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Research Article

EFFECTS OF LIFE STAGES, SPAWNING AND FIGHTING BEHAVIOURS ON BIMODAL RESPIRATION IN SIAMESE FIGHTING FISH, *BETTA SPLENDENS* (REGAN)

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ABSTRACT

Bimodal respiration was studied in Siamese fighting fish *Betta splendens* in relation to life stages, spawning and fighting behaviors. The rates of aquatic and total respiration of test animals were decreased with an advancement of life stages from fry to adult. However, the trend was reversed in aerial respiration. The mean percentage of aquatic respiration was 100, 96, 45 and 46 as against aerial respiration 0, 4.4, 55 and 53 in the fry, fingerlings, adolescent and adult stages of *B. splendens* respectively. There was no significant ($P>0.05$) difference found on per-cent aquatic or aerial respiration in adolescent and adult stages of *B. splendens*. Male individuals exhibited the more metabolic rate (aquatic, aerial and total respirations) at pre- and post-spawning significantly ($P <0.01$) as compared to female individuals. Aerial respiration was high in male *B. splendens* prior to fight between themselves while after fighting, aquatic and total respirations were significantly ($P<0.05$) increased in male individuals. After fighting, aerial respiration was drastically declined to 16 and 19 times in the loser and winner male *B. splendens* while aquatic respiration was increased to 3 times in both males.

Keywords: Air breathing organ, Respiratory transition, Aggressive male, Energetic cost, *Betta splendens*.

INTRODUCTION

The flamboyant Siamese fighting fish, *Betta splendens* has attracted the interest of fish hobbyists for over a century due to its behavioral characteristics, convenient size, hardiness and especially its exquisitely brilliant and spectacular colour variations. It grows to a maximum size of 6cm and comes to the surface waters to take air using its labyrinth organ. The fry are totally dependent on their gills; the labyrinth organ which is involved in aerial respiration develops at 3 to 6 weeks of age, depending on the general growth rate, which can be highly variable. The metabolic rate of animal is dependent on various factors such as temperature, nutrition, and season, sex, stage in life cycle, body weight and activity (James, 2009). Bimodal oxygen uptake in relation to body weight was studied in few cultivable organisms (Ojha *et al.*, 1979; Sampath *et al*., 2007; Vasumathi, 2001). However, there is no literature on the bimodal respiration in an ornamental fish *Betta splendens* as a function of its life stages (fry, fingerlings, adolescent and adult), and spawning and fighting behaviors. *B. splendens* is an obligatory air breathing fish and found to be a very good research tool. Hence, the present study has been undertaken to study the bimodal respiration in *B. splendens* and also to find out the critical transitional state of shifting aquatic breathing to aerial breathing as a function of life stages.

MATERIALS AND METHODS

In the present study, there are three series of experiments were conducted.

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Series 1. Bimodal respiration in relation to life stages: Healthy and active fishes of four different stages, fry (0.0095 mg), fingerlings (49 mg), adolescent (296 mg) and adult (723 mg) *B. splendens* were selected from the laboratory bred brooders. The test animals were starved for 24 hr prior to commencement of the experiment. The chosen 4 stages of fish was separately introduced into the respiratory chambers (fry and fingerlings reared in 500 ml; others in 750 ml capacity) and allowed 1 hr for acclimation. Triplicates were maintained for each stage. The size and number of animals per animal chamber (1-20) were varied to achieve a similar ratio of animal weight at different stages. Simple respire manometric technique was followed to estimate aquatic and aerial respiration (bimodal) of fish simultaneously. Aerial respiration of fish was measured by multiplying the constant (calibrated value) with the rise of indicator fluid in the capillary tube of manometer. For the estimation of aquatic respiration, 10ml of water was drawn at every hour through the outlet and dissolved oxygen content was measured following the unmodified winklers method. Oxygen level at the end of each hour was considered as the initial reading for the next hour estimation. The difference between the initial and final oxygen contents represented the aquatic respiration of the animals. After three hours, the water was changed. The next set of reading was taken after 1hr like previously. The rate of oxygen consumption was calculated using the following formulae.

$$\text{Rate of aquatic respiration } (\text{ml } O_2\ g^{-1}h^{-1}) = \frac{\text{Volume of oxygen consumed from water (ml } h^{-1})}{\text{Weight of the fish (g)}}$$

$$\text{Rate of aerial respiration } (\text{ml } O_2\ g^{-1}h^{-1}) = \frac{\text{Volume of oxygen consumed from air (ml } h^{-1})}{\text{Weight of the fish (g)}}$$

$$\text{Rate of total respiration } (\text{ml } O_2\ g^{-1}h^{-1}) = \frac{\text{Volume of oxygen consumed from air+water (ml } h^{-1})}{\text{Weight of the fish (g)}}$$

Series 2. Spawning behavior on bimodal respiration: Active individuals of male and female adolescent (296 mg) and adult (723 mg) staged *B. splendens* were collected from the laboratory bred brooders. They were separately reared in 750 ml respirometer chamber and then bimodal respiration was estimated at prior to commencement of the spawning. The male and female individuals of adolescent and adult staged *B. splendens* were separately paired and reared in circular jars in the ratio of 1:1. After spawning, both male and female fish were isolated quickly and transferred to the same respirometer chamber and estimated the bimodal respiration like first series of experiment. Triplicates were also maintained.

Series 3. Fighting behavior on bimodal respiration: Adolescent and adult staged male *B. splendens* were selected from the laboratory bred brooders. They were separately reared in 750 ml respirometer chamber and estimated the bimodal respiration (aquatic, aerial and total) and it is considered as respiration prior to fighting. Thereafter, two males of adolescent and adult staged *B. splendens* were reared together in two circular tanks separately and allow them to fight with one another. When the fighting was completed, they were immediately transferred to separate respirometer chambers and estimated the bimodal respiration like 1st series of experiment and it is considered as respiration after fighting. Student "t" test was used to determine the significance of mean values between different life stages of *B. splendens.* Correlation and regression were applied following the least square method (Zar, 1984).

RESULTS

The rate of aerial respiration was high in adolescent stage of *B. splendens* and it significantly ($P < 0.05$) declined in adult stage, however aerial respiration did not occur in fry and just begin at fingerling stage (Figure1). A significant and negative correlation was obtained between the rate of total or aquatic respiration and life stages/body weight; however aerial respiration showed the positive trend (Table 1). In the present study, 296 mg body weight is the adolescent stage at which the air breathing organ gets completed (Plate 1) in *B. splendens* because at stage/body weight, the oxygen consumption through aquatic respiration decreased by 50% and at the same time the aerial respiration crossed over 50% level (Figure 2). However the large sized *B. splendens* (729 mg) consumed more oxygen from air (53%) than from water (47%) of its total volume of oxygen consumed (0.0159 ml $O_2\ g^{-1}\ hr^{-1}$).

During spawning, male *B. splendens* exhibited a higher respiration (aquatic, aerial and total respiration) than it's female. Besides test animals registered high respiration at pre-spawn as compared to post spawn. The rate of aerial respiration of male *B. splendens* at pre-spawn was 0.095 ml $O_2\ g^{-1}\ hr^{-1}$ and it significantly ($P < 0.01$) declined to act 0.37 $O_2\ g^{-1}\ hr^{-1}$ at post spawn. A similar trend was observed in the female and in the percentage of aerial respiration also (Table 2).

In male *B. splendens*, prior to fighting, the rate of aerial respiration was significantly ($P < 0.05$) higher than their aquatic respiration. After fighting, aerial respiration drastically declined to 16 and 19 times in the loser and winner male *B. splendens* while aquatic respiration increased to 3 times in both males (Table 3).

Table 1. Regression functions of aquatic, aerial and total respirations rates in *Betta splendens* as a function of life stages. The correlation coefficient (r) and its significant levels (P) are given (df = 23).

Parameters	Y = a + bx	r Value	Significant
Rate of aquatic respiration	Y = 0.42 – 0.09X	-0.927	P < 0.01
Rate of aerial respiration	Y = - 0.04 + 0.04X	+0.652	P < 0.05
Rate of total respiration	Y = 0.38 – 0.06X	-0.836	P < 0.05
Per-cent aquatic respiration	Y = 124.14 - 20.88x	+0.810	P < 0.05
Per-cent aerial respiration	Y = - 24.14 + 20.88x	0.810	P < 0.05

Table 2. Aquatic, aerial and total respirations (mlO_2 g^{-1} h^{-1}) *Betta splendens* during pre spawn and post spawn. Each value is the mean ($\overline{X}$ ± SD) performance of six observations.

Parameters	Spawning behavior			
	Pre-spawn		Post-spawn	
	Male	Female	Male	Female
Rate of aquatic respiration	0.084± 0.007	0.058± 0.002	0.061± 0.002	0.052± 0.002
Rate of aerial respiration	0.095± 0.005	0.074± 0.003	0.037± 0.003	0.016± 0.002
Rate of total respiration	0.179± 0.008	0.132± 0.004	0.099± 0.002	0.068± 0.003
Per–cent aquatic respiration	46.95± 2.18	44.02± 1.62	62.12 ± 2.09	76.54 ± 1.82
Per–cent aerial respiration	53.76± 1.64	56.00± 1.67	37.88 ± 2.07	23.46 ± 1.82
Student's 't' test				
	Pre-spawn male Vs pre-spawn female		Post-spawn male Vs post- spawn female	
Rate of aquatic respiration	t = 7.88; P < 0.01		t = 6.92; P < 0.01	
Rate of aerial respiration	t = 8.08; P < 0.01		t = 13.13; P < 0.01	
Rate of total respiration	t = 11.75; P < 0.01		t = 19.38; P < 0.01	

Table 3. Effect of the fighting behavior on the rates of aquatic and aerial and total respirations (ml O_2 g^{-1} h^{-1}) in males *Betta splendens.* Each value is the mean ($\overline{X}$ ± SD) performance of six observations. Values in parenthesis (+ or -) represents the per-cent difference over before fighting behavior).

Parameters	Before fighting		After fighting	
	Male – I	Male – II	Male – I(Loser)	Male – II(Winner)
Rate of aquatic respiration	0.081 ± 0.001	0.079 ± 0.004	0.228 ± 0.014(+156%)	0.258 ± 0.005(+263%)
Rate of aerial respiration	0.096 ± 0.004	0.098 ± 0.001	0.0063 ± 0.0001(-94%)	0.005 ± 0.0001(-94%)
Rate of total respiration	0.176 ± 0.004	0.177 ± 0.003	0.234 ± 0.014(+27%)	0.263 ± 0.020(+94%)
Per-cent aquatic respiration	48.27 ± 1.00	47.49 ± 1.12	96.98 ± 0.14	97.26 ± 0.25
Per-cent aerial respiration	52.27 ± 1.13	52.51 ± 1.12	2.74 ± 0.14	2.30 ± 0.19
Student's 't' test				
	Before fighting		After fighting	
	Male I Vs Male II		Male I Vs male II	
Rate of aquatic respiration	t = 0.80; P > 0.05		t = 4.48; P < 0.01	
Rate of aerial respiration	t = 1.50; P > 0.05		t = 16.88; P < 0.01	
Rate of total respiration	t = 0.46; P > 0.05		t = 2.24; P < 0.05	
Per–cent aquatic respiration	t = 0.34; P > 0.05		t = 4.37; P < 0.01	
Per–cent aerial respiration	t = 1.16; P > 0.05		t = 4.19; P < 0.01	

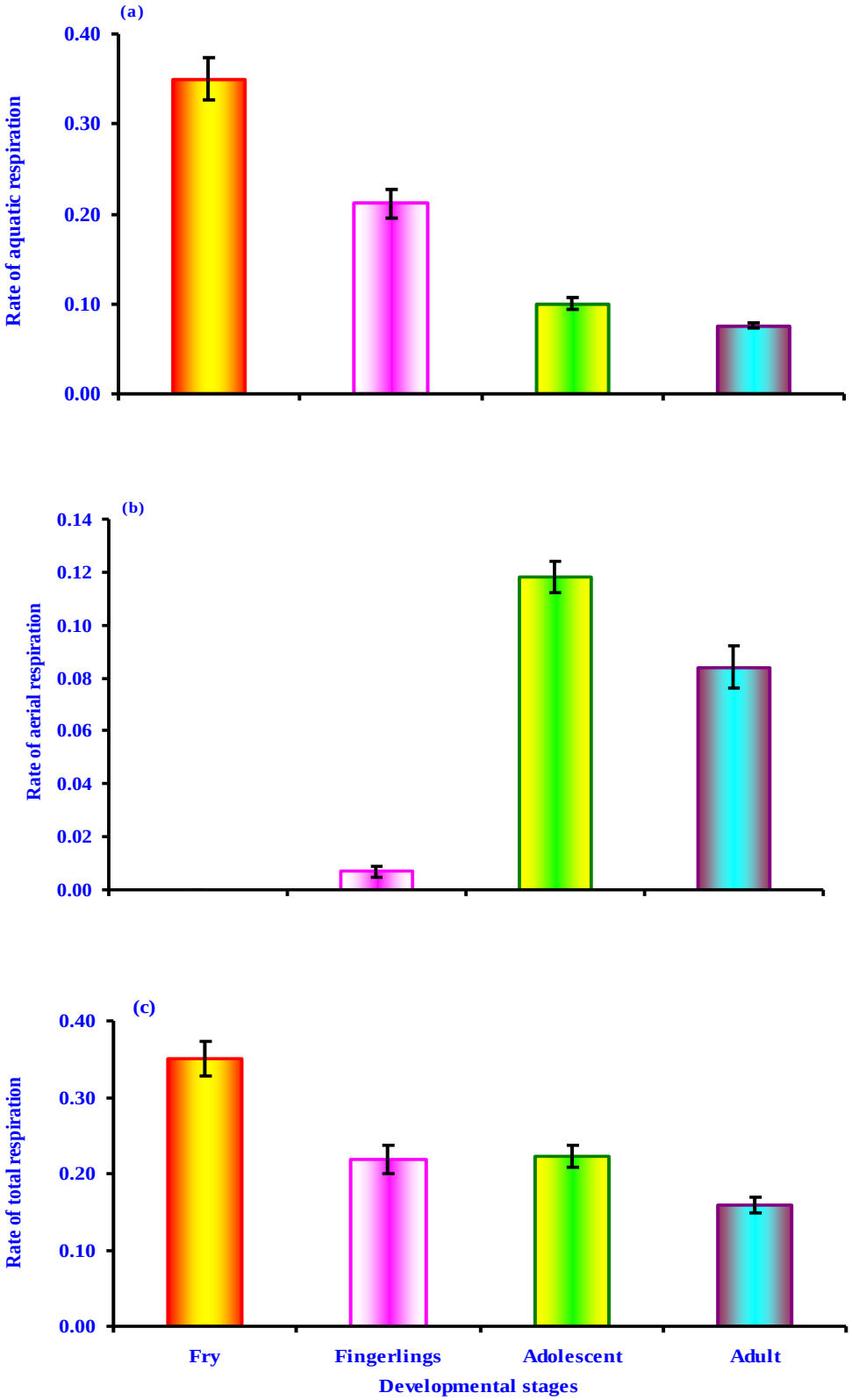

Figure 1. Effect of developmental stages on the rates of aquatic (a), aerial (b) and total (c) is respirations (mlO_2 g^{-1} h^{-1}) in the *Betta splendens.*

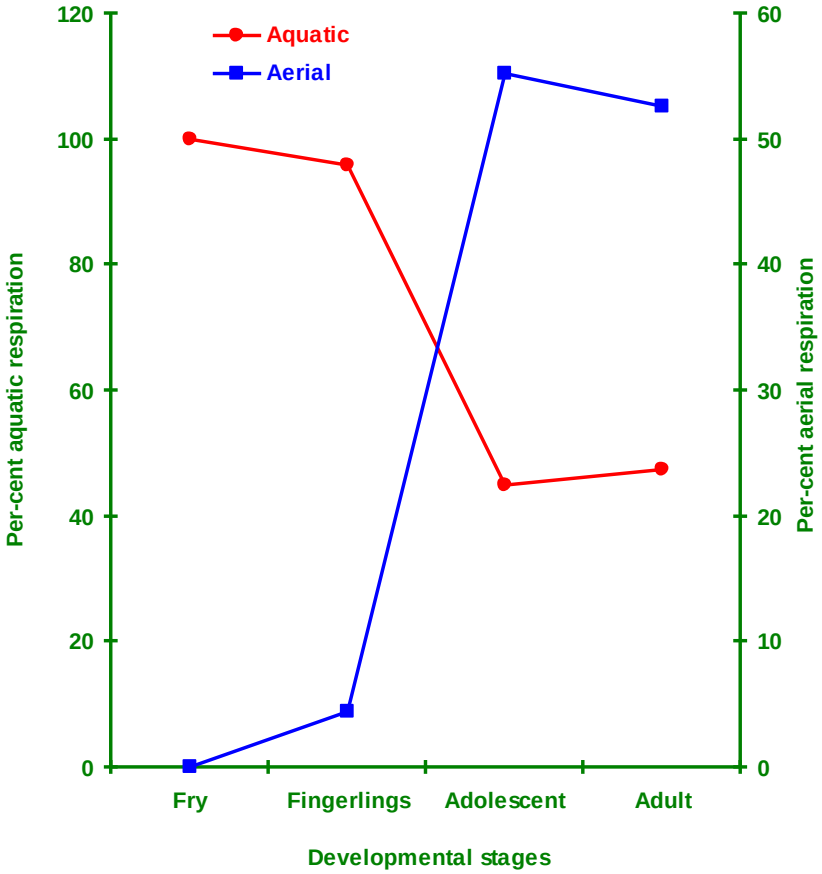

Figure 2. Effect of developmental stages on the percent of the aquatic and aerial respirations animals (mlO_2 g^{-1} h^{-1}) in *Betta splendens* as a function of developmental stages.

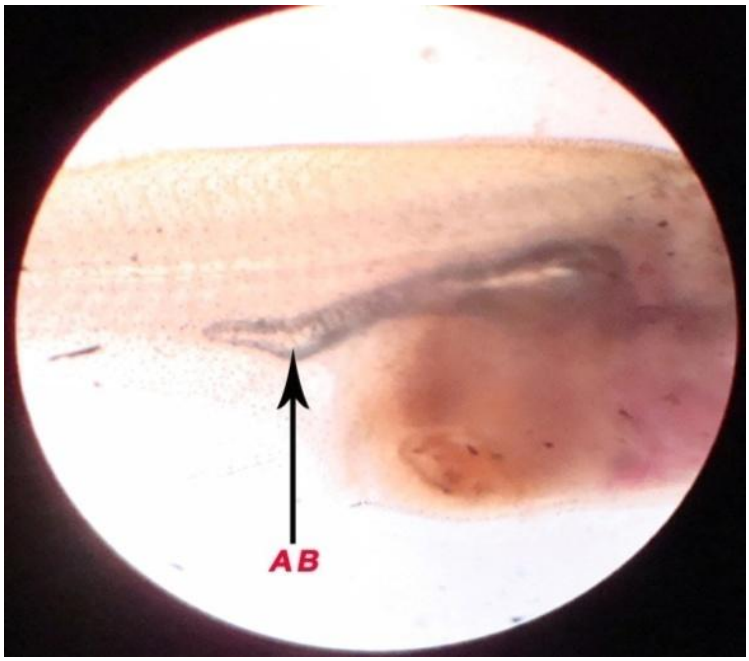

Plate 1. *Betta splendens* is showing the development of air bladder (AB).

DISCUSSION

The present study reveals that, the rate and percentage of aquatic respiration showed a declining trend while aerial respiration showed an increasing trend as a function of life stages in *B. splendens*. It indicates the shifting of respiration from aquatic to aerial during the development of *B. splendens* which is known as respiratory transition. Vasumathi, (2001) reported that the stage at which aerial respiration crosses to 50% of total respiration is the critical stage when the development of air breathing organ is completed.

Sex is one of the most important factors which modify the metabolic rate. The male fish registered the higher metabolic rate than female during its courtship behavior. It might be due to the more aggressive nature towards revolving around the female, stretching its caudal fin to attract the female, chasing and biting the female and collect all the eggs and squeezed them into the bubble nest at post-spawning.

The fighting males are intensively concentrated on fighting with each other and even refrained from coming to the surface and gulp atmospheric air and thereby total respiration evidently declined. In this circumstance, fighting males are compelled to utilize aquatic oxygen for active metabolism which in turn enhanced the aquatic respiration. Castro *et al*. (2006) was found that the metabolic cost increased in aggressive *B. splendens* males only after fighting which supports the observation made in the present study.

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