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Gill Dimensions of a Hillstream Fish, *Botia lohachata* (Pisces, Cobitidae)

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The gill filaments borne by the hypobranchial and epibranchial elements in the anterior hemibranch of a gill arch are larger than the posterior hemibranch. The filaments are of similar size in the ceratobranchial region of the gill arch.

PAS and alcian blue positive acidophil cells occur in the gill head region and at the free ends of the gill filaments. The gill secondary lamellae are wedge-shaped structures in which the diffusion distance measures 1.71 μm. Comparative study on the four functional gills revealed a greater lamellar area and consequently a gill surface area for the first gill arch and a closer packing of the gill lamellae in the fourth arch.

Gill dimensions in relation to body weight were: Total gill area=913.3 $W^{0.699}$; Total length of gill filaments=661.5 $W^{0.281}$; Secondary lamellae/mm =77.96 $W^{-0.005}$; Average area of secondary lamellae=0.0175 $W^{0.414}$.

Key Words: *Botia lohachata*, **Respiratory surface area, Gill lamella**

Introduction

The gill surface area in a number of water-breathing teleosts has been examined and the relationship existing between various parameters of the gill and the body size has been established by a number of workers (Putter 1909, Price 1931, Gray 1954, Oliva 1960, Nowogrodzka 1963, Hughes 1960, 1966, 1972, 1976, Hughes & Gray 1972, Muir & Hughes 1969 and Niimi & Morgan 1980). Measurements on gill dimension among obligatory or facultative air breathing forms are known for *Anabas testudineus* (Saxena 1962 and Hughes et al. 1973), *Heteropneustes fossilis* (Hughe set al. 1974), *Macrognathus aculeatus* (Ojha & Munshi 1974) and gars (Landolt & Hill 1975).

Schottle (1931) measured the gill surface area of *Boleophthalmus boddaerti*, *B. viridis* and four species of *Periophthalmus*. The gill surface area of these and other fishes such as *Blennius pholis* (Milton 1971), *Mnierpes macrocephalus* (Graham 1973), *Periophthalmus cantonensis* and *Boleophthalmus chinensis* (Tamura et al. 1976) indicates that both inter as well as intraspecific variation occur in the respiratory surface area of the gill depending on their habit and habitat.

Information on gill dimension of hill stream fishes is scanty. Some of the loaches investigated so far are *Nemacheilus barbatus* and *Cobitis taenia* (Robotham 1979) and *Lepidocephalichthys guntea* (Singh et al. 1981). The present investigation aims at describing the gill dimension of a small-sized freshwater loach—*Botia lohachata* which inhabits torrential streams of Himalayas flowing through the territory of Nepal and north-eastern parts of India.

Material and Methods

Gills were fixed in aqueous Bouin's solution and their dimensions were measured by dividing each gill arch into different sections at an interval of 5 or 10 filaments. An average filament length was determined for each section. The total number of gill filaments and the number of secondary lamellae/mm occurring along the length of each filament were counted under Ermascope. The average bilateral surface area of a secondary lamella was determined with the help of camera lucida diagrams made on hand cut sections of the same from the tip, middle and base of a filament.

The gill area was calculated by employing the equation of Muir and Hughes (1969):

$$Gill\ area = 2\ (L/d')\ bl$$

where L=total length of filaments; $2/d'$=secondary lamellae on both sides of gill filaments; and bl=average bilateral surface area of the secondary lamellae

Measurements of the gill dimensions were made on 34 specimens of *Botia lohachata* (Cypriniformes, cobitidae) in the weight range of 0.33—4.5 g and body length 3.5–7.5 cm. The data were then reduced to ten average body weights. Various gill parameters (Y) such as total filament length, number of secondary lamellae/mm, average area of the secondary lamellae and total gill area were plotted on a double logarithmic grid against the body weight (W) and analysed using the equation,

$$Log\ Y = Log\ a + b.\ Log\ W$$

The morphological estimate of the diffusing capacity of the gill was obtained by employing the modified Fick equation (Hughes 1972).

$$Diffusing\ capacity = \frac{Vo_2}{\Delta Po_2} = \frac{K.A.}{t}$$

where Vo_2 = O_2 uptake; ΔPo_2 = driving force across the respiratory surface; K = Krogh (1919) permeation coefficient (= 0.00015 $ml/min/\mu m/cm^2/mm$ Hg) for connective tissue; A = functional or effective gill area (cm^2) i.e. gill area/g body weight; t=thickness of the barrier or water/blood pathway (μm);

In the equation, $\frac{Vo_2}{\Delta Po_2}$ gives the physiological estimate whereas $\frac{K.A.}{t}$ gives the morphological estimate of the diffusing capacity.

The fixation of the gills in aqueous Bouin's solution caused nearly 5% shrinkage and the data presented here for gill dimensions have not been corrected for this shrinkage. However, such a shrinkage did not affect the estimate for the number of secondary lamellae since its effects were compensated for automatically by a corresponding increase in the frequency of the secondary lamellae.

Histological preparations of the gill were made on materials fixed either in Zenker or Bouin's fluid. Paraffin sections of the gill (4–6 μm) were stained in haematoxylin-eosin, 0.02% toluidine blue in 0.1 m phosphate-citrate buffer (pH 3.0) for 30 min at 20°C (Spicer 1960), PAS (Pearse 1960) or PAS in combination with alcian blue (Mowry 1956). Blood

Table 1 *Measurements on gill parameters of* B. lohachata *(average weight 2.021 g)*

Gills	Filament length (mm)	Secondary lamellae/mm on one side	Length of inter branchial septum (mm)	Secondary lamellar width/blood channel length (mm) at three regions of the gill		
				1	2	3
1	1.53	38.25	0.74	0.189/0.303	0.222/0.328	0.083/0.265
2	1.52	38.22	0.72	0.173/0.319	0.187/0.365	0.076/0.294
3	1.40	39.19	0.69	0.116/0.355	0.112/0.328	0.114/0.309
4	1.24	39.90	0.57	0.131/0.291	0.161/0.330	0.158/0.355

supply of the individual secondary lamella was studied on thick frozen sections of the gill stained in Giemsa.

Results

The gill filaments borne by the hypobranchial and epibranchial elements in the anterior hemibranch of a gill arch are larger in comparison to the posterior-hemibranch but the filaments of both the hemibranchs are almost of similar size in the ceratobranchial region of the gill (figures 1–4).

The secondary lamellae arranged on either side of a gill filament are wedge-shaped structures. The lamellar length and width vary on different gill arches and even on the same gill arch depending on the position of the gill filament (table 1). Two afferent blood channels (marginal blood channels) run almost parallel at the outer margin of a secondary lamella and supply blood to it. Distribution of blood to the secondary lamella takes place through a number of capillaries—like branches arising from the marginal blood channels and running obliquely from afferent to efferent blood vessels.

The epithelium covering a secondary lamella remains separated by characteristic pillar cells (9.3×3.1 μm) and the spaces between them constitute the blood channels. The harmonic mean of the water-blood pathway is 1.71 μm and the morphological estimate of the diffusing capacity 0.08 ml/min/μm/cm^2/mm Hg.

Taste-buds are present in the gill head region but not in the interbranchial septum of the gill. Large number of mucous cells occur in the epithelial layer and acidophil cells in both the epithelial and sub-epithelial layers of the gill. Acidophil cells present near the free end of the gill filament differ in their staining properties when stained with PAS-alcian blue.

The results on gill dimension are summarised in tables 1–3. Expressions relating these to body size are set out in tables 4–5. In table 6, comparison of the slopes and intercepts alongwith their standard deviations for the different parameters of the four gill arches has been made. Gill area and other parameters of the gills of *B. lohachata* have been compared with those of other fishes (table 7).

The number of gill filaments increases with the body size (table 2) with an average of 533 occurring on a fish size of 2.02 g±0.43 or 5.83 cm±0.39. Other parameters of the gills such as the filament length, average bilateral area of the secondary lamellae, their number as well as the total gill surface area are directly correlated with the body size (figures 5 & 6). However, the increase/g body weight and the slopes of the regression lines are different for the various parameters referred to above (table 4).

For example, 0.699 is the slope for the gill area (mm²), 0.291 for the total filament length (mm) and 0.414 for the average area of the secondary lamellae.

The respective slopes of the regression lines for the weight specific gill area (gill area/g body weight) and the number of secondary lamellae/mm on both sides of a gill filament in relation to body weight (g) are −0.299 and −0.005 respectively. The weight specific gill area or the functional gill area and the density of the secondary lamellae (secondary lamellae/mm) are inversely related to body size.

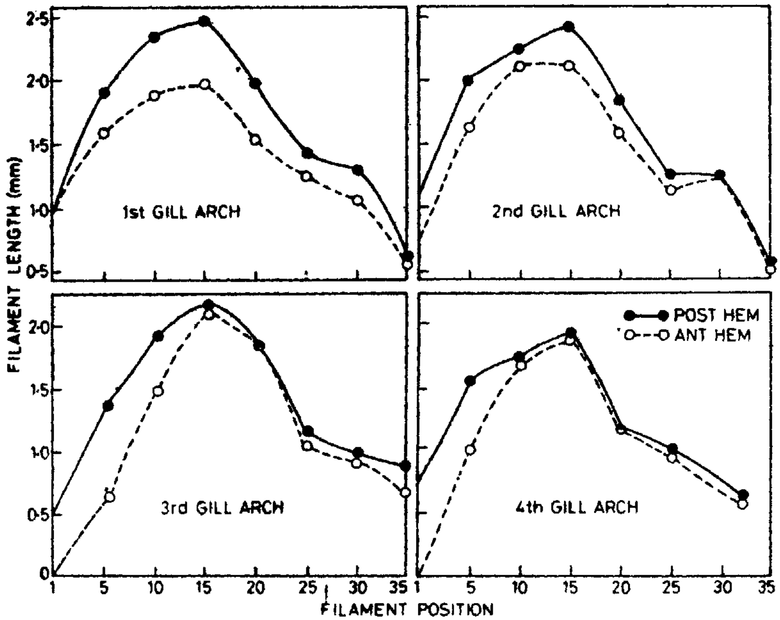

Figure 1–4 Filament length at different positions of gill arches 1–4

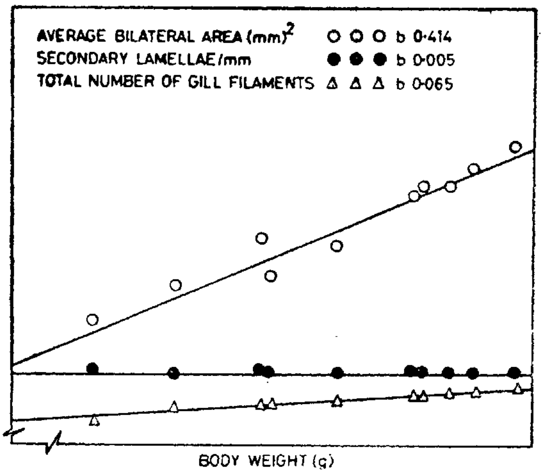

Figure 5 Log/log plot of average bilateral area, number of secondary lamellae/mm and total number of gill filaments in relation to body weight

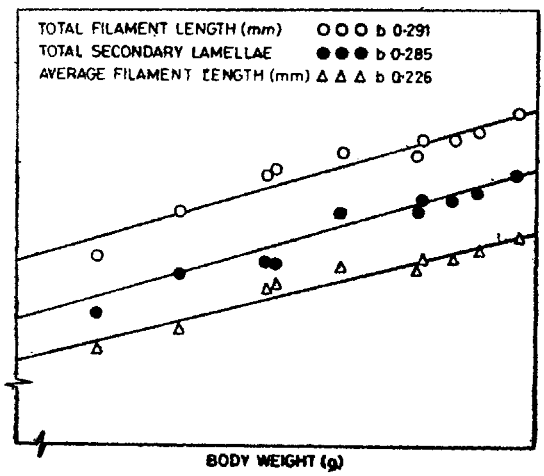

Figure 6 Log/log plot of average and total filament length and number of secondary lamellae in relation to body weight

Table 2 *Gill parameters of* B. lohachata

Body weight (g)	Body length (cm)	Total no. of filaments	Average length of filaments (mm)	Total length of filaments (mm)	Secondary lamellae/mm on both sides of a filament	Total no. of secondary lamella	Av. surf. area of a secondary lamellae $(mm)^2$	Total gill area $(mm)^2$	Gill area/g body weight mm^2/g
0.33	3.5	464	0.92	429.07	78.90	33855.62	0.011	375.79	1138.75
0.55	4.4	514	1.08	556.94	76.78	42765.87	0.014	623.14	1132.98
0.93	5.4	520	1.34	700.10	79.86	55916.48	0.019	1081.69	1163.11
1.0	5.0	526	1.36	717.74	77.43	55575.27	0.015	841.39	841.39
1.5	6.0	536	1.52	816.56	76.72	62654.14	0.019	1177.71	785.14
2.4	6.4	544	1.48	806.58	78.47	63296.97	0.025	1621.77	675.73
2.5	6.7	544	1.59	868.24	78.16	67866.77	0.026	1798.73	719.49
3.0	6.4	546	1.58	863.50	76.93	66438.00	0.027	1792.68	597.56
3.5	7.1	560	1.62	911.09	78.41	71440.88	0.029	2140.77	611.65
4.5	7.4	576	1.79	1032.37	76.14	78607.34	0.034	2681.17	595.81
20.2	58.3	5330	14.32	7702.23	777.85	598417.34	0.222	14134.86	8261.65
2.021	5.83	533	1.432	770.223	77.785	59841.734	0.022	1413.48	826.16
SD 1.382	1.241	30.437	0.262	139.399	1.166	1846.091	0.007	720.02	233.96
SE 0.437	0.392	9.625	0.082	44.082	0.369	583.799	0.002	227.69	73.98

Table 3 *Comparative parameters of the gills on the four different arches of* B. lohachata *(Body weight 0.33–4.5 Mean weight 2.02 ± 0.437 SE)*

Parameters	Gills			
	1	2	3	4
Total number of filaments	120–154 138±2.96	120—150 135±.263	120–148 136±2.40	104—128 121±2.04
Average length of filament (mm)	1.01–2.00 1.52±0.16	0.95—1.84 1.51±0.09	0.92—1.94 1.40±0.08	0.80—1.64 1.24±0.07
Total length of filaments (mm)	121.2—296.0 212.9±16.9	114.8—268.3 207.4±17.3	111.2—287.9 193.3±15.2	83.4—204.0 152.1±11.1
Secondary lamellae/mm on both sides of a filament	73.38—78.40 76.5±0.44	73.85—78.27 76.4±0.57	75.44—80.78 78.3±0.59	77.21—83.5 79.8±0.65
Total number of secondary lamellae	9,414—22,895 16,270±1,035	9,002—20,024 16,041±373.3	8,986–21,719 15,135±388.6	6,612—16,110 12,141±879.3
Average area of secondary lamellae	0.014—0.035 0.024±0.002	0.012—0.035 0.023±0.002	0.010—0.031 0.020±0.002	0.009—0.034 0.021±0.003
Total gill area (mm^2)	144.6— 734.5 416.3±66.2	115.8—714.5 392.7±62.3	92.0—678.6 334.8±55.6	64.1—551.03 281.0±48.9
Gill area/g body weight	163.2—438.2 248.9±26.5	158.7—351.1 232.5±22.7	105.3—297.9 198.0±20.1	113.9—241.1 160.1±15.4

Table 4 *Regression analysis of the different parameters of the gills in relation to body weight (g)*

Body wt. (W) vs	Equation	Correlation Coefficient
Number of filaments	$\text{Log } y = 2.7138+0.0651 \text{ Log } W$ $y = 517.4\ W^{0.0651}$	0.927
Average length of filaments	$\text{Log } y = 0.1066+0.2913 \text{ Log } W$ $y = 1.278\ W^{0.2913}$	0.961
Total length of filaments	$\text{Log } y = 2.8205+0.2913 \text{ Log } W$ $y = 661.5\ W^{0.2913}$	0.965
Secondary lamellae/mm	$\text{Log } y = 1.8919-0.0055 \text{ Log } W$ $y = 77.96\ W^{-0.0055}$	0.842
Total number of secondary lamellae	$\text{Log } y = 4.7124+0.2856 \text{ Log } W$ $y = 51570.0\ W^{0.2856}$	0.700
Average area of secondary lamellae	$\text{Log } y = -1.7548+0.4142 \text{ Log } W$ $y = 0.01758\ W^{0.4142}$	0.975
Total gill area	$\text{Log } y = 2.9606+0.6999 \text{ Log } W$ $y = 913.3\ W^{0.6999}$	0.987
Gill area/g body wt.	$\text{Log } y = 2.9577-0.2999 \text{ Log } W$ $y = 907.1\ W^{-0.2999}$	0.936

Table 5 *Regression analysis of the different parameters of the gills in relation to body length (cm)*

Body Length (L) vs	Equation
Number of filament	$\text{Log } y = 2.5429+0.2425 \text{ Log } L$ $y = 348\ L^{0.2425}$
Average length of filament	$\text{Log } y = -0.388+0.8419 \text{ Log } L$ $y = 0.407\ L^{0.8419}$
Total length of filament	$\text{Log } y = 2.0552+1.0844 \text{ Log } L$ $y = 113.6\ L^{1.0844}$
Secondary lamellae/mm on both sides	$\text{Log } y = 1.8223-0.01603 \text{ Log } L$ $y = 66.42\ L^{-0.01603}$
Total secondary lamellae	$\text{Log } y = 3.9605+1.0652 \text{ Log } L$ $y = 9131.0\ L^{1.0652}$
Average area of secondary lamellae	$\text{Log } y = -2.76+1.4252 \text{ Log } L$ $y = 0.0017\ L^{1.4252}$
Total gill area	$\text{Log } y = 3.9792+2.5609 \text{ Log } L$ $y = 9532.0\ L^{2.5609}$
Gill area/g body wt.	$\text{Log } y = 3.6578-1.0002 \text{ Log } L$ $y = 5447.0\ L^{-1.0002}$

Table 6 *Comparison of the slopes (b) and intercepts (log a) alongwith their standard deviations (Sb, Sa) for the different parameters of the four gill arches (1–4) of* Botia lohachata

Parameters	Gill 1	Gill 2	Gill 3	Gill 4
Total number of filaments	0.076 ±0.0267 2.1248±0.0094	0.069 ±0.0241 2.1237±0.0085	0.061 ±0.0221 2.1227±0.0078	0.049 ±0.0220 2.0745±0.0077
Average filament length	0.226 ±0.0689 0.1338±0.0924	0.244 ±0.0843 0.1277±0.0296	0.222 ±0.0812 0.0979±0.0285	0.221 ±0.0836 0.0453±0.0294
Total length of filaments	0.294 ±0.1052 2.2601±0.0370	0.314 ±0.1110 2.2512±0.0391	0.284 ±0.1022 2.2206±0.0360	0.276 ±0.1015 2.1187±0.0357
Secondary lamellae/mm	−0.007 ±0.0076 1.8850±0.0026	−0.007 ±0.0093 1.8845±0.0032	−0.005 ±0.0094 1.8956±0.0033	+0.002 ±0.0100 1.9014±0.0035
Total number of secondary lamellae	0.297 ±0.1033 4.1432±0.0363	0.301 ±0.1028 4.1369±0.0362	0.277 ±0.0999 4.1160±0.0351	0.272 ±0.0100 4.0213±0,0351
Average bilateral area of secondary lamella	0.359 ±0.1240 −1.7027±0.0436	0.373 ±0.1306 −1.7277±0.0459	0.395 ±0.1423 −1.7802±0.0501	0.480 ±0.0998 −1.7875±0.0351
Total gill area	0.654 ±0.2208 2.4406±0.0777	0.675 ±0.2282 2.4092±0.0803	0.674 ±0.2358 2.3363±0.0830	0.753 ±0.1741 2.2338±0.0613
Gill area/g body weight	−0.345 ±0.1207 2.4406±0.0425	−0.323 ±0.1579 2.4090±0.0556	−0.325 ±0.1295 2.3363±0.0456	−0.245 ±0.1105 2.2341±0.0389

Table 7 *Comparison of the gill parameters in some water-breathing fishes*

Fish species	Number of sec. lamellae/mm on one side for 1 g fish	Gill area for 1 g fish (mm²)	Gill area/g body weight	Slope of the gill area	References
Micropterus dolomieu		865	330	0.78	Price 1931
Gray's intermediate		1392	399	0.82	Ursin 1967
Katsuwonus pelamis	30.43	5218	1320	0.85	Muir & Hughes 1969
Opsanus tau	15.99	560.7	214	0.79	Hughes & Gray 1972
Cabitis taenia	45.50	—	507.9	—	Robotham 1979
Nemacheilus barbatus	36.40	—	316	—	,,
Stizostedion vitreum vitreum		224.6	—	1.07	Niimi & Morgan 1980
Salmo gairdneri (Richardson)	—	156.2	—	1.05	,,
Botia lohachata	38.72	913.3	907	0.69	Present authors

Comparative measurements on various parameters of the four gill arches are given in (table 3). The first two gill arches possess almost equal number of gill filaments and secondary lamellae but the total surface area of the first gill arch is greater (416.3 mm²) than the second (392.7 mm²). The surface area and consequently the surface area/g body weight is highest in the first (416.3 mm²) and lowest in the fourth gill arch (181.05 mm²). Further, the total filament length is largest in the first (212.9 mm) and smallest in the fourth gill arch (152.1 mm). However, the number of secondary lamellae/mm on both sides of a gill filament is greater in the fourth (79.8) than the first gill arch (76.5). The slopes for the gill area, average area of the secondary lamellae and the number of secondary lamellae/mm are comparatively higher in the fourth gill arch than the others (table 6). However, these slopes do not differ significantly from one another as their standard errors are larger than the difference in their regression coefficients.

Discussion

Taste-buds present in the gill-head region are advantageous for the fish as they come first in contact with the circulating water in the branchial chamber and help determine the chemical nature of the food and water passing over it. However, taste-buds are lacking from the gill filaments unlike that of *Lepidocephalichthys guntea* (Singh 1966, 1969) belonging to the same family.

Muir and Brown (1971) reported the blood channel length in the middle of the secondary lamella in a number of teleosts to vary from 0.25 to 2.4 mm and the lamellar width from 0.12 to 0.86 mm. In *Botia lohachata*, the marginal (peripheral) blood channel length varies from 0291 to 0.365 mm and the secondary lamellar width from 0.076 to 0.222 mm. These figures are well comparable to other water breathing teleosts and stand closer to those reported for another cobitid fish, *Lepidocephalichthys guntea* (Singh et al. 1981).

Morphological estimate of the diffusing capacity (0.08 ml) is similar to other cobitids but is greater in comparison to the gills of other Indian teleosts. For example, 0.08 ml is known to be diffusing capacity for the gills of *Lepidocephalichthys guntea* (Singh et al. 1981), 0.02 ml for *Saccobranchus fossilis* (Hughes et al. 1974) and 0.014 ml for *Anabas testudineus* (Hughes et al. 1973).

Adaptation in the gills of *Botia* for an active mode of life in a hill stream habitat is well documented by presence of a large number of secondary lamellae/mm (38.9/mm) on either side of a gill filament and consequently a greater respiratory surface area of the gill in comparison to other water breathing fishes (table 6). The number of gill filaments (533) is also greater in comparison to that of *L. guntea* (Singh et al. 1981). The length of a gill filament is lower (1.4322 mm) in comparison to that of *Lepidocephalichthys* (1.473 mm), *Saccobranchus* (2.42), *Anabas* (1.70) and the gars (4.47). The number of filaments as well as their length in *Botia* though not well correlated with increase in the body length, are not independent of fish length as reported by Robotham (1979) for loaches.

The number of secondary lamellae/mm on each side of a gill filament of the fish is higher in comparison to *L. guntea* (Singh et al. 1981) and three species of tunny (Muir & Hughes 1969). Hughes (1966) reported the number of secondary lamellae/mm on each side of a gill filament to be 31 in active and pelagic mackerel, 11–12 in sluggish and benthic species and 22 in Gray's intermediates. The number of secondary lamellae in *Botia lohachata* indicates an active mode of life for the fish. Still greater number of secondary lamellae/mm in the two species of cobitids, *Cobitis taenia* and *Noemachelius barbatus* have been reported by Robotham (1979). The greater number of secondary lamellae/mm (39.9) in the fourth gill arch than the first (38.25) indicates their spacing more apart in the latter and closely packed together in the former.

The bilateral surface area of a secondary lamella in *Botia lohachata* is almost similar to gars (Landolt & Hill 1975) but is smaller than *L. guntea* (Singh et al. 1981), *Saccobranchus fossilis* (Hughes et al. 1974) and *Anabas testudineus* (Hughes et al. 1973). Further, the total gill area of the fish is greater in comparison to many water and air breathing fishes but is less than *Thunnus* (Muir & Hughes 1969) and *Coryphaena hippurus* (Hughes 1970).

The slope of the regression line for the gill area (0.699) does not differ much from that of water or air breathing teleosts. The gill area/g body weight is higher in smaller specimens and suggests a higher metabolic rate for the younger fishes. Price (1931) also suggested a higher metabolic rate correlated with higher weight specific gill area for the young *Micropterus*. A higher gill area/g body weight in the weight range of 0.33 to 1.5 g which is normal average size of the fish usually found abundantly in nature, seems to be related to the efficiency of the gill required for higher metabolic activity in an oxygen-rich hill stream.

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