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DIFFUSING CAPACITY OF GILLS AND SWIMBLADDER OF A FRESHWATER FEATHERBACK, *NOTOPTERUS NOTOPTERUS* (PALLAS) IN RELATION TO BODY WEIGHT.

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Abstract: *The diffusing capacity of the bimodal gas exchange organs of Notopterusnotopterus has been studied as it gives an idea of the efficiency of respiratory membranes for gaseous exchange. 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 cells. The air-blood diffusion barrier is composed of a single layer of epithelial cells and an underlying layer of blood capillaries. The harmonic mean of the thickness of water-blood (gills) and air-blood (swimbladder) diffusion barrier for Notopterusnotopterus were measured to be 1.327µm and 1.705µm whereas the respective arithmetic mean was found to be 1.629µm and 1.984µm. The diffusing capacity (Dt: $mlO_2.min^{-1}.mmHg^{-1}$) increases by a power of 0.71283 and 0.73517 respectively for gills and swimbladder with unit increase in body weight, whereas the weight specific diffusing capacity (Dt_1: $mlO_2.min^{-1}.mmHg^{-1}.kg^{-1}$) decreases by the slope value of -0.28714 and -0.26483 respectively for water and air breathing organs of Notopterusnotopterus. The estimated value for 1.0g fish i.e. intercept 'a' for the respiratory organs gills and swimbladder were computed to be 0.00058 and 0.00017 $ml0_2.\ min^{-1}.mmHg^{-1}$ respectively.*

Key Words: *Diffusing capacity, Body weight, The water-blood and air-blood diffusion barrier Notopterusnotopterus.*

1. INTRODUCTION:

In respiratory physiology, the diffusing capacity is an important phenomenon which determines the efficiency of various respiratory membranes for gaseous exchange. The effective respiratory surface area and the thickness of diffusing barrier i.e. water-blood barrier and air-blood barrier are the most important parameters to be considered in determining the diffusing capacity of gases through the respiratory interface. The amount of oxygen or carbon dioxide diffusion across the respiratory surface in unit time is directly proportional to the respiratory surface area and inversely proportional to the diffusion barrier.

The diffusing capacity of the bimodal gas exchange machinery has been studied by many workers viz. Hughes et al., (1973, 1974), Dube and Munshi (1974), Ojha and Munshi (1976). Hughes (1976), Dandotia (1978), Hakim et al., (1978), Chaudhary (1979). Hughes et al., (1992) and Roy and Munshi (1992,1996) used the harmonic mean of water-blood barrier and stereological methods in association with electron microscopy for the measurement of diffusing capacity of the respiratory organs of certain air-breathing fishes of India. The following workers deserve special mention in determining the diffusing capacity of some hill stream fishes viz. Sharma et al., (1982) in *Botialohchata*; Ojha et al., (1982) in *Garralamta*; Rooj (1984) in *Noemacheilusrupicola*; Singh et al.,(1988) in *Botiadario* and Subba (1999) in *Glyptothoraxtelchitta*. The present work is an attempt to elucidate the possible functional relationship between the diffusing capacity of the dual breathing organs (gills and swimbladder) and body weight in a freshwater feather back *NotopterusNotopterus.*

2. MATERIALS AND METHODS:

Live specimens of*Notopterusnotopterus*were procured from the river Ganga and local ponds near Bhagalpur. They were brought to the Post-Graduate Department of Zoology, T.M. Bhagalpur University, Bhagalpur and were maintained in large plastic pools. The specimens were then transferred to the laboratory in glass aquaria for about two weeks with aeration facility on. The gills and swimbladder were dissected out and put into saline water to remove the adhering mucus and blood etc. and then fixed into freshly prepared Bouin's fixative for 18 hrs, decalcified in 5% HNO_3 in 70% ethanol, processed as usual to cut 5-6 µm thick paraffin sections. The sections were dewaxed and stained in Eosin/Haemotoxylin and oil immersion photomicrographs were taken from various levels. The maximum and minimum diffusion distances were measured directly from the photomicrographs and the actual values of the diffusion distance were obtained by dividing the measured thickness of magnification. The arithmetic and harmonic means of diffusion distances were calculated. Modified Fick's equation (Hughes, 1972; Weibel, 1972) used to calculate the diffusing capacity of the respiratory organs. The modified Fick's equation is as follows:-

$$VO_2 = K.A.\ \Delta PO_2 \quad \text{(i)}$$

$$\text{or, } VO_2/\Delta PO_2 = K.A./t \quad \text{(ii)}$$

$$\text{or, } Dt = K.A./t \quad \text{(iii)}$$

$$TO_2 = VO2/\Delta PO_2 \quad \text{(iv)}$$

Where,

VO_2 = Oxygen uptake ($mlO_2.min^{-1}$)
K = Krogh's permeation coefficient (for frog's connective tissue at 20ºC i.e., 0.00015 ($mlO_2.cm^{-2}.\mu m^{-1}.min^{-1}.mmHg^{-1}$)
A = Respiratory (Gill /swimbladder) surface Area (cm^2) taken from previous chapter of thesis.
ΔPO_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-

$$Dt = aW^b$$

Where,

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

3. DISCUSSION:

In purely water breathing teleosts, the diffusion barrier 3-6 µm has been reported by Hughes and Grimstone (1965), Newsted (1967) and Hughes and wright (1970). Munshi and Singh (1968) calculated the water-blood pathway of gills of certain water-breathing and air-breathing teleosts of India. Hughes (1970) found very low diffusion barrier (0.533 to 0.598) for active fishes like, Tunny. In *Notopterusnotopterus*, the harmonic mean of the water-blood diffusion distance was computed to be 1.327 µm which is very close to juveniles of *Labeorohita*(1.32µm, Pandey et al., 1989). However, the value of *N. notopterus* is slightly higher than *Cirrhinusmrigala (*1.290 µm, Roy and Munshi, 1987*).*The value 1.327 µm estimated for *N.notopterus* when compared with other air-breathing fishes, it is quite thin viz, *Anabas testudineus*(10.00 µm, Hughes et al., 1973),*Clariasbatrachus*(7.67 µm, Munshi et al., 1980), *Channastriata*(6.978 µm, Choudhary, 1979),*Heteropneustesfossilis*(3.58µm, Hughes et al.,1974), *Channapunctatus* (2.03µm,Hakim et al. ,1978).Although,the thickness of diffusing barrier of water breathing organ of *Notopterus chitala* was reported to be quite thin (1.179µm by Kumari et al., 2020). The above finding suggests that the water breathing organ of *Notopterusnotopterus* is less efficient in gaseous exchange than the gills of *Notopterus chitala* while more efficient than other air-breathing fishes of India.

In *Notopterusnotopterus*, the slope(b) value for Dt and Dt_1 have been found to be 0.71283 and -0.28714 suggesting that in smaller fishes the efficiency of water breathing organ is more in comparison to the higher weight group of fishes. The gill diffusing capacity Dt_1 for a 100 g fish comes to be 0.15505 which is lower in comparison to the values for major carps like *Cirrhinusmrigala*(0.5891), *Catlacatla*(0.7416) and higher than the weight specific diffusing capacity of air-breathing fishes like *Anabas testudineus*(0.0071), *Heteropneustesfossilis*(0.0242), *Notopterus chitala* (0.17860) but very close to the hill stream fish *Glyptothoraxtelchitta*(0.1675).

4. AIR- BLOOD DIFFUSION DISTANCE:

The air- blood diffusion distance and water-blood diffusion distance were calculated to be 1.705 and 1.327 respectively. This indicates that the water breathing organ provides better respiratory surface than those of the air-breathing organ. The similar trend has been reported in other species of *Notopterus.*Although,the value of 1.705 is higher when compared with other Indian air-breathing fishes except *Channastriata*but closer to *Heteropneustesfossilis* (1.605 µm). The weight specific diffusing capacity (Dt_1) of the swimbladder of a 100 g *Notopterusnotopterus* has been estimated as 0.05019 which is lower than *N.chitala*(0.05865), *A.testudineus* (0.0539), *A.cuchia*(0.165) however, lower than *C.gachua* (0.0366),*C . Striata*(0.0254) etc. Thus, it can be inferred from the above findings that swim bladder of *Notopterusnotopterus* holds the intermediate position in gaseous exchange efficiency.

4. RESULTS:

4.1 Water blood diffusion barrier:

The water-blood diffusion barrier in the secondary lamellae consists of an outer single layer of epithelium, middle the basement membrane and innermost flanges of pillar cells. The harmonic mean($\bar{x}$h) of the thickness of water-blood diffusion barrier of different regions of secondary lamellae for *Notopterusnotopterus* was measured to be 1.327. The arithmetic mean for the same was found to be1.629.

Relationship between body weight and gill diffusing capacity(Dt) (mlO2.min-1.mmHg-1):

The diffusing capacity of the gills of *Notopterusnotopterus* increased from 0.00170 to 0.02179 with increase in body weight from 5.0 to 175.0g (Tab-1). Log-log plots of the body weight and the diffusing capacity for first, second, third, fourth and total gill arches were plotted against the respective body weights , they gave straight lines with the slopes 'b' of 0.72923, 0.71382, 0.69670, 0.72160 and 0.71283 respectively (Tab-2, Fig-1). The value of gill diffusing capacity for 1, 10, 100 and 1000 g *Notopterusnotopterus* were estimated to be 0.00058, 0.00300, 0.01550 and 0.08004 respectively (Tab-3). The relationship between two variables could be represented as follows-

$$Dt = 0.00058.W^{0.71283}$$

There was a significant and positive correlation between the two variables.

Relationship between body weight and weight specific diffusing capacity (Dt_1) ($mlO_2.min^{-1}.mmHg^{-1}.Kg^{-1}$).

The log-log plots between the body weight and weight specific diffusing capacity for first, second, third, fourth and total gill arches when plotted, gave straight lines with the slopes 'b' of -0.27077, -0.28618, -0.30330, -0.27840 and -0.28714 respectively (Tab-2, Fig-2). The weight specific diffusing capacity (Dt_1) were calculated to be 0.58184, 0.30035, 0.15505 and 0.08004 respectively for 1,10,100 and 1000 g body weight of *N. notopterus*. The intercept 'a', slope 'b' and correlation coefficient 'r' for the total gill arches were calculated to be 0.58184, -0.28714 and 0.98682respectively.

4.2.Air blood diffusion barrier

In *Notopterusnotopterus* air blood diffusion barrier was consists 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 swim bladder was calculated to be 1.705 µm while arithmetic mean was found to be 1.984 µm.

Relationship between body weight and swimbladder diffusing capacity (Dt) ($mlO_2.min^{-1}.mmHg^{-1}$)

The swimbladder diffusing capacity (Dt) in *Notopterusnotopterus* has shown increasing trend with increase in body weight (Tab-1) . The intercept 'a', slope 'b' and correlation coefficient 'r' for diffusing capacity(Dt) were found to be 0.0001, 0.73317 and 0.099138 respectively (Tab-2). The diffusing capacity valuesfor 1,10,100 and 1000g fishes were estimated to be 0.00017, 0.00092, 0.00502 and 0.02723 respectively. When log-log graphs plotted between the two variables gave straight lines with the slope of 0.73517. The relationship between two variables could be represented as follows-

$$Dt = 0.00017.W^{0.73517}$$

Relationship between body weight and weight specific swimbladder diffusing capacity (Dt_1) ($mlO2.min^{-1}.mmHg^{-1}.kg^{-1}$)

The weight specific diffusing capacity of swimbladder (Dt_1) for 1, 10, 100 and 1000 g *Notopterusnotopterus* were computed to be 0.16994, 0.09236, 0.05019 and 0.02728 respectively. The relationship between the two variables is as follows-

$$Dt_1 = 0.16994.W^{-0.26483}$$

The correlation coefficient 'r' value of 0.9388 indicates a highly significant but negative correlation between two variables(Tab-2). The log-log plots gave straight lines when plotted against respective body weight (Fig-3).

Table-1:Gill and swim bladder diffusing capacity for different weight groups of *Notopterusnotopterus*.

Body weight (g)	Gill area (cm^2)	Diffusion capacity		Swim bladder area (cm^2)	Diffusion capacity	
		Dt. ($mlO_2.min^{-1}.mmHg^{-1}$)	Dt_1 ($mlO_2.min^{-1}.mmHg^{-1}.Kg^{-1}$)		Dt. ($mlO_2.min^{-1}.mmHg^{-1}$)	Dt_1 ($mlO_2.min^{-1}.mmHg^{-1}.Kg^{-1}$)
5.0	15.04940	0.00170	0.34023	5.40000	0.00048	0.09501
15.4	39.28544	0.00444	0.28836	17.87000	0.00157	0.10209
49.0	86.57119	0.00979	0.19971	35.30000	0.00311	0.06338
98.0	135.38534	0.01530	0.15616	53.73000	0.00473	0.04823
175.0	192.79905	0.02179	0.12453	81.19000	0.00714	0.04082

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 *Notopterusnotopterus*.

Body weight vs diffusing capacity	Intercept (a)		Slope (b)		Correlation coefficient (r)	
	Value	S.E.	Value	S.E.		
A. GILL						
Dt. ($mlO_2.min^{-1}.mmHg^{-1}$)						
1st Gill Arch	0.00017	0.05699	0.72923	0.03436	0.99668	($p<0.001$)
2nd Gill Arch	0.00016	0.03577	0.71382	0.02157	0.99863	($p<0.001$)
3rd Gill Arch	0.00015	0.07987	0.69670	0.04816	0.99290	($p<0.001$)
4th Gill Arch	0.00011	0.07479	0.72160	0.04510	0.99419	($p<0.001$)
Total Gill Arches	0.00058	0.04508	0.71283	0.02718	0.99782	($p<0.001$)
Dt_1 ($mlO_2.min^{-1}.mmHg^{-1}.Kg^{-1}$)						
1st Gill Arch	0.16780	0.05699	-0.27077	0.03436	0.97668	($p<0.001$)
2nd Gill Arch	0.16056	0.03577	-0.28618	0.02157	0.99158	($p<0.001$)
3rd Gill Arch	0.14644	0.07987	-0.30330	0.04816	0.96419	($p<0.001$)
4th Gill Arch	0.10634	0.07479	-0.27840	0.04510	0.96281	($p<0.001$)
Total Gill Arches	0.58184	0.04508	-0.28714	0.02718	0.98682	($p<0.001$)
B. SWIM BLADDER						

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Dt. ($mlO_2.min^{-1}.mmHg^{-1}$)	0.00017	0.09302	0.73517	0.05609	0.99138	($p<0.001$)
Dt_1($mlO_2.min^{-1}.mmHg^{-1}.Kg^{-1}$)	0.16994	0.05609	-0.26483	0.05609	0.93882	($p<0.001$)

Table 3: Computed diffusing capacity values for 1,10,100 and 1000 g fishes *(Notopterusnotopterus*) along with their 95% C.L.

Respiratory organs	Diffusing capacity	1 g		10 g		100 g		1000 g	
		Value	95% C.L.	Value	95% C.L.	Value	95% C.L.	Value	95% C.L.
Total Gill Arches	Dt. ($mlO_2.min^{-1}.mmHg^{-1}$)	0.00058	0.00042 0.00081	0.00300	0.00177 0.00510	0.01550	0.00748 0.03213	0.08004	0.03164 0.20244
	Dt_1 ($mlO_2.min^{-1}.mmHg^{-1}.Kg^{-1}$)	0.58184	0.41815 0.80960	0.30035	0.17687 0.51005	0.15505	0.07481 0.32133	0.08004	0.03164 0.20244
Swim Bladder	Dt. ($mlO_2.min^{-1}.mmHg^{-1}$)	0.00017	0.00009 0.00034	0.00092	0.00031 0.00275	0.00502	0.00112 0.02258	0.02728	0.00402 0.18508
	Dt_1 ($mlO_2.min^{-1}.mmHg^{-1}.Kg^{-1}$)	0.16994	0.08595 0.33598	0.09236	0.03097 0.27542	0.05019	0.01116 0.22577	0.02728	0.00402 0.18508

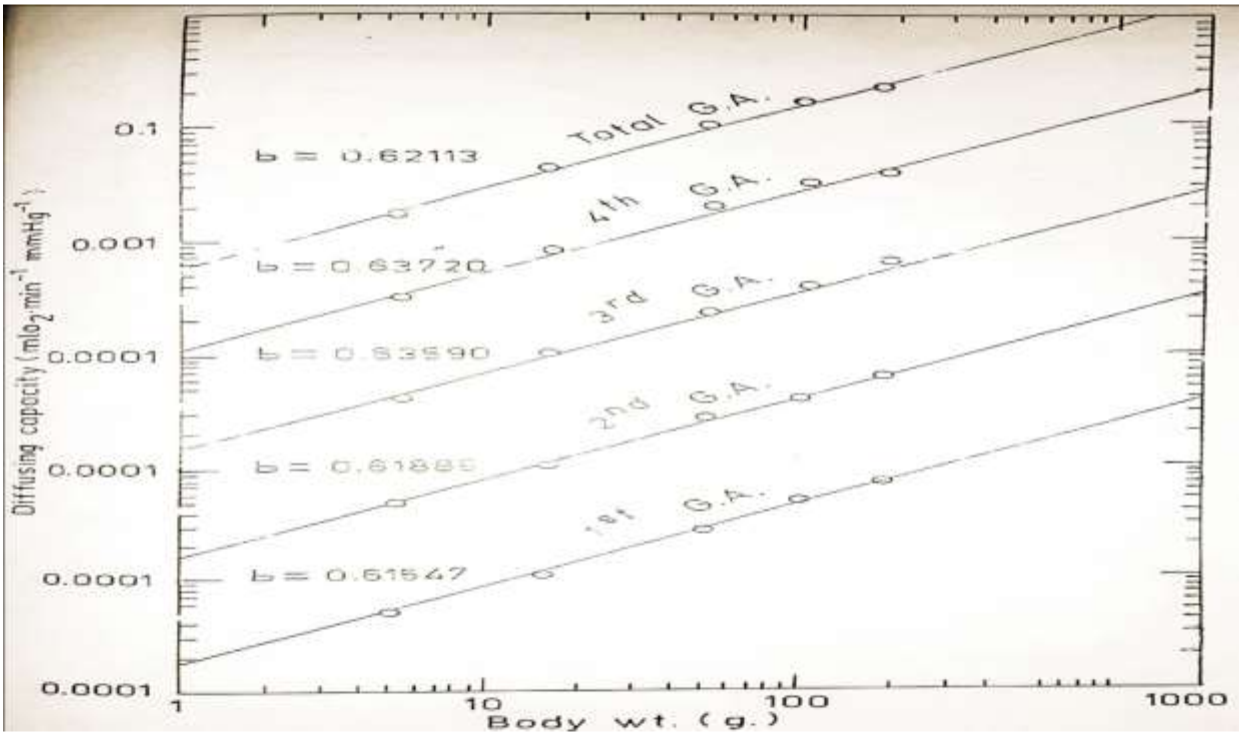

Figure 1.Log/log plots showing the relationship between body weight and diffusing capacity of *N. notopterus.*

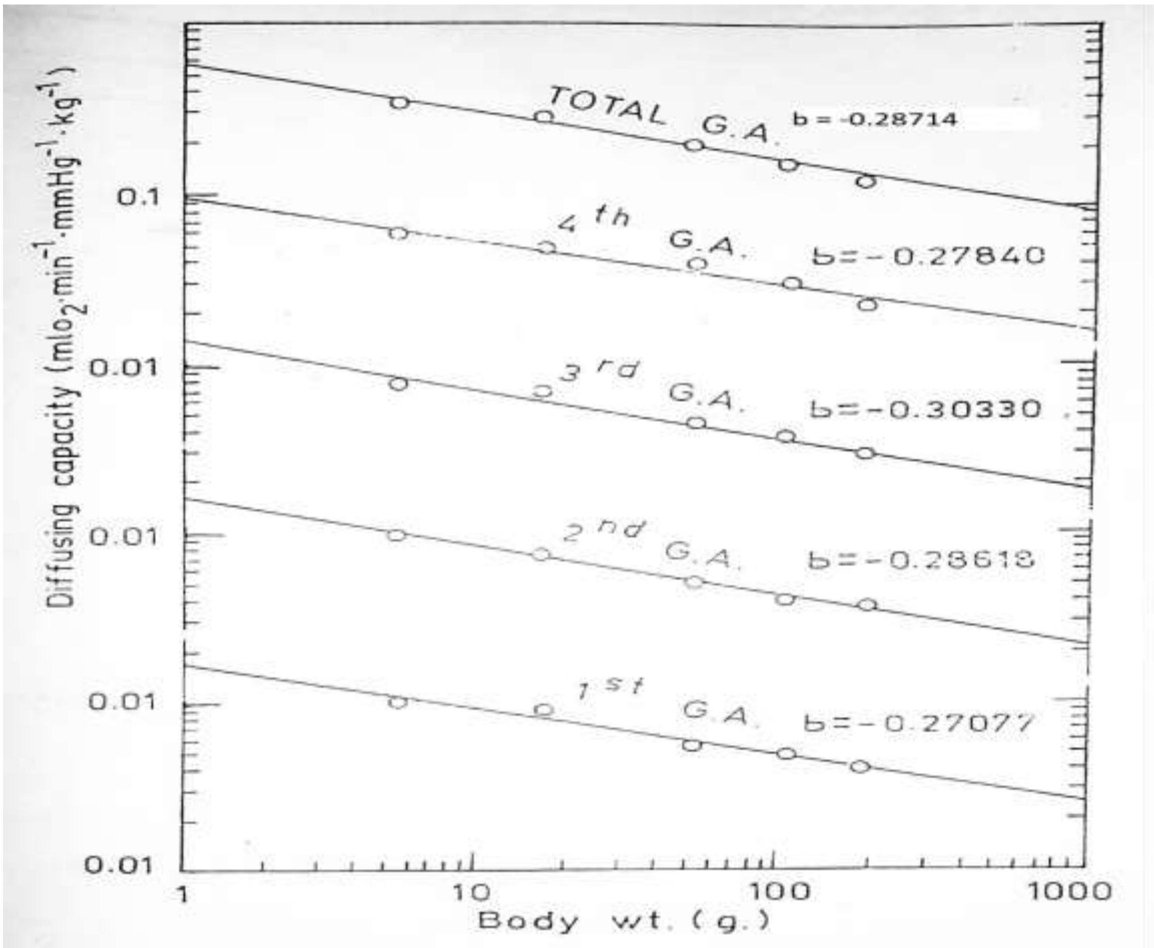

Figure 2. Log/log plots showing the relationship between body weight and weight specific diffusing capacity of *N. notopterus.*

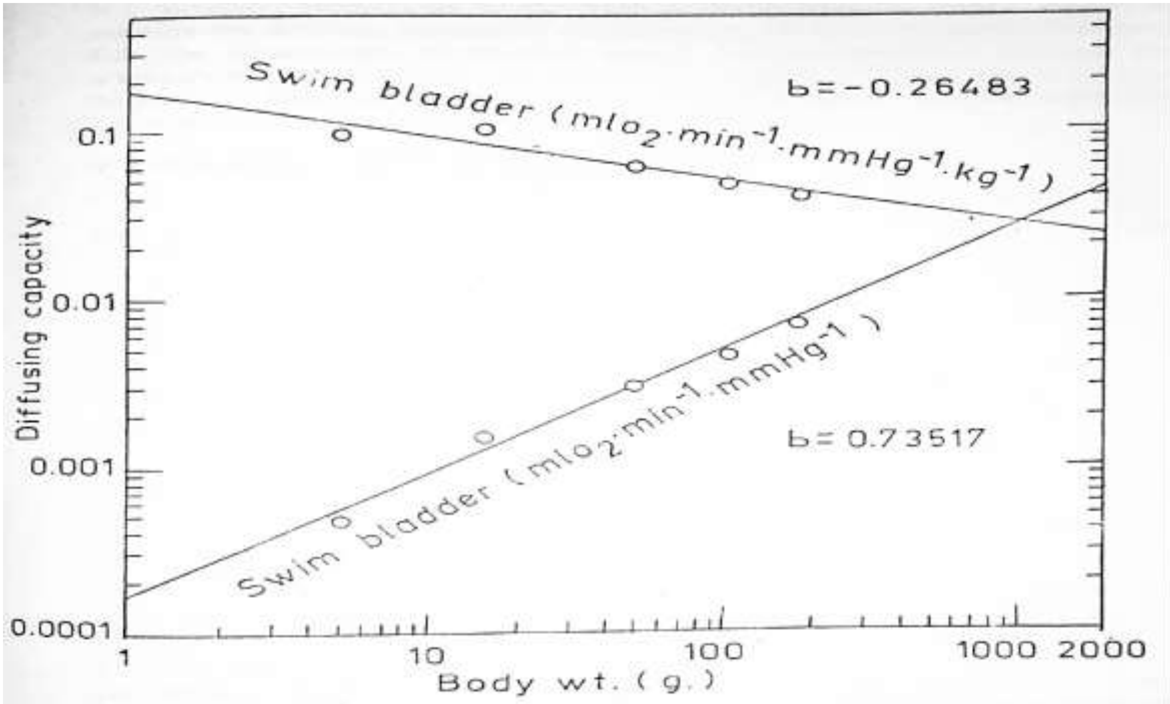

Figure 3. Log-log plot showing body weight and diffusing capacity (Dt, $mlO_2.min^{-1}.mmHg^{-1}$) and (Dt_1, $mlO_2.min^{-1}.mmHg^{-1}Kg^{-1}$) of swimbladder in *Notopterusnotopterus.*

5. CONCLUSION:

The above finding suggests that the water breathing organ of *Notopterusnotopterus*is more efficient in gaseous exchange than the air breathing organ swimbladder.

6. ACKNOWLEDGEMENT

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