

Respiratory Metabolism as a Function of Oxygen Tension at Different Life Stages of Blue Morph, *Pseudotropheus lombardoi*

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Abstract

Oxygen consumption per unit weight is an objective and versatile characteristic describing the level of metabolism. The metabolic rate gives an indication of energy expenditure on the life supporting functions associated with growth, feeding and reproduction. The present study was conducted to determine the effect of oxygen tension on respiratory metabolism on different life stages of *Pseudotropheus lombardoi* and the cost of parental care in mouth brooding is estimated by their metabolic rate during their oral incubation. Metabolic rate decreased linearly at statistically significant rate with increasing biomass. In relation to sex, the metabolic rates of males were higher than in females. During mouth brooding, metabolic rate of *P. lombardoi* was 70% more than the non-mouth brooding female fish and 40% more than the spent fish.

Introduction

Metabolism is a physiological process reflecting the energy expenditure of living organisms and hence their food requirement. The metabolic rate of fish is usually measured by their rate of oxygen consumption. Information on oxygen consumption is not only useful in comparative physiology, but in fish culture and fishery management as well. It provides insight in solving the problems associated with rearing and transporting live fish. In general, fishes are able to utilize 80% of oxygen dissolved in water. This figure goes down to 10% under stressful conditions of low dissolved oxygen, reduced gill function and water temperature (Shepherd and Bromage, 1988). It has been reported as a valuable index of the overall physiological activity of animals and is generally used as an indicator under stress conditions either due to pathological state of the animal or adverse, ambient environmental conditions (Rao and Padmavathi, 2004). Rate of gas exchange correlated with survival, productivity and growth. Fish respiratory function is controlled by the medullary respiratory centre, which generates rhythmical stimuli responsible for the functioning of branchial system (Klyszeiko et al. 2003). The centre receives information from branchial O₂ and CO₂/pH

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chemoreceptors that are sensitive to the concentration of respiratory gases concentration in both blood and water (Sundin et al. 2000), from proprioceptors sensing changes in respiratory muscles activity (Ballintijn and Alink, 1977), and from mechanoreceptors that measure the speed of water flow through the gill (Burlison and Milsom, 1995).

Mouth brooding cichlids incur metabolic cost in providing oxygen to the developing embryos and in removing the metabolic wastes from them. Live bearing fishes also have additional metabolic demand for the formation of placental analogs and or uterine nutrient secretions and for the increased work for the cardiac system which thereby increased ventilation rate (Blackburn et al. 1985). The experimental fish, *P. lombardoi* (Blue morph) take approximately 6 months to reach sexual maturity. The metabolic activity at each stage of their lifetime gives an idea about its biological function. Moreover, the sex differentiation in *P. lombardoi* occurs at a stage when it reaches about 4cm and weighs about 1.0g. As maturation of the fish plays an important role in recruitment, the estimation of metabolic rate of these fishes during maturation is also imperative. In addition, these fishes are mouth brooders. Keeping all these parameters in mind, the present study was designed to find out their metabolic activity during different developmental stages of *P. lombardoi*.

Materials and Methods

The metabolic activity at each stage of their life cycle gives an idea about its biological function. So their respiratory metabolism was estimated every month during the entire period of development.

Respirometer

Respirometers used in the present study was constructed after Job (1957). The respirometers were of closed type model. They were wide mouthed conical flasks of 575 mL capacity closed with a one-holed rubber stopper fixed with a thistle funnel. The thistle funnel stem went at least 2/3 into the flask which served as the inlet. The outlet was a side glass tube fixed closed to the bottom of the flask to which a rubber tube was attached and was kept closed with a pinch cock.

Experimental Design

Developmental stages of the experimental animal as small as 0.01g to the maximum of 14.0g were chosen for the investigation. For our convenience the various stages of the fish are named as stage I (0.015 ± 0.002 g), stage II (0.080 ± 0.015 g), stage III (0.13 ± 0.02 g), stage IV (1.1 ± 0.02 g), stage V (4.7 ± 0.15 g) and stage VI (8.23 ± 0.15 g). Before starting the experiment, the experimental animals were starved for a period of at least 24 hr to avoid the influence of recent feeding on metabolism. Every time a new experiment was started with the introduction of three healthy fish (stage I-V) and one fish (stage VI) into the respirometer with little stress. Respirometers were

tightly closed after filling them with tap water. After the introduction of the fish into the respirometers, sampling of water was done with an interval of 30 min.

At the time of sampling the pinch cock was released and a few drops of water were deliberately allowed to flow down as it might be the residual water in the rubber tube and the samples were collected in the microwinkler's bottle. To compensate water loss from the respirometer, while sampling, a small quantity of water was poured into it through the thistle funnel. Necessary correction was also made for the water added to the respirometer through the thistle funnel before sampling. Water from the respirometer was sampled every 30 min and continued up to 3 hr or till the experimental animal showed signs of asphyxiation inside the respirometer.

The experimental water samples were analysed for the dissolved oxygen content using unmodified Winkler's method (Ellis et al.1946). The mean value was converted into pO_2 (mmHg) at the experimental temperature following Pierce (1973). As the respirometer is a closed type, the fish were exposed initially to a higher partial pressure of oxygen and then slowly exposed to lower pO_2 .

Formulae

$$\text{mg } O_2 \cdot l^{-1} = \frac{N \times \text{mL of titrant} \times 8 \times 1000}{V_R - V} \times \frac{V_S}{V_R}$$

where, N = Normality of $Na_2S_2O_3$ (0.001 N)

V_S = Volume of sample titrated

V_R = Volume of reagent bottle

V = Volume of reagents added

$$pO_2 = \frac{X \times 160}{7.63}$$

$$\text{Where, } X = \frac{O_2 \text{ in control sample} + O_2 \text{ in experimental sample}}{2}$$

7.63 = Oxygen saturation level at 30 °C

From the data obtained, a graph was drawn for the rate of O₂ consumption as a function of different O₂ tension. Regression analysis and two way ANOVA was carried out using MS Excel.

Results

The metabolic rates of juvenile *P. lombardoi* decreased with decreasing partial pressure in the respirometer. In other words, the metabolic rate increased linearly with increasing pO₂. It is interesting to mention here that the metabolic rate of stage III fish showed a significant reduction of 6-7 times than stage I fish. But in stage II, the metabolic rate did not show wide variation when compared with stage III fish (Table 1).

Table 1. The metabolic rate (mgO₂·g⁻¹·hr⁻¹) of juvenile (stage I, II & III) *P. lombardoi* as a function of partial pressure of oxygen (mmHg).

Size Groups	pO ₂ (mmHg)				
	80	90	100	110	120
Stage I	1.4	2.6	3.4	4	4.5
Stage II	0.2	0.65	1.21	1.61	1.9
Stage III	0.1	0.40	0.57	0.70	0.8

The metabolic rates of male *P. lombardoi* invariably showed an increasing trend as the pO₂ increased. For example, in stage VI, the metabolic rate increased from 0.1 mgO₂·g⁻¹·hr⁻¹ to 0.27 mgO₂·g⁻¹·hr⁻¹ as the pO₂ increased from 90 to 110 mmHg (Table 2). Similar trend was also observed in other stages of fish.

The metabolic rates of females also exhibited a similar trend as shown by the male counterparts of the same age group. Invariably, the metabolic rates increased linearly as the pO₂ increased. The metabolic rate in stage IV fish gave a value of 0.1 mgO₂·g⁻¹·hr⁻¹ to 1.75 mgO₂·g⁻¹·hr⁻¹ as the pO₂ increased from 95 to 120 mmHg (Table 3).

The metabolic rates during incubation followed a positive linear relationship with increasing oxygen tension. The metabolic rate in the incubating fish was found to be increased from 0.16 mgO₂·g⁻¹·hr⁻¹ to 0.41 mgO₂·g⁻¹·hr⁻¹ as the pO₂ increased from 90 to 120 mmHg (Table 4 and Fig. 1).

When metabolic rate of the mouth brooding fish was compared with the spent fish, the metabolic activity of the former was higher than the spent fish. For example, the metabolic rate of mouth brooding fish at 90 mmHg was 0.16 mgO₂·g⁻¹·hr⁻¹ while for the spent fish, it was 0.12 mgO₂·g⁻¹·hr⁻¹. In general, the rate of oxygen consumption recorded in all oxygen tension for spent fish was lesser than this mouth brooding counterpart (Table 4 and Fig.1).

Table 2. The metabolic rate ($\text{mgO}_2\cdot\text{g}^{-1}\cdot\text{hr}^{-1}$) of male (stage IV, V & VI) *P. lombardoi* as a function of partial pressure of oxygen (mmHg).

Size groups	pO ₂ (mmHg)					
	95	100	105	110	115	120
Stage IV	0.1	0.4	0.9	1.3	1.6	1.75
Stage V	0.22	0.27	0.32	0.36	0.41	0.44
Stage VI	0.04	0.12	0.15	0.18	0.25	0.28

Analysis of two way ANOVA revealed that, the biomass of the experimental species affected the rates of oxygen consumption more significantly than the variation in the partial pressure of oxygen as the F values were higher between size groups than between pO₂. Regression analysis was carried out to find out the relationship between pO₂ and metabolic rate and the regression lines also showed a linear trend of increased metabolic rate with increasing oxygen tension.

Table 3. The metabolic rate ($\text{mgO}_2\cdot\text{g}^{-1}\cdot\text{hr}^{-1}$) of female (stage IV, V & VI) *P. lombardoi* as a function of partial pressure of oxygen (mmHg).

Size groups	pO ₂ (mmHg)				
	90	95	100	105	110
Stage IV	0.4	0.91	1.4	1.8	2.12
Stage V	0.3	0.45	0.49	0.55	0.59
Stage VI	0.1	0.16	0.21	0.24	0.27

Table 4. The metabolic activity ($\text{mgO}_2\cdot\text{g}^{-1}\cdot\text{hr}^{-1}$) of *P. lombardoi* during mouth brooding as a function of partial pressure (mmHg).

Variations	pO ₂ (mmHg)				
	90	100	110	115	120
Mouth Brooding Fish	0.16	0.22	0.32	0.38	0.41
Spent Fish	0.12	0.22	0.23	0.24	0.25

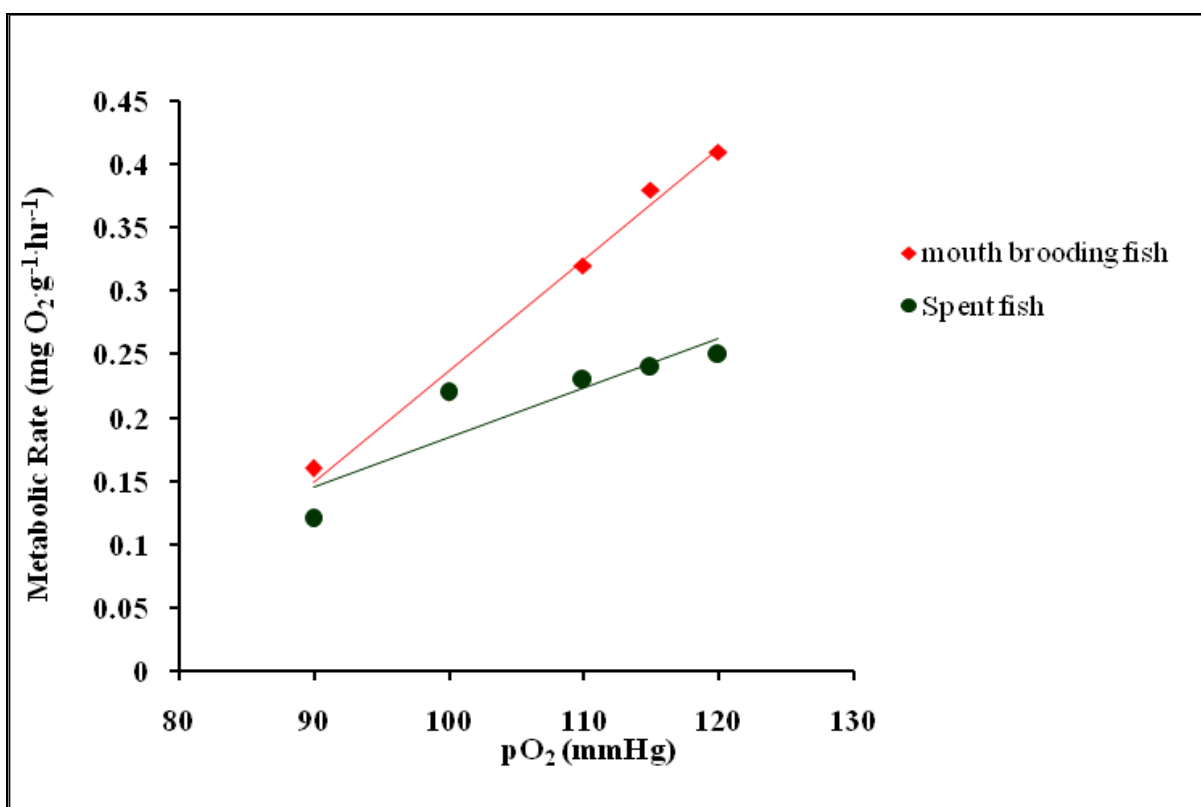


Fig. 1. The metabolic activity ($\text{mgO}_2\text{g}^{-1}\text{hr}^{-1}$) of *P. lombardoi* during mouth brooding.

Discussion

Metabolism corresponds to the energetic cost of all the processes required to maintain life including ventilation, circulating body fluids, protein synthesis, maintaining ionic gradients and osmotic work (Johnston, 1993).

Influence of size group on metabolic rate

The observed data in the present investigation suggest that fish undergo metamorphic, metabolic and ecological changes from larvae to adult. The results of the present investigation also revealed the fact that as the size group increased the metabolic rate decreased. The metabolic rate decreased linearly at statistically significant rate with increasing biomass in all stages of fish. It is noteworthy to mention here that at 80 mmHg, a 14 fold reduction in metabolic rate at stage III was observed when compared with stage I of juveniles (Table 1). The diminishing trend of rate of oxygen consumption per unit weight increase is in conformity with the findings of Job (1959), Narayanan and Ramachandran (2001), Klyszejko et al. (2003) and Thanalakshmi et al. (2004). The explanation for this gradual decrease in metabolic rate which follows the increase of body dimensions could also be due to the influence of negative exponential correlation by growth pattern,

increase of enzyme as related to body mass and disproportionate increase of tissue of low metabolic activity such as skeleton, fatty and connective tissue as opined by Prosser and Brown (1961).

Influence of pO₂ on metabolic rate

There was a good correlation between the pO₂ and the metabolic rate. The metabolic rate invariably showed an increasing trend as the pO₂ increased irrespective of the different stages of fish. Linear relationship between the partial pressure of oxygen and the rate of oxygen consumption clearly indicate that the partial pressure of oxygen influences the rate of oxygen consumption of fish. Similar trends have also been reported by various authors in various tropical animals such as *Tilapia mossambica* (Job, 1969), *Poecilia reticulata* (Narayanan and Ramachandran, 2001) and *Cyprinus carpio*, *Heteropneustes fossilis* and *Mystus keletius* (Jeyaseeli and Narayanan, 2004).

Influence of sex on metabolic activity

The experiments revealed possible differences in oxygen consumption in relation to sex. The metabolic rates of males were higher than in females and declined with increasing biomass in both sexes (Table 2 and 3). At 95 mmHg partial pressure, the male fish consumed oxygen nearly 10 times higher than that of female of equal weight. This finding agrees with the results previously reported by Kazakov and Khalyapina (1981). The present deviation of metabolic rate of males and females of *P. lombardoi* might be due to its aggressive behaviour. Though male and female were well known for their aggressiveness, compared with the female the males were more aggressive as reported by Naish and Ribbink (1990). Fry (1971) also pointed out that a major component of metabolic energy expenditure can be swimming activity. Thus, the active aggressive behaviour of male fish could be the reason for their higher metabolic rate.

Influence of breeding activity on metabolic activity

In general, the breeding activity is a costly phenomenon which increases the metabolic activity in all living organisms. Comparative measurements of the respiration rate of gestating and non-gestating fish indicate the changes in mass-specific metabolic rate with development. In mouth brooders, the cost of incubation was even higher than the live bearers and oviparous fishes. The current study clearly shows that the mouth brooding fish has higher metabolic activity than the mature female fish and the spent fish (Fig. 4). The metabolic rate of mouth brooding *P. lombardoi* was 70% more than the non-mouth brooding female fish and 40% more than the spent fish. The results of this study are in agreement with earlier works on gestating female *Sebastes schlegeli* (Boehlert et al. 1991) and in the sailfin molly *Poecilia latipinna* (Timmerman and Chapman, 2003). For example, *S. schlegeli* increased its metabolic rate to the tune of 68% just prior to parturition; while the sailfin molly *P. latipinna* had a metabolic rate 27% higher than non-gestating females, while the live bearing surf-perches (*Rhacochilus vacca* and *Embiotoca lateralis*) increased their metabolic rate by 53% during late gestation (Webb and Brett, 1972). They have reported that

metabolic activity in live bearing fishes increased with embryonic development so that embryos prior to parturition might have a higher mass-specific oxygen requirement than maternal tissues resulting in temporary increase in the total routine oxygen requirement of the female. The experimental fish, *P. lombardoi*, is a mouth brooding cichlid. During mouth brooding, it refuses to take feed. It churns the eggs constantly and that churning activity might increase the metabolic activity.

Conclusion

Metabolic rate is a manifestation of energy expenditure on the various life-supporting activities associated with growth, feeding and reproduction. The present study substantiates the basic concept that juveniles show higher metabolic activity than adults. In mouth brooders, the energy requirement is more for incubating their young ones in their mouth rather than the live bearers and oviparous fishes. From the present study, it reveals that the cost of oral incubation for the experimental fish, *P. lombardoi* was 70%. Further, it could be stated that the energy needed by the incubating fish for their routine activity in spite of being dormant was conserved to be utilized for incubation. Thus, the cost of incubation may be more than the calculated 70%.

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