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CADMIUM TOXICITY IMPACT ON BIOMODAL RESPIRATION OF AIR BREATHING FISH *CHANNA PUNCTATUS* (BLOCH)

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Abstract

Physiological adjustments associated with the transition from aquatic to aerial respiration in vertebrates have been of interest to physiologists for centuries. The aquatic-to aerial transition has obvious implications for gas transport but also has dramatic effects on the physiology of ion regulation, acid–base balance and nitrogenous waste excretion. The values of oxygen uptake from aerial and aquatic and in both respirations of control and treated fish (*Channa punctatus*) exposed to sublethal concentration of cadmium (29mg/l) for long term periods (7, 15 and 30 days). The percent contribution of two modes of respiration are also different in cadmium treated fish when compared to control fish ,the proportion of oxygen uptake from aquatic and aerial respiration are 44.60, 54.68, 44.53, 54.74 and 44.20, 55.07 in control and 46.15, 52.88, 46.15, 52.74 and 44.59, 55.41 in cadmium treated fishes after 7,15 and 30 days exposure, respectively. The percent contribution of two mode of respiration are also different in cadmium treated fish when compared to control. The rate of biomodal O_2 consumption of sublethal concentration of cadmium treated fishes were found to shows a significant declinethis could be due to the failure of gaseous exchange in gills or depression in gill ventilation,disintegration of respiratory epithelium,mucous secretion over the gill and decreased RBC count as aresult of which absorption of oxygen from the surrounding was adversely affected.The altered functional respiratory activity of the gill was due to the direct contact with the cadmium toxicant medium and also interaction between the metal and gill tissue,which may altered the permeability of the gill membrane.

Key Words: *Channa punctatus,* Cadmium, Aquatic respiration and Aerial respiration.

Introduction

Heavy metals are selectively toxic even at low concentration and affect the survival of fishes by interfering with respiratory metabolism (David and Ray, 1966). The metabolic rate as reflected by respiration is considered to be an important parameter in determining the physical well being. Oxygen availability in aquatic habitats is a major ecological factor influencing the distribution of fish. The fish has limited potentialities to survive out of water. Higher humidity enhances the survival rate and minimizes evaporative water loss. Humidity plays an important role in the survival capacity of fish (Mallikaraj *et al*., 2011). Air breathing fish is commonly believed to have arisen as an adaptation to aquatic hypoxia. The effectiveness of air breathing for tissue oxygen supply depends on the ability to avoid oxygen loss as oxygenated blood from the air breathing organ passes through the gills (Scott *et al*., 2017). Most of the air breathing fishes use a dual system of gas exchange through the gills /skin system in water and accessory organs from air. When the environment is less favourable for aquatic respiration, air breathing organ becomes a more important alternative in the air breathing fishes. The morphometric respiratory potential of gills compared to the stomach in obtaining oxygen for aerobic metabolism is *Pterygoplichthys anisitsi*, a facultative air breathing fish (Andre and Marisa, 2016). The role of the first pair of gill arches in the control of cardiorespiratory responses to normoxia and hypoxia in the air breathing responses were predominantly mediated by receptors in the first pair of gill arches (Belao *et al*., 2015). Biomodel (aquatic and aerial) oxygen consumption of the air breathing fish, *Anabas testudines* exposed to lindane (Bakthavathsalam and Srinivasa Reddy, 1983). Crucian carp (*Carassius carassius*), common carp (*Cyprinus carpio*) goldfish (*Carassius auratus*) and mangrove killifish (*Kryptolebias marmoratus*) exhibit reversible gill remodeling in accordance with changes in O_2 demand or availability, whereas *Arapaima gigas* exhibits a permanent remodeling of lamellar structure in association with a developmental transition from water-to air-breathing (Brauner *et al*., 2004). Physiological adjustments associated with the transition from aquatic to aerial respiration in vertebrates have been of interest to physiologists for centuries, with extant air-breathing. fishes providing a glimpse into how aerial respiration must have arisen (Burggren and Johansen, 1986). Among fishes, air-breathing is thought to have evolved as many as 67times (Graham, 1997), and the degree of aerial dependence among air-breathing fishes varies greatly. Facultative air breathers depend upon air-breathing to augment gill oxygen uptake during periods of hypoxia, which may involve changes as minor as increased vascularization of the buccal cavity so that O_2 can be removed from air held in the mouth. However, at the other extreme are air-breathing fishes that are completely dependent upon aerial respiration and drown without access to the surface, even when water is well oxygenated. Generally in facultative air breathers, as the oxygen tension in the water drops, aerial respiration increases and the metabolic demand for oxygen is satisfied to a greater extent by aerial oxygen uptake (Gee and Braham 1978, Stevens and Holeton 1978, McMahon and Burggren 1987). During air breathing ,blood is oxygenated at the air breathing organ before passing through the gills, therefore the gills could be a site of oxygen loss to the hypoxic environment (Brauner *et al*., 1995). Hence an attempt has been made in the present study, to evaluate the effect of sublethal concentration of cadmium on biomodal respiration of the air breathing fish *Channa punctatus* under different periods of exposure (7,15 and 30 days).

Materials and Methods

Collection and maintenance of the experimental animals

Healthy adult *Channa punctatus* were collected from the ponds in and around Chidambaram. They were acclimatized for a period of 15 days in large plastic tank before they are used for experiments. The fishes of 12 ±2 cm in length and 15±2gm in weight were used for experimental studies. Feeding stopped two days before the commencement of the experiments to reduce the excretory products in the experimental tank (Karthigarani and Navaraj, 2012, Punitha *et al*., 2014).

Biomodal Respiration

Respiratory studies were programmed, using simple respirometer involving the principles of manometric techniques. Aerial and aquatic gas exchange were measured simultaneous, similar to that designed by Reddy and Natarajan (1970).The respiratory chamber had tight fitting two hole Bakelite screw type cork. The lower portion of the respiratory chamber contained 500ml water provided the medium for aquatic respiration and the upper portion of the chamber (350 ml) provided a gas phase for aerial gas exchange. The possibility of oxygen diffusion from air into the water was relatively slight (Hughes and Singh, 1970). Also a polythene sheet cut exactly to the size of circumference of the bottle was used to minimize the diffusion, and the fish could easily lift out of this sheet to break air. In the air space enclosed an injection bottle containing filter paper soaked in 20 percent KOH served as CO_2 absorbent. Two glass tubes (5 mm bore) passed through the holes of the cork. One tube was externally connected to the closed arm of the manometer, by means of a tight short rubber tubing and the inner end of that tube was kept above the water column. The second tube which touches the bottom of the respiratory chamber served both for letting in water and for collecting water samples. The connection were made air tight by applying a thick smear of plaste cone together with pinch corks. Care was taken to see that sufficient volume of water and air medium were provided for the respiration of the fish .Pressure changes in the respiratory chamber due to extraneous factors were corrected by a thermobarometer (TB).

Both aquatic and aerial gas exchanges were measured simultaneously for 30 minutes. Oxygen content in water before and after the experiment was measured by estimating dissolved oxygen content using Winkler's method (Welsh and Smith, 1960). Oxygen consumed from the gas phase was calculated using the method described in the manometric techniques by Umbreit *et al*., 1959. Values of oxygen consumption were expressed in ml/g/hr. Measurements were taken after the fish had adjusted to the respiratory chamber for 2 to 3 hours and the duration of the experiment never exceeded half an hour. The respiratory chamber was thermostated by immersion in the temperature controlled water bath.

Statistical Analysis

The observed results were analyzed for statistical significance of differences using one way analysis of variance (ANOVA), according to the methods of Sokal and Rohlf (1973).

Result

The values of oxygen uptake from aerial, aquatic and in both respirations of control and treated fish, *Channa punctatus* with sublethal concentration of cadmium (29mg/l) for long term periods(7,15 and 30 days) and their percentage change over control are given in table-1. In control, when *C. punctatus* is allowed to respire simultaneously both from toxic free water and air, the total oxygen uptake are 1.39 ± 0.028, 1.37 ± 0.024 and 1.38 ± 0.032 ml/g/hr during the periods of 7, 15 and 30 days, respectively. Out of which 0.76 ± 0.015, 0.75 ± 0.016 and 0.76 ± 0.018 ml/g/hr are obtained from aerial phase and 0.62 ± 0.013, 0.61 ± 0.009 and 0.61 ± 0.014 ml/g/hr from aquatic phase over the periods of 7, 15 and 30 days, respectively.

In the fish exposed to Cd the oxygen uptake from aerial phase is decreased to a maximum of 0.41 ± 0.008 ml/g/hr, showing a decline of 46.05 per cent after post exposure period of 30 days and in the same experiment the oxygen uptake during early exposure periods of 7 and 15 days are also parallely decreased to 0.55 ± 0.014 and 0.48 ± 0.012 ml/g/hr, exhibiting a reduction of 27.63 per cent and 36.00 per cent, respectively. Also from the aquatic phase the oxygen uptake is declined significantly to 22.59%, 31.15% and 45.90%, respectively at 7, 15 and 30 days of exposure. Similarly the total oxygen uptake by fish also is decreased to 25.184, 33.586 and 46.386 during the period of 7, 15 and 30 days, respectively. The percent contribution of two modes of respiration are also different in Cd treated fish when compared to control fish. The aquatic phase shows the maximum rate when compared to aerial phase at all periods of exposure as compared to that control the proportion of oxygen uptake from aquatic and aerial respiration are 44.60, 54.68. 44.53, 54.74 and 44.20, 55.07 in control and 46.15, 52.88, 46.15, 52.74 and 44.59, 55.41 in Cd treated fishes after 7, 15 and 30 days exposure, respectively. (Table.2).

Table 1 : Bimodel O_2 uptake of *C. punctatus* exposed to sublethal concentration (29 mg/l) of Cd after different exposure period.

Mode of respiration	**Duration of treatment (days)**			**F - value**	**C.D of treatment group**
	7	**15**	**30**		
Aquatic					
Control	0.62 ± 0.0128	0.61 ± 0.00914	0.61 ± 0.0413		0.0027
Treated	0.48 ± 0.0168	0.42 ± 0.0162	0.33 ± 0.0165	1863.00**	0.0033
% change over control	-22.59	-31.15	-45.90		0.0047
Aerial					
Control	0.76 ± 0.0152	0.75 ± 0.0157	0.76 ± 0.0177		0.0029
Treated	0.55 ± 0.0140	0.48 ± 0.0123	0.41 ± 0.00846	1284.34**	0.0036
% change over control	-27.63	-36.0	-46.05		0.0051
Total					
Control	1.39 ± 0.0280	1.37 ± 0.0248	1.38 ± 0.0321		0.0028
Treated	1.04± 0.0307	0.91 ± 0.0285	0.74 ± 0.0250	6429.73**	0.0034
% change over control	-25.18	-33.58	-46.38		0.0048

Values expressed in ml of oxygen consumption / g /hr
Date represent mean of six individual ± S.D.
C.D. Critical difference indicates
** Significant at 0.01 level of F test.

Table 2 : Percent contribution of each mode respiration to total respiration in the fish *C. punctatus*

Animal group	**Mode of respiration**	**Exposure period (days)**		
		7	**15**	**30**
Control	Aquatic	44.60	44.53	44.20
	Aerial	54.68	54.74	55.07
Treated (exposed at 29mg/l of Cd)	Aquatic	46.15	46.15	44.59
	Aerial	52.88	52.74	55.41

Discussion

An examination of the respiratory rate as an index of metabolic activity of animal. A bimodal pattern of respiration using gills and air breathing organs is normally employed by air breathing fish. The relative importance of the two modes of respiration differs in obligate and facultative air breathers and it is reflected structurally. In *A. testudineus,* the gill shows a lesser functional efficiency with reduced surface area, while the air- breathing organ have a vascularized large surface area for efficient gas exchange from air (Hughes *et al.,* 1973).

In the present study, when control *C. punctatus* is maintained under toxicant free water, the average total oxygen utilization is found to be 1.38 ml/g/hr out of which 0.61 ml/g/hr for aquatic respiration and 0.76 ml/g/hr for aerial respiration over 30 days of exposure. This result indicate that the fish *C. punctatus* consume more oxygen from air (55.72%) than from water (44.28% of its total volume of oxygen during dual respiration. In some previous observation the obtained oxygen from air by obligate air breathers is 78% in *Electrophorus electricus* (Farber and Rahn, 1970): 89% in *Symbranchus marmoratus* (Johansen, 1976) and 80% in *A. testudineus* (Reddy and Natarajan, 1970). Also in the present study, *C. punctatus* consumes more amount of oxygen (56%) from air which is falling in the same line with the above observations. The above result indicates that the gill with lesser effectiveness in oxygen exchange from water which may be due to greater thickness of gill epithelium thereby increases the diffusion distance between blood and water. Generally, the obligate air breathers succumb more air from atmosphere to total submersion under water even when oxygen rich (Johnson, 1968).

The toxic substances can affect fish productivity and survival value through their effect on fish respiration. The data (Table -1) in the present study reveals the effect of Cd on the consumption of oxygen from aerial and aquatic respiration of *C. punctatus* under control and sublethal concentration of Cd. The result in oxygen consumption for *C. punctatus* indicates that the Cd as decreases the Oxygen consumption in both aquatic and aerial mode of respiration as the fish was subjected to the sublehal concentration of Cd toxicity for long term periods (7, 15 and 30 days). The inhibition of oxygen consumption from water through gills were increased with increasing period of exposure. The drop in oxygen consumption by gill can result in oxygen deflect and also losses its effective mechanism due to 'Histoxi anoxia' in which gill tissue not only suffer from diffusion of oxygen but also losses its effective mechanism for removing Co_2 from blood. Such condition has been frequently encountered in fishes exposed to heavy metals (Sharma *et al.,* 1985) believes that the entry of toxicant is mainly to the gill which affect the performance of the function of respiration.

Further, the inhibition of oxygen consumption through gill tissue may be due to the 'coagulation film anoxia' (Koundinya and Ramamurthi, 1978) in which mucous is last from the gills as a result of which absorption of oxygen from the surrounding is adversely affected. This could also be true in the present study, where the greater secretion of mucous on the entire body of fish under Cd stress. Srivastava (1982) observed cumulative poisoning by heavy metals like Cu, Cd and Hg, even at low concentrations for a prolonged period possibly by acting through coagulation of mucous on gill, caused damage to respiratory surface resulting hypoxia.

In the present study, the reduced oxygen uptake by *C. punctatus* could also be suggested as sequel to gill damage under Cd stress which coincide with the result of gill respiratory epithelium, damage when exposed to Cd. The route of O_2 uptake by fish from water is through gill to the blood, passing through the epithelium of the secondary lamellae the basement membrane and the pillar cells (Hughes and Shelton, 1962). The reduction of oxygen uptake throughout the exposure periods get positive correlation with damage of tissue. A number reports are available on the histopathological changes produced in gill and other tissue of fishes exposed to metals, which may result in the alteration of oxygen consumption rate (Palanichamy and Baskaran, 1995; Pandey *et al.,* 1997 and Karuppamy, 2000b). Mallaredy (1988) observed decreased rate of oxygen consumption and opercular movements in *C. Carpio* exposed to malathion on 15th day. He suggested that the reason for decreased oxygen consumption was due to alteration in the gill architecture, gill damage and decreased haemoglobin content. Injury to the red blood corpuscles and alteration in haemoglobin – oxygen binding capacity have also been suggested as reason for drop in oxygen uptake in fish exposed to industrial effluents (Murthy *et al.,* 1986). Haniffa and Porchelvi (1985) they have pointed outs that the dissolved solid inorganic salts present in the distillary effluent, might have interfered in the respiration of *S. mossambicus* by gill mucous asphyxiation and inhibition of enzyme system at mitochondrial level and resulted in decrease of oxygen consumption.

The result of reduced level of oxygen uptake from surfacing air by the test fish due to the fishes were mostly remained suspended in lower layer of water and its surfacing frequency also parallely with negative correlation to the exposure periods. Such decrease of aerial respiration may be due to the loss of muscular coordination treated with neurotoxic nature of cadmium. Srivastava (1982) pointed out that the damage in the nervous system Causing lack of muscular coordination in fish due the heavy metal Cd. Similar reduction in aerial respiration has been reported in *Mystus vittatus* to thiodan (Gopalakrishna Reddy and Gomathy, 1997). Also an increase in aerial with decrease in aquatic respirarion of fish at the initial period (24 hrs) of exposure under toxicants have been reported in *Ryoplaxgangetia* to Cu, Zn and Hg (Savant and Amte, 1992), *A. testudineus* to monocrotophos (Santhakumar *et al.*, 2000) and *C. punctatus* to phenyl mercuric acetate (Karuppasamy, 2000b).

Water breathing is energetically more expensive than breathing air. Under severe hypoxic conditions, O_2 uptake by the stomach is more efficient than by the gills, although the stomach has a much lower diffusing capacity. Thus, *P. anisitsi* uses gills under normoxic conditions but the stomach may also support aerobic metabolism depending on environmental conditions (Andre and Marisa, 2016). *Anguilla* leaves the water and emerges on to land for long periods it faces a number of problems which ultimately can be solved only be return to the water. These problems are O_2 uptake and CO_2 loss, ammonia loss and desiccation. Oxygen availability in aquatic habitats is a major ecological factor influencing the distribution of fish. The fish has limited potentialities to survive out of water. Higher humidity enhances the survival rate and minimizes evaporative water loss. Humidity plays an important role in the survival capacity of fish. At 35 % - 40% relative humidity an *A. bengalensis* survive at 3h-5h. (Mallikaraj *et al.*, 2011). *Arapaima gigas* undergoes a transition from water- to air-breathing during development, resulting instriking changes in gill morphology. In small fish (10·g), the gills are qualitatively similar in appearance to another closely related water-breathing fish (*Osteoglossum bicirrhosum*) (Brauner *et al*., 2004). Air breathing frequency in *H. littorale* increased significantly from 2 to 28 breaths/h as the oxygen tension in the water decreased from 137 to 105 mmHg. The metabolic rate of fish deprived access to air decreased significantly at a PO_2 of 56mm Hg relative to control values and reached a minimum of 8mg $kg^{-1}h^{-1}$ at30 mm Hg. his metabolic rate was maintained for several hours and no mortalities were observed. Thus, air breathing frequency was influenced by the oxygen level in the water, but *H. Littorale* was capable of a large reduction in during hypoxia when denied access to air (Brauner et al.,1995).

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