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Erythrocyte Count and Haemoglobin Concentration of some Tropical Fishes

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Table 1: Indian marine fishes : Erythrocyte and leucocyte counts and haemoglobin concentration. Each value represents the mean (±SD) of a minimum of 10 values obtained from 4 to 5 individuals.

Sex and Weight (g)		Erythrocytes (million/mm³)	Leucocytes (thousand /mm³)	Haemoglobin (%; ml/100 ml)
			Boleophthalmus boddaerti	
Male	(31 g)	3.48±0.23	15.20±0.48	16.3±1.2
Female	(30 g)	2.96±0.19	15.11±1.01	15.2±0.6
Young	(2 g)	2.66±0.38	15.23±0.86	12.8±1.8
Mean		3.03±0.27	15.18±0.78	14.8±1.2
			Therapon jarbua	
Male	(35 g)	2.86±0.22	14.38±0.79	8.9±1.2
Female	(37 g)	2.43±0.22	15.10±1.00	8.4±1.7
Young	(4 g)	2.16.±0.18	14.96±0.56	6.9±0.9
Mean		2.48±0.21	14.81±0.78	8.1±1.3
			Rastrelliger kanagurta	
Male	(62 g)	2.64±0.23	15.00±1.23	7.6±2.3
Female	(50 g)	2.30±0.19	14.92±0.99	7.5±1.1
Young	(5 g)	2.19±0.32	15.16±0.35	7.0±0.9
Mean		2.38±0.25	15.03±0.86	7.4±1.4

Table 2: *Boleophthalmus boddaerti* : Changes in blood properties of the male (30 g) forced to aquatic or aerial breathing. Each value represents the mean (± SD) of a minimum of 10 values obtained from 3 to 4 individuals.

Period (hr) after forced to	Erythrocytes (million / mm³)	Leucocytes (thousand / mm³)	Haemoglobin (%; ml / 100 ml)
	In individuals allowed to breathe in water and air		
	3.48±0.23	15.20±0.48	16.3 ± 1.2
	In individuals allowed aquatic breathing alone		
12	3.28±0.48	15.26±0.58	13.0±1.4
24	3.10±0.26	15.48±0.63	11.9±1.6
36	3.22±0.62	15.21±0.80	12.6±2.2
48	3.30±0.50	15.40±0.67	11.8±1.9
	In individuals allowed aerial breathing alone		
12	3.60±0.44	14.98±0.86	11.0±1.2
24	3.46±0.46	15.36±1.10	12.1±2.0
36	3.32±0.51	15.21±0.85	11.3±1.6
48	3.35±0.33	15.22±1.13	11.3±1.3

Table 3: *Tilapia mossambica* : Changes in blood properties of individuals (6 g) exposed to different physiological stress of salinity and Po_2. Each value represents the mean (± SD) of a minimum of 10 values observed for 4 to 5 individuals.

Physiological stress	Erythrocytes (million /mm³)	Haemoglobin (%; ml/100 ml)
	Individuals exposed to salinity	
0.3%	3.18±0.40	7.2±0.26
7%	2.86±0.31	7.1±0.58
14%	2.53±0.12	7.0±0.81
21%	2.18±0.20	6.8±0.70
28%	2.22±0.13	7.3±0.65
	Individuals exposed to Po_2 of	
152 mm Hg	3.06±0.23	7.0±0.85
98 mm Hg	3.11±0.30	6.9±0.41
76 mm Hg	2.89±0.18	8.6±0.39
48 mm Hg	3.03±0.25	8.1±0.42
35 mm Hg	2.76±0.22	8.5±0.44

fishes such as *Tilapia* spp. (Badawi and Said, 1971) and Labeo spp. (Hattingh, 1973). Such correlations were also apparent in the marine species *B. boddaerti* (r = 0.756; P 0.02), *T. jarbua* (r = 0.805; P 0.01) and *R. kanagurta* (r = 0.788; P 0.02) also.

In all three species studied, values obtained for blood properties such as erythrocyte counts haemoglobin concentration were the highest for males, intermediate for females and least for juveniles. The reason for the sex-dependent changes in the blood properties is not at present clear. With increasing body weight, the gill area per unit weight of the fish is known to decrease; for instance, the area decreased from 440 mm^2/g live weight in a 3 g rockskipper *Mnierpes macrocephalus* to 295 mm^2/g in a 6 g individual (Graham, 1973). The larger individuals may possibly compensate the decreasing gill area by increasing erythrocyte counts and haemoglobin concentration.

Leucocyte counts of the fishes studied averaged 15,000/mm^3. Surprisingly, very few authors have given total leucocyte counts for fishes (Farghaly *et al.*, 1973; McLeay, 1975). Total leucocyte counts differ very little with changes in body weight, size and sex in the 3 species (Table 1), and when *B.boddaerti* was forced to breathe only in water or air (Table 2).

Boleophthalmus boddaerti:

With the view to study the changes in the blood characteristics of the mudskipper, which were restricted to aquatic breathing alone, healthy male individuals (31 g) were kept in aquaria containing aerated sea water (24% S; 30°C); suitable plastic wire meshes were introduced into the aquaria so as to prevent the individuals from breathing atmospheric air. To force *B. boddaerti* to breathe only from the atmospheric air, a few other individuals were rolled in wet cloth with their heads exposed, (sea water, 24% S) and kept in terraria at 30°C.

As mentioned earlier, the erythrocyte counts and the haemoglobin concentration were 3.5 million/mm^3 and 16.3% in *B. boddaerti*, which was given free access to breathe in water as well as in air. From these high levels the values decreased to 3.3 million/mm^3 and 13% (Table 2), when the individual was allowed to breathe only in water; the corresponding values for the individuals restricted to aerial breathing alone were 3.6 million/mm^3 and 11%. Subsequently the erythrocyte count stabilised around 3.2 million/mm^3 and the haemoglobin concentration around 12% in these individuals. The rockskipper *M. macrocephalus* is reported to stabilise the O_2 uptake level within 4 to 6 hr after it was forced to respire only in water or in air (Graham, 1973). An important feature to be noted was that the blood characteristics were similar in individuals allowed to breathe in water or in air. Perhaps this may explain the fact that the mudskipper *Periophthalmus sobrinus* (Teal and Carey, 1967) and the rockskipper *M. macrocephalus* (Graham, 1973) stabilised their O_2 uptake, when forced to respire only in water or air.

Whereas *B. boddaerti* forced to breathe only in water was quite normal even after 48 hrs, that allowed to breathe only in air succumbed to death after 51 ($\pm$ 2.8 SD) hrs exposure. Nevertheless, the ability of *B. boddaerti* to tolerate aerial exposure is greater than that recorded for the mudskipper *P. sobriunus* (37 hr; Teal and Carey, 1967) and for the Chilean clingfish *Sicyases sanguineus* (40 hr; Gordon *et al.*, 1970). A possible explanation for the death of these fishes is the loss of body weight due to evaporation of water, especially from the branchial chamber. *B. boddaerti* lost 23$\pm$3.7% of body weight, when succumbed to death. This value compares with that reported for the mudskipper *P. sobriunus* (22%; Teal and Carey, 1967) and the rockskipper (25%; Graham, 1973). Another reason is that these fishes could not eliminate CO_2 for want of sufficient water and accumulated CO_2 in the blood and body tissues beyond a critical level. Air-breathing amphibious fishes are known to have difficulties with aerial excretion of CO_2 (Johansen, 1970). For instance, rate of aerial CO_2 release from *M. macrocephalus* forced to respire aerially was much lower than that of O_2 consumption (Graham, 1973). The possibility that *B. boddaerti* succumbed to death for want of sufficient O_2 uptake, appears to be ruled out by the observations of Teal and Carey (1967) and Graham (1973). These authors found that the mudskipper and the rockskipper procured cutaneously 40 to 48% of the total oxygen requirement, when allowed to breathe only in water or only in air or in both. These amphibious skippers (including *B. boddaerti*) have well developed cutaneous vascularization and hence could very effectively respire cutaneously. Further work is in progress to know whether CO_2 accumulation or water loss is the primary reason for the death of the mudskipper *B. boddaerti*.

Tilapia mossambica:

To study the effect of salinity, the euryhaline freshwater fish *T. mossambica* (6+0·5 g) was chosen. Healthy individuals were gradually acclimatized to 7, 14, 21 and 28% S at 30°C; the aquaria were continuously aerated (Po_2: around 150 mm Hg) and the fish were fed on mutton once a day.

The mean values obtained for the blood characteristics of *T. mossambica* kept in freshwater were 3.2 million erythrocytes/mm³ and 7.2% haemoglobin (Table 3). The following values (million mm³) are available in the literature for erythrocyte counts of different species of *Tilapia*: 1.7 to 2.1 for *T. zilli* (Farghaly *et al.*, 1973), 0.9 for *T. galilaea*, 1.2 for *T. nilotica*, 1.3 for *T. kurea* and 1.6 for *T. zilli* (Badawi and Said, 1971). Uniformly, the Egyptian *Tilapia* Spp have lower erythrocyte counts than the Indian *Tilapia*; however, the Egyptian *Tilapia* Spp compensate the lower counts by increasing the size of erythrocytes. Haemoglobin concentration (%; ml / 100 ml) was 7.2 for *T. mossambica*, 5.8 to 7.6 for *T. zilli* (Farghaly *et al.*, 1973), 6.3 for *T. galilaea*, 6.5 for *T. nilotica*, 8.2 for *T. kurea* and 8.7 for *T. zilli* (Badawi and Said, 1971). Since Badawi and Said (1971) and Farghaly *et al.* (1973) have not described the feeding history and other conditions, to which the test Tilapias were exposed to, it is difficult to compare the value of *T. mossambica* to any one of them. Even the capture methods and handling stress are known to affect the blood characteristics in fish (Bouch and Ball, 1966; Hattingh, 1973).

With increasing salinity there was a steady decrease in erythrocyte counts of *T. mossambica* from 3.2 million/mm³ in freshwater to 2.2 million/mm³ in 28%.S, but there was no statistically significant change in the haemoglobin concentration. Farghaly *et al.* (1973) reported the following values for erythrocytes (million/mm³) : 2.1 at 30°C (possibly in freshwater), 1.7 in freshwater (possibly at 16°C?), 1.7 at 10%.S, 2.0 at 20%.S and 2.2 at 40%.S; the corresponding values for haemoglobin concentration were 7.7, 5.8, 6.2, 6.5 and 7.4%. From the last 4 values, one may infer that there is a steady increase in the erythrocyte counts and haemoglobin concentration with increasing salinity. But it becomes confusing, when the first values are considered. More information is required before any conclusion is drawn.

Effect of Po_2 of water

To follow the changes in blood characteristics of *T. mossambica* kept in non-aerated aquaria, a number of individuals (kept and fed in separate aquaria containing freshwater at 30°C) was sacrificed on the O, I, II, III and IV day; on these days, the Po_2 in the non-aerated aquaria averaged 152, 98, 76 48 and 35 mm Hg. Hence, it has been possible to follow the changes in the blood characteristics as a function of (fluctuating and) decreasing Po_2.

Individuals sacrificed from aquaria containing freshwater fully saturated with O_2 on the 0 day exhibited 3.1 million erythrocytes/mm³ and 7% haemoglobin. The erythrocyte counts fluctuated around 3 million/mm³ during the subsequent 4 days, when the Po_2 decreased rapidly from 152 to 35 mm Hg (Table 3). However, the haemoglobin concentration showed a statistically significant increase to 8.4% on the II, III and IV day, after water change when the Po_2 was 76, 48 and 35 mm Hg respectively. The set of experiment was repeated twice, each time on a minimum of 4 or 5 individuals/day for each group and consistent values were obtained. Hence, Po_2 level lower than 76 mm Hg induces adaptive changes in the blood characteristic of *T. mossambica*, namely increase in haemoglobin concentration.

The tropical air-breathing fishes inhabiting freshwater swamps, where the Po_2 levels are usually below 60 mm Hg (Willmer, 1934), exhibit higher blood oxygen capacities than aquatic breathers found in more oxygenated water. However, the compensatory changes in the blood characteristics of *T. mossambica* to the decreasing Po_2 appear to be partial in view of the fact that a 5 g *T. mossambica* decreased its O_2 uptake from 2.25 ml/hr at the Po_2 of 150 mm Hg to 0.67 ml/hr in freshwater holding a Po_2 of 50 mm Hg at 30°C (Job, 1969). In Job's respirometer, *T. mossambica* had no access to oxygen from air. In our aquaria, *T. mossambica* stayed at the water surface and commenced breathing air orally from the II day onwards when the Po_2 decreased below 75 mm Hg. Such Po_2-induced behavioural changes are not uncommon among other teleosts (e. g. Moss and McFarland, 1970). Hence, it is very likely that the Po_2-induced changes in blood characteristics may become fully compensated in *T. mossambica*, which has access to breathe in water and in air. Such adaptive changes in the blood characteristics and behaviour of *T. mossambica* appear to be of great survival value only within the range of Po_2 between 75 and 30 mm Hg; for, more than 50% of *T. nilotica* exposed to the Po_2 of about 30 mm Hg at 24°C succumbed to death within 24 hour (Mahdi, 1973).

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