected fishes than in the uninfected ones. Statistical analysis revealed significant differences (P<0.05) between the haematological indices of the infected and uninfected clariid species of the Anambra River System.

The activities of AST, ALT and ALP were found to be significantly (P<0.05) higher in the infected fishes than in the uninfected ones. The levels of creatinine and urea showed significant differences (P<0.05) in both infected and uninfected fish species (Table 2).

Table 1: Effect of parasites on the haematological profile of the infected clariid fishes

	Clarias gariepinus		
Parameters	**Infected**	**Uninfected**	**P-values**
RBC (cells/mm^3)	4.16±0.07	7.79±0.08	0.0001*
PCV (%)	24.61±0.32	37.56±0.23	0.0001*
Hb (g/dl)	7.03±0.11	10.86±0.11	0.0001*
WBC (cells/mm^3)	1.20x10^4± 1.53 x 10^2	7.50x10^3±4.7 x 10^1	0.0001*
	Clarias anguilaris		
RBC (cells/mm^3)	4.16±0.09	7.70±0.10	0.001*
PCV (%)	24.75±0.50	37.78±0.25	0.001*
Hb (g/dl)	7.01±0.17	10.75±0.14	0.001*
WBC (cells/mm^3)	1.21x10^4±2.06 x 10^2	7.57x10^3±5.32 x 10^1	0.001*

*Significant difference (P<0.05) across the horizontal rows

Table 2: Effect of parasites on the biochemical parameters of some clariid species of the Anambra River system

	Clarias gariepinus		
Parameters	Infected	Uninfected	p-values
AST (U/L)	95.85±0.36	73.13±0.38	0.001*
ALT (U/L)	130.0±1.18	93.27±0.52	0.001*
ALP (U/L)	75.92±0.37	53.03±0.32	0.001*
Creatinine (U/L)	1.62±0.02	1.02±0.01	0.001*
Urea (U/L)	38.21±0.23	24.62±0.28	0.001*
	Clarias anguilaris		
AST (U/L)	95.35±0.57	73.31±0.44	0.001*
ALT (U/L)	129.1±1.56	94.59±0.6	0.001*
ALP (U/L)	75.59±0.50	52.91±0.41	0.001*
Creatinine (U/L)	1.61±0.02	1.02±0.41	0.001*
Urea (U/L)	37.87±0.38	24.48±0.35	0.001*

*significant different (P < 0.05) across the horizontal rows

Discussion

Blood is a good bio-indicator of the health of an organism [33]. It also acts as a pathological reflector of the whole body. Hence, haematological parameters are important in diagnosing the functional status of the fish (host) infested by parasites [33] and also to evaluate the physiological condition and nutritional state of fish [34].

In the present study, all the haematological parameters studied varied significantly ($p<0.05$) between the infected and the uninfected fishes. This observation supports the works of many researchers. An increase in the lymphocyte count in *Heteroponeutus fossilis* infected with *Lucknowia indica* has been reported [35]. A higher degree of eosinophilia was observed in *Clarias batrachus* carrying helminth infections [36]; and [37] reported decreased RBC, Hb and PCV from fishes infected with hemoparasites.

The reduction in RBC count, haemoglobin (Hb) value and packed cell volume (PCV) in infected fishes may be as a result of the parasitic infestation that often leads to anaemia [38]. Furthermore, the parasites simply acted as stressors and during the primary stages of stress, the PCV are altered due to the release of catecholamine (an enzyme) which mobilizes RBCs to swell as a result of fluid entry into the intracellular compartment [39]. Similar results were recorded by [40] and [41] in *Clarias gariepinus* that are naturally infected with *T. mukasai*. [42] and [43] also reported that the severe anemia that lead to the reduction of RBC, PCV and Hb of the infected fishes maybe as a result of chronic liver inflammation which causes depression of erythrogenesis. On the other hand, significant increase was observed in WBC of the infected fishes when compared to those of the uninfected fishes. According to [44] and [40], the increase in WBCs count occurred as a pathological response since WBCs play a major role during infestation by stimulating the haemopoietic tissues and immune system to produce antibodies and chemical substances which work as defense agent against infection. [45] has made a similar report.

The activity of the serum ALP in the parasite infected clariid species of the Anambra River System revealed a significant increase when compared to the uninfected fishes. This finding is in agreement with the observations of [46] and [47] who reported increase in ALP in *Fasciola* infected monkey. They argued that the elevation in the ALP activity may be as a result of the penetration of the parasite into the bile duct.

Alanine aminotransferase is remarkably specific for liver function since aspartate aminotransferase is mostly present in kidney [48]. The activities of AST and ALT were significantly higher ($P<0.05$) in the infected fishes when compared to the uninfected fishes. A similar result was observed by [49] who reported that AST, ALT and Urea showed significant increase in *O. niloticus* infected with external protozoa and monogenetic trematodes. [50] reported that blood serum AST, ALT enzyme activities, creatinine and Urea values were increased in *Trichodina* infected *Clarias gariepinus*. [51] reported that the blood serum AST, ALT, creatinine and urea values were elevated in the infected *O. niloticus* and *C. gariepinus*. The increase in the activity of AST and ALT in the serum of the infected fishes revealed that the parasites had effect on the parenchymous tissues and skeletal musculature [52], which probably may have altered the permeability [53] and integrity of cell organelles as reported by [54].

The pattern of ALT and AST activities observed in this study are biochemical symptoms tending towards liver cytolysis, indicating disturbance in the structure and integrity of cell organelles like endoplasmic reticulum and membrane transport system [55]. Alterations in their activities may have adverse effects on the amino acid metabolism of the tissues and consequently the intermediates needed for gluconeogenesis [53].

Serum urea and creatinine levels were observed to be elevated in the present study due to parasitic infestation of fishes. [56] reported that creatinine leaves the muscles and enters the blood where it is a waste product largely from the muscle breakdown. It is removed by filtration through the glomeruli of the kidney and excreted as urine. The increase in the level of creatinine in the infected fishes may be as a result of the alteration of the muscles structure of the infected fishes by parasites [52]. [51] also reported elevated levels of creatinine and urea in fishes infected with protozoan parasites.

Urea is a principal end product of protein catabolism. It is a waste product metabolized in the liver and excreted by the kidney. The increases in the urea values in the infected fishes may be due to gill dysfunction (caused by protozoa mainly found in the gills) as urea are mainly excreted through the gill [57]. Also, these findings may be attributed to the inflammatory reactions of intoxication produced by the parasites in the infected fishes [58, 51].

The role of condition indices as stated by [59] is to quantify the health of individuals in a population or to tell whether a population is healthy relative to other populations. In this study, the condition factors of the parasitized clariid fishes were significantly different ($P<0.05$) from the non-parasitized ones. The reduction in the condition factor of the parasitized fishes may be as a result of the dependence of the parasites on the fish for its nutrition [58], thereby reducing the nutrient available for their fish hosts. Parasites are also known to act as stressors to their hosts [45] and as such, causing discomfort to their hosts. This finding is in agreement with the observation of [60] who reported a decrease in the condition factor of bluegill sunfish (*Lepomis macrochirus*) due to parasite load.

Hepatosomatic index (HSI) is the general measurement of the overall condition of fish or the growth status of the liver [61] and can be an excellent predictor of adverse health condition in fish [62]. In this study, there was a positive correlation between parasite load and HSI. The HSI of the infected fishes were significantly higher ($p<0.05$) than those of the uninfected fishes. In fact, the correlation matrix plot of the influence of parasite prevalence on HSI tended towards unity ($r = 0.913$ and 0.916 for *C. gariepinus* and *C. anguilaris* respectively). This significant increase observed in the liver weight of the infected fishes may be as a result of the migration of the larvae of the cestode and nematode worms into the liver of the fish, a finding that is corroborated by reports of [63] and [45]. Also some parasites are known to utilize their host tissues and organs for reproduction thereby making the infected cells become hypertrophic and able to accommodate proliferating parasites (xenoma) [64].

References

1. Iyaji FO, Eyo JE. Parasites and their freshwater fish host. Bio-Research 2008; 6(1):328-338.
2. Bichi AH, Dawaki SS. A Survey of the ectoparasites on the gills, skin and fins of *Oreochromis niloticus* at Bagauda Fish Farm, Kano, Nigeria. Bayero Journal of Pure and Applied Science 2010; 3(1):83-86.
3. Ekanem AP, Eyo VO, Sampson AF. Parasites of landed fish from Great Kwa River, Calabar, Cross River State, Nigeria. International Journal of Fisheries and

Aquaculture 2011; 3(12):225-230.
4. Bichi AH, Yelwa SI. Incidence of piscine parasites on the gill and gastrointestinal tract of *Clarias gariepinus* (Teugels) at Bagauda Fish Farm, Kano. Bayero Journal of Pure and Applied Sciences 2010; 3(1):104-107.
5. Hassan AA, Akinsanya B, Adegbaju WA. Impacts of helminth parasites on *Clarias gariepinus* and *Synodontis clarias* from Lekki Lagoon, Lagos, Nigeria. Report and Opinion 2010; 2(11):42-48.
6. Lafferty KD. Ecosystem consequences of fish parasites. Journal of Fish Biology 2008; 73:2083-2093.
7. Nmor JC, Egwunyenga AO, Ake JEG. Observation of the intestinal helminths parasites of cichlids in the upper reaches of River Orogodo, a freshwater body in Delta State, Southern Nigeria. Tropical Freshwater Biology 2004; 13:131-136.
8. Echi PC, Eyo JE, Okafor FC. Co-parasitism and morphormetrics of three clinostomatids Digenea: Clinostomatidae; in *Sarotherodon melanotheron* from a tropical freshwater lake. Animal Research International 2009a; 6(2):982-986.
9. Echi PC, Okafor FC, Eyo JE. Co-parasitism and morphormetrics of three clinostomatids Digenea: Clinostomatidae; in *Tilapia guineensis* Bleeker, 1862 from Opi lake, Nigeria. Bio-Research 2009b; 7(1):432-436.
10. Fedato RP, Simonato JD, Martinez CBR, Sofiaa SH. Genetic damage in the bivalve mollusk *Corbicula fluminea* induced by the water-soluble fraction of gasoline. Mutation Resources 2010; 700:80-85.
11. Caruso G, Genovese L, Maricchiolo G, Modica A. Haematological, biochemical and immunological parameters as stress indicators in *Dicentrarchus labrax* and *Sparus aurata* farmed in off-shore cages. Aquaculture International 2005; 13:67-73
12. Oriakpono O, Hart A, Ekanem W. Acute haematological response of a cichlid fish *Sarotherodon melanotheron* exposed to crude oil. Journal of Toxicology and Environmental Health Sciences 2012; 4(9):151-155.
13. Yaji AJ, Auta J. Sub-lethal effects of monocrotophos on some haematological indices of African catfish *Clarias gariepinus* (Teugels). Journal of Fisheries International 2007; 2(1):115-117.
14. Azugo WI. Ecological Studies of the Helminth Parasites of the Fish of Anambra River System M. Phil Thesis, University of Nigeria Nsukka 1978, 178.
15. Ezenwaji NE, Aguigwo JN, Ilozumba PCO, Ezenwaji HMG. Helminth endo-parasites of Mochokids in a tropical rainforest river system. Animal Research International 2005; 2(2):346-352.
16. Nwani CD, Oti EE, Odo GE, Ezenwaji HMG. Endoparasitic helminthes of four *Mormyrid* species (Osteichthyes: Mormyridae) from a West African flood river system. Pakistan Journal of Biological Sciences 2008; 11(2):202-207.
17. Odo GE, Onoja SO, Onyishi GC. The biology of *Parachanna obscura* (Osteichthyes: Channidae) in Anambra river, Nigeria. International Journal of Fisheries and Aquaculture 2012; 4(8):154-169.
18. Nwani CD. Length-weight relationship and condition factor of *Distichodus* species of Anambra River. Animal Research International 2006; 3(2):461-465.
19. Odo GE. Studies on the Ecology of Macro Invertebrate Fauna as Fish Food in Anambra River Basin, Nigeria. Ph.D. Thesis University of Nigeria, Nsukka, Nigeria 2004, 192.
20. Odo GE, Nwani CD, Eyo JE. The fish fauna of Anambra River Basin, Nigeria species abundance and morphometry. Revista de Biologia Tropical 2009; 57(1-2):177-186.
21. Idodo-Umeh G. Freshwater Fishes of Nigeria (Taxonomy, Ecological Note, Diet and Utilization). Idodo-Umeh Publishers Edo State, Nigeria 2003.
22. Olaosebikan BD, Raji A. Field Guide to Nigeria Freshwater Fishes. Federal College of Freshwater Fisheries Technology New Bussa 1998.
23. Ayanda OI. Comparative parasitic helminth infection between cultured and wild species of *Clarias gariepinus* in Ilorin, North-Central Nigeria. Scientific Research and Essay 2009; 4(1):18-21.
24. Paperna I. Parasites, Infection and Diseases of Fishes in Africa-an Update. Central Institute for Freshwater Aquaculture (CIFA) Technical FAO, Rome 1996, 31.
25. Hamish DR. Fish Disease Manual. Vet-Aqua International Ireland 2010.
26. Yamaguti S. Systema Helminthum: The Acanthocephala. Interscience Publisher New York 1963.
27. Pouder DB, Curtis EW, Yanong RPE. Common freshwater fish parasites pictorial guide: Sessile ciliates, 2011. *http:edis.ifas.ufl.edu/FA-107*. 12 April 2014.
28. Lucky Z. Methods for the Diagnosis of Fish Diseases. Amerno publishing Co., PVT, LTD, New Delhi, Bombay, Calcutta and New York 1977.
29. Abudu JO, Sofola DA. Relationship between red cell mass and packed cell volume in Nigeria primigraviadae. Nigerian Journal of Physiological Sciences 1994; 10(1/2):13-21.
30. Blaxhall G, Diasley N. Routine haematological methods for use with fish blood. Journal of Fish Biology 1973; 5:771-941.
31. Bessey OA, Lowry OH, Brock MJ. A method for rapid determination of alkaline phosphatase with five cubic millimeters of serum. Journal of Biological Chemistry 1946; 164:321-329.
32. Jenkins JA. Fish Bioindicators of Ecosystem condition at the Caleasieu Estuary, Louisiana. National Wetlands Research Center USGS, Lafayette 2004.
33. Joshi PK, Bose M, Harish D. Changes in certain haematological parameters in siluriod catfish, *Clarias batrachus* (L.) exposed to cadmium chloride. Pollution Resources 2002; 21(2):119-122.
34. Chagas EC, Val AL. Effeito da vitmina C no ganho de peso e em parametros hematologicos de tambaqui. Pesquisa Agropecuaria Brasileira. Brasilia 2003; 38(3):397-402.
35. Sexena KK, Chauchan RRS. Effect of parasitic infection on the blood of *Heteropneustus fossilis* (Bloch). Bioved 1993; 4:41-43.
36. Sinha KP. Haematological manifestation in *Clarias batrachus* carrying helminth infection. Journal of Parasitic Diseases 2010; 24:167-170.
37. Shahi N, Yousuf AR, Rather MI, Ahmad F, Yaseen T. First report of blood parasites in fishes from Kashmir and their effect on the haematological profile. Open Veterinary Journal 2013; 3(2):89-95.
38. Martins ML, Tavares-Dias M, Fujimoto RY, Onaka EM, Nomura DT. Haematological alterations of *Lepomis macrocephalus* (Osteichthyes: Aniskidae) in fish pond.

Arq. Brasileiro Medical and Veterinary Zootenecnia 2004; 56:640-646.
39. Chocchia G, Motais R. Effect of catecholamines on deformability of red blood cells from trout: relative roles of cyclic AMP and cell volume. Journal of Physiology 1989; 412:321-332.
40. Hassen FEZM. Studies on Diseases Caused by Henneguya Infection. Ph.D. Thesis, Faculty of Veterinary Medicine Suez Canal University, Egypt 2002.
41. Ismail GAE. Histopatological and Physiological Studies on Naturally Infected Catfish, *Clarias gariepinus* with Trypanosomes. M.Sc. Thesis, Faculty of Science Cairo University, Egypt 2003.
42. Lofty HS, Mahmoud SM, Abdel-Gawad MA. Some studies on Fascioliasis in Mid-Egypt. Agricultural Research 2003; 81(2):209-227.
43. Omran HH, El-Kholony KH. The influence of Nitroxynil on clinical, biochemical and haematological parameters in Egyptian buffaloes affected with liver flukes in Sharkia governorate. Egyptian Journal of Agricultural Research 2003; 81(2):739-751
44. Lebola SL, Saunders DK, Crawford TG. Observation on blood viscosity in striped bass, *Morone saxatilis* (Walbaum) associated with fish hatchery condition. Kansas Academy of Science 2001; 104:183-184.
45. Pinky K, Qureshi TA, Rekha S, Susan M, Bilal A. Histopathological and haematological investigations on *Nandus nandus* (Ham.) parasitized by metacercariae of *Clinostomum complanatum* (Rudolphi, 1819). International Journal of Environmental Sciences 2012; 2(3):1324-1330.
46. El-Aziz MZA, Emara SA, Salem FS. Clinopathological studies on fasciolaisis among sheep in Giza province. Egyptian Journal of Veterinary Science 2002; 36:75-86.
47. Ahmed MI, Ambali AG, Baba SS. Haematological and biochemical responses of Balami sheep to experimental *Fasciola gigantic* infection. Journal of Food, Agriculture and Environment 2006; 4(2):71-74.
48. Witthwaskid P, Panthong A, Kanjanapothi D, Taesothikul T, Lertprasertsuke A. Acute and sub-acute toxicity of saponin mixture isolated from *Schefflera leucantha* viguuire. Journal of Ethnopharmacology 2003; 89:115-121.
49. Younis AAE. Effect of some ectoparasites on the blood and serum constituents of *Oreochromis niloticus* fish with referring to treatment. Beni Suif. Veterinary Medical Journal 1999; 9(3):341-351.
50. Osman HAM, Ismaiel MM, Abbas TW, Ibrahim TB. An approach to the interaction between Trichodiniasis and pollution with Benzo-apyrene in catfish (*Clarias gariepinus*). World Journal of Fish and Marine Sciences 2009; 1(4):283-289.
51. Mahmoud AE, Mona SZ, Abdel RY, Dsouky HA, Osman KAH, Ottia AAZ. Study on clinopathological and biochemical changes in some freshwater fishes infected with external parasites and subjected to heavy metals pollution in Egypt. Life Science Journal 2011; 8(3):401-405.
52. Egbu FM. Prevalence of Bovine Cysticercosis and Fascioliasis in Slaughtered Cattle in Nsukka Zone and the Haematological, Biochemical and Histopatological Changes in Response to Active Infection. M. Sc. Thesis. University of Nigeria, Nsukka, Nigeria 2011.
53. Kabir MA. Sublethal Effects of tobacco (*Nicotiana tobaccum*) leaves dust on enzymatic activities of *Heteroclarias* (a hybrid of *Heterobranchus bidorsalis* and *Clarias gariepinus*). Jordan Journal of Biological Sciences 2009; 2(4):151-158.
54. Adamu KM, Iloba KI. Effect of sublethal concentrations of portland cement powder solution on the amino tranferases of the African catfishes {*Clarias gariepinus* (Burchell, 1822)}. Acta Zoologica Lituanica 2008; 18(1):50-54.
55. Dere E, Polat F. The effect of paraquat on the activity of some enzymes in different tissues of mice (*Mus musculus*). Turkish Journal of Biology 2001; 25:323-332
56. Kabir MA, Ovie K. Effects of sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust on some biochemical parameters of hybrid (*Clarias gariepinus* and *Heterobranchus bidorsalis*). Brazilian Archive of Biology and Technology 2011; 54(1):183-196.
57. Murray RKD, Rranne PAM, Rodwell VW. Harper's Biochemistry. Norwalk, Connecticut Los Altos, California 1990.
58. Mehra HR. The Fauna of India and the Adjacent Countries 1991, 1.
59. Stevenson RD, Woods WA. Condition indices for conservation: new uses for evolving tools. Integrative and Comparative Biology 2006; 46(6):1169-1190.
60. Bryan DN, Luca MC. Relationship between condition factors, parasite load and paternity in bluegill sunfish, *Lepomis macrochirus.* Environmental Biology of Fishes 2004; 71:297-304.
61. West HJ. Evaluation of total plasma bile-acid concentrations for the diagnosis of hepatobiliary disease in horses. Research of Veterinary Science 1989; 46(2):264-270.
62. Adams SM, McLean RB. Estimation of largemouth bass, *Micropterus salmoides,* lacepede, growth using liver somatic index and physiological variables. Journal of Fish Biology 1985; 26:111-126.
63. Sommerville C. The pathology of *Haplorchis pumilio* (Looss, 1895) infection in cultured Tilapias. Journal of Fish Diseases 2000; 5:243-250.
64. Food and Agriculture Organization of the United Nations (FAO). Diagnostics, Prevention and Therapy of Fish Diseases and Intoxications. In: Svobodova Z, Vykusova B (editors). Manual for International Training Course on Fresh-water Fish Diseases and Intoxifications: Diagnostics, Prophylaxis and Therapy. Research Institute of Fish Culture and Hydrobiology Vodrary, Czechodovakia 1991.