*INT. B. BIOL. BIOTECH.*, 10 (2): 279-282, 2013.

# EFFECT OF DIFFERENT WATER pH ENVIRONMENTS ON HEMATOLOGICAL PARAMETERS OF *TILAPIA MOSSAMBICUS* (PETERS, 1852)

**Naeemuddin Arain[1]* and Abdul Rauf[2]**

[1]*Department of Zoology, Federal Urdu University for Arts, Science & Technology, Gulshan-e-Iqbal campus, Karachi, Pakistan.*
[2]*Department of Zoology, Govt. Superior Degree Science College, Shah Faisal Colony, Karachi, Pakistan*

## ABSTRACT

The effect of different water pH environments on hematological parameters of juvenile *Tilapia mossambicus* was investigated after three weeks of exposure to different acidic ($p^H$5.5 and 6.5) and alkaline ($p^H$ 8.5 and 9.5) enviroments. The results obtained indicated a significant decrease (P<0.05) in the erythrocyte count, hemoglobin concentration, hematocrit, lymphocyte count and significant increase (P<0.05) in total leukocyte count, monocytes and neutrophils of fish exposed to acidic pH 5.5 and alkaline pH of 9.5. The values of mean corpuscular volume decreased significantly in fish exposed to pH level of 5.5 only, whereas the values of mean corpuscular hemoglobin and mean corpuscular hemoglobin concentrations were not significantly different in all exposed fish groups when compared with controls. On the other hand, the variations in hematological parameters of fish exposed to pH levels of 6.5 and 8.5 were not significantly different from controls. These hematological alterations indicated that change in water pH might have caused physiological stress to the fish that can affect the growth and survival of fish in natural environment.

**Keywords:** Water quality, pH, Tilapia mossambicus, Hematological parameters

## INTRODUCTION

Natural and humanly induced environmental changes (temperature, pH, salinity, photoperiod and pollutants) in water bodies could affect physiological response of aquatic animals including fish. Hematological variables are considered good physiological indicators and frequently used to assess stress level and healthy state of fish exposed to toxicants (Affonso *et al*., 2002). There have been numerous studies regarding effect of different water quality parameters on hematological parameters of fish. It regards, effect of salinity on the hematology of *Acipenser naccarii* (García-Gallego and Sanz, 2002) and *Cyprinus carpio* (Hafez amini and Oryan 2002), thermal stress on the hematology of gilthead sea bream, *Sparus aurata* (Sala-Rabanal *et al.*, 2003) and photoperiod on rainbow trout, *Oncorhynchus mykiss*, (Valenzuela, 2008) and common carp *Cyprinus carpio (*Ruchin, 2006). Water pH is an important water quality parameter which plays a significant role to maintain homeostasis in fish. Increase and decrease in water pH levels can disturb acid-base balance, ion regulation, excretion of ammonia (Wilkie *et al.*, 1993; Jensen and Brahm, 1995; Parra and Baldisserotho, 2007), growth and cause physiological stress in fish which may lead to death (Zanibomi-Filho *et al*., 2009). The Mozambique tilapia, *Tilapia mossambicus*, is an exotic fish introduced in Pakistan from Malaya, in 1951, but due to its omnivorous food habit and adaptability to adverse environmental conditions it has become abundant in reservoirs (Naik, 1973). Tilapia farming has witnessed vast expansion in developed and developing countries, because of its robust growth rate and hardy characteristics. It can be commercially farmed in ponds, in cages in lakes or rivers, or in water tanks or raceways. In this context, the present study was carried out to examine effects of different water pH on the hematological parameters of *Tilapia mossambicus.* Such information would provide a better understanding of the physiological mechanisms of this species.

## MATERIALS AND METHODS

A group of juvenile tilapia (*Tilapia mossambicus*) of mean length 9.8 ± 1.4 cm; mean weight 66.11 ± 5.21 g were purchased from Masha-Allah fish hatchery Thatta, Karachi, Pakistan, and transported to the laboratory in aerated plastic containers. Fish were kept in a 300 L fiber glass tank for one week to acclimatize to the laboratory conditions. Water in the tank was aerated continuously and changed daily. Fish were fed with a commercial fish food twice a day (2% of body weight) but starved one day prior to the experiment. To determine the lethal values of extreme acidic and alkaline pH, fish were transferred in 80 L glass aquaria each containing ten fish and were exposed to seven different pH levels (4.5, 5.5, 6.5, 7.4, 8.5, 9.5, and 10.5) for 48 hours. The experiment was run in triplicate. The water quality parameters of experimental water are given in Table 1 and were determined using the standard methods (APHA, 1989). After determining the lethal limits of acidic and alkaline pH, fish were exposed to four different sub-lethal pH values (5.5, 6.5, 8.5, and 9.5) for three weeks to investigate their hematological response. Stock solution of Sodium bicarbonate ($Na_2CO_3$) and glacial acetic acid were used to create different acidic and alkaline conditions in the aquaria. The pH in the aquaria was measured using a digital pH meter (Milwaukee pH600, Bright Medi-Weld Appliances, India) and water quality parameters were maintained the same as used to determine lethal pH values. Ten fish were transferred in 80 L glass aquaria and three replicates were used for each pH value. Two control aquaria containing same number of fish and but having a pH of 7.4 were also run in parallel as controls. . Water in the aquaria was renewed daily to minimize the accumulation of metabolic wastes. Fish were fed twice a day, but feeding was stopped 24 h prior to the blood sampling. After three weeks of exposure, one fish from each replicate of different pH values was caught using a small dip net causing minimum disturbance in the aquarium. Blood was collected in a 18 G needle attached to a plastic syringe by cardiac puncture and transferred into plastic vials containing EDTA as an anticoagulant and used for hematological examinations. Hematological parameters examined included, erythrocyte count (RBC), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leucocyte count (WBC), lymphocytes (Lymp), Neutrophils (Neut), Monocytes (Mono) and were determined according to the unified methods for hematological examination of fish (Svobodova *et al*., 1991).

Data was analyzed by one way analysis of variance (ANOVA) to test the significant difference among different hematological parameters in experiment fish groups and controls. All statistical analyses were carried out using SPSS 17.0 computer programme (SPSS Inc. Chicago, USA) and $P<0.05$ was considered statistically significant.

## RESULTS

The upper and lower lethal pH limits for juvenile tilapia (*Tilapia mossambicus*) were recorded as 10.5 and 4.5, respectively. Fish mortalities were recorded within four hours of exposure to these pH levels. At these pH levels damaged respiratory epithelium and a thick layer of mucous was observed on the skin of fish. Compared to the control, fish exposed to sub-lethal pH levels exhibited jerking movements, restlessness, increased opercular movement and hanging vertically in the aquaria containing water of 5.5, 8.5 and 9.5 pH levels, respectively. The hematological response of test fish after three weeks exposure to different pH levels is depicted in Table 1. Compared to the controls there was a significant decrease in the erythrocyte count, hemoglobin concentration, hematocrit and lymphocyte count of fish exposed to acidic pH 5.5 and alkaline pH of 9.5 ($P<0.05$). A significant reduction ($P<0.05$) in the mean corpuscular volume of fish was observed in fish exposed to pH level of 5.5 only; whereas the values of mean corpuscular hemoglobin and mean corpuscular hemoglobin concentrations also showed a declining trend in all experimental fish groups but could not reach statistical significance. Total leukocyte count, monocytes and neutrophils of exposed fish showed an increasing trend in fish exposed to more alkaline and acidic pH levels as compared to controls but these parameters reached statistical significance in fish exposed to pH 5.5 and 9.5 only ($P<0.05$). On the other hand, the variations in these hematological parameters of fish exposed to pH levels of 6.5 and 8.5 were not significantly different from controls.

Table 1. Quality parameters of the experimental water.

| pH | Temperature °C | Dissolved $O_2$ (mg/l) | Free $CO_2$ (mg/l) | Chloride (mg/l) |
|---|---|---|---|---|
| 4.5 | 30.11 ± 1.22 | 5.8 ± 0.25 | 1.49 ± 0.54 | 65.21 ± 18.25 |
| 5.5 | 30.32 ± 1.30 | 6.0 ± 0.31 | 1.40 ± 0.35 | 52.69 ± 12.58 |
| 6.5 | 30.22 ± 1.47 | 6.1 ± 0.38 | 1.35 ± 0.28 | 19.32 ± 10.32 |
| 7.4 | 30.40 ± 1.62 | 6.4 ± 0.45 | 1.21 ± 0.11 | 15.28 ± 13.36 |
| 8.5 | 30.28 ± 0.82 | 6.7 ± 0.50 | 1.31 ± 0.41 | 14.65 ± 21.41 |
| 9.5 | 30.53 ± 1.12 | 6.9 ± 0.38 | 1.45 ± 0.33 | 14.85 ± 14.32 |
| 10.5 | 30.08 ± 1.34 | 7.2 ± 0.22 | 1.65 ± 0.52 | 13.95 ± 10.84 |

Table 2. Hematological variations in juvenile tilapia (*Tilapia mossambicus*) after three weeks exposure to different pH levels.

| Parameters | Controls | Experimental fish groups | | | |
|---|---|---|---|---|---|
| | pH 7.4 | pH 5.5 | pH 6.5 | pH 8.5 | pH 9.5 |
| RBC ($10^6/mm^3$) | $2.95 \pm 0.14^a$ | $1.58 \pm 0.75^b$ | $2.64 \pm 0.41^a$ | $2.79 \pm 0.35^a$ | $1.45 \pm 0.52^b$ |
| Hb (g/dl) | $8.21 \pm 1.20^a$ | $5.72 \pm 1.16^b$ | $7.48 \pm 1.05^a$ | $6.5 \pm 0.85^a$ | $4.98 \pm 0.55^b$ |
| Hct (%) | $18.21 \pm 0.31^a$ | $14.29 \pm 0.47^b$ | $17.19 \pm 0.65^a$ | $17.36 \pm 0.42^a$ | $13.98 \pm 0.74^b$ |
| MCV (fl) | $220.14 \pm 5.66^a$ | $1.88 \pm 2.55^b$ | $1.99 \pm 3.41^a$ | $205.32 \pm 4.25^a$ | $208.45 \pm 5.11^a$ |
| MCH (pg) | $25.17 \pm 1.22^a$ | $23.58 \pm 1.59^a$ | $22.75 \pm 1.19^a$ | $24.88 \pm 1.74^a$ | $21.37 \pm 1.87^a$ |
| MCHC (%) | $33.52 \pm 3.58^a$ | $31.52 \pm 4.20^a$ | $29.74 \pm 3.46^a$ | $32.85 \pm 1.49^a$ | $30.25 \pm 4.6^a$ |
| WBC ($10^3/mm^3$) | $21.82 \pm 1.37^a$ | $27.91 \pm 3.58^b$ | $23.53 \pm 2.58^a$ | $24.11 \pm 2.41^a$ | $28.22 \pm 3.73^b$ |
| Lymp (%) | $55.31 \pm 6.20^a$ | $45.85 \pm 4.89^b$ | $51.94 \pm 6.77^a$ | $52.75 \pm 5.95^a$ | $44.54 \pm 3.67^b$ |
| Neut (%) | $31.22 \pm 3.84^a$ | $39.27 \pm 4.55^b$ | $33.96 \pm 6.31^a$ | $38.48 \pm 3.43^b$ | $32.28 \pm 4.55^a$ |
| Mono (%) | $2.98 \pm 0.41^a$ | $4.39 \pm 0.22^b$ | $3.15 \pm 0.12^a$ | $3.08 \pm 0.10^a$ | $4.55 \pm 0.29^b$ |

Figures followed by a dissimilar letter within in a row are significantly different.

## DISCUSSION

In the present study, the acidic pH of 4.5 and alkaline pH of 10.5 was found to be lethal for the test fish after 48 h of exposure, whereas test fish exhibited sever physiological stress after three weeks exposure to 5.5 and 9.5 pH levels as indicated by significant hematological alterations. The lethal acidic pH of 5.5 and alkaline pH of 10.5 for the test fish are in consistence with the previous dealing with the critical and lethal pH levels of different fish species. Metelev *et al*., (1983) reported fish mortality after 24 h exposure to below 5.0 pH in carps and trout, similarly 24 h exposure to alkaline pH of 9.2 for perch, 10.4 for roach, 10.7 for pike and 10.8 for carps have been reported critical for fish survival. Fish mortalities at extreme pH limits may be attributed to the severe damage to their gills epithelium, resulting in suffocation and accumulation of $Co_2$ in the body. Igram and Wares (1979) reported inhibition of oxygen diffusion, ionic and acid/base balance deterioration caused by acid pH in fish. Murthy *et al*., (1981) reported lethal pH limits of 3.7 and 10.3 for 10 g *T. mossambicus***.** Slightly higher lethal pH levels for the same test fish in the present study may be attributed to the age and weight of the fish that may have increased the resistance of fish to acidic and alkaline pH levels.

In the present study the reduction in erythrocyte count, hemoglobin and hematocrit in *T. mossambicus* after exposure to extreme acidic and alkaline pH support previous reports dealing with the effect of pH on hematological parameters of other fish species. It regards, decrease in the RBC, hemoglobin and pack cell volume of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* exposed to different acidic and alkaline pH levels (Das *et al*., 2006). Allin and Wilson, (1999) and Akinorkimi *et al*., (2012) have also reported similar hematological alterations in Juvenile *Oncorhynchus mykiss* and *Tilapia guineensis*, respectively. The reduction in RBC count, hemoglobin, hematocrit and mean corpuscular volume of *T. mossambicus* following exposure to extreme acidic (5.5) and alkaline (9.5) pH in the present study may be attributed to erythrocytic type of anemia with subsequent erythroblastosis. Previous studies have showed that pH induced hemodilution following impairment of osmoregulation across gill epithelium results in the shrinkage of RBC and hemolysis leading to erythroblastosis and anemia (Bornhart *et al*., 1983; Das *et al*., 2004; Akinrotimi *et al*., 2007). The decrease in erythrocyte count, hemoglobin, hematocrit and MCV after exposure to extreme pH levels indicated that the test fish was under stress and had decreased oxygen carrying capacity. In such hypoxic conditions fish usually respond by increasing erythrocyte production or oxygen carrying capacity of hemoglobin (Martinenz and Souza, 2002). In the context of significant decrease in these parameters at extreme pH of 5.5 and 9.5, the non- significant decrease at moderate pH of 6.5 and 8.5 in the test fish may be attributed to the compensatory response of the fish to overcome hypoxic condition. Similar compensatory response has also been reported in *C. carpio*, following exposure to moderate pH levels (Ghanbari *et al*., 2012); and similar to our findings this compensatory response in *C. carpio* could not sustained against the higher pH levels and resulted in macrocytic type of anemia. Leukocytes regulate immunological function in organisms and their number increase as a protective response of the body during stress, through increased release of leukocytes from spleen in the blood circulation and stimulation of leukopoeitic process (Palikova *et al*., 1999). In the present study the significant increase in total leukocytes, lymphocytes, monocytes and neutrophils of juvenile *T. mossambicus* after three weeks of exposure to acidic and alkaline pH levels is in consistence with the other reports. Akinrokimi *et al*. (2012) reported increased total leukocyte counts, lymphocytes and monocytes count in *T. guineensis* exposed to varying pH levels of water and attributed it to the stress induced protective response of the fish. In contrast to our findings, Ghanbari *et al* (2012) reported a significant decrease in total leukocytes in juvenile *C. carpio* following exposure to 5.0 and 9.0 pH levels and attributed this to the susceptibility of *C. carpio* to greater change in water ph and weakening of leukopoeitic process at such higher acidic and alkaline pH of water. In agreement of our findings, decreased lymphocytes at extreme acidic and alkaline pH of water have also been reported in *Sarotherodon melanotherin* (Akinrotimi *et al*., 2007) and *T. guineensis* (Akinrotimi *et al*., 2009). The increase in leukocytes, neutrophils and monocytes of *T. mossambicus* in present study may be attributed to the result of stress induced stimulation of leukopoeitic process in fish following exposure to different pH levels. The decrease in lymphocytes of test fish may be attributed to the re-trafficking of cells to lymphoid tissues as a result of altered acid/base balance and ionic regulation during exposure to different pH levels of water, similar lymphonemia has also been reported in *C. catla*, *L. rohita* and *C. mrigala* exposed to different acidic and alkaline pH levels (Das *et al*., 2006).In conclusion, the results of this study show that pH changes in aquatic medium is an example of environmental parameters that significantly influenced hematological parameters of *T. mossambicus*.

## ACKNOWLEDGEMENTS

This work was supported by the Dean research grant 2204/20/2012 of Federal Urdu University for Arts, Science and Technology, Karachi, Paksitan. Authors are grateful to Miss. Qurat-ul-Ain Aslam of Bahria University, Islamabad for her suggestion to improve manuscript.

## REFERENCES


Affonso, E.G., V.L.P. Polez, C.F. Corrêa, A.F. Mazon, M.R.R. Araujo, G. Moraes and F.T. Ratin (2002). Blood parameters and metabolites in the teleosts fish *Colossoma macropomum* exposed to sulfide or hypoxia. *Comp. Biochem. Physiol*., 133: 375-382.

Akinrotimi O.A., O.M.G. Abu, E.J. Ansa, O.M. Edun and O.S. George (2009). Haematological responses of *Tilapia guineensis* to acute stress. *Int. J. Nat. Appl. Sci*., 5: 338-343.

Akinrotimi, O.A, E.J. Ansa, K.N. Owhonda, D.N. Onunkwo, P.E. Anyanwu, O.M. Edun, J.Y. Opara and P.T. Cliffe (2007). Effects of transportation stress on haematological parameters of black chin tilapia *Sarotherodon melanotherin*. *J. Anim. Vet. Adv*., 6: 841-845.

Akinrotimi, O.A., J.Y. Opara and I.F. Ibemere (2012). Changes in haematological parameters of *Tilapia guineensis* exposed to different water pH environments. *Innovat. Sci. Engi.*, 2: 9-14.

Allin, C.J. and R.W. Wilson (1999). Behavioral and Metabolic effects of chronic exposure to sub-lethal aluminum in acidic soft water in juvenile rainbow trout *Oncorhynchus mykiss*. *Can. J. Fish. Aquat. Sci*., 56: 670-678.

APHA (1989). *Standard methods for the examination of water and waste water*, 17$^{th}$ edition. American Public Health Association, Washington, DC, pp. 10-203.

Bornhart, M.I, M.A. Wallace and L.M. Lusler (1983). Red blood cells. In: *Biomedical research applications of scanning electron microscopy*. (Hodges, G.M. and K.E. Corr eds.). Academic press, New York. pp. 171-243.

Das, P.C., S. Ayyappan and J. Jena (2006). Haematological changes in the three Indian major carps, *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) exposed to acidic and alkaline water pH. *Aquaculture*, 235(1-4): 633-644.

Das, P.C., S. Ayyappan, J.K. Jena and B.K. Das (2004). Acute toxicity of ammonia and its sub lethal effects on selected haematological and enzymatic parameters of mrigal, *Cirrhinus mrigala* (Hamilton). *Aquacult. Res*., 35: 134-143.

García-Gallego, M and A. Sanz (2002). Physiological changes of sturgeon, *Acipenser naccarii*, caused by increasing environmental salinity. *J. Exp. Biol*., 205: 3699-3706.

Ghanbari, M., M. Jami, J.D. Konrad and K. Wolfgang (2012). Long-term effects of water pH changes on hematological parameters in the common carp (*Cyprinus carpio* L.). *Afr. J. Biotech*., 11(13): 3153-3159.

Hafez amini, P and S.H. Oryan (2002). Effects of NaCl stress on hematocrit and hemoglobin in common carp (*Cyprinus carpio*). *Iran. Fish. J.*, 3: 13-22.

Igram, R. and W. D. Wares (1979). Oxygen consumption in the fathead minnow (*Pimephales promelas*). Effects of pH, osmotic pressure and light level. *Comp. Biochem Physiol*., 62: 895 897.

Jensen, F.B and J. Brahm (1995). Kinetics of chloride transport across fish red blood cell membranes. *J. Exp. Biol*., 198: 2237-2244.

Martinez, C.B.R. and M.M. Souza (2002). Acute effects of nitrite on ion regulation in two neotropical fish species. *Comp. Biochem. Physio*., 133: 151-160.

Metelev, V. V., N. G. Kanaev and N. G. Dzasokhova (1983). *Water Toxicology*. Kolos Publisher, Moscow.

Murthy, V.K., P. Reddanna and S. Govindappa (1981). Hepatic carbohydrate metabolism in *Tilapia mossambica* (Peters) acclimated to low environmental pH. *Can. J. Zoo*., 59(3): 400-404.

Naik, I. U. (1973). Studies on *Tilapia mossambicus* (Peters) in Pakistan. *Agric. Pakistan*, 24: 47 76.

Palikova, M., J. Mares and J. Jirasek (1999). Characteristics of leukocytes and thrombocytes of selected sturgeon species from intensive breeding. *Acta Vet. Brno.* 68: 259-264.

Parra, J.E.G and B. Baldisserotto (2007). Effect of water pH and hardness on survival and growth of fresh water teleosts. In: *Fish Osmoregulation*. (Baldisserroto B, J.M. Mancera and B.G. Kapoor Eds.). Science publishers, Enfield.

Ruchin, A. B. (2006). Effect of Light on White Blood Cell Count in Carp *Cyprinus carpio* L. Seriya *Biologicheskaya*, 5: 634-637.

Sala-Rabanal, M., J. Sánchez, A. Ibarz, J. Fernández-Borràs, J. Blasco and M.A. Gallardo (2003). Effects of low temperatures and fasting on hematology and plasma composition of Gilthead Sea bream (*Sparus aurata*). *Fish Physiol. Biochem*., 29: 105-115.

Svobodova, Z., D. Pravda and J. Palackova (1991). *Unified methods of haematological examination of fish*. Research Institute of Fish Culture and Hydrobiology, Vodnany.

Valenzuela, A.E., V.M. Silva and A.E. Klempau (2008). Effects of different artificial photoperiods and temperatures on haematological parameters of rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol. Biochem*., 34: 159-167.

Wilkie, M.P., P.A. Wright, G.K. Iwama and C.M. Wood (1993). The physiological responses of the Lahontan cutthroat trout (*Onchorhynchus clarkihenshawi*), a resident of highly alkaline Pyramid lake (pH 9.4) to challenge at pH 10. *J. Exp. Biol*. 175: 173-194.

Zaniboni-Filho, E., A.P.O. Noner, D.A. Reynalte-Tataye and R.L. Serafini (2009). Water pH survival. *Fish Physiol. Biochem*., 35: 151-155.


(*Accepted for publication April 2013*)