

Impact of Environmental Acidic pH on Oxygen Consumption in an Air Breathing Murrel Fish, *Channa gachua*

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Abstract

Fish are amongst the most diverse groups dwelling in fresh waters of the world. Among them, several groups developed air breathing mechanisms to avoid low oxygen constraints, which are common in fresh waters and more than 1200 species described so far. Such diversity is reflected also in feeding habits, fish size, swimming performance and breathing patterns, among others. The obligatory air breathing habit in the family channidae is an adaptation to low oxygen environments. Fish of this family are capable of using their stomach as a gas exchanger. Water breathing fish, which are highly active are not supposed to be hypoxia tolerant fishes but are often found in hypoxic environments. The present study was aimed to investigate the total oxygen consumption of fresh water fish *Channa gachua* during exposure and acclimation to sub lethal acidic pH (5.0) medium. The oxygen consumption of fish significantly depleted in all the days of exposure to sub lethal acidic pH 5.0 starting from first day to fourteen days of exposure. The rate of total oxygen consumption depletion was higher on first day. From the second day onwards a gradual decreasing trend was observed in the depletion up to 14th day of exposure. The unit of oxygen consumption of fish depends on exposure to sub lethal pH medium exhibited significant depletion when compared to control. The rate of depletion was maximum in the initial days of exposure and reduced gradually in twelfth and fourteenth day of exposure. But at day 14th no significant decrease was observed in both the parameters of the experimentation. This observation indicates that the fish was capable of regulating their metabolic modulations and physiological functions through acclimation process to be the possible reason for the successful survival of fish in acidifying water. The reduced oxygen consumption of fish on exposure to sub lethal acidic medium confirms the prevalence of hypoxic condition in the surrounding medium.

Keywords: Acidic pH, oxygen consumption, *Channa gachua*.

Introduction

Most of the water bodies are polluted by heavy load of industrial effluents, sewage and chemicals of industries in metropolitan cities. Water is essential for all living organisms to perform all life functions but if aquatic organisms are exposed to toxic water contents then their physiology and biochemical processes are affected badly. All these chemicals interact in various ways with cells and they can be detected by chemical analysis only. Water quality i.e. hardness, oxygen, temperature, pH etc. has a marked influence on the expression of toxicity as reported by Qaisur and Shamim (2014). The bimodal breathing system may be defined as the one in which an organ or organs of the fish at a given stage of life history utilizes both water and air in its gas exchange mechanisms. The same environmental pressure that caused the development of different air breathing mechanisms is considered to induce long and short term adaptations in fishes (Qaisur and Sadhu, 2012). These adaptations are the result of adjustments in biochemical, physiological and molecular characteristics and enable the fish of the river to survive episodes of hypoxia. Environmental stress is of prime importance during the course of exposure of an animal to different acidic media. The recommended pH range for aquaculture is 6.5 to 9.0. The fish and other vertebrates have an average

blood pH of 7.4 and fish blood comes into close contact with water (1 or 2 cell separation) as it passes through the blood vessels of the gills and skin. A desirable range for pond water pH would be close to that of fish blood (i.e. 7.0 to 8.0). Fish may become stressed and die if the pH drops below 5 (e.g. acidic run off) or rises above 10 (e.g. low alkalinity combined with intense photosynthesis by dense algal blooms phytoplankton or filamentous algae (Boyd, 1979). The acidic medium is known to negatively influence to the most organisms and therefore low pH of aquatic medium is an important aspect of environmental pollution. The oxygen consumption in fish has been considered as an index for denoting the intensity of metabolism as metabolic activity of an organism is increased by its oxygen utilization (Bijay *et al.*, 2003). Respiration is an essential physiological activity of all the living organisms by which they obtain energy for carrying out all metabolic activities of the body (Krishna, 1981). The rate of oxygen consumption is the basic physiological parameter serves as an indicator of physiological stress (Bhaskar and Govindappa, 1985) and changes in the oxygen consumption could be used to evaluate any change that would occur in the metabolism due to alterations in the environment. Such stresses like acidic medium have been studied by Sreenivasa *et al.*, (2008); pesticides by Roberts *et al.* (1987); heavy metals by Venkata *et al.* (2007); pulp mill effluents by Reeta *et al.* (2011); hydro carbons by Percy (1977) and natural stresses like temperature and salinity by Bashamohideen (1984). Thus variations in respiratory activity have been used as sensitive indicators of stress in fish exposed to pollutants (Bashamohideen, 1984 and Qaisur, 2011). The above studies unequivocally suggest that the sensitive indicator of stress like oxygen consumption in fishes exposed to pollutants is considerably increased and decreased but studies involving the oxygen consumption of the whole animal with reference to sub-lethal effect of acidic media is very few in fishes which are of great commercial and economical value. Hence, an attempt is made in the present investigation to study the influence of acidic media on certain aspects of oxygen consumption in an air breathing fish *Channa gachua* following exposure to the sub lethal concentration of acidic medium at different exposure periods.

Materials and Methods

Live specimens of *Channa gachua* were procured from local fish dealers at Hazaribag (Latitude 25° 59'N and Longitude 85° 22'E) and maintained in large glass aquaria size (90x60x60cm) with continuous flow of water. The specimens were fed on chopped goat liver daily during a minimum acclimation period of 15 days in the laboratory. Routine oxygen consumption from air and still water was measured in a closed glass respirometer containing 3 litres of water (initial O₂ content = 6.5 mg O₂/ litre and pH = 7.2) and 0.51 mL of air (**Figure 1**). The fish had free access to air through a small semicircular hole (10 cm diameter) in a disc float. Carbosorb (B.D.H) or KOH in a petridish placed on the float to absorbed CO₂. Thus the fish could exchange gases with water by way of its gills as well as with the air using the supra branchial chamber. The air phase of respirometer was connected to a differential manometer. Movement of the manometer fluid follow uptake of oxygen when the CO₂ is absorbed by Carbosorb (KOH). The fish were acclimated to the respirometers for at least 12 hours before the readings were taken. The concentration of dissolved oxygen in the water was estimated by Winkler's volumetric method Welch (1948). The oxygen uptake through gills was calculated from the difference between the oxygen levels of the ambient water in the respirometer before and after the experiment and the reading of volume of water in the respirometer. The oxygen uptake from air was measured and calculated from the reading of volume change in the manometer and by the use of the combined gas law equations and vapour pressure (Qaisur and Sadhu, 2012). Mean values of CO₂ of a series of observations, on each fish at standard temperature pressure dry and standard errors were calculated. The experiments were conducted at 29.0 ± 1.5°C. The pH of the ambient water was measured by an electronic pH meter systronics.

The respiratory chambers were thermostated by immersion in a temperature controlled water bath. However, sexually mature *Channa gachua* of almost same weight group 70-90 grams were used. The experimental individuals were divided into different groups on the basis of body weight and sex. The fish was kept in large aquaria with continuously flowing dechlorinated water to acclimatize them to laboratory conditions of 12 hours per day for fifteen days before they were used for experimentation. This fish is known for its adaptability to laboratory conditions and suitability to acidic media (Bhaskar and Govindappa, 1985). Hence, this fish was selected as the experimental model for the present investigation. As the smaller fish are considered to be more sensitive to toxic pollutants rather than larger one (Anderson and Weber, 1975) small sized fish weighing around $200 \pm 50\text{g}$ were selected for the present investigation. After 24 h of the stipulated period, the fish were immediately used for the estimation of oxygen consumption and unit oxygen consumption in different aquatic pH media. The acidic media with different pH ranging between 3.0 and 7.0 with variability of 0.5 were prepared by adding 1 N hydrochloric acid to water. 1 N solution of hydrochloric acid was prepared separately with distilled water (pH= 7.0) soft water chlorides ($110 \text{ mg/L} \pm 10$) carbonates ($16\text{mg/L} \pm 2$) bicarbonates ($400 \text{ mg/L} \pm 50$) and hardness (35mg/L as $\text{CaCO}_3 \pm 4$). The 1 N HCL was added for the purpose of obtaining different acidic media mentioned above and their pH was verified with pH meter Elico India. As the chloride ions are least toxic, hydrochloric acid was used widely for the preparation of acid media Jones (1968).

The water media having different pH range of 3.0 to 7.0 with variability of ± 0.5 pH were taken. For each pH medium in the above range 10 separate tanks were maintained with fish each tank containing 10 individuals. The fishes were fed daily at 8 a. m with commercial fish feeds and the medium in the tanks was changed at 9 a.m. The pH of the media was maintained constantly by employing special dropping system during experimentation. The number of fish died in each of the above media was recorded at regular intervals of time up to 96 h. The percentage of mortality was calculated on the basis of individuals that died at each time interval. The media where the mortality of the fish was observed were taken as lethal media and the others where there was no mortality were taken as sub lethal media. The oxygen consumption was determined during the temporal sequence of exposure starting from the first day till the maintenance of constant oxygen consumption by the fish on the 10th day to 14th day respectively. The sub lethal acidic media (pH= 5.0) was employed as an ambient medium for acclimatization of the fish. The fish were taken into the respiratory chamber containing the same pH medium at 2.00 p.m. on alternate days and the oxygen consumption was determined within a period of 1hour. After this the fish were transferred into their respective tanks. The oxygen consumption was determined during the temporal sequence of exposure starting from the first day till the maintenance of constant oxygen consumption by the fish on the 10th day to 14th day. Similarly control fish in the neutral pH= 7.0 were maintained and their oxygen consumption was also studied individually over the temporal sequence of time as done in the case of fish maintained under sub lethal acidic media. The oxygen consumption of fish was determined by the static method. The unit oxygen consumption was calculated by dividing the oxygen consumption by weight of the fish and represent as $\text{mg O}_2/\text{kg/h}$. All the values of-t- below 5 percent levels are considered as not significant and the value above 5 percent level is considered as significant.

Results and Discussion

The pH of water plays an important role in regulation of growth and development of aquatic animals. The present study was aimed to find out the relationship between environmental acidification on the growth and development of *Channa gachua*. The measurement of oxygen consumption becomes a marker to understand the overall changes that takes place in the whole body on exposure to any stress condition. Several investigators (Sailaja *et al.*, 2009; Chavan

et al., 2011; Sathick *et al.*, 2011; Sindhe *et al.*, 2011) reported that the level of whole oxygen consumption was an index for metabolic modulations that take place in the body on exposure to acidic stress.

The data presented in **Tables 1 & 2**, show changes in the total oxygen consumption and unit oxygen consumption of the fresh water fish during exposure to sub lethal acidic (pH=5.0) media. The total oxygen consumption was significantly decreased on first day of exposure to sub lethal acidic (pH=5.0) medium and exhibited a non-significant change over control on 14th day of exposure. This observation indicates that the fish was capable of regulating their metabolic modulations and physiological functions through acclimation process to compensate the acidic stress imposed by the external media, which might be the possible reason for the successful survival of fish in acidified water. Decrease in total oxygen consumption might be due to hypoxic condition prevailed in external media. Another reason might be due to reduced gaseous exchange through gill surface because of excessive formation of mucous Suchismita and Gupta (2012). The other alternative could be the reduced oxygen carrying capacity of the blood due to altered blood pH on exposure to acidic media. Several investigators also reported earlier in supporting to the present study that decreased ability of fish to extract oxygen from low pH waters Packer and Dunson (1972) and Sailaja *et al.*, (2009). Investigator like Qaisur and Sadhu (2012) in *Channa gachua* experimentally shown that one of the causes of thermal death is the osmoregulatory failure at temperature extremes. In a changed environmental condition organism tries to adapt and adjust to sustain its life is called acclimatization. It must try to prevent or minimise changes in its body temperature is called thermoregulation. Changes in the environment and its composition compel the fish to alter its body physiology and biochemistry in order to cope up with this novel threat.

Reduced oxygen consumption of fish *Channa gachua* on exposure to sub lethal acidic media confirms that prevalence of hypoxic condition in the surrounding media. Since the unit oxygen consumption also exhibited similar depletion trend it suggests the possibility of existence of hypoxic condition. The unit oxygen consumption of fish also exhibited non-significant change on 14th day of exposure. This observation supports and forms a basic evidence for the perfect type of acclimation by adults. Since the total oxygen consumption in animals forms a marker for the general metabolism the stabilization of total oxygen consumption on 14th days exposed fish reflects the maintenance of normal oxygen consumption on par with the control fish. Hence, two weeks period of exposure to fish to acidic media made successful in survival through acclimation process, failure of such a regulatory mechanism, might be responsible for the mortality of fish in lethal pH media. In view of acclimation of fish to acidic media provided better metabolic adjustments at whole animal level reveal the better survival value. Thus, the changes in oxygen consumption could reflect the effect of acidic media on the aquatic fauna especially in fishes. Information on temperature tolerance and resistance is of much value in judging the eco-physiological characteristics of fishes. Qaisur and Shamim (2017) reported that in *Channa gachua* the behavioural characteristics are obviously sensitive indicators of toxicant effect. It is necessary however, to select behavioural indices of monitoring that relate to the organisms behaviour in the field in order to derive a more accurate assessment of the hazards that a contaminant may pose in natural system, should be considered for species forming social organizational. If social interactions are not considered, only a certain portion of a population may be protected, and the toxicity of contaminant may be under estimated and also primarily influence modulations in the level of oxygen consumption. This also provides a platform to establish tolerable limits and safe levels of toxic agents for the biota of aquatic environment and to save the residue imbalance in aquatic bio-ecological cycles which help in involving bio detector monitoring.

Table 1: Changes of oxygen consumption during different days of exposures in *Channa gachua*.

S.No.	Time in Days	Control (pH < 7.0)	Sub-lethal (pH < 5.0)	Significant value P<0.001
1	1	0.2780±0.0510	0.1675±0.0104	-39.74
2	2	0.2730±0.0160	0.1528±0.0115	-44.02
3	4	0.2784±0.0144	0.1722±0.0092	-38.14
4	6	0.2752±0.0181	0.1912±0.0128	-30.52
5	8	0.2719±0.0161	0.2081±0.0156	-23.46
6	10	0.2792±0.0169	0.2145±0.0144	-23.17
7	12	0.2821±0.0150	0.2391±0.0154	-15.24 N. S*
8	14	0.2791±0.0122	0.2391±0.0154	-44.02 N.S*

Mean ± SD, + and - indicates percentage increase and decrease p<0.001 and 'NS' indicates non-significant.

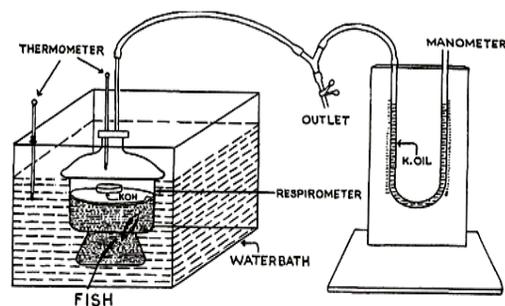


Figure 1: Experimental set up for the measurements of dual mode of oxygen uptake in *Channa gachua*.

Table 2: Changes of oxygen consumption during different day of exposure in *Channa gachua*.

S.No.	Time in Days	Control (pH < 7.0)	Sub-lethal (pH < 5.0)	Significant value P<0.001
1	1	3.7252±0.0221	2.8420±0.1310	-23.70
2	2	3.8224±0.0425	2.6051±0.1202	-31.84
3	4	3.7235±0.0421	2.9752±0.1081	-20.09
4	6	3.7210±0.0521	3.0244±0.0951	-18.72
5	8	3.8314±0.0512	3.1652±0.0951	-17.38
6	10	3.7935±0.0131	3.2826±0.0710	-13.46
7	12	3.7624±0.0831	3.3789±0.1710	-10.19
8	14	3.7416±0.0626	3.5914±0.1430	-4.01 N.S*

Mean ± SD, + and - indicates percentage increase and decrease, p<0.001 and 'NS' indicates non-significant.

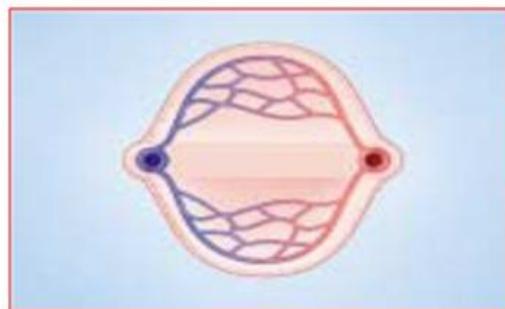


Figure 1: Gills of *Channa gachua* showing large respiratory surface area and counter current exchange.

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