## Impact of Thickness of Diffusion Barrier on the Efficiency of Respiratory Organs in Relation to Body Weight in Freshwater Featherback, *Notopterus Chitala(Ham.)*

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#### ABSTRACT

The present investigation is carried out to throw light on the oxygen uptake efficiency of water breathing organ, gills and air breathing organ, swim bladder which depends on the surface area and the thickness of the diffusion barrier of the respiratory membranes.

The water-blood diffusion barrier in the secondary lamellae composed of an outer layer of epithelium, a thin basement membrane and the innermost layer of flanges of pillar cell. The air-blood diffusion barrier is composed of a single layer of epithelial cells and an underlying layer of blood capillaries. The water-blood and air-blood diffusion barrier were calculated to be 1.179  $\mu$ m and 1.439  $\mu$ m respectively in Notopterus chitala.

In Notopterus chitala, the diffusing capacity of gills increased from 0.00094 and 0.07208 ml02 min-1 mmHg-1 and of swim bladder from 0.00036 to 0.02446 with gradual increase in body weight from 1.2 to 1435.0 g The slope value (b) were found to be 0.62113 and 0.64957 respectively for waterbreathing and air-breathing organs.

The weight specific diffusing capacity decreased from 0.78379 to 0.05023 and 0.30056 and 0.01705 mlO2 min-1 mmHg-1kg-1respectively for gills and swim bladder of

Notopterus chitala for the same body weight range. The slope value (b) were calculated to be -0.37887 and -0.35043 respectively for water-breathing and air breathing organ both.

The estimated value for 1.0 g fish i.e, intercept (a) for respiratory organ were computed to be 1.02236 and 0.29452 respectively.

KEYWORDS: Diffusing capacity, Body weight, Notopterus chitala

#### INTRODUCTION

Diffusing capacity is a physiological parameter defined as the quantity of oxygen that passes across a membrane system in unit time for given partial pressure difference. This parameter depends upon two important variables- (i) the water-blood or the air-blood diffusion distance and (ii) the dimensions of the respiratory surface (Hughes, 1970) reported that the amount of oxygen or carbon dioxide diffusing across the respiratory surface in unit time is directly proportional to the respiratory area and inversely proportional to the diffusion barrier. A great deal of work has been done on the diffusion capacity of the biomodal gas exchange machinery of Anabas testudineus(Hughes et al., 1973), Heteropneustesfossilis (Hughes et al., 1974), Anabas testudineus (Dube and Munshi, 1974), Macrognathusaculeatum(Ojha and Munshi, 1976) etc. Biswas et al., 1981 determined the diffusing capacity of the gills of an eustarine goby, Boleophthalmusboddaerti. The gill diffusing capacity of a freshwater major carp in relation to body weight was also studied by Roy and Munshi (1987). The contribution of the following workers deserve special mention for determining the diffusing capacity of hill stream fishes- Botialohchata (Sharma et al., 1982), Botiadario (Roy and Munshi, 1988), Glyptothoraxtelchitta(Subba, 1999) etc.

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Hughes et al., (1992) and Roy and Munshi (1992, 1996) used the harmonic mean of water-blood barrier and stereological method in association with electron microscopy for the measurement of diffusing capacity of the respiratory organs of certain air-breathing fishes of India. So far, no attempts has been made to study in detail the possible functional relationship between the diffusing capacity of bimodal gas exchange machinery and the body weight in freshwater featherback*Notopterus chitala.* The present investigation is an attempt to determine the possible functional relationship between the diffusing capacity of the bimodal gas exchange machinery i.e, gills and swim bladder and body weight in freshwater featherback. *Notopterus chitala.* 

#### **MATERIALS AND METHODS**

Live specimens of *Notopterus chitala* of different body weight were brought by the fishermen from river Ganga and from local ponds. They were maintained in the laboratory conditions for about two weeks with aeration facility on. The gills and swim bladder were excised and fixed in Bouin's fixative, decalcified in 5% HNO3, processed as usual to cut 5-6  $\mu$ m thick paraffin sections. Sections were processed, stained with haemotoxylin and eosin and oil immersion

photomicrographs were taken from various level. The maximum and minimum diffusion distances were measured directly from the photomicrographs and the actual values of the diffusion distances were obtained by dividing the measured thickness by magnification. The arithmetic and harmonic means of diffusion distances were calculated. Modified Fick's equation (Hugher, 1972; Weibel, 1972) used to calculate the diffusing capacity of the respiratory organs. The modified Fick's equation is as follows-

 $VO_2 = K.A. \Delta PO_2$  (i) or,  $VO_2/\Delta PO_2 = K.A./t$  (ii) or, Dt = K.A./t (iii)  $TO_2 = VO2/\Delta PO_2$  (iv)

#### Where,

 $VO_2 = Oxygen uptake (mlO_2.min^{-1})$ 

- $\label{eq:Komplexity} \begin{array}{ll} \mbox{K} & = \mbox{Krogh's permeation coefficient tissve at } 20^0\mbox{C i.e.,} \\ & 0.00015\ mlO_2.\mbox{cm}^{-2}.\mbox{\mu}\mbox{m}^{-1}.\mbox{mmHg}^{-1} \mbox{)} \end{array}$
- A = Respiratory (Gill/ swimbladder) surface Area (cm<sup>2</sup>) taken from previous chapter of thesis.
- $\Delta PO_2$  = Difference of oxygen tension between water/air and blood (mmHg).
- t = thickness of water /air-blood pathway (µm)

The respiratory surface area, together with diffusion distance and the value for permeation coefficient were applied to equation (iii) to calculate the diffusing capacity (Dt).Regression analysis using logarithmic transformation was made to establish the relationship between the diffusing capacity and body weight. The relationship was expressed by the following allometric equation-

Dt = aW<sup>b</sup>

Where,

- Dt = Diffusing capacity
- W = Body weight (g) of fish
- a = Intercept (value for 1 g fish)
- b = slope value

#### RESULTS

#### Water - blood diffusion barrier

The water – blood diffusion barrier in the secondary lamellae consists of an outermost- single layer of epithelium, middle–the basement membrane and innermost layer of flanges of pillar cells. The harmonic mean ( $\bar{x}h$ ) of the thickness of water – blood diffusion barrier of different region of secondary lamellae was calculated to be 1.179 µm, while arithmetic mean of the data obtained for the thickness was found to be 1.461 in *Notopterus chitala*.

## Relationship between Body weight and Gill diffusion capacity (Dt) (mlO<sub>2</sub>.min<sup>-1</sup>.mm Hg<sup>-1</sup>)

The diffusing capacity of the gills of *Notopterus chitala* increased from 0.00094 to 0.07208 with increase in body weight from 1.2 to 1435.0 g (Tab-1). Log-log plots of the body weight and the diffusing capacity for 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and total gills arches gave straight lines with slopes of 0.61547, 0.61885, 0.63590, 0.63720 and 0.62113 respectively. (Tab-2 Fig-1). There was significant and positive correlation between the two variables. The relationship may be expressed as follow-

 $Dt = 0.00102.W^{0.62113}$ 

Or log Dt = log 0.00102 + 0.62113 .log W

## Relationship between Body weight and Weight specific diffusion capacity (Dt<sub>1</sub>) (mlO<sub>2</sub>.min<sup>-1</sup>.mm Hg<sup>-1</sup>.Kg<sup>-1</sup>)

The relationship between the body weight and the weight specific diffusion capacity of gills showed a highly significant but negative correlation between them. The log-log plots of the two variables gave a straight line with the slopes - 0.38453, -0.38115, -0.36410, -0.36280 and -0.37887 for the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and total gill arches respectively (Tab 2 Fig 2). The intercept 'a' values for all the four gill arches separately and also when taken together were found to be 0.28317, 0.29013, 0.21813, 0.15367 and 1.02236 respectively. The weight specific diffusing capacity for 1.0, 10.0, 100.0, 1000.0 and 2000.0 g were estimated to be 1.02236, 0.42730, 0.17860, 0.07465 and 0.05741 (mlO<sub>2</sub>.min<sup>-1</sup>.mmHg<sup>-1</sup>.Kg<sup>-1</sup>) respectively (Tab 3). The relationship between total diffusing capacity (Dt<sub>1</sub>) and body weight can be expressed by following equation:

Dt<sub>1</sub> = 1.02236.W<sup>-0.37887</sup>

#### Air - blood diffusion barrier

The air- blood diffusion barrier of *Notopterus chitala* composed of a single layer of epithelial cells and an underlying layer of blood capillaries. The harmonic mean  $(\bar{x}h)$  of the thickness of air – blood diffusion barrier from different regions of the swimbladderwas calculated to be 1.439 µm while arithmetic mean was found to be 1.851 µm

#### Relationship between Body weight and swimbladder Diffusing capacity (Dt) (mlO<sub>2</sub>.min<sup>-1</sup>.mm Hg<sup>-1</sup>)

The swimbladder diffusing capacity (Dt) of *Notopterus chitala* increased from 0.00036 to 0.02446 with increase in body weight from 1.2 to 1435.0 g (Tab 1). The value for 1.0, 10.0, 100.0, 1000.0 and 2000.0 g fishes were found to be 0.00029, 0.00131, 0.00586, 0.02617 and 0.04105 (mlO<sub>2</sub>.min<sup>-1</sup>.mmHg<sup>-1</sup>) respectively. The allometric equation for the two variables could be expressed as

Dt = 0.00029.W<sup>0.64957</sup>

The log-log plot between the two variables gave a straight line with a slope of 0.64957 and the intercept 0.00029.

# Relationship between Body weight and the weight specific swimbladder Diffusion capacity (Dt<sub>1</sub>) (mlO<sub>2</sub>.min<sup>-1</sup>.mmHg<sup>-1</sup>.Kg<sup>-1</sup>)

The relationship between the two variables indicated a highly significant but a negative correlation (r = 0.94453; p<0.001). The log - log graph when plotted gave a straight line with a slope of -0.35043 and the intercept obtained was 0.29452(Tab 2 Fig 3). The relationship between the two variables could be expressed as –

#### $Dt_1 = 0.29452.W^{-0.35043}$

The weight specific diffusing capacity of swimbladder for 1.0, 10.0, 100.0, 1000.0 and 2000.0 g fishes were found to be 0.29452, 0.13142, 0.05865, 0.026617 and 0.02053 mlO<sub>2</sub>.min<sup>-1</sup>.mm Hg<sup>-1</sup>.Kg<sup>-1</sup> respectively (Tab 3).

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Body weight (g)		Diffusio	n Capacity	Swim	Diffusion Capacity		
	Gill area (cm²)	(Dt) (mlO <sub>2</sub> /min /mmHg)	(Dt <sub>1</sub> ) (mlO <sub>2</sub> /min/ mmHg/kg)	Bladder Area (cm²)	(Dt) (mlO <sub>2</sub> /min /mmHg)	(Dt <sub>1</sub> ) (mlO <sub>2</sub> /min/ mmHg/kg)	
1.2	7.39270	0.00094	0.78379	3.46000	0.00036	0.30056	
5.2	19.67084	.00250	0.48128	4.84000	0.00050	0.09702	
9.8	28.09494	0.00357	0.36474	10.24000	0.00107	0.10892	
17.9	48.83931	0.00621	0.34713	18.33000	0.00191	0.10674	
32.0	77.02790	0.00980	0.30625	29.04000	0.00303	0.09460	
50.0	108.95028	0.01386	0.27723	49.05000	0.00511	0.10226	
82.5	156.90882	0.01996	0.24198	62.22000	0.00649	0.07862	
100.0	175.72696	0.02236	0.22357	74.52000	0.00777	0.07768	
136.0	230.86005	0.02937	0.21597	88.16000	0.00919	0.06757	
190.0	231.77661	0.02949	0.15520	98.25000	0.01024	0.05390	
251.0	240.57714	0.03061	0.12194	100.28000	0.01045	0.04165	
525.0	332.47184	0.04230	0.08057	146.04000	0.01522	0.02900	
1000.0	475.23577	0.06046	0.06046	206.38000	0.02151	0.02151	
1435.0	566.52528	0.07208	0.05023	234.66000	0.02446	0.01705	

Table 1: Gill and swim bladder diffusing capacity for different weight groups of Notopterus chitala

 Table 2: Intercept(a), slope(b) along with their standard error (S.E.) and correlation coefficient(r), of the relationship of body weight and diffusing capacity Notopterus chitala.

Body weight Vs	Intercept(a)		Slop	e(b)	Correlation			
diffusing capacity	Value S.E.		Value	S.E.	Coeffi	Coefficient(r)		
H	ð .•	A .Gil	RD .	N S				
Dt.(mlO <sub>2</sub> /min/mmHg)	- Into	rnationa	Lournal	S S				
1st Gill Arch 🧧 🗧	0.00028	0.06681	0.61547	0.03289	0.98329	(p<0.001)		
2 <sup>nd</sup> Gill Arch 🎽 🧧	0.00029	0.06888	0.61885	0.03391	0.98245	(p<0.001)		
3 <sup>rd</sup> Gill Arch 🏼 🖉 🚬	0.00022	0.06034	0.63590	0.0297	0.98715	(p<0.001)		
4th Gill Arch 🛛 🚺 🦻	0.00015	0.04789	0.63720	0.02358	0.99188	(p<0.001)		
Total gill arches 🏹	0.00102	0.05540	0.62113	0.02727	0.98862	(p<0.001)		
Dt1.(mlO2/min/mmHg/kg)				88				
1 <sup>st</sup> Gill Arch	0.28317	0.06681	-0.38453	0.03289	0.95879	(p<0.001)		
2 <sup>nd</sup> Gill Arch	0.29013	0.06888	-0.38115	0.03391	0.95564	(p<0.001)		
3 <sup>rd</sup> Gill Arch	0.21813	0.06034	-0.36410	0.02970	0.96230	(p<0.001)		
4 <sup>th</sup> Gill Arch	0.15367	0.04789	-0.36280	0.02358	0.97558	(p<0.001)		
Total gill arches	1.02236	0.0554	-0.37887	0.02727	0.97028	(p<0.001)		
B. Swim Bladder								
Dt.(mlO <sub>2</sub> /min/mmHg)	0.00029	0.07145	0.64957	0.03517	0.98285	(p<0.001)		
$Dt_1.(mlO_2/min/mmHg/kg)$	0.29452	0.07145	-0.35043	0.03517	0.94453	(p<0.001)		

#### Table 3: Computed diffusing capacity values for 1, 10, 100, 1000 and 2000 g fishes (Notopteruschitala) along with their.

Respiratory	Diffusing	1g		10g		100g		1000g		2000g	
organs	capacity	Value	95% C.L.	Value	95% C.L.	Value	95% C.L.	Value	95% C.L.	Value	95% C.L.
Total Gill Arches	Dt.(mlO <sub>2</sub> /min /mm Hg)	0.00102	0.00077 0.00135	0.00427	$0.00282 \\ 0.00647$	0.01786	$0.01029 \\ 0.03100$	0.07465	$0.03750 \\ 0.14858$	0.11481	0.05535 0.23815
	Dt <sub>1</sub> .(mlO <sub>2</sub> /min /mmHg/kg)	1.02236	0.77429 1.34661	0.42730	$0.28224 \\ 0.64694$	0.17860	$0.10288 \\ 0.31004$	0.07465	$0.03750 \\ 0.14858$	0.05741	$0.02768 \\ 0.11907$
Swim Bladder	Dt.(mlO <sub>2</sub> /min /mm Hg)	0.00029	$\begin{array}{c} 0.00021 \\ 0.00042 \end{array}$	0.00131	$\begin{array}{c} 0.00077 \\ 0.00224 \end{array}$	0.00586	$0.00288 \\ 0.01195$	0.02617	0.01077 0.06359	0.04105	$0.01602 \\ 0.10520$
	Dt <sub>1</sub> .(mlO <sub>2</sub> /min /mmHg/kg)	0.29452	0.20580 0.42149	0.13142	0.07698 0.22438	0.05865	0.02879 0.11945	0.2617	0.01077 0.06359	0.02053	0.00801 0.5260



Fig 1. Log/log plots showing the relationship between body weight and diffusing capacity of N.chital



Fig 2. Log/log plots showing the relationship between body weight and weight specific diffusing capacity of *N.chitala.* 



Fig 3. Log-log plot showing body weight and diffusing capacity (Dt, mlO<sub>2</sub>.min<sup>-1</sup>.mmHg<sup>-1</sup>) and (Dt<sub>1</sub>, mlO<sub>2</sub>.min<sup>-1</sup>.mmHg<sup>-1</sup>Kg<sup>-1</sup>) of swimbladder in *Notopterus chitala*.

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#### DISCUSSION

Hughes (1970) has shown that the thickness of diffusion barrier varies greatly in its dimension with the activity of fishes and ecological condition of the habitat. The waterblood diffusion distance was very low (0.533 to 0.598µm) in very active fishes like Thunnusalbacares reported by Hughes (1970). In Notopterus chitala the water- blood diffusion distance was measured to be 1.179 which is closer to Indian water breathing fishes like Cirrhinusmrigala (1.290µm, Roy and Munshi, 1987), juveniles of Labeorohita (1.32µm, Pandey, 1988) etc. However, the value of diffusion distance of gills of Notopterus chitala was quite thin when compared with Indian air-breathing fishes viz, 3.58µm in Heteropneustesfossilis (Hughes et al., 1974);7.67µm in Clariasbatrachus (Sinha, 1977);2.03µm in Channa punctatus (Hakim et al., 1978) and 6.978µm inChannastriata (Chaudhary, 1979) and 1.327µm in Notopterus notopterus (Kumari, 2003). From this finding, it can be inferred that the gill for gaseous exchange is more efficient in comparision to most of the air breathing fishes.

The slope of the regression line (b) of the diffusing capacity (Dt) of *Notopterus chitala* increases by a power of 0.62113 with unit increase in body weight. As, the slope value is less than one, thus the weight specific diffusion capacity (Dt<sub>1</sub>)decreases by a power of -0.37887 with increase in body weight. It is evident from the above findings that the gills of smaller fishes are more efficient than higher weight group of fishes. Variations in the slopes of the regression lines of different gill arches due to heterogenous growth patterns of different gill arches.

The value of weight specific diffusing capacity  $(Dt_1)$  for 100 g of *N. chitala* was found to be 0.17860 which is closer to the area value of hill stream fish *Glyptothoraxtelchitta* (0.1675, Subba, 10) 1999) but lower than *Garralamta* (0.1982, Rooj, 1984). When this value was compared with purely water breathing fishes, it was found to be lower than Cirrhinusmrigala (0.5891, Roy and Munshi, 1986), Cattacatta (0.7416, Kunwar, 1984) and Labeorohita (0.3061, Pandey, 1988). However, the value calculated for N. chitala was higher than most of the Indian air-breathing fishes viz. Anabas testudineus (0.0071, Hughes et al., 1973, ) Channa punctatus (0.0530, Hakim et al., 1978), Channagachua (0.0382, Dandotia, 1978) and Heteropneustesfossilis (0.0242, Hughes et al., 1974). Similary, the value is higher than another featherback, Notopterus notopterus (0.15505, Kumari, 2003). These findings suggest the better oxygen uptake efficiency of this fish in comparison to most of the Indian air-breathing fish.

#### AIR-BLOOD DIFFUSION DISTANCE

The diffusion barrier of the swimbladder of *Notopterus chitala* was calculated to be 1.439 which is quite thick in comparison to the diffusion barrier of the gill (1.179). This finding clearly indicates that the water breathing organ provides better respiratory surface to this fish. Similar trend has been observed when compared with *Notopterus notopterus* (1.705 as air-blood diffusion distance and 1.327 as water – blood diffusion distance, Kumari, 2003). However, the value (1.439) is very close to the value of the accessory respiratory organ of *Channastriata* (1.359, Hughes and Munshi, 1973 b). The air-blood diffusion distance of *N. chitala* is higher than the other air- breathing fishes like. *Amphipnouscuchia* (0.435, Hughes and Munshi, 1973),

*Clariasbatrachus* (0.550, Sinha, 1977), *Anabas testudineus, Channa punctatus* (0.21 and 0.78 respectively, Hughes and Munshi, 1973 a), *Channagachua* (0.080, Dandotia, 1978). Thus, the air- breathing organ of *N. Chitala* is less efficient in gaseous exchange as compared to most of the air-breathing fishes of India except *Heteropneustesfossilis* (1.605µm) and *Notopterus notopterus* (1.705µm).

It is evident from the computed data on the regression lines of the diffusing capacity (Dt) that in this featherback, diffusing capacity increases by a power of 0.64957. The weight specific diffusing capacity of the swimbladder for a 100 g *Notopterus chitala* was found to be 0.05865 which is less than the value obtained for the gills (0.17860). The above finding clearly indicates that the gills of *N.chitala* is more efficient in gaseous exchange than the swimbladder. This value (0.05865) was found to be closer to the respiratory membrane of the suprabranchial chamber of a 100 g Anabas testudineus (0.0539, Hughes et al., 1973). However, the value is higher than the air- breathing organs of most of the air-breathing fishes like Amphipnouscuchia (0.165, Hughes et al., 1974 b), Channagachua (0.0366, Dandotia, 1978) Heteropneustesfossilis (0.0288, Hughes et al., 1974 a) etc. except the dendritic organ of *Clariasbatrachus* (0.0773, Hughes et al., 1974 b) and suprabranchial chamber of Channa punctatus0.0753, Hakim et al., 1978). Hence, it is clear that the swimbladder of Notopterus chitala is more efficient in comparison to most of the air-breathing fishes.

The morphometrically calculated VO<sub>2</sub> for a 100 g *N. chitala* was found to be 1071.6 through gills and 351.9 through swimbladder is much higher than the actual oxygen uptake (31.385 through gills and 82.069 through swimblader) (Kumari, 2003). Hence, in *N. chitala* the oxygen uptake through gills and swimbladder is almost 34 and 4 times higher respectively than the actual oxygen uptake at 27.5  $\pm$  1.0°C. A 5-10 times more oxygen uptake by the fishes in active condition was reported by Alexander (1967). Thus, it can be inferred that the morphometrically estimated oxygen uptake may be the maximum oxygen uptake capacity of the fish.

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