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RESPIRATORY BEHAVIOUR OF CLARIAS BATRACHUS, LINN.DURING EXPERIMENTAL PLUMBISM

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ABSTRACT

Cat Fishes like Clarias batrachus, are gifted with an accessory respiratory device that makes them adapted to pollute or oxygen deficit water bodies. They regularly visit to the surface to engulph air. In the present piece of study, the fish was exposed to various sub-acute and chronic concentrations of Lead acetate. They were monitored constantly for respiratory behaviour. The results revealed enhanced surfacing decreased opercular beat.

Keywords: Lead acetate, *Clarias batrachus*, Plumbism, Respiratory behavior

INTRODUCTION

Lead is an important stress or pollutant that can modulate neuro-behavioural activities of human being as well as in other organisms. Perhaps more than any other poison, lead (Pb2+, the ionic form most commonly used in research) from residential, commercial, and industrial sources (Silbergeld & Patrick, 2005; Del Bene Davis, 2007; Chen et al., 2011; Mielke et al., 2011) conjures up images of lower IQs, behavioral disorders, and sensory-motor deficits – and with good reason. This pollutant has been associated with a range of childhood behavioral problems at ever lower levels of exposure (Bellinger, 2004, 2008; Olympio et al., 2009).

Fishes are ideal sentinels and test organisms for behavioral assays of various stress factors and toxic chemical exposure due to their ecological relevance in many natural systems (Little et al., 1990). In addition, fish may bio-accumulate various contaminants and/or play a role in food web bio-magnification (Rout and Naik 2013a). Behavioral endpoints in fish, as well as other organisms, are valuable tools to discern and evaluate effects of environmental stress. These endpoints of exposure are important because they integrate endogenous and exogenous factors that can link biochemical and physiological processes, and can provide insights into individual- and community-level effects of environmental contamination.

In the present piece of work it has been tried with *Clariasbatrachus*, Linn. a common air breathing cat fish of the locality with good economic potential to observe the respiratory behavior of the fish in different concentrations of lead acetate for three days, seven days for sub acute study as well as for 105 days in one fixed concentration for chronic study.

MATERIAL AND METHODS

Experimental Design

Large-sized (120-200 gms.) fishes were collected from culture ponds of village Deopada in Bhadrak district of Odisha and aclimatized for seven days in the laboratory aquaria as reported earlier (Rout and Naik 2013b). For short-term studies, different sub acute concentrations of lead acetate [Pb (CH_3-$COO)_2$], Johnson and Sons, Ltd., London, 1990]

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were choosen after obtaining LC_{50}(500 ppm) and LT_{50} (45 days for25pm, 40 days for 50 ppm, 37days for 75 ppm., 35 days for 100 ppm, 30days for 125ppm and 28 days for 150ppm.

For behavioral studies the protocols of the Course manual of IISER Kolkata (2012) and Webber et.al. (2013) were followed strictly.

For the study different parameters, the entire experimental set up was divided in to 3 sets. The 1st two sets meant for sub acute treatment for 3 days (48hrs) and 7 days respectively, while the 3rd set was meant for chronic 15 ppm exposure to about 105 days.

For sub acute treatments of 3 days and 7 days, fishes in group were selected and each group kept in separate aquaria demarcated for control, 25 ppm, 50 ppm, 100 ppm, 125 ppm and 150 ppm of lead acetate respectively.

The 3rd set included Chronic treatment 15 ppm of lead acetate for 105 days with 15 days interval. For this 8 fishes were taken in each group for controls, 1st day, 15days, 45days, 60days, 75days, 90days and 105days respectively.

PLATE-1: Pathological observations-C

i) Lateral movement of the fish just before death.

ii) Magnified view of the gill movement of the fish just before death.

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Respiratory Behaviour

I. Opercular beats: The number of opercular beats was studied taking stopwatch and the number was control for invidual fishes, kept in control and treated aquaria.

$$\text{Frequency of opercular beat} = \frac{No\, of\, beats}{Minutes}$$

II. Surfacing: Since the fish is an obligating air breaths, it visits to the water surface to gulf air. Frequency of surfacing = No of visits to surface / hr.

Statistical Analysis

Standard Deviation

The Respiratory behaviors of the fishes were expressed by standard deviation following Banerjee (2004).

Students T-Test

Unpaired t – test was used the level of significance for the respiratory behaviours following Chainy et.al. (2008).

RESULTS

After the fishes were transferred to the aquaria of the experimental sets, they were treated variously and monitored regularly to find any stress in them. The sub-acute treatments were found to be more pungent to the fishes with well-marked tendency of phobia to the medium. The respiratory behaviour sharply varies from those of control ones (Table.1, 2 and 3). The frequency of opercular beats decreases and that of the surface visit (surfacing) increases in all the sets (Fig.1, 2 and 3). The opercular beats in sub acute cases reduced by 50% than the control, while in chronic aquaria the reduction is up to 65%. On the other hand, surfacing increased many fold. Before death the fishes showed lateral movement (plate-I).

Table 1. Respiratory behaviour of the fish *Clarias batrachus*, *Linn*. During different concentrations of sub-acute Lead toxicity for 3 days

Concentrations of Lead Acetate (ppm)	*Opercular beats/min (Mean ± S.D.)*	*Surfacing/min (Mean ± S.D.)*
Control (0)	30.0 ± 6.43	6 .0± 0.1
25	25.5 ± 2.35†	12.5 ±2.27┴
50	22.7 ± 1.52┴	15.0 ± 3.13┴
75	19.9 ± 2.65┴	20.4 ± 2.17†
100	15.3 ± 2.65*	22.3 ±1.16*
125	14.2 ± 1.62*	24.5 ± 1.23*
150	12.2 ±1.10*	24.6 ± 2.31*

*p< 0.001,┴ p< 0.005,†p<0.05

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Table 2. Respiratory behaviour of the fish *Clarias batrachus*, *Linn.*during different concentrations of sub-acute Lead toxicity for 7 days

Concentrations of Lead Acetate (ppm)	*Opercular beats/min (Mean ± S.D.)*	*Surfacing/min (Mean ± S.D.)*
Control (0)	32.0 ± 6.33	6 .0± 0.1
25	27.5 ± 2.25†	13.5 ±2.27┴
50	24.7 ± 1.02*	18.0 ± 3.13┴
75	22.6 ± 1.15┴	20.7 ± 2.17*
100	20.3 ± 2.35*	25.8 ±1.16*
125	18.8 ± 3.72*	27.5 ± 1.23*
150	15.5 ±2.10*	30.6 ± 2.31*

*p< 0.001,┴ p< 0.005,†p<0.05

Table 3. Respiratory behaviour of the fish *Clarias batrachus, Linn.*during 15 ppm chronic Lead toxicity for 105 days

Period of exposure in days	*Opercular beats/min (Mean ± S.D.)*	*Surfacing/min (Mean ± S.D.)*
01C	30.5± 5.54	7.4 ±0.8
E	34.4± 5.14	20.5 ±2.41
15C	31.6 ±4.43	6.2 ±1.26
E	28.2 ±3.54†	8.5 ±0.6†
30C	29.9±2.76	6.7 ±0.7
E	25.5± 1.13†	13.1 ±1.42*
45C	30.4 ±4.56	6.8± 0.2
E	23.2 ±3.31*	15.4± 2.14†
60C	30.7 ±6.43	7.3 ±1.23
E	18.1 ±1.25┴	17.2 ±1.25┴
75C	29.5 ±5.44	6.4 ±1.2
E	16.6 ±1.87*	19.8± 2.35*
90C	30.2 ±3.26	6.4 ±1.1
E	14.2 ±1.12*	21.6 ±3.1*
105C	30.6 ±3.43	6.3 ±1.28
E	10.1 ±1.6*	24.1± 3.54*

* p< 0.001,┴ p< 0.005,†p<0.05, C = Control, E = Experimental

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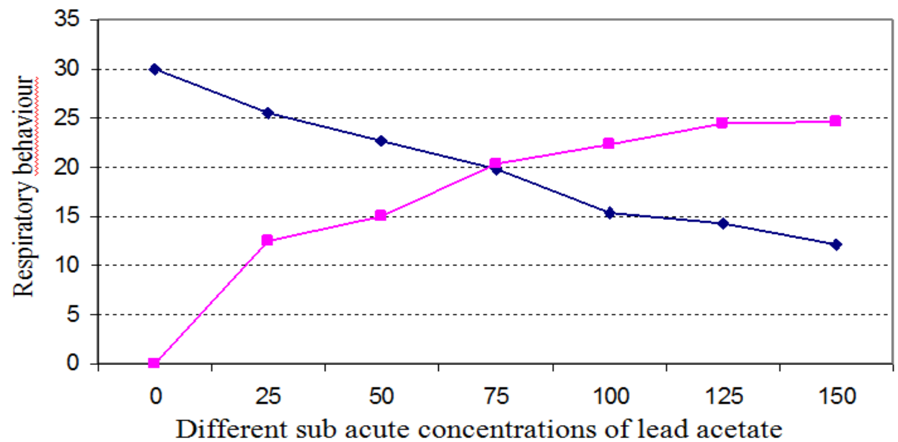

Figure 1. Respiratory behaviour of the fish *Clarias batrachus*, *Linn.* During different concentrations of sub-acute Lead toxicity for 3 days

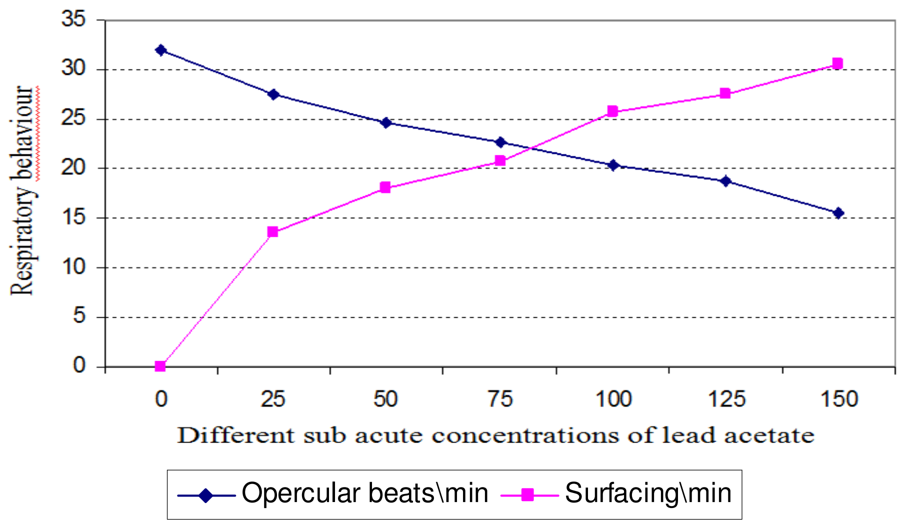

Figure 2. Respiratory behaviour of the fish *Clarias batrachus*, *Linn.*during different concentrations of sub-acute Lead toxicity for 7 days

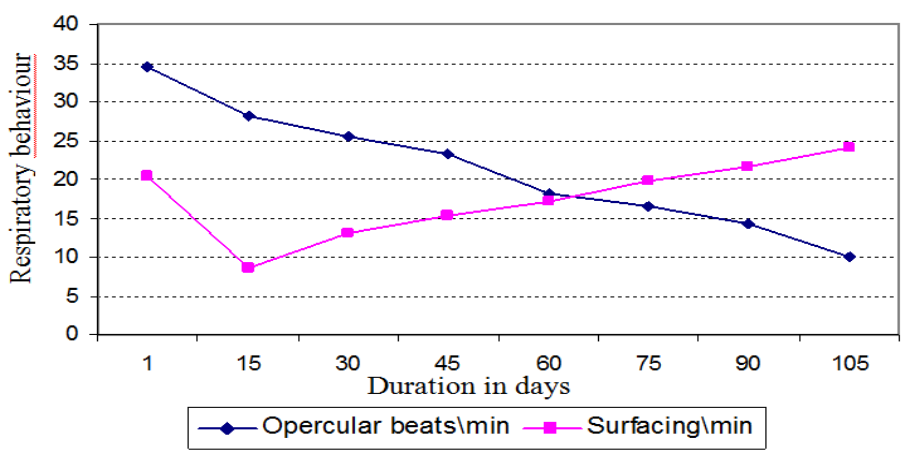

Figure 3. Respiratory behaviour of the fish Clarias batrachus, *Linn.*during 15 ppm chronic Lead toxicity for 105 days

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DISCUSSIONS

A universal reaction to low oxygen is increased ventilation of the gills. In the same way that people start to breathe faster in order to bring more air to their lungs, fishes increase the rate of water flow through their gills. A convenient measure is the rate at which gill covers open and close under different levels of dissolved oxygen. Thus three-spined sticklebacks have been reported to increase their rate of gill cover movements from 95 to 165 per minute after the oxygen content of their water was experimentally cut down to half of its normal level. One could also hypothesize that ram-ventilating sharks (fishes that bring oxygenated water to their gills by swimming with their mouths open) would increase their swimming speed and would open their mouths wider when oxygen is scarce. This is indeed what has been observed. In the lab, researchers normally reduce oxygen levels by blowing bubbles of a neutral gas – usually nitrogen – into the water; nitrogen then becomes much more abundant than oxygen and displaces it as a dissolved gas. One has to be mindful of confounding variables in experiments of this kind: disease, stress and strenuous activity can also make fishes – like people – breathe faster. To eliminate the potential effect of these variables, it is important to work with animals that are consistently healthy, calm and not fidgety. Moreover, fishes are ectotherms (coldblooded), which means that warm temperatures can raise their metabolism and consequently their breathing rate also. Comparisons between oxygen levels must therefore be done at similar water temperatures.

The present study has shown that how *Clarias batrachus* can survive the respiratory stresses which it might encounter its normal environment as earlier studied (Scott & Sloman 2004). Survival may be achieved in several ways which is collectively called as adaptation. The sub acute and chronic treatments have similar effects but in different ways. While the sub acute responses are sudden, those increase with increase with increase in doses, during chronic responses they appear slowly but cumulatively.

Behavioural abnormalities in a subject is dependent on several extrinsic and intrinsic factors. Here the fish shows abnormality mainly in respiratory activities (Table-1 and 2 and Fig. 1-3) and (Plate-I). *Clarias batrachus* regularly raises its snout above the water surface to engulf air for aerial respiration. Forced air exposure prevents normal branchial respiration. Hence to compensate for the O_2 deficiency, gasping of air by the fish increases as soon as they are placed out of water or it is in a toxic medium (Chandra & Banerjee 2003,Gulf state 2008). After encountering an area of low oxygen content, fish often start to swim rapidly and to zig-zag all over the place. This is probably an adaptive response to escape the danger zone. However, if hypoxic conditions persist, most fish will greatly curtail their general activity. The advantage of inactivity in the face of hypoxia is simple: less muscular work means less need for oxygen. Lead-exposed fish were also extremely more active in the aquaria than the control group as previously reported in the fish Gillichthys mirabilis (Somero et.al. 1977). Behavioural toxicology is a tool for hazard assessment of water pollution and the behavioural changes seen are indicative of internal disturbances of the body functions such as inhibition of enzymes, impairment of neural transmission and disturbances in metabolic pathways (Shah and Altindag, 2005). The abnormal behavioural manifestations exhibited by the fishes on exposure to lead are similar to that reported for other toxicants like cadmium and chromium. (Olaifa et al. 2003; Vutukuru, 2005; Tawari – Fufeyin et al. (2008). Like other heavy metals, lead is manifestly accumulative and cannot be excreted. Therefore consumption of fish that have already accumulated quantities of lead could result in bio-magnification further up the food chain, reaching their maximum in predators. In conclusion, it is clear that lead is highly toxic and warrants further scientific work to be focused on developing sustainable and

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environmentally friendly aqua cultural practices. Further, the changes seen in the behavioural parameters could be used as a tool for bio-monitoring.

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REFERENCES

[1] Bellinger, D.C. (2011). The protean toxicities of lead: new chapters in afamiliar story. *International Journal of Environmental Research and Public Health*, *8*, 2593–2628.

[2] Chainy, G.B.N., G. Mishra & P.K. Mohanty.(2008): Biostatistics Theory and Applications. Kalyani Publishers, Ludhiana. P.310

[3] Chandra Sunita, T. K. Banerjee, (2003). Histopathological Analysis of the Respiratory Organs of the Air-breathing Catfish *Clarias batrachus* (*Linn.*) exposed to the Air. Acta Zool. *Taiwanica, 14*(1), 45-64.

[4] Chen, A., Dietrich, K.N., Huo, X. & Ho, S. (2011). Developmental neurotoxicants in e-waste: an emerging health concern. *Environmental Health Perspectives*, *119*, 431–438.

[5] Del Bene Davis, A. (2007). Home environmental health risks. *Online Journal of Issues in Nursing*, *12*(2),

[6] Gulf state marine fisheries (2008). Online reference. Univ. of Southern Mississippi

[7] Hughes, G. M., Perry, S. F.& Brown V. M. (1979). A morphometric study of effects of nickel, chromium and cadmium on secondary lamellae of rainbow trout. *Water Res., 13*, 665-679.

[8] IISER, Indian Institute of Science Education and Research (2012). Course manual for Science Academie's Refresher Course in Experimental Biology.Dec-19-31.

[9] Little, E. E. & Finger, S. E. (1990). Swimming behavior as an indicatorof sublethal toxicity in fish. Environ. Toxicol. *Chem. 9*, 13–19.

[10] Mielke, H.W., Laidlaw, M.A. & Gonzales, C.R. (2011). Estimation of leaded (Pb) gasoline's continuing material and health impacts on 90 US urbanized areas. *Environment International*, *37*, 248– 257.

[11] Olympio, K.P., Gonçalves, C., Günther, W.M. & Bechara, E.J. (2009). Neurotoxicity and aggressiveness triggered by low-level lead in children: a review. *Revista Panamericana de Salud Pública*, *26*(3), 266–275.

[12] Olaifa, F. E. et al., (2003) Toxic stress of lead on *Clarias gariepinus* (African catfish) fingerlings. *African J. of Biomedical Res.* V (6), 101-104.

[13] Rout Prafulla Chandra and Naik BN (2013a). Kinetics of Lead and bio-concentration factor (BCF) in different tissues of *Clarias batrachus* during experimental Plumbism. *IJSRP, 3*(8), 325-340.

ISSN: 2186-8476, ISSN: 2186-8468 Print
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[14] Rout Prafulla Chandra, Naik BN (2013 b).Tracing out Correlation between Blood Lead and Heamatological Parameters in *Clarias batrachus, Linn*. During Experimental Plumbism. *IJCR, 5*(9).

[15] Scott, G. R.& Sloman, K. A. (2004): The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. *ELSVIER Aquatic Toxicology*, 68, 368-392.

[16] Silbergeld, E.K. & Patrick, T.E. (2005). Environmental exposures, toxicologic mechanisms, and adverse pregnancy outcomes. *American Journal of Obstetrics and Gynecology*, *192*(Supplement), S11–S21.

[17] Shah, S.& Altindag, A. (2005) Alterations in the immunological parameters of Tench (Tinca tinca L) after acute and chronic exposure to lethal and sub lethal treatments with mercury, cadmium and lead. *Turk J Vet Anim Sci. 29*, 1163-1168.

[18] Somero, G. N., Yancey, P. H., Chow, T. J. & Snyder, C. B.(1977): Lead effects on tissue and whole organism respiration of the estuarine teleost fish, Gillichthys mirabilis. *Arch Environ Contam Toxicol., 6*(2-3), 349-54.

[19] Tawari – Fufeyin, P. et al.,(2008) Changes in the catfish *Clarius garipinus* exposed to acute cadmium and lead poisoning. *Biosci Res. Commun, 20*(5), 271-276.

[20] Vutukuru, S.S. (2005) Acute effects of hexavalent chromium on survival oxygen consumption, haematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*, *Int. J. Envirion.Res. Public Health, 2*(3), 456-462.

[21] Webber, D. N., Hesselbalch, R., Kane, A. S., Petering,D. H., Petering, L.& Berg, A. (2013). Minows as a classroom model for Human Environmental Health. *American Biology Teacher, 75*(3), 203-209.