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Influence of Sex on Haematological Response of *Clarias gariepinus* Juveniles Treated with Atrazine and Metalochlor

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Abstract

A total of 180 *Clarias gariepinus* juveniles comprising of 90 male and female each, were exposed to different concentrations of combined atrazine and metolachlor (0.00 mg/L-control, 0.01, 0.02, 0.03, 0.04 and 0.05 mg/L) for 14 days to assess the influence of sex on the response of the fish to this chemical under laboratory conditions. The result obtained revealed that the female fish in the control group had an increase value of haemoglobin (Hb) than the male, while in the experimental group the values of Hb in male fish were consistently higher than the females. The comparative values of red blood cell (RBC) indicated that the female fish had higher values of RBC than the male in all concentrations of exposure. In packed cell volume (PCV), female fish were higher than the males. In assessment of male and female response to the toxicant under consideration, the values of white blood cells (WBC), neutrophils and monocytes increased significantly with increasing concentrations of the chemical with the female values higher than the males. Also, the values of lymphocytes, platelets, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) in the exposed fish indicated a significant reduction, which was more noticeable in female than the male fish. However, mean corpuscular haemoglobin concentration (MCHC) values were within the same range for both sexes. Overall, it appears that the males were more responsive to the stress of chemical exposure than the females.

Keywords: Catfish; Sex; Toxicants; Blood; Aquatic systems; Water pollution; Atrazine; Metalochlor

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Introduction

Water contamination has become an issue in recent times, causing a great damage to the aquatic ecosystems. Wastes generated by industries and homes ultimately find their way into the aquatic environment. Most of these water bodies have become polluted consequent of consistent discharge of these refuse into them and making it unfavourable for aquatic organism especially fish [1]. Fresh and marine water pollution by toxicants released from industrial discharge is a regular source of public health concern especially in urban centers [2]. These pollutants range from herbicides as runoffs from farms, to organic pollutants, such as polycyclic aromatic hydrocarbons from oil explorative activities, and heavy metals from industries [3]. The run offs from farms are a compound combination of a number of chemical components [4] and have high level of oxidant capability [5]. According to Makinde [6] most of these run offs from farms and fumigation use, consists of a large number of toxic substances, such as arsenic, benzene, chlorine, dioxins, ethylene oxide, formaldehyde, and methanol.

The release of chemicals into the aquatic environment results in some changes, which may threaten functional attributes, the integrity and existence of aquatic organisms, especially fish [7]. Recently, haematological parameters have become promising biomarkers in measuring the effects of chemical pollutant in fish. Blood samples can regularly be obtained from test organisms, thus allowing the use of non-destructive approach in effect assessment [8-10]. Typically, haematological parameters are non-specific in their responses towards chemical stressors. Nevertheless, they may provide important information in assessment studies, by providing an indication as to the general

physiology and health status of the organism under investigation [11,12].

Numerous authors have reported the toxicity and haematological alterations in fish exposed to different chemicals in the laboratory [13-15]. Moreover, the use of hematological strategies in fisheries studies is developing rapidly, as it is very essential in toxicological research which bring about tracking and envisaging fitness situations of the fish [16,17]. In view that fish are closely related with the aqueous surroundings, the blood will bring to light some alterations inside the fish quickly than any physiological assessment parameters [18]. Contaminants which include herbicides, pesticide and industrial effluent are well-known to cause drastic changes in the haematological parameters of fish [19-21].

Evaluation of blood variables is a vital tool for assessment of fish physiological conditions [22,23]. Estimation of haematological indices for detection of chemical induced stress in the system of the fish is a common practice among fishery scientists. Fish blood parameters vary in different species which may be attributed to genetic variation, nutritional status, sex, age, and strain due to capture, handling and sampling procedures [24,25]. For this reason, it is essential to evaluate influence of sex on haematological parameters of fish, so as to ascertain its influence in the face of any stressor such as chemicals. The influence of sex factor is widely recognized in various diseases, but its molecular basis, particularly how sex-biased gender behave in response to toxico-pathological changes is poorly understood. Hence, this study focused on the influence of sex on haematological response of C*larias gariepinus* juveniles treated with atrazine and metalochlor under laboratory conditions

Material and Methods

The experiment was conducted at the Toxicity laboratory in African Regional Aquaculture Centre, Aluu, Rivers State, Nigeria. One Hundred and eighty (180 juveniles) of *C. gariepinus* of (mean length 8.74 ± 2.64 cm and mean weight 56.68 ± 1.81 g) comprising of 90 male and female fish each were procured from the center and taken to the laboratory where they were acclimatized for a period of seven days. After acclimatizing the fish to laboratory conditions, 10

fish were introduced individually into 18, aquaria tanks of 1.5 m × 1 m × 0.5 m dimension, containing 0.00 (control), 0.10, 0.20, 0.30, 0.40 and 0.50 mg/L of Delta Force **(**metalochlor/atrazine). Each treatment and control had three replicates and lasted for a total of 14 days. The solution for each concentration was replenished on daily basis, with freshly prepared solution of Delta Force **(**metalochlor/atrazine).

Haematological analysis methods as described by Blaxhall and Daisley [26] were used in the assessment of the various blood parameters or otherwise stated. Red blood cell (RBC) was analysed using haemocytometer; while the packed cell volume (PCV) was estimated using micro haematocrit tubes after centrifuging for five minutes. The hemoglobin content of the blood was evaluated by cyanomethaemoglobin method. The white blood cells (WBC) were determined using improved Neubauer counter. The values of thrombocytes were estimated using the Rees and Beeker method [27]. The differential counts (neutrophils, lymphocytes, eosinophils and monocytes) were assessed by dropping thoroughly mixed blood film on clean microscope slides and allowed to dry. The slides were then fixed in methanol and stained with leishman stain. The counting was done based on different cell types and recorded. The values of haematological indices were calculated using the method of Rusia and Sood [28].

OCC=Hb x 1.25

$$MCV=\frac{HCT}{RBC}\times 10$$

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

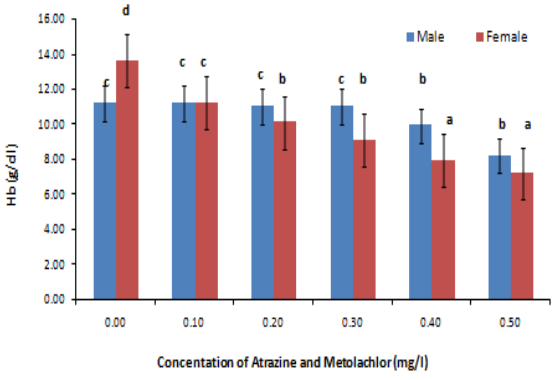
$$MCHC=\frac{Hb}{PCV}\times 10$$

Source: Seiverd [27].

Data obtained from the study were collated and analyzed using Microsoft excel statistical package.

Results

The results of the haematological parameters of male and female of *C. gariepinus* exposed to Atrazine and Metalochlor for 14 days are shown in **Figures 1 to 11**. The result on the **Figure 1** indicates that the female *C. gariepinus* in the control group had an increase value of Hb than the male, while in the experimental group the values of Hb in male fish were consistently higher than the females **(Figure 1).** The comparative values of RBC revealed that the female fish had higher values of RBC than the male in all concentrations **(Figure 2)**. In **Figure 3**; the values of PCV in female fish were higher than the male. In assessment of male and female response to the toxicant under consideration, the values of WBC, neutrophils and monocytes increased significantly



Bars with the different superscripts are significant (p<0.05)

Figure 1 Comparative values of Haemoglobin (Hb) in *C. goriepinus* juveniles exposed to Atrazine and Metolachlor.

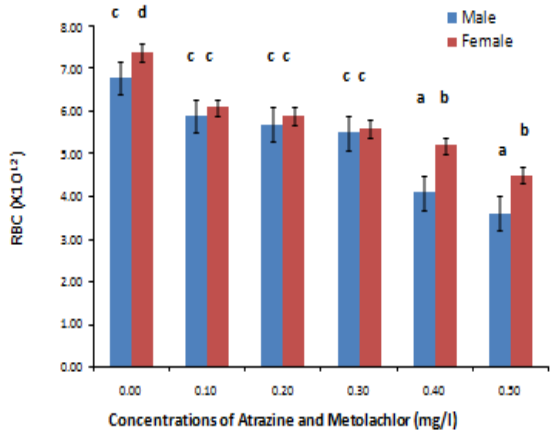


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Figure 2 Comparative values of Red Blood Cell in *C. gariepinus* juveniles exposed to Atrazine and Metolachlor.

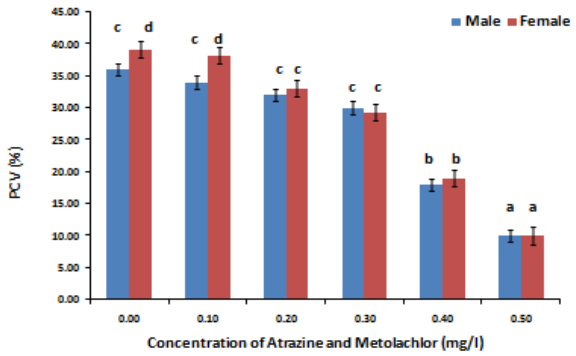

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Figure 3 Comparative values of Red Blood Cell in *C. gariepinus* juveniles exposed to Atrazine and Metolachlor.

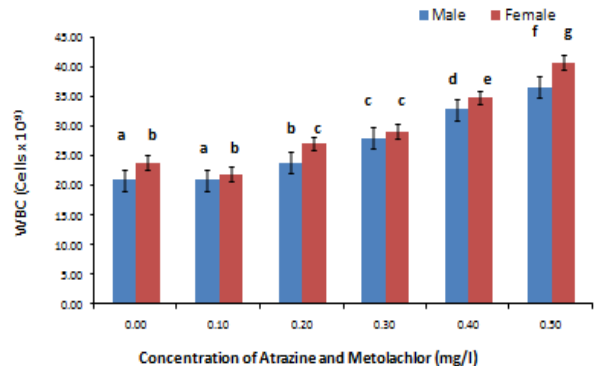

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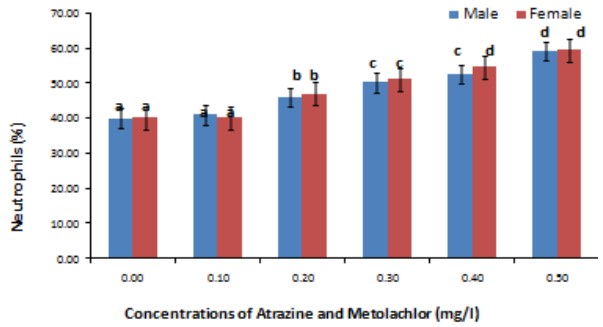
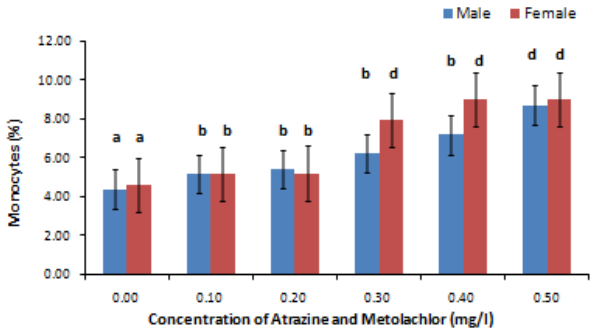
Figure 4 Comparative White blood cell (WBC) values in *C. goriepinus* juveniles exposedto Atrazine and Metolachlor.



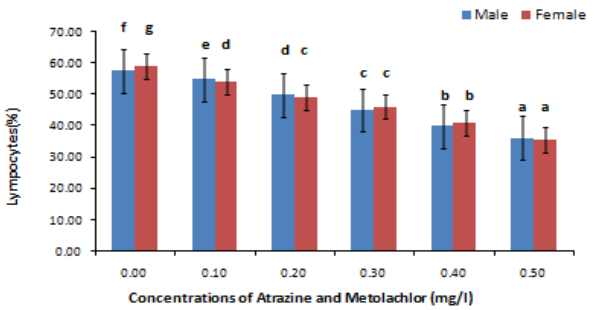
Bars with the different superscripts are significant (p<0.05)

Figure 5 Comparative Neutrophils values in *C. goriepinus* exposed to Atrazine and Metolachlor.



Bars with the different superscripts are significant (p<0.05)

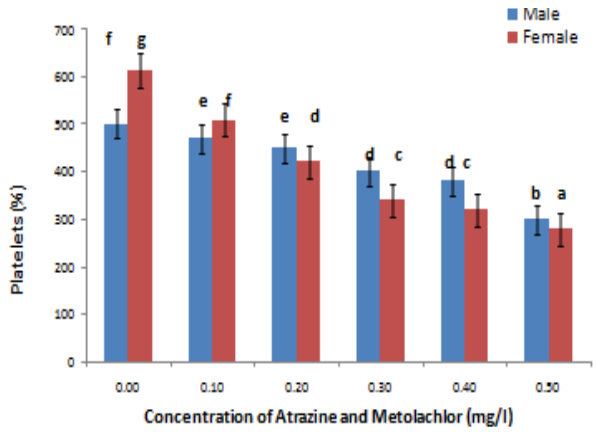
Figure 6 Comparative values of Monocytes in *Clarias goriepinus* juveniles exposed to Atrazine and Metolachlor.



Bars with the different superscripts are significant (p<0.05)

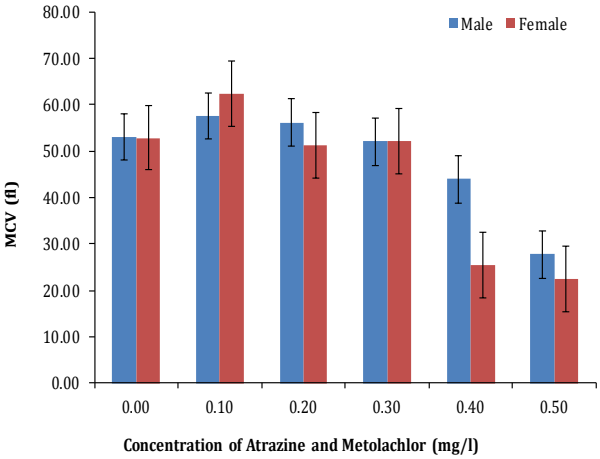
Figure 7 Comparative values of Lympocytes in *C. goriepinus* juveniles exposed to Atrazine and Metolachlor.

with increasing concentrations of the chemical **(Figures 4-6)**. Comparatively, the values of lymphocytes platelets and MCV in the exposed fish indicated a significant reduction that was more noticeable in female than the male fish **(Figures 7-9)**. The values of MCH were lower in female fish than the male especially at higher concentrations of the chemical **(Figure 10).** The MCHC



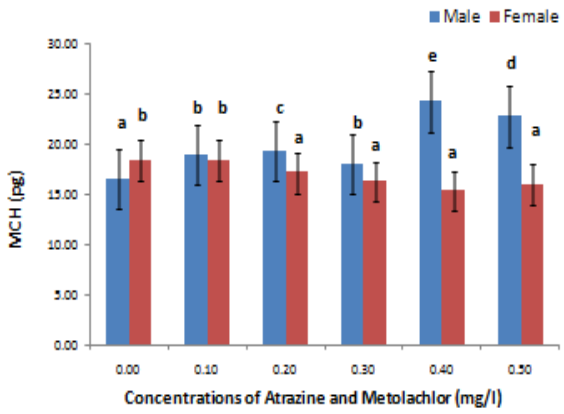
Bars with the different superscripts are significant ($p<0.05$)

Figure 8 Comparative values of Platelets in *C. goriepinus* exposed to Atrazine and Metolachlor.



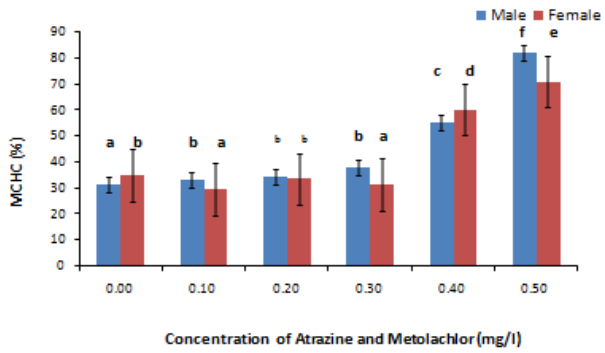
Bars with the different superscripts are significant ($p<0.05$)

Figure 9 Comparative values of MCV in *C.gariepinus* juveniles exposed to Atrazine and Metolachlor



Bars with the different superscripts are significant ($p<0.05$)

Figure 10 Comparative MCH values in *C. goriepinus* juveniles exposed to Atrazine and Metolachlor.



Bars with the different superscripts are significant ($p<0.05$)

Figure 11 Comparative valus in MCHC of *C. goriepinus* exposed to Atrazine and Metolachor.

values were lower between 0.00 to 0.30 mg/L of the chemical, higher values were recorded between 0.40 and 0.50 mg/l of the chemical in both male and female fish **(Figure 11).**

Discussion

Changes in hematological parameters might have been brought about by combined atrazine and metalochlor as an anemic condition due to decreased synthesis of Hb and RBC number in hemopoietic organs. The reduction of RBC is mainly due to development of hypoxic condition during the treatment which intern leads to increase in destruction of RBC or decrease in rate of formation of RBC due to non-availability of Hb content in cellular medium [29]. The damaging of toxicant on erythrocyte may be secondary, as resulting from a primary action of toxicant on erythropoietic tissues on which there exists a failure in red cell production and or due to increase in the erythrocyte destruction [30]. Moreover, Adhikari [31] also reported that the RBC count and Hb concentration decrease may depend on, sex as observed in this study.

Sex differences in haematology of fish would constitute a plausible physiological mechanism behind behavioral differences between males and females in response to chemicals. Evaluation of influence of sex response is necessary in assessment of fish haematology. In the evaluation of the blood parameters of *Clarias batrachus* exposed to toxins the authors [32,33] found that males always had appreciably higher PCV values than the females and advised that during blood analysis, parameters should be separated on the basis of sex to avoid attributing sex related variations to other elements. This statement agrees with that of Spirita [34] who reported wide variations in Hb, PCV and RBC indices between male and female zebra fish exposed to akyl benzene . Furthermore, in this study, disparity was recorded in the values of the various blood parameters between male and female in the fish exposed to atrazine and metalochlor. Similar observations had been made in other fish species and were attributed to intrinsic factors not sex [35,36].

Our study revealed sex differences between all groups of exposed fish. The male fish showed a wide range in most of

the parameters than female fish. This also have been reported by Akinrotimin [37] who found sex differences between male and female *Sarotherodon melanotheron* exposed to acclimation . Also, Gabriel *et al*. [21] found a high erythrocyte count in males than females of of *Clarias gariepinus* exposed to cypermethrin in the laboratory. This appears to be related to activity of sexes, male being more active and also appears to be associated to be more responsive to the chemical. However, the result obtained in this study indicates that after exposure to the chemical, the female fish consistently had higher values of Hb, RBC, PCV, WBC, neutrophils, monocytes and lymphocytes than the males, but the reverse was the case with platelets, MCV, MCH and MCHC. It appears then that the males are more responsive to the stress of chemical exposure than the females. These differences on hematological data recorded in this study depend on different needs of oxygen in male and female fish, especially in reproduction period. For instance, the values of Hb are higher in male than female, while that of WBC is higher in female than male. Higher values of some hematological indices for male than females are caused by varying activity of erythropoietin. Testosterones, the male sex hormones, stimulate its production while estrogens, the female hormones, have a suppressing effect on erythropoietin, which is responsible for of some blood parameters [38]. This conclusion is in line with other authors [39,37] who observed the same trend in fish exposed to different types of stressors in culture medium.

Exposure of *C. gariepinus* to combined effects of atrazine and metalochlor indicated a consistent elevation in the values of WBC which is more pronounced in female fish than the males. The result agrees with the report of Anyanwu [12] in exposure of black jaw tilapia to different salinity gradients. Increase in WBCs count occurred as a pathological response since these WBCs play a great role during infestation by stimulating the haemopoietic tissues and the immune system by producing antibodies and chemical substances working as defense against infection [40]. The higher values of WBC in the female fish indicated that is WBCs in the system of the fish respond immediately to the change in medium due to xenobiotic transformation, an indication that female fish is able to cope with the toxic stress more than the males.

Furthermore, some of the haematological traits indicative of stress-coping style appear to be influenced by sex as differential count levels is higher in male fish than the female, an indication of aggressive behavior noticeable in male fish when compared to the female. Our analyses revealed striking observation that sex-influenced hematology in *C. gariepinus* exposed to toxicants exhibited generally similar expression behavior in response to toxicological perturbations. Intriguingly, the extent of these alterations in blood indices also increased with the increasing concentrations of the chemical. As this will determine the severity or susceptibility of fish to a given toxico-pathological state, suggesting the importance of sex-biased haematological parameters in playing active roles in predicting physiological functions of the fish.

Conclusion

The present investigation showed that combined Atrazine and metalochlor caused noticeable alterations in the haematological parameters in *C. gariepinus* which suggest that the pesticide may weaken the immune system and result in severe physiological problem and ultimately lead to the death of fish. Taken together, these findings support the view that distinct physiological stress-coping styles are present in teleost fish, and influence its haematological indices. This study revealed that the sex of fish may exert some degrees of influence on some of hematological characteristics *C. gariepinus* and hence the need to reckon with these factors in the assessment and reporting of the hematological indices of this fish species at the resting state or in the face of any stressor such as chemicals.

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