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The Effect of Different Acclimation Temperatures on Oxidative Muscle Proportions of the Tilapia *Oreochromis mossambicus*

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Abstract - This study investigated the effect of acclimation temperature (16, 25, 30 and 35°C) on oxidative muscle proportions of yolk-sac larvae, feeding larvae and fry stages of the tilapia *Oreochromis mossambicus*. Over the thermal range tested, the proportions were highest for the yolk-sac stage and significant decline occurred in feeding larvae. The proportions were significantly higher for the fry stages at 16, than at 25, 30 and 35°C. Changes in oxidative muscle proportions and succinic dehydrogenase activity at different acclimated temperatures may explain the positive compensation of oxidative metabolism in *O. mossambicus* myotomal muscle during different developmental stages.

Many poikilothermic species show complete or partial compensation in metabolic rate and movement following acclimation to different temperatures (Hazel and Prosser 1974). It has been suggested that the compensations which help maintain movement in either cold or warm temperatures are changes in the enzyme activity of muscle cells (Sidell 1980) and in the proportions of muscle fiber types (Johnston and Lucking 1978). Since succinic dehydrogenase exhibits marked compensation for temperature following a period of cold acclimation in a wide variety of organisms, it has been used in several metabolic-compensation studies (Hazel 1972).

Fish species that remain active over a wide thermal range show compensatory changes that permit some constancy of energy output after temperature acclimation (Shaklee et al. 1977). Tilapias are eurythermal tropical warmwater fishes. *O. mossambicus* is characterized by a rapid rate of acclimation to changes in water temperature (Allanson and Noble 1964). Thus, this experiment compares oxidative metabolism changes in the myotomal muscle of *O. mossambicus* during different early developmental stages, in relation to different acclimation temperatures.

O. mossambicus broodstock (35-55 g) were obtained from the Stirling University tropical laboratory and acclimated 4-5 weeks at 25, 30 and 35°C in three 100-l aquaria filled with tap water and constantly aerated and filtered. Water temperature was adjusted using water heaters. The broodstock were fed once daily with dry pellets until satiation. The yolk-sac larvae were collected from the mouths of females and reared in a 10-l holding tank adequately aerated. The larvae and fry collected from females acclimated to 25°C were maintained in a separate aquarium and acclimated to 16°C water temperature. The larvae and fry were fed *ad libitum* with dry fine particulated food, twice daily.

Table 1. Test of significance for oxidative muscle proportions of *Oreochromis mossambicus* at different developmental stages and temperatures.

Stages*	Probability	Temperature (°C)	Probability
I - II	<0.001	16-25	<0.001
I - III	<0.001	16-30	<0.001
I - IV	<0.001	16-35	<0.001
II - III	<0.001	25-30	<0.001
II - IV	<0.001	25-35	<0.001
III - IV	<0.001	30-35	>0.010

*Stages are defined in Fig. 2.
Number of samples for each stage = 9.

Four acclimation temperatures (16, 25, 30 and 35°C) were tested on four developmental stages: yolk-sac (stage I: 7.3-7.8 mm), first-feeding larvae (stage II: 10.8-15.2 mm), fry (stage III: 17-20 mm) and large fry (stage IV: 25-30 mm). Table 1 lists the numbers of samples examined for each treatment.

For all stages, 20 µm sections were cut through the post-anal region (Fig. 1), at -21°C in a cryostat. The sections were stained for succinic dehydrogenase activity using the nitro-blue tetrazolium (NBT) method (Pearse 1972), and photographed onto tracing paper and the outlines of the oxidative muscle proportions were traced. The area occupied by oxidative muscle was measured in cm^2 using a tablet digitizer interfaced with a computer.

ANOVA and Student's t-test were used to detect differences in the oxidative muscle proportions within the four developmental stages.

Maximum oxidative muscle proportions were observed in the yolk-sac stage and at 16°C. With the complete absorption of the yolk, the proportions declined in all stages. At the third stage (fry), there was a marked increase in the proportions at 16, 25 and 35°C, but a decrease at 30°C. At the fourth stage, the highest proportions were at 16 and 30°C, respectively. While at 25 and 35°C, they decreased. The data are presented in Fig. 2. Table 1 lists the statistical significance in the muscle proportions at different stages and temperatures.

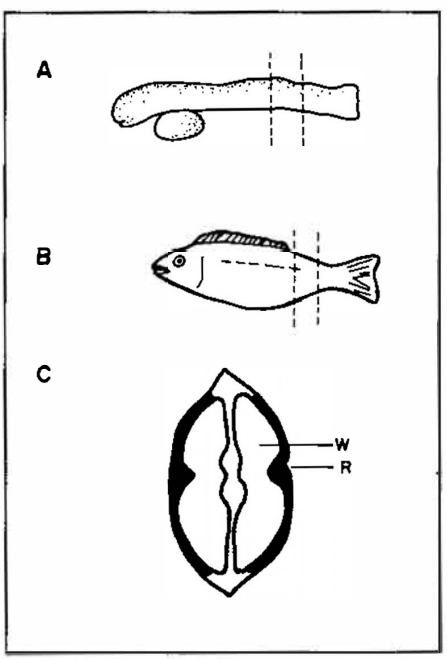

Fig. 1. Diagrammatic representation of sectioning site (post-anal) in (a) yolk-sac, (b) fry stage and (c) transverse section through the post-anal region. W(white/glyolytic muscle fiber), R(red/oxidative) muscle.

Yolk-sac larvae had high oxidative muscle proportions at all four acclimated temperatures, with highest values at 16°C. Batty (1984) suggested that early larval stages in herring rely on red (oxidative) muscle for oxygen consumption due to cutaneous respiration and undeveloped gills, which could explain the higher oxidative muscle proportions at the yolk-sac stage in *O. mossambicus*. Moerland and Sidell (1986) suggested that the proliferation of oxidative fibers during cold acclimation permits the fish to swim faster and ameliorates the impact of cold temperatures on sustained swimming capacity. Increased oxidative enzyme with cold acclimation may also offset the effects of low temperature on the

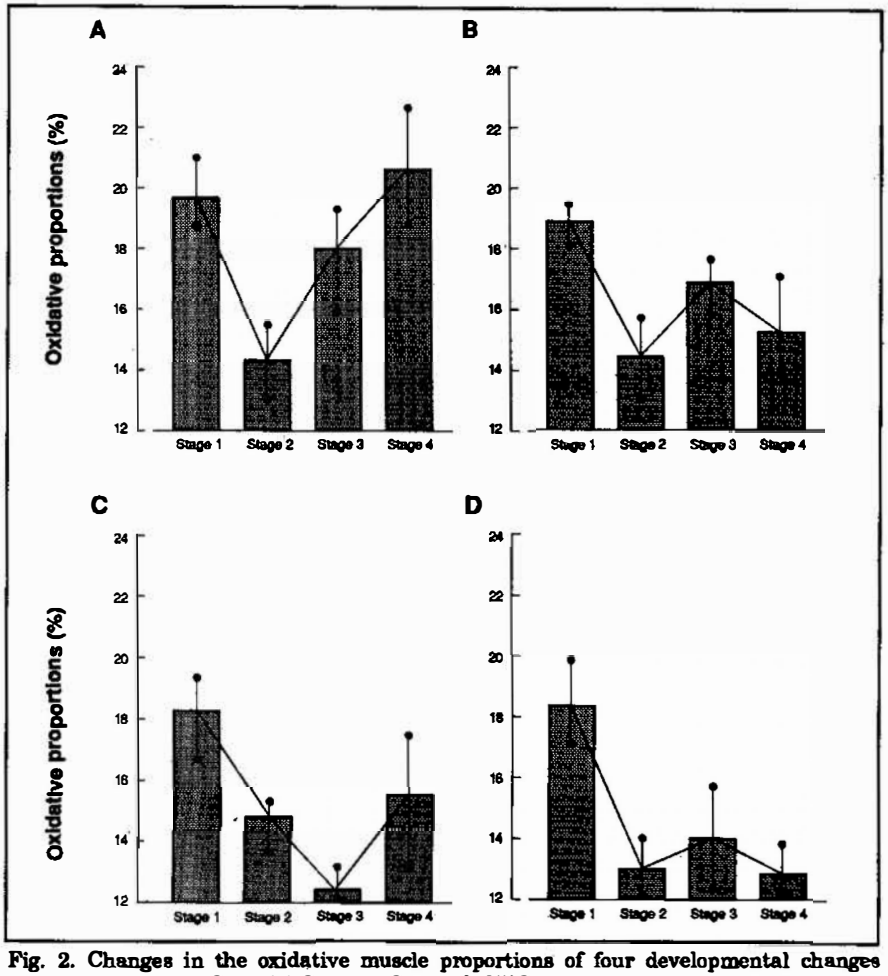

Fig. 2. Changes in the oxidative muscle proportions of four developmental changes acclimated to (a) 16°C, (b) 25°C, (c) 30°C and (d) 35°C.

*Histograms represent the mean ± SD of the fiber proportions.

Stage (I) Yolk-sac (7.3-7.8 mm)
Stage (II) Feeding larvae (10.8-15.2 mm)
Stage (III) Fry (17-20 mm)
Stage (IV) Fry (25-30 mm)

aerobic energy (ATP) supply (Johnston and Wokoma 1986). Succinic dehydrogenase activity increased during the yolk-sac stage over the thermal range tested (Al-Marzouk 1988). This suggests that the increased proportions of oxidative muscle with cold acclimation in *O. mossambicus* could conserve the sustained swimming ability and enhance the capacity for energy supply by oxidative metabolism.

El-Fiky et al. (1987) found that in the larval stage of cyprinids, the unicellular red-layer (RL) of muscle fiber contracts from the dorsal and ventral sides towards the lateral region, thereby decreasing their contribution to total muscle mass. The same developmental pattern has been observed in *O. mossambicus* (Fig. 3). This suggests that the decreased proportions of oxidative muscle at the second stage for all four acclimated temperatures were due to the decreased red layer contribution and the development of glycolytic muscle fibers. The lowest proportions found at 35°C could be due to the faster development of glycolytic fibers and growth of the larvae.

Fig. 2. illustrates a significant increase in the oxidative muscle proportions during the third stage at 16, 25 and 35°C, but not at 30°C. The latter may be due to the high proportions of glycolytic fibers (Al-Marzouk 1988) and to a delay in sustained swimming ability. The higher proportions at the other acclimated temperature could be due to the higher metabolic rate in the oxidative muscle, which is necessary to supply the energy needed to sustain swimming (Lin et al. 1973).

At the fourth stage, the proportions increased continuously at 16 and 30°C, temperatures at which the fry rely more on aerobic metabolism to sustain swimming and enhance ATP production. Moreover the glycolytic-muscle proportions decreased at these temperatures, but

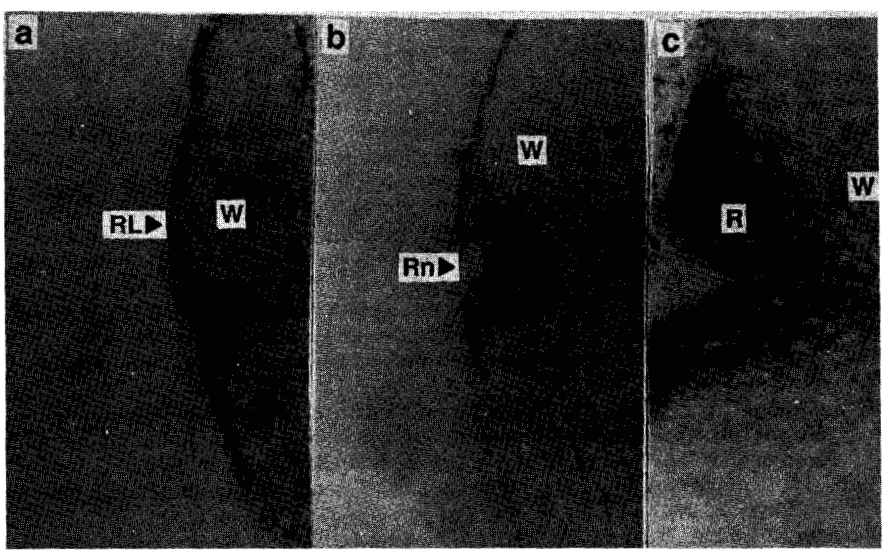

Fig. 3. Transverse sections illustrating the development of red-layer in (a) yolk-sac, (b) feeding larvae and (c) fry. RL (red-layer), Rn (nascent red muscle fibers arising from red layer by splitting), W (white muscle) and R (red muscle).

increased at 25 and 35°C, when the fry did not need high aerobic capacity to maintain swimming performance (Al-Marzouk 1988). Therefore, the part played by the aerobic red muscle decreased and the share of anaerobic white muscle increased.

Rome et al. (1985) suggested that poikilotherms can achieve some independence from the effect of seasonal changes in environmental temperature by modifying their musculature. Thus, it can be concluded that the muscle tissue of *O. mossambicus* fry and larvae show metabolic reorganization and adaptive response to changes in temperature, and can therefore maintain the metabolic requirements for certain activities such as feeding and swimming.

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