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Influence of Different Calcium Levels and Low pH of Water on the Plasma Electrolyte Regulation of a Fresh Water Teleost Fish *Cyprinus carpio* Var. *communies*, (*Linnaeus*, 1958)

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Abstract: The present study investigated low pH with calcium and without calcium treatment on gill histology and ionoregulation of fish, *Cyprinus carpio* exposed to low pH 4.0 with low (normal water) calcium 6 mg/L and low pH 4.0 with added calcium 15 mg/L treatment for a period of 96 h. The light microscopic studies of the processed low pH with low calcium treatment, exposed gills showed marked histological alterations. The lesions of the gills included hypertrophy of the filament and lamellar cells; hyperplasia of the filament and lamellar cells, and deformities of gill arches, filaments and lamellae were seen. But in low pH with high calcium treatment the gill lesins were minimum such as, hypertrophy, hyperplasia and proliferation of chloride cells. In low pH with (normal water) low calcium treatment, plasma ionic levels (Na^+, K^+ Cl^-) decreased as the exposure period extended. In all the three experiments sodium level showing steep decrease ranging from 0.405, 21.382 to 8.411 at the end of 24 h to 32.965, 57.350 and 18.915 at the end of 96 h, respectively. Low pH with high calcium treatment, the fish exhibited only a minor depression in the plasma ionic levels showing minimum decrease of 4.952, 20.128 and 7.702 at the end of 96 h, respectively. Impact on gills and ionoregulation were minimum in low pH with added calcium level due to the ameliorative effects of calcium. The significant alteration in both the histology and electrolyte levels can serve as a biomarker of pollutant exposure and effects.

Key words: Chloride, gill histology, plasma Sodium, Potassium

INTRODUCTION

In acidified waters, the toxic effects may be exerted through disturbances to a host of physiological functions including histological alteration, ionoregulatory failure, fluid volume disturbance, hemoconcentration, acid-base homeostasis and oxygen uptake and transport (McDonald *et al*., 1980; Milligan and Wood, 1982; Carmona *et al*., 2004; Chezhian and Sivakumari, 2006). Fish are very vulnerable to disturbances in their environment due to the intimate contact of the skin and gills with the surrounding water (McDonald and Wood, 1993; Fernandes *et al*., 2007). Given that the gills are the major sites of osmotic and ionic regulation in fish, any changes in gill morphology may result in perturbed osmotic and ionic status (Carmona *et al*., 2004).

The impact of metals, as well as other pollutants, on aquatic biota can be evaluated by toxicity tests, which are used to detect and evaluate the potential toxicological effects of chemicals on aquatic organisms (Martinez *et al*., 2004). Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and gonads (Dutta, 1996). A histological investigation may therefore prove to be a cost-effective tool to determine the health of fish populations, hence reflecting the health of an entire aquatic ecosystem. These cells participate in various functions, such as gas exchange, maintenance of blood acid–base balance and ionic regulation (Goss *et al*., 1998; Fernandes *et al*., 1998; Sturla *et al*., 2001; Ovie *et al*., 2008).

In constant contact with the water, the gill is a sensitive primary target for a variety of insults including heavy metals (Hinton *et al*., 1992). Exposure of fish to heavy metals may also result in variable degrees of ion regulatory disruption, and plasma ion levels may be employed for quantifying toxic effects of metals during acute exposure (Mayer *et al*., 1992). In freshwater fish, osmotic water influx and diffusive losses of ions such as Na^+ and Cl^- are compensated for by the excretion of large volumes of dilute urine and active uptake to replace ions lost by the gills (Evans *et al*., 1999).

Low pH apparently interferes with ionic regulation in fishes and reduce the survival time of fishes in acidified water bodies (Wood and McDonald, 1982). Pickford *et al*. (1966) reported that hard water improves the survival of

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euryhaline and stenohaline teleosts and reduce the energy expenditure required for the maintenance of ion balance (Eddy, 1975). Now-a-days neutralization of acidified waters by the addition of external calcium has been employed to restore and protect endangered fisheries in several rivers (Rosseland and Skogheim, 1984), were calcium is the important ion determining fisheries status more important, impact than pH. Laurén and McDonald (1985) observed a large stimulation of the passive efflux of NaCl (reflecting an increase in permeability) in freshwater rainbow trout, an effect which presumably was due to displacement of Ca^{++} by Cu^{++} in tight junctions of the gill epithelium. An increased passive ion influx in seawater cod would elevate plasma [Na^{+}] and [Cl^{-}], and an increased water loss would lead to haemoconcentration.

Ionoregulatory disturbances are reduced or eliminated when the pH of the water is raised by external calcium (Leivestad and Muniz, 1976; Rosseland *et al*., 1984). Jagoe and Haines (1990) reported that neutralization of acid water diminishes or eliminates plasma ion losses caused by acid stress, where it follows that changes in gill tissue associated with acid exposure should be prevented or reduced by external calcium. Hence in the present study, an attempt was made to study the effect of low pH on gill histology and ionoregulation in a freshwater fish, *Cyprinus carpio* and the protective role of external calcium against the acid stress.

MATERIALS AND METHODS

Specimens of *Cyprinus carpio* were collected from the local fish farm and acclimatized to the laboratory conditions for fifteen days. The experiment were done at CAS in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu- India in 2009. Water was changed daily and fish were fed *ad libitum* with rice bran and groundnut oilcake twice a day. For experimental studies, fish ranging from 8-12 cm in length and weighing 10-12 g were selected. The physico-chemical parameters of the water was estimated according to APHA (1976) and are as follows: Dissolved Oxygen- 6.2±0.02 mg/L; pH 7.2±0.2; Temperature- 25.0±2.0ºC; Salinity- 0.2±0.07 ppm; Total hardness- 14±2.0 mg/L; Calcium- 4.0±0.1mg/L; Magnesium- 8.0±2.0 and Total alkalinity- 180 (165-185) ppm.

Preliminary studies were carried out to find out the survival/mortality of fish in acid water with low (normal water calcium) calcium level. For this 20 L tubs were taken each filled with 15 L of water. Then, 15 fish were introduced in to each tub. A common control was also maintained. The survival time of fish in all the pH ranges were monitored. Only in pH 4.0 and above, the fish exhibited 80-150% survival up to 96 h. Based on the above observations, pH 4.0 was selected.

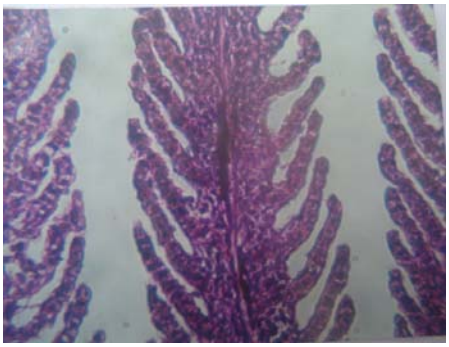
For low pH with high calcium studies, 5 tubs were taken The pH of the water was noted and it was lowered to 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 by adding 0.1N sulphuric acid drop by drop and different calcium nitrate levels (5, 15, 15, 20and 25 mg/L) were added. Then 15 fish were introduced in to each tub. The mortality/survival time of fish in each tub was noticed. Based on the above observation 15 mg/L was taken as the high calcium level. Because only in 15 mg/L of calcium oxide 100% survival of fish was noted. In above 15 mg/L the percent survival of fish showed a declining trend when the exposure period extended.

For treatment-I a large glass tank of 150 L capacity with 150 L of water was taken. The pH of the water in the tank was noted (7.2) and it was lowered to pH 4.0 by adding 0.1 N. H_2SO_4 drop by drop. Then 150 fish released to the above tank for a period of 96 h.

In Treatment-II another experimental tank of 150 L capacity with 150 l of water and pH was lowered to pH 5.0 by adding 0.1 N. H_2SO_4 drop by drop. Then 15 mg/L of calcium nitrate was added to the experimental tank then 150 fishes were released to the tank. Fish ranging from 8-12 cm in length and weighing 10-12 g were selected to above treatments for a period of 96 h. A common control (pH 7.2) was also maintained. Fish were fed *ad libitum*. Toxicant renew daily. No mortality was observed throughout the experimental period. At the end of every 24 h, 25 fishes from each experimental tank were collected and blood was drawn from the heart region by cardiac puncture with heparin as an anticoagulant and centrifuged at 9000 rpm for 20 min. and clear plasma was collected for the analysis of sodium (Trinder, 1951; Maruna, 1958) potassium (Sunderman and Sundarman, 1959; Tietz, 1970) and chloride (Schoenfeld and Lewellen, 1964). For histopathological studies, gills were subsequently dissected using a sterile scalpel, and were rinsed with distilled water in order to remove the adhering body fluid and were fixed in Bouin's fixative and later processed following the methods of Pearse (1968), Roberts (1978) and Humason (1979). All the above values were analysed statistically.

RESULTS AND DISCUSSION

Plate 1 shows the changes in the gills of *Cyprinus carpio* var. *communis* when exposed to treatment-1 and II. In the present study, the control fish, primary lamellae appeared normal and mucus free with well-defined secondary lamellae branched from them (Plate 1A). In the fish exposed to 24 h. acid treatment the result revealed that the gills showed the magnitude of hypertrophy, swelling and proliferation of chloride cells were observed (Plate 1B). When the fish was exposed to 48 h. acid treatment the hyperplasia, fusion of secondary lamellae, lifting up of the epithelium lamellar fusions and disintegration of epithelial cells were seen (Plate 1C).



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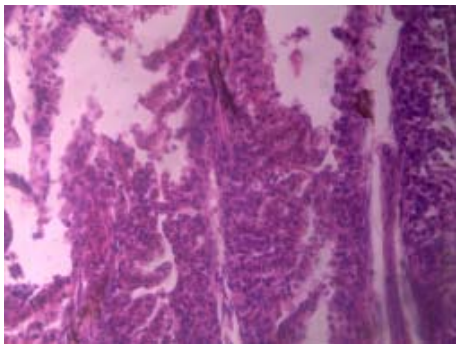

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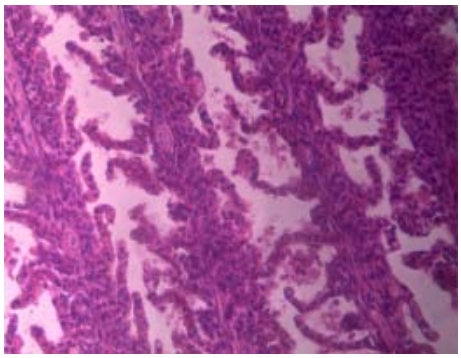

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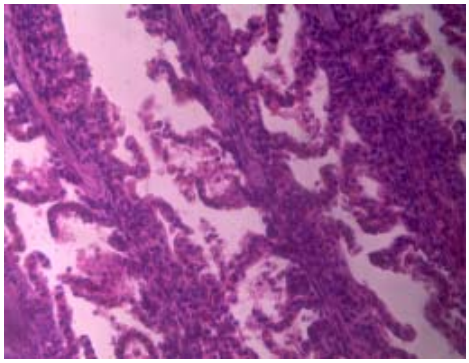

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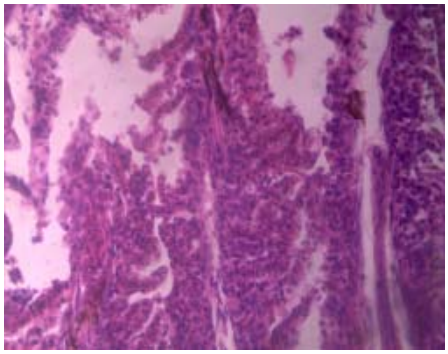

E

Plate 1: Photographs showing the transverse section of gills of fish *Cyprinus carpio* exposed to low pH concentration (magnification 400X), A- Control B- 24h low pH treated fish gill, C-48h low pH treated fish gill, D-72h low pH treated fish gill and E- 96h low pH treated fish gill.

In 72 h acid treatment, in fish hyperplasia, fusion of secondary lamellae and disintegration of epithelial cells were seen (Plate 1D); but in 96 h acid treatment, disintegration of epithelial cells, desquamated epithelium, hemorrhage and complete damage of epithelial cells of lamellae and necrosis were observed (Plate 1E). Whereas in acid with high calcium treated fish's gills showing very minimum gill alterations like that of proliferation of chloride cells, hyperplasia and hypertrophy disintegration of epithelial cells of lamellae were observed (Plate 2A, B, C and D).

Table 1 show the changes in the plasma sodium level of fish exposed to treatment-I. The plasma sodium and chloride level were decreased as the exposure period (24, 48, 72 and 96) extended showing minimum percent decrease of 0.405, 21.382 and 8.411 at the end of 24 h. Whereas the maximum percent decrease of 32.965, 57.350 and 18.915 observed at the end of 96 h. In both treatments potassium level where increased through out the experiment showing minimum percent decrease 21.382 in low pH and high calcium treatments, respectively at the end of 24 h. whereas the maximum percent decrease of 57.350 was observed at the end of 24 and 96 h, respectively. In addition, the changes in plasma electrolyte levels in treatment-II were more or less equal to that of the control. In treatment-2 the plasma sodium and chloride level were decreased as the exposure period (24, 48, 72 and 96) extended showing minimum percent decrease of 0.405, 21.382 and 8.411 at the end of 24 h. Whereas the maximum percent decrease of 32.965, 57.350 and 18.915 observed at the end of 96 h. In both treatments potassium level where increased through out the experiment showing minimum percent decrease 21.382 in low pH and high calcium treatments,

Table: 1: Changes in the blood plasma Sodium, Potassium and Chloride levels of fish *Cyprinus carpio var. communis* exposed to Low pH and added calcium concentration for 96 h

Parameters	Exposure period (h)	Control	Treatment-I	t-test	Treatment-II	t-test
Sodium (Na^+)	24	152.903±0.759	61.280±0.223 (-0.405)	52.615*	95.166±0.176 (-7.518)	9.930*
	48	152.239±1.418	89.790±0.134 (-12.302)	8.844*	99.848±0.924 (-2.834)	1.499
	72	119.386±0.655	98.030±1.418 (-17.780)	13.578*	112.481±0.157 (-5.667)	15.034*
	96	111.753±.463	74.913±0.144 (-32.965)	75.978*	156.219±0.159 (-4.952)	11.305*
Potassium (K^+)	24	13.568±0.206	15.667±0.173 (-21.382)	15.784*	12.068±0.307 (-11.055)	4.057*
	48	13.077±0.004	8.499±0.018 (-35.008)	248.277*	11.228±0.179 (-14.139)	15.327*
	72	13.208±5.608	5.808±0.157 (-56.027)	1.319	15.741±0.038 (-18.678)	0.439
	96	15.829±0.027	6.751±0.098 (-57.350)	89.305*	12.643±0.038 (-20.128)	68.346*
Chloride (Cl^-)	24	98.036±0.033	89.790±0.571 (-8.411)	14.417*	94.760±0.207 (-3.342)	2.872*
	48	99.848±0.924	85.790±0.954 (-14.079)	15.585*	95.801±0.315 (-4.053)	4.261*
	72	94.760±0.207	79.767±0.659 (-15.822)	21.704*	89.708±1.747 (-5.331)	15.629*
	96	95.166±0.176	77.165±0.314 (-18.915)	50.008*	87.836±0.269 (-7.702)	22.802*

Values are mean ± of five individual observations, denotes percent decrease over control; *: values are significant at 5 % level; *: degrees of freedom at 8 $t_{0.05}$ = 2.306

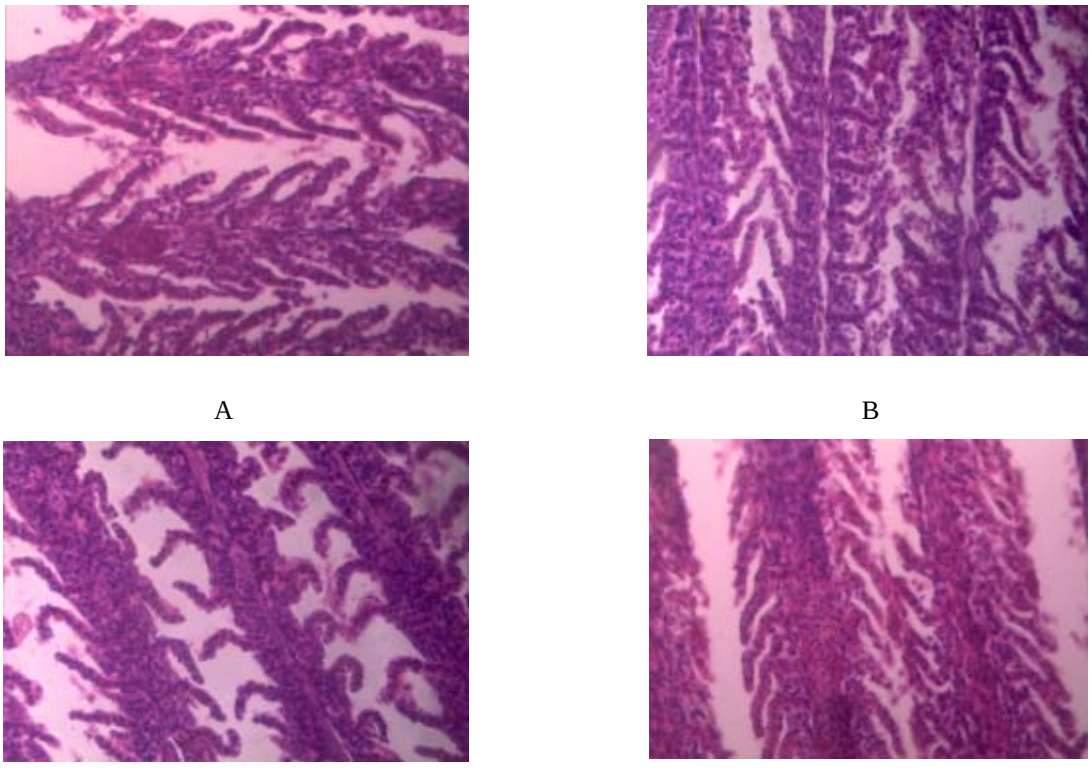

Plate 2: Photographs showing the transverse section of gills of fish *Cyprinus carpio* exposed to calcium concentration (magnification 400X), A-24 h calcium treated fish gill, B-48 h calcium treated fish gill, C-72 h calcium treated fish gill and D- 96 h calcium treated fish gill

respectively at the end of 24 h. whereas the maximum percent decrease of 57.350 was observed at the end of 24 and 96 h, respectively. In addition, the changes in plasma electrolyte levels in treatment-II were more or less equal to that of the control.

The fundamental toxic mechanism of direct acid stress in the gills is the disturbance of electrolyte regulatory process (Wood and McDonald, 1982). According to Wood *et al*. (1988) the acidotic conditions resulting from the uptake of hydrogen ions from the water inhibit the active uptake of sodium and chloride ions and stimulate the loss of electrolytes through both passive efflux and the stimulation of changes in the transepithelial potential at the gill membrane.The intimate contact of gill with polluted water may lead to alterations in the normal gill epithelium (Skidmore and Tovell, 1972) reported that many noxious compounds and ions have been shown to damage the respiratory epithelium of gills. Gill irritation was observed by (Lewis and Peters, 1956) in fish, in waters of pH 4.0 - 4.5. Gill lesions can be divided into two groups *ie*, the direct deleterious effects of the irritants (Temmink *et al*., 1983) and the defence responses of the fish (Morgan and Tovell, 1973). In the present study rupture, necrosis of gill epithelium of fish may be due to direct deleterious effect of low pH. However, hyperplasia, hypertrophy, lamellar fusion and mucus secretion and sloughing of gills may be defense responses of the fish to low pH.

Lamellar fusion could he protective as it diminishes the amount of vulnerable Gill Surface in fish (Mallatt, 1985). Fusion of Secondary lamellae and Swelling of primary and secondary lamellae increases the diffusion distance (Tietge *el al*., 1988) and reduced surface area (Smith and Haines, 1995). Fusion of primary lamellae at the distal end and thickened and shortened secondary lamellae observed in the present study may be involved in reducing the impact of metal toxicity supporting the observation of the above authors.

Leivestad and Muniz (1976) reported that brown trout *Salmo trutta* exposed to an acid pluse at spring showed reduced plasma sodium and chloride concentrations. They further reported that the ionic imbalance caused by acid stress appears to result from changes in both the branchial membrane permeability and branchial ion transport mechanisms. Ultsch *et al*. (1981) reported that decreased level of sodium and chloride in rainbow trout *Salmo gairdnery* exposed to pH 4.82 may be due to partial inhibition of influx. In the present study, the significant reduction in plasma sodium and chloride level during low pH treatment may be due to partial inhibition of influx or direct acid stress on the gills. When rainbow trout and carp were exposed to pH 4.0, the plasma K^+ level increased (McDonald *et al*., 1980; Ultsch *et al*., 1981; Chezhian and Sivakumari, 2006). Irwin *et al*. (1960) reported that the increase in plasma potassium during low pH treatment could possibly be due to cation exchange of H^+ and K^+ between intracellular and extracellular space. Nevillie (1980) suggested that plasma potassium may be released by the breakdown of erythrocyte which is more fragile to the lower blood pH.

In the present study, significant increase in plasma potassium level may be due to cation exchange of H^+ and K^+ between intercellular and extracellular space or erythrocyte swelling leading to hemolysis and contamination of plasma with extracellular K^+ or a reduction in the extracellular space supporting the views of the above authors. McDonald (1983) reported that exposure of rainbow trout in low pH (4.3) in soft water (Ca^{2+} = 300 μ equiv/l) led to a more pronounced plasma ionic disturbance compared to that developing at the same pH in moderate hard water (Ca^{2+} = 1600 μ equiv/l). The principal sites of interaction of calcium and low pH are the gill mechanisms for the regulation of sodium and chloride (McDonald, 1989). Playle *et al*. (1989) reported that the presence of H^+ ion the external environment inhibits active uptake of Na^+ and Cl^- at the gill and stimulates passive effluxes through Paracellular channels, perhaps by displacement of Ca^{2+} from the tight junctions.

Calcium binds to the surface of cells and the main binding groups are oxy anions. McWilliams (1983) reported that calcium binds to gills at two distinct types of sites, but binding at only one type is clearly involved in altering membrane permeability. He also noted that surface-bound calcium is removed from the gills more rapidly under acid than under neutral conditions. Tight binding of hardness may be a significant physiological adaptation for survival in acidic, low hardness waters which prevent loss of plasma electrolytes. In the present study, minimum ionic disturbance during low pH with high calcium treatment may be due to binding of calcium in the gills thereby reducing gill permeability. Hence, environmental hardness plays a significant role in protecting fish from acidified waters.

CONCLUSION

The exotic scale carp, C*yprinus carpiovar*. *communis* is considered to be one of the chief edible fishes in all region. In Indian condition not much work on the toxic effect of low pH on fish with reference to Indian context. Hence, an attempt was made to assess toxic impact of low pH on above said fish and the ameliorative effect of external calcium on acid toxicity under tropical conditions. The light microscopic studies of the processed low pH with low calcium treatment, exposed gills showed marked histological alterations. The lesions of the gills included hypertrophy of the filament and lamellar cells; hyperplasia of the filament and lamellar cells, and

deformities of gill arches, filaments and lamellae were seen. But in low pH with high calcium treatment the gill lesions were minimum such as, hypertrophy, hyperplasia and proliferation of chloride cells. In low pH with (normal water) low calcium treatment, plasma ionic levels (Na^+, K^+ Cl^-) decreased as the exposure period extended. Low pH with high calcium treatment, the fish exhibited only a minor depression in the plasma ionic levels showing minimum decrease of 4.952, 20.128 and 7.702 at the end of 96 h, respectively. The significant alteration in both the histology and electrolyte levels can serve as a biomarker of pollutant exposure and effects. In the present study, increased survival of fish in high calcium level may be due to neutralization of acid toxicity or protection of membrane permeability or reduced ion loss across the gill or preferential ion regulation may be operating individually or in any fermentation combination.

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REFERENCES

APHA, AWWA and WPCF, 1976. In: Standard Methods for the Examination of Water and Waste Water. 14th Edn., American Public Health Association, Washington, USA.

Carmona, R., M.G. A-Gallego, A. Sanz, A. Domezain and M.V. Ostos-Garrido, 2004. Chloride cells and pavement cells in gill epithelia of Acipenser naccarii: ultrastructural modifications in seawater-acclimated specimens. J. Fish Biol., 64: 553-566

Chezhian, A. and K. Sivakumari, 2006. Influence of external calcium on the toxicity of low pH and phosphamidon in relation to ionic regulation of *Cyprinus carpio var.*communis (*Linnaeus*, 1758). Indian J. Fish., 53(1): 59-65.

Eddy, F.B., 1975. The effect of calcium on gill potentials and on sodium and chloride fluxes in the goldfish, *Carassius auratus*. J. Comp. Physiol., 96: 131-142

Dutta, H.M., J.S.D. Munshi, P.K. Roy, N.K. Singh, S. Adhikari, J. Killius, 1996. Ultrastructural changes in the respiratory lamellae of the catfish, *Heteropneustes fossilis* after sublethal exposure to melathion. Environ. Poll., 92: 329-341.

Evans, D.H., P.M. Piermarini and W.T.W. Potts, 1999, Ionic transport in the fish gill epithelium. J. Exp. Zool., 283: 641-652.

Fernandes, M.N., S.A. Perna and S.E. Moron, 1998. Chloride cell apical surface changes in gill epithelia of the armoured catfish *Hypostomus plecostomus* during exposure to distilled water. J. Fish Biol., 52: 844-849.

Fernandes, C., E.A. Fontaı́nhas-Fernandes, S.M. Monteiro and E.M.A. Salgado, 2007. Changes in plasma electrolytes and Gill Histopathology in Wild Liza saliens from the Esmoriz-Paramos Coastal Lagoon, Portugal. Bull. Environ. Cortom. Toxicol., 79: 302-305.

Goss, G.G., S.F. Perry, J.N. Fryer and P. Laurent, 1998. Gill morphology and acid–base regulation in freshwater fishes. Comp. Biochem. Physiol., 119A: 107-115.

Hinton, D.E., P.C. Baumann, G.R. Gardner, W.E. Hawkins, J.D. Hendricks, A. Murchelano and M.S. Okihiro, 1992. Histopathological Biomarkers. In: Huggett, R.J., R.A. Kimerle, P.M. Mehrle-Jr. and H.L. Bergman (Eds.), Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress. Lewis Publishers, Boca Raton, Fl.

Humason, G.L., 1979. Animal Tissue Techniques. 4th Edn., W.H. Freeman and Company, San Francisco, USA, pp: 3-331.

Irwin, R.O.H., S.J. Stunders, M.D. Milne and M.A. Carwford, 1960. Gradients of potassium and hydrogen ion in potassium - deficient voluntary muscle. Clin. Sci., 20: 1-18.

Jagoe, C.H. and T.A. Haines, 1990. Morphometric effects of low pH and limed water on the gills of Atlantic salmon, *Salmo salar*. Can. J. Fish Aquat. Sci., 47: 2451-2456.

Laurén, D.J. and D.G. McDonald, 1985, Effects of copper on branchial ionoregulation in the rainbow trout, *Salmo gairdneri* Richardson. J. Comp. Physiol., 155(B): 636-644.

Leivestad, H. and Muniz, P. 1976. Fish kills at low pH in a Norwegian river. Nature (London), 1259: 391-392.

Lewis, W.M. and C. Peters, 1956. Coalmine slag drainage. Ind. Waste (Chicago), 1: 145-147.

Maruna, R.F.L., 1958. Determination of serum sodium by the magnesium uranyl acetate. Clin. Chem Acta., 2: 581-585.

Martinez, C.B.R., M.Y. Nagae, C.T.B.V. Zaia and D.A.M. Zaia, 2004. Acute morphological and physiological effects of lead in the neotropical fish *prochilodus lineatus.* Bran. J. Biol., 64(4): 798.

Mayer, F.L., D.J. Versteeg, M.J. Mckee, L.C. Folmar, R.L. Graney, D.C. McCume and B.A. Rattner, 1992. Physiological and Nonspecific Biomarkers. in Biomarkers. Biochemical, Physiological, and Histological Markers of Anthropogenic Stress'. In: Kimerle, R.J., R.A. Mehrle, P.M. Jr and H.L. Bergman (Eds.), Proceedings of the Eighth pellston Workshop, Keystone, Colorado, 23-28 July, Lewis Publishers, Boca Raton, USA, pp: 5-85.

Mallatt, J., 1985. Fish gill structural changes induced by toxicants and other irritants: A statistical review. Can. J. Fish. Aquat. Sci., 42: 630-648.

McDonald, D.G., 1983. The interaction of environmental calcium and low pH on the physiology of the rainbow trout, *Salmo gairdneri.* I. Branchial and renal net ion and H^+ fluxes. J. Exp. Biol., 152: 123-140.

McDonald, D.G., H. Hobe and C.M. Wood, 1980. The influence of calcium on the physiological responses of the rainbow trout, *Salmo gairdneri*, to low environmental pH. J. Exp. Biol., 88: 109-131.

McDonald, D.G., J.P. Reader and T.R.K. Dalziel, 1989. The combined effects of pH and trace metals on fish ionoregulation. In: Morris, R., D.J.A. Brown, E.W. Taylor and J.A. Brown (Eds.), Acid Toxicity and Aquatic Animals. Society for Experimental Biology Seminar Series. Cambridge University Press, Cambridge, England, UK, pp: 221-242.

McDonald, D.G. and C.M. Wood, 1993. Branchial Mechanisms of Acclimation to Metals in Freshwater Fish. In: Rankin, J. (Ed.), Fish Ecophysiolgy. Chapman and Hall, London.

McWilliams, P.G., 1983. An investigation on the loss of bound calcium from the gills of the brown trout, *Salmo trutta*, in acid media. Comp. Biochem. Physiol., 74A: 107-116.

Morgan, M. and P.W.A. Tovell, 1973. The Structure of the gill of the trout (*Salmo gairdneri*) (Richardson). Zellforch Mikrosk Anat., 142: 147-162.

Milligan, C.L. and C.M. Wood, 1982. Disturbances in hematology, fluid volume distribution and circulatory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. J. Exp. Biol., 99: 397-415.

Nevillie, C.M., 1980. The effects of environmental acidification on rainbow trout. Ph.D. Thesis, University of Toronto, Toronto, Canada.

Ovie, K.S., 2008. Electrolytes response to sublethal concentrations of Zotassium permanganate in the African catfish: *Clarias gariepinus* (Burchell, 1822). Int. J. Integrative. Biol., 5(1): 67.

Pearse, A.G.E., 1968. Histochemistry: Theoretical and Applied. 3rd Edn., J and A Churchill Ltd., London, pp: 13-102.

Pickford, G.E., P.K.T. Pang, J.G. Stanley and W.R. Fleming, 1966. Calcium and freshwater survival in the euryhaline cyprinodonts, *Fundulus kansee* and *Fundulus heteroclitus*. Comp. Biochem. Physiol. 18: 503-509.

Playle, R.C., G.G. Goss and C.M. Wood, 1989. Physiological disturbances in rainbow trout (*Salmo gairdneri*) during acid and aluminum exposures in soft water of two calcium concentrations. Can. J. Zool., 67: 314-324.

Roberts, R.J., 1978. The Pathophysiology and Systemic Pathology of Teleosts, and Laboratory Methods. In: Fish Pathology. 1st Edn., Bailliere Tindall, London, UK, pp: 235-246.

Rosseland, B.O. and O.K. Skogheim, 1984. A comparative study on salmonid fish species in acid aluminium-rich water II. Physiological stress and mortality of one and two years old fish. Fresh Water Research. National Swedish Board of Fisheries Rep., 61: 1580-1591.

Rosseland, B.O., O.K. Skogheim, H. Abrahamsen and D. Matzow, 1984. Survival and reduction of physiological stress of Atlantic salmon, *Salmo salar* L. Smolts in an acidic river through slurry liming. Norwegian ministry of environment. Kalking-sprosjektet Rep., 17-84: 24.

Schoenfeld, F.G. and C.J. Lewellen, 1964. A colorimetrie method for determination of serum chloride. Clin. Chem., 10: 533.

Smith, T.R. and T.A. Haines, 1995. Mortality, growth, swimming activity and gill morphology of brook trout, *Salvelinus fontinalis* and Atlantic salmon, *Salmo salar*, exposed to low pH with and without aluminium. Environ. Pollut., 90: 33-40.

Skidmore, J.F. and W.A. Towel, 1972. Toxic effects of zinc sulphate on the gills of raibow trout. Water Res., 6: 217-230.

Sturla, M., M.A. Masini, P. Prato and C. Grattarola, 2001. Mitochondria-rich cells in gills and skin of an African lungfish, Pro topterus annectens. Cell Tissue Res., 303: 351-358.

Sunderman, F. and F.W. Sunderman, 1959. Nickel metabolism in health and disease. Ann. New York Acad. Sci., 199: 300.

Temmink, J.B., P. Bowmeister, P. Dejong and J. Van den Berg, 1983. An ultrastructural study of chromate-induced hyperplasia in the gill of rainbow trout (*Salmo gairdneri*). Aquat. Toxicol., 4: 165-179.

Tietge, J.E., R.D. Johnson and H.L. Bergman, 1988. Morphometric changes in gill secondary lamellae of brook trout (*Salvelinus fontinalis*) after long-term exposure to acid and aluminium. Can. J. Fish. Aquat. Sci., 45: 1643-1648.

Tietz, N.W., 1970. Fundamentals of Clinical Chemistry. W.B. Saunders Company, Philadelphia, pp: 696.

Trinder, P., 1951. A rapid method for the determination of sodium in serum. Analyst, 76: 596-599.

Ultsch, G.R., M.E. Ott and N. Heister, 1981. Acid-base and electrolyte status in carp, (*Cyprinus carpio*), exposed to low environmental pH. J. Exp. Biol., 93: 65-80.

Wood, C.M. and D.G. McDonald, 1982. Physiological Mechanisms of Acid Toxicity to Fish. In: Johnson, R.E. (Ed.), Acid Rain/Fisheries. Proceedings of the International Symposium on Acid Precipitation and Forest. Impacts in North-Eastern North America. American Fisheries Society, Bethesda, Maryland, USA, pp: 197-226.

Wood, C.M., B.P. Simons, D.R. Mount and H.L. Bergman, 1988. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*) 2. Blood parameters by cannulation. Can. J. Fish. Aquat. Sci., 45(9): 1597-1605.

Wood, C.M., R.C. Playle, B.A. Simons, G.G. Goss and D.G. McDonald, 1988a. Blood gases, acid-base status, ions, and hematology in adult brook trout (*Salvelinus fontinalis*) under acid/aluminum exposure. Can. J. Fish. Aquat. Sci., 45(9): 1575-1586.