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Influence of ambient oxygen on respiratory and substrate level metabolism in freshwater fish, *Cirrhinus mrigala*

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Abstract
In the present study experiments were conducted to find out oxygen consumption, ammonia excretion and ammonia quotient (AQ) in a freshwater fish, *Cirrhinus mrigala* acclimated sand tested at 28 ± 1 ºC, subjected to hypoxic condition. The rate of oxygen consumption in *Cirrhinus mrigala* a normoxia and hypoxia at normoxia was 145.2 mg/kg/hr, which decreased with the duration of experiment and decrease in ambient oxygen consumption from 5.2 to 3.6 mg/l was found to be higher than that of under hypoxic condition. The normoxic rate of ammonia excretion was found to be 26.19 mg/kg/hr and the hypoxic rate of ammonia excretion was observed range of 4.82 to 5.3 mg/kg/hr. Lactic acid in muscle, gill and liver tissues were found to be 122.61, 23.20 and 26.81 m. mole/g respectively, whereas the hypoxic lactic acid content in muscle, gill and liver tissues were found to be 226.72, 30.94 and 50.90 m.mole/g respectively.

Keywords: Dissolved oxygen, *Cirrhinus mrigala*, Ammonia quotient (AQ), Metabolism, glycogen

Introduction
Aquaculture is being looked upon to serve the needs of under nourished people in developing countries. It is estimated that about 10 million tonnes of fish is required annually to meet the present day demand of fish protein for 1000 million people of India against the current annual production of only 3.0 million tonnes comprising about 1.8 million tonnes of marine fish and 1.2 million tonnes of freshwater fish. Oxygen is one of the most important components of surface water for maintenance and growth of aquatic organisms, which utilize oxygen for aerobic respiration. Metabolism and growth of fishes are dependent on the availability of ambient oxygen (Daudaraft and Shumway, 1970; Davis, 1975; Kutty, 1981) [8, 10]. The demand of oxygen is often reported as a cause for mass mortality in both wild and farm reared fishes (Moore, 1942). For studying energy metabolism, measurement of oxygen consumption method is well recognized, most satisfactory and the easiest. Oxygen consumption and ventilation in freshwater major carp was studied by Roy and Munshi (1988) [16] and they reported that the rate of oxygen update in *Cirrhinus mrigala* declined with the increase in oxygen concentration but decreased with decrease in oxygen concentration. The Nitrogen excretion as ammonia N was used to obtain an estimate of the amount of protein utilized (Kutty, 1972; Kutty and Mohamed, 1975) [12, 13] whereas the total N excretion was considered for computing protein degradation in fish (Sukumaran, 1976). Kutty (1971) [11] observed that the oxygen consumption did not change markedly with reduction in ambient oxygen in *Mugil cephalus.* whereas temperature salinity, dissolved oxygen, dissolved carbon-di-oxide, pH, light, pressure, water currents among the factors the most important one was activity, which accompanied with other factors, like temperature caused about a twenty fold change in metabolism of fish (Brett, 1964; Groves, 1979). Some metabolic adaptation of 5 species of south Indian *Marine teleosts namely Caronix carangen*, *Chorinemus lysan*, *Chanos chanos*, *Synagris furosus* and *Gerrel lecidus* were studied by Ameer Hamsa and Kutty (1972) [12]. Oxygen consumption in freshwater catfish, *Mystus gulio* was studied by Natarajan (1980) [14]. *Mystus gulio* consumed 120.12.ml/kg/hr of oxygen in water without assess to air and 70.38ml/kg/ hr of oxygen in water with assess to air. Under completely aerial condition, fish consumed 26-30 ml/kg/hr of oxygen from air and when the fish was kept in water with access to air it consumed

22.41ml/kg/hr of oxygen. Peer Mohamed and Kutty (1980) studied the influence of ambient oxygen on random activity in four freshwater teleosts, *Rhinomugil corsula, Carassias auratus., Tilapia mosammbica and Puntius sarana* at various ambient oxygen concentration below air saturation of two temperatures 30 ºC and 35 ºC. Padmavathy and Sukumaran (1991) [15] studied the influence of ambient oxygen on the metabolism of *O. mossambicus* showed the increase in blood glucose level, with the decrease of glycogen in muscle and liver during the Ist hour of experiment. During recovery period, the level of glycogen increased in all tissues, the increase being marked in muscle and liver, indicating the regaining of glycogen. Brobery and Ristoffersson (1983) [6] studied the oxygen consumption and lactate accumulation in the intra ovarian embryo and young of viviparous fish *Zoarces viviparous* in relation to decreasing water oxygen concentration. Blood lactate concentration in fish increased up to 2-4 hrs after exercise and restored to normal level with in 12-24 hrs was investigated by Wood and Perry (1985) [19]. The present study involves simultaneous measurements of metabolic rate in *C. mrigala* under hypoxia (below 50% air saturation) and subsequent recovery condition. Besides, the changes in oxygen consumption and Ammonia excretion, glycogen concentration and lactate production in muscle and liver and blood glucose levels under normoxia, hypoxia and recovery period were studied.

Materials and Methods

The experimental fish, ***Cirrhinus mrigala*** was collected from the state fisheries hatchery at Kadana. They were acclimatized to the laboratory condition by stocking them in large tanks of 500 L capacity at 28 $\pm$ 1^{0}C with continuous aeration. The fish were fed daily with a formulated diet.

Taxonomic position:	***Cirrhinus mrigala***
Grade	- Pisces
Class	- Osteichthyes
Subclass	- Actinopterygii
Division	- Neopterygii
Sub Division	- Teleostein
Order	- cypriniformes
Family	- cyprinidae
Sub family	- Cyprininae
Tribe	- Labeonini
Sub tribe	- Labeones
Genus	- *Cirrhinus*
Species	- *mrigala* (Hamilton-Buchanan)

Experimental design

The experiment was carried out in 2 liter round bottom flask covered at the top using cork with inlet and outlet. The experimental fish weighing about 28.6 $\pm$ 0.85g was introduced into the flask. To prevent the diffusion of atmospheric oxygen into the water, liquid paraffin was evenly spread on the water surface.

The experiments were done into two sets. First set to study the respiratory metabolism of the *Cirrhinus mrigala* exposed to hypoxic condition and the other to study the substrate metabolism of the fish, *Cirrhinus mrigala* exposed to hypoxic condition.

Respiratory metabolism

The rate of oxygen consumption and ammonia excretion were estimated in the water at 0 hrs and for every one hour interval until the fish begins to lose its equilibrium. The water with drawn for analysis was compensated with fresh water.

Substrate metabolism

In order to study the extent of involvement of substrate in metabolism under hypoxic condition, the blood samples were collected from caudal vein puncture for the analysis of glucose and then the fish were dissected and the muscle, liver and gill tissues were taken out for the analysis of lactic acid and glycogen.

Analytical methods

1. Dissolved Oxygen

The Winkler;s method was followed for the estimation of dissolved oxygen (APHA, 1955) [1].

2. Ammonia

Ammonia nitrogen in water samples was estimated by phenol hypochlorite method (Solozzano, 1969). Ammonia free distilled water was used for the preparation of chemicals for estimating ammonia. The ammonia quotient (The relation between the volume of ammonia excreted and the volume of oxygen consumed) was also calculated.

Biochemical methods

1. Estimation of Blood Sugar

The blood glucose content was estimated by the modified method of Carrol *et al*. (1965) [7]. One ml of blood was taken in a centrifuge tube and 3.5ml distilled water, 0.2ml of 8% zinc sulphate, and 0.2ml barium hydroxide reagent were added and centrifuged at 3000 rpm. Then 100µl filterate taken in a test tube and marked as a test sample and distilled water (used as blank 0.1 to 0.9 ml of glucose solution) was taken in the test tubes as standard. The volume of all the test tubes were made up to 1ml with distilled water. 4ml of anthrone reagent was added to all the test tubes and the tubes were placed in a boiling water bath for 10 minutes and then cooled to room temperature. The colour was read at 620nm in UV spectrophotometer against the blank.

2. Estimation of Muscle, Liver and Gill Glycogen

The glycogen in the muscle, gill and liver was analyzed by slightly modified method of Corrol *et al*. (1956). About 1.5g of muscle or liver or gill tissue was accurately weighed. The tissues were placed in centrifuge tube containing 2ml KOH and heated in a boiling water bath for 20 minutes with occasional shaking. The tubes were cooled in ice and 0.2ml of ethanol was added and centrifuged at 1000rpm. The supernatant was discarded and the precipitated glycogen was dissolved in 5ml of water on gentle warming. Then the content was diluted to 10ml with distilled water.

One ml of sample glycogen solution was taken in the test tube and 1ml of HCl was added to it. A marble was placed on the top of the test tube and heated in boiling water bath for 2 hrs. Then one drop of phenol red indicator was added and neutralized carefully with NaOH until the indicator changed from pink through orange to yellow colours. The content of tubes was diluted to 5ml with distilled water and glucose was determined by Anthrone method.

3. Estimation of Muscle, Liver and Gill Lactic Acid

Lactic acid content was estimated by modified method of Barker and Summerson (1941) [2]. After isolation of tissues 10% homogenates of muscle or liver or gill tissue was taken

and 5 ml of the homogenates was centrifuged at 1000 rpm for 15 minutes. To 1.0ml of supernatant 1.0 ml of 20% copper sulphate solution was added. The contents were mixed and the volume was made up to 10.0 ml with distilled water. To it 1g of powdered calcium hydroxide was added. The tubes were kept aside for 1 hour at room temperature giving intermittent shaking and later centrifuged. To 1.0ml of sapernatant, 0.05ml of 4% copper sulphate solution and 6.0 ml concentric sulphuric acid were added. The contents were mixed well by lateral shaking and then boiled in a water bath for 6 minutes. After cooling 0.1ml of p-hydroxy diphenyil solution was added to the solution and kept at room temperature for 30 minutes. After cooling, the colour was read against a reagent blank at 560nm. 0.2 to 1.0/mg ml of lithium lactate was taken as standard. The blank and the standard received the same treatment as that of samples.

Results

In the present study experiments were conducted to find out oxygen consumption, ammonia excretion and ammonia quotient (AQ) in a freshwater fish, *Cirrhinus mrigala* acclimated sand tested at 28 $\pm$ 1 ºC, subjected to hypoxic condition. In addition the levels of glycogen and lactate in muscle, liver, gill tissues of the fish exposed to hypoxic condition and subsequent recovery in well aerated water, besides the blood glucose level under above condition were analysed.

Respiratory Metabolism

The data on the rate of oxygen consumption in mrigal a normoxia and hypoxia are presented in table 1 and graphically shown in fig. 1 and 2. It is clear from the table 1 and fig. 1 that above the oxygen consumption in *Cirrhinus mrigala* at normoxia was 145.2 mg/kg/hr, which decreased with the duration of experiment and decrease in ambient oxygen. It is evident from figure 2 that the decrease in oxygen consumption from ambient oxygen 5.2 to 3.6 mg/l was found to be higher than that of under hypoxic condition.

The values of ammonia excretion of freshwater fish, *Cirrhinus mrigala* acclimated and tested at 28 $\pm$ 1ºC are given in table 2 and fig. 3 & 4. It is clear from the table that ammonia excretion decrease with increase in the duration of experiment as has been seen in the case of oxygen consumption. The normoxic rate of ammonia excretion was found to be 26.19 mg/kg/hr and the hypoxic rate of ammonia excretion was observed to be 4.82 to 5.3 mg/kg/hr. It is evident from the fig. 4 that the rate of ammonia excretion increased with increase in ambient oxygen. The increase in the rate of ammonia excretion from ambient oxygen 2.2 to 5.2 was found to be steeper than that of in lower ambient oxygen which clearly indicate the increase of protein utilization in from exposed to ambient oxygen above 50% air saturation. The rate of ammonia excretion steeply increased with increase in ambient oxygen level.

The values of ammonia quotient of freshwater fish, *Cirrhinus mrigala* acclimated at 28 + 1ºC was calculated from the values of oxygen consumption and ammonia excretion and are presented in table 3 and fig. 5. It is clear from table 3 that ammonia quotient showed the same trend of as that of the rate of ammonia excretion.

The normoxic ammonia quotient (AQ) was found to be 0.194, where the hypoxic ammonia quotient was found to be 0.1. In fig. 6 the AQ values are plotted against ambient oxygen. It is clear from the graph that the AQ values increased with increase in ambient oxygen with a slight decrease at the ambient oxygen level of 2.2 mg/l.

Influence of ambient oxygen on substrate level of in tissues

The data on glycogen content in muscle, gill and liver and blood glucose of *Cirrhinus mrigala* subjected to hypoxia and subsequent recovery are given table 4 and graphically shown in fig. 7. It is evident from table that there was a decrease of glycogen content in all tissues studied in mrigal from normoxia to hypoxia but in the case of blood glucose there was a steep increase from normoxia to hypoxia. During the recovery period there was an increase in the glycogen content of muscle, gill and liver tissues but there was reduction in blood glucose recovery period fig. 7.

The data on lactic acid content in muscle, gill and liver tissues of freshwater fish, *Cirrhinus mrigala* subjected to hypoxia and subsequent recovery are given in table 5 and fig. 8. The table 5 and fig. 8 showed that the normoxic lactic acid in muscle, gill and liver tissues were found to be 122.61, 23.20 and 26.81 m. mole/g respectively, whereas the hypoxic lactic acid content in muscle, gill and liver tissues were found to be 226.72, 30.94 and 50.90 m.mole/g respectively. The increase in lactic acid during hypoxia in muscle tissue was greater than that of in gill and liver tissues fig. 8. During recovery period the decrease of muscle tissue was higher than that in gill and liver tissues.

Discussion

The metabolic rates such as oxygen consumption, ammonia excretion and ammonia quotient (A.Q.) and the substrate level changes in muscle, liver and gill tissues of a freshwater fish, *Cirrhinus mrigala* subjected to hypoxic condition were studied and discussed here under.

The rate of oxygen consumption as a measure of total metabolism decreased with the decrease in ambient oxygen, when the experimental fish was exposed slowly to lower ambient oxygen concentrations (Fig. 1 & 2), indicating that the total metabolism of the fish was found to be reduced due to lack of oxygen in ambient water. This type of reduction in total metabolism during hypoxic condition was also observed by Kutty and Peer Mohamed (1975) [13] in freshwater mullet; Kutty (1972) [12] in tilapia; Sukumaran and Kutty (1977) [18] in catfish. They observed that the rate of oxygen consumption in fishes reduced under hypoxic condition, because the fishes are not able to derive sufficient oxygen through respiration due to lack of ambient oxygen in water. The same trend was observed in *mrigal* in the present study.

The rate of ammonia excretion and ammonia quotient (A.Q), a measure of protein degradation, showed the same trend as that of rate of oxygen consumption (Fig 3& 5), suggesting that the relative protein utilization was also decreased with decrease in ambient oxygen. In previous studies of Kutty (1972) [12], Kutty and Peer Mohamed (1975) [13] and Sukumaran and Kutty (1977) [18], the rate of ammonia excretion increased with the decrease of ambient oxygen, whereas in the present study reverse trend of ammonia excretion was noticed, probably due to the lesser utilization of protein for energy production under hypoxic condition. The fish might have reduced its activity, when it was exposed to adverse condition such as low ambient oxygen and thereby conserve energy; especially energy from protein source which is most costly substrate as far as fish is concerned.

The reduction of glycogen content in tissues viz. muscle, liver and gill during hypoxic condition in mrigal is probably due to the utilization of glycogen for energy through anaerobic

pathway. This is also evident from the accumulation of lactic acid in muscle tissue of mrigal under hypoxic condition (Table5). Among the tissues, the muscle glycogen showed a drastic reduction from normaxic to hypoxic condition and the lactic acid level was also higher in muscle than in other tissues. This clearly indicates that the anaerobic glycolysis takes place more in muscle tissue than in other tissues as observed in other fishes (Sukumaran, 1977; Padmavathy and Sukumaran 1996; Black *et al*., 1957) [18, 3].

The reduction of glycogen in liver tissue of mrigal under hypoxic condition (fig.7) may be due to the utilization liver glycogen to muscle through blood for compensating the glycogen loss in the muscle. This is also supported by the fact that the blood glucose level showed an increase under hypoxic condition (fig 7). These observations in mrigal very well coincides with the observations in other freshwater fishes such as freshwater mullet, (Sukumaran, 1976); rohu (Padmavathy and Sukumaran, 1991) [15] and gold fish (Walker and Sehansem, 1977) [18]. The lactic acid production in the muscle tissue of mrigal subjected to hypoxia was much higher than in other tissues (table 5), suggesting that the fish derives more energy through anaerobic metabolism, when the total aerobic metabolism got reduced due to lack of sufficient oxygen in ambient water during hypoxic condition. This is also supported by the fact that the rate of oxygen consumption was found to be much reduced under hypoxic condition (Fig. 1 & 2), indicating less energy derivation through aerobic metabolism.

Table 1: Influence of ambient oxygen on oxygen consumption (mg/kg/hr) of freshwater fish, *Cirrhinus mrigala* acclimated and tested at 28±1ºC. Each value is an average of four individual observations with a standard deviation.

Hours of experiment	O_2 consumption mg / kg / hr
1	145.20 ± 25.21
2	76.51 ± 3.48
3	54.34 ± 5.65
4	32.16 ± 7.82

Ambient oxygen mg / l	O_2 consumption mg / kg / hr
1.2	32.16 ± 7.82
2.2	54.34 ± 5.65
3.6	76.51 ± 3.48
5.2	145.20 ± 25.21

Table 2: Influence of ambient oxygen on ammonia excretion (mg/kg/hr) of freshwater fish, ***Cirrhinus mrigala*** acclimated and tested at 28±1ºC. Each value is an average of four individual observations with a standard deviation.

Hours of experiment	Ammonia excretion mg / kg / hr
1	26.19 ± 6.85
2	14.92 ± 11.50
3	4.80 ± 3.09
4	5.29 ± 2.72

Ambient oxygen mg / l	Ammonia excretion mg / kg / hr
1.2	5.29 ± 2.72
2.2	4.80 ± 3.09
3.6	14.92 ± 11.50
5.2	26.19 ± 6.85

Table 3: Influence of ambient oxygen on (AQ) ammonia quotient of freshwater fish, ***Cirrhinus mrigala*** acclimated and tested at 28±1ºC. Each value is an average of four individual observations with a standard deviation.

Hours of experiment	AQ
1	0.194 ± 0.08
2	0.188 ± 0.14
3	0.083 ± 0.04
4	0.152 ± 0.04

Ambient oxygen mg / l	AQ
1.2	0.152 ± 0.04
2.2	0.083 ± 0.04
3.6	0.188 ± 0.14
5.2	0.194 ± 0.08

Table 4: Influence of ambient oxygen on glycogen content of muscle, gill and liver tissue and blood glucose of freshwater fish, ***Cirrhinus mrigala*** subjected to hypoxia and subsequent recovery. Each value is an average of four individual observations with a standard deviation

	Glycogen m. mol/g			
Tissue	**Muscle**	**Gill**	**Liver**	**Blood (glucose)**
Normoxia	8.77 ± 0.57	5.96 ± 0.59	103.12 ± 7.67	4.22 ± 0.72
Hypoxia	4.54 ± 0.45	3.11 ± 0.90	21.32 ± 4.40	13.85 ± 1.72
Recovery	5.37 ± 0.50	4.25 ± 0.35	44.73 ± 2.93	5.23 ± 0.64

Table 5: Influence of ambient oxygen on lactic acid content of muscle, gill and liver tissue of freshwater fish, *Cirrhinus mrigala*, subjected to hypoxia and subsequent recovery. Each value is an average of four individual observations.

	Lactic acid m, mol/g		
Tissue	**Muscle**	**Gill**	**Liver**
Normoxia	122.61 ± 36.75	23.20 ± 2.59	26.81 ± 2.13
Hypoxia	226.76 ± 51.86	30.94 ± 4.13	50.90 ± 4.29
Recovery	83.56 ± 4.32	28.70 ± 5.44	21.72 ± 3.07

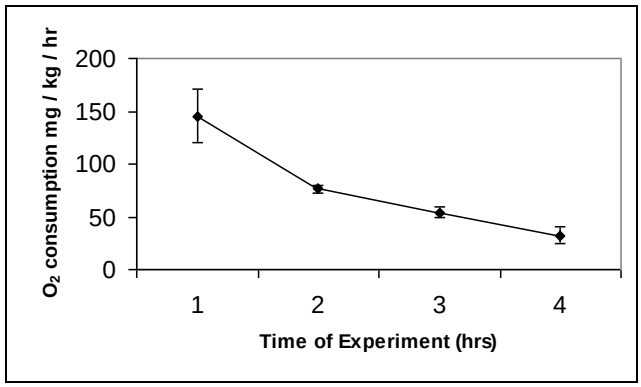

Fig 1: Influence of ambient oxygen on oxygen consumption (mg/kg/hr) of freshwater fish, ***Cirrhinus mrigala*** acclimated and tested at 28±1ºC. Each value is an average of four individual observations with a standard deviation.

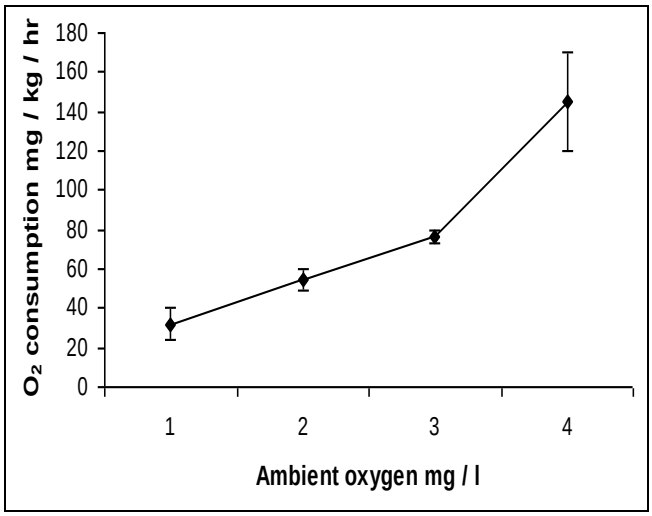

Fig 2: Rate of oxygen consumption (mg/kg/hr) is plotted against ambient oxygen (mg/l). Each value is an average of four individual observations with a standard deviation.

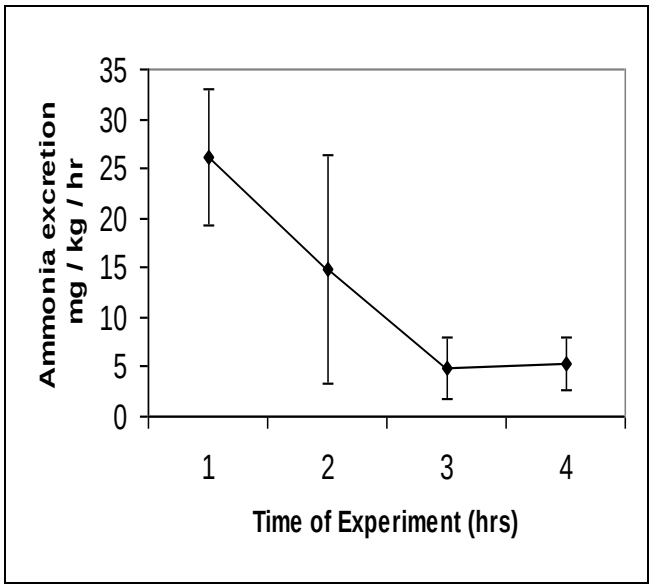

Fig 3: Influence of ambient oxygen on ammonia excretion (mg/kg/hr) of freshwater fish, *Cirrhinus mrigala,* acclimated and tested at 28±1ºC. Each value is an average of four individual observations with a standard deviation.

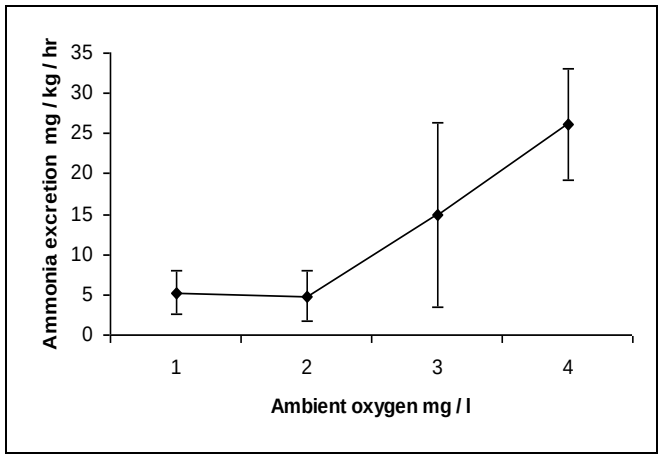

Fig 4: The rate of ammonia excretion (mg/kg/hr) plotted against ambient oxygen (mg/l). Each value is an average of four individual observations with a standard deviation.

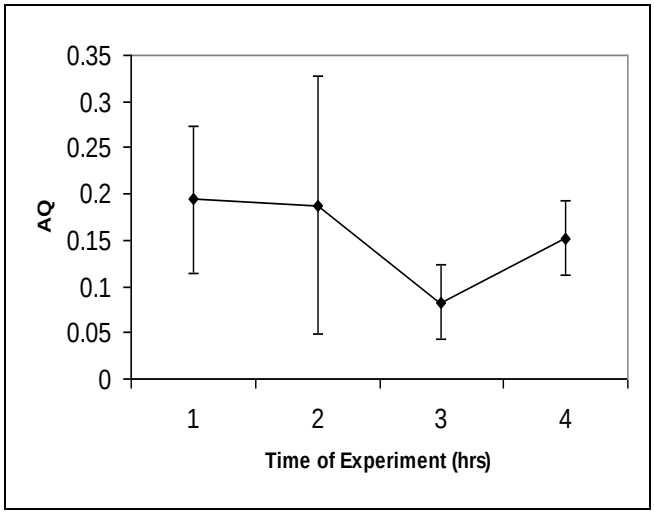

Fig 5: Influence of ambient oxygen on ammonia quotient (AQ) of freshwater fish, *Cirrhinus mrigala* acclimated and tested at 28±1ºC. Each value is an average of four individual observations with a standard deviation.

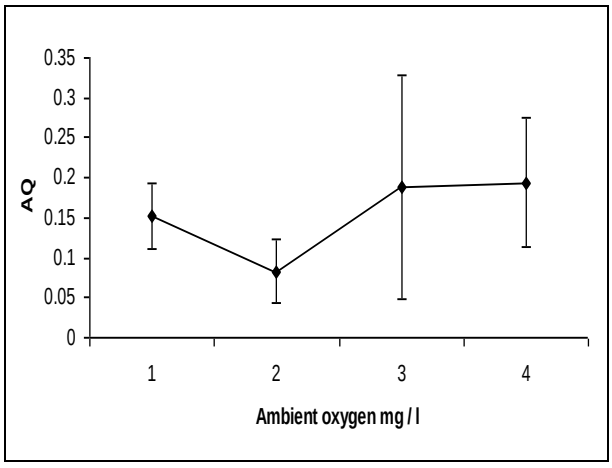

Fig 6: Effect of ambient oxygen on (AQ) of freshwater fish. Each value is an average of four individual observations with a standard deviation.

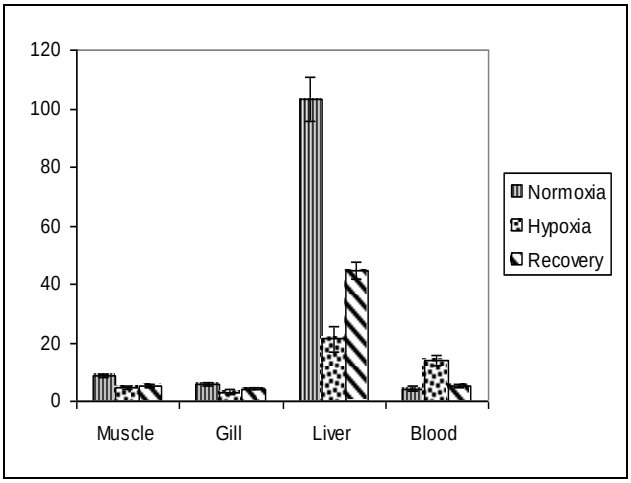

Fig 7: Influence of ambient oxygen on glycogen content of muscle, Gill, liver tissue and blood glucose of freshwater fish ***cirrhinus mrigala*** subjected to hypoxia and subsequent recovery. Each values average of four individual observation.

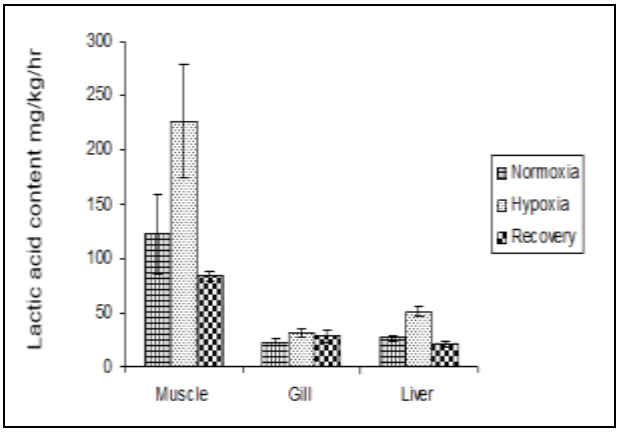

Fig 8: Influence of ambient oxygen on lactic acid content of muscle, gill and liver tissue of freshwater fish, *Cirrhinus mrigala* subjected to hypoxia and subsequent recovery. Each value is an average of four individual observations.

References

1. APHA (American Public Health Association) Standard Methods for examination of Water, Sewage and industrial wastes. Am.Publ. Health Org. New York, 10th Edn 1955, 522.
2. Barker SB, Summerson WH. The colorimeter determination of lactic acid in Biological material: J.Biol; chem 1941;138:535-605.
3. Black EE. Alteration in the blood level of lactic acid in certain salmonid fishes following muscular activity in Kamloops trout, *Salmo gairderi* J. Fish Res. Bd. Can., 1957;14:117-134.
4. Black EC, Robertson AC, Parker RR. Some aspects of carbohydrate metabolism in fish. In comparative physiology of carbohydrate metabolism in Heterothermic animals (A.W. Martin ed.) 1961, 89-122. Seattle: University of Washington Press.
5. Breet JB. Sutherland. Respiratory metabolism of Pumpkin seed (*Leopmis gibbosus*) in relation to swimming speed. J.Fish Res. Bd Can. 1965;22:405-409.
6. Broberg A, Ristofferesson R. Oxygen consumption and lactate accumulation in the intra ovarian embryos and young of viviparous fish, *Zoascer viviparous* (L) in relation to decreasing water oxygen concentration Ann. Zool. FENN 1983;20(4):301-308.
7. Carrol NR, Longly W, Rao JH. The determination of glycogen in liver and muscle by on of anthrone reagent J.Bio-chem 1965;220:583-593.
8. Davis JC. Minimal dissolved oxygen requirement of aquatic life with emphasis on Canadian species: A review. J. Fish. Res. Bd. Can 1975;32:2295-2332.
9. Karuppannan NV. Studies on locomotory metabolism of *Tilapia mossambica* (Peters) Ph.D., thesis, Madurai University, Madurai, India 1972.
10. Kutty MN. Energy metabolism in mullet in Aquaculture of grey mullets. -ed. O.H. Oren (London: Cambridge University Press) 1981, 219
11. Kutty MN, Karuppan NV, Narayanan M, Mohamed MP. Maros-Schulek technique for measurement of carbon-di-oxide production in fish and respiratory quatient in *Tiplapia Mossambica* I. Fish Res. Bd. Can. 1971;28:1342-1344.
12. Kutty MN. Respiratory quatient and ammonia excretion *Tilapia mossambica*. Marf. Biol 1972;16:126-133.
13. Kutty MN, Per Mohamed. Metabolic adaptation of mullet, *Rhinomugil Corsula* (Hamilton) with special reference to energy utilization. Aquaculture 1975;15:252-270.
14. Natarajan GM. Observation on the oxygen consumption in Indian air- breathing fishes. IV.Oxygen consumtion in the black snake head, *Ophiocephatus stratus* (bloch). Comphysical, Ecol 1980;5:19-21.
15. Padmavathy T, Sukumar N. Influence of amobient oxygen on the metabolism of Tilapia *Orechromis Mossambicus* M.Sc Thesis, Tamilnadu vetenary and Animal sciences University, Tuticorin 1991.
16. Roy PK, Munish JSD. Oxygen consumption and ventilation rate of freshwater major carp, *Cirrhinus Mrigala*. J. Envt. Biol 1988;9:5-13.
17. Sukumaran N, Kutty MN. Quantitative studies on muscle and liver glycogen utilization during sustained swimming in freshwater mullet *Rhinomugil Corsula* (Hamilton) Pro.Indian Natn Sci. Acad 1986;52:471-475.
18. Sukumaran N, Kutty MN. Oxygen consumption and ammonia excretion of a cat fish, *Mystus armatus*. Proc. Indian Acad. sci 1977;86:195- 206.
19. Wood CM, Perry SF. Respiratory, circulatory and metabolic adjustment to exercise in fish. In circulation, Respiration and metabolism ed. R.Gilles. Barlin: Springer-verlang 1985, 1-22.