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Studies on blood volume in a teleostean fish, *Mystus bleekeri* in relation to body weight in summer and winter seasons

***Akash Garain and Rajendra Mistry**

Department of Zoology,
St Columba's College ,
HAZARIBAG (JHARKHAND) INDIA
*Corresponding Author
Email-akashgarain@gmail.com

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ABSTRACT

The total blood volume, relative (%) blood volume and haematocrit value of *Mystus bleekeri* of six different weight groups ranging from 24 to 76 grams were studied in winter and summer months at water temperatures of 18°C and 28°C. The total blood volume at both temperatures increased from lower to higher weight groups. The correlation coefficient between total blood volume and body weight were 0.8587 at 18°C and 0.9946 at 28°C. Total blood volume in all weight groups was greater at the higher temperatures and most results were statistically significant. The relative blood volume and haematocrit decreased from lower to higher groups at both temperatures.

Figures : 03 References : 18 Tables : 03
KEY WORDS : Blood volume, *Mystus bleekeri,* Summer, Winter months

Introduction

The direct method for blood volume determination was first employed[18] in 1858 but it is only recently that improved techniques have been devised to study the blood volume of fishes[8]. Effects of sex and maturity on blood volume in) *Chimaera colliei*, *Squalus sucklii* and Raja species[2] were investigated. The blood volume of wild and pond carps and the blood volume of Salmonids[4,7] were measured.

Workers[10] considered wider parameters and compared the blood volume in animals with open and closed circulatory systems . Another worker[17] while studying the body fluids in Osteichthyes discussed their phylogenetic and ecological implications in aquatic vertebrates and measured the fluid compartments in sea lamprey and marine Chondrichthyes. The effects of environmental temperature and thermal acclimatization on the body fluid and blood volume of fishes have received relatively little attention from fish biologists. The works[6] on environmental temperature and the body fluid systems of freshwater teleosts and on the effect of exercise on the distribution of blood to various organs of rainbow trout are of special interest .

In aquatic animals like fishes, gaseous exchange takes place between blood and water through the gills and body surfaces. In some tropical freshwater fishes special structures have evolved in response to exceptional environmental conditions. The accessory respiratory organs enable the fish to tolerate oxygen depletion in water or to live out of water for short periods. The blood of terrestrial animals and fishes function as carrier of respiratory gases mainly bound to haemoglobin in erythrocytes and also dissolved in blood plasma[1] Therefore, not only the haemoglobin concentration per unit volume of blood, but also the determination of total blood volume of a particular animal is important. It is of much interest for respiratory physiologists to know if there is any relationship between total blood volume and respiratory surface area of fishes.

The present investigation on *Mystus bleekeri* was undertaken to study the effect of seasonal temperature on the absolute and relative blood volume and its relationship with the body weight.

Material and Methods

Fishes of different size and weight were collected from the ponds of Hazaribag but mostly though the local fish dealers. They were transported to laboratory and kept in glass aquarium. The animals were kept under intensive care and were fed on chopped goat liver. Weights of each group were maintained almost constant in the laboratory.

Quantitative measurements of blood volume were made during November when the average water temperature of aquaria was 18°C, and in April when the average water temperature rose to 28°C. Fishes of different sizes were divided into six weight groups *viz.,* 24-25 g,30-

TABLE- 1 : ***Mystus bleekeri:*** **relationship between blood volume and haematocrit value at water temperature of and with significance level (t-test) for different weight groups**

Parameter	**At 18º water temparature**		**At 28º water temperature**		
	Mean body weight(g)	**T.B.V(ml)**	**Mean body weight(g)**	**T.B.V(ml)**	**Value of P**
Total Blood Volume	25.0 (1-.080)	0.356(0.056)	24.5(0.645)	0.487(0.050)	P 0.025(S)
	30.0 (1.235)	0.460(0.050)	31.0(0.504)	0.563(0.054)	P 0.050(S)
	42.0 (0.590)	0.642(0.061)	41.0(1.235)	0.654(0.566)	P 0.500(NS)
	53.0 (1.470)	0.824(0.060)	50.0(0.925)	0.738(0.057)	P 0.100(NS)
	61.0 (1.080)	0.872(0.039)	62.0(1.130)	0.966(0.067)	P 0.050(S)
	73.0 (0.925)	0.970(0.800)	76.0(1.585)	1.215(0.037)	P 0.001(HS)
Relative Blood volume (per 100g body weight)	25.0 (1-.080)	1.424(0.224)	24.5(0645)	1.948(0.200)	P 0.010(S)
	30.0 (1.235)	1.533(0.166)	31.0(0.405)	1.816(0.175)	P 0.100(NS)
	42.0 (0.590)	1.528(0.146)	41.0(1.235)	1.595(0.136)	P 0.500(NS)
	53.0 (1.470)	1.526(0.111)	50.0(0.925)	1.466(0.127)	P 0.500(NS)
	61.0 (1.080	1.429(0.069)	62.0(1.130)	1.541(0.106)	P 0.250(NS)
	73.0 (0.925)	1.328(0.121)	76.0(1.585)	1.598(0.049)	P 0.010(S)
	Mean body weight	Haematocrit	Mean body weight(g)	Haematocrit	
Haematocrit value (%)	25.0 (1-.080)	42.0(1.260)	24.5(0645)	46.7(1.235)	P 0.050(S)
	30.0 (1.235)	42.0(0.980)	31.0(0.405)	41.5(1.165)	P 0.500(NS)
	42.0 (0.590)	38.0(1.365)	41.0(1.235)	39.0(0.915)	P 0.500(NS)
	53.0 (1.470)	36.5(1.215)	50.0(0.925)	38.0(1.215)	P 0.250(NS)
	61.0 (1.080	33.0(0.930)	62.0(1.130)	36.0(1.585)	P 0.025(S)
	73.0 (0.925)	29.0(1.600)	76.0(1.585)	33.0(0865)	P 0.050(S)

T.B.V= Total blood volume; D=Significant; HS=highly significant and NS=Non significant. Figures in brackets show standard error (+)

TABLE- 2 : *Mystus bleekeri* correlation coefficient and equations showing the blood relationship of blood volume and body weight at different temperatures

Parameter analysed	Equations	Correlation coefficient(r)
1. Body weight vs. total blood volume at28°C $\pm$ 1°C.	$\log Y = -1.4305 + 0.7880 \log W$ or $\log Y = 0.371\ W$	0.997
2. Body weight vs. total blood volume at18°C $\pm$ 1°C.	$\log Y = 1.5143 + 0.8571 \log W$ Or $\log Y = 0.0243\ W$	0.8567
3. Body weight vs. total blood volume per gram body weight at28°C $\pm$ 1°C.	$\log Y = 1.4300 + 0.8157 \log W$ Or $\log Y = 0.0371\ W$	-0.8157
4. Body weight vs. total blood volume per gram body weight at18°C $\pm$ 1°C.	$\log Y = 1.6707 + -0.1059 \log W$ Or $\log Y = 0.02131\ W$	-0.5087

31 g, 41-42g , 50-51g, 61-62 g and 73-76 g. Four animals from each weight group were used for these experiments.

The blood volume of fishes has been measured by the following methods, (a) bleeding and haemoglobin washout[2,7,18] (b) Evans blue (T-1824) and haematocrit[1,8,10,13,17] (c) albumin I^{131} and haematocrit[1] d) albumin I^{131} and Cr^{51} labelled red blood cells.

In the present investigation blood volume was determined by dye dilution technique using Evans blue (T-1824) and haematocrit. The fishes were anaesthetized[2] by MS 222 (Sandoz) and were dissected carefully to expose the heart without causing any injury to either heart or blood vessels. In making an estimation of blood volume, a sample of blood was obtained before dye injection in order to determine plasma colour and haematocrit value. Then a known amount of Evans blue together with sodium citrate as an anticoagulant was injected into the bulbus arteriosus. The dye was left to diffuse properly within the blood stream and a blood sample was taken 30 minutes after injection. The concentration of dye in the plasma was measured by photoelectric colorimeter and from this value, plasma volume was determined. The haematocrit value gives the relative volumes of plasma to corpuscles and from these data the total blood volume of a particular fish was calculated[3].

Worker[13] injected T-1824 directly into the circulation of *Oncorhynchu kisutch* 0. nerka and Salmo gairdneri and allowed a minimum mixing time of 60 minutes. Without any major operative procedure, it is extremely difficult to inject the dye directly into the circulatory system of *Mystus bleekeri.*

A worker suggested that the dye could be injected into the sinus of fishes[4] but the author failed to achieve consistency with this method, even in the same weight groups. The present method of injecting the dye into a living fish in air probably has little effect on blood volume estimation in this species as these air-breathing fishes can live out of water for quite some time provided they are kept moist. Further, the plasma volume calculated for 30 and 60 minutes were identical and in the anesthetized condition the excretion slope of dye was consistent for 30 minutes. Therefore, a minimum mixing time of only 30 minutes was allowed in the present investigation. The extent of any shock reactions was not evaluated.

The results obtained for total blood volume, relative (%) blood volume and haematocrit values at 18°C and 28°C were compared. Multiple (Student's) t- tests were carried out to study the level of significance.

The blood volume data of the present work were calculated around the body weight of 10, 20, 30, 40, 50, 60, 70, 80 and 90 grams. The coefficients of regression and correlation were computed through logarithmic transformations of data to study the relationship existing between total blood volume *vs* body weight

TABLE- 3 : *Mystus bleekeri:* total blood volume per gram body weight in weight group of at 18ºC±1ºC and 28ºC±1ºC.

Mean body weight(g)	Total blood volume(ml) per gram body weight at 18ºC± 1ºC	Mean body weight (g)	Total blood volume(ml) per gram body weight at28ºC±1ºC
25	0.0142	24.5	0.0198
30	0.0163	31	0.0181
42	0.0152	41	0.0159
53	0.0155	50	0.0147
61	0.0126	62	0.0155
75	0.0132	76	0.0159

Results

A. Total Blood Volume and Body Weight: At water temperatures of both 18°C and 28°C, the total blood volume increased from lower to higher weight groups (Table- 1). When the total blood Volume of corresponding weight groups at both temperatures were compared, it was found that the total blood volume at 18°C was less than at 28 C. The results for body weights of 24 25g, 30 -31 g and 61-62g (Table-1) were found to be significantly different (P <0.025, 0.050 and 0.050 respectively). The results for the highest body weight group (73-76 g were highly significant (P<0.001) while the results for 41 -42 g and 50 - 53 g body weights were non-significant.

Mean values of total blood volume and body weight

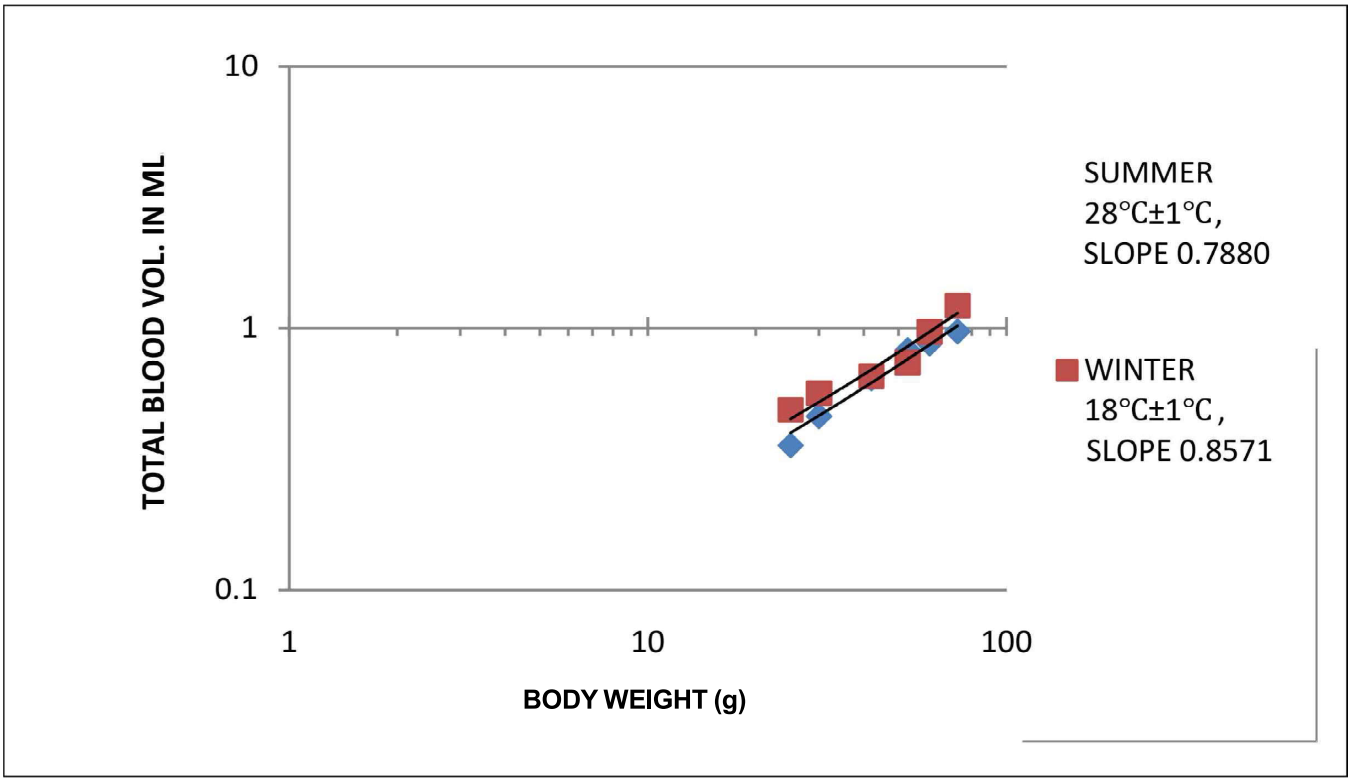

Fig. 1: Total blood volume plotted against body weight at 28ºC± 1ºC and 18ºC± 1ºC water temperature on log/log co-ordinates. Each point for given weight group

were plotted for each weight group on log co-ordinates. Straight lines were fitted by the least square regression method for both temperatures (Fig. 1). The regression line had a slope (b) of 0.857 at (18°; 28°) and 0.788 at 28 (Table-2). The relationship between total blood volume (Y) and body weight (W) followed the general equation ã= aW^b or log Y=log a+b log W (Table-2).

With unit increase in the body weight of *Mystus bleekeri,* the total blood volume increased by a fractional power of 0.857 at 18° C and 0.788 at 28°C Table-2). The coefficient of correlation (r) has been calculated to be 0.8587 at 18°C and 0.9947 at 28°C. It is evident that high degree of correlation exists between the total blood volume and body weight at both temperatures but is greater at the higher temperature.

B. Relative (%) Blood Volume (RBV) and Body Weight: The relative or percentage blood volume has been calculated for each weight group separately from actual observations that the measured blood volume of each fish was converted to a value for 100 g body weight.

The relative (%) blood volume for the blood volume for different weight groups at 18°C and 28°C is given in Table-1. With very few exceptions,relative blood volume decreased from lower weight groups to higher weight groups at both temparatures.When the RBV for the two temparatures were compared,the value at 18°C appeared to be less than that at 28°C.The results for the lowest weight group(24-25g) and for the highest weight group(73-76 g) were significantly different(P< 0.010 and 0.010 repectively) but difference between other weight groups were non-significant (Table-1).

The blood volume per gram body weight at both temperature was calculated for each weight group(Table 3).When these values were plotted against the respective weight groups on log/log co-ordinates ,a straight line was obtained (Fig.2, Table 2).The coefficients of correlation(r) were 0.5087 at 18°C and 0.8157 at 28°C.

C.Haematocrit and body weight: The Haematocrit or packed cell volume is the relative volume of corpuscle to plasma and is usually expressed as a percentage.

At both temperatures haematocrit decreased from the lower to the higher weight groups (Table-1). At 18°C all the haematocrit values were found to be lower than those at (Table-1, Fig. 3). Only the results for weight groups 24-255. 61-62 g and 73-76 g showed a significant difference between values for the two temperatures (P<0.050, 0.025, and 0.050 respectively).

Discussion

One of the main results of the present investigation was to show that the total blood volume of *Mystus bleekeri*

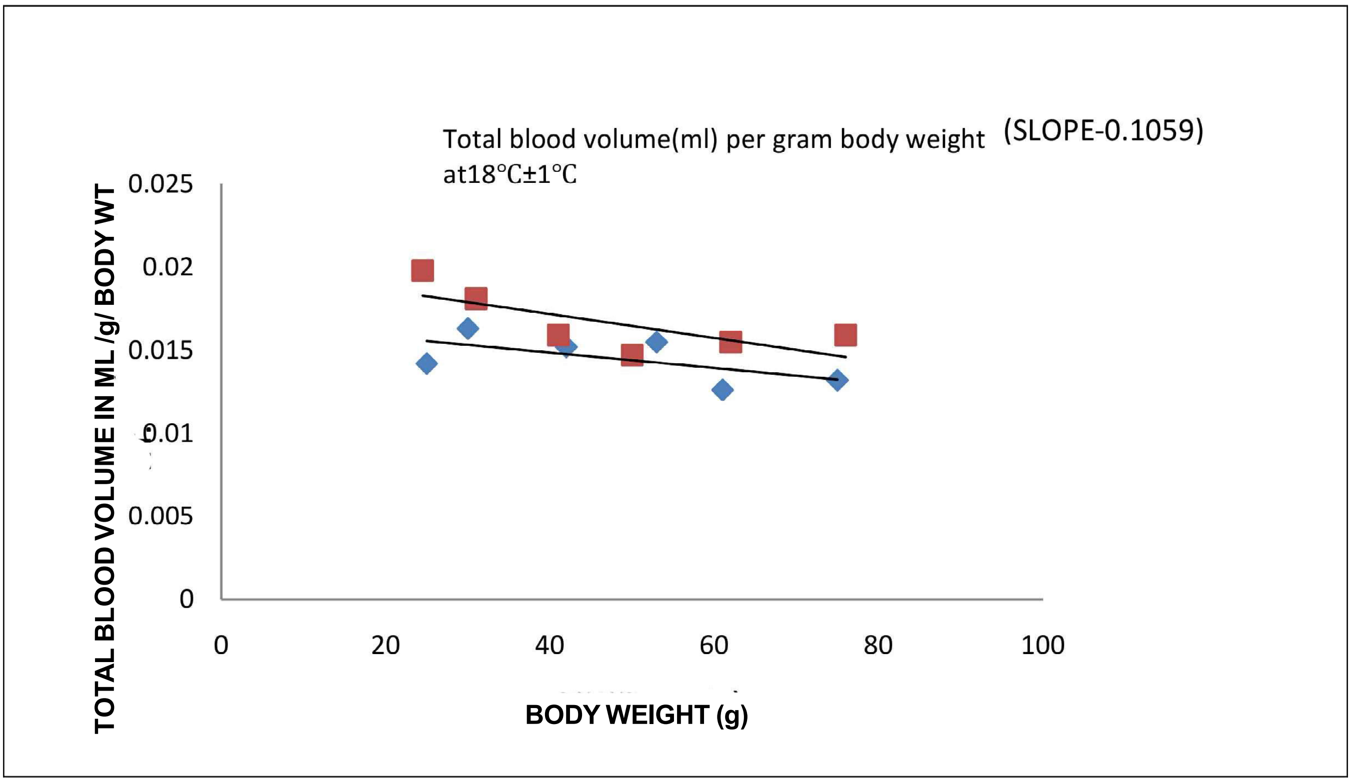

Fig. 2 : Showing blood volume of *M.bleekeri* per gram body weight at 28ºC± 1ºC and 18ºC± 1ºC water temperature plotted against body weight on log/log co-ordinat

increased with body weight during both the winter and summer months, but that the relative volume (*i.e.* blood volume/100 g body weight) and also the haematocrit decreased both in the winter and summer months. Similar observations have been made earlier[16] for humans and they reached the conclusion that there was a definite relationship between blood volume and age[14]. Further, they observed that "in infants the blood volume was greater in proportion to the body weight than in the adult" and the blood volume/unit body weight decreased with age in birds. It is well known that the metabolic rates for younger animals are higher in most vertebrates when expressed/ unit body mass. Thus the relationships between blood volume and metabolic rate against body mass showed similar trends both in Heteropneustes and higher vertebrates. Workers[4] observed in *Anabas testudineus* and in *H. fossilis* higher values for the erythrocyte count and haemoglobin concentrations in younger fish when expressed/gram body weight[15,16].

It has also been found that the total blood volume of *Mystus bleekeri* is greater in summer than in winter (Table-1). The t-test indicated significant differences for most of the weight groups. Like total blood volume, blood volume/unit body mass and haematocrit for different weight groups were greater than at lower water temperatures. Environmental conditions such as high temperature, muscular exercise and emotional excitement cause increase in blood volume and in the case of acute exposure to cold, the total water content of the body remains unaltered, whereas there is loss of water from the blood to the tissues[16]. There was difference in the blood volume of thermally-acclimatized *Salvenlinus fontinalis*[6].

Differences in total blood volume between different weight groups of fishes, however was not uniform during the winter and summer months. The correlation coefficient (r) between total blood volume and body weight was higher (09947) at 28°C than at 18°C (0.8587) but the slope of the regression line (b) was less (0.7880) at 28°C than at 18°C (b=0.8571). The change in blood volume stimulated by the environmental temperature might be regarded as a physiological adjustment to general metabolic compensations in these poikilothermic animals.

There was a higher blood volume in gravid females. The normal breeding season of July-August and these experiments were carried out in November and April. From the maturity index, it may be concluded that the specimen investigated were not gravid.

The average relative of percentage blood volume in *Mystus bleekeri* at 18°C was 1.461 ml (1.328-1.533) and 1.660 ml (1.466-1.948) at 28°C, which is quite low compared to the findings of other authors for other marine and freshwater teleosts. The present values for relative blood volume are in agreement with earlier observations

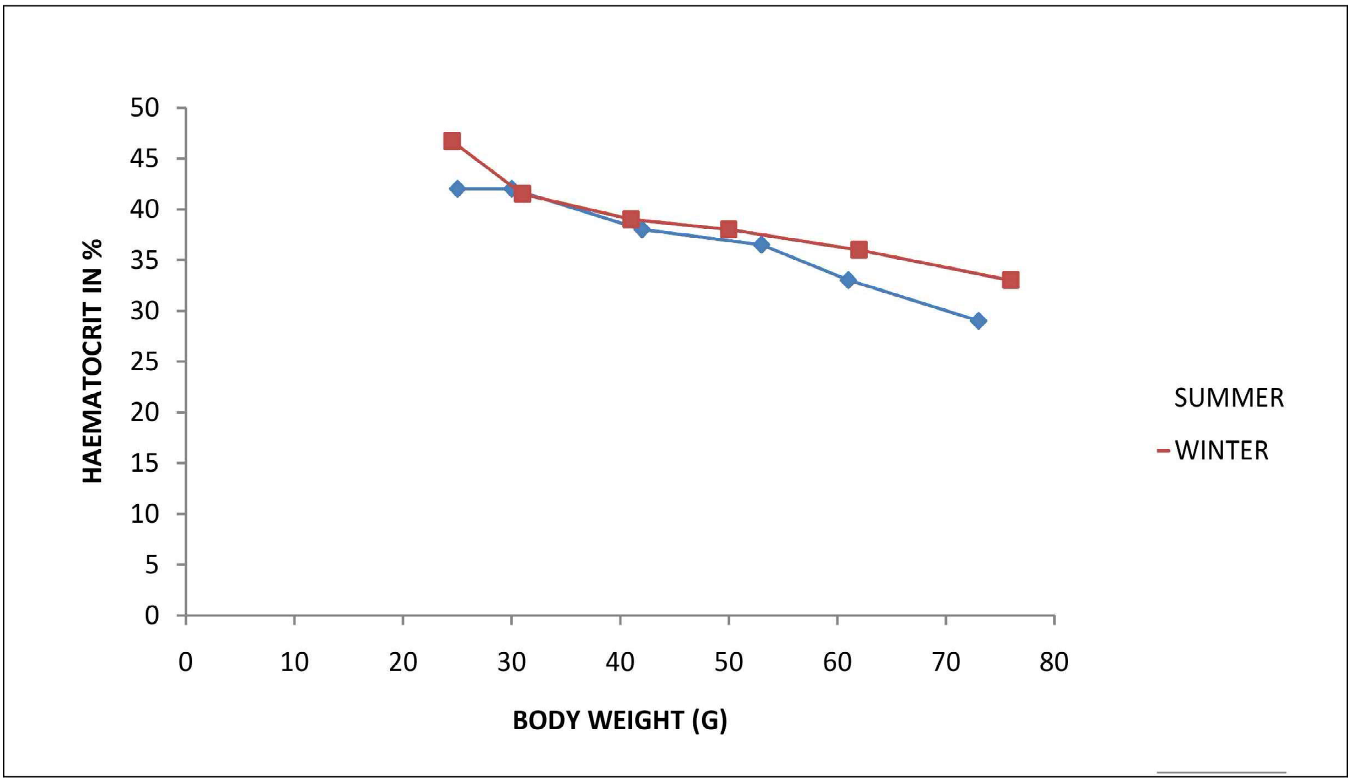

Fig. 3: *Mystus bleekeri* : Plot of haematocrit values against body weight(S.E) at 28ºC± 1ºC and 18ºC± 1ºC water temperature

in *Cyprinus tinca* (1.78%) and *Perca fluviatilis* (1.28%) determined by bleeding the animal[18] and for *Ameiurus natalis* (1.26%) using the T-1824[10]. Other investigators reported blood volume to be approximately 3% of body weight in trout, cod and carpas[1,7,17]. It was observed that the blood volume of *Condrichthyes* was about 6.6% of body weight, whereas the blood volume of *Chondrostei*, *Holostei* and *Teleostei* was approximately 3% of body weight and the evolutionary trend among fish appeared to be towards lower blood volumes.

The explanation for such an evolutionary trend may be associated with the development of a more rapid circulation of the blood and is accompanied by more efficient mechanisms for the return of venous blood from the time. A smaller blood volume and shorter circulation time would also speed up all functions which depend upon substances transmitted in the blood. The oxygen-carrying properties of blood will be one of these functions and must be associated with adaptive changes in the properties. The precise way in which these functions are adapted among Indian air breathing fish has not yet been fully investigated. The relatively high haematocrit and consequently high oxygen-carrying capacity would fit with such an interpretation. The low values for blood volume obtained in the present work would be suspected now a days if they were obtained with water-breathing telcosts and in the absence of cannulation procedure. Until these results have been confirmed using cannulated fish, which will be difficult for this species, perhaps the absolute values should be treated with some caution, even though results obtained using the direct method are almost identical. Nevertheless the trends indicated, especially in relation to body weight and temperatures are almost certainly valid.

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