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Diffusing capacity of gills of a hill-stream fish *Glyptothorax telchitta* (Hamilton-Buchnan) in relation to body weight

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Abstract

The diffusing capacity of the gills of *Glyptothorax telchitta* was determined in relation to body weight by calculating harmonic mean $\overline{X}$ of the thickness of water blood diffusion barrier from different regions of the secondary lamellae. The harmonic mean $\overline{X}$ was 1.7202 μm, whereas the arithmetic mean was found to be 2.607 μm, which was more than the harmonic mean. The thickness of the water blood pathway varied from 0.77 to 7.70 μm. The diffusing capacity Dt (ml O_2 min^{-1} $mmHg^{-1}$) has been found to increase with increasing body weight by a power of 0.7008. As the slope of the diffusing capacity is less than 1.0, the weight specific diffusing capacity Dt_1 (ml O_2 min^{-1} $mmHg^{-1}$ Kg^{-1}) decreases with increasing body weight by a power of -0.2996.

Keywords: Body weight, diffusion barrier, harmonic mean, secondary lamellae, water blood pathway.

Introduction

Diffusing capacity (Dt) is an important physiological parameter that provides knowledge of the efficiency of respiratory organs for oxygen diffusion. The thickness of the diffusion barrier inversely affects the gas transfer through the system. The role of two important parameters such as the water blood distance and the respiratory surface area help in determining the effectiveness of the fish in gas exchange. The water-blood pathways in the gills of fishes consist of outer epithelial cells, the middle basement membrane, and the inner flanges of the adjacent pillar cells (Newstead, 1967).

Studies of the thickness of the water-blood barrier of the secondary gill lamellae in fishes have been worked out by Schultz (1960), Newstead (1967), Hughes and Munshi (1968), Munshi and Singh (1968), Hughes and Wright (1970), Munshi *et al.* (1980), Biswas *et al.* (1981), Ojha *et al.* (1982), Rooj (1984), Singh and Munshi (1985), and Roy and Munshi (1987).

In case of a water breathing fish,

$Dt = VO_2/APO_2$

where, Dt = Diffusing capacity

VO_2= Oxygen uptake across the respiratory organs and

APO_2= The mean difference in oxygen tension between the respiratory media and the blood of the respiratory surfaces

As $VO_2 = \frac{K.A.}{t} \Delta PO_2$

Then $= \frac{VO2}{\Delta\ PO2} = \frac{K.A.}{t}$

where,

A = respiratory area (cm^2)

t = diffusion distance or thickness of water blood pathway (μm) and

K = Krogh's permeation co-efficient (for frog connective tissue at 20°C i.e., 0.00015 m1 $O_2\ cm^{-2}.\ \mu m^{-1}\ min^{-1}\ mmHg^{-1}$

Hughes (1970) and Weibel (1970) were the pioneer workers to give detailed accounts of the diffusing capacity in fish gill and a mammalian lung respectively. Hughes (1972a) introduced the method of determining diffusing capacity more elegantly in fish respiratory structure. He reported that in a tench, *Tinca tinca* the diffusing capacity of the gills per unit body weight decreased with an increase in body weight. Hughes (1972b) determined the diffusing capacity of the gills of an ice fish, *Chaenocephalus aceratus*.

Hughes *et al*. (1973) and Hughes *et al*. (1974a, b) computed the diffusing capacity of the bimodal gas exchange machinery of the climbing perch, *Anabas testudineus;* a catfish, *Heteropneustes fossilis* and the eel *Amphipnous cuchia* respectively. Dube and Munshi (1974) calculated the diffusing capacity in relation to body weight, in a climbing perch, *Anabas testudineus*.

The diffusing capacity of the gills of *Latimeria chalumnae* was estimated by Hughes (1976). Hughes and Perry (1976) made a detailed comparative study of diffusing capacity of the gills of trout from unpolluted (controlled) and those procured from polluted waters, by using stereological methods. Ojha and Munshi (!976) calculated the diffusing capacity of the gas exchange machinery of a freshwater mud eel, *Macrognathus aculeatum* and observed a decreasing trend in diffusing capacity of the gill per unit body weight, with increasing body weight.

The diffusing capacity of a freshwater air-breathing fish, *Clarias batrachus* was calculated by Sinha (1977). Dandotia (1978), Hakim *et al*. (1978), and Choudhary (1979) computed the diffusing capacity of three freshwater murrels, *Channa gachua*, *Channa punctata* and *Channa striata* respectively. Diffusing capacity of the gills of an estuarine goby, *Boleophthalmus boddaerti* was determined by Biswas *et al.* (1981). Singh and Munshi (1985) gave a detailed account of diffusing capacity of the gills in a freshwater goby, *Glossogobius giuris*. Roy and Munshi (1987) estimated the diffusing capacity (oxygen uptake efficiency) of a freshwater major carp, *Cirrhinus mrigala* in relation to body weight. More contributions to the measurements of diffusing capacity of gills area made by Sharma *et al.* (1982) in *Botia lohachata*, Rooj (1984) in *Garra lamta* and *Noemacheilu srupicola*, Ojha *et al*. (1985) *in Mystus vittatus*, Singh (1990) in *Xenentodon cancila*, and Yadav *et al*. (1990) in *Periophthalmadon schlosseri*.

Emphasis was given to the use of harmonic mean of water-blood barrier and stereological method in association with electron microscopy. Such methods have been applied in the measurement of diffusing capacity by different authors (Munshi *et al.*, 1989;

Hughes *et al*., 1992; Roy and Munshi, 1992, 1996).

The present investigation deals with the measurement of the water-blood barrier and the relationship between the diffusing capacity of gills and body weight in a freshwater hill-stream fish, *Glyptothorax telchitta*.

Material and Methods

Live specimens of different weight groups of *Glyptothorax telchitta* were collected from two fish-catching sites (Tribeni and Barhkshetra) of the Saptakoshi river, Nepal. Fishes were anesthetized with benzocaine then the gills were taken out carefully. Small pieces of the gill arches were fixed in freshly prepared Bouin's and Zenker's fixatives and brought to the Ichthyology Research laboratory of the Post Graduate Department of Zoology, T.M. Bhagalpur University for further study.

The fixed materials were decalcified with acid alcohol (5% conc. HNO_3), processed and embedded in paraffin wax and 6µm thick horizontal sections were acquired. The sections were stained with haematoxylin and eosin. Photomicrographs of sections of the gill lamellae were taken. The minimum and maximum water/blood diffusion distances were measured directly from the photomicrographs. The actual thickness of the diffusion distances were procured by dividing the measured thickness by the magnification and their harmonic means (Xh) were secured.

Diffusing capacity of the gills

The diffusing capacity of gills was determined from gill area (A) and thickness of water blood barrier (t) with the help of modified Fick's equation (Hughes, 1972; Weibel, 1972) which is as follows:

As $VO_2 = = \frac{K.A.}{t} \Delta PO_2$ ……………………….i

or $Dt = \frac{VO2}{\Delta PO2} = \frac{K.A.}{t}$ ……………………….ii

or $Dt = \frac{K.A.}{t}$ …………………………………iii

Where,

VO_2 = the rate of oxygen consumption (mI O_2.min $^{-l}$)

K^2 = Krogh's permeation coefficient (for connective tissue of frog at 20°C i.e., 0.00015 m1 O_2.cm^{-2}. cm-^{1}. min$^{-'}$.mmHg$^{-'}$)

ΔPO_2 = difference of oxygen tension between water and blood (mmHg);

t = thickness of water-blood pathway (µm)

A = dimension of gill area (cm^2), taken from Subba (1999).

The values of gill dimensions and harmonic mean of the water blood pathways were applied to equation (iii) for the computation of diffusing capacity. Regression analysis using logarithmic transformations was carried out to find out the relationship between body weight and diffusing capacity. The relationship was expressed by the allometric equation:
$Dt = aW^b$

or Log Dt = log a + b. log W
where, Dt = the diffusing capacity, a = intercept value (value for 1g fish), b = slope value, and W = body weight (g)

Results

The water-blood diffusion barrier in the secondary lamellae of *Glyptothorax telchitta* consists of a usual outermost single-layered epithelium, thin basement membrane and the innermost layer of flanges of pillar cells. The water-blood thickness in the far gill arches did not differ significantly. The calculated harmonic mean (Xh) of the thickness of the water-blood diffusion barrier from different regions of the secondary lamellae was 1.7202 µm. The thickness of the water-blood pathway varied from 0.77 µm to 7.700 µm. The arithmetic mean of the data obtained for the thickness came to be 2.60468 m while the harmonic mean (X h) was lesser i.e. 1.7202 µm.

Diffusing capacity of the gills

The diffusing capacity for different gill arches as well as the total gill arches increased with an increase in body weight. However, weight-specific diffusing capacity showed a decreasing trend with an increase in body weight. The gill diffusing capacity (Dt) of the total gill arches of *Glyptothorax telchitta* ranged from 0.001089 to 0.007425 $m10_2$. min^{-1}. mmHg' with an increase in body weight from,1.75g to 31.80g. The weight-specific diffusing capacity (Dt_1) for total gill arches decreased from 0.62236 to 0.23350 $ml0_2$, min-^{1}. $mmHg^{-1}.kg^{-1}$ for the fishes of the same body weight.

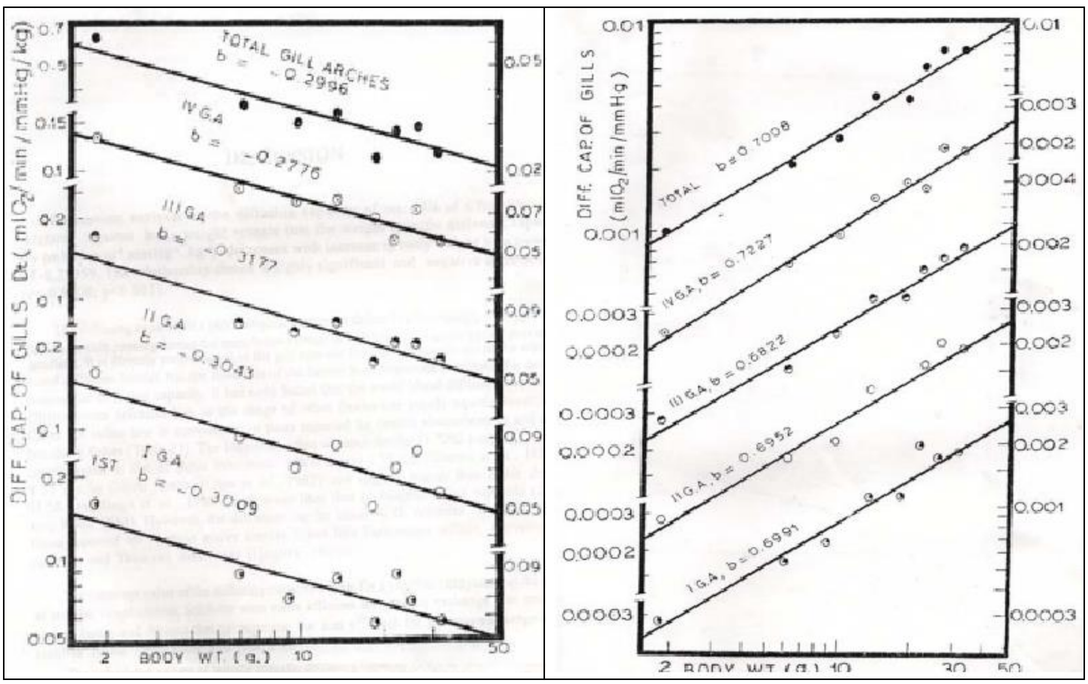

Figure 1. Log/log plots showing the relationship between body weight and weight specific diffusing capacity (Dt $mlO_2.min^{-1}$. $mmHg.Kg^{-1}$) for different and total gill arches in *G. telchitta*

Relationship between body weight and the diffusing capacity (Dt)

The diffusing capacity decreased significantly with an increase in body weight from 1.75g to 31.80g. The diffusing capacity for the first, second, third, and fourth gill arches increased from 0.000283 to 0.001826, 0.000280 to 0.001894, 0.000290 to 0.001937 and 0.000235 to 0.001768, respectively. The increase has been found to be highly significant which is clear from the values of correlation coefficients ($r=0.9703$; $p<0.001$, $r=0.9600$; $p<0.001$; $r=0.9836$; $p<0.001$ and $r=0.9897$; $p<0.001$) (Table1). The slope of the regression lines relating to body weight and diffusing capacity for the first, second, third and fourth gill arches were 0.699108, 0.695232, 0.682250 and 0.722677, respectively. When all the arches were taken together, the slope value was 0.700831 (Table 1). The log/log plots of these values always gave a straight line (Fig. 1). The intercept (log 'a') values were 0.00016942, 0.00016934, 0.00017473 and 0.00015119 for the first, second, third and fourth gill arches respectively and 0.00066439 when all the gill arches were taken together (Table 1). The relationship may be expressed as follows:

$Dt = 0.000664\ W^{\circ.70083}$ or
Log Dt = log 0.000664 + 0.70083.1ogW

Table 1. Table showing intercept (a) regression coefficient (b), correlation coefficient (r) and allometric equation for the relationship between the diffusing capacity of gill per unit time ($mlO_2\ min^{-1}.\ mnHg^{-1}$) and body weight. (SE is standard deviation of intercept a and slope b)

Gill Arch	Body weight Vs diffusing capacity	Intercept (a) Value	Intercept (a) SE	Regression Coefficient (b) Value	Regression Coefficient (b) SE	Correlation Coefficient (r)
1. Dt ($mlO_2\ mn^{-1}\ mmHg^{-1}$)						
1st	0.00016942	±0.077800	0.699108	±0.071164	0.970293	(P<0.001)
2nd	0.00016934	±0.095145	0.695232	±0.082729	0.960049	(P<0.001)
3rd	0.00017473	±0.058831	0.682250	±0.051154	0.983550	(P<0.001)
4th	0.0001511	±0.049019	0.722677	±0.042622	0.989725	(P<0.001)
Total	0.00066439	±0.059807	0.700831	±0.052003	0.983881	(P<0.001)
2. Dt1 ($mlO_2\ mn^{-1}\ mmHg^{-1}$)						
1st	0.1694090	±0.081845	-0.300878	±0.071164	-0.865272	(P<0.001)
2nd	0.169680	±0.096211	-0.304325	±0.083656	-0.829486	(P<0.001)
3rd	0.174728	±0.058832	-0.317749	±0.051154	-0.930280	(P<0.001)
4th	0.151215	±0.048946	-0.277584	±0.042559	-0.936159	(P<0.001)
Total	0.66542	±0.059576	-0.299595	±0.051801	-0.920818	(P<0.001)

The weight specific values for 1, 10 and 100g fish came to be 0.000664, 0.003336 and 0.016733 $m10_2.min^{-1}.\ mmHg^{-1}$, respectively (Table 2). The diffusing capacity decreased significantly with an increase in body weight.

Table 2. Computed diffusing capacity values for 1, 10 and 100g fishes along with their 95% confidence limits

Gill Arches	Diffusing Capacity	1.0g Value	1.0g 95% CL	10.0g Value	10.0g 95% CL	100.0g Value	100.0g 95% CL
1st	Dt ($mlO_2\ min^{-1}\ mmHg^{-1}$)	0.000169	0.000269 0.00107	0.000847	0.002007 0.000358	0.004238	0.014987 0.001199

	Dt_1 (mlO_2 min^{-1} $mmHg^{-1}kg^{-1}$)	0.169410	0.268661 0.106825	0.084735	0.200659 0.035782	0.042382	0.149869 0.011985
2nd	Dt (mlO_2 min^{-1} $mmHg^{-1}$)	0.000169	0.000289 0.000099	0.000839	0.002287 0.000308	0.004161	0.018067 0.000958
	Dt_1 (mlO_2 min^{-1} $mmHg^{-1}kg^{-1}$)	0.169681	0.291778 0.098676	0.084199	0.231996 0.030563	0.041781	0.184416 0.009466
3rd	Dt (mlO_2 min^{-1} $mmHg^{-1}$)	0.000175	0.000243 0.000125	0.000841	0.005620 0.000452	0.004045	0.010027 0.001631
	Dt_1 (mlO_2 min^{-1} $mmHg^{-1}kg^{-1}$)	0.174728	0.243399 0.125432	0.084065	0.156221 0.045237	0.040445	0.100267 0.016314
4th	Dt (mlO_2 min^{-1} $mmHg^{-1}$)	0.000151	0.000199 0.000115	0.000798	0.001338 0.000476	0.004216	0.008983 0.001979
	Dt_1 (mlO_2 min^{-1} $mmHg^{-1}kg^{-1}$)	0.151216	0.199234 0.114771	0.079802	0.133634 0.047655	0.042114	0.089633 0.019787
Total Gill Area	Dt (mlO_2 min^{-1} $mmHg^{-1}$)	0.000664	0.000931 0.000474	0.00336	0.006264 0.001777	0.016733	0.042162 0.006657
	Dt_1 (mlO_2 min^{-1} $mmHg^{-1}kg^{-1}$)	0.665420	0.930833 0.475686	0.333811	0.625216 0.178226	0.167458	0.419941 0.066777

Relationship between body weight and the weight-specific diffusing capacity of gills (Dt_1)($m10_2.min^{-1}.mmHg^{-1}.kg^{-'}$):

The relationship between the body weight and the weight-specific diffusing capacity of gills indicated a high but negative correlation between them (Table 1) which suggested a decrease in Dt_1 with an increase in body weight. The log/log plots always gave straight lines for all four gill arches individually and also when taken together, with slopes of -0.300878, -0.304325, -0.317749 and -0.277584 for the first, second, third and fourth gill arches respectively and -0.299595 when considered together (Fig. 1).

In all the cases the diffusing capacity of gills per unit body weight decreased with the increase in body weight. The intercept (log a) values were found to be 0.169409, 0.169680, 0.174728, 0.151215 and 0.665420 for the first, second, third, fourth and total gill arches respectively (Table 1). The allometric equation for the relationship between weight-specific diffusing capacity and body weight is as follows:

$Dt_1 = 0.66542\ W^{-0.29959}$

OT, $\text{Log } Dt_1 = \log 0.66542 - 0.29959. \log W.$

The weight specific diffusing capacity for 1, 10 and 100g fishes came to be 0.665420, 0.333811 and 0.167458 ($m10_2.min^{-1}$ $.mmHg^{-1}$. kg^{-1}) respectively (Table 2).

Discussion

Regression analysis of the diffusing capacity of the gills of *Glyptothorax telchitta* against body weight revealed that the weight-specific diffusing capacity ($mI0,.min^{-1}mmHg^{-1}$ kg^{-1}) decreases with increase in body weight by a power of -0.29959. The relationship showed a highly significant and negative correlation ($r=-0.9208$; $p<0.001$).

The diffusing capacity is a physiological parameter, defined as the quantity of oxygen or carbondioxide passing across the membrane system in unit time for a given partial pressure gradient. It is directly proportional to the gill area but inversely proportional to the water-blood diffusion harrier. So, the thickness of the barrier is an important parameter for determining the diffusing capacity. It has been found that the water-blood diffusion barrier in *Givptothorax telchitta* lies in the range of other freshwater purely aquatic-breathing fishes but is rather low in comparison to those reported for certain elasmobranchs and air-breathing fishes (Table 2). The harmonic mean of the tissue barrier (1.7202 μm) is approximately equal to that of *Botia lohachata* where it was 1.71 μm (Sharma *et al.,* 1982); 1.75 m in *Garra lamta;* (Ojha *et al.,* 1982) and slightly higher than *Botia Dario* (1.58 μm; Singh *et al.,* 1988); and lower than that of *Noemacheilus rupicola* (2.25. μm. Rooj, 1984). However, the diffusion barrier tissue in *G. telchitta is* thicker than those reported for certain active marine fishes like *Euthynnuss affinis. Katsuwonus pelamis* and *Thunnus albacares* (Hughes, 1970).

The intercept value of the diffusing capacity (value for a 1.0g fish) suggests that the gills of smaller *Glyptothorax telchitta* were more efficient for gaseous exchange than smaller *Garra lamta* and *Noemacheilus rupicola* but less efficient for gaseous exchange than smaller *Botia lohachata* and *Botia dario.*

As the body weight of this fish always remains below 100.00g, it is judicious to com- pare the values of diffusing capacity of 10.0g fishes. The weight specific diffusing *capacity* ($m10_2$. min^{-1}.$mmHg^{-1}$. kg^{-1}) of a 10.0g *G. telchitta* for the first to fourth gill arches were calculated to be 0.084735; 0.084199; 0.08065 and 0.07980 respectively. This suggested that the diffusing capacity of the first to third gill arches were somewhat similar whereas the fourth gill arch had lower Dt. In *Garra lamta* of same body weight (10g), the third gill arch had the highest diffusing capacity (0.08003 $m10_2$. min^{-1}. $mmHg^{-1}$. Kg^{-1}). But, in case of *Noemacheilus rupicola,* the first gill arch showed the highest diffusing capacity (0.10581 $ml0_2$. min^{-1}. $mmHg^{-1}$. Kg^{-1}) while the fourth gill arch had the lowest one (Rooj, 1984). In *Botia dario* also the highest intercept value (0.30524 $m10_2$. min^{-1}. $mmHg^{-1}$ Kg^{-1}) was found for the first gill arch and the lowest (0.1854 $m10_2$.min^{-1} $mmHg^{-I}$. Kg^{-1}) for the fourth gill arch (Singh *et al.,* 1988). The above data clearly indicated that the diffusing capacity of *Glyptothorax telchitta* was similar to that of *N. rupicola* and *Botia dario.*

The slope of the regression line relating to body weight and diffusing capacity for the total gill arches in *Glyptothorax telchitta* was calculated to be 0.70083 which showed that with an unit increase in body weight, the diffusing capacity increases by a power 0.70083 (Table 1). As the slope value for diffusing capacity and body weight relationship in *G. telchitta* is less than one (=0.70083) the weight specific diffusing capacity increases with increase in body weight. Different gill arches also showed variations in the slope values of regression lines (Table 1). These differences in slope values of different gill arches suggested that the growth pattern in all the four gill arches were not in *G. telchitta* was found to be -0.2996 which approximates the value of *Botia dario* (-0.284; Singh *et al.,* 1988). In *Garra lamta,* the value was -0.12029, while the respective value for *N. rupicola* was 0.30059 (Rooj, 1984). This indicated that weight specific diffusing capacity in *N. rupicola* increases with body weight. Modified Fick's equation suggests *a way to* estimate the morphometric oxygen uptake. In *G. telchitta* was found to be -0.2996 which approximates the value of *Botia dario* (-0.284; Singh *et al.,* 1988). In *Garra lamta,* the value was -0.12029, while the respective value for *N. rupicola* was 0.30059 (Rooj, 1984). This indicated that weight specific diffusing capacity in *N. rupicola* increases with body weight. Modified Fick's equation suggests *a way to* estimate the morphometric oxygen uptake. Same was in *Garra lamta.* Dt = VO_2 □ K.A.

In *Glyptothorax telchitta,* the slope value for the total diffusing capacity was less than 1, so the total weight-specific diffusing capacity ΔPO_2 t

By rearranging the equation VO_2 is equal to Dt. ΔPO, where, VO = Oxygen uptake min^{-1} weight specific diffusing capacity decreased by a power of -0.29959 with unit increase in body weight. While comparing the slope values obtained for different gill arches, the efficiency of the fourth gill *arch was* found to decrease at a slower rate than rest of the gill arches (Table 1).

In *Garra lamta* and *Noemacheilus rupicola* the slope (b) value for total gill arches were 0.87771 and 1.34528 respectively. However, slope values of more than 1 were recorded for the first and second gill arches of *Garra lamta* (Rooj, 1984). The weight specific slope value for the total gill arches (m10.min-I), Dt = Diffusing capacity ($m10_2.min^{-1}.mrnHg^{-'}$) and ΔPO_2 = partial pressure gradient (mmHg). As no direct measurement is available fo ΔPO_2, by assuming the difference in $AP0_2$ of water and afferent blood as 100 mmHg, the $V0_2$ for 100g fish was estimated theoretically to be 1004.748 ($m10_2.Kg^{-1}.h^{-1}$). The corresponding values computed for the gill arches 1-4 (Table 2). The ΔPO_2 also changes with the ambient condition. Lamella consists of different lamellar channels and it has been argued that not all the channels except marginal ones are always engaged in active gaseous exchange.

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