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Growth and survival status in the mosquitofish *Gambusia affinis* exposed to mild hypoxia

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Abstract

This study examined whether chronic exposure to mild hypoxic condition can limit growth and survival in the mosquitofish *Gambusia affinis*. Exposure of fish to mild hypoxic conditions (4.0 mg O₂/L) for a period of 25 days resulted in reduction in mean body weight gain, specific growth rate (SGR), survival rate and protein efficiency ratio (PER), whereas the feed conversion ratio (FCR) remained high compared to those of controls on day 25. On the other hand, re-exposure of fish to normoxic condition (between days 26-40) after initial exposure to hypoxia for 25 days partially improved the mean body weight gain, SGR, PER and FCR values, but not survival rate. These results indicate that chronic moderate hypoxic condition causes reduction in growth and survival rate by interfering with the efficacy of feed conversion into biomass in *G. affinis*. It is suggested that monitoring of optimum dissolved oxygen levels is critical for attaining maximum growth and survival in mosquitofish culture.

Keywords: Growth, survival, mosquitofish, specific growth rate, moderate hypoxia.

1. Introduction

Fish may have reduced fitness, low egg production, poor quality offspring, and a higher risk of mortality due to poor environmental conditions [1]. Low dissolved oxygen (DO) concentration (<2 mg/L) in water masses due to natural processes alone or in combination with anthropogenic processes [2] is a serious problem to the life of fish. The low concentration of DO in natural waters can lead to a condition called hypoxia for most fish [3]. Hypoxia has negative effect on growth and survival of fish [4]. Severe hypoxia-induced reduction in growth rate is observed in many estuarine fishes such as the black sea bass *Centropristis striata* [5], the southern flounder *Paralichthys lethostigma* [6] and the spot croaker *Leiostomus xanthurus* [7]. On the other hand, suppressive effect of moderate hypoxia on growth is reported only in few estuarine species such as juvenile summer flounder *Paralichthys dentatus* and winter flounder *Pseudopleuronectes americanus* [8]. However, growth and survival in relation to moderate oxygen deprivation has not received much attention in viviparous fish. The mosquitofish *Gambusia affinis* is a viviparous species, which reproduces until 20-34 months of age. The development period for fry inside the mother is approximately 28 days, which may fluctuate depending on stress and environmental conditions [9]. The influence of mild hypoxia on growth and survival is not known in this species. Therefore, the aim of the present study was to determine the growth and survival of the mosquitofish *G. affinis* exposed to moderate hypoxic condition.

2. Materials and methods

2.1 Experimental procedure

The female *G. affinis* weighing 0.25-0.27 g were collected from the ponds in Karnatak University Campus, Dharwad, brought to the laboratory and reared in plastic tubs measuring 60 cm diameter with 30 cm depth under natural photoperiodic conditions (11.45 ± 0.5 L; 12.15 ± 0.5 D) at a stock density of ten fish in four liters of water per tub for one month prior to the commencement of experimentation. The fish were fed twice everyday with commercially available food pellets (Taiya pet feed, Chennai, India) at the rate of 4% of the body weight. Fifty female fish with regressed abdomen (post-parturition) were identified and used for the experimentation.

A group of fish (n=10) were sacrificed on the day of commencement of the experiment and served as initial controls. The remaining fish were divided into four groups. The first group was maintained in normoxic condition (7.25 ± 0.26 mg/L) for a period of 25 days (25d controls), whereas the second group were exposed to moderate hypoxic condition (4.0 ± 0.1 mg/L) for a period of 25 days. The fish in the third group were also exposed to hypoxia initially for 25 days and then kept in normoxic condition until 40 days (hypoxic recovery group). Parallel controls were maintained for 40 days in normoxia (40d controls). The tubs were provided with aerator and aquatic plants to ensure the supply of oxygen for 25d and 40d controls and recovery group (between 26-40 days). For these groups, recirculation of water was done on alternate days to keep up normoxia, whereas for the hypoxic fish, the low DO level was achieved by not providing aerators to water. The temperature (25.15 ± 2.25 °C) was not artificially manipulated.

2.2 Growth and survival status

To assess the growth and survival status, different parameters such as mean body weight gain, Specific Growth Rate (SGR), Condition Co-efficient (k), Stock Survival (S), Feed-Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) were calculated. The formulae (after Kozłowski *et al.* 2012) used to calculate the above parameters are given below.

- Mean body weight gain = mean final body mass - mean initial body mass
- $SGR = 100 \times \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Rearing period days}}$
- $K = 100 \times \frac{\text{Body weight (g)}}{\text{Body length (cm)}}$
- $S = 100 \times \frac{\text{Final abundance}}{\text{Initial abundance}}$
- $FCR = \frac{\text{Weight of food consumed}}{\text{Final Stock Biomass} - \text{Initial Stock Biomass}}$
- $PER = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Quantity of protein feed}}$

3. Results and discussion

Reduction in growth rate following exposure to severe hypoxia was observed in many estuarine fishes such as the black sea bass *Centropristis striata* [5], the southern flounder *Paralichthys lethostigma* [6], the spot croaker *Leiostomus xanthurus* [7] and the gulf killifish *Fundulus grandis* or the Mummichog *F. heteroclitus* [10, 11]. The severity of hypoxia necessary to suppress growth has often been shown to be temperature-dependent in some species. For example, a significant growth limitation was observed under even

moderate levels of hypoxia (3.5 - 5.0 mg/L) associated with high temperature in juvenile summer flounder and winter flounder [8]. The present study reveals growth retardation in response to mild hypoxia for the first time in a viviparous species. While the temperature was not artificially manipulated, the mean body weight gain (Fig. 1A) and SGR (Fig. 1B) were lower in hypoxic group compared to those of 25d and 40d controls. Although these values were slightly higher in hypoxic recovery group compared to hypoxic fish, they were lower compared to both 25d and 40d controls suggesting that there was a lag in the growth due to mild hypoxic environment.

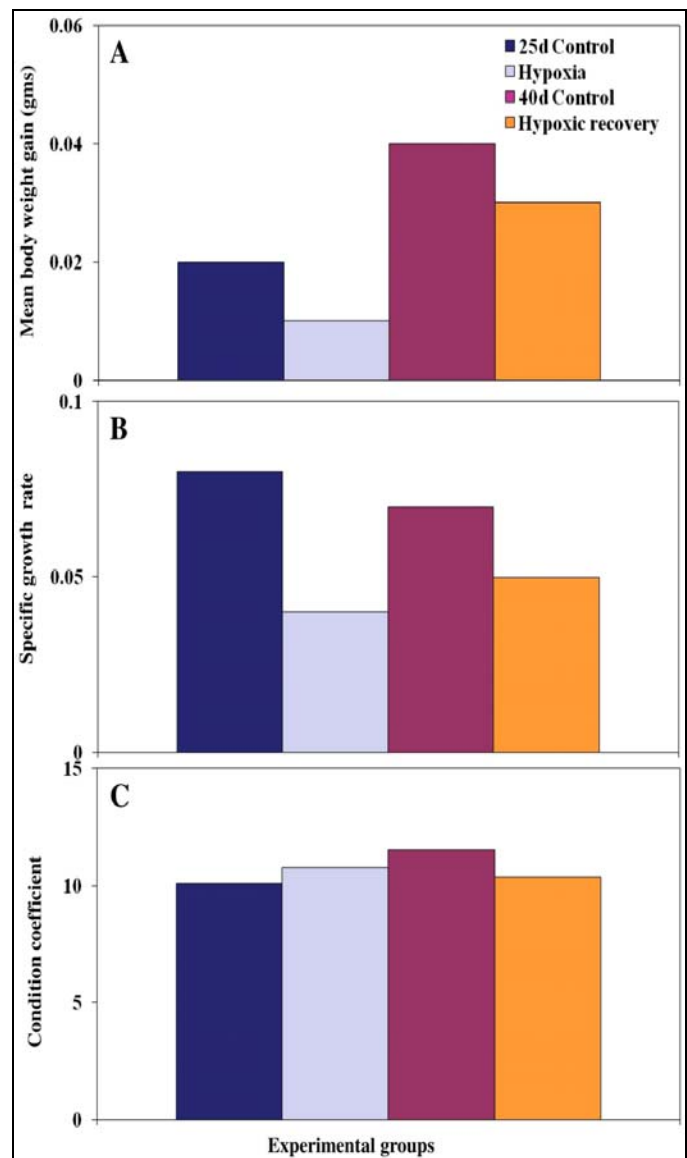


Fig 1: (A-C): Effect of hypoxia on mean body weight gain (A), specific growth rate (B) and condition coefficient (C) in *G. affinis*

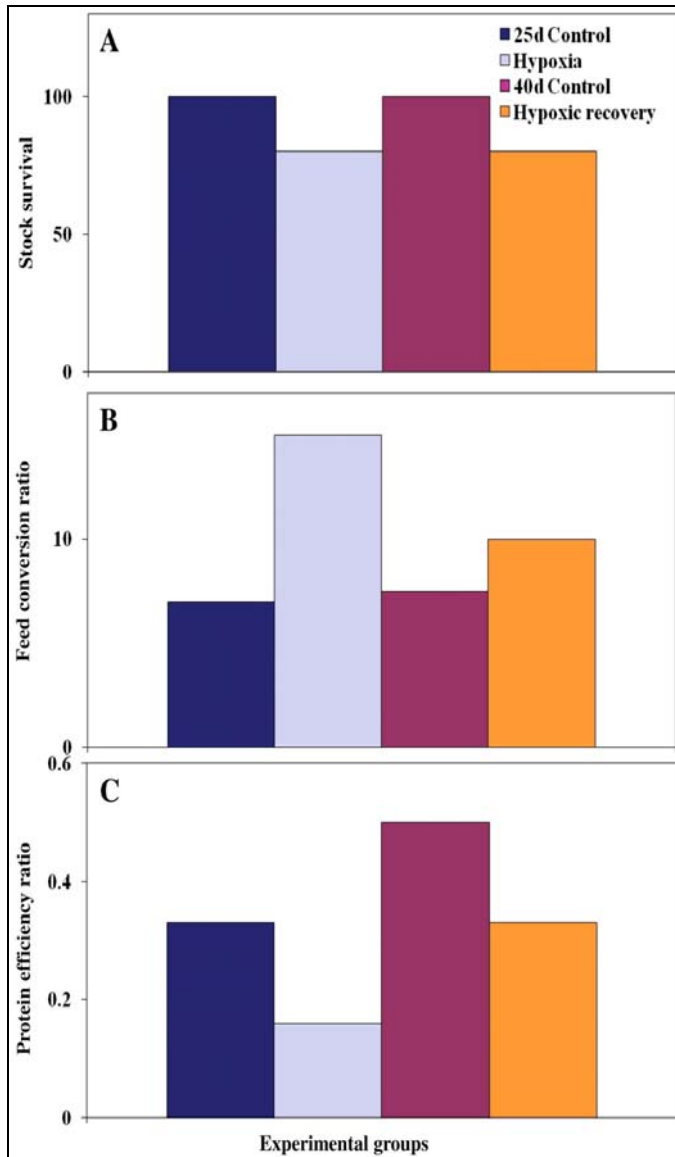


Fig. 2 (A-C): Effect of hypoxia on stock survival (A), feed conversion ratio (B) and protein efficiency ratio (C) in *G. affinis*.

Condition factor has been used as an indicator of health in fish [12]. The condition factor provides information on the variation of fish physiological status [13]. In the present study, it appears that the rearing conditions were not much detrimental to the survival of the fish as the condition co-efficient remained almost same for all experimental groups (Fig. 1C). Furthermore, natural aquatic ecosystems have long been associated with fish deaths due to hypoxic condition [14, 15, 16]. A decrease in the survival rate was observed in the zebrafish *Danio rerio* exposed to severe hypoxia (0.9 mg O₂/L) [17], whereas exposure of the same species to moderate hypoxic condition (4.3 mg O₂/L) did not affect growth and survival [18]. In the present study, the lowest survival rate (80%) was recorded in fish exposed to mild hypoxia and hypoxic recovery group in contrast to 100% survival rate in both 25d and 40d controls (Fig. 2A). These results indicate that the moderate hypoxic condition affects the survival rate of the mosquitofish shortly within 25 days of initial rearing period, and that the mortality is prevented once normoxia is reestablished. Good nutrition is important for the production of healthy and high quality fish [19]. Survival might be affected due to the metabolic response of fish to prolonged periods of food deprivation [20]. As most of the oxygen consumed by fish is

used for ATP production through the electron transport chain in normoxic condition [21], it is probable that the metabolic energy balance might be affected in fish exposed to low oxygen conditions, which might interfere with growth. However, in the present study, it is unlikely that the growth was retarded due to non-availability of food as the fish were fed daily twice to satiation.

The FCR is a measure of an animal's efficiency in converting feed mass into body mass. It is known that lower FCR values indicate better efficiency as observed for other fishes [22, 23]. In the present study, feeding the mosquitofish with food pellets consisting of 32% crude protein resulted in higher FCR in fish exposed to hypoxia compared to 25d and 40d controls, whereas it remained slightly higher in hypoxic recovery group compared to hypoxia alone group (Fig. 2B). In addition, the PER was lowest in fish exposed to hypoxia compared to 25d and 40d controls (Fig. 2C). While highest PER was noticed in 40d controls compared to other groups, this value was slightly higher in hypoxic recovery group compared to hypoxia alone group. Taken together, these results suggest that the retardation in fish growth might be related to decrease in efficacy of making use of food as energy source under moderate hypoxic conditions.

4. Conclusion

This study shows that moderate hypoxic condition causes reduction in the growth and survival rate, whereas re-exposure of hypoxic mosquitofish to normoxic condition partially improves the mean body weight gain, specific growth rate, FCR and PER but not survival rate. Therefore, it is suggested that monitoring of optimum dissolved oxygen levels is critical for attaining maximum growth and survival in mosquitofish culture.

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