Effects of Environmental Stressors on the Hematological Indices of Some Freshwater Teleosts

Nellie C. Lopez
Institute of Biology
College of Science

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ABSTRACT

Changes in blood values were observed in young adult freshwater fishes exposed to environmental stressors. *Clarias batrachus* (TL=221–391 mm; 68.7–371.4 g) starved for 34, 47 and 53 days showed significant reductions in lymphocyte counts. Lymphocyte percentages decreased while neutrophil percentages increased in starved fish. *Oreochromis niloticus* (TL=105–172 mm; 19.1–77.6 g) exposed for 30 days to 10 and 20 ppm $ZnSO_4$ concentrations at pH 6.7–7.2 showed consistent reductions in total WBC and lymphocyte counts but insignificant changes in RBC-related values. *O. niloticus* (TL=56–124 mm; 4.0–21.7 g) exposed for 96 h to 10, 20, and 30 ppm $ZnSO_4$ concentrations showed a general tendency of reduction in RBC-related values and total and relative WBC counts.

Exposure of *O. niloticus* (TL=56–106 mm; 2.9–14.2 g) for 96 h to 5, 10, and 20 ppm $ZnSO_4$ at low ph (ph 3.1–3.8) elicited marked increases in neutrophil counts and reductions in lymphocyte and RBC counts. *O. niloticus* (TL=94–154 mm; 13.2–54.4 g) subjected to 24 h and 48 h crowding showed increased hematocrit, neutrophilia, and lymphopenia. In *Ophicephalus striatus* (TL=259–306 mm; 123.2–192.8 g) stressful effects of crowding and handling were less evident from the blood values obtained after 24 h and 48 h exposure.

INTRODUCTION

Hematological parameters are being used in fish laboratories to assess the health of fishes (1, 2) and monitor stressful effects of various conditions such as exposure to toxicants (3, 4, 5, 6, 7, 8) and trauma associated with

handling and transport (10). Changes in blood parameters are often quick responses to environmental or physiological alterations which may be useful as indicators of adverse consequences of stressors and provide an integrated measure of the physiological status of the fish (11).

Very little studies have been published on the hematology of fishes from the Philippines (12, 13, 14). The present work reports on the changes in the blood values of local fishes exposed to a number of environmental stressors.

MATERIALS AND METHODS

Source and Maintenance of Fishes

Young adult freshwater fishes belonging to three species were collected live during the period October 1988 to November 1990. *Clarias batrachus* (TL=221–391 mm; 68.7–371.4 g) were obtained from Nepa Q Mart and Farmers Market while *Oreochromis niloticus* (TL=56–172 mm; 2.9–77.6 g) were collected from freshwater ponds in Quezon City. Fish were transported to the laboratory in plastic pails and aerated plastic bags.

Fishes were held at low densities in glass aquaria supplied with aged tap water. *C. batrachus* were held in 10- and 15-gal aquaria with each containing 1–2 and 5–7 fish, respectively. *O. niloticus* were acclimated in aerated 15- and 30-gal aquaria, each holding a maximum of 20 and 40 fish, respectively; testing was done using the 15-gal aquaria with 10–13 fish in each. *O. striatus* were held in aerated 15-gal aquaria, each holding 4–5 fishes.

Fishes were acclimated for a minimum period of 2 weeks before testing except for the test groups of *O. striatus* which were not acclimated to include their handling from the market to the laboratory as part of the test. The control group of *O. striatus* were acclimated.

During acclimation, *C. batrachus* and *O. niloticus* were fed twice daily with a mixture of fish meal and rice bran, *O. striatus*, with chopped cooked shrimps. Excess food and waste matter were removed daily and aquarium water changed every 3 days. (No overt diseases were encountered during the study.)

Water temperature, pH and dissolved oxygen (D.O.) were measured at least once a week (before the aquarium water was changed) during acclimation and prolonged experiments, at least once (on the day of blood sampling) during the 24–96 h tests. An ordinary laboratory thermometer, a pH meter (CG 810, TOA Electronics Ltd. Japan) and a D.O. meter (YSI Model 54 A, Yellow Springs Instrument Co., Ohio) were used.

Blood collection and Hematological techniques

Fish were caught individually in a hand net and blood samples withdrawn immediately from the caudal circulation with a disposable syringe and hypodermic needle rinsed with 10% EDTA (disodium ethylenediamine tetra acetic acid, AR, BDH Chemicals Ltd., England).

RBC counts (x 10^6 mm^{-3}) were done by standard method with the aid of the Improved Neubauer Counting Chamber (Boeco, Austria) and using Yokoyama's solution as diluting fluid (15).

Total WBC counts (x 10^3 mm^{-3}) were determined by the indirect method (16) from blood smears stained panoptically (17) using Jenner's solution (18) as fixative. Relative WBC counts were done from stained smears similar to the method of Lucky (17) for differential counts. The percentages were then used to determine absolute numbers of lymphocytes and neutrophils. Hematocrit values (%) were determined by the microhematocrit method (19). Heparinized capillary tubes (75 mm long, 1.1–1.2 mm I.D.; Chase Instruments Corp., N.Y.), plastic sealing clay (Seal-Ease, Clay Adams, N.Y.), a clinical model centrifuge, and a vernier caliper were used.

Hemoglobin concentrations (g/100 ml) were determined by the cyanmethemoglobin method (19). Drabkin's Reagent and Hemoglobin Standard (Medical Center Trading Corp., Manila) were used. Readings were made at 540 nm on a Spectronic 21 (B & L) spectrophotometer.

Lengths and widths (microns) of mature erythrocytes from panoptically stained smears were measured with a calibrated ocular micrometer under the oil immersion objective of a compound microscope (Carl Zeiss). A total of 30 cells were taken at random on one slide for each fish.

Statistical Analysis

Means + 1 S.E.M. were determined. Significant differences ($P<0.05$) between groups were tested by the Student's test.

Starvation

Blood samples were collected from a total of 30 *Clarias batrachus* (TL=222–391 mm; 68.7–371.4 g) after 0 (control), 34, 47, and 53 days starvation. Control fish were not fed 12–24 h before blood sampling. Water temperature was 26.5°C, pH was 6.7–6.9 and D.O. 0.8–2.6 cc/l. Fecal matter and wastes were remove daily and aquarium water changed every 3 days during the experiment.

Prolonged exposure to $ZnSO_4$ concentrations

Fifty-four young adult *Oreochromis niloticus* (TL=105–172 mm; 19.1–77.6 g) were exposed to aerated aged tap water (control), 10 ppm, and 20 ppm $ZnSO_4$ concentrations for 30 days (72-h LC50 = 31.6 ppm). Aquarium water temperature was 26–27°C, pH was 6.9–7.2, and D.O. 2.3–4.4 cc/l. Fish were fed twice daily. Unconsumed food and feces were removed daily. Water was changed every 3 days. All fish were starved 12–24 h before sampling.

96-h exposure to $ZnSO_4$ concentrations at near neutral pH

A total of 54 *Oreochromis niloticus* (TL=56–124 mm; 4.0–21.7 g) were placed in aerated aged tap water (control), and 10, 20, and 30 ppm $ZnSO_4$ concentrations for 96 h. Water temperature of aquarium water ranged from 26–28°C, pH from 6.7–7.2, and D.O. from 1.8–4.4 cc/l. Feces were removed daily but fish were not fed and aquarium water not changed during the exposure period.

96-h exposure to $ZnSO_4$ concentrations at low pH

Forty-five *O. niloticus* (TL=56–106 mm; 2.9–14.2g) were distributed in aerated aged tap water (control), and in 5, 10, and 20 ppm $ZnSO_4$ concentrations made acidic by the addition of 5 ml concentrated nitric acid to each aquarium containing 50 ml water. Water temperature ranged from 26.0-26.5°C and D.O. from 4.5–6.0 cc/l. pH in the control aquarium ranged from 6.8 to 7.1, in the test aquaria, from 3.2 to 3.8. Fish were not fed and water was not changed during the exposure period, but feces were removed daily.

Handling and Crowding

A total of 39 *O. niloticus* (TL=94–154 mm; 13.2–54.4 g) were exposed to 0 (control), 24 h, and 48 h crowding stress. Fish acclimated for 2 weeks in a 15-gal aquarium containing 50 ml aerated aged tap water served as the control. Acclimated fish transferred to 15-gal aquaria with only 2.0 cm depth of aerated aged tap water and held for 24 h and 48 h, respectively, served as test groups. Water temperature in the aquaria was 25.5°C, pH ranged from 6.4–6.8, and D.O. 1.7 to 2.5 cc/l. Control fish were not fed 24 h before blood sampling; test fish were not fed during the test period. Feces were removed daily but water containing test fish was not changed during the exposure period.

Sixteen *Ophicephalus striatus* obtained from the market were transferred to 15-gal aquaria containing aerated aged tap water. Control fish were acclimated for 2 weeks with twice daily feeding in 40 ml water changed every 3 days; fish were starved 24 h before blood sampling. Test groups were newly collected fish held for 24 h and 48 h, respectively, in 2.0 cm depth of water; fish were not fed and water not changed during the exposure period but feces were removed daily. Water temperature of aquarium water was 26.5°C, and D.O. ranged from 6.8–7.1 cc/l.

RESULTS AND DISCUSSION

Starvation

All starved fish survived the test period except for one fish in the 34 day-starvation group.

As shown in Table 1, most of the blood values decreased from the control levels in *Clarias batrachus* starved for 34 and 47 days. After 34 days starvation, significant reduction from the control level was observed in the lymphocyte count (from 11.6 ± 1.1 thousand mm^{-3} to 7.6 ± 0.9 thousand mm^{-3}). RBC lengths (RBC-L) and RBC widths (RBC-W), however, increased significantly, RBC-L, from 9.9 ± 0.2 μ to 11.2 ± 0.3 μ and RBC-W from 8.9 ± 0.2 μ to 10.6 ± 0.3 μ.

After 47 days starvation, reductions from the control levels in RBC count (from 2.8 ± 0.2 million mm^{-3} to 1.5 ± 0.01 million mm^{-3}), total WBC count (14.3 ± 0.5 thousand mm^{-3}), and lymphocyte count (6.8 ± 0.1 thousand mm^{-3}) were significant. Hemoglobin concentration, neutrophil count, and RBC length and width were not significantly different from those of the control.

After 53 days starvation, lymphocyte count (4.9 ± 1.6 thousand mm^{-3}) and RBC width (7.7 ± 0.2 μ) were significantly lower than those of the control but the other blood values were not significantly different.

Lymphocyte percentages decreased from the control level (54%) in fish starved for 34 days (43%), 47 days (47%), and 53 days (19%). Neutrophil percentages, however, increased from the control level (46%) after starvation for 34, 47, and 53 days (57%, 53%, and 81%, respectively).

Present results indicate that the most consistent change exhibited by starved *C. batrachus* was depressed lymphocyte count. RBC-related values (hematocrit, hemoglobin and RBC count) decreased initially but returned to control levels as starvation continued. Previous investigators (10) found

that leucocytopenia, lymphopenia, and neutrophilia were characteristic hematological responses to stress in fish regardless of the nature of stressor. The characteristic pattern of change in blood values in stressed fish was observed in the present study of starved *C. batrachus.*

Mahajan and Dheer (19) reported similar results from *Channa punctatus.* A sharp decline in RBC and total leucocyte counts, and lymphocyte percentages, but consistent increase in neutrophil percentages were observed after 5 weeks starvation.

Weinberg et al. (21) reported starvation-induced anemia in kissing guorami (*Helostoma temminckí*) starved for 9 days, and anemia-induced erythropoiesis beginning day 11 through 15, when a decrease was observed. The reduction in RBC-related values after 34 and 47 days starvation in *C. batrachus* observed in the present study, and return to control levels after 53 days starvation also suggest suppressed erythropoiesis induced by starvation, and erythropoiesis induced by anemia, respectively. The larger red cells in fish starved for 34 and 47 days, and smaller cells in the 53 days starved fish are consistent with this explanation. A trend towards increased cell size and volume occurs during differentiation and maturation of erythroid elements in fish (21). Starved fish with suppressed erythropoietic activity will possess mainly larger, adult circulating red cells; with anemia-induced erythopoietic activity, size of red cells decrease reflecting the appearance of younger cells.

Prolonged exposure to $ZnSO_4$ concentrations

Table 2 shows that young adult *Oreochromis niloticus* exposed for 30 days to 10 ppm $ZnSO_4$ concentration exhibited insignificant changes from the control values in hematocrit, hemoglobin concentrations, RBC count and RBC lengths and widths, and neutrophil counts. However, significant reductions in total WBC counts (from 13.9 ± 1.2 thousand mm^{-3} to 6. ± thousand mm^{-3}) and lymphocyte counts (from 12.3 ± 1.2 thousand mm^{-3} to 5.7 ± 0.7 thousand mm^{-3}) were observed. At 20 ppm, RBC related values were not significantly different from the control, but total WBC count (8.0 ± 2.6 thousand mm^{-3}) and lymphocyte count (6.1 ± 2.1 thousand mm^{-3}) were significantly reduced. In comparison to the control, lymphocyte percentages decreased (from 88% to 76%) in fish exposed to 20 ppm $ZnSO_4$, while neutrophil percentages increased (from 10% to 24%).

McLeay (7) found depression of leucocyte-thrombocyte counts and unchanged RBC counts in salmon exposed to zinc; the decline in WBC-T counts was attributed to reduced number of circulating small lymphocytes.

Present findings in *O. niloticus* support the sugestion of Belova (8) that a decrease in number of circulating lymphocytes is a more reliable indication of unfavorable environmental conditions and stress than hematocrit or erythrocyte counts.

96-h Exposure to $ZnSO_4$ concentrations at near neutral pH

As shown in Table 3, exposure of *O. niloticus* for 96 h to 10, 20, and 30 ppm $ZnSO_4$ at near neutral pH resulted in significant reductions in hemoglobin concentrations from the control level (from 5.5 g/100 ml to 4.9, 4.9, and 4.8 g/100 ml, respectively). Decreased total WBC and lymphocyte counts were also observed, the reduction being significant for the lymphocyte count in the 10 ppm-exposed fish (from 4.7 ± 0.9 thousand mm^{-3} to 1.9 ± 0.4 thousand mm^{-3}). RBC count was significantly lower in the 10 ppm-exposed fish (from 1.9 ± 0.1 million^{-3}) but changes were not significant in the 20 ppm and 30 ppm fish groups. Changes in hematocrit, neutrophil counts and RBC sizes were not significant except for the lower RBC length in the 20 ppm group (from $10.7 \pm 0.2\ \mu$ to $10.1 \pm 0.2\ \mu$).

The general tendency of the blood values from *O. niloticus* exposed to $ZnSO_4$ for 96 h to decrease from the control levels indicate stress response of fish to zinc exposure. Metal exposure induces changes in hematological parameters, generally because of changes in blood water content (11). Hemodilution after zinc exposure was observed in *Colisa fasciatus* (Mishra and Srivastava 1979, 1980 cited in 11). In the present study, decreased blood values after 96 h exposure to zinc was indicative of hemodilution.

Lymphocytopenia, a characteristic hematological response to an unspecific stressor, was also observed in the present work (10 ppm-exposed fish). McLeay (7) also reported decline in WBC-thrombocyte and unchanged RBC counts in coho salmon exposed to 0.5 LC50 and greater concentrations of zinc for 24 h.

The insignificant differences in most blood values in the 20 and 30 ppm treated fish after 96 h in the present study may be an indication of a tendency to recovery. In the dogfish, *Scyliorhinus canicula*, exposed for 96 h to sublethal levels of cadmium, Tort and Torres (11) observed that blood values (i.e. RBC count, leucocrit) which had changed after 24 h showed a tendency to return to control levels after 96 h treatment. Further studies need to be done to determine initial hematological effects to zinc exposure in *O. niloticus*.

96-h Exposure to $ZnSO_4$ concentrations at low pH

Table 4 shows the blood values from *O. niloticus* exposed to $ZnSO_4$ concentrations at low pH (pH 3.2D3.8). Hemoglobin concentrations. (from 5.5 ± 0.1 g/100 ml to 6.4 ± 0.4 g/100 ml), hematocrit (from 21.8 ± 0.7% to 23.0 ± 2.2%), and RBC counts (from 1.9 ± 0.1 milllion mm^{-3}) increased from control values at 5 ppm but decreased at higher concentrations. Total WBC counts (from 6.1 ± 1.0 thousand mm^{-3} to as high as 15.2 ± 4.0 thousand mm^{-3}) increased in the exposed fish due to increased neutrophils (from 1.4 0.3 thousand mm^{-3} to as high as 10.4 ± 3.7 thousand mm^{-3}). Lymphocyte counts decreased significantly in the 20 ppm treated fish (from 4.7 ± 0.9 to 0.9 ± 0.2 thousand mm^{-3}). Lymphocyte percentages decreased consistently in the treated fish (from 77% to 32%, 23% and 14%, respectively) while neutrophil percentages increased (from 23% to 68%, 77% and 86%, respectively). RBC lengths and widths did not change significantly.

Present results indicate that at low pH, $ZnSO_4$ concentrations of 5 ppm or greater elicit the characteristic stress response in *O. niloticus*. Reduced lymphocyte counts noted in *O. niloticus* exposed to $ZnSO_4$ concentrations at near neutral pH in the present study, were also observed at low pH, but in addition, significantly reduced RBC count and increased numbers of neutrophils were manifested. These changes may be an indication of increased toxicity of $ZnSO_4$ to *O. niloticus* at low pH. Everall et al. (22) reported that zinc toxicity to brown trout (*Salmo trutta*) increased below pH 5.

Crowding and Handling

O. niloticus exposed to 24 h and 48 h crowding (Table 5) exhibited significant changes in blood values, namely, increased hematocrit (from 21.2 ± 1.9% to 27.1 ± 1.5%, and 23.1 ± 1.8%, respectively) and neutrophil counts (from 2.0 ± 0.9 thousand mm^{-3} to 8.2 ± 1.1 and 6.5 ± 1.3 thousand mm^{-3} respectively) and reduced lymphocyte counts (from 7.2 ± 1.9 thousand mm^{-3} to 2.2 ± 0.3 and 2.8 ± 0.4 thousand mm^{-3}, respectively). Lymphocyte percentages decreased (from 78% to 21% and 30%, respectively) while neutrophil percentages increased (from 22% to 78% and 70%, respectively). Other changes observed after 24 h crowding were insignificant increases in hemoglobin concentration, RBC counts, total WBC counts, RBC lengths and RBC widths. After 48 h, significant increase in hemoglobin concentration (from 5.1 ± 0.3 g/100 ml to 6.0 ± 0.1 g/ml) was also observed.

Ophicephalus striatus exposed to 24 h and 48 h handling and crowding (Table 6) exhibited reductions in all blood values except RBC lengths and widths which increased. The changes in hemoglobin concentrations and RBC counts were not significant but the reductions in hematocrit (from 51.2 ± 3.6% to 41.4 ± 2.8% and 40.2 ± 2.6%, respectively) were significant. Total WBC counts (from 94.9 ± 22.5 thousand mm^{-3} to 59.1 ± 24.4 and 69 ± 15.9 thousand mm^{-3}, respectively) decreased with the reduction in the numbers of lymphocytes and neutrophils. Lymphocyte percentages also decreased from the control level in the 24 h and 48 h exposed fish (from 18% to 13% and 12%, respectively), while neutrophil percentages increased (from 82% to 87% and 88%, respectively).

The above changes in *O. niloticus* exposed to crowding indicate that they were stressed. The changes observed after 24 h were also observed after 48 h exposure. The expected lymphopenia and neutrophilia characteristic of stressed fish (10) were manifested by exposed fish. McLeay and Gordon (8) also observed significant depression in WBC-thrombocyte count and elevated hematocrit in rainbow trout exposed to 96 h crowding. Well, et al. (9) reported erythrocyte swelling and increased hematocrit and hemoglobin concentrations in giant Antarctic cod exposed to severe agitational stress. Present results, i.e., increased RBC lengths, RBC widths, hematocrit and hemoglobin concentrations in exposed *O. niloticus* may be due to erythrocyte swelling.

Stressful effects of handling and crowding on *Ophicephalus striatus* were less evident from the blood values. However, the general tendency of blood values to decrease especially the total WBC and lymphocyte counts and the increase in the neutrophil percentages may be an indication of stress.

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Table 1. Blood Values of Starved *Clarias batrachus**

	Control Mean ± S.I.	34 days Mean ± S.E.	47 Days Mean ± S.E.	53 Days Mean ± S.E.
Fish Length (mm)	303 ± 11 (19)	249 ± 7 (5)	291 ± 11 (3)	367 ± 7 (3)
Fish Weight (g)	187.4 ± 19.4 (19)	89.1 ± 7.2 (5)	153.9 ± 4.0 (3)	272.3 ± 21.5 (3)
Ht (%)	42.1 ± 1.7 a (19)	41.4 ± 3.2 a (5)	37.6 ± 1.7 a (3)	45.7 ± 1.6 a (3)
Hb (g/100 ml)	8.5 ± 0.4 a (20)	7.6 ± 0.3 a (5)	8.3 ± 0.2 a (3)	8.5 ± 0.7 a (3)
RBC ($x\ 10^6\ mm^{-3}$)	2.8 ± 0.2 a (19)	2.9 ± 0.2 a (5)	1.5 ± 0.01 b (3)	2.8 ± 0.2 a (3)
WBC ($x\ 10^3\ mm^{-3}$)	22.4 ± 2.1 a (17)	18.1 ± 1.4 a (5)	14.3 ± 0.5 b (3)	18.8 ± 2.6 ab (3)
Lymphocytes ($x\ 10^3\ mm^{-3}$)	11.6 ± 1.1 a (17)	7.6 ± 0.9 b (5)	6.8 ± 0.1 b (3)	4.9 ± 1.6 b (3)
Neutrophils ($x\ 10^3\ mm^{-3}$)	10.8 ± 2.0 ab (17)	10.5 ± 1.6 ab (5)	7.6 ± 0.2 a (3)	13.9 ± 1.4 b (3)
RBC–L (μ)	9.9 ± 0.2 a (16)	11.2 ± 0.3 b (5)	10.2 ± 0.2 b (5)	9.5 ± 0.4 a (3)
RBC–W (μ)	8.9 ± 0.2 a (16)	10.6 ± 0.3 b (5)	10.6 ± 0.2 a (3)	7.7 ± 0.2 c (3)

*Sample size in parentheses; values with the same letters are not significantly different at 5% level.

Table 2. Blood Values of *Oreochromis niloticus* exposed to 10 ppm and 20 ppm $ZnSO_4$ for 30 days*

	Control Mean ± S.E.	10 ppm Mean ± S.E.	20 ppm Mean ± S.E.
Fish Length (mm)	134 ± 3 (34)	136 ± 3 (15)	140 ± 2 (5)
Fish Weight (g)	38.9 ± 2.3 (34)	39.8 ± 2.7 (15)	42.0 ± 3.6 (5)
Ht (%)	28.2 ± 0.8 a (32)	29.6 ± 1.5 a (15)	26.4 ± 2.9 a (5)
Hb (g/100 ml)	6.5 ± 0.1 a (29)	6.4 ± 0.3 a (13)	6.0 ± 0.6 a (5)
RBC ($x\ 10^6\ mm^{-3}$)	1.9 ± 0.1 a (29)	2.1 ± 0.1 a (14)	1.4 ± 0.4 a (5)
WBC ($x\ 10^3\ mm^{-3}$)	13.9 ± 1.2 a (22)	6.4 ± 0.9 b (14)	8.0 ± 2.6 b (5)
Lymphocytes ($x\ 10^3\ mm^{-3}$)	12.3 ± 1.2 a (22)	5.8 ± 0.8 b (14)	6.1 ± 2.1 b (5)
Neutrophils ($x\ 10^3\ mm^{-3}$)	1.4 ± 0.5 a (22)	0.6 ± 0.2 a (14)	1.9 ± 0.9 a (5)
RBC-L (μ)	11.3 ± 0.1 a (16)	11.1 ± 0.1 a (13)	11.0 ± 0.2 a (5)
RBC-W (μ)	8.6 ± 0.2 a (16)	8.6 ± 0.2 a (13)	8.4 ± 0.2 a (5)

*Sample size in parentheses; values with the same letters are not significantly different at 5% level.

Table 3. Blood Values of *Oreochromis niloticus* exposed to $ZnSO_4$ for 96 h*

	Contrrol Mean ± S.E.	10 ppm Mean ± S.E.	20 ppm Mean ± S.E.	30 ppm Mean ± S.E.
Fish Length (mm)	75 ± 3 (18)	88 ± 4 (18)	81 ± 6 (10)	78 ± 7 (8)
Fish Weight (g)	6.5 ± 0.6 (18)	10 ± 1.2 (18)	8.5 ± 2.0 (10)	7.2 ± 1.2 (8)
Ht (%)	21.8 ± 0.7 a (16)	20.8 ± 1.3 a (17)	24.2 ± 1.4 a (10)	22.9 ± 1.2 a (9)
Hb (g/100 ml)	5.5 ± 0.5 a (16)	4.9 ± 0.2 b (16)	4.9 ± 0.1 b (10)	4.8 ± 0.1 b (9)
RBC ($x\ 10^6\ mm^{-3}$)	1.9 ± 0.1 a (18)	1.2 ± 0.1 b (18)	1.8 ± 0.1 a (10)	2 ± 0.1 b (9)
WBC ($x\ 10^3\ mm^{-3}$)	6.1 ± 1.0 a (15)	3.5 ± 0.6 a (13)	4.8 ± 0.7 a (8)	5.7 ± 1.1 a (8)
Lymphocytes ($x\ 10^3\ mm^{-3}$)	4.7 ± 0.9 a (15)	1.9 ± 0.4 b (13)	3.7 ± 0.5 a (8)	4.4 ± 0.9 a (8)
Neutrophils ($x\ 10^3\ mm^{-3}$)	1.4 ± 0.3 a (15)	1.5 ± 0.6 a (13)	0.8 ± 0.2 a (8)	1.3 ± 0.4 a (8)
RBCL-L (μ)	10.7 ± 0.2 a (10)	10.3 ± 0.2 a (9)	10.1 ± 0.2 b (9)	10.5 ± 0.1 a (9)
RBC-W (μ)	7 ± 0.1 a (10)	7.1 ± 0.2 a (9)	7.3 ± 0.2 a (9)	7.4 ± 0.2 a (9)

*Sample size in parentheses; values with the same letters are not significantly different at 5% level.

Table 4. Blood Values of *Oreochromis niloticus* exposed to low ph and varying ZnS04 concentrations for 96 h*

	Control Mean ± S.E.	5 ppm Mean ± S.E.	10 ppm Mean ± S.E.	20 ppm Mean ± S.E.
Fish Length (mm)	75 ± 3 (18)	90 ± 2 (6)	87 ± 4 (4)	70 ± 1 (11)
Fish Weight (g)	6.5 ± 0.6 (18)	10.8 ± 0.7 (6)	8.7 ± 0.9 (4)	5 ± 0.2 (11)
Ht (%)	21.8 ± 0.7 a (16)	23 ± 2.2 a (5)	18.6 ± 1.0 a (3)	20 ± 2.3 a (11)
Hb (g/100 ml)	5.5 ± 0.1 ac (16)	6.4 ± 0.4 b (6)	4.8 ± 0.3 c (3)	6.3 ± 0.4 ab (8)
RBC ($x 10^6 mm^{-3}$)	1.9 ± 0.1 a (18)	2.1 ± 0.1 b (6)	1.2 ± 0.1 c (3)	1.1 ± 0.1 c (11)
WBC ($x 10^3 mm^{-3}$)	6.1 ± 1.0 a (15)	15.2 ± 4.0 b (5)	9.2 ± 4.6 ab (3)	6.4 ± 2.1 ab (6)
Lymphocytes ($x 10^3 mm^{-3}$)	4.7 ± 0.9 a (15)	4.8 ± 1.1 a (5)	2.1 ± 1.1 ab (3)	0.9 ± 0.2 b (6)
Neutrophils ($x 10^3 mm^{-3}$)	1.4 ± 0.3 a (15)	10.4 ± 3.7 b	7.1 ± 3.5 ab (3)	5.5 ± 2.0 ab (6)
RBC-L (μ)	10.7 ± 0.2 a (10)	10.4 ± 0.2 a (6)	9.7 ± 0.4 a (5)	10.4 ± 0.2 a (7)
RBC-W (μ)	7 ± 0.1 a (10)	7.1 ± 0.2 a (6)	6.8 ± 0.2 a (5)	7.1 ± 0.3 a (7)

*Sample size in parentheses; values with the same letters are not significantly different at 5% level.

Table 5. Blood Values of *Oreochromis niloticus* exposed to 24h and 48h handling*

	Contrrol Mean ± S.E.	24 h Mean ± S.E.	48 h Mean ± S.E.
Fish Length (mm)	127 ± 8 (9)	119 ± 3 (21)	112 ± 4 (9)
Fish Weight (g)	35.9 ± 5.9 (9)	26.8 ± 2.4 (21)	23.1 ± 1.8 (9)
Ht (%)	21.2 ± 1.9 a (8)	27.1 ± 1.5 b (20)	30.3 ± 2.4 b (9)
Hb (g/100 ml)	5.1 ± 0.3 a (8)	5.2 ± 0.1 a (20)	6 ± 0.1 b (9)
RBC ($x\ 10^6\ mm^{-3}$)	1.2 ± 0.1 a (8)	1.4 ± 0.1 a (20)	1.5 ± 0.1 a (9)
WBC ($x\ 10^3\ mm^{-3}$)	9.2 ± 1.7 a (8)	10.5 ± 1.2 a (20)	9.3 ± 1.3 a (8)
Lymphocytes ($x\ 10^3\ mm^{-3}$)	7.2 ± 1.9 a (8)	2.2 ± 0.3 b (20)	2.8 ± 0.4 b (8)
Neutrophils ($x\ 10^3\ mm^{-3}$)	2.0 ± 0.9 a (8)	8.2 ± 1.1 b (20)	6.5 ± 1.3 b (8)
RBC-L (μ)	10.6 ± 0.3 a (9)	10.9 ± 0.2 a (21)	11.2 ± 0.1 a (9)
RBCW (μ)	7.9 ± 0.3 a (9)	8 ± 0.1 a (21)	8.4 ± 0.3 a (9)

*Sample size in parentheses; values with the same letters are not significantly different at 5% level.

Table 6. Blood Values of *Ophicephalus striatus* exposed to 24h and 48h handling*

	Control Mean ± S.E.	24 h Mean ± S.E.	48 h Mean ± S.E.
Fish Length (mm)	274 ± 4.0 (4)	271 ± 5.0 (6)	282 ± 6.0 (6)
Fish Weight (g)	146.9 ± 10.3 (4)	154.8 ± 4.8 (6)	173.9 ± 7.0
Ht (%)	51.2 ± 3.6 a (4)	41.4 ± 2.8 ab (6)	40.2 ± 2.6 b (6)
Hb (g/100 ml)	7.9 ± 0.3 a (4)	7.2 ± 0.4 a (6)	7.6 ± 0.3 a (5)
RBC) x 10^6 mm^{-3})	3.7 ± 0.2 a (4)	3.8 ± 0.2 a (6)	3.6 ± 0.1 a (6)
WBC (x 10^3 mm^{-3})	94.9 ± 22.5 a (4)	59.1 ± 24.4 a (6)	69 ± 15.9 a (6)
Lymphocytes (x 10^3 mm^{-3})	17.5 ± 4.5 a (4)	7.9 ± 3.1 a (6)	8.4 ± 2.3 a (6)
Neutrophils (x 10^3 mm^{-3})	77.4 ± 18.7 a (4)	51.2 ± 21.9 a (6)	60.6 ± 14.1 a (6)
* RBC–L (μ)	8.6 ± 0.2 a (4)	9.4 ± 0.05 b (5)	9.9 ± 0.1 c (5)
RBC–W (μ)	6.7 ± 0.3 a (4)	7.3 ± 0.2 a (5)	7.5 ± 0.2 a (5)

*Sample size in parentheses; values with the same letters are not significantly different at 5% level.